Understanding and managing canine distemper virus as a disease threat to Amur tigers

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Abstract

The endangered population of Amur tigers (*Panthera tigris altaica*) in the Russian Far East (RFE) faces an increasing risk of extinction due to infection with canine distemper virus (CDV). Short-lasting CDV infections are unlikely to be maintained in small populations of species with limited connectivity like tigers, where viruses fade out as susceptible hosts are depleted. Multi-host pathogens can persist in more abundant host species that can act as reservoirs of infection for threatened populations. This study combines assessments of host demography, serology and viral phylogeny to establish the relative contribution of domestic dogs and small bodied wild mesocarnivores to the maintenance of CDV, and as sources of infection for tigers. No antibodies were detected among tigers sampled prior to 2000 (n=19), but were measured in 35.7% of tigers in subsequent years (n=56), with at least five discrete transmission events occurring in one well-monitored population. Viral sequences from three tigers and one Far Eastern leopard (*P. pardus orientalis*) aligned within the Arctic-like clade of CDV, and shared recent common ancestry with viruses from 22 other wild carnivores from the region. Extensive spatial mixing of wild carnivore lineages suggested long chains of transmission consistent with a maintenance population. The exposure of tigers following 2000 coincides with increases in sable (*Martes zibellina*) numbers and hunting pressure, which could lead to greater pathogen prevalence and potential for spill over from a wild reservoir. The ratio of humans to dogs in rural areas in the RFE are among the lowest in the world (1.73), but the overall number of dogs has been stable during the period of increased CDV exposure in tigers. The only CDV sequence obtained from dogs shared high identity with Asia-4 clade viruses from dogs in Thailand, and was distantly related to wildlife sequences from the RFE. Serum antibodies were detected in dogs in all 26 communities where households were surveyed, but seroprevalence was higher in remote, less densely populated areas, suggesting possible transmission from wildlife. Although the maintenance of CDV in Russian dogs remains unconfirmed, the strong support for a wildlife reservoir limits options for managing the impact of CDV on tiger populations. The high turnover of large and often inaccessible populations of mesocarnivores combines with limitations in vaccine safety, efficacy and delivery, to render the control of CDV in a wildlife reservoir untenable. Managing the impact of CDV on Amur tigers must therefore focus on restoring the size and integrity of remaining tiger populations to withstand future outbreaks. The safety and efficacy of vaccine products for tigers should also be investigated, for use in low coverage vaccination strategies that could enhance the long-term persistence of tiger populations.

Table of Contents

List of Tables

List of Figures

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Sabrina at a mark tree, the evening before we met (Photo: L. Kerley, Zoological Society of London)

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Chapter 1 Introduction

Abstract

Infectious disease is capable of inducing declines in wildlife populations, and occasionally results in their extinction. Threatened populations are at greater risk from pathogens that are transmitted through density independent processes, including those contracted during social interaction (frequency dependent transmission), vector-borne diseases, or those that spillover from reservoirs of infection. With small, often fragmented populations, large carnivores are particularly vulnerable to the effects of infectious disease, especially multi-host pathogens like rabies and canine distemper virus (CDV). Recently CDV has been diagnosed in Amur tigers (*Panthera tigris altaica*) in the Russian Far East, threatening population viability, and indicating a need to consider management options. Available strategies include measures to 1) reduce disease incidence in the reservoir, 2) reduce spillover from the reservoir to the threatened host, or 3) reduce transmission within the threatened host itself. The use of vaccines to reduce transmission is more challenging in wild than in domestic host populations, and must address issues of vaccine safety, efficacy and delivery. Selection of control strategies should be based on epidemiological understanding of the disease system concerned, particularly the identity of host populations that contribute to the maintenance of the pathogen. This thesis assesses the relative contribution of domestic and wild carnivores to the maintenance of CDV in the Russian Far East, and their likely roles as sources of infection for Amur tigers. These findings will be interpreted in the context of potential control measures, to inform management recommendations aimed at minimizing the threat to remaining populations of Amur tigers.

Infectious disease and extinction

We are living through an age where species extinction is estimated at 100-1,000 times that of pre-Anthropocene levels (Pimm et al. 1995). Measured against human-mediated drivers such as habitat modification, over-exploitation, invasive species and climate change, infectious disease has been considered to be a relatively minor contributor to population decline and extinction (De Castro and Bolker 2005, Mace et al. 2008). While the importance of these anthropogenic drivers is not in question, infectious disease has been implicated in a growing list of species declines (Skerratt et al. 2007, McCallum et al. 2009,

Lorch et al. 2011). In many cases pathogens have had a profound effect on population size, but there are few examples where they have resulted in extinction (Thorne and Williams 1988, Carlton et al. 1991, Pounds et al. 1997, Schloegel et al. 2006). This chapter will examine the circumstances where infectious disease can increase the extinction potential of a population, and apply these observations to the case of canine distemper virus (CDV) and Amur tigers (*Panthera tigris altaica*) in the Russian Far East. With reference to potential management strategies to reduce the impact of CDV, the chapter will outline the key information gaps, which will be addressed as the main objectives of this thesis.

In many simple host-parasite systems, transmission follows a density dependent process where the parasite is unable to reduce host numbers to zero. Declines in host population through reduced survival or fecundity lead to a decrease in host density, which slows rates of transmission and enables host numbers to recover. However, under certain circumstances pathogens are able to threaten population survival, even where subject to density dependence, or where transmission occurs in a density independent manner (summarized in Table 1.1). Even in density dependent situations, a pathogen can threaten the survival of a host population, by reducing it to a size where extinction may occur due to stochastic processes. Without intervention, it is likely that outbreaks of CDV would have led to the extinction of the black-footed ferret (*Mustela nigripes*), whose populations had previous been reduced through habitat fragmentation and declining prey resources (Williams et al. 1988, Thorne and Williams 1988).

Populations can also be threatened indirectly, when pathogens reduce the availability of a critical resource such as food. The loss of eelgrass (*Zostera marina*) from the Atlantic seaboard of North America due to infection with the *Labyrinthula* slime mould, deprived the eelgrass limpet (*Lottia alveus*) of food and habitat, resulting in its extinction (Carlton et al. 1991). Other examples include the impact of Lagomorph infections on prey availability for Iberian lynx (*Lynx pardinus*, Castro and Palma 1996, Ferrer and Negro 2004), the role of *Yersinia* in the collapse of prairie dog colonies, which support black-footed ferrets (Thorne and Williams 1988), and the recovery of East African lions (*P. leo*) following the control of rinderpest virus (Packer et al. 2005). These examples demonstrate an ‘ecological cascade’, illustrating the profound effects that could occur wherever disease affects the structure of an ecosystem.

Table 1.1. Mechanisms and examples of risk factors that can precipitate the decline or extinction of threatened species due to infectious disease.

Extinction mechanism	Examples		Effects	Source
	Host	Pathogen		
Small host population - Stochastic effects (e.g. genetic bottlenecks, reduced immunity, sex imbalance), or Allee effects	Black-footed ferret	Canine distemper virus	Extinction likely	Thorne and Williams 1988
	Ethiopian wolf	Rabies	Decline	Laurenson et al. 1998
Frequency dependent infection - Transmission independent of host density (e.g. through sexual contact, or social structure)	Koala	*Chlamydia pecorum / C. pneumoniae*	Decline	McCallum 2012
	Tasmanian devil	Tasmanian devil facial tumour	Decline	McCallum et al. 2009
Vector-borne disease - Where survival of the vector is independent of declining host species	Hawaiian honeycreepers	Avian malaria /Avian poxvirus	Decline & extinction	van Riper et al. 1986
	Yellow-billed magpie	West Nile virus	Decline	Crosbie et al. 2008
Biotic reservoirs - Sympatry with more abundant maintenance host species	Christmas Island rodents	Trypanosoma spp.	Extinction	Wyatt et al. 2008
	Great apes	Ebola virus	Decline?	Leroy et al. 2004
	Arctic fox	*Otodectes cynotis*	Decline	Goltsman et al. 1996
Abiotic reservoir - Environmental stability of the pathogen	North American bats	*Pseudogymnoa-scus destructans*	Decline	Lorch et al. 2011
	Amphibians	*Batrachochytrium dendrobatidis*	Decline & extinction	Skerratt et al. 2007
Indirect - Pathogen depletion of resource on which host depends	Eel grass limpet	Labyrinthula spp.	Extinction	Carlton et al. 1991
	Iberian lynx	Rabbit haemorhhagic disease virus /myxoma virus	Decline	Castro and Palma 1996

The potential for pathogens to threaten host populations increases in situations where transmission continues despite a decline in host density. The frequency of certain behaviours such as sexual or territorial interactions, are not always mediated by the density of hosts, leading to opportunities for frequency dependent transmission (Begon et al. 2002,

Swinton et al. 2002). Aggressive encounters between Tasmanian devils (*Sarcophilus harrisii*) during reproduction and when feeding are unaffected by density, and facilitate the transmission of Tasmanian devil facial tumour disease (TDFTD), even in the face of widespread declines (McCallum et al. 2009). Modelling of sarcoptic mange transmission in British foxes (*Vulpes vulpes*) also suggests a frequency dependent process (Devenish-Nelson et al. 2014), which if applicable to Otodectic mange, could explain the near extinction of Arctic foxes (*V. lagopus*) on Mednyi Island (Goltsman et al. 1996). Despite the compelling theoretical linkage between frequency dependent transmission and host extinction, there are few examples of this occurring in the real world (De Castro and Bolker 2005). This may be due to a switch from frequency dependence to density dependence when populations reach very low levels (Ryder et al. 2007), or intermediate modes of transmission that operate in different temporal or ecological contexts (Smith et al. 2009, Morters et al. 2012).

The presence of biotic or abiotic reservoirs represents another mechanism that allows pathogens to avoid density dependent effects on a threatened host population (Begon and Bowers 1995, Woodroffe 1999, Haydon et al. 2002, Viana et al. 2014). The fungal pathogens *Pseudogymnoascus destructans* (responsible for white-nose syndrome in North American bats), and the chytrid fungus *Batrachochytrium dendrobatidis* (implicated in the decline and extinction of over 200 species of amphibians, Skerratt et al. 2007), both utilize environmental reservoirs and alternate hosts as sources of infection for dwindling populations (Murray et al. 2009, Lorch et al. 2011, 2013). A key element in reservoir-based systems is the capacity of the pathogen to infect multiple host species, thus expanding the pool of susceptible individuals, ensuring maintenance of infection and a continual source of 'spillover' for the declining population (Woodroffe 1999, Haydon et al. 2002).

Population declines and extinctions are most likely where several factors converge to create a 'perfect storm' of circumstances. Table 1.2 summarizes a series of cases, where infectious disease has driven declines, or extinction of wild populations. From this it is evident that small populations are particularly at risk to the effects of infectious disease, both through their inherent vulnerability to stochastic events, but also where genetic bottlenecks have increased their susceptibility to infection. It is this genetic similarity that

Table 1.2. Illustrates the multifactorial contributors that can lead to population decline and extinction from infectious disease

Example	Pathogen	Small host population	Frequency dependent infection	Vector-borne disease	Biotic reservoirs	Abiotic reservoir	Indirect	Source
Black-footed ferret	Canine distemper virus	✓			✓			Thorne and Williams 1988
Ethiopian wolf	Rabies virus	✓			✓			Laurenson et al. 1998
Ethiopian wolf	Canine distemper virus	✓			✓			Gordon et al. 2015
Koala	Chlamydia pecorum / C. pneumoniae	✓	✓					McCallum 2012
Tasmanian devil	Tasmanian devil facial tumour	✓	✓					McCallum et al. 2009
Amphibians	Batrachochytrium dendrobatidis		✓			✓		Skerratt et al. 2007
Hawaiian honeycreepers	Avian malaria /Avian poxvirus			✓	✓			van Riper et al. 1986
Christmas Island rats	Trypanosomes			✓	✓			Wyatt et al. 2008
Great apes	Ebolavirus				✓			Leroy et al. 2004
Arctic fox	Otodectes cynotis	✓			✓			Goltsman et al. 1996
North American bats	Pseudogymnoascus destructans					✓		Lorch et al. 2011
Eel grass limpet	Labyrinthula spp.	?					✓	Carlton et al. 1991
Iberian lynx	Rabbit haemorhhagic	✓					✓	Castro and Palma 1996
Iberian lynx	Feline leukaemia virus	✓	?		✓			López et al. 2009

allowed TDFT to proliferate within a population of devils so similar, that they have no immunological capability to recognize the transmissible tumour as foreign (McCallum 2008). Another feature of these case studies is the prominence of exotic infections, for which threatened hosts have yet to evolve defenses. In most cases these introductions follow the movement of pathogens along human transport networks, or introduction from domestic or pest species (Daszak et al. 2000, Cunningham et al. 2003). As human populations continue to rise, and gain in mobility, such ‘pathogen pollution’ events are likely to continue, and infectious disease may become a more prominent feature of species decline and extinction.

Infectious disease threats to wild carnivores

Several aspects of wild carnivore ecology and life history make their populations particularly sensitive to the effects of infectious disease (Murray et al. 1999, Purvis et al. 2000, Cardillo et al. 2014). Carnivores require larger areas, and generally occur at lower densities than species that meet their nutritional needs at lower trophic levels (Lindstedt et al. 1986). Habitat fragmentation limits the areas available for carnivores, leading to small and isolated populations that are susceptible to stochastic extinction (Crooks 2002), an effect that disproportionally affects predators of large body size (Crooks 2002, Cardillo et al. 2014). Predatory behaviour also brings carnivores into conflict with humans, which increases mortality along the edges of populations that extend beyond the boundaries of protected areas (Woodroffe and Ginsberg 1998). The combined effects of small population sizes, and barriers to dispersal in multi-use landscapes also lead to genetic homogenization, with deleterious effects on immunity (O'Brien et al. 1985, Roelke et al. 1993, Pokorny et al. 2010).

Pathogens affecting threatened carnivores tend to have a wide host range, ensuring a continued source of infection, in areas of sympatry with abundant susceptible hosts (Table 1.3, Murray et al. 1999; Woodroffe 1999). Domestic dogs are often considered to be the most likely sources of infection for threatened populations (Gascoyne et al. 1993, Kat et al. 1995, Roelke-Parker et al. 1996, Randall et al. 2004), but an abundance of susceptible wildlife could be equally important in some areas (Weiler et al. 1995, Craft et al. 2009, Viana et al. 2015). With high rates of mortality, rabies virus has been particularly prominent in the decline and extinction of wild carnivore populations (Gascoyne et al. 1993, Kat et al. 1995, Hofmeyr et al. 2000, Randall et al. 2004). Outbreaks of CDV are also commonly reported in wild carnivores (Table 1.3), but only in the case of the black-footed ferret was extinction considered a likely sequel (Thorne and Williams 1988). With pathogens like CDV, which invoke a strong protective immunity in a cohort of recovered animals (Greene and Appel 2006), survivors may be sufficient to repopulate an area once an outbreak has abated (Prager et al. 2012).

Table 1.3. Pathogens associated with the decline of endangered wild carnivores.

Species	Pathogen	Multi-host pathogen?	Source
African wild dog	Rabies	Yes	Cleaveland and Dye 1995
African wild dog	Rabies	Yes	Kat et al. 1995
African wild dog	Rabies	Yes	Scheepers and Venke 1995
African wild dog	Rabies	Yes	Hofmeyer 2000
Ethiopian wolf	Rabies	Yes	Laurenson 1998
Bat-eared fox	Rabies	Yes	Maas 1993
Blanford's fox	Rabies	Yes	McDonald 1993
Black-footed ferret	Canine distemper virus	Yes	Thorne and Williams 1988
Lion	Canine distemper virus	Yes	Roelke-Parker et al. 1996
Ethiopian wolf	Canine distemper virus	Yes	Gordon et al. 2015
Santa Catalina Island fox	Canine distemper virus	Yes	Timm et al. 2009
Baikal seal	Canine distemper virus	Yes	Grachev et al. 1989
Harbour seal	Phocine distemper virus	Yes	Dietz et al. 1989
Iberian lynx	Feline leukaemia virus	Yes	López et al. 2009
Grey wolf	Parvovirus (suspected)	Yes	Peterson et al. 1998
Mednyi Arctic fox	*Otodectes cynotis*	Yes	Goltsman et al. 1996
Sea otter	*Toxoplasma gondii*	Yes	Conrad et al. 2005
African wild dog	Anthrax	Yes	Creel 1995

Conservation status of tigers

Global populations of tigers are at an all time low, with numbers of reproductive females in the wild now fewer than 1,000 individuals (Walston et al. 2010). Pressure from agriculture, industry and urbanization has fragmented tiger habitat, such that remaining populations occupy less than 7% of their former range and more than half of the world's tigers are confined to habitat islands containing 25 or fewer animals (Sanderson et al. 2006, Walston et al. 2010). Despite their high profile, tiger declines are continuing, with the number of countries that support breeding populations falling from thirteen in 2006, to eight by 2015 (Goodrich et al. 2015). Only 21% of current tiger range is under some form of protection, although many of these reserves are severely constrained by shortages in budget, management, and enforcement (Forrest et al. 2011). Even in suitable habitat, tigers face a variety of threats, including competition with humans for prey resources, direct poaching to meet the demand for their body parts and retaliation due to conflicts with humans (Walston et al. 2010, Goodrich et al. 2015).

The Amur tiger subspecies once occupied vast areas of temperate forest in northeast Asia, extending from the Far East of Russia south through the Korean Peninsula, and west across Manchuria. In the early Twentieth Century carnivore control measures and a trade fueled by the demand from zoological collections drove Amur tiger numbers to as few as 20-30 individuals by the 1940s (Kaplanov 1948). Hunting of tigers was outlawed in 1947, and

collection for captivity was banned in 1956 (Smirnov and Miquelle 1999). Aided by the establishment of strictly-protected areas ('zapovedniks'), tiger numbers began to climb and by 2005 there were estimated to be between 331 and 393 adult and subadult Amur tigers, although the population may now have plateaued, or possibly slipped into decline (Miquelle et al. 2007, 2011).

In 2003, CDV was diagnosed in a tigress from the Russian territory of Khabarovskii Krai (province); the first time the disease had been detected in a wild tiger (Quigley et al. 2010). A further two cases were confirmed in 2010, with others suspected, leading to concerns that disease may represent a new threat to the population (Seimon et al. 2013, Gilbert et al. 2015). All three of these tigers were severely debilitated, with two dying spontaneously while in care, and one being euthanized (Gilbert et al. 2015). Analysis of 40 serum samples collected from tigers between 1992 and 2004 found antibodies to CDV in a further five tigers, all sampled from 2000 onward, and it was proposed that the virus may be newly emerging in the population (Goodrich et al. 2012, Seimon et al. 2013). Further details describing the initial detection of CDV in tigers in Primorskii are provided in Appendices I and II. A population viability analysis, incorporating an epidemiological SIR-D (susceptible, infected, recovered/dead) compartmentalized model predicted that CDV could increase the extinction potential of Amur tigers (Gilbert et al. 2014). The model exposed tigers to CDV through predation from a reservoir consisting of domestic dogs and/or wild carnivores, as well as during regular, but infrequent contact with other tigers. The model outcome was sensitive to increases in CDV prevalence (from 0.6% – 6.2%), and contact with the reservoir (1.65 – 3.87 contacts/tiger/year) with 50-year extinction risk probability increasing from 6.3% to 55.8% for a starting population of 18 tigers, compared to a control population without CDV (Gilbert et al. 2014). The model also indicated a disproportional outcome for small populations with a fifty-year extinction probability for a population of 25 tigers that was 1.65 times greater in the presence of CDV than control populations (Gilbert et al. 2014).

Several features of tiger ecology increases the risk represented by infectious disease. The Amur tiger population is small, and thinly dispersed with breeders maintaining large territories that mostly exclude tigers of the same sex (390 ±136 km^2 for females, and 1,385 ±539 km^2 for males, Goodrich et al. 2010). Thus, most tigers range beyond the boundaries of protected areas where they are at an increased risk from encounters with humans

(Woodroffe and Ginsberg 1998), with anthropogenic factors implicated in at least 80% of Amur tiger deaths (Miquelle et al. 2005, Goodrich et al. 2008). Deaths from CDV are thought to represent an additive cause of mortality (Robinson et al. 2015), placing a further burden on an already stressed population. In comparison to other solitary felids, tigers breed later in life and have a longer birth interval, which reduces their resilience to modest increases in mortality, such that populations take longer to recover after declines (Chapron et al. 2008).

Introduction to Morbilliviruses and canine distemper virus

Canine distemper virus (CDV) is a member of the family Paramyxoviridae, and the genus *Morbillivirus*, which includes some of the most important pathogens in human history. Morbilliviruses are single stranded, non-segmented, negative sense, RNA viruses encoded by genomes of between 15,690 to 16,050 nucleotides in length (Greene and Appel 2006, Nambulli et al. 2016). In addition to CDV, there are currently five species recognized within the genus: measles virus, rinderpest virus, *peste des petits ruminants* virus (PPRV), phocine distemper virus (PDV) and cetacean morbillivirus (CeMV). Also, a novel feline *Morbillivirus* (FeMV) has recently been described in domestic cats in Hong Kong, mainland China, Japan and the United States (Woo et al. 2012, Furuya et al. 2014, Sharp et al. 2016). However, the clinical presentation and genetic structure of FeMV differs from other Morbilliviruses, and its true relationship has yet to be resolved (Nambulli et al. 2016, Sharp et al. 2016).

Morbilliviruses are highly infectious, and share a similar pathogenesis. Due to their relative fragility, Morbilliviruses are generally transmitted horizontally through direct contact via infected aerosols, urine or faeces, although vertical transmission has been documented in humans, dogs and cetaceans (Krakowka et al. 1977, Fernández et al. 2008, Giusti et al. 2013). All Morbilliviruses are lymphotropic, and alveolar macrophages and dendritic cells in the lung serve as the most likely route of entry into the host (Lemon et al. 2011). The viral haemagglutinin (HA) glycoprotein binds to host cell receptors, and together with the fusion (F) glycoprotein mediates fusion and cell entry. The main host receptor utilized during early infection is the CD150, or signaling lymphocyte activation molecule/F1 (SLAM), present on B and T-lymphocytes and dendritic cells (Tatsuo et al. 2000, 2001,

von Messling et al. 2006, Melia et al. 2014). The virus then spreads systemically, via the lymphatic and blood system (Ludlow et al. 2014). Death of lymphocytes at this stage can result in profound leucopoenia and immunosuppression, the extent of which strongly influences the outcome of infection (Greene and Appel 2006). Once established, Morbilliviruses progressively make use of the nectin-4 receptor (also known as poliovirus receptor-like 4) to gain entry into epithelial cells (Muhlebach et al. 2011, Noyce et al. 2012, Birch et al. 2013, Melia et al. 2014). Infection of epithelial cells is associated with clinical disease including respiratory signs, erythema and fever, as well as transmission to other susceptible hosts (Sawatsky et al. 2012). During later stages of infection, viruses can invade the central nervous system, causing a demyelinating leukoencephalomyelitis, leading to progressive neurological signs including myoclonus, ataxia, plegia and seizures (Beineke et al. 2009, Ludlow et al. 2012, Duignan et al. 2014). In dogs infected with CDV, mortality rate varies with viral strain, with in excess of 25 to 75% of infections being subclinical, and mortality of up to 50% of dogs that develop disease (Appel 1987, Greene and Appel 2006). Dogs that survive infection develop a long lasting immunity that can remain protective for the remainder of their lives (Greene and Appel 2006).

Morbilliviruses show a propensity for infecting multiple host species (Table 1.4). The host range of CDV is the widest of all Morbilliviruses, and is the only member of the genus where natural infections of both terrestrial and marine species have been recorded (Table 1.4). Susceptibility to CDV is not confined to carnivores, with mortality reported in peccaries (*Tayassu tajacu*, Appel et al.1991), rodents (Origgi et al. 2013), and primates (Yoshikawa et al. 1989, Sun et al. 2010, Sakai et al. 2013). Species susceptibility to infection is mainly mediated through the conformation of the SLAM receptor, the structure of which appears to have coevolved with that of the Morbillivirus HA-genes (Ohishi et al. 2010).

Several features of Morbillivirus biology have important implications for disease control. All Morbilliviruses exist as a single serotype, and vaccines are capable of stimulating strong, and long-lasting immunity in susceptible hosts (Greene and Appel 2006). The devastating impact of rinderpest virus on agro-economies and food security motivated a global vaccine-based control programme, which was successful in eradicating the virus by 2011 (Food and Agriculture Organisation of the United Nation and World Organisation for Animal Health 2011, Morens et al. 2011, Roeder 2011). Plans are now underway for a

similar eradication effort focused on PPRV (Anderson et al. 2011, Baron et al. 2011). However, unlike rinderpest (which was maintained in cattle), the wider host range of PPRV may present a challenge to control efforts, as the virus may be maintained in a number of host species. Similar issues apply to CDV, and even local elimination projects must address the potential of viral maintenance in both domestic and wild species. For a pathogen of limited zoonotic or economic importance, the prospects for widespread CDV control are remote due to limited financial incentives.

Canine distemper virus in Felids

The first case of CDV in a tiger was diagnosed in a captive Bengal tiger in the United States in 1979 (Blythe et al. 1983). Subsequently a number of other reports describing sickness and mortality in captive tigers have been published (Appel et al. 1994, Gould and Fenner 1983, Konjević et al. 2011, Nagao et al. 2012, Zenker et al. 2001), and further cases were identified in a retrospective review of archived specimens (Myers et al. 1997). Cases have typically presented with neurological signs (seizures, ataxia, paresis), anorexia, gastrointestinal signs (vomiting and diarrhea), and less frequently respiratory disease. To date, the only reports of CDV in wild tigers have involved those in Russia (Quigley et al. 2010, Seimon et al. 2013), although an anecdotal report from India may also have included wild tigers (ProMED, 2014).

Rates of morbidity and mortality are difficult to infer from many published accounts of CDV outbreaks in captive tigers and other large felids but approximately 50% of clinically affected tigers have died (Table 1.5). In many cases the number of tigers (or other large felids) present within the collection are not stated, and/or serological data were not collected to assess exposure of contact animals. Death can occur during early stages of infection, which are characterized by anorexia, diarrhea, vomiting and respiratory signs. Survivors can go on to develop neurologic signs, or these can develop spontaneously without evidence of prior disease (Appel et al. 1994). In most cases initial signs of enteric and respiratory disease last from a few days to approximately two weeks, with neurologic signs evident from two to five months later (Blythe et al. 1983, Nagao et al. 2012). Most tigers that develop neurologic signs are either die or are euthanized (Table 1.5). However, one case exhibited progressive neurologic signs for a period of 16 months before being euthanized (Blythe et al. 1983), although the severe clinical signs would have prevented

Table 1.4. Summary of the host range of Morbillivirus species currently recognised by the International Committee on Taxonomy of Viruses, and the pathology and immunological responses they elicit.

Morbillivirus	Order	Family	No. of species	Clinical disease/ pathology	Antibodies	Source
Canine distemper virus	PROBOSCIDEA	Elephantidae	1	No	Yes	(Oni et al., 2006)
	PRIMATES	Cercopithecidae	3	Yes	Yes	(Qiu et al., 2011; Sun et al., 2010)
	RODENTIA	Sciuridae	1	No	Yes	(Origgi et al., 2013)
	CARNIVORA	Felidae	16	Yes	Yes	(Appel et al., 1994; Munson et al., 1997)
		Viverridae	5	Yes	No	(Chandra et al., 2000; Machida et al., 1992)
		Hyaenidae	1	Yes	Yes	(Alexander et al., 1995; Haas et al., 1996)
		Canidae	27	Yes	Yes	(Mueller et al., 2011; Woodroffe et al., 2012)
		Ursidae	6	Yes	Yes	(Nagao et al., 2012)
		Otariidae	1	Yes	No	(Barrett et al., 2004)
		Odobenidae	1	No	Yes	(Philippa et al., 2004)
		Phocidae	6	Yes	Yes	(Grachev et al., 1989; Kennedy et al., 2000)
		Mustelidae	18	Yes	Yes	(Keller et al., 2012; Williams et al., 1988)
		Mephitidae	1	Yes	No	(Gehrt et al., 2010; Helmboldt and Jungherr, 1955)
		Procyonidae	4	Yes	Yes	(Hoff et al., 1974; Kazacos et al., 1981)
		Ailuridae	1	Yes	No	(Bush et al., 1976; Itakura et al., 1979)
	ARTIODACTYLA	Suidae	1	No	Yes	(Kamao et al., 2012; Suzuki et al., 2015)
		Tayassuidae	1	Yes	Yes	(Appel et al., 1991; Noon et al., 2003)
		Cervidae	1	No	Yes	(Kamao et al., 2012; Suzuki et al., 2015)
Phocine distemper virus	CARNIVORA	Otariidae	7	No	Yes	(Duignan et al., 2014)
		Odobenidae	1	No	Yes	(Duignan et al., 2014)
		Phocidae	12	Yes	Yes	(Duignan et al., 2014)
		Mustelidae	1	Yes	Yes	(Duignan et al., 2014)

Morbillivirus	Order	Family	No. of species	Clinical disease/ pathology	Antibodies	Source
Cetacean morbillivirus	CETACEA	Balaenopteridae	2	Yes	Yes	(Van Bressem et al., 2014)
		Delphinidae	18	Yes	Yes	(Van Bressem et al., 2014)
		Phocoenidae	1	Yes	Yes	(Van Bressem et al., 2014)
		Physeteridae	2	Yes	Yes	(Van Bressem et al., 2014)
		Ziphiidae	1	Yes	No	(Van Bressem et al., 2014)
Measles virus	PRIMATES	Cebidae	2	Yes	Yes	(Munson, 2001)
		Callitrichidae	>1	Yes	Yes	(Munson, 2001)
		Aotidae	1	Yes	Yes	(Munson, 2001)
		Pitheciidae	1	Yes	Yes	(Munson, 2001)
		Cercopithecidae	>6	Yes	Yes	(Munson, 2001)
		Hylobatidae	>1	Yes	Yes	(Munson, 2001)
		Hominoidea	4	Yes	Yes	(Munson, 2001)
Rinderpest virus	ARTIODACTYLA	Suidae	4	Yes	Yes	(Kock, 2006; Plowright, 1982)
		Hippopotamidae	1	No	Yes	(Plowright, 1982)
		Giraffidae	1	Yes	Yes	(Kock, 2006; Plowright, 1982)
		Bovidae	>29	Yes	Yes	(Kock, 2006; Plowright, 1982)
Peste de petits ruminants virus	ARTIODACTYLA	Cervidae	1	Yes	No	(Munir, 2014)
		Bovidae	19	Yes	Yes	(Munir, 2014)
	CARNIVORA	Felidae	1	No	No	(Balamurugan et al., 2012)

Table 1.5. Summary of clinical findings in published accounts of canine distemper virus infection in captive tigers and other large felids.

Species	Clinical cases		Number exposed	Clinical summary	Source
	Died	Recovered			
Tiger	2	0	2?	At 4 weeks old, both cubs presented with anorexia, diarrhoea and vomiting. One died after 1 week. The other recovered, but after 5 months developed progressive neurological signs, which continued until euthanasia after 16 months.	Blythe et al. 1983
Tiger	1	0	?	Presented with diverse neurological signs (tremors, hypermetric gait, incoordination), which progressed for 2 months, after which the tiger was euthanised.	Gould and Fenner 1983
Tiger	2	4	?	At Shambala Preserve, a 4 month old tiger was admitted with anorexia, respiratory signs and seizures. Seven months later a six month old tiger developed similar signs and was euthanized. Four tigers with respiratory signs survived.	Appel et al. 1994
Tigers, lions, leopards and a jaguar	17 (including 4 tigers)	18 (number of tigers unspecified)	74	Of 74 large felids at Wildlife Waystation, 35 developed respiratory, enteric and/or neurologic signs presenting in two ways: 1) Six cats presented with acute onset neurologic signs. One survived. 2) 29 cats presented with diarrhoea (1-2 weeks), and respiratory signs. 14 recovered with supportive care and remaining 15 cats developed neurologic signs (lasting from 1-2 days, up to 2 weeks), of which 12 died or were euthanized.	Appel et al. 1994
Tiger	5	2	?	Sudden onset of "mostly" progressive neurological signs lasting "several weeks to months". CDV confirmed on conjunctival swab.	Zenker et al. 2001
Tiger	2	0	4?	Gasterointestinal signs in two 6 month old cubs lasting 2-4 days before death. Mother and one cub appeared unaffected.	Konjević et al. 2011
Tiger	3	9	22	12/22 tigers presented with diarrhoea, vomiting and respiratory signs, of which 2 died after 1-2 weeks. One survivor later died with neurologic signs afer 2 months.	Nagao et al. 2012

the tiger surviving without supportive care. The extended period of infection in this case, may be analogous to a syndrome in domestic dogs referred to as ‘old dog encephalitis’ in which replication defective virus persists in the cerebral hemispheres and brainstem of mature dogs that have otherwise recovered from infection earlier in life (Greene and Appel 2006).

The status of CDV in the wider Felidae family is complex, with clinical disease and mortality described in two of the twelve extant genera (*Panthera* and *Lynx*, Appel et al. 1994, Daoust et al. 2009, Meli et al. 2010, Origgi et al. 2012, RoelkeParker et al. 1996, Seimon et al. 2013), and antibodies detected from a further six without apparent clinical disease (*Acinonyx*, *Caracal*, *Felis*, *Prionailurus*, *Leopardus* and *Puma*, Fiorello et al. 2007, Ikeda et al. 2001, Munson et al. 2004, Thalwitzer et al. 2010, Uhart et al. 2012). Appel *et al.* (1974) demonstrated limited replication of CDV in experimentally challenged domestic cats, but a failure to transmit infection to other cats, or dogs (Appel et al. 1974). It has been suggested that the substitution of a single amino acid to the binding epitope of the CD150 receptor, may explain the variable susceptibility seen in Canidae and Felidae (Ohishi et al. 2014). Comparison of the amino acid residues of the Morbillivirus binding epitope of canid and felid CD150 molecules revealed substitutions at nine of 34 positions, of which three or four conferred an alteration in charge that could affect viral binding. A substitution at one of these nine positions (position 76) differentiated large felids (tigers, lions, and clouded leopards *Neofelis nebulosa*), from small felids (domestic cats, and two subspecies of leopard cat *Prionailurus bengalensis*). The positively charged residue found in large felids is also carried by domestic dogs, and may contribute to the susceptibility of these species, while the uncharged residue in small felids may limit the binding of wild type virus (Ohishi et al. 2014).

Managing infectious disease in wild populations

The first priority in approaching the management of infectious disease is to identify specific objectives for the programme, as these are the fundamental yardstick against which interventions should be measured. Outside of domestic animal or public health environments, the absolute control of an infectious disease is often unrealistic, and objectives are directed at limiting the impact on a defined host (Blancou et al. 2009). In the case of threatened species, interventions are usually intended to reduce the likelihood that

populations will decline to extinction due to the effects of an infectious disease. Since extinction is more likely in small populations, or those where isolation prevents recolonization following an outbreak, an obvious mitigation strategy may be to improve connectivity through the use of wildlife corridors. This approach is not without controversy, and it has been argued that corridors could facilitate the spread of a pathogen within a larger metapopulation leading to a wider extinction (Hess 1996). Modeling approaches have indicated that this is unlikely in wild situations, where infections spillover from an abundant reservoir (as in CDV), particularly when the force of infection is low (Gog et al. 2002, McCallum and Dobson 2002).

Approaches to actively controlling the impact of an infectious disease on a threatened population will depend on the epidemiology of the pathogen concerned (Cleaveland et al. 2007, McCallum 2012). Broadly, strategies focus on 1) reducing disease incidence in the reservoir (whether biotic or abiotic), 2) reducing spillover from the reservoir to the threatened host, or 3) reducing transmission or mortality within the threatened host population itself (Woodroffe 1999, Haydon et al. 2002, Laurenson et al. 2005). For pathogens with a biotic reservoir like CDV, reservoir-targeted strategies aim to moderate transmission by reducing the number of susceptible animals in the population, either through culling or vaccination (Woodroffe 1999, McCallum 2012). Culling of reservoir populations is rarely practical, or socially acceptable, and can have unforeseen consequences on the burden of disease (Donnelly et al. 2003, Streicker et al. 2012). Vaccination of domestic dogs is feasible, and can disrupt spillover into threatened populations (Cleaveland et al. 2006, Viana et al. 2015). Likewise, measures to isolate a domestic dog reservoir from a threatened population could be successful, either with physical barriers such as fences, or curtailment of activities (such as hunting) that facilitate contact between dogs and threatened species (Laurenson et al. 2005). Situations where wildlife contributes to the reservoir are more problematic, raising issues of vaccine safety, and efficacy as products are rarely tested on wild species, and responses may differ greatly from that of domestic animals. Delivery of vaccine doses to wild animals can also be challenging, due to the availability of environmentally stable vaccine products, and the logistical difficulty of administering doses to a sufficient proportion of the population to achieve control. Also, elimination of pathogens circulating on wide spatial scales cannot be achieved by local vaccination, which must then continue indefinitely, bringing considerable cost implications, or risking large outbreaks if coverage is disrupted (Laurenson et al. 2005).

Where the identity of the reservoir is unknown, or its vaccination or isolation is impractical, increasing the immune status of the threatened host may be the only management option available. This strategy has generally been applied in 'emergency situations', where ongoing outbreaks are likely to result in unacceptable losses (Gascoyne et al. 1993, Hofmeyr et al. 2000, Randall et al. 2006, Knobel et al. 2008, Timm et al. 2009). These interventions can prove controversial, with claims that vaccination hastened the extinction of African wild dogs (*Lycaon pictus*) in the Serengeti (Burrows 1991, Burrows et al. 1994), despite a lack of supporting evidence (Woodroffe 2001). The availability of safe and efficacious vaccines is a major constraint (Laurenson et al. 2005, Connolly et al. 2013), particularly where opportunities to deliver booster doses are limited. However, in emergency situations, even low coverage strategies, which vaccinate a subset of the affected population can reduce the extinction probability substantially (Haydon et al. 2006).

How pathogens are maintained

Identifying reservoir populations is an important precursor to the selection of control measures, and is aided by clear terminology in conceptualizing reservoir systems. For a population to maintain a pathogen indefinitely, it must exceed a critical community size (CCS), below which pathogen 'fade out' is likely due to the depletion of susceptible hosts over time (Bartlett 1960). In Tanzania outbreaks of CDV have occasionally resulted in large-scale mortality of lions, but the population is numerically insufficient to maintain the virus indefinitely, with outbreaks requiring spillover from more abundant reservoir hosts (Roelke-Parker et al. 1996, Viana et al. 2015). A reservoir is therefore one or more epidemiologically connected populations in which the pathogen can be permanently maintained and from which infection is transmitted to a defined target (such as an endangered population, Haydon et al. 2002). Individual populations that exceed the CCS, and can, therefore, maintain infection indefinitely are termed maintenance populations, although several non-maintenance populations could act synergistically to form a maintenance community. Finally, a source population is that which transmits infection directly to the target, and may either be a maintenance population, or be connected to the maintenance population as a transmission link to the target.

Although conceptually attractive, the CCS is extremely hard to estimate for free-living populations, therefore researchers must rely on numerous lines of evidence to identify likely maintenance populations (Viana et al. 2014). Analysis of data on population size and demographics may give an indication of their potential to maintain a pathogen. Generally, maintenance is more likely in populations that are large, well mixed, with a high rate of turnover. For short-lasting infections, like CDV, the detection of pathogens can be challenging but the presence of antibodies can provide useful indicators of disease incidence and prevalence, particularly in reference to the age structure of the sampled population. For rapidly evolving pathogens, chains of infection can give rise to genetically distinguishable strains, providing a means of tracing transmission between hosts, and an assessment of pathogen diversity in possible maintenance populations. Individually, these lines of evidence may be inadequate to describe reservoir structure, particularly for multi-host pathogens (Viana et al. 2014). But when interpreted collectively, they may be sufficient to identify likely contributors to a maintenance population, and inform appropriate control measures.

Study rationale

Prior to this study, CDV had been detected in three cases involving sick tigers that displayed severe and progressive neurological signs (Quigley et al. 2010, Seimon et al. 2013), and that a further five tigers carried neutralizing antibodies indicating prior infection with the virus (Goodrich et al. 2012). In addition, model simulations predicted that CDV infection could have an impact on the viability of tiger populations (Gilbert et al. 2014), but the scale of this impact was dependent on the identity of reservoir species responsible for maintaining infection, the prevalence of infection within that population, and the frequency of transmission to tigers. Amur tigers coexist with domestic dogs and a number of wild carnivore species that are susceptible to CDV (Table 1.6), and represent potential contributors to CDV maintenance. The study utilized multiple lines of evidence including published, unpublished and newly collected material to assess the structure and demography of host populations, their serological patterns of CDV exposure, and phylogenetic relatedness of viruses from respective host groups. This information was used to determine which of the potential reservoir species may be contributing to the maintenance of CDV in the ecosystem of the Russian Far East, and whether these viruses were genetically related to those found in the tiger population.

Table 1.6. Carnivores of Primorskii Krai, adapted from Voloshina *et al.* (1999).

Family	English common name	Scientific name	Status
Canidae	Raccoon dog	*Nyctereutes procyonoides*	Common
Canidae	Grey wolf	*Canis lupus*	Common
Canidae	Red fox	*Vulpes vulpes*	Common
Ursidae	Brown bear	*Ursus arctos*	Common
Ursidae	Asiatic black bear	*Ursus thibetanus*	Common
Mustellidae	Asian badger	*Meles leucurus*	Common
Mustellidae	Sable	*Martes zibellina*	Common
Mustellidae	Yellow-throated marten	*Marte flavigula*	Common
Mustellidae	Wolverine	*Gulo gulo*	Rare
Mustellidae	Ermine	*Mustela erminea*	Rare
Mustellidae	Least weasel	*Mustela nivalis*	Common
Mustellidae	Siberian weasel	*Mustela sibrica*	Common
Mustellidae	American mink*	*Neovison vison*	Common
Mustellidae	River otter	*Lutra lutra*	Common
Felidae	Leopard cat	*Prionailurus bengalensis*	Southern Primorye
Felidae	Eurasian lynx	*Lynx lynx*	Common
Felidae	Amur tiger	*Panthera tigris altaica*	351-393 mature individuals
Felidae	Far Eastern leopard	*Panthera pardus orientalis*	Southwest Primorye
Otariidae	Northern sealion	*Eumetopias jubatus*	Rare
Otariidae	Northern fur seal	*Callorhinus ursinus*	Common
Otariidae	Spotted seal	*Phoca largha*	Common
Otariidae	Ringed seal	*Pusa hispida*	Uncertain

*Introduced non-native.

The overall goal of this thesis is to compile an epidemiological evidence base that can be used by natural resource managers in the Russian Far East to select appropriate strategies for managing the effects of CDV on Amur tiger populations. At the outset, it was acknowledged that there would be considerable challenges inherent in reconstructing a complete epidemiological understanding of such a complex multi-host pathogen, with less than three years of fieldwork available, in an ecosystem where the disease was largely unstudied. For these practical reasons, it was decided to focus on the gaps in our understanding that were of greatest relevance to decision-makers. In view of the practical limitations in controlling CDV in a wild reservoir, substantial involvement of wildlife in CDV maintenance was considered to have the greatest influence on available control measures. Therefore, determining whether wild hosts were important contributors to the maintenance of viruses sharing genetic identity with those found in tigers was a key focus of the project. Conversely, if wildlife were not involved in CDV maintenance, then control of the infection in domestic dogs may be the most cost effective means of mitigating the impact on tiger populations. Therefore, simultaneously the study aimed to determine

whether CDV was being maintained in dog populations, and to assess factors including patterns of ownership, dog demography, and vaccination status that would be essential to the design of possible control programmes. Findings were interpreted collectively to produce a qualitative picture of CDV epidemiology in the region and inform management recommendations aimed at minimizing the threat to remaining populations of Amur tigers.

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Chapter 2 Project overview

In 2003, the first case of canine distemper virus (CDV) in a free ranging Amur tiger (*Panthera tigris altaica*) was diagnosed in a sick tiger that died in the Russian Far East (RFE, Quigley et al. 2010). A further two fatal cases were confirmed in 2010, and questions were raised about the possible emergence of CDV in tigers, and the impact this may be having on population viability (Seimon et al. 2013). Analysis of tiger serum samples (n=40) collected between 1992 and 2004, found CDV-neutralizing antibodies in six tigers including the 2003 case (Goodrich et al. 2012). All of the exposed tigers were among 22 individuals sampled between 2000 and 2004, suggesting that the virus may have been a recent arrival in the ecosystem, or had recently adapted to enable infection of tigers. A population viability analysis that incorporated an epidemiological SIRD compartmentalized model ('susceptible-infected-recovered/dead') found that CDV could reduce the viability of Amur tiger, and disproportionally affected small populations (Gilbert et al. 2014). The present study was conceived as a means of augmenting the limited understanding of CDV ecology in the Russian Far East, providing information that could inform management decisions to limit the impact of the virus on Amur tiger populations.

Research strategy

Identifying reservoirs of infection is a challenging proposition. In situations that preclude experimental manipulation, it may be unrealistic to reach more than a presumptive conclusion on the identity of maintenance hosts. This is particularly true for multi-host pathogens like CDV, where several host species or populations may be contributing to pathogen persistence as members of a maintenance community. In the timeframe of a project of this scale, a qualitative understanding of CDV maintenance was considered a realistic outcome, and would be reached through examination of numerous lines of evidence. Determining the involvement of wildlife in CDV maintenance was a particular priority, due to the limits this would place on management options. To maximise available sources of evidence, a strategy was adopted that would capitalize on all forms of existing information and diagnostic material, as well as performing novel field research designed to fill gaps in current understanding. Specific approaches included:

1. *Assessment of host population size, structure and demography* – Pathogen maintenance is more likely in large, well-mixed populations, with high rates of turnover, where immunologically naïve young provide a ready supply of susceptible hosts. The study aimed to characterize the composition and biology of the domestic dog population in Primorskii Krai, focusing on rural areas with the greatest opportunity for contact with tigers.

2. *Molecular characterization of CDV in potential host populations* - Rapidly evolving pathogens like CDV accumulate mutations that can be used to infer likely transmission patterns, and assess the diversity of strains circulating in potential maintenance populations. Partial sequence data had previously been published from cases of CDV in three tigers from the RFE (114-430 bp in length, Seimon et al. 2013). Tissues from these tigers were available as a resource for possibly obtaining more extensive sequence data, as well as further untested archived samples, and novel samples collected during the course of the project. A variety of phylogenetic tools were considered, including topological interpretation of phylogenetic trees, use of molecular clock models, and more detailed inference of statistical parsimony networks (Templeton et al. 1992, Lembo et al. 2007), to provide varying degrees of insight into possible chains of transmission. The complexity of analytical techniques is largely dependent on the quality and quantity of available sequence data. Therefore, a broad strategy was adopted, analyzing a wide range of sample types, from tigers, other large carnivores, mesocarnivores and domestic dogs in order to maximize available sequence data and the conclusions that could be drawn from them.

3. *Patterns of exposure to CDV in host populations* – The detection of pathogens like CDV, where infections are short-lived, can be very challenging, particularly in wildlife populations that are difficult to sample. In these situations, detection of antibodies indicative of prior exposure can be an informative sign that a pathogen is circulating in a population. Antibodies to CDV remain detectable for prolonged periods that may extend to years, or even the lifetime of the host (Greene and Appel 2006). In these situations, exposure of young animals can be a useful indicator of recent infection (once maternal antibodies titres have waned at approximately 12-14 weeks, Greene and Appel 2006), and a means for comparing relative incidence on different temporal and spatial scales.

Study area

The range of the Amur tiger currently comprises approximately 155,000 km^2 of the Far Eastern maritime territories of Primorskii Krai, and Khabarovskii Krai (Figure 2.1, Hebblewhite et al.2014). Tigers in Russia have traditionally been monitored using snow track counts, a method that provides the most reliable index of population trends given the constraints of working in remote and challenging terrain (Hayward et al. 2002). Based on this approach the most recent estimate of adult and subadult Amur tigers numbers in the RFE was between 331 and 393 individuals in 2005 (Miquelle et al. 2007). Although recently extirpated as a breeding species from the Jewish Autonomous Region, and Amurskaya Oblast, dispersing individuals (particularly males), continue to be reported there, and a recent translocation programme in both territories shows some promise of future restoration (including one case of confirmed breeding in the Jewish Autonomous Oblast by 2015). Small numbers of Amur tigers (<20) continue to be recorded in Jilin and Heilongjiang Provinces of northeastern China, but these mostly represent an extension of the Russian population across the border (Hebblewhite et al. 2012). Although small numbers of samples were obtained from wild carnivores in Khabarovskii Krai and Amurskaya Oblast, this study primarily focused on the occurrence of CDV in carnivores in Primorskii Krai, as the territory contains the majority of the extant tiger distribution.

Primorskii Krai runs approximately 900 km north-south along the spine of the low Sikhote-Alin Mountain chain (which rises to a height of 1,200 m above sea level). The territory is bordered by the Ussuri River and China to the west, and the Sea of Japan to the east, extending south to a short common border with North Korea. The landscape is dominated by taiga forest characterized by Korean pine (*Pinus koraiensis*), birch (*Betula* sp.) and Mongolian oak (*Quercus mongolica*), which constitutes suitable habitat for wild boar (*Sus scrofa*), sika deer (*Cervus nippon*) and red deer (*C. elaphus*) that constitute the main prey for Amur tigers (Hebblewhite et al. 2014). Mean monthly temperatures vary from -13.1 °C in January to 19.5°C in August, with 80% of precipitation (mean 650-800 mm) occurring between April and November. The coast provides a moderating influence on winter temperatures, which average approximately 10 °C higher on the eastern slope than the west.

The national census conducted in 2010 registered a human population of 1,956,497 in

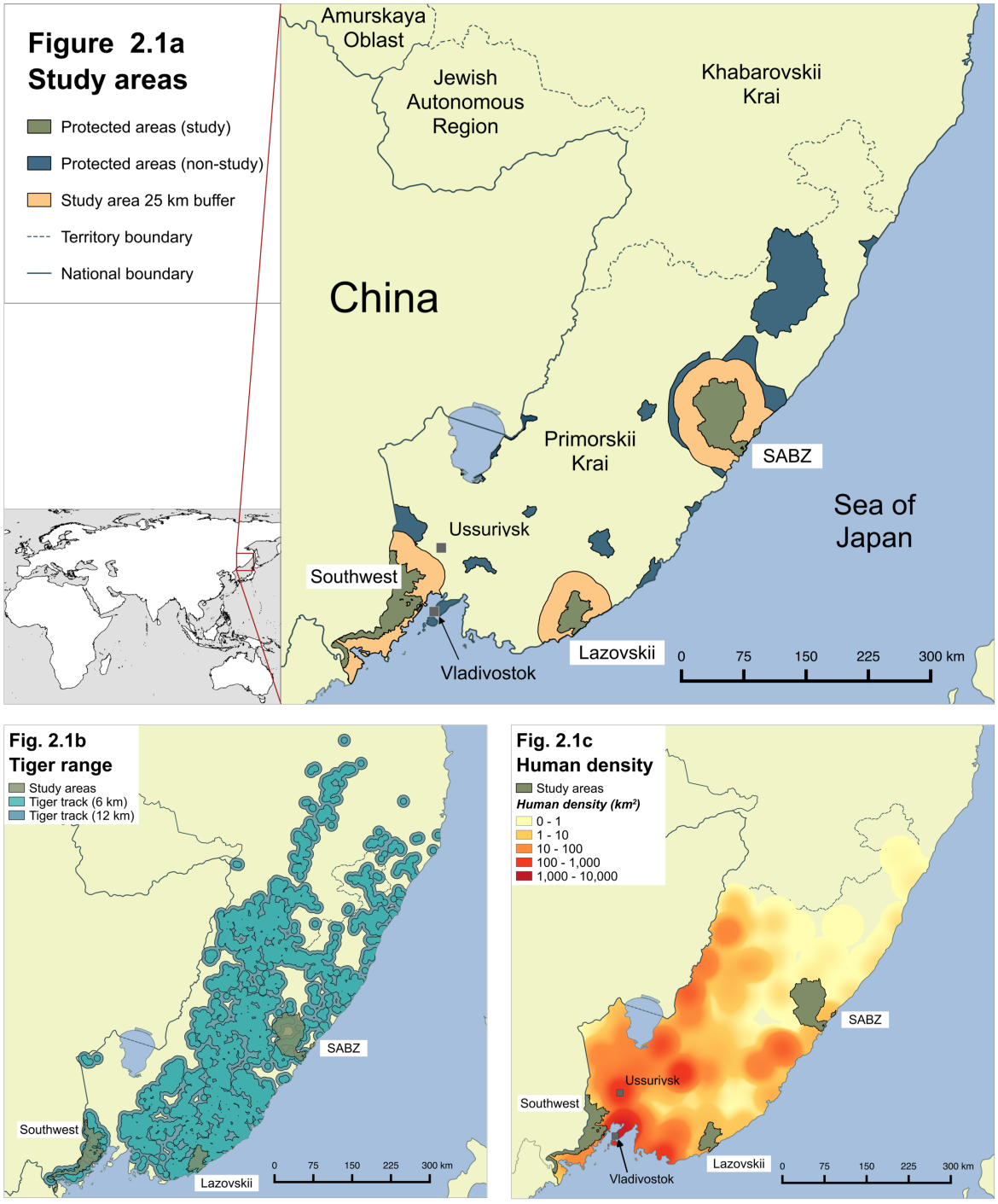

Figure 2.1. a) Map of the Russian Far East, including main primary study areas defined by 25 km buffers around three main protected areas. These included Southwest Primorskii (centred on the 'Land of the Leopard' National Park), Lazovskii (centred on Lazovskii Zapovednik) and SABZ (centered on the Sikhote-Alin Biosphere Zapovednik). Other (non-study) protected areas are also illustrated, and include zapovedniks (strictly protected areas) and zakazniks (sanctuaries); b) Geographic distribution of the Amur tiger, based on 6 km and 12 km buffers of snow track locations recorded in Primorskii Krai and Khabarovskii Krai during February and March 2005 (Hebblewhite et al. 2014); and c) human population densities based on the 2010 national census (Russian Federal State Statistics Service 2011), represented as a heatmap coloured according to the number of people per 1 km^2 based on a smoothing radius of 40 km.

Primorskii Krai, of which 76% resided in urban areas, with the remainder in small rural settlements (Figure 2.1b, Russian Federal State Statistics Service, 2011). Populations are concentrated along the Ussuri River valley, and a development corridor that roughly connects the cities of Vladivostok and Ussuriysk in the south. This region of high population density acts as an effective barrier to the movement of tigers between the main Sikhote-Alin Mountain population, and a smaller population (16-21 individuals, Pikunov et al. 2003) in Southwest Primorskii, that extends into the low Changbaishan Mountains along the border with China (Henry et al. 2009). Away from urban centers, people reside in small rural communities, relying heavily on natural resources (including fish, wildlife and forest products) and smallholding cultivation for subsistence. Approximately 7% of tiger habitat receives some form of protection (Miquelle et al. 2005), and are free from human settlement. Much of the remaining forest has been selectively logged, and essentially all non-protected areas are utilized for hunting of ungulates, and in many areas also fur-bearing mesocarnivore species.

Although archived samples originated from many locations, prospective surveys of domestic dogs and wild mesocarnivores in 2013 and 2014 focused on three study areas that centered on protected reserves that support Amur tigers (Figure 2.1). These were selected to reflect a range of human population densities, with the strictly protected Sikhote-Alin Biosphere Zapovednik (SABZ, N44.8°, E135.7° to N45.7°, E136.8°) representing an area of low human habitation, Lazovskii Zapovednik (N42.9°, E133.7° to N43.4°, E134.2°) with a moderate human population, and Southwest Primorskii, centered on the Land of the Leopard National Park (N42.5°, E130.4° to N43.8°, E131.7°), which is surrounded by high numbers of human settlements. No settlements are found within the protected areas themselves, but do occur in the immediate vicinity of park boundaries. Study areas were defined by plotting a 25 km buffer around the boundaries of each protected area using R (R Development Core Team 2015), and were clipped to exclude marine areas, and non-Russian territory.

Project partners

This diverse project was primarily funded by the Morris Animal Foundation and the Biotechnology and Biological Sciences Research Council, and has relied on contribution from a large number of organizations and individuals. A full list of all contributors is

provided in the Acknowledgement section, along with specifics of their roles and activities. For brevity, the following is limited to a list of primary project partners and their respective contributions:

1. *Wildlife Conservation Society (WCS)* – Formerly known as the New York Zoological Society, WCS is based in the Bronx Zoo, and has maintained a national programme in Russia since 1992. Their work has focused on the conservation of Amur tigers and other threatened species in Primorskii. This has included intensive studies of tiger ecology in the Sikhote-Alin Biosphere Zapovednik (including placement of telemetry collars), and the handling of tigers as part of conflict resolution programmes. Samples collected as part of this work (1992-2014) were archived in Russia and at the Bronx Zoo and served as a critical resource to the success of the project. The author was employed by WCS for the duration of the project period.

2. *Institute of Biology and Soil Sciences, Far Eastern Branch of the Russian Academy of Sciences (IBSS)* – A research institute based in Vladivostok, with an active programme of ecological studies focused on terrestrial and marine ecosystems throughout the Far Eastern region. Researchers with IBSS contributed to many aspects of field and laboratory-based activities throughout the project. Archived samples collected from Southwest Primorskii during 2007 and 2008 (in collaboration with the National Cancer Institute, MY, USA) represented an invaluable resource for serological analyses.

3. *Zoological Society of London (ZSL)* – Based at the Regents Park Zoo, ZSL have been active in conservation in Primorskii since 2001. The organisation has been collaborating with Lazovskii Zapovednik on a programme of tiger monitoring and protection since 2006. Participation from ZSL included all research in Lazovskii Zapovednik and surrounding areas, as well as many aspects of field surveys in other areas.

4. *University of Glasgow (UoG)* – The project was supervised by Prof. Sarah Cleaveland, and Dr. Louise Matthews, at the Institute of Biodiversity, Animal Health and Comparative Medicine. Members of the MRC-Center for Virus

Research at the Institute of Infection, Immunity and Inflammation also provided support in aspects of serology, molecular characterization and Illumina sequencing.

Ethical review and permitting

A detailed 'Animal Involvement Justification' was included within the successful project proposal reviewed by the Morris Animal Foundation, and included all aspects of animal handling and sample collection from domestic and wild carnivores. The Institutional Care and Use Committee (IACUC), at the Bronx Zoo (Appendix III), performed an ethical and technical review of animal restraint/immobilization and sample collection techniques from wild carnivores. Approval for questionnaire surveys and sample collection from domestic dogs was provided by the State Veterinary Inspection, Primorskii Krai (Appendix IV). Project information was shared with all householders participating in household questionnaire surveys, and copies of signed informed consent forms were retained from all dog owners who agreed for samples to be collected from their dogs.

Project challenges

At the outset of the project, very little was known about CDV in the Russian Far East. Anecdotally, local veterinarians reported that CDV was "common" in domestic dogs in the region, but the only publications relating aspects of CDV epidemiology in Russia, in either the Russian or international literature related to the outbreaks affecting Baikal seals (*Phoca sibirica*) 2,000 km west of Primorskii Krai (Grachev et al. 1989, Mamaev et al. 1995, Butina et al. 2010), or the tigers in Primorskii Krai itself (Quigley et al. 2010, Goodrich et al. 2012, Seimon et al. 2013). Due to this lack of baseline information, the project was designed to achieve a coarse understanding of CDV epidemiology in the study area, and focused on questions of greatest relevance to management decisions. With two years available for fieldwork (2012-2014), it was necessary to prioritize the potential host populations to focus on, and decide on the spatial scale on which to operate. Although a longitudinal approach, where a population is sampled repeatedly over an extended timescale provides a more detailed picture of disease incidence, this option was rejected in favour of a geographically extensive sampling strategy. This wider approach was selected in order to obtain a more representative assessment of CDV epidemiology across a large

portion of the tiger distribution, and including a range of domestic dog densities (that was assumed to follow a coarse index of human population densities).

The use of archived samples enabled an assessment of CDV exposure over a wider timescale, and greatly increased the sample size available, particularly for large carnivore species. However, this introduced some limitations and possible biases to the available dataset. Large carnivore samples had been collected as part of research programmes (e.g. telemetry), or from animals captured during incidents of conflict resolution. Although extremely valuable, this opportunistic sample set had several shortcomings from the perspective of an epidemiological study. The number of samples available from different locations and time points was often insufficient to provide meaningful comparison. Potential biases may have arisen based on the exposure history of animals captured during research studies versus those in conflict situations. Furthermore, in an effort to maximize the number of samples for analysis, it was necessary to foster collaboration with multiple groups of researchers. In addition to the differences in study design, there was also variation in the data that accompanied archived samples, which limited the analyses that were possible. For instance, researchers used different approaches to categorize the age of animals, and in the case of mesocarnivores, this was often lacking. Wherever possible, data analysis has taken account of these shortcomings, and limited conclusions to findings that can be supported by available data.

Many of the wild carnivores that form the base of this study are covered by regulations governing natural resources and endangered species that either slowed, or prevented planned activities. For instance, delays in the approval of permits covering the capture of mesocarnivores in one study area (SABZ) greatly reduced the availability of samples for comparison with other sites. Limitations in accessible laboratory resources within Russia required that samples be exported in multiple shipments, for analysis in international facilities. Each shipment required a lengthy process to acquire permits satisfying both domestic and international regulations (e.g. CITES). In one case, an application was denied, preventing the inclusion of important samples in the analyses. Similarly, shipment of archived samples between the United States and the United Kingdom proved a convoluted process, and in the case of some older samples, insufficient paperwork prevented the export of material from some animals. For this reason serological analyses were performed in two laboratories, which introduces questions of comparability. To

minimize this a full set of results were obtained from at least one laboratory for each of the three main host populations (domestic dogs, wild mesocarnivores and large carnivores), ensuring comparability within groups, even if comparison between groups remains an issue.

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Chapter 3 Domestic dog demography and patterns of ownership in Primorskii Krai

Abstract

The structure and demography of domestic dog populations determine their potential to maintain pathogens such as canine distemper virus (CDV), and must be accounted for when designing control programmes. Identifying CDV reservoirs and the potential for inter-species transmission has become a priority following the recent death of several Amur tigers from CDV in the Russian territory of Primorskii Krai. Most dogs in the territory are owned, as extreme winter temperatures reduce survival of feral dogs. This questionnaire-based study collected baseline information on domestic dog demography and patterns of ownership adjacent to three protected areas that support tiger populations, and the nearby city of Ussuriysk. The primary objectives focus on aspects of ownership that 1) influence the potential for dogs to maintain CDV, 2) identify opportunities for CDV transmission between dogs and other species, including tigers, and 3) provide recommendations for the design of CDV control programmes. Features favouring CDV maintenance included high domestic dog densities within settlements (up to 673.4 domestic dogs/km^2 in the city), high canine reproductive rate, and low canine vaccination coverage with only 11.4% of dogs (n =2,369) receiving a CDV vaccination in the previous year. Inter-settlement movement of dogs (which may enhance dissemination of CDV) was generally low, primarily limited to young dogs, with 28.8% (n=2,332) sourced in other communities, and no movement reported for 93.1% following their acquisition (n=2,379). A large proportion of dogs were restricted from unsupervised movement, with only 16.9% given freedom to move and mix with other dogs on a daily basis. Overall 41.0% of dogs (n=2,324) were taken to forested areas where contact with wildlife could occur, and owners reported anecdotal accounts of direct interaction with tigers and wild mesocarnivores. Residence type was the strongest predictor of dog ownership, and vaccination strategies targeting households in cottages versus apartment buildings would reach greater numbers of dogs.

Introduction

Following the death of several free-ranging Amur tigers (*Panthera tigris altaica*) from the Morbillivirus canine distemper virus (CDV) (Quigley et al. 2010, Seimon et al. 2013), concern has been growing as to the impact this may have on the species' conservation status (Gilbert et al. 2014). With an estimated 331-393 adult and subadult wild Amur tigers (Miquelle et al. 2007), the population is likely to be too small to maintain CDV without transmission from more abundant susceptible hosts. Tigers coexist with a range of susceptible wild carnivore species, but in the human context, domestic dogs constitute the most conspicuous susceptible host in the Russian landscape. In many parts of the world, domestic dogs are considered the most important host of CDV, often contributing to the maintenance of the virus where vaccination is not routinely practiced (Greene and Appel 2006, Acosta-Jamett et al. 2011, 2015, Belsare et al. 2014). In others, CDV outbreaks continue to occur among susceptible wild carnivores despite routine dog vaccination, suggesting that sylvatic cycles in CDV maintenance may be important in some areas (Craft et al. 2008, Almberg et al. 2010, Prager et al. 2012, Woodroffe et al. 2012, Viana et al. 2015).

The maritime province of Primorskii Krai runs approximately 900 km north-south along the spine of the Sikhote-Alin mountain chain in the Far East of Russia. The national census conducted in 2010 registered a human population of 1,956,497, of which 76% resided in urban areas, largely concentrated in the south and west of the territory, with the remainder in small sparsely distributed rural settlements (Russian Federal State Statistics Service 2011).

Several key factors might contribute to the potential for domestic dogs to act as maintenance hosts for CDV in Primorskii. Fundamentally, the capacity for a population to maintain a pathogen indefinitely is dependent on it exceeding a critical community size (CCS), below which stochastic extinction of the pathogen is likely (Bartlett 1960). The size of the CCS is dependent on features of pathogen biology, particularly the reproductive number (R_0, the mean number of secondary infections arising from an infected individual in a freely mixing, fully susceptible population), the biology of the host, including the rate at which new susceptible animals are born into the population, and host contact. A reduction in R_0, or birth rate will typically increase CCS, as it increases the threshold of

susceptibles required to initiate an epidemic (Earn et al. 2000, Metcalf et al. 2013). Field estimates of R_0 and the CCS are lacking for CDV in domestic dog populations. Estimates for the related measles virus in human populations consistently fall within the range of 250,000 to 400,000 people (Bartlett 1957, 1960, Black 1966). However, some features of host biology (such as the higher rate of canine reproduction), may suggest a lower CCS for CDV, while others (such as limitations in social contacts) may increase it.

The distribution of domestic dogs in Primorskii is not homogenous, but rather animals are spatially clustered among settlements of varying size. Movement of dogs between these settlements would give rise to a metapopulation structure that would influence their capacity to maintain CDV (Grenfell and Harwood 1993). In the epidemiological context, metapopulations consist of multiple subpopulations of susceptible hosts that represent patches of 'habitat' for pathogens, and are loosely connected (e.g. through host movement) to enable the dispersal of the infection between patches (Grenfell and Harwood 1993). Morbilliviruses have played an important role in the development of metapopulation theory, particularly through factors that influence the persistence and spatiotemporal distribution of measles outbreaks (Bartlett 1957, Grenfell and Harwood 1993, Grenfell et al. 2001), and phocine distemper virus (Swinton et al.1998). A metapopulation might include patches of varying size, with some exceeding the CCS acting as a source of infection for smaller outlying patches, that experience outbreaks and fadeout as susceptible hosts are depleted (Bartlett 1957, Grenfell and Harwood 1993, Grenfell et al. 2001). Theoretically, other metapopulations might consist of patches that lie below the CCS, but exhibit a host demography and structure that could enable persistence (Swinton et al. 1998). Therefore, information on host demography and patch connectivity is an important precursor to understanding the potential for a metapopulation to maintain a pathogen, indefinitely. This information can also be valuable in the construction of spatially explicit models, to inform the design of locally relevant vaccination programmes.

Estimation of the CCS has remained a challenging proposition for most disease systems, but information on host populations and structure can provide a qualitative indicator of their potential to maintain a pathogen. Therefore, a better understanding of the demographic factors that might influence viral persistence is an important part of assessing the potential contribution of dogs to CDV maintenance in Primorskii. Key features include the number of dogs, their population density in settlements of varying size, estimates of

turnover and population trend, based on rates of reproduction and survival, and the movement of dogs within and between settlements.

As a multi-host pathogen, the transmission of CDV between dogs and other susceptible host species or communities may play an important role in the dynamics of the virus. In Primorskii there are two main wild populations to consider that can be broadly categorized based on their relative body size. Large-bodied wild carnivores, notably Amur tigers, but also leopards (*Panthera pardus orientalis*), Eurasian lynx (*Lynx lynx*), grey wolves (*Canis lupus*), brown bear (*Ursus* arctos) and Asiatic black bear (*U. thibetanus*) occur in low densities in populations that likely fall below the CCS. The environmental stability of CDV is relatively low, and transmission is likely to require direct contact between an infectious and susceptible host (Appel 1987, Greene and Appel 2006). In the case of tigers, direct interactions with domestic dogs are likely to be limited to incidents of predation, which might occur on the periphery of settlements, or when dogs encroach on tiger habitat for purposes such as recreation or hunting.

There are also several species of medium and small-bodied wild carnivores (from here on referred to as 'mesocarnivores') that are found widely throughout Primorskii. These abundant mesocarnivore species include red foxes (*Vulpes vulpes*), raccoon dogs (*Nyctereutes procyonoides*), Asian badgers (*Meles leucurus*) and sable (*Martes zibellina*). Interactions between dogs and mesocarnivores may be more complex than between dogs and large carnivores, and include agonistic interactions, such as encounters over common food resources. Although CDV infection is common among many species of mesocarnivore throughout the world, it can be difficult to quantify their contribution to CDV maintenance, particularly where the infection is poorly controlled in domestic dogs. In the United Kingdom, routine vaccination has almost eliminated CDV from domestic dogs (Walker et al. 2014), and infection is now rare or absent in Eurasian badger (*M. meles*) populations (Delahay and Frölich 2000). In contrast, CDV infections continue to be detected in dogs, badgers and other mesocarnivores on mainland Europe (Alldinger et al. 1993, Benetka et al. 2011, Origgi et al. 2012, Nouvellet et al. 2013), indicating the continued circulation of CDV in domestic and wild hosts. In Tanzania, large scale vaccination of dogs around the Serengeti National Park reduced the size of domestic CDV outbreaks, but exposure continued in lions (*P. leo*), suggesting that wild carnivores remained a source of infection for lions (Viana et al. 2015). Together, these observations

suggest that the role of mesocarnivores in CDV maintenance may vary with location, and emphasizes the importance of interactions between dogs and wild carnivores to understanding the local epidemiology of CDV.

The availability of effective vaccines against CDV for dogs has contributed to efficient control of the disease in many parts of the world, leading to near elimination where dogs are routinely inoculated (Norris et al. 2006, Walker et al. 2014). This contrasts with areas where uptake of vaccines is low, and CDV is allowed to circulate unchecked (Cleaveland et al. 2000, Millán et al. 2013). The most widely used vaccines are based on modified live viruses, for which a primary course of two doses 2 to 4 weeks apart is recommended once maternal antibodies have waned at 12-14 weeks (Greene and Appel 2006). Thereafter booster doses are recommended at least every three years due to reports that older vaccinated dogs may contract infections (Greene and Appel 2006), although other studies were unable to find a decline in protective titers as much as 9 to 15 years later (Bohm et al. 2004). Therefore, it is important to assess the level of reported CDV vaccination in the dog population in Primorskii, and to determine the reasons why some owners may fail to vaccinate their dogs. Looking ahead to the possible design of vaccination programmes, it would be useful to identify determinants that could be used to predict the distribution of dogs among households, in order to improve the targeting of control measures, and predictors of higher vaccination coverage.

The ultimate objective of this chapter is to determine whether the structure and demography of the domestic dog population in Primorskii Krai favours the maintenance of CDV, and to assess opportunities for viral transmission with populations of tigers and other wild carnivores. In anticipation that domestic dogs may prove to be maintaining CDV, and acting as an important source of infection for tigers, this chapter also assesses the current usage of CDV vaccines, as a basis for informing any future vaccination programmes. Although the maintenance of CDV would require the dog population to exceed a CCS, it is not possible to accurately predict how large this population would need to be, as the size of the CCS itself depends on the structure and population dynamics of the host (Viana et al. 2014). However, it is possible to make some general predictions about the features of a population that would favour maintenance. Large populations are clearly more likely to exceed the CCS required to maintain a pathogen, and therefore estimates of population size represent a critical starting point when assessing maintenance potential. Maintenance also

requires a pool of susceptible hosts, which must be replenished fast enough to avoid pathogen extinction through stochastic processes during the troughs between outbreaks. As CDV invokes an immunity that may persist for the lifetime of those that survive infection, replenishment of the susceptible pool is dependent on the introduction of naïve juveniles, and therefore maintenance is favoured in a population that reproduces rapidly, and where vaccination coverage is low. For pathogens like CDV, where transmission requires direct contact, populations must be well mixed to insure that $R_0>1$, thus high dog densities and a freedom to move and interact with other dogs would be key features of a CDV maintenance population. Finally, for hosts that are distributed within a metapopulation structure, local persistence may be impossible yet regional maintenance can be achieved through recolonization ('rescue effects') where local outbreaks occur asynchronously. For a dog population distributed in settlements, these rescue effects require a loose connectivity, with at least some movement of dogs between settlements.

The objectives of this chapter are therefore to

1. Describe the features of dog ownership and demography that would favour the maintenance of CDV in the domestic dog population of Primorskii Krai, including assessments of:
    i. Dog population size.
    ii. Dog population density, at settlement and landscape levels.
    iii. Dog population turnover (rates of reproduction and mortality).
    iv. Dog movement within settlements.
    v. Dog movement between settlements.
2. Determine whether there have been changes in the numbers of dogs in Primorskii Krai during the preceding decade that might explain the apparent increase in tiger exposure to CDV since 2000.
3. Document opportunities for inter-specific transmission, when dogs come into direct or indirect contact with tigers and other wild carnivores, including assessments of:
    i. Dog access to forested areas.
    ii. Anecdotal reports of contact between dogs and tigers and/or other wild carnivores.
4. Assess the levels of CDV vaccination reported by owners, and reasons for failing to vaccinate.

Extreme winter temperatures in Primorskii, which regularly dip below -15 C limit opportunities for dogs to subsist without human care, and as a result feral dogs are limited or absent, particularly outside urban centers. For this reason, this study concentrated on owned dogs, as these constitute the majority of dogs within the province.

Methods

The surveys focused on three study areas that centered on protected reserves that support Amur tigers (Figure 3.1). These were selected to reflect a range of human population densities, with the strictly protected Sikhote-Alin Biosphere Zapovednik (SABZ, N44.8°, E135.7° to N45.7°, E136.8°) representing an area of low human habitation, Lazovskii Zapovednik (N42.9°, E133.7° to N43.4°, E134.2°) with a moderate human population, and Southwest Primorskii, centered on the Land of the Leopard National Park (N42.5°, E130.4° to N43.8°, E131.7°), which is surrounded by high numbers of human settlements. No settlements are found within the protected areas themselves, but do occur in the immediate vicinity of park boundaries. Study areas were defined by plotting a 25 km buffer around the boundaries of each protected area using R (R Development Core Team 2015), and were clipped to exclude marine areas, and non-Russian territory.

Dog densities were expected to correlate with human distribution, and so human census data were used for the selection of study settlements (Butler and Bingham 2000, Gompper 2014). Human census data was available for 2002 and 2010, with populations distributed among 83 extant settlements within the three 25 km buffers in 2010 (consisting of four around SABZ, 15 around Lazovskii and 64 in Southwest Primorskii, Table 3.2). A random selection of 26 settlements was identified for the survey, consisting of three settlements around SABZ, seven around Lazovskii, and 16 in Southwest Primorskii. Settlements were assigned to categories based on the number of residents reported in the 2010 census as villages (consisting of 1,000 people or fewer), towns (consisting of more than 1,000 people, but fewer than or equal to 10k), large towns (consisting of more than 10k people but fewer than or equal to 100k), or cities (more than 100k people). Selected settlements included 22/71 villages, 3/11 towns, and the only large town within the study areas. The study areas contained no cities, therefore the city of Ussuriysk situated less than 20 km from the Southwest Primorskii study area was also included in order to collect data describing dog ownership in urban areas.

Figure 3.1. Map illustrating the location of study areas Sikhote-Alin Biosphere Zapovednik (SABZ), Lazovskii Zapovednik, and Southwest Primorskii. Protected areas are illustrated in green, and 25 km buffers in orange. Study settlements are illustrated in red, and non-study settlements in yellow. The city of Ussuriysk is indicated by a blue square.

Target household sample sizes were selected using the 'pwr' package in R (Champely 2015). A minimum sample size of 389 households per study area was selected using a two-tailed test to detect differences in proportional responses to questions of 10%, with an expected response of 50% (which maximizes required sample size), at a power of 80% and a 95% significance level (Figure 3.2).

A questionnaire was developed based on guidelines published by the World Health Organization and the World Society for the Protection of Animals (1990), and was designed to collect information on dog and cat ownership patterns and demography.

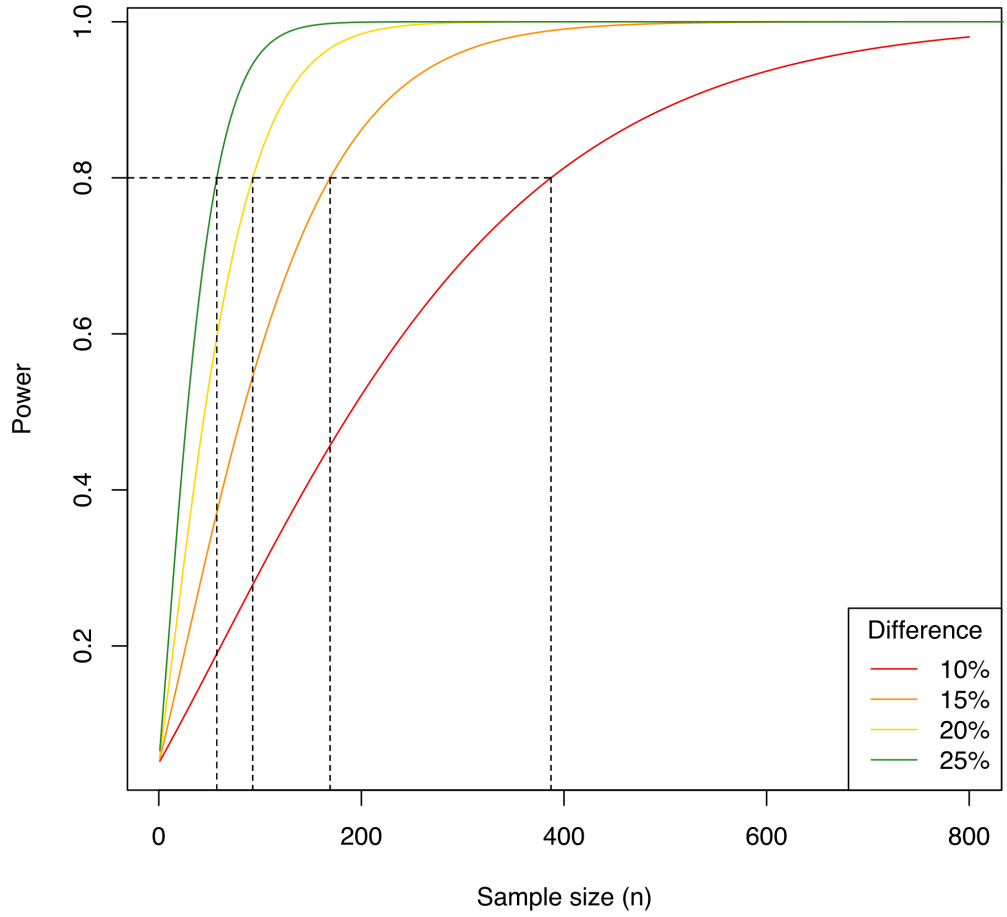

Figure 3.2. Illustrates the relationship between sample size and power, for detecting differences in proportional responses during questionnaire surveys of 10-25%, at a 95% significance level.

Questionnaires were developed following an initial pilot survey in June and July 2012, consisting of preliminary surveys of households in Southwest Primorskii and SABZ to assess the receptiveness of dog owners, and identify key questions. Questions were translated into Russian, and then back-translated to ensure consistency. Draft questionnaires were then adapted to produce a final questionnaire following initial trials among householders in SABZ during November 2012. Final questionnaires were used during visits to all 26 settlements during November 2012, June 2013 and October 2014. Interviewers visited all residences in villages, explained project objectives to householders and requested their compliance in completing the surveys irrespective of whether they owned dogs. In towns and large towns, it was impractical to survey all residences. In these settlements a subset of residences were randomly generated using the guidelines published by the World Society for the Protection of Animals (2007). Briefly, settlement maps were prepared, dividing the town into blocks, each containing a similar number of households (approximately 20-30, based on a visual assessment using Google Earth imagery). Blocks

were assigned one of four colours, such that no adjoining blocks were represented by the same colour. Survey households were identified by randomly selecting one of the colours, thus ensuring a random selection of households across the whole settlement. Once all blocks of a given colour had been visited, interviewers moved on to blocks of an additional randomly selected colour as time permitted, to maximize numbers of residences visited.

Interviews were conducted in Russian, by teams of at least two veterinary undergraduate students from the Primorskaya State Academy of Agriculture. Teams were supervised by the author on a rotational basis, to improve consistency in interview technique. Interviewers were trained to avoid leading questions and were encouraged to maintain awareness for inconsistencies, and to revisit points of confusion by approaching questions in a different manner until answers were resolved.

Interviews consisted of a list of questions that were completed in a semi-structured manner, allowing householders the freedom to answer inquiries on a conversational basis in any order they wished, before returning to unanswered sections. Questions were designed to obtain data at the level of the household, and on individual dogs and cats currently living in the residence. Details of questions pertaining to cats will not be reported further here, but are provided in Appendix V. Household data included residence type (typically apartment or cottage), the number of adult and child residents (<18 years old), and the number of dogs, and other domestic animals currently owned. Householders were also asked how many dogs they owned ten years ago to identify any temporal trends in dog ownership. Data collected on individual dogs included their age, sex, history of neutering and reason (or reasons) for ownership. Additional questions focused on the reproduction of female dogs, including current reproductive status (i.e. whether pregnant or lactating), number of litters within the last 12 months, number of pups in the last litter, and fate of all puppies in the last litter (e.g. alive and present, given away, died through trauma or sickness, euthanized etc.). Other questions addressed the movement of dogs, including their origin (local or from other settlements), frequency of movement beyond the settlement (never, rarely, at least annually, at least monthly, at least weekly), and freedom to roam unsupervised beyond the property boundaries (never, rarely/sometimes, part of the day, or all day). Owners were also asked whether they took their dogs to the forest, to assess their opportunity for contact with tigers. Data on vaccination history was limited to verbal responses, as vaccination certificates were rarely available and included questions on

whether dogs had received vaccines against CDV and rabies, and the approximate date of last vaccination. Those owners who reported that their dogs had not been vaccinated within the last year were asked for their reasons. The full questionnaire is provided in Appendices VI and VII.

An urban questionnaire was prepared to collect more limited data describing ownership within the city of Ussuriysk, and focused on assessing the numbers of dogs and cats owned by city residents (Appendix VIII). In such a large community it was considered impractical to survey a representative sample of households through door-to-door visits, therefore surveys were conducted among people out in the community in areas where most residents were assumed to visit. This included passers by and shoppers interviewed along major thoroughfares, transport hubs and outside popular groceries. Surveyors approached every adult passer-by to request their compliance in completing a short survey, and respondents were assumed to comprise a representative cross section of the city population. Interviewees were asked to provide their settlement of residence, the number of adults and children within their household, the type of residence (cottage or apartment), the number of dogs and cats currently owned, and the number of dogs owned a decade earlier.

Once data had been entered into electronic format, errors and inconsistencies were identified using the package ‘editrules’ in R (de Jonge and van der Loo 2013), and then corrected with reference to the original questionnaires. The human population growth was estimated for each study settlement using the census figures from 2002 and 2010. These growth estimates were used to extrapolate the 2010 census figures, to obtain estimated population sizes at the start of the study on 6 November 2012. Ratios of humans to dogs in surveyed households were used to estimate total numbers of dogs in each settlement, and also used as the basis for extrapolating numbers of dogs in each study area and the whole province at the start of the study in 2012. For this purpose, the distribution of human to dog ratios for settlements of each size category were obtained by resampling the data using a subsample of 100 households in each settlement size category, through 1,000 bootstrap replicates. These were fitted to a gamma distribution, which was then used to estimate the number of dogs in all settlements in each study area, and throughout Primorskii, across 1,000 replicates. Dog densities were calculated at the landscape and settlement level, by dividing the number of dogs estimated in each study area or settlement with their respective areas. The area of each settlement was obtained by calculating 95% kernel

distributions (based on an *ad hoc* smoothing parameter for a bivariate normal kernel, Worton 1989) around all surveyed households using the package 'adehabitatHR' in R (Calenge 2006). Household locations were not collected during the urban questionnaire survey, and so the area of Ussuriysk was estimated using the polygon tool in Google Earth Pro version 6.0.1.2032 (beta).

Expected dog lifespan was estimated using the methods outlined in Caughley (1977). Briefly, tables of life history were prepared for dogs, listing the numbers of live animals recorded in each age category (by year), and the number of deaths in each age category in the preceding year. The survivorship function L_x for each age category was calculated using the formula:

$$L_x = \frac{l_x + l_{x+1}}{2}$$

Where $l_x + l_{x+1}$ is the total number of animal-years lived by the cohort from age x to x+1, which is divided by two to give L_x at the midpoint of the year. The total number of animal years from year x until all animals had died (T_x) was then calculated according to:

$$T_x = \sum l_x - l_{x-1}$$

Life expectancy for age category x (e_x) was then calculated using:

$$e_x = \frac{T_x}{L_x}$$

Mean life expectancy was therefore estimated at the life expectancy for age class 0 years.

Before constructing multivariable models, a subset of cleaned data was prepared from the raw data, which excluded outcome variable entries where information on the appropriate predictor variables was incomplete. Data were tabulated for cleaned and raw data subsets, to identify any changes in the distribution of observations for each predictor variable that

may have occurred during the cleaning process (Appendices IX, X, XI, XII). Cleaning had little effect, amounting to ≤1.7% difference between raw and cleaned datasets for each non-numeric predictor variable.

Multivariate generalized binary logistic regression models were prepared to identify explanatory variables that were significantly associated with the following outcome variables: dog ownership ('dog owning' or 'non-dog owning' at the household level), recent CDV vaccination ('vaccinated' or 'not vaccinated' within the previous year, at the individual dog level), origin ('local' or 'non-local' at the individual dog level), and roaming ('roaming allowed' or 'roaming never allowed' at the individual dog level). Explanatory variables included study area, community type, residence type, number of people within residence (adults and children), presence of children, cats, poultry, and livestock, age, gender, whether the dog was a guard, a hunting dog, or a companion animal, and origin. Details of explanatory variable categories used for each outcome variable are provided in Table 3.1. Settlement was used as a random effect for the outcome variable dog ownership, and household was used as a random effect for recent CDV vaccination, origin and roaming. Models were prepared using a forward selection process, and Akaike information criterion (AIC) values were used to assess model quality. The final model was identified when addition of explanatory variables did not reduce AIC values further. Odds ratios were estimated as a measure of association between explanatory and outcome variables expressed within 95% confidence limits.

Results

A total of 2,576 rural questionnaires were completed across the 26 study settlements, and 1,461 urban questionnaires in the city of Ussuriysk. These included 523 households in the SABZ study area, 616 in Lazovskii and 1,437 in Southwest Primorskii (Table 3.2). Census figures from 2002 and 2010 were used to project the number of residents in each settlement on the first day of the study in 2012. Due to negative population growth, the projected 2012 population for one very small settlement (Poyma) was estimated to be zero, and since this site was still settled in 2013, the population from the 2010 census was used. Mean survey coverage across the 26 study settlements, based on the number of people in surveyed households as a percentage of total people projected in 2012 was 62.8% (SD

Table 3.1. Explanatory variables used to assess the domestic dog outcome variables ownership (Own.), vaccination (Vacc.), origin (Orig.), and roaming (Roam.), using multivariate generalized binary logistic regression models.

Explanatory variable	Variable type	Levels	Outcome variables			
			Own.	Vacc.	Orig.	Roam.
Study Area	Categorical	Southwest*	✓	✓	✓	✓
		Lazovskii				
		SABZ				
Community Type	Categorical	Village*	✓	✓	✓	✓
		Town				
		Large town				
Residence type	Categorical	Apartment*	✓	✓	✓	✓
		Cottage				
People in house	Numeric	Number of people	✓	✓	✓	✓
Children in house	Categorical	No*	✓	✓	✓	✓
		Yes				
Cat owner	Categorical	No*	✓	✓	✓	✓
		Yes				
Poultry owner	Categorical	No*	✓	✓	✓	✓
		Yes				
Livestock owner	Categorical	No*	✓	✓	✓	✓
		Yes				
Age	Numeric	Age in months				✓
Gender	Categorical	Female*		✓	✓	✓
		Male				
Guard dog	Categorical	Non-guard*		✓	✓	✓
		Guard				
Hunting dog	Categorical	Non-hunter*		✓	✓	✓
		Hunter				
Companion dog	Categorical	Non-companion*		✓	✓	✓
		Companion				
Source	Categorical	Local*		✓		✓
		Non-local				

* Indicates reference level for categorical variables.

54.2%). Coverage was lowest in the largest settlements surveyed, Plastun (6.2% with 5,123 people projected in 2012), and Slavyanka (10.4% with 13,776 people projected in 2012). The number of people in surveyed households exceeded the projected 2012 population figures in two of the smallest survey settlements (Poyma and Razanovka), suggesting that the populations in these settlements may have stabilized or increased after the 2010 census. A total of 1,156 respondents to the urban questionnaire reported residence in Ussuriysk (79.1%, n = 1,461), with remaining respondents residing in 82 other settlements. The Ussuriysk respondents represented households containing 0.73% of the population of 158,067 people projected to reside in Ussuriysk in 2012.

Dogs and cats were the most commonly kept domestic animals in the rural households surveyed, with the exception of chickens (Table 3.3). Median human to dog ratios were 1.73 in villages (SD=0.81, n=22), and 2.47 in towns (SD=0.62, n=3). Only one large town (Slavyanka) was present within the study area, which had a human to dog ratio of 10.21. The human to dog ratio in the city of Ussuriysk was 5.05. There was a greater number of dogs in Southwest Primorskii, than Lazovskii and SABZ (Table 3.2). Across the whole of Primorskii there were estimated to be 467,224 dogs, (CI: 442,549-496,933) and 555,778 cats, (CI: 518,890-594,211), owned by a projected 1,926,447 people at the start of the survey in 2012 (Table 3.2).

The three study areas were calculated to cover 11,964 km^2 in SABZ, 5,138 km^2 in Lazovskii, and 7,965 km^2 in Southwest Primorskii, which equated to overall dog density estimates of 0.3 dogs/km^2 (CI: 0.3-0.4 dogs/km^2), 1.1 dogs/km^2 (CI: 1.0-1.3 dogs/km^2) and 2.5 dogs/km^2 (CI: 2.3-3.0 dogs/km^2) respectively. Dog density within the settlements themselves varied widely (Table 3.4, mean=120.0 dogs/km^2, SD=103.4), and was highest in Slavyanka, the only large town in the study areas, with 458.5 dogs/km^2, and in the city of Ussuriysk with a density of 673.4 dogs/km^2.

The number of dogs owned by surveyed households across the 26 study settlements was 2,438, and these households reported owning a total of 1,862 dogs a decade earlier. This equated to a mean human to dog ratio of 3.14 (SD=1.68) in 2002, and 2.36 (SD-1.79) during the survey in 2012, indicating a per capita increase in dog ownership. However, since the combined human populations of all study settlements declined from 36,827 people in 2002, to 32,519, the total number of dogs present in the study settlements changed little during this time. The projected number of dogs present across all 26 study settlements was 8,248 in 2002, and 8382 in 2012, equating to an increase of only 1.62%.

Overall 57.4% of residences visited were dog-owning households (DOHH, n=2,576), and mean number of dogs per DOHH was 1.65 (SD=1.04, n=1,479), with 41.9% of DOHH supporting more than one dog. The best fit multivariate model found that dog ownership was most likely in households that were cottages, and those which also owned cats, poultry and other livestock, and increased with the number of people in the residence (Table 3.5, Appendix XIII). Conversely, dog ownership was less likely in towns and large towns than

Table 3.2. Summary of survey coverage in the three study areas, Sikhote-Alin Biosphere Zapovednik (SABZ), Lazovskii Zapovednik, and Southwest Primorskii. Human populations within each study area are derived from national censuses conducted in 2002 and 2010, and are extrapolated to estimate human populations at the start of the survey on 6 November 2012. Mean human to dog and mean human to cat ratios for villages, towns, and large towns are derived from survey data, and used to estimate dog and cat numbers at the start of the survey in 2012, with 95% confidence intervals based on a bootstrap analysis.

Study Area	Number of settlements in study area	Number of settlements surveyed	Number of households surveyed	Number of people in surveyed households	Human population census in 2002	Human population census in 2010	Estimated human population in 2012	Estimated dog population in 2012	Estimated cat population in 2012
SABZ	4	3	523	1,260	11,355	9,800	9,399	3,896 (CI: 3,379 to 4,491)	4,032 (CI: 3,439 to 4,742)
Lazovskii	15	7	616	1,555	17,516	14,235	13,390	5,869 (CI: 5,241 to 6,636)	5,917 (CI: 5,159 to 6,880)
Southwest Primorye	64	16	1437	3,732	61,231	58,374	57,638	19,914 (CI: 18,608 to 21,435)	20,777 (CI: 19,183 to 22,494)
Primorye total	653	26	3,732*	9,869*	2,071,210	1,956,497	1,926,947	467,224 (CI: 442,549 to 496,933)	555,778 (CI: 518,890 to 594,211)

*Includes households surveyed in Ussuriysk as part of urban questionnaire.

Table 3.3. Numbers of people and animals in surveyed households in each study area.

	Southwest	Lazovskii	SABZ	Total
People	3,732	1,555	1,260	6,547
Dogs	1,210	739	489	2,438
Cats	1,194	729	517	2,440
Pigs	271	147	11	429
Cattle	283	82	37	402
Sheep	145	5	1	151
Goats	75	19	6	100
Horses	67	2	6	75
Rabbits	328	233	226	787
Chickens	5,957	3,706	1,963	11,626
Ducks	134	104	53	291
Geese	314	75	59	448
Turkeys	51	45	25	121
Guineafowl	0	10	0	10
Quail	10	0	0	10
Parrots	0	1	0	1
Lizards	0	1	1	2

in villages. Owners stated one or more reasons for keeping dogs (n=2,396 dogs), the most common of which was for guarding property (67.5 %), followed by companionship (38.3 %), or for hunting (5.8 %).

Mean age was 4.49 (SD=3.82, n=1,652) for male dogs, and 4.57 years (SD=3.87, n=740) for females. The expected lifespan of dogs at birth (mean age at death) was estimated to be 3.39 years, but dogs surviving to one year of age have a mean life expectancy of 6.66 years old. A population pyramid illustrating the age and sex distribution of dogs in the survey, exhibited a wide base typical of a rapidly reproducing population, and moderately convex sides indicating a high death rate (Figure 3.3). The dog population was heavily skewed toward males, with a male:female ratio of 2.24:1, and was a reflection of the selective euthanasia of female puppies as the primary means of population control. Only 0.5% of male dogs (n=1,653), and 1.8% of females (n=741) were reported to have been sterilized.

Owners reported on the breeding status of 703 bitches over the previous year, of which 612 were of breeding age (taken to be 1 yr or older). Of these, 279 bred at least once in the last year, representing 45.6 % of breeding age females. These bitches had a total of 365 litters in the previous year, with a mean litter size of 4.5 (SD=2.4, n=305). Owners reported that

Table 3.4. Summary of descriptive statistics for survey settlements in each of the three study areas: Sikhote-Alin Biosphere Zapovednik (SABZ), Lazovskii Zapovednik (LZ), and Southwest Primorskii (SW).

Study area	Settlement name	Census 2010	No. households surveyed	No. people in survey households	No. dogs in survey households	Dog density (dogs/km^2)	Apartments (%)	CDV vaccine coverage (%)	Rabies vaccine coverage (%)
SABZ	Plastun	5,350	130	318	92	205	63.1	19.2	20.7
SABZ	Taejnoye	67	17	40	32	35	0	0	0
SABZ	Terney	3,590	376	902	365	100.1	5.6	42.9	58.5
SABZ	**TOTAL**	**9,007**	**523**	**1260**	**489**	**0.3**	**19.7**	**35.8**	**47.4**
LZ	Chistovodnoye	106	17	36	31	28.3	0	42.9	20
LZ	Danilchenkovo	176	19	66	25	127.5	0	0	0
LZ	Kamenka	154	29	63	50	63.9	0	50	50
LZ	Kievka	518	59	161	88	114.7	0	36.8	46.7
LZ	Kishenevka	160	43	114	57	52.6	0	14.3	0
LZ	Lazo	3,434	443	1,104	478	231.5	21.7	45.3	44.8
LZ	Svobodnoe	26	6	11	10	42.9	0	33.3	0
LZ	**TOTAL**	**4574**	**616**	**1555**	**739**	**1.1**	**15.6**	**40.3**	**38.7**
SW	Baranovskii	439	103	241	71	118	55.3	38.5	84.1
SW	Bezverkhovo	889	74	231	93	141.3	18.9	41	46
SW	Devatyy-Val	658	104	286	89	123.4	28.9	21.9	25
SW	Krounovka	594	85	208	128	189.5	0	54.3	39.3
SW	Lebedinoe	21	2	7	5	73.7	0	0	0
SW	Nejhino	435	88	242	130	227.6	0	17.6	27.3
SW	N. Lvovskoe*	148	49	111	79	38.7	0	43.3	89.6
SW	Olenevod	823	152	439	174	276.1	32.9	35.8	56.2
SW	Ovchinnikovo	71	14	33	31	64.5	0	40	0
SW	Perevoznoye	284	76	193	85	40.2	13.2	50	58.3

Study area	Settlement name	Census 2010	No. households surveyed	No. people in survey households	No. dogs in survey households	Dog density (dogs/km^2)	Apartments (%)	CDV vaccine coverage (%)	Rabies vaccine coverage (%)
SW	Poyma	6	4	9	8	20	0	80	80
SW	Razanovka	47	31	75	65	1	0	31.2	60
SW	Slavyanka	14,036	564	1,430	140	458.5	94.2	40.3	40.9
SW	Steklozavodsky	151	25	67	20	125.7	36	45.5	75
SW	Sukhanovka	76	15	29	9	12	73.3	0	0
SW	Timofeevka	260	51	131	83	209.6	0	28	37
SW	**TOTAL**	**18,938**	**1,437**	**3,732**	**1,210**	**2.5**	**49.5**	**36.4**	**47.5**

* Nikolo Lvovskoe

the majority (48.8%, n=1,354) of pups were given away, with 33.6% being euthanized, 7.6% being kept by the owners, 6.4% dying of accidental causes, 1.6% dying of sickness, and 1.9% where outcomes were reported as “other” or “unknown”. Owners reported only eight stillbirths. Factoring-in the breeding age females for which breeding status had not been reported (n=28), the surveyed population produced an estimated 1,736 puppies in the previous year. Considering the 33.6% of puppies that are euthanized, this equated to a per capita breeding rate of 0.48 births per dog per year (n=2,426).

Table 3.5. Odds ratios with 95% confidence intervals (CI) for explanatory variables predicting dog ownership, based on the best fit multivariate generalized binary logistic regression model, with settlement as a random variable.

Explanatory variable	Variable type	Levels	Odds ratio	CI: 2.5%	CI: 97.5%	p value
Residence type	Categorical	Apartment*	1	-	-	-
		Cottage	10.280	7.597	14.091	<0.01
Cat owner	Categorical	No*	1	-	-	-
		Yes	2.484	1.997	3.090	<0.01
Poultry owner	Categorical	No*	1	-	-	-
		Yes	2.703	1.998	3.699	<0.01
People in house	Numeric	1 person*	1	-	-	-
		Per additional person	1.306	1.189	1.435	<0.01
Livestock owner	Categorical	No*	1	-	-	-
		Yes	2.860	1.536	5.947	<0.01
Community Type	Categorical	Village*	1	-	-	-
		Town	0.674	0.496	0.931	0.01
		Large town	0.668	0.417	1.093	0.07

* Indicates reference level for categorical variables.

Survey respondents reported 308 dogs that died in the previous year. This equated to a per capita mortality rate of 0.13 deaths per dog per year (n=2,426), which combined with productivity from births, equated to an overall growth rate of 0.35 dogs per individual per year (ignoring the effects of migration). Owners reported that “sickness” was the most frequent cause of death (38.7%, n=308), with “distemper” (“*чума*”) explicitly mentioned in 51/119 cases. Other common causes of death included “road accidents” (14.0%), “senescence” (10.4%), and “anthropogenic” causes (9.7%, including “poisoning”). Two households in Lazo reported that tigers had killed one of their dogs during visits to the forest within the last year (0.6%). In addition, one householder in the settlement of Chistovodnoye reported that a tigress had repeatedly entered her property on the edge of

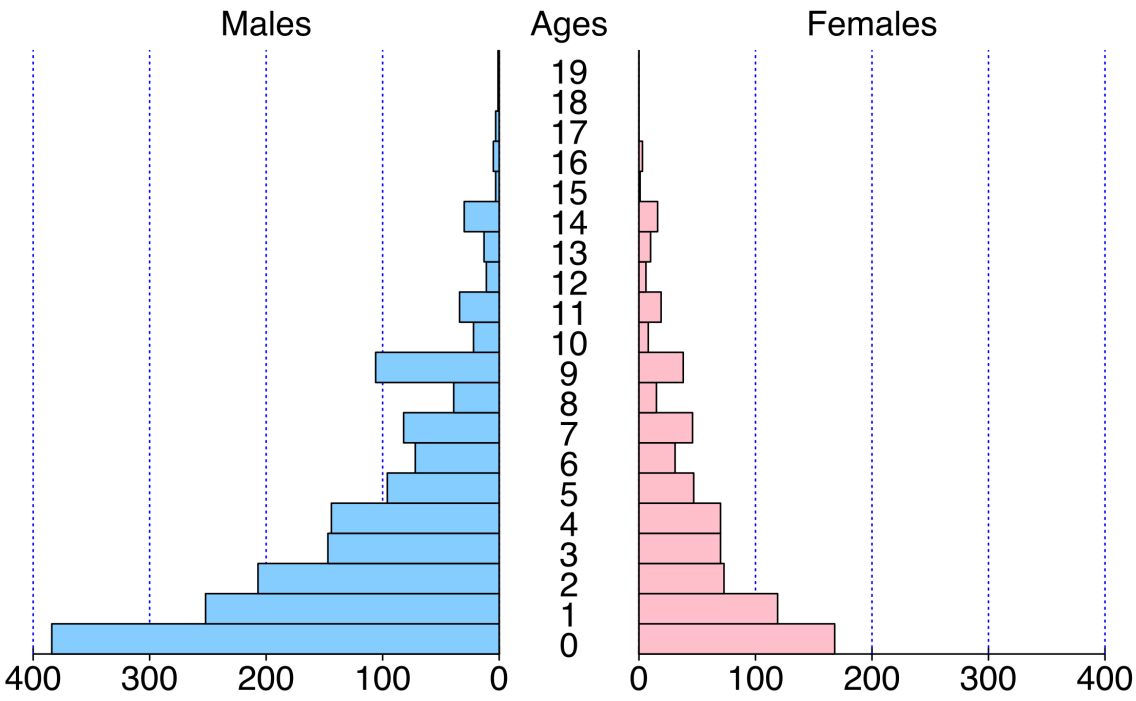

Figure 3.3. An age pyramid illustrating the frequency distribution of dogs at yearly age intervals, generated using the R package 'pyramid' (Nakazawa 2014).

the village, and killed seven of her dogs over approximately seven years (although none of these deaths occurred within the preceding 12 months, and so these are excluded from the mortality figures).

Owners were able to provide information on the origin of 2,332 dogs. Of these, 28.8% had originated from other settlements, 19.3% from other districts, 1.5% from other provinces, and 0.2% from other countries (including China, the United States, Australia and Belarus). The distance between origin, and current location for dogs originating from other settlements was highly left skewed, with a median distance of 51.5 km, and a mean of 222.8 km (SD=783.5, n=670). The best fit multivariate model indicated that a dog was less likely to have originated in another settlement if it was a guard dog, lived in a town or a large town, and resided in a cottage (Table 3.6, Appendix XIV). Although inclusion of study area improved the fit of the model, it was not significant as an explanatory variable.

Owners reported that the majority (93.1 %, n=2,379) of dogs were never taken to other settlements, with 2.6% travelling rarely, 2.6 % at least annually, 1.3 % at least monthly,

and only 0.4 % on a weekly basis. Owners were also asked about the unsupervised movement of dogs within settlements beyond their property boundaries. Most dogs (65.4 %, n=2,379) were reported never to roam without supervision, while 17.7 % reported to roam rarely or sometimes, 10.8 % to be allowed to roam for part of the day, and 6.1 % were allowed the freedom to roam at all times. Many of the owners who allowed their dogs to roam rarely, or sometimes noted that they only allowed them to roam during the winter, when their neighbours were not tending their produce gardens.

Table 3.6. Odds ratios with 95% confidence intervals (CI) for explanatory variables predicting dog origin, based on the best fit multivariate generalized binary logistic regression model, with household as a random variable.

Explanatory variable	Variable type	Levels	Odds ratio	CI: 2.5%	CI: 97.5%	p value
Guard dog	Categorical	No*	1	-	-	-
		Yes	0.513	0.381	0.682	<0.01
Community Type	Categorical	Village*	1	-	-	-
		Town	0.416	0.254	0.668	<0.01
		Large town	0.250	0.121	0.494	<0.01
Residence Type	Categorical	Apartment*	1	-	-	-
		Cottage	0.280	0.150	0.504	<0.01
Study Area	Categorical	Southwest*	1	-	-	-
		Lazovskii	1.136	0.736	1.753	0.56
		SABZ	1.219	0.688	2.167	0.50

* Indicates reference level for categorical variables.

The best fit multivariate model indicated that dogs were less likely to roam in the Lazovskii study area, or if they resided in towns or large towns, or had been sourced in another settlement (Table 3.7, Appendix XV). Roaming was more likely for companion dogs, from households with cat ownership, and in older dogs.

Owners reported taking 41.0% of dogs to visit forested areas (n=2,324 dogs), where encounters with tigers or other wild carnivores could potentially occur. In addition to the reported incidents of predation by tigers, several owners reported direct contact between their dogs and mesocarnivores. The owner of one dog, with visible skin lesions consistent with sarcoptic mange and bite wounds, noted that the infection had been contracted during a fight with a raccoon dog. Another owner reported a similar incident, whereby their dog contracted mange from a raccoon dog and subsequently died following an infestation with fly larvae.

Table 3.7. Odds ratios with 95% confidence intervals (CI) for explanatory variables predicting dog roaming, based on the best fit multivariate generalized binary logistic regression model, with household as a random variable.

Explanatory variable	Variable type	Levels	Odds ratio	CI: 2.5%	CI: 97.5%	p value
Community Type	Categorical	Village*	1	-	-	-
		Town	0.6615	0.4649	0.9462	0.02
		Large town	0.2448	0.1503	0.3835	<0.01
Study Area	Categorical	Southwest*	1	-	-	-
		Lazovskii	0.4055	0.2951	0.5511	<0.01
		SABZ	0.9845	0.6495	1.4838	0.94
Companion dog	Categorical	No*	1	-	-	-
		Yes	1.6868	1.4026	2.0289	<0.01
Source	Categorical	Local*	1	-	-	-
		Non-local	0.729	0.5989	0.8851	<0.01
Cat owner	Categorical	No*	1	-	-	-
		Yes	1.3969	1.1129	1.7617	<0.01
Age	Numeric	1 month	1	-	-	-
		Per additional month	1.0022	1.0003	1.0041	0.02

* Indicates reference level for categorical variables.

Owners reported that 32.0% of dogs had been vaccinated against CDV within their lifetime, with only 11.4% of dogs immunized within the preceding 12 months (n =2,369). Similar vaccination rates were reported against rabies, with 34.0% having been inoculated at least once in their lifetime, and 16.8% within the last 12 months (n=2,367). Levels of vaccination study area were significantly different (chi-square = 57.467, $p<0.01$, df = 2), with 39.3%, (CI: 36.5-42.2%, n=1,169) of dogs vaccinated in Southwest Primorskii, 23.9%, (CI: 20.9-27.2%, n=719) in Lazovskii, and 26.4%, (CI: 22.6-30.6%, n=481) in SABZ. The best fit multivariate model found that dogs were more likely to have been vaccinated during the previous year if they had been sourced in another settlement, but vaccination was less likely for guard dogs (Table 3.8, Appendix XVI). Owners who gave one or more reasons for not vaccinating against CDV in the last year attributed this to: “unaware of benefit” (58.3%, n=1,237), “lack of local facilities” (17.9%), “lack of time” (6.8%), “lack of awareness of locations offering vaccination” (5.7%), “cost” (2.3%), “inability to handle” (1.8%), “unaware of yearly need” (1.1%), or “other” reasons (14.8%).

Table 3.8. Odds ratios with 95% confidence intervals (CI) for explanatory variables predicting vaccination of dogs within the previous year, based on the best fit multivariate generalized binary logistic regression model, with household as a random variable.

Explanatory variable	Variable type	Levels	Odds ratio	CI: 2.5%	CI: 97.5%	p value
Source	Categorical	Local*	1	-	-	-
		Non-Local	3.56	1.27	10.9	0.02
Guard dog	Categorical	No*	1	-	-	-
		Yes	0.25	0.0684	0.793	0.02

* Indicates reference level for categorical variables.

Discussion

Domestic dogs are a ubiquitous part of village life in rural Primorskii, with among the highest levels of dog ownership (i.e. the lowest human to dog ratios) anywhere in the world. The mean human to dog ratio of 1.95 recorded in villages was roughly equivalent to those recorded in rural areas in Latin America (including 1.5 to 1.87 in Argentina [Cardinal et al.2006; Gurtler et al.2007], 1.5 in Bolivia [Fiorello et al.2006], 1.1 to 2.1 in Chile [Acosta-Jamett et al.2010; Silva-Rodríguez et al.2010], and 1.7 to 2.6 in Mexico [Fishbein et al.1992; Ortega-Pacheco et al.2007]), and was marginally lower than elsewhere in Asia (e.g. 3.1 in Cambodia [Ly et al.2009]), and considerably lower than those recorded in Africa (e.g. 5.3 to 14 in Tanzania [Knobel et al.2008a]). These ratios lead to high densities of dogs within settlements, however, dogs are distributed in a highly aggregated manner across the landscape, and so their impact on wild species may be considerably lower than these figures suggest. For instance, the dog densities of 0.3 to 2.4 dogs/km^2 estimated in the three study areas in Primorskii compare to 5.72 to 7.17 dogs/km^2 estimated in Serengeti District of Tanzania, which were thought to be the source of 1994 outbreak of CDV among the Serengeti lions (Cleaveland et al. 2000). Furthermore, the Primorskii figures are liable to over-estimate dog densities in tiger habitat, due to the exclusion of dogs from protected areas.

The strongest predictor of dog ownership was residence type, with more dogs owned by cottage households than apartments. Apartments become more common with the increasing size of settlements, representing 15.3% of households in villages (SD 26.7, n=22), 30.3% in towns (SD=29.9, n=3), 94.3% in the large town of Slavyanka, and 79.8% in the city of Ussuriysk. This may be due to restrictions in space, the comparative difficulty of outdoor access, limitations in areas suitable for exercising dogs, or the greater security

in buildings with a single common access (and therefore reduced need for guard dogs). Interestingly, the presence of other animals (cats, poultry and livestock) was a strong predictor of dog ownership. One possible explanation is that dogs are kept to prevent predation of livestock and poultry by other dogs or mesocarnivores, although it should be noted that the latter are rarely observed in settled areas due to high levels of persecution and the value of their pelts (Dronova and Shestakov 2005). Alternatively, people with an affinity for animals may be more likely to keep dogs, as well as consider the care of poultry and livestock a worthwhile endeavor. In Tanzania, Knobel et al. (2008) also found a strong association between dog and livestock ownership. In that study only 12.7% of those surveyed reported the primary reason dog ownership as protection of livestock, and 23.5% as protection of crops, suggesting that dogs were primarily kept for reasons other than increasing agricultural productivity.

Although growth rate estimates based on birth and death rates suggest a markedly growing population, it is likely that the actual growth rate falls somewhat lower than this. At the level of the household, dog ownership has increased over the last decade at a mean annual rate of 2.73%. This increase falls considerably below the calculated annual growth rate of 34.9% based on reported rates of breeding and mortality. One possible explanation for this is the tendency for owners to under-report dog mortality during the first year of life. Owners reported that 41.5% of pups were either euthanized, or died due to accident or sickness. This would equate to 720 of the estimated 1,736 pups born in the previous year, yet householders reported just 101 mortalities of dogs less than 12 months old. The disparity is only slightly greater than the estimated 547 pups that would have been euthanized, and it is possible that owners are reluctant to report euthanized pups in their annual mortality figures. Another explanation for under-reporting of deaths during the first year of life may be that owners do not perceive pups and juveniles as equivalent to older dogs. There is an expectation of high mortality in this age class, and pups have had less time to bond with their owners, therefore their deaths do not have the same emotional impact as it would for older dogs.

Despite the increase in dog ownership over the last ten years, the total numbers of dogs in the three study areas have remained relatively steady (increasing by only 0.9% during the decade). This was due to a declining human population in study settlements, as urbanization drives a depletion of rural areas in Primorskii Krai. Notably, the ten year trend

in dog ownership was affected by residence type. Owners currently residing in apartments reported keeping 26.7% fewer dogs than a decade earlier, compared to an increase of 32.3% reported by owners in cottages. Although the survey questionnaire did not account for changes in residence type over the preceding decade, it is likely that urbanization would increase apartment occupancy. This would suggest that the changing picture of dog ownership is complex, with more dogs being kept by fewer households in rural areas, while net urban migration is likely to be driving an overall decline in dogs being kept within the province as a whole.

The prominence of sickness among reported causes of mortality was notable (38.7%, n=308), particularly given the frequency with which “distemper” was explicitly mentioned (51/119 cases of sickness). This certainly suggests that infectious disease may be common in dog communities in Primorskii, but attempts to relate this to the true incidence of distemper may be misleading. Due to the limited access to veterinary care, and particularly to confirmatory diagnostics, few (if any) of the reported distemper cases were likely to refer to anything more than a presumptive diagnosis. It is possible that owners may have been predisposed to mention “distemper” specifically if interviewers had used the word themselves when introducing the project when requesting survey compliance. Furthermore, in common with the etymology of the word ‘distemper’ in English, the Russian word for ‘distemper’ (“*чума*” - čúma) equates to the word for ‘plague’, and may be used by laypersons as a catchall for different causes of sickness in dogs. The use of the English words ‘cold’ or ‘flu’ used in reference to respiratory disease in humans is a similar example.

Although more than a quarter of dogs originated outside their current settlement, only a small proportion (6.9%) travelled to other settlements in later life. Assuming that most rural settlements fall below the CCS, this movement of young dogs represents the most likely means of introducing CDV from other dog populations. Infection with CDV is most common in young dogs, after maternal antibody titers wane at three months of age, and up to 75% of infections can be subclinical (Greene and Appel 2006). Once introduced, the spread of CDV through a community is facilitated by the ability of infected dogs to interact with susceptible animals. In Primorskii, relatively few dogs were free to roam within settlements, with 65.4% never allowed to leave the owner’s property unsupervised, and only 16.9% roaming on a daily basis. Movement was particularly restricted for dogs living

in apartments, with 78.7% (n=169) never leaving the apartment unsupervised. By comparison, in Chile, Acosta-Jamett *et al.* (2010) found that 27% of dogs in cities, and 67% of dogs in rural areas had freedom to roam, with as many as 92.2% roaming in another rural area of the same country (Sepúlveda et al. 2014). Similarly 90% of dogs surveyed in Kenya were allowed to move unsupervised for substantial periods of time (Kitala et al. 2001). The level of movement restriction in the present study was roughly equivalent to the 68.3% of dogs restricted in an urban area of Taiwan (Weng et al. 2006). These restrictions in dog movement would hinder the capacity for CDV to spread within communities, an effect that would be particularly evident among dogs living in large towns and cities, where apartments predominate.

Vaccination has been very effective in controlling CDV, to the point of near elimination among domestic dogs in some parts of the world (Bohm et al. 2004, Norris et al. 2006). Despite its importance, as few as 30-50% of dogs are estimated to be vaccinated against CDV and other canine infections in developed countries, with far fewer in developing nations (Day et al. 2010). By comparison, the 32.0% of dogs in Primorskii that had received a CDV vaccine at least once in their lives, and 11.4% within the previous year, while low, is not far below the range that could be expected within a country with good levels of veterinary care. More encouraging was the observation that five communities in Southwest Primorskii reported recent rabies vaccination coverage in excess of 60%, with one community reaching an impressive coverage of 89.6% of dogs (Nikolo-Lvovskoe in 2013). These levels were reached due to an intensive rabies vaccination campaign that took place in parts of Southwest Primorskii immediately prior to our survey, following a recent human rabies exposure, and highlighted the potential of well organized, government sponsored vaccination programmes. Increased vaccination in dogs sourced from other settlements indicated by the best fit multivariate model, may have reflected a higher value of these animals, with people more willing to travel to obtain a desirable breed. Conversely, the lower rates of vaccination of guard dogs in the model may reflect lower value, greater difficulty in handling, or a perception that disease is less likely in dogs that are often chained up.

Few studies have attempted to estimate the CCS that would be required to ensure the persistence of CDV. Empirical studies estimating the CCS of human measles, consistently produced figures in the region of 250,000 to 400,000 (Bartlett 1957, Black 1966), however,

rates of human reproduction and contact are quite different from those of domestic dogs, and so these figures will not apply to CDV. A single-species model based on the coyote population in the Greater Yellowstone Ecosystem estimated a minimum CCS of at least 50,000 to 100,000 animals to achieve a moderate persistence scenario for CDV of >50% within ten years (Almberg et al. 2010). These figures are an order lower that the province-wide estimate of 467,224 dogs made for the whole of Primorskii. However, within this metapopulation, the single largest community (Vladivostok) had an estimated dog population of approximately 100,000 dogs, for which contact is likely to be far more restricted than a free-ranging population of coyotes. For the purposes of comparison, there may be value in estimating the population of free-ranging wild carnivores over an equivalent area to the dog population estimates made here. Using conservative density estimates of the region's most common wild carnivore species, extrapolated across the 155,000 km^2 of habitat occupied by tigers (Hebblewhite et al. 2014), mesocarnivores may number between 196,850 and 585,900 animals (Table 3.9). While the margins of these estimates are quite wide, it is notable that they bracket the projections made for domestic dogs, emphasizing that the role of wild carnivores in CDV maintenance should not be ignored.

Despite the uncertainty surrounding the CCS needed to support CDV in domestic dog populations, certain features of ownership and demography make maintenance more or less likely. Among features favouring CDV maintenance are the high densities of dogs, particularly in larger settlements, the high rate of reproduction, and the relatively low level of vaccination coverage. The tendency to move dogs during the early stages of life would also promote spread to communities that fall below the CCS. These effects would be countered by the lack of movement of mature dogs between settlements, and the comparatively low rates of unrestricted movement, particularly in large populations where dogs are found at high densities.

The maintenance of CDV in domestic dogs only becomes relevant to the health of tigers in circumstances where contact is sufficient to enable dogs to transmit infection to the tigers directly, or to other susceptible wildlife that may act as a source of infection. With 41.0%of dogs from rural and urban areas being taken to the forest, and 5.8% of dogs being used for hunting, there are clearly many opportunities for wildlife interaction. Anecdotal reports of tigers predating dogs illustrate the opportunity for direct transmission. Furthermore, two

owners reported that their dogs contracted mange (assumed to be *Sarcoptes scabei* infection) during agonistic interactions with raccoon dogs, suggesting a degree of contact

Table 3.9. Published density estimates (animals/km^2) for the four most abundant mesocarnivore species in Primorskii Krai. Low and high density estimates are based on non-urban settings within published sources, giving preference to Russian sources where available. Density estimates are not available for Asian badgers, so the range quoted refers to the closely related Eurasian badger (*Meles meles*), to which the taxon was formerly considered conspecific. Density estimates are extrapolated across the 155,000 km^2 distribution of the Amur tiger to produce low and high population estimates.

Species name	Low density estimate	High density estimate	Low population estimate	High population estimate	References
Sable *Martes zibellina*	0.04	0.67	6,200	103,850	(Stroganov, 1969)
Asian badger *Meles leucurus*	0.4	1.5	62,000	232,500	(Larivière and Jennings, 2009)
Red fox *Vulpes vulpes*	0.49	1.13	75,950	175,150	(Heydon et al., 2000)
Raccoon dog *Nyctereutes procyonoides*	0.34	0.48	52,700	74,400	(Ward and Wurster-Hill, 1990)
Total			**196,850**	**585,900**	

that would likely enable transmission of CDV. The frequency of these interactions is difficult to assess, and in the case of hunting dogs it is worth noting that hunting season in Primorskii runs from 1 November through 15 January, a period coinciding with the hibernation of badgers and raccoon dogs, which may reduce opportunities for direct contact. Conversely, cold temperatures at this time may extend the viability of CDV (Greene and Appel 2006, Ballmann Acton 2007), raising a possible role for indirect transmission in the epidemiology of the virus.

Attempts to control CDV in the dog population in Primorskii should focus on raising the herd immunity by increasing vaccination coverage. In the face of limited resources, vaccination programmes should adopt strategies that maximize the number of unvaccinated dogs that are inoculated using the funds available (Knobel et al. 2008). In Primorskii, the strongest predictor of dog ownership is residence type, with 80.1% of cottage households owning at least one dog, compared to just 16.9% of apartment households. Cottages are also likely to house a greater number of dogs than apartments. Therefore, in large towns

and cities, vaccination programmes should concentrate on districts where cottages predominate, as strategies targeting apartments provide a low return for investment. However, this targeting strategy would be difficult to apply to most villages, as cottages represent the predominant, or only house type in most settlements of this size.

While only 11.4% of dogs had received vaccinations within the previous year, a further 20.6% of dogs had been vaccinated at earlier time points. The level of protection afforded by these earlier vaccinations could have an important bearing on the overall level of herd immunity. A study that measured antibody titers in 144 dogs in England, which had been vaccinated at least three years previously found that 71.5% still had protective titers, with a further 18.1% found to have borderline titers where protection was possible, but could not be assured (Bohm et al. 2004). Other researchers have found that CDV antibody titers declined significantly after two years (Jóźwik et al. 2004), and in India dogs vaccinated more than a year earlier were as likely to contract CDV as unvaccinated dogs (Latha et al. 2007). This disparity could relate to the effectiveness of vaccine products, or variation in protocols used. It would therefore be valuable to measure the relationship between antibody titers and time since vaccination in dogs in Primorskii, to determine whether protection is adequate in this population.

At a population level, vaccination reduces the number of dogs that are susceptible to contracting CDV infections. When an infected individual enters a population where the number of susceptible dogs exceeds $1/R_0$, the incidence of infection is liable to increase, a situation that favours the onset of an outbreak. Consequently, vaccination programmes aim to attain a coverage of $1-1/R_0$, in order to keep the proportion of susceptible animals below this threshold. Assuming that vaccination against CDV protects dogs indefinitely, the level of vaccination reported in study settlements would be sufficient to control outbreaks if the R_0 of CDV were 1.47 or lower in dog populations in Primorskii. The value of R_0 is not absolute, and will vary with population structure and other factors. Few studies have attempted to estimate R_0 for CDV in field situations. A model based on surveillance data from Italian foxes was used to estimate R_0 to be 1.26 in that population (Nouvellet et al. 2013), however, this may have little relevance to domestic dogs living in higher densities in human settlements. In Tanzania, the success of dog vaccination in controlling rabies, but not CDV was attributed to the higher R_0 of the latter (Viana et al. 2015). Estimates of R_0 for rabies among domestic dogs in Tanzania is approximately 1.2, and has been

consistently found to be <2 among dogs in other parts of the world (Hampson et al. 2009), suggesting that the R_0 for CDV may exceed the 1.2 to 2.0 range. If this applied to rural Primorskii, then the R_0 of CDV may exceed the 1.47 threshold of protection afforded by the vaccination that has been reported alone. Further work to assess the levels of protective antibodies (both from vaccination and natural infection), as well as research to estimate R_o in the population would be required to assess whether measures to increase vaccination coverage would be desirable.

Additional measures might complement vaccination programmes to control CDV in the dogs of Primorskii. The majority (58.3%) of people who had not vaccinated their dogs within the previous year reported that they were unaware of the purpose for doing so. This might indicate a place for outreach programmes, to inform owners of the value of vaccination. Given the potential importance of young dogs in the movement of CDV, advice on the vaccination of puppies after 12 weeks might reduce the spread of CDV between settlements.

Conclusion

The levels of dog ownership in the far eastern province of Primorskii are among the highest anywhere in the world, with among the lowest recorded human to dog ratios. However dog populations are highly aggregated, and densities are extremely low in rural landscapes where tigers live. Although the number of dogs kept per person has increased during the previous decade in rural areas, this has been offset by a trend toward urbanization. Thus the overall number of dogs in the province has changed little during the period since 2000 when CDV exposure in tigers appears to have increased. Several factors, including high local densities, high reproductive rate, and relatively low vaccination coverage could favour the maintenance of CDV. However, the limited movement of dogs between settlements, and restrictions on dog movement within settlements will reduce the capacity of CDV to infect news hosts, and reduce R_0. The influence of these factors on the capacity for CDV to be maintained in the dog population may be advanced through the development of epidemiological models, and serological studies to determine the spatial and temporal patterns of CDV exposure. In the event that the domestic dog population is capable of maintaining CDV, opportunities do exist for transmission to tigers and other susceptible carnivores. However, it should be noted that the estimated dog population in

the province of almost half a million animals may be numerically similar to that of susceptible mesocarnivores. Given these numbers it would be prudent to consider the possible contribution of mesocarnivores to CDV maintenance before attempting to control tiger infections through vaccination of domestic dogs alone.

Author contribution

The author conceived and developed the overall study design, and secured primary sources of funding for the project. Permission to conduct the study was secured from the State Veterinary Inspection by the author and colleagues with this Institute of Biology and Soil Sciences (IBSS, Appendix IV). The author designed questionnaire surveys, and trained local veterinary undergraduate students from the Primorskaya State Academy of Agriculture (PSAA), and a veterinarian with IBSS to implement them. All questionnaire data were collected by PSAA and IBSS interviewers under the supervision of the author. The author performed all data entry, analysis and interpretation of results.

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Chapter 4 Molecular characterization of canine distemper virus in Amur tigers and the wider carnivore community in Primorskii Krai

Abstract

Canine distemper virus (CDV) negatively impacts the population viability of Amur tigers *Panthera tigris altaica* in the Russian Far East but little is known about transmission links between tigers, other wild carnivores and domestic dogs. This study used conventional and Illumina sequencing to generate complete and partial sequence data from haemagglutinin and fusion gene sequences of viruses from tigers (n=3, 2003-2013), six other wild carnivore species (n=23 2011 to 2015) and a domestic dog (n = 1, 2016) in the Russian territory of territory of Primorskii Krai. Phylogenetic trees were constructed using maximum likelihood methods. All wild carnivore viruses, including tigers, clustered within the Arctic-like clade of CDV, which is widely distributed in northern latitudes but rare among domestic animals in neighbouring countries. By contrast, the two domestic dog viruses clustered within the Asia-4 clade, and shared highest identity to a virus from Thailand. CDV sequences from tigers and other large wild carnivores shared recent common ancestors with those obtained from mesocarnivores, and viruses from disparate locations were well mixed across sublineages. These results suggest that CDV sublineages circulate among a wide range of wild carnivore hosts across relatively large geographic scales. Although samples sizes were very limited, this study also indicates that CDV sublineages circulating in wildlife are distinct from the viruses isolated from domestic dogs in the same region. These results indicate that wild carnivores represent the most likely source of CDV for tigers and that control measures directed only at domestic dogs are unlikely to be effective in mitigating the impact of infection on Amur tiger populations in the Russian Far East.

Introduction

Based on the findings from Chapter 3, it is evident that some aspects of the domestic dog population in Primorskii Krai may favour the maintenance of CDV (particularly the large dog population, rapid reproduction, and relatively low vaccine coverage), while others may inhibit it (including restricted movement within and between settlements). It was also noted that numbers of dogs and wild mesocarnivores might be roughly comparable, suggesting that either population could be important contributors to the CDV reservoir in the region. Anecdotal reports of direct contact between dogs and tigers, as well as between dogs and mesocarnivores suggest potential routes for CDV transmission between these populations. With 41.0% of dogs given access to the forest, this contact could be occurring on a regular basis. Although owners reported that sickness was the most common cause of dog mortality, with specific mention of distemper in many cases, this can only be taken as anecdotal support for the presence of CDV in the dog population. Therefore the primary objective of Chapter 4 is to diagnose cases of CDV in the dog, mesocarnivore and large carnivore populations, and to assess the genetic relatedness of viruses in each, as an indication of potential transmission pathways.

Canine distemper virus (CDV) is an enveloped negative single-stranded RNA virus within the family Paramyxoviridae and genus *Morbillivirus*. The pathogen is capable of infecting a diverse range of host species, particularly within the order Carnivora (Deem et al. 2000). Consequently, CDV has a near global distribution, and has been detected in both terrestrial and marine ecosystems, with infections in both domestic and wild animal hosts (Harder and Osterhaus 1997, Craft et al. 2008). This capacity to transmit between species presents a particular threat to endangered populations, as more abundant reservoir host species can act as a continued source of infection (Woodroffe 1999, Viana et al. 2014). The recent detection of CDV in Amur tigers *Panthera tigris altaica* in the Russian Far East is an example of this (Quigley et al. 2010, Seimon et al. 2013), with the virus increasing the extinction potential of individual tiger populations (Gilbert et al. 2014). With population estimates of between 331 and 393 adult and subadults (Miquelle et al. 2007), and low intra-species connectivity (Goodrich et al. 2010) it is unlikely that tigers can maintain CDV without transmission from other species. As CDV infections generally have short-lasting incubation and infectious periods (Greene and Appel 2006), intra-species transmission chains between tigers are likely to be short, and restricted to infrequent contacts between territorial males and females, or mothers and cubs (Gilbert et al. 2015).Therefore tiger

infections are more likely to follow transmission through ‘spill over’ from more abundant susceptible hosts, such as domestic dogs or wild mesocarnivores, with which they are sympatric.

Due to the low copy fidelity of many pathogen genomes (particularly those of RNA viruses), mutations are accumulated on a similar timescale to transmission, which can provide insights on the dynamics of infection in host populations. At a fundamental level, viruses that spread inefficiently in a target population following ‘spill over’ from a reservoir, will share a recent common ancestor with viruses in the source population (Viana et al. 2014). By obtaining sequence data from pathogens across an epidemiological system, it may also be possible to retrace more complex transmission routes using techniques adapted from genealogy studies (Bedford et al. 2010, Mather et al. 2013), or the construction of parsimony networks (Templeton et al. 1992, Lembo et al. 2007). A major limitation in these approaches is the availability of representative sequences from across the reservoir system. Where no samples are available from source populations, infections in the target may be incorrectly attributed to transmission from other populations where sequences have been obtained (Beerli 2004, Viana et al. 2014). Nevertheless, as long as available sample sets are evaluated critically, they may provide a useful means of estimating possible routes of transmission. The genome of CDV accumulates substitutions at a relatively high rate (4×10^{-4}, nucleotide substitutions per site per year, Panzera et al.2015). Sequence data collected at different time points can be used to estimate the rate of nucleotide substitution using molecular clock models of evolution, which enables the time to most recent common ancestor (TMRCA) for a set of sequences to be estimated (Drummond et al. 2003). Given that intra-species transmission chains among tigers are likely to be short, it would follow that tiger viruses should share a recent common ancestor to those found in maintenance hosts, or sources of infection (Viana et al. 2014).

The phylogeny of sequences across a host population may also be used to infer its potential for maintaining the pathogen. By definition, maintenance comprises the long term persistence of a pathogen, and is therefore likely to be characterized by long transmission chains within the host population (Haydon et al. 2002, Viana et al. 2014). These long chains of transmission give rise to multiple lineages that are genetically distinguishable, and over time disseminate spatially through infection of neighbours, and movement of infected animals across the landscape giving rise to a diverse and geographically well-

mixed pathogen gene pool across the maintenance population (Viana et al. 2014). Similar genetic patterns can arise where spill over occurs regularly, giving rise to a spatially well-mixed pattern of pseudo-endemicity (Viana et al. 2014). As a result of this, spatial-mixing of genetic lineages is not a definitive identifier of a maintenance population; nonetheless it can be useful as supporting evidence within the wider framework of an epidemiological investigation.

Viral sequence data can also be used to identify adaptive changes that may influence the biology of the virus. With no evidence that Amur tigers have been exposed to CDV prior to 2000, it has been proposed that the virus may be newly emerging in the tiger population (Goodrich et al. 2012, Seimon et al. 2013). This could arise through an increase in viral exposure (e.g. through an expanding viral distribution, or increasing incidence within the reservoir population), or through an adaptive change in the virus itself, which increases the potential for tiger infection. The sequencing of viruses from tigers, and other carnivores in the Russian Far East may therefore produce important information about adaptive mutations that may affect species susceptibility.

The CDV genome is 15,690 base pairs (bp) long, and includes genes that encode for six structural proteins: the nucleocapsid (N), phospho- (P), matrix (M), fusion (F), haemagglutinin (HA) and large- (L) proteins. In addition, the P-gene encodes for two non-structural proteins, V and C, which modify host immune response (Nakatsu et al. 2008). The HA-gene shows the greatest variation within the CDV genome, and has been used to classify the virus into distinct clades that roughly approximate to <96% similarity at the amino acid level (Bolt et al. 1997, Martella et al. 2006). Currently 15 clades have been proposed (excluding vaccines), which are largely restricted to geographic regions (Haas et al. 1997, Bolt et al. 1997, Iwatsuki et al. 1997, Zhao et al. 2010, Radtanakatikanon et al. 2013, Espinal et al. 2014, Sarute et al. 2014, Riley and Wilkes 2015). Analysis of the F-gene has supported this phylogeographic clustering, although available data are more limited (Lee et al. 2013, Sarute et al. 2013, Romanutti et al. 2016).

Most research has focused on the envelope glycoproteins HA (responsible for cell binding and host specificity), and F (responsible for entry into host cells, and the formation of syncytia, through the fusion of neighbouring cells). The HA-protein can bind to two host

receptors: the signalling lymphocyte activation molecule family 1 (SLAM/F1, also known as CD150) on T and B lymphocytes, and the nectin-4 receptor (also known as polioviruslike receptor 4, PVLR-4) on epithelial cells (Tatsuo et al. 2001, Noyce et al. 2012). Once bound, conformational changes to the HA-protein stimulate the F-protein to mediate cellular entry. Viral affinity for SLAM/F1 receptors is necessary for entry into new hosts, and is at least partially responsible for the lymphodepletion and immunosuppression that characterizes CDV infections (von Messling et al. 2006). Epithelial infection, mediated via nectin-4 entry, plays a key role in pathogenesis, causing respiratory, gastrointestinal and systemic signs, and may also be linked to neurological disease that can follow in later stages of infection (Sawatsky et al. 2012), although other as yet unidentified receptors may also play a role (Sato et al. 2012). Host susceptibility is primarily determined by the affinity of the HA and SLAM/F1 proteins, influenced by variation in the structural and physical properties of both molecules (McCarthy et al. 2007, Ohishi et al. 2014).

Non-synonymous mutations at two positions of the HA-gene have been postulated as influencing the host specificity of CDV (McCarthy et al. 2007, Nikolin et al. 2012b). An analysis of 73 CDV HA-gene sequences by McCarthy *et al.* (2007) identified amino acid substitutions at position 530 (from glycine [G] or glutamic acid [E], to either arginine [R], aspartic acid [D], or asparagine [N]), and position 549 (from tyrosine [Y] to histidine [H]) that appeared to favour infection in non-canid hosts (McCarthy et al. 2007). However, a more recent analysis of a larger dataset (comprising 139 sequences) found no support for the involvement of position 530 in host adaptation, with amino acid identity at this position largely conserved within geographic regions irrespective of species (Nikolin et al. 2012b). The analysis also found a more complex correlation at position 549, with domestic dogs showing a strong bias toward Y residues (Y/H ratio of 71/1), a weak bias toward Y residues in wild canids (23/11), and a weak bias toward H in non-canids (13/20). Within a single host species, the Y549H substitution can be acquired through just three passages of an attenuated CDV 549Y strain through a ferret infection model, and was shown to be associated with an increase in virulence (von Messling et al.2003). The rapid acquisition of this substitution suggests that at least in ferrets, the Y549H substitution may be highly adaptive. *In vitro* experiments have determined that dog-like (549Y) strains were more efficient at infecting cell lines carrying dog SLAM/F1 receptors, than those bearing lion or cat SLAM/F1 receptors. Conversely, non-dog-like (549H) strains were more generalist, infecting all three cell lines at a moderate efficiency (Nikolin et al. 2012a). This finding

implies that generalist non-dog-like (549H) strains might be adaptive in a multi-host ecosystem, whereas specialist dog-like (549Y) strains might have a selective advantage wherever domestic dogs are numerically dominant.

The sequence identity of additional regions of the HA and F genes are known to influence viral function (e.g. receptor binding regions and cleavage sites), and pathogenicity (N-Glycosylation, Table 4.1, Figure 4.1). In comparison, relatively little is known about the adaptive importance of internal replication proteins, but experimental studies have indicated that they may have a role to play in host adaptation. Infection of ferrets with a recombinant virus bearing the envelope proteins from an attenuated parent (of dog origin), with the internal proteins (N, P and L) from a virulent virus caused clinical signs of intermediate severity to those of the parent viruses (von Messling et al.2003). Each of the internal proteins in the recombinant virus bore two substitutions that were absent in the attenuated parent, suggesting that some, or all may play a role in virulence, however any effect on host specificity is unknown. Likewise, a single substitution of a cysteine residue for a tyrosine at position 267 of the non-structural V-protein has been associated with the adaptation of dog origin CDV to infect human cell lines (Otsuki et al. 2013). Whether these *in vitro* findings have implications for inter-species transmission of CDV in the wild is currently unknown, however, substitution within replicating proteins are known to affect host range in other pathogens, such as highly pathogenic avian influenza virus (Czudai-Matwich et al. 2014).

The objective of this study was to obtain CDV sequence data from tigers, other wild carnivores and domestic dogs in Primorskii Krai that could contribute to an understanding of the epidemiology of the virus in the territory. In particular, molecular data were interpreted to assess the relative importance of domestic and wild carnivores as likely sources of infection for the target population of Amur tigers, as this information is essential for prioritizing control strategies. Sanger sequencing was used to obtain sequences of the external HA and F genes, and Illumina sequencing was employed to obtain wider genome coverage from viruses obtained from tigers. In particular Illumina sequencing was used to attempt to obtain more extensive sequences from the previously confirmed cases identified

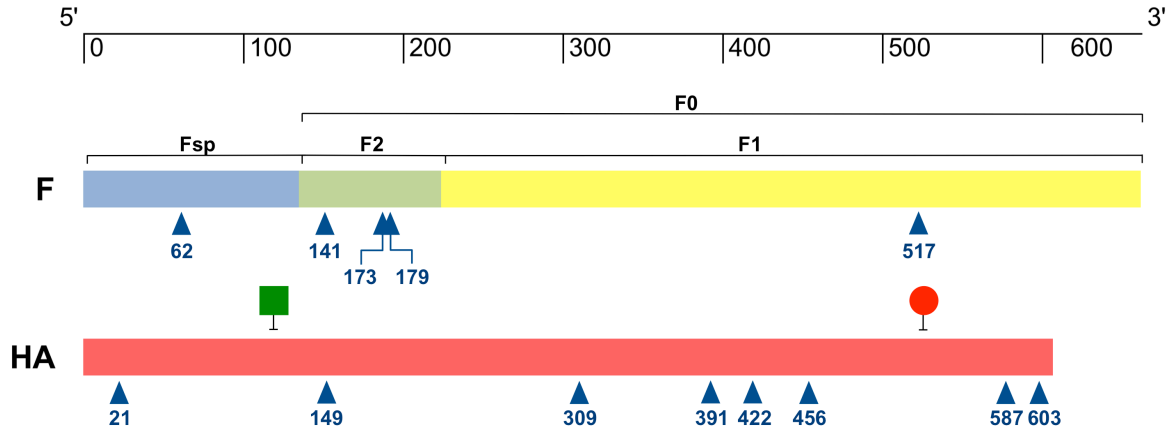

Figure 4.1. Map of key amino acid residues influencing the structure and function of the envelope glycoproteins fusion (F) and haemagglutinin (HA) of canine distemper virus. The scale indicates amino acid residue position. Primary cleavage of the F protein between residues 135 and 136 results in the fusion signal peptide (Fsp) and F0 protein. Secondary cleavage of F0 between residues 224 and 225 produces proteins F2 and F1. Positions of motifs for attachment of N-glycan side chains are shown in blue triangles. The green square indicates the K-L-N-E-I motif at positions 110-114 that is critical to interaction between the HA and F proteins. The red circle indicates key residues (525Y, 526D, and 529R) in the receptor-binding site.

by Seimon et al. (2013, particularly PT61/Pt 2004, Pt 2010-2 and PT56/Pt 2010-3[1]) as well as a suspected case PT90/Pt-2010-1, from which no sequence could be amplified using conventional approaches. Sequence data were used to describe the post-translational identity of viral glycoproteins, focusing on amino acid motifs that influence protein function and structure (including receptor binding sites, and the presence of N-glycan motifs). The phylogenetic relatedness of viruses was interpreted both within the global context, using a worldwide dataset of CDV sequences, and locally within Primorskii Krai. At a local level, the relatedness of viruses from different host species, locations and time points was used to inform the epidemiology of CDV in the region. Results were used to prioritize potential control strategies that could reduce the impact of CDV on the viability of tiger populations in the Russian Far East.

In particular, this chapter will test the following statements and hypotheses:

1. Species that contribute to the maintenance of CDV will be commonly infected with the virus. The study will test the null hypothesis that CDV infections will not be

[1] Throughout this chapter the tigers whose tissues were submitted for Illumina sequencing will be identified using the conventional PT identifier codes used elsewhere in this thesis (e.g. PT61), together with the identifiers used by Seimon et al. (2013, e.g. Pt 2004) to ensure clarity.

Table 4.1. Functionally important amino acid residues and motifs in the canine distemper virus (CDV) haemagglutinin (HA) protein, and fusion (F) protein.

Amino acid identity	Importance	Source
HA-Protein		
K-L-N-E-I motif at position 110-114	Conserved in all CDV viruses, and critical to HA and F protein interaction.	Lee et al. 2008
525Y, 526D, 529R	Critical to efficient binding to host signalling lymphocyte activation molecule (SLAM) receptors.	Zipperle et al. 2010
G/E530R/D/N	Proposed as adapative for host receptor binding in non-domestic carnivores, but questioned during recent analyses.	McCarthy et al. 2007, Nikolin et al. 2012
Y549H	Proposed as adapative for host receptor binding in non-canids (may be adaptive in non-domestic canids).	McCarthy et al. 2007, Nikolin et al. 2012
N-X-T/S motif (X = any residue except proline) found at N21, N149, N309,N391, N422, N456, N587 and N603 in wild viruses	Potential N-glycosylation site for attachment of oligosaccharide side chains. Affect protein folding. Reduced glycocylation reduces pathogenicity.	Sawatsky and von Messling 2010
F-Protein		
A|QIHW motif at position 135-136	Essential for peptidase cleavage between Fsp and F0 proteins. Fsp down-regulates fusion activity, stabilizing the virus and influences neuropathogenesis	von Messling and Cattaneo, 2002
RXK/RR motif at position 224-225	Essential for peptidase cleavage of F0 to profuce F2 and F1proteins.	von Messling and Cattaneo, 2002
N-X-T/S motif (X = any residue except proline) found at N62, N141, N173, N179, and N517	Potential N-glycosylation site for attachment of oligosaccharide side chains. Affect protein folding.	Lee et al. 2013

identified in dogs and/or wild mesocarnivores, as they are not contributing to the maintenance of CDV in Primorskii Krai.

2. Even if viruses are found among dogs and/or mesocarnivores, these populations may not be acting as sources of infections for tigers, if their viruses are not closely related to those found in tigers. The study will test the null hypothesis that tiger viruses are not closely related to those obtained from dogs and/or mesocarnivores, as they are not acting as sources of infection for tigers.

3. Long chains of CDV transmission will be an important indicator of a maintenance population, and give rise to a multiple genetic lineages with a geographically well-mixed phylogeny. The study will test the null hypothesis that viral sequences from disparate locations will not group together on phylogenetic branches that share a recent common ancestor.

4. Substitutions at position 549 of the HA glycoprotein may play a role in host adaptation. The study will test the null hypothesis that no wildlife viruses will carry the Y549H substitution that has been proposed as favouring infection in non-dog hosts.

Methods

Sample collection from domestic dogs

Domestic dog samples were obtained from two sources: 1) clinically healthy dogs sampled during household surveys (Chapter 3), and 2) dogs presented for treatment at veterinary clinics. Household samples were collected from all dogs whose owners consented to sample collection during household surveys in the study areas of Southwest Primorskii, Lazovskii and Sikhote-Alin Biosphere Zapovednik (SABZ, described in more detail in Chapters 2 and 3). Nasal swabs were preserved in 300 µl of RNAlater stabilizing reagent (Qiagen Inc., Valencia CA), and whole blood was collected from the cephalic vein into vacutainers containing EDTA as an anticoagulant. All samples were then frozen (at -20 Celsius or lower) until analysis.

State and private veterinarians in rural and urban areas agreed to participate in the collection of clinical samples from sick dogs using a broad case definition, to maximize the chances of detecting CDV infections. Veterinarians were requested to collect conjunctival and nasal swabs in RNAlater from all dogs displaying any combination of upper respiratory disease, oculonasal discharge, gastrointestinal disease, and/or neurological signs. Participating veterinarians were based in the city of Vladivostok and the districts of Lazovskii, Ussuriyskii, Nadezhdinskii, Khankayskii, Khantaiskii, Dalengorskii, Partizanskii, and Arsenevskii.

Sample collection from wild mesocarnivores

Tissue samples were obtained from small-bodied wild carnivores (mesocarnivores) with the assistance of state hunting inspectors in the towns of Ternei (Terneiskii district), Lazo (Lazovskii district) and Bikin (Pozharskii district). These inspectors made contact with local fur trappers authorized to capture fur-bearing species during the winter hunting seasons of 2011/2012, 2012/2013, and 2013/2014. Trappers provided skinned carcasses, or heads, which were frozen at -20 Celsius until sample collection. Samples were collected from specimens obtained during the 2011/2012 and 2012/2013 hunting seasons in the following manner. Frozen skulls were bisected, to facilitate the collection of approximately 30 µg of tissue from the region of the hippocampus, which was then frozen at -20 Celsius in 1 ml of RNAlater. Where present, 50 µg of lung tissue was pooled with brain tissue from corresponding animals. Utensils and surfaces were disinfected with a 20% solution of household bleach (3% sodium hypochlorite) between each specimen. Techniques were modified for specimens obtained during the 2013/2014 hunting season to reduce the potential for cross contamination, with approximately 30 µg of frozen brain tissue obtained by curette via the foramen magnum.

Additional mesocarnivore samples were obtained from dead animals encountered opportunistically, including road traffic accidents, or animals found dead in the forest. Nasal swabs were also collected from anesthetized mesocarnivores captured during serosurveillance (Chapter 5). Archived blood products from mesocarnivores (including whole blood, blood clots and other cellular blood samples) were also selected from animals with CDV neutralizing antibodies at a titre of at least 1:16 (Chapter 5).

Sample collection from large-bodied wild carnivores

Tissue samples were collected opportunistically from large-bodied wild carnivores during necropsy examinations, and preserved in the same manner as described for dead mesocarnivores. In addition, frozen tissue samples and blood products were obtained from the archives of the Wildlife Conservation Society, Bronx NY. Blood products were also selected for RNA extraction from animals with measurable titres of serum antibodies of at least 1:16 (Chapter 5).

Formalin-fixed paraffin embedded (FFPE) blocks of tissue that had previously been analaysed by Seimon *et al.* (2013) were selected for further extraction, with the objective of extending published sequences. Following the animal codes used by Seimon et al. (2013), these included the confirmed CDV cases PT61/Pt 2004, Pt 2010-2 and PT56/Pt 2010-3 (which previously yielded the CDV sequences: KC579363 [PT61/Pt 2004; partial H gene], KC579361 [PT61/Pt2004; partial P gene], and KC579362 [PT56/Pt2010-3; partial H gene]), and from suspected case PT90/Pt 2010-1.

Archived tiger scat samples collected in SABZ during 2009 and 2010 (an area and period when CDV had been detected in at least one Amur tiger, Appendix I, Gilbert *et al.* 2015) were prioritized for extraction. Samples had been individually bagged, and had been frozen at -20 Celsius for a period of up to three years. Approximately 50 µg was suspended in 1 ml of RNAlater, and frozen at -20 Celsius prior to RNA extraction.

Sanger sequencing

In preparation for RNA extraction, samples were centrifuged at 14,000 r.p.m. for five minutes, to facilitate the removal of RNAlater. Tissue samples were macerated using a disposable pestle in 200 µl of Buffer RLT Plus (Qiagen Inc., Valencia CA), with 1% ß-Mercaptoethanol and 5 µl of 3U proteinase-K, vortexed regularly and incubated for one to three hours at 56 Celsius, until completely homogenized. RNA was extracted from tissue homogenates using the AllPrep DNA/RNA Mini Kit (Qiagen Inc., Valencia CA) following manufacturer's instructions. Extraction of RNA from nasal swabs, whole blood, blood clots and scat samples was performed using the QIAamp Cador Pathogen kit (Qiagen Inc., Valencia CA), using manufacturer's instructions.

All extracts were initially screened by qPCR for a 114 bp fragment of the P-gene based on previously described protocols (Scagliarini et al. 2007). Reactions were performed using the QIAGEN OneStep RT-PCR kit (Qiagen Inc., Valencia CA), and primers CDVF4 and CDVR3, and a TaqMan probe reporting in the FAM channel (Table 4.2). All reactions included a negative control, and either a modified live virus vaccine, or a synthetic CDV sequence as a positive control. Reactions were performed using a Bio-Rad Minopticon Cycler through 45 cycles, with a transcription step of 20 minutes at 50 Celsius, and an

annealing step of 30 seconds at 60 Celsius. Wells with characteristic amplification curves of cycle threshold <38 were considered to be positive. Samples that tested positive in at least one of three wells then underwent additional rounds of reverse transcription polymerase chain reaction (RT-PCR) amplification, using primer sets for a 429 bp fragment of P-gene (using primers Morb1/Morb2, Table 4.2), and a 291 bp fragment of the HA-gene (with primers TSCDVH2-F/ TSCDVH3-R, Table 4.2). Reactions were performed using a Bio-Rad Minopticon Cycler through 45 cycles, with a transcription step of 30 minutes at 50 Celsius, and an annealing step of 60 seconds at 45 Celsius. Products were separated by electrophoresis on a 1.5% agarose gel, and all extracts that produced bands of the expected molecular weight using both sets of primers were prioritized for further amplification and sequencing.

Prioritized extracts were amplified by RT-PCR, using four sets of primers that covered the whole HA-gene modified from Müller *et al.* (2011) (1F/1R, 2Farctic/2Rarctic, 3Farctic/3R, 4Farctic/4R, Table 4.2). Reactions were performed using a Bio-Rad Minopticon Cycler through 45 cycles, with a transcription step of 30 minutes at 50 Celsius, and an annealing step of 55 seconds at 45 Celsius. Products were separated by electrophoresis on a 2.0% agarose gel, and bands of the expected molecular weight were cleaned using the ExoSAP-IT reagent (Affymetrix, Santa Clara, CA), and directly sequenced in the forward and reverse directions (Genewiz Inc., South Plainfield, NJ).

For positive samples that did not yield full-length HA-gene consensus sequences, DNA cloning techniques were employed to obtain additional sequences. Further DNA cloning was used to obtain near full-length F-gene sequences for all samples from which full-length HA-genes were sequenced. RNA extracts were used to prepare cDNA for DNA cloning using the ProtoScript First Strand cDNA Synthesis Kit (New England Biolabs, Ipswich, MA) following manufacturer's protocols, excluding the optional RNA denaturation step. The first strand DNA product was then amplified using Q5 High-Fidelity DNA Polymerase (New England Biolabs, Ipswich, MA) and primers RusCDV5primUTR and RusCDV5primUTR (HA-gene) and CDV5primUTR and CDV3primUTR (F-gene) (Table 4.2) in a VERITI 96 well fast thermocycler (Applied Biosystems) through 35 cycles (with a denaturation step of 98 Celsius for 10 seconds, an

Table 4.2. Primer and probe sequences used in the detection of canine distemper virus (CDV). Nucleotide start and end positions are based on CDV strain A75/17 (GenBank accession no. AF164967). Restriction sequences used in cloning are indicated in red font.

Primer target	Primer/probe name	Primer sequence	Start position	End position	Source
P-gene (partial)	CDVF4	GTCGGTAATCGAGGATTCGAGAG	2,206	2,228	(Scagliarini et al., 2007)
	CDVR3	GCCGAAAGAATATCCCCAGTTAG	2,319	2,297	
	CDVProbe	6FAM-ATCTTCGCCAGAATCCTCAGTGCT-MGBNFQ	2,274	2,251	
P-gene (partial)	MorbF	ATGTTTATGATCACAGCGGT	2,132	2,151	(Barrett et al., 1993)
	MorbR	ATTGGGTTGCACCACTTGTC	2,560	2,541	
HA-gene (partial)	TSCDVH2-F	TACTGAGTCCAATTTAGTGGTGTTGCC	8,593	8,619	Unpublished
	TSCDVH3-R	CATGAGAATCTTATACGGAC	8,883	8,864	
HA-gene (complete)	1F	GGGCTCAGGTAGTCCARCAA	7,060	7,079	(Müller et al., 2011)
	1R	CCTCCGGAGAGTGCTGATAA	7,611	7,592	
	2Farctic	GTGAGACAATTGGGATCAGA	7,539	7,558	Modified from (Müller et al., 2011)
	2Rarctic	TGGGTGAGCGACAGGTGTCA	8,098	8,079	
	3Farctic	TGGGAATCTTTGGGGCAACA	8,037	8,056	Modified from (Müller et al., 2011)
	3R	TCCATAATCTGGGATGTTTGAA	8,580	8,559	
	4Farctic	ATCCCTCATGTGTTATCATT	8,501	8,520	Modified from (Müller et al., 2011)
	4R	GACCTCAGGGTATAGAATCTGG	9,071	9,050	
HA-gene (complete) (cloning)	RusCDV5primUTR	GCTCTGGTAGGAGAGCAATG	7,062	7,081	Unpublished
	RusCDV5primUTR	GTCCAATTGAGATGTGTATCATCATACT	8,931	8,904	
	AmurtigercdvHsalF	GGATGTCGACACCATGCTCTCCTACCAAGATAAGGT	7,076	7,101	Unpublished
	AmurtigercdvHnot1R	GGATGCGGCCGCTCAAGGTTTTGAACGGTTACATGAG	8,902	8,878	
F-gene (complete) (cloning)	CDV5primUTR	ACAAGCCTCATGCACAAGGAAAT	4926	4948	Unpublished
	CDV3primUTR	GTGACTAGAGTGATTCAGAGTG	6,937	6,916	
	cdvFsal1Fwd	GGATGTCGACATGCACAAGGAAATCC	4,935	4,950	Unpublished
	cdvFnot1R	GGATGCGGCCGCTCAGAGTGATCTTACATAG	6,923	6,905	

annealing step of 50-72 Celsius for 30 seconds, and an extension step of 72 Celsius for 20 seconds). A second round of DNA amplification was performed using primers AmurtigercdvHsalF and AmurtigercdvHnot1R (HA-gene) and cdvFsal1Fwd and cdvFnot1R (F-gene) (Table 4.2), to tag PCR products with recognition sites for the SalI and NotI restriction enzymes (New England Biolabs, Ipswich, MA). DNA fragments were then ligated into the pVR1012 DNA vaccine plasmid, for cloning in *Escherichia coli*. Cloned DNA was purified and directly sequenced in the forward and reverse direction using a Pacific Biosciences PacBio RS II sequencer (operated by GATC Biotech, UK).

Illumina sequencing

Blocks of FFPE tissues were sectioned using a microtome at a thickness of 10 µm, and deparaffinized using xylene. Ten sections per sample were extracted using the RNeasy FFPE kit (Qiagen Inc., Valencia CA). Synthesis of first strand cDNA was performed using Maxima H Minus Reverse Transcriptase (Thermo Fisher Scientific, Inc., Waltham, MA), from which double strand cDNA was generated using NEBNext mRNA Second Strand Synthesis Module (New England Biolabs, Ipswich, MA), and purified using Agencourt AMPure XP beads (Beckman Coulter, Brea, CA) at a 0.9 ratio. A KAPA library preparation kit (KAPA BioSciences, Wilmington, MA) was used to prepare the cDNA for Illumina sequencing with the following modifications to reduce sample input requirements. End-repaired cDNA was purified with AMPure XP beads at a 0.9 ratio to remove fragments shorter than 150 bp, and a ‘with-bead’ approach was used to reduce the sample losses during subsequent elution steps and tube transfer (Fisher et al. 2011). Adaptor concentration was reduced 100-fold, in order to maintain a favourable ratio of adaptor to sample DNA during ligation and prevent the formation of adaptor dimers. Adapter-ligated DNA was amplified with real-time PCR using a KAPA Hifi Real-time library amplification kit, on an ABI 7500 cycler. Index tags were added using either NEBnext multiplex oligonucleotides (New England Biolabs, Ipswich, MA), or equivalent oligonucleotides synthesised by TruGrade processing (Integrated DNA Technology, Coralville, IA) to reduce tag crossover. Amplified DNA was purified using AMPureXP beads and eluted in a final volume of 15 µl. Library DNA concentration was assessed using a Qubit 2.0 fluorometer, and an Agilent 2200 TapeStation was used to verify the final size profile of amplified library DNA and ensure no carry-over of primer dimers. Up to six DNA libraries with appropriate index tags were pooled, and 2x150 nt paired end

sequence data sets were generated on an Illumina MiSeq platform using 300-cycle v2 reagents.

Reads were mapped against all 30 full-length CDV genomes currently available on GenBank using Tanoti (http://www.bioinformatics.cvr.ac.uk/tanoti.php), a blast guided reference based short read aligner. Consensus genomes were built from assemblies using SAM2consensus, a consensus calling programme.

Phylogenetic analyses

Sequence data were analysed in three ways:

1) Global phylogenies were estimated to assess geographic clade designation for all full length HA-genes obtained in this study, and a worldwide dataset including all full length HA-genes published on GenBank (maintained by the National Center for Biotechnology Information).

2) Local phylogenies were estimated for all full length HA-gene and F-genes obtained in this study, to assess the relatedness of viruses from different host species, and spatial, and temporal scales.

3) A molecular clock model was fitted using all HA genes obtained in this study, together with closely related HA-genes (>1,500 bp) from the global dataset.

The worldwide dataset was obtained using a BLAST search based on the full genome sequence of wild-type CDV strain A75/17 (GenBank accession AF164967). Information on host species and date of collection for each sequence was extracted from GenBank metadata, or source publications. Sequences lacking host information, or specifying passage in cell culture, or *in vitro* recombination were removed from the dataset (although vaccine sequences were retained). Sequences were aligned using the MUSCLE algorithm using the software Geneious (version 8.1.8) and were edited manually. Worldwide datasets were trimmed to the bases equivalent to the A75/17 strain, with bases 7,079 to 8,902 indicating the HA-gene (length 1,824 bp).

Appropriate nucleotide substitution models were identified using jModeltest 2 version 2.1.8 (Guindon and Gascuel 2003, Darriba et al. 2012), with best fit models selected based on lowest Akaike information criterion (AIC) scores. Best fit models were used in the construction of maximum likelihood phylogenetic trees using the Geneious phyML plug-in, and 500 bootstrap replicates to determine branch support (Guindon and Gascuel 2003).

The viral evolutionary rate was inferred by fitting a molecular clock model to sequence alignments using a Bayesian Markov chain Monte Carlo (MCMC) approach. Preliminary analyses used the software Path-O-Gen v1.4 (Rambaut et al. 2016) to ensure that sequences were evolving in a clock-like manner. Analyses were performed using the BEAUti/BEAST package v1.8.3 (Drummond et al. 2012) under a relaxed molecular clock model with branch rates drawn from a lognormal relaxed clock distribution (to allow for variation in evolutionary rate in each branch, Drummond et al. 2006), using a normally distributed clock prior with mean of 4×10^{-4} nucleotide substitutions per site based on the results of Panzera *et al.* (2015), with a wide standard deviation of 0.4 nucleotide substitutions per site, and truncated to between 0 and 2.0. The Bayesian skyline model was used as a flexible demographic prior, and two independent MCMC chains were run for 10^7 iterations. Convergence was assessed using TRACER v1.6, and skyline plots were examined to determine a suitable burn-in period.

Results

The number of samples analysed from all sources are summarized in Table 4.3. Of the 633 healthy dogs sampled during the household surveys, owners reported that 472 were unvaccinated. Among the wild carnivore species sampled, sable (*Martes zibellina*) specimens were the most abundant species represented in the sample set.

Sanger sequencing

Of 1,664 samples tested (representing 1,424 individuals and 35 scats), 75 were found to be positive for the 114 bp fragment of the CDV P-gene by qPCR. Full-length HA-gene sequences were obtained for 11 of these by assembling consensus sequences from the four

Table 4.3. Summary of samples tested for the presence of canine distemper virus, and of haemagglutinin (HA) and fusion (F) genes sequenced.

Survey	Species common name	Species scientific name	Sample type	n	HA	F
Household surveys						
	Domestic dog	Canis familiaris	Nasal swab	633	0	0
			Whole blood*	205	0	0
Clinic surveys						
	Domestic dog	Canis familiaris	Conjunctival swab	75	<1**	0
Dead wild carnivores						
	Leopard cat	*Prionailurus bengalensis*	Tissue	30	0	0
	Eurasian lynx	*Lynx lynx*	Tissue	4	0	0
	Leopard	*Panthera pardus*	Tissue	1	<1**	0
	Tiger	*Panthera tigris*	Tissue	3	1	0
	Grey wolf	*Canis lupus*	Tissue	2	1	0
	Raccoon dog	*Nyctereutes procyonoides*	Tissue	27	1	1
	Red fox	*Vulpes vulpes*	Tissue	9	0	0
	Asiatic black bear	*Ursus thibetanus*	Tissue	1	0	0
	Sable	*Martes zibellina*	Tissue	518	17†	9
	Yellow-throated marten	*Martes flavigula*	Tissue	3	0	0
	Siberian weasel	*Mustela sibrica*	Tissue	27	1	1
	American mink	*Neovison vison*	Tissue	4	0	0
	River otter	*Lutra lutra*	Tissue	3	0	0
	Asian badger	*Meles leucurus*	Tissue	5	1	1
	Unidentified	Unidentified	Tissue	1	1	0
Wildlife surveys						
	Sable	*Martes zibellina*	Nasal swab	2	0	0
	Asian badger	*Meles leucurus*	Nasal swab	17	0	0
	Leopard cat	*Prionailurus bengalensis*	Nasal swab	8	0	0
	Raccoon dog	*Nyctereutes procyonoides*	Nasal swab	10	0	0
Archived blood						
	Eurasian lynx	*Lynx lynx*	Serum	1	0	0
	Leopard	*Panthera pardus*	Whole blood /clots	2	0	0
	Tiger	*Panthera tigris*	Whole blood /clots	20	1	0
	Raccoon dog	*Nyctereutes procyonoides*	Serum	6	0	0
	Asiatic black bear	*Ursus thibetanus*	Serum	2	0	0
	Brown bear	*Ursus arctos*	Whole blood /clots	1	0	0
	Asian badger	*Meles leucurus*	Serum	5	0	0
Illumina sequencing						

Tiger	*Panthera tigris*	Formalin fixed paraffin embedded tissue blocks	4	1§	1§
Scat survey					
Tiger	*Panthera tigris*	Faeces	35	0	0
		TOTAL	1,664	25†	13

*The 205 whole blood samples were analysed from a subset of the 633 dogs tested during passive surveillance, and so do not represent additional individuals.
**Refers to a partial length sequence.
† Figure includes an HA gene from which a gap of 442 base pairs could not be sequenced.
§ Full virus genome obtained from one animal.

primer sets that spanned the HA-gene, and a further 12 were obtained from DNA cloning. An additional partial HA-gene sequence was obtained with a 442 bp gap (KX708733, Table 4.4). Corresponding full-length F-gene sequences were successfully obtained for 12 of the 23 full-length HA-genes. Sequences were submitted to GenBank under accession numbers KX708710-KX708733 for the HA- gene, and KX708734- KX708745for the F-gene. In addition, a 429 bp fragment of the P-gene, and a 528 bp fragment of the H-gene was obtained from the sick leopard that died in 2015. Also, in 2016 a 390 bp fragment of P gene, and a 529 bp fragment of the HA was obtained from the sick domestic dog in Vladivostok. Further sequencing is underway to obtain full HA gene sequences from both of these viruses.

Little information was available on the health of many of the wild carnivores that tested positive for CDV infection. Most viruses were detected in sables and a Siberian weasel that were captured in the wild by fur trappers, and it is unknown whether ill health may predispose animals to capture. Several viruses were detected in animals that were found dead, including a raccoon dog (that died in a vehicle collision), an Asian badger (found dead in the forest), and a grey wolf (which also showed signs consistent with a severe infestation with *Sarcoptes scabei*, including near total alopecia), and it is possible that CDV infection could have contributed to death in these cases.

Virus was also detected from two tigers, and a leopard that were diagnosed during the course of the study. Infection with CDV was not suspected in either of the two tiger cases prior to diagnosis, indicating that some clinically affected animals may fall outside the case

definition used in Russia (typified by neurological signs including a loss of fear and aggression, approachability, ataxia and sensory deficits). One of these animals had died following a gunshot wound, but its body was otherwise intact (suggesting the death was not linked to poaching). The second tiger was sampled as a 13 month old cub, that was repeatedly observed along a roadside, displaying an indifference to vehicles. At the time this behaviour was attributed to juvenile naivety. The cub disappeared four months after sampling, and was assumed to have been poached. The leopard case showed clinical signs consistent with advanced neurological stages of CDV infection, and died despite 17 days of supportive care in a rehabilitation center. Further details of these cases are presented in Appendix II.

Illumina sequencing

Libraries were prepared using FFPE tissues from four tigers: PT61/Pt 2004 (brain), PT90/Pt 2010-1 (lymph node) and Pt 2010-2 (lymph node), and PT56/Pt 2010-3 (brain), from which between 5,482,492 and 10,872,522 reads were obtained. These reads were mapped against 30 full-length CDV genomes available on GenBank. Among these, two samples from PT61/Pt 2004 had approximately 95,000 reads that mapped to the CDV genome. Samples from PT90/Pt 2010-1 and Pt 2010-2 had ten or fewer mapped reads, whereas the sample from Pt 2010-2 had approximately 780 mapped reads. Of thirty references, the sequence KF914669, derived from a dog in Italy in 2013 was found to share the greatest identity, and was used for subsequent viral genome assembly using Tanoti. The coverage and depth of assemblies are summarized in Table 4.5. The consensus sequence fragments from PT56/Pt 2010-3 were distributed throughout the genome and varied in length from 74 bp to 327 bp. These included a fragment of 106 bp within the HA-gene, and two sections within the F-gene including a 57 bp section within the Fsp region, and 93 bp in the F1 region. Only 10 reads obtained from PT90/Pt 2010-1 mapped to KF914669, equating to 3.6% of the genome, but no overlapping contigs were found. None of the reads from Pt 2010-2 mapped to reference strain KF914669. The sequence for PT61/Pt 2004 was submitted to GenBank (KX774415, Table 4.4).

Infections with CDV had previously been confirmed in both PT61/Pt 2004 and PT56/Pt 2010-3 (Seimon et al. 2013), but these results greatly increased the coverage from the former of these. The limited reads from PT90/Pt 2010-1 represented the first confirmation

Table 4.4. A summary of complete (COMP) and partial (PART) haemagglutinin gene (HA) and fusion gene (F) sequences obtained from carnivores in the Russian Far East. Includes host species, location of origin (KH = Khabarovskii Krai, TY = Terneiskii district, PZ = Pozharskii district, LZ = Lazovskii district, SW = Southwest Primorskii, VL = Vladivostok), and GenBank accession number.

Animal ID	Species	Study area	HA gene	F gene	Accession number (HA)	Accession number (F)
HNT20	Unidentified small carnivore	TY	COMP	-	KX708732	-
PT61/Pt 2004	Amur tiger	KH	COMP	COMP	KX774415	KX774415
PT79	Amur tiger	TY	COMP	-	KX708720	-
FUR0056	Sable	TY	COMP	COMP	KX708721	KX708734
FUR0061	Sable	TY	COMP	COMP	KX708722	KX708735
FUR0074	Grey wolf	TY	COMP	-	KX708711	-
FUR0076	Sable	TY	COMP	-	KX708712	-
FUR0134	Sable	TY	COMP	COMP	KX708713	KX708736
FUR0140	Sable	PZ	COMP	COMP	KX708710	KX708737
FUR0141	Sable	TY	COMP	-	KX708714	-
FUR0188	Sable	PZ	COMP	COMP	KX708723	KX708738
FUR0192	Sable	PZ	COMP	COMP	KX708724	KX708739
FUR0207	Sable	TY	COMP	COMP	KX708715	KX708740
FUR0244	Sable	PZ	COMP	COMP	KX708716	KX708741
FUR0251	Siberian weasel	PZ	COMP	COMP	KX708717	KX708742
FUR0258	Sable	PZ	COMP	COMP	KX708725	KX708743
FUR0309	Asian badger	SW	COMP	COMP	KX708718	KX708744
FUR0310	Amur tiger	SW	COMP	-	KX708726	-
FUR0319	Raccoon dog	SW	COMP	COMP	KX708727	KX708745
FUR0326	Sable	LZ	PART	-	KX708733	-
FUR0332	Sable	LZ	COMP	-	KX708719	-
FUR0336	Sable	LZ	COMP	-	KX708728	-
FUR0364	Sable	LZ	COMP	-	KX708729	-
FUR0378	Sable	LZ	COMP	-	KX708730	-
FUR0596	Sable	TY	COMP	-	KX708731	-

Table 4.5. Summary of Illumina sequence data obtained from formalin-fixed tiger tissues in paraffin embedded blocks.

Tiger identifier	Total reads	Number of reads mapped to reference (KF914669)	Percentage coverage to reference (KF914669)	Depth of coverage to reference (KF914669)
PT61/Pt 2004	10,872,522	95,021	99.20%	806
PT90/Pt 2010-1	7,143,772	10	3.60%	0
Pt 2010-2	6,571,996	0	0.00%	0
PT56/Pt 2010-3	5,482,492	816	16.50%	7

that this tiger was infected with CDV at the time of its death. This eleven year old male was responsible for a fatal attack of a local fisherman, and was subsequently killed by Russian authorities (Appendices I and II).

The consensus sequence from PT61/Pt 2004 was analysed to identify unique non-synonymous substitutions, by aligning it against all 51 full CDV genomes available on GenBank (Appendix XVII). Non-coding regions were trimmed, and individual genes were translated for direct comparison (Table 4.6). Gaps in the PT61/Pt 2004 consensus prevented the translation of amino acid residues in the F-protein (3 residues), HA-protein (1 residue) and large protein (16 residues, Table 4.7). A stop codon at residue number 55 on the matrix protein was likely due to a sequencing error. The PT61/Pt 2004 consensus included 76 unique amino acid substitutions, which were not present in the other 51 published full genomes (Tables 4.6, and 4.7). The non-structural C-protein had the highest proportion of unique substitutions (2.9%), followed by the structural L-protein (1.9%). Unique residues were concentrated in three regions near the 3' end of the L protein (residue positions 1,714–1,783, 1,867–1,890, 2,026–2,084, Figure 4.2).

Phylogenetic analyses

A global phylogeny was prepared from a dataset of 521 full-length HA-genes sequences were obtained from GenBank (excluding sequences lacking host information, or where passage in cell culture or *in vitro* recombination were specified). Additionally, two unpublished HA sequences were obtained from Arctic foxes (*Vulpes lagopus*) sampled in Barrow, Alaska in 2012 and 2014 (samples donated by K. Beckmen, Alaska Dept. of Fish and Game, amplified by E. Dubovi, Cornell University, and sequenced by P. Duprex, Boston University). All sequences from Primorskii, Alaska and the global dataset were aligned, and found to have pairwise nucleotide identities of 87.2-100%. Pairwise amino acid identity was 81.5-100%. A transitional model with a gamma distribution (TIM1+G) was found to be optimal, and was used to generate a maximum likelihood tree (Figure 4.3). All HA sequences from Primorskii wildlife, and Alaska clustered within the Arctic-like clade in the global dataset. Primorskii sequences were all markedly different from that of the Onderstepoort vaccine (pairwise nucleotide identity of 91.3-91.7%, and pairwise amino acid identity of 89.1-90.1%).

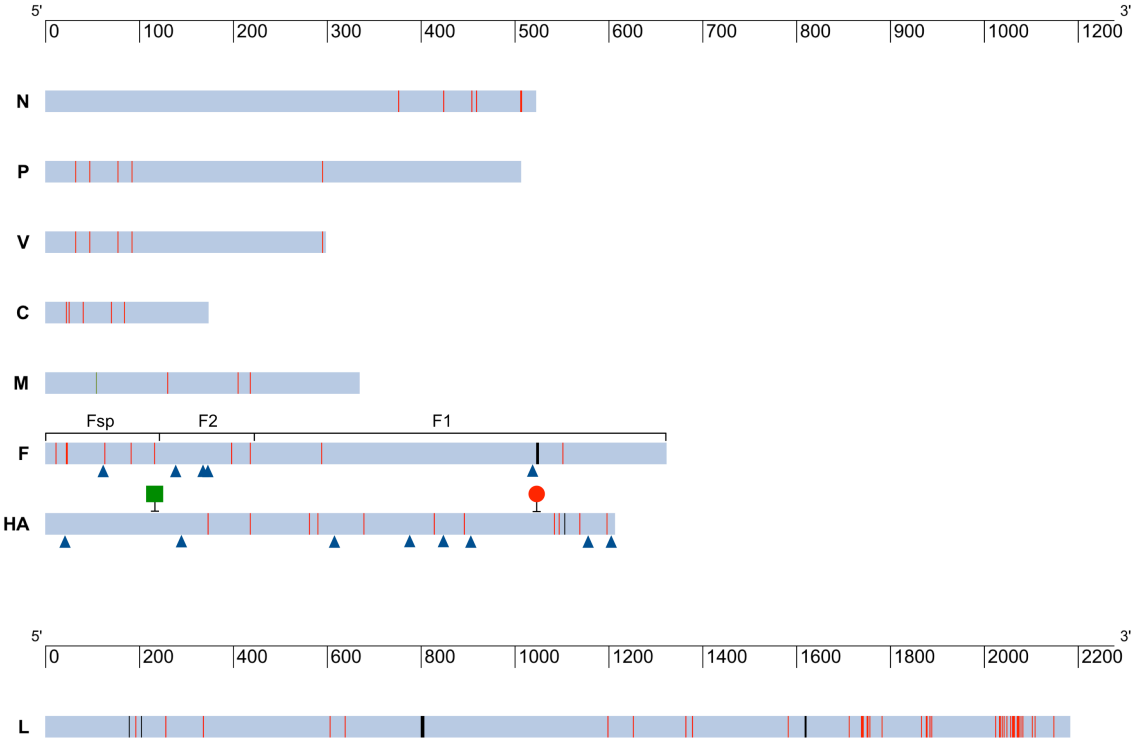

Figure 4.2. Protein map of PT61/Pt 2004, illustrating the position of unique residues (red), residues that could not be translated due to gaps in the genome (black), and a stop codon within the M gene (green), which is likely to represent a sequencing error. Positions of motifs for attachment of N-glycan side chains are shown in blue triangles. The green square indicates the K-L-N-E-I motif at positions 110-114 that is critical to interaction between the HA and F proteins. The red circle indicates key residues (525Y, 526D, and 529R) in the receptor-binding site.

Table 4.6. Summary of unique amino acid residues in coding regions of the PT61/Pt 2004 consensus sequence compared to all 51 other full genome sequences published on GenBank

Protein	Unique residues in PT61/Pt 2004	Gaps in PT61/Pt 2004 consensus	Protein length	Percentage of unique residues
N	6	-	523	1.1%
P	5	-	507	1.0%
V	5	-	299	1.7%
C	5	-	174	2.9%
M	3	-	335	0.9%
F	10	3	662	1.5%
HA	11	1	607	1.8%
L	41	16	2,184	1.9%
Total	96	20	4,818	2.0%

Table 4.7. Unique amino acid residues within the PT61/Pt 2004 consensus sequence that were not represented within 51 other full genome sequences published on GenBank.

Protein	Substitutions	Gaps in PT61/ Pt 2004 consensus genome
N	377N, 425P, 455L, 460A, 507E, 508I	-
P	33N, 48I, 78P, 93P, 296G	-
V	33N, 48I, 78P, 93P, 296G	-
C	23R, 26T, 41S, 71Q, 85S	-
M	131N, 206S, 219I, 294S	-
F	12Y, 23D, 24N, 64S, 92K, 117V, 199T, 219S, 295D, 552L	524, 525, 526
HA	174G, 219T, 282I, 291A, 340S, 415T, 447F, 543Q, 548M, 570N, 599K	554
L	193S, 257K, 337I, 607F, 639S, 1200N, 1254A, 1366K, 1379K, 1584G, 1714F, 1739P, 1741T, 1744P, 1753P, 1754L, 1757T, 1783Q, 1867P, 1877P, 1878T, 1885P, 1890T, 2026R, 2034A, 2036D, 2039E, 2043S, 2049V, 2057S, 2061A, 2064C, 2065G, 2071G, 2073D, 2075R, 2080V, 2084C, 2103H, 2110H, 2149P	180, 205, 206, 801, 802, 803, 804, 805, 806, 807, 808, 809, 1619, 1620, 1621, 1622

All published sequences in the Arctic-like clade that exceed 1,500 bp are summarized in Appendix XVIII. A sequence from a dog in Italy in 2005 (DQ226088) identified most closely with the Primorskii wildlife sequences, with a pairwise nucleotide identity of 97.7-98.4% and an amino acid identity of 96.7-97.7%. A similar level of identity was found with an Arctic-like sequence (EF445052), obtained in 2005 from a farmed fox from the Chinese province of Heilongjiang (which borders Primorskii), which shared a pairwise nucleotide identity of 97.5-98.4% and an amino acid identity of 96.3-97.3% with Primorskii wildlife viruses.

The P-gene fragment from a Primorskii dog shared the greatest nucleotide identity (98.7%) with an Asia 4 clade sequence obtained from a dog in Thailand in 2007 (AB299204). The 539 bp region of the HA-gene from this dog also showed highest identity with Asia 4 clade viruses, sharing 98.4% nucleotide identity with AB301065 and AB301066 also collected from Thai dogs in 2007. The partial HA-gene from the leopard shared 99.8% nucleotide identity with a sequence from the Arctic-like clade obtained from an Asian badger (FUR0309) in Southwest Primorskii during 2013.

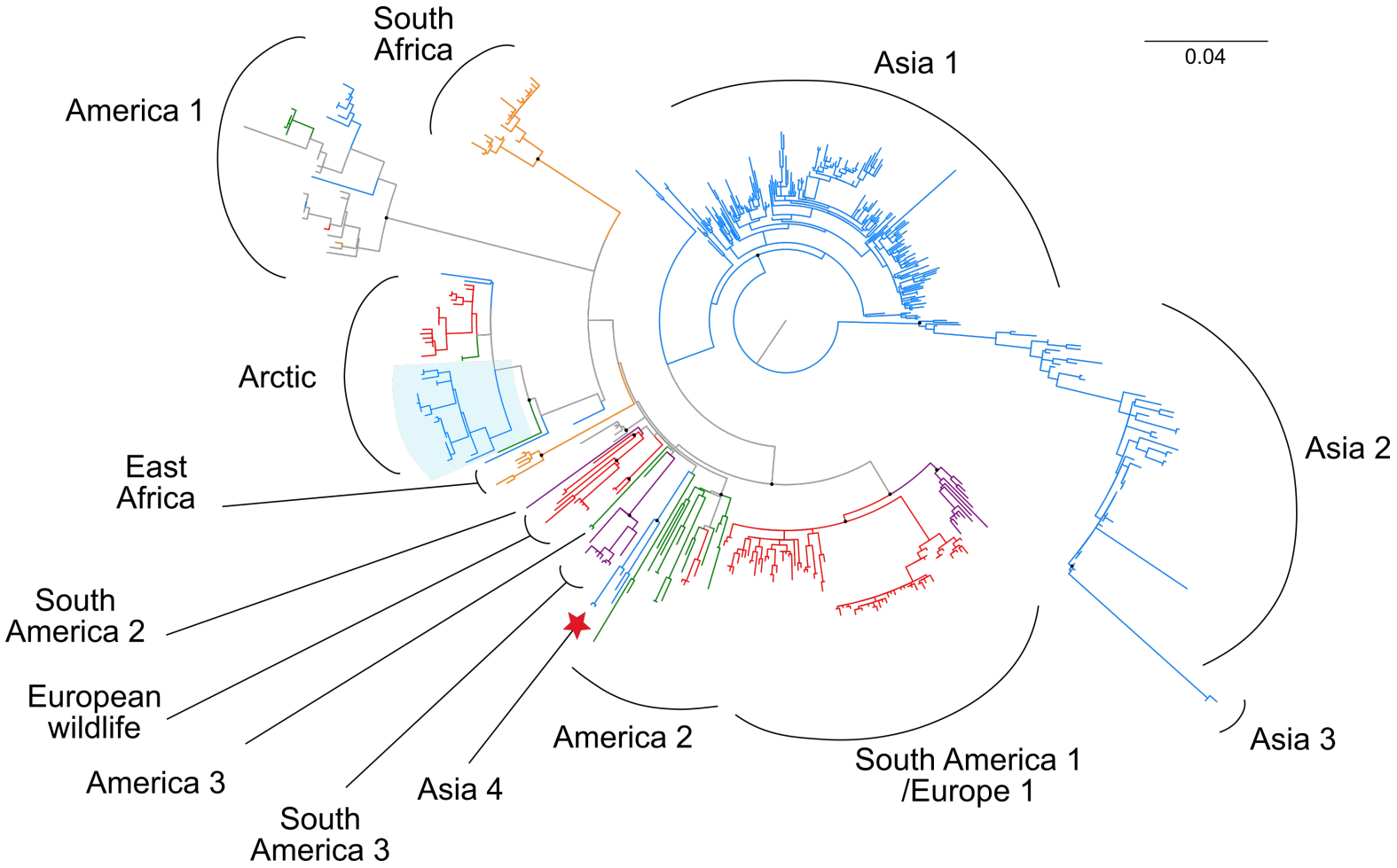

Figure 4.3. Maximum likelihood phylogenetic tree constructed using Primorskii and worldwide sequences of the complete haemagglutinin gene of canine distemper virus. obtained in Russia, and 512 worldwide obtained from GenBank. Trees were constructed using the software Geneious v8.1.8 and the PHYML plug-in, with the transitional substitution model with gamma distribution, and 500 replications to obtain bootstrap branch support. Major nodes with >0.80 support are illustrated using a black point. Colours are used to indicate continent of origin (Asia = blue; Europe = red; North America = green; Latin America = purple; Africa = orange). Blue shading indicates Primorskii wildlife sequences, and the position of the dog sequence is noted by a red star.

The global HA dataset included large numbers of sequences from countries in the vicinity of Primorskii Krai, including China (n=167 sequences), Republic of Korea (n=33), and Japan (n=57). The majority of viruses in China fell within the Asia-1 clade (92.2%, n=167), with the remainder identified as Arctic-like (1.8%), Asia-3 (1.2%), Asia-4 (1.8%) or America-1 (3.0%, which were likely of vaccine origin). All three Arctic-like sequences from China originated from the northeast of the country, in provinces bordering Primorskii Krai (Heilongjiang and Jilin). Although 95 non-domestic species were sampled in China it was unclear whether any of these represented free-ranging animals, with most likely originating from fur farms or zoological collections. Other countries in the East Asian region where CDV sequences were reported from wild species (particularly raccoon dogs) included the Republic of Korea and Japan, and all of these aligned within either the Asia-1 or Asia-2 clades. No viruses from the Arctic-like clade have been detected in the Republic of Korea or Japan.

Local phylogenies were prepared using maximum likelihood methods for the 24 Primorskii wildlife HA-gene and 13 F-gene sequences obtained using Sanger and Illumina methods using substitution models of Hasegawa, Kishino and Yano (HKY) and a transversion model with proportion of invariable sites (TVM+I) respectively (Figure 4.4). The topology was found to be consistent for both trees. Sequences for HA and F-genes were found to have an identity of 97.9-100% and 97.5-100% respectively, with an amino acid identity of 96.8-100% for both genes.

Older sequences from tigers sampled in 2003 (PT61/Pt 2004), and 2006 (PT79) branched closer to the base than more recent sequences obtained between 2011 and 2015. Recent sequences branched into two main clades; hereafter designated as clade 1 and clade 2 (Figure 4.4A). Sample locations are indicated in Figures 4.4C-D. Sequences in Clade 1 included sables and a Siberian weasel that were collected in Bikin (during 2012/13), sables from Terneiskii (during 2012/13) and Lazovskii (during 2013/14). Clade 2 branched into three subclades; 2.1 comprising an Asian badger and a raccoon dog from Southwest Primorskii (2012/13), an unidentified wild carnivore and a sable from Terneiskii (from 2011/12 and 2013/14 respectively), and 2.2 comprising a tiger from Southwest Primorskii (2013/14), and a sable from Lazovskii (2013/14), and 2.3 comprising two sable and a grey wolf from Terneiskii (2012/13).

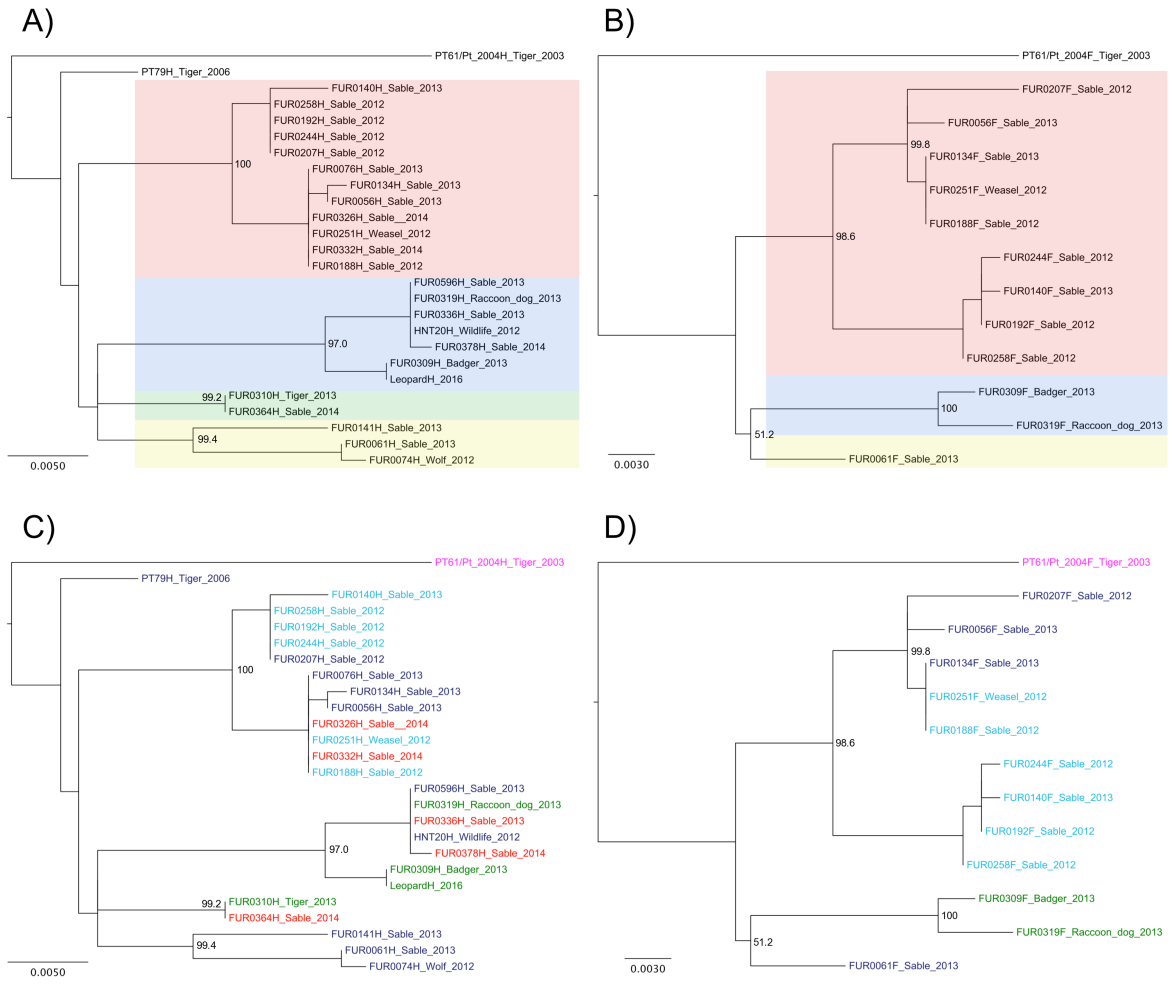

Figure 4.4. Phylogenetic trees constructed using complete haemagglutinin (HA) gene (A & C), and fusion (F) gene (B & D) of canine distemper viruses obtained in Russia. Trees were constructed using maximum likelihood methods, with substitution models Hasegawa, Kishino and Yano (HKY) (HA-gene) or a transversion model with proportion of invariable sites (F-gene), and 1,000 replications to obtain bootstrap branch support. Informal clade designations are highlighted in A and B (clade 1 = red, subclade 2.1 = blue, subclade 2.2 = green, subclade 2.3 = yellow). Use of colour indicates location of origin in C and D (Khabarovskii Krai = pink, Terneiskii district = dark blue, Pozharskii district = light blue, Lazovskii district = red, Southwest Primorskii = green).

A molecular clock model was fitted to an alignment of all HA-genes obtained from Primorskii wildlife, together with all 38 Arctic-like sequences from GenBank and Alaska exceeding 1,500 bp (Appendix XVIII). Results from BEAST analysis showed a molecular clock rate of 7.5×10^{-4} nucleotide substitutions per site for these sequences (95% Highest Posterior Density (HPD): 4.8×10^{-4} - 1.0×10^{-3}). The Alaskan lineage was estimated to have diverged from the Eurasian clade in 1986 (95% HPD: 1981-1992), and the Primorskii viruses were estimated to have diverged from the European viruses in 1989 (95% HPD: 1984-1994, Figure 4.5). The Primorskii subclades were estimated to have diverged between 2005 (95% HPD: 2003-2008) and 2007 (95% HPD: 2004-2010, Figure 4.5).

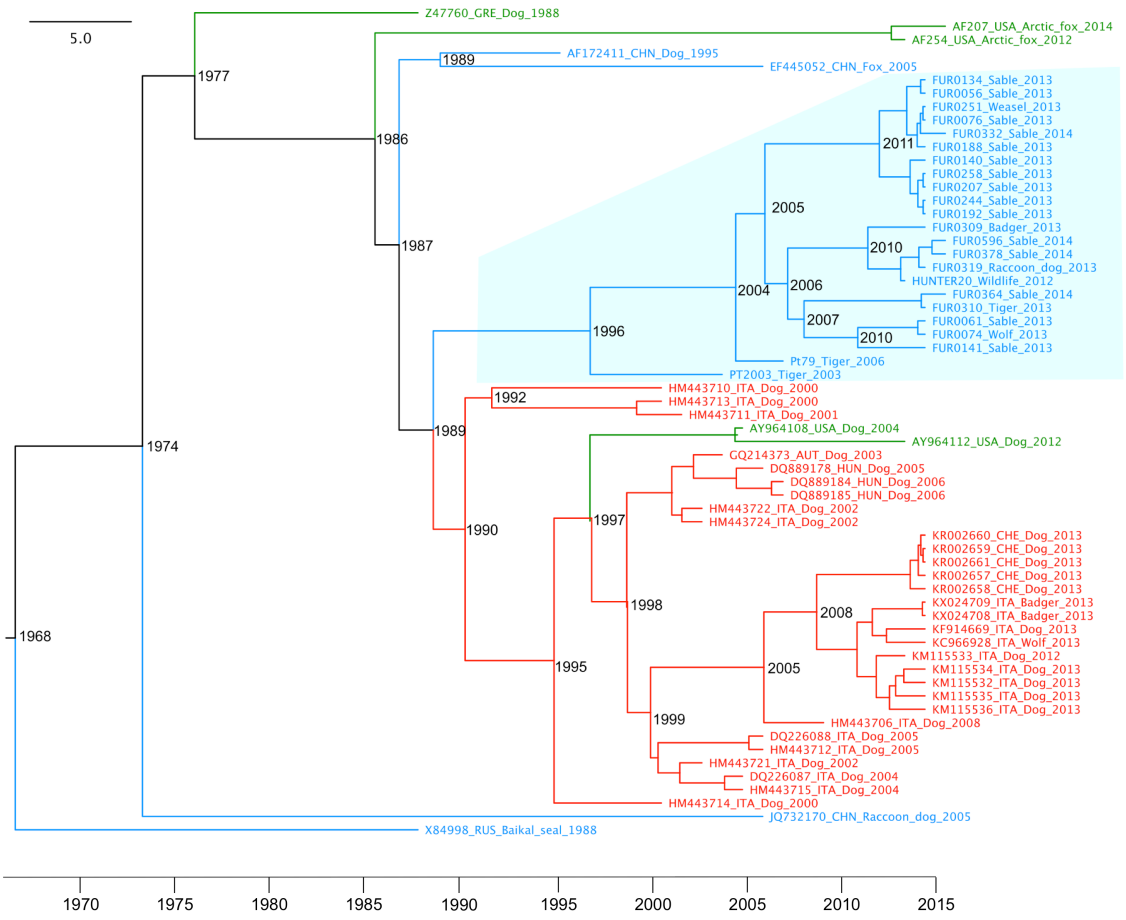

Figure 4.5. A time-calibrated phylogeny generated in 'BEAST' using haemagglutinin gene sequences ≥ 1,500 base pairs for all available canine distemper viruses in the Arctic-like clade. Markov Chain Monte Carlo chains were performed using BEAST v1.8.3 under a relaxed molecular clock model with branch rates drawn from a log normal distribution, a normally distributed clock prior with mean of 4 x 10^{-4} nucleotide substitutions per site, with a standard deviation of 0.4 nucleotide substitutions per site, and truncated to between 0 and 2.0. Colours are used to indicate continent of origin (Asia = blue; Europe = red; North America = green). Blue shading indicates Primorskii wildlife sequences.

Translated HA-genes from Primorskii were all found to have N residues at amino acid position 530, which had been proposed as enhancing infection of non-dog hosts. All Primorskii wildlife sequences carried the dog-like Y residue at position 549 of the HA-protein. This residue is shared by all other Arctic-like viruses with the exception of those from two Italian Eurasian badgers (KX024708 and KX024708), and an Italian dog (KM115535). Motifs representing potential sites for N-glycosylation (N-X-S/T) were found in all Primorskii sequences at positions N21, N149, N391, N422, N456, N587 and N603, as well as the alternative N-glycocylation site (N-Y-C) at N152. However, potential N-glycan N309 was only found in sequences from PT61/Pt 2004, PT79, all representatives of Primorskii subclades 2.1, 2.2 and a sable FUR0141. As expected, all Primorskii viruses

bore the residues Y525, D526, and R529 that are critical to efficient SLAM-binding (Zipperle et al. 2010), and the characteristic K-L-N-E-I motif at positions 110-114, that are conserved in all CDV viruses, and are understood to be critical to interaction between the H and F proteins (Lee et al. 2008).

All Primorskii fusion proteins included the cleavage motifs at residues 135/136, and 224/225. The sequence from PT61/Pt 2004 shared all five N-glycosylation sites that are found in the Onderstepoort vaccine strain (at N residues 62, 141, 173, 179, and 517). All other Primorskii viruses lacked the N-glycan site at residue 62, as has been recorded in other Asian strains (Lee et al. 2013).

Discussion

The detection of CDV in tigers and a leopard in 2003, 2006, 2010, 2013 and 2015, confirms that infection in these threatened populations is occurring on a regular basis. All viruses from large carnivores fell within the Arctic-like clade of CDV, and aligned closely to sequences obtained from mesocarnivores from Primorskii. While sequences from tiger viruses obtained in 2003 and 2006 diverged at basal positions in the Primorskii lineage, the sequence from the tiger in 2013 shared a recent common ancestors with sequences obtained from mesocarnivores (TMRCA of 1.2 years). Although the species identity of hosts infected with these MRCAs remains unknown, the short time elapsed suggests a likely wildlife origin. By contrast, the only virus detected in domestic dogs belonged to the Asia-4 clade of CDV, and is only distantly related to sequences detected in wildlife. Although Arctic-like CDV was not detected within the dog population, the presence of undetected Arctic-like viruses among Primorskii's dogs cannot be excluded. However, considering the comparative ease with which Arctic-like viruses were detected in wildlife over a wide area, it seems unlikely that the strain is common in dogs, suggesting a limited role in viral maintenance.

Arctic-like viruses are rare in neighbouring China, with the only examples having been sequences from provinces in the northeast of the country neighbouring Primorskii Krai. Although the Chinese fur-farming industry is mainly based in northeastern provinces, Asia-1 viruses predominate in this sector (81/86 sequences), and only three Arctic-like

sequences have been reported (Appendix XVIII, Zhao et al. 2010). If domestic dogs were important to the maintenance of Arctic-like viruses then a wider distribution might be expected, as sequences from dog hosts have been obtained from throughout China. Heilongjiang and Nei Mongolia lie at the southern limit of the taiga forest found throughout Primorskii, which extends north into Eastern Siberia and west toward Lake Baikal. While the ranges of some wild carnivore species (such as Asian badger and red fox) extend south beyond the taiga zone, others such as sable reach their southern limit in the Manchurian forests in this region (Monakhov 2011). The distribution of Arctic-like viruses in China might therefore be a consequence of the ranges of wildlife hosts, and their absence in the rest of the country suggests that they are unlikely to be maintained in domestic populations.

The wide distribution of Arctic-like CDV suggests a mode of dispersal that differs from other more geographically restricted clades, and comprises large parts of the world where wild carnivores outnumber dogs. With the exception of the America-1 strain used in vaccines, the Arctic-like clade is the most geographically dispersed of all CDV lineages (Figure 4.3). Sequences within this clade have been detected from Europe (Austria, Hungary, Switzerland and Italy), Greenland, Russia (including Lake Baikal), China and the United States (Alaska and Missouri, Appendix XVIII). This wide distribution contrasts with that of most other lineages, which are found in relatively limited geographical areas (Figure 4.3, Bolt et al.1997; Martella et al.2006). In North America, Europe and China, Arctic-like viruses are sympatric with other major lineages, raising the question of why these viruses should disseminate so readily, while other co-circulating strains remain relatively localised. Alternate modes of dispersal could occur along anthropogenic routes (e.g. movement of infected dogs), via wildlife-mediated transmission, or a combination of the two. The more northerly range of Arctic-like viruses, particularly in areas of low human habitation in Russia, Alaska and Greenland may hint at a role for wild carnivores. However, dispersal along human transport routes has also been documented (Nambulli et al. 2016), and might be a more plausible explanation for two Arctic-like viruses in Missouri that are placed basal to the European Arctic-like clade (Figure 4.5). Of the published Arctic-like sequences, 80% (n=49, Appendix XVIII) were obtained from domestic dogs, but this may reflect the comparative ease of sampling a domestic animal versus a wild one. However, while their role in the maintenance and dispersal of Arctic-like viruses may be open to debate, the inclusion of dogs among known hosts suggests some involvement in the epidemiology of the clade. Although current data may be

insufficient to definitively assess the relative roles of dogs and wildlife in the dispersal of the Arctic-like clade, it is clear that its distribution is distinct from other CDV viruses, and the ease with which it moves through sparsely populated regions may suggest an important role for wildlife in its epidemiology.

Wildlife viruses from Primorskii are spatially well-mixed (Figures 4.4C and D), suggesting long chains of transmission in wild species, which supports the case for wildlife maintenance (Viana et al. 2014). By definition, chains of transmission in maintenance populations will be long, or in some cases indefinite. Over time, the spatial distribution of individual viral lineages may change within the limits of host population structure, due to the dispersal of infected animals, or more gradually through infection between neighbours. Conversely, outbreaks in non-maintenance populations, that rely on spillover from a more abundant reservoir will tend to have much shorter chains of transmission, resulting in a more localized distribution, that dies out quickly. The topology of phylogenetic trees constructed using both HA and F-genes indicates a high degree of spatial mixing of viruses infecting mesocarnivores in Primorskii (Figure 4.4). Of the main Primorskii wildlife subclades, three of the four lineages include sequences representing two or more sampling areas. The clustering of sequences from disparate geographical locations suggests that CDV is circulating among mesocarnivores over a wide spatial area, and is consistent with their contributing to the maintenance of the virus.

Previous studies have proposed two amino acid substitutions on the HA glycoprotein that may promote infection in non-dog hosts (G/E530R/D/N and Y549H, McCarthy et al. 2007, Nikolin et al. 2012b). Although all wildlife sequences from Primorskii carried an asparagine residue at position 530 (proposed as favouring infection in non-dog hosts), the importance of this residue has since been dismissed as it relates more strongly to phylogeographic clade than host identity (Nikolin et al. 2012b). By contrast, all CDV sequences from wildlife in Primorskii carried a tyrosine (Y) residue at position 549 of the HA glycoprotein, which is more common in domestic dogs (McCarthy et al. 2007, Nikolin et al. 2012b), and has been shown by *in vitro* experiments to be ‘dog adaptive’ (Nikolin et al. 2012a). This experimental work suggests that 549Y is more efficient at infecting dog cell lines than viruses carrying the more generalist Y549H substitution (which is adapted to infecting cell lines from a wider range of host species). If these findings were to apply to a wild situation, it might be assumed that 549Y could only persist in a maintenance

community containing large numbers of domestic dogs, where it had a selective advantage. Conversely, a maintenance community dominated by wildlife species should favour the selection of the more generalist Y549H substitution, which is more efficient at replicating in a wide range of species. While there is a bias in the global non-canid dataset toward 549H, it is not invariably present. This could be interpreted in several ways. Experiments have shown that the Y549H substitution is rapidly acquired during passage in ferrets (von Messling et al. 2003), so the presence of 549Y in a non-canid could simply reflect a virus contracted directly from a dog host. Alternatively, the importance of the Y549H substitution in promoting infection across a wider range of hosts may have been overstated, and other factors are preventing its selection in a reservoir where wild carnivores predominate. This might arise if 549Y were also adaptive in another, as yet unidentified wild carnivore that is well represented in the Primorskii reservoir system. Unless future studies are able to demonstrate extensive involvement of Primorskii dogs in the circulation of Arctic-like CDV, it seems that the second explanation is most likely, and the virology of the Y549H mutation is more complex than has been realized.

The only mutations recorded in Primorskii viruses at positions of known functional significance were those affecting the N-glycocylation of the HA-protein. The presence of N-glycan chains on the HA-glycoprotein of CDV affects the structure and virulence of viruses (Sawatsky and von Messling 2010), with removal of N-glycans associated with a reduction in pathogenicity (Sawatsky and von Messling 2010). The HA-gene of most wild-type CDVs includes at least eight motifs for potential N-glycosylation (with asparagine residues at positions 21, 149, 309, 391, 422, 456, 587, and 603, Sawatsky and von Messling 2010), with almost all Asia-1 clade viruses carrying an additional motif at N584 (Iwatsuki et al. 1997). All of these N-glycan motifs were found in Primorskii viruses, with the exception of 309N, which was absent in 13/24 viruses. All Arctic-like viruses form other pars of the world, and viruses of all clades arising from *Panthera* species possess an N-glycan motif at position 309N. Whether the loss of this motif incurs any functional change on the virus is unknown, and it is notable that only some of the potential glycosylation sites carry N-glycans in wild-type viruses (Sawatsky and von Messling, 2010).

Residues on the HA glycoprotein within the receptor binding site all carried typical signatures (Y525, D526, and R529), and the K-L-N-E-I motif at positions 110-114 (critical to interaction between the HA and F proteins, Lee et al.2008) was also intact. All F-

glycoproteins carried the cleavage motifs at residues 135/136, and 224/225. The sequence from PT61/Pt 2004 shared all five N-glycosylation sites on the F-protein that are found in the Onderstepoort vaccine strain (at N residues 62, 141, 173, 179, and 517). All other Primorskii viruses lacked the N-glycan site at residue 62, as has been recorded in other Asian strains (Lee et al. 2013). Although only one full genome was sequenced from Primorskii, clusters of amino acid substitutions within the L-protein suggest that positive selection may be occurring at these sites. To date, virtually all research on substitutions that influence host adaptation have focused on the external HA-protein, however the present findings suggest that internal proteins, particularly the L-protein warrant further attention.

The molecular clock model used to describe substitution rates within the HA-genes, provided a detailed account of the evolution of Arctic-like CDV viruses, with high posterior support for most nodes. From this, it was evident that the main geographic subclades appear to have diverged within a fairly discrete time period, with viruses from China and the subclades in Europe, Alaska, and Primorskii all diverging between 1986 and 1989. This period coincided with two major events in the history of the Morbillivirus genus: the first report of Arctic-like CDV in Baikal seals (*Phoca sibirica*, Grachev et al.1989; Osterhaus et al.1989), in 1988, and the first recorded outbreak of CDV congener, phocine distemper virus (PDV) that affected harbour seals in the North Sea (Heide-Jorgensen et al. 1992). While we don't know where the most recent common ancestor (MRCA) of the main Arctic-like subclades originated, the Holarctic-wide distribution of progeny lineages is suggestive of a northern origin, where lines of dispersal into Europe, Asia and North America are shortest. Coincidentally, PDV is also thought to have originated in the Arctic waters of the Barents Sea, and been carried south in a large scale movement of harp seals (*Pagophilus groenlandicus,* Dietz et al.1989). It is tempting to speculate that the timing of these two outbreaks, and of the divergence of Arctic-like CDV may not have happened by chance, and similar drivers could have been responsible for all three events. Possible drivers could include sudden increases in the size of reservoir populations, changes in animal movement patterns (e.g. driven by changes in food availability, or climatic extremes), or a combination of factors that increase contact between populations of susceptible species.

On a more local level, the divergence of the main subclades of wildlife viruses in Primorskii during the mid-2000s suggests an epizootic may have occurred at this time. The

viruses within Primorskii subclade 1 diverged from those in subclade 2 in approximately 2005, with 2.1, 2.2 and 2.3 all diverging around 2006-2007. This radiation of CDV diversity might suggest the occurrence of a local epizootic in Primorskii during the mid-2000s, which gave rise to all modern sublineages. The first case of CDV in a wild tiger, which occurred during late 2003, falls within the 95% HPD estimates for this divergence, and may have been part of this epizootic (Quigley et al. 2010, Seimon et al. 2013). The apparent lack of tiger cases before this date might therefore reflect a true expansion of the virus into Primorskii, rather than a failure to detect CDV cases prior to 2003.

It is important to recognize that interpretation of trees produced using a molecular clock model is heavily influenced by the availability of sequences used in its construction. For instance, a previous study applied a molecular clock model to 208 published CDV sequences from throughout the world (Panzera et al. 2015). While the timing of divergence within the Arctic-like clade in that study was similar to that found here, the author's interpretation of worldwide CDV dispersal is limited, as they fail to recognise the shortcomings of their available dataset. While an Arctic-like strain from Greenland in 1988 (Z47760), and more recent Italian strains may have diverged from a MRCA in approximately 1967, that does not imply that the virus moved directly between Greenland and Italy at that time. Had a greater number of sequences been available from a wider geographic area the conclusions may have been quite different. An alternate and perhaps more likely explanation would involve an MRCA at a third site, perhaps in the Arctic, that gave rise to more southerly viral lineages in Greenland and Italy. Similar errors are made when assuming the direction of viral transmission occurs from domestic to wild hosts, based on a dataset that is biased to domestic dogs (which are more readily sampled). While the distinction may appear to be slight, it does have major implications for interpreting potential routes of dispersal, and whether this is achieved through anthropogenic or wildlife transmission chains.

The detection of the Asia-4 clade viruses from sick domestic dogs in Vladivostok was unexpected. This recently described clade was initially detected in Thailand, and has also been found in three dogs in Central China (Radtanakatikanon et al. 2013, Bi et al. 2014). The Russian virus shares the greatest identity with the Thai viruses (>98%). However, comparatively little sequencing of CDV has been performed in the Southeast Asian region, and so the true geographic range of Asia-4 is currently unknown. Notably, Thailand is a

common tourist destination for Russian citizens, (with over 1.73 million visitors recorded in 2013, Tourism Authority of Thailand, 2014), which could represent a potential mode of introduction for exotic viral clades into the Far East. However, it should be noted that Russian regulations only permit then importation of dogs from countries that are free from diseases including rabies and tuberculosis (The Embassy of the Russian Federation to the United Kingdom of Great Britain and Northern Ireland 2016). As rabies and tuberculosis occur in both Thailand and China, as well as much of the rest of East Asia, it is unclear how Asia-4 clade viruses may have reached Vladivostok by legal means.

The circumstances surrounding the deaths of the three new tiger cases identified in this study differed from those detected previously, and may suggest a need to expand the case definition for CDV in wild tigers. Previous cases have exhibited an unusual fearless demeanor, with animals behaving in a non-aggressive manner (Quigley et al. 2010, Seimon et al. 2013). Initially there was no reason to suspect the involvement of CDV in the deaths of either of PT79, or the tiger found dead in 2013 (Appendix II). Both of these tigers were suspected or confirmed to have died during encounters with people. In retrospect, it seems likely that aberrant behaviour related to CDV infection, could have contributed to the human encounter that led to their deaths. Unprovoked tiger attacks are also very rare in Russia (Miquelle et al. 2005), and so the detection of CDV in a lymph node from PT90/Pt 2010-1, a tiger that attacked and killed a fisherman, suggests that infections could have implications for human safety. Expansion of the case definition, to include tigers involved in incidents of poaching, or human-tiger conflict, could lead to the detection of further cases in Russia and elsewhere. As part of this, CDV testing should be included as part of a routine health screen for live or dead tigers handled as part of human-tiger conflict incidents. These observations also suggest that the benefits of successful CDV management strategies may extend beyond tigers, and also have implications for human safety.

From a conservation perspective, the detection of CDV in a tiger and a Far Eastern leopard in the Southwest Primorskii region is a particular cause for concern. This region supports the entire population of Far Eastern leopards (approximately 60 individuals), and approximately 16-21 tigers, that are genetically isolated from the larger population in the Sikhote Alin Mountains (Chapter 2, Henry et al.2009; Pikunov and Miquelle, 2003). These populations are of particular importance, as they represent founders for the gradual

recolonization of northeastern China (Hebblewhite et al. 2012, Wang et al. 2016). Population viability models have indicated that CDV increases the likelihood of extinction of tiger populations, an effect that disproportionally affects isolated areas supporting fewer individuals (Gilbert et al. 2014). The 2015 leopard case also represents the first report of CDV infection in a free-ranging leopard, and the diagnosis highlights a new threat to the conservation of this critically endangered subspecies.

Conclusion

Since 2003, there have been regular cases of CDV infections affecting Amur tigers throughout their range in the Russian Far East. The detection of CDV in a clinically affected Far Eastern leopard represents the first case in this subspecies, and a new threat to its conservation. The detection of CDV in several species of wild mesocarnivore (particularly sable) supports the hypothesis that they are involved in the maintenance of the virus, while the detection of a single domestic dog infection is less convincing. However, for short-lasting infections like CDV, it can be challenging to detect infected individuals, limiting the conclusions that can be drawn on the species contributing to CDV maintenance. For such transient infections, the detection of CDV neutralizing antibodies can provide a more complete assessment of exposure at a population level, and will be the focus of Chapter 5. Viral sequences from tigers and leopards clustered closely to viruses obtained from wild mesocarnivores, and were only distantly related to those obtained from domestic dogs, supporting the hypothesis that infections in these big cats are likely contracted from wildlife sources. Contemporary wild carnivore viruses in Primorskii diverged during the early 2000's, suggesting an epizootic event at that time, and coincident with the first recorded case in a wild Amur tiger. Since then, CDV has become spatially mixed in wild mesocarnivore populations, suggesting long chains of transmission and providing further support to the hypothesis that the virus is being maintained in wild carnivore communities across large geographic scales. All wildlife viruses carried a tyrosine residue at position 549 on the HA glycoprotein, which has been proposed as favouring infection in domestic dogs which does not support the hypothesis that the Y549H substitution is adaptive to non-dog infections.

Given the prominence of wildlife in CDV epidemiology in this region, control measures that target domestic dogs are likely to have little effect on controlling infection or preventing transmission to tigers. Options for controlling infection in mesocarnivores are limited. Currently, all available CDV vaccines must be delivered parentally, thus achieving meaningful coverage in abundant and free-ranging wildlife is an impractical proposition. Likewise, there is no prospect of reducing contact between tigers and mesocarnivores that might act as sources of infection. Control strategies must therefore target the tigers themselves, raising the immune status of the population sufficient to withstand periodic transmission from the CDV reservoir.

Author contribution

The author conceived and developed the overall study design, and secured primary sources of funding for the project. The author also obtained ethical approval for the study from the Institutional Care and Use Committee of the Wildlife Conservation Society (WCS, Appendix III). Permission to collect samples from dogs was secured from the State Veterinary Inspection by the author and colleagues with this Institute of Biology and Soil Sciences (IBSS, Appendix IV). Permission to collect samples from wildlife was secured from the Ministry of Natural Resources and Environment by WCS. Archived samples were collected by WCS, IBSS, National Cancer Institute (NCI) and the Zoological Society of London (ZSL). Additional contemporary wildlife samples were collected by the author, and veterinarians with ZSL and IBSS. Veterinary and Endangered Species permits to enable the export of samples were obtained by the author, with the assistance of staff with IBSS, ZSL, WCS and the University of Glasgow (UoG). Nucleic acid extraction and polymerase chain reactions were performed by the author, and researchers with WCS, IBSS, and the UoG.. The preparation of libraries for Illumina sequencing were performed by researchers with UoG, with the assistance of the author. Bioinformatics were performed by researchers at UoG. The author performed all data entry, analysis and interpretation of results.

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Chapter 5 Patterns of canine distemper virus infection in susceptible carnivores in Primorskii Krai

Abstract

For acute short-lasting infections such as canine distemper virus (CDV), serological data often provide the only feasible means for assessing spatial and temporal patterns of infection in free-living wildlife populations. This study aimed to describe patterns of CDV exposure in Amur tigers (*Panthera tigris altaica*), and other wild and domestic carnivores in the Russian Far East using archived and newly collected samples. Serum samples were obtained from tigers and other large carnivores (from 1992-2014), small-bodied wild mesocarnivores (from 2005-2014), and domestic dogs (from 2012-14). Samples with neutralizing antibody titers ≥1:16 were considered positive. Exposure to CDV was widespread in tigers, mesocarnivores and dogs. No antibodies were detected in tigers sampled prior to 2000 (CI: 0.0-20.9%, n=19), but were detected in 35.7% of tigers sampled from 2000-2014 (CI: 23.7-49.7%, n=56). However, the detection of antibodies in two Far Eastern leopards (*P. pardus orientalis*) and a brown bear (*Ursus arctos*) in two locations in 1993-4 indicated the presence of CDV in wild habitats prior to 2000. Seroprevalence was significantly higher among unvaccinated dogs in the remotely located Sikhote-Alin Biosphere Zapovednik (SABZ, 41.4%, CI: 32.4-50.9%, n=116), than Lazovskii (29.5%, CI: 22.8-37.2%, n=166), and Southwest Primorskii (15.9%, CI: 11.1-22.3%, n=182, chi-square = 23.918, $p<0.01$, df = 2). Evidence of recent outbreaks (based on detection of antibodies in dogs less than one year old) was found in a greater proportion of settlements in SABZ (3/3 settlements, 100.0% CI: 30.9-100.0%,), than in Lazovskii (3/6 settlements, 50.0%, CI: 18.8-81.2%,), and Southwest Primorskii (2/12 settlements, 16.7%, CI: 2.9-49.1%,). Seroprevalence was higher in unvaccinated dogs with access to the forest (34.2%, CI: 26.8 to 42.4%, n=152), compared to those that did not (25.0%, CI: 19.4 to 31.6%, n=208), but this difference was not significant at the 95% confidence level (chi-squared = 3.1922, df = 1, p = 0.074). Age stratified seroprevalence data were used to estimate the force of infection (0.114, ±0.019 cases per dog per year), and effective reproductive number (R_e, between 1.39 ±0.068 and 1.48 ±0.063). Only 40.8% of dogs reported to have received CDV vaccines were found to have antibodies, which may indicate low immunogenicity of vaccines, deficiencies in vaccine administration protocols, or

inaccurate reporting by owners. Given that only 23.9-39.3% of dogs were reported to have received vaccines, population immunity at these levels would not be sufficient to control CDV with an R_e greater than 1.11-1.19. Overall these findings suggest that CDV is circulating widely among wildlife in the Russian Far East, which limits options for controlling infection in tigers through domestic dog vaccination. Consideration may need to be given to vaccination of tigers themselves.

Introduction

An understanding of the factors that contribute to the maintenance of a pathogen in an ecosystem is key to the design of rational control strategies for managing infectious disease. The recent detection of canine distemper virus (CDV) in free-ranging Amur tigers (*Panthera tigris altaica*, Seimon et al. 2013), has been predicted to negatively impact population survival (Gilbert et al. 2014), highlighting a need to identify potential control strategies. Fundamentally, interventions aim to reduce the number of cases within a defined 'target' population (in this case tigers), and either curtail transmission within the target, or disrupt infections transmitted from other source populations (Woodroffe 1999, Haydon et al. 2002, Viana et al. 2014). Selection of appropriate control measures requires an understanding of the relative contribution of different populations to the maintenance of a pathogen, or as sources of infection to the target. Identifying disease reservoirs is complex, and relies on multiple sources of evidence (Viana et al. 2014). Case information derived from molecular studies provides data on active infections, and the phylogenetic relationship of pathogens infecting different individuals (Chapter 4). However, for transient infections such as CDV, molecular studies may provide limited information on the patterns of exposure across populations, as the likelihood of detecting an infected individual is often small. The immune response of hosts that have recovered from infection may be measurable for many times longer than the infection itself. In the case of CDV, which invokes a long lasting immune response (Greene and Appel 2006), serological techniques that detect the presence of neutralizing antibodies can provide a more complete picture of pathogen exposure across affected populations. The objective of this chapter is to use serology to augment the results of molecular approaches described in Chapter 4, to describe the patterns of CDV exposure among tigers and other domestic and wild carnivores in the Russian Far East.

Consisting of just 331 to 393 adult and subadult animals (Miquelle et al. 2007), occupying an area of 155,000 km^2 (Hebblewhite et al. 2014), it is unlikely that the size and population structure of the Amur tiger would be sufficient to maintain CDV infections in the long term. The extent of tiger-tiger contact is greatest between mothers and cubs, with periodic interactions between males and females of breeding age (Goodrich et al. 2010, Quigley et al. 2010). Breeding Amur tigers are territorial, with male breeders occupying territories that encompass between 1 and 5 female territories (Goodrich et al. 2010). Direct contact between territory holders of the same sex is limited, and particularly in the case of males may be violent in outcome. At independence, female cubs often inherit a portion of their mother's home range, while males tend to roam more widely in search of a territory of their own. Relatively little is known about the movements and social interactions of non-breeding tigers, but considering the risks inherent in violent encounters, it is likely that opportunities for direct contact are limited until tigers secure a territory of their own. For these reasons, opportunities for intra-specific transmission between tigers are relatively limited. At most, a breeding male might infect each of the female territory holders in his home range, which in turn could infect any offspring present, but there is little opportunity for infection to spread to neighbouring breeding networks. The possibility of indirect transmission is a notable caveat, particularly if CDV virions remained viable for an extended period in the cold of the Russian winter (Appel 1987, Ballmann Acton 2007), and could theoretically lead to further intra-specific spread, via urine-marked scent posts, or contamination by roving non-breeders. However, even if this were to occur, the low density of the Russian tiger population suggests that opportunities for such events may be limited, and most tiger-tiger transmission chains are destined to fade out.

For these reasons, transmission from other species is likely to play an important part in CDV infections in tigers. Wildlife have been implicated as reservoirs and sources of infection for other populations of threatened carnivores (Craft et al. 2008, Woodroffe et al. 2012, Viana et al. 2015), and the Russian taiga forest supports a diverse array of susceptible wild hosts. Some larger bodied species, such as brown bear (*Ursus arctos*), Asiatic black bear (*U. thibetanus*), Eurasian lynx (*Lynx lynx)*, and the 'Critically Endangered' and range-restricted Far Eastern leopard (*P. pardus orientalis*) exhibit social structures not dissimilar to that of the tiger, occurring at low densities, with few intra-specific contacts, that may limit opportunities for transmission. Smaller-bodied mesocarnivore species occur at much higher densities where direct interactions may be more likely. Most abundant among these are raccoon dogs (*Nyctereutes procyonoides*), red

fox (*Vulpes vulpes)*, leopard cat (*Prionailurus bengalensis*)*,* Asian badger (*Meles leucurus*), sable (*Martes zibellina*), and Siberian weasel (*Mustela sibirica*). Several of these species, such as Asian badgers are more social, providing greater opportunity for intra-species transmission, as well as from other species through contact associated with shared resources of food and shelter (Stroganov 1969, Kowalczyk et al. 2008). In addition, the use of urine, faeces and anal secretions to mark food resources and territory (Henry 1977, Wells and Bekoff 1981, Kruuk et al. 1984) also represents a potential mode for indirect transmission of virus.

In numerical terms, domestic dogs may be the most abundant susceptible host species for CDV in the RFE (Chapter 3). Studies focused on domestic dog demography and patterns of ownership have found features of the dog population that could both favour, and hinder its potential to maintain CDV (Chapter 3). Rates of dog ownership by people living in rural areas are very high, and local densities within human settlements are likely to far exceed those of any other susceptible species in the region, which could favour CDV maintenance (Chapter 3). However, the infrequency of dog movement between settlements, restrictions on roaming behaviour and the impact of vaccination may hinder CDV transmission, acting to slow its spread across the dog metapopulation. The analysis of samples collected from 633 apparently healthy dogs during household surveys, and 75 sick dogs at veterinary clinics, led to the detection of only one CDV sequence (Chapter 4). However, due to the relatively short duration of CDV infections in dogs (Greene and Appel 2006), it is possible that the virus may be more widespread than these results suggest. Therefore, the use of serology to detect antibodies in unvaccinated dogs would give an indication of prior exposure to the virus, which could be more informative in the inference of viral epidemiology in this host. Furthermore, serological studies would provide insights into the immune status of the domestic dog population, that could be of considerable value to the design of vaccination programmes to reduce the impact of the virus on this host, and potentially also on the tigers.

Direct and indirect contact between dogs and wildlife may be relatively common, representing a potential opportunity for CDV transmission. Ownership surveys found that 5.8% of dogs were used for hunting, and owners reporting that 41.0% of dogs (n=2,324) are taken to the forest for this and other recreational purposes (Chapter 3). Anecdotally, owners have reported direct contact between their dogs and wild carnivores (Chapter 3),

and predation of dogs by tigers has been well documented in Russia (Smirnov and Miquelle 1999, Miller 2012). Given these direct interactions, as well as the possibility of indirect transmission, domestic dogs could be important hosts of CDV, and a possible source of infection for tigers.

Any, or all of this diverse array of susceptible species could be contributing to the maintenance of CDV in the Russian ecosystem. For a pathogen to persist, each infected individual in a population must on average give rise to one, or more secondary cases. As long as these conditions are met, the identity of secondary hosts, whether the same or different species is unimportant to viral maintenance, and frequent inter-species contact could give rise to very complex maintenance communities. Disentangling these host relationships is challenging, and may not be possible, even with very intensive sampling programmes, and long study periods. A more practical strategy might take a more qualitative approach, assessing whether exposure in the target population is occurring in particular localities or time periods, or the relative contributions made by different sectors of the maintenance community. The goal of this study is to use serological techniques to assess the exposure of species in Primorskii Krai that are susceptible to CDV, and use this to augment existing sequence data (Chapter 4) to assess patterns of exposure. An assessment of the likely contribution of domestic dogs to CDV maintenance, versus that of wild carnivores is of particularly importance to the selection of control strategies, given the comparative ease of increasing vaccination coverage in domestic settings.

Specific objectives and hypotheses included:

1. Through measurement of CDV neutralizing antibodies, assess the relative exposure of Amur tigers, and other domestic and wild carnivores to identify species that may contribute to CDV maintenance. While definitive conclusions on the maintenance of a pathogen cannot be draw solely from serological data, a maintenance population would be expected to exhibit a high seroprevalence, involving animals of all age classes, over a wide geographic area.

2. Assess the temporal exposure of Amur tigers to identify any long term trends in the incidence of infection. Previously CDV exposure has been detected in six tigers sampled between 2000 and 2004, whereas no antibodies were detected in tigers sampled between 1992 and 1999 (n=19, Goodrich et al. 2012). An important

objective was to use additional samples and supporting data to obtain a more detailed account of tiger exposure over a longer time period, with particular attention to:

a. Determine the status of CDV in Primorskii Krai prior to 2000. To test the hypothesis that CDV was absent from Primorskii Krai between 1992 and 1999, which could explain the lack of exposure of tigers during this period.

b. Reconstruct the exposure history of the intensively studied population of tigers in the Sikhote-Alin Biosphere Zapovednik (SABZ), to estimate the regularity of exposure, and assess evidence for tiger to tiger transmission.

3. Assess the factors influencing the exposure of unvaccinated domestic dogs to CDV. In particular, test the hypothesis that seroprevalence, and frequency of outbreaks would be greater in Southwest Primorskii than in Lazovskii and SABZ, where dog numbers and density were progressively lower.

4. Assess the effectiveness of CDV vaccination of domestic dogs. Vaccination represents the main approach to controlling CDV in domestic dog populations. The capacity for vaccination programmes to invoke a protective immunity is therefore central to their success. A key study objective was therefore to measure the prevalence of protective antibody titres in vaccinated dogs.

5. Estimate force of infection and effective reproductive number (R_e) of CDV infection in domestic dogs. Serological data can be used to estimate the mean force of infection (λ, the rate at which susceptible individuals acquire an infectious disease), and the effective reproductive number (R_e, the mean number of secondary cases arising from each infectious animal in a population that is partially immune, e.g. through use of vaccination). By fitting a catalytic model, to observed serological data stratified by host age, and incorporating plausible rates of CDV mortality, it is possible to obtain mean estimates for λ, and R_e for a population (Vynnycky and White 2010). Assessments of R_e are valuable in assessing desired levels of vaccination coverage in programmes to control infection, and for informing risk models to estimate the transmission of CDV from dogs to tigers (Gilbert et al. 2014).

Methods

Serum samples were acquired from several sources, focusing on the three study areas described in Chapter 3: SABZ (with low dog density), Lazovskii Zapovednik (with intermediate dog density) and Southwest Primorskii (with high dog density, Figure 5.1). Samples from large carnivores included some collected outside these locations, and were defined as originating in 'non-study areas'.

Samples from tigers and other large-bodied wild carnivores were obtained from the archives of the Wildlife Conservation Society, Bronx, New York, and were collected from wild carnivores captured in Primorskii Krai (124 animals), neighbouring Khabarovskii Krai (six animals) and Amurskaya Oblast (one animal) between November 1992 and November 2014. These included samples from 40 tigers described previously by Goodrich *et al.* (2012). Animals were captured either for research purposes (e.g. the placement of telemetry collars), or in response to human conflict situations (such as encroachment on human settlements, or following predation of domestic animals). Research animals were immobilized using either a combination of ketamine hydrochloride (10 mg/kg for tigers) and xylazine hydrochloride (0.8 mg/kg for tigers), or '*Zoletil*'® Virbac (tiletamine and zolazepam, 4-8 mg/kg depending on species), delivered using an injectable dart from a helicopter (research animals), or on the ground to animals captured using Aldrich foot snares (research and conflict animals) (Goodrich et al. 2001). A proportion of the research animals were followed throughout their lives, and so age was known with a high degree of certainty. For others, age was estimated based on tooth wear and gum recession. All animals were considered clinically healthy at the time of capture, with the exception of one tigress (PT61/Pt 2004), infected with CDV (described previously in Chapter 4 and in Quigley et al. 2010, and Seimon et al. 2013). Blood was collected through venipuncture of femoral veins, and serum harvested on the day of capture, through centrifugation of clotted blood at 2,500 rpm for 10 minutes. Serum samples were stored at -20 Celsius for up to eight years prior to export, after which they were transferred to -80 Celsius, and shipped using dry ice for analysis.

Serum samples from small-bodied mesocarnivores were obtained from animals captured specifically for this project, and from archived material. Captures took place in Lazovskii Zapovednik (during May 2013, and October/November 2013), and SABZ (April/May

Figure 5.1. Map illustrating the three primary study areas (white labels): Sikhote-Alin Biosphere Zapovednik (SABZ), Lazovskii Zapovednik (Lazovskii) and Southwest Primorskii (Southwest). Protected areas are indicated in dark green, and 25 km buffers in orange. The three districts (Rayons) where mesocarnivore tissue specimens were collected from fur-trappers (Chapter 4) are indicated in olive (Lazovskii Rayon, Terneiskii Rayon and Pozharskii Rayon).

2014). Animals were captured using folding cage traps (manufactured by Tomahawk, Hazelhurst, WI, and Havahart, Lititz, PA). Between 26 and 50 folding cage traps were utilized in Lazovskii, and 21 in SABZ. Traps were set along riparian and coastal forests where camera trap footage had indicated the presence of wild carnivores, and were checked once daily. Captured animals were immobilized using '*Zoletil*'® Virbac (3-5 mg/kg, depending on species), and blood was collected from the cephalic or jugular veins. Animals were examined for signs of clinical disease, and age was estimated based on tooth wear and gum recession. Serum was separated by centrifugation of clotted blood at 2,500 rpm for 10 minutes, and was then transferred to liquid nitrogen for storage in the field.

Samples were maintained on -23 Celsius ice packs during export, and were transferred to -80 Celsius freezers prior to analysis.

Archived material included samples collected in Southwest Primorskii during 2007-8 (by the Institute of Biology and Soil Sciences of the Far Eastern Branch of the Russian Academy of Sciences, Vladivostok; and the National Institutes of Health/National Cancer Institute, Frederick, Maryland, IBSS/NCI), Lazovskii Zapovednik during 2008-9 (by the Zoological Society of London, ZSL), and from SABZ between 2005 and 2011 (by the Wildlife Conservation Society). Archived samples had been collected from mesocarnivores using cage traps (IBSS/NCI, ZSL and WCS), and padded leg-hold traps (IBSS/NCI only, Meek et al. 1995) deployed in a similar manner to those captured specifically for the purposes of this project, and were therefore considered comparable.

Samples were collected from dogs during visits made to 19 communities in the Southwest Primorskii and SABZ study areas during June and July 2012, and in 26 settlements in all three study areas during household questionnaire surveys between November 2012 and June 2014 (described in detail in Chapter 3). Owners were asked for details of all individual dogs in their household, including age, time since their last distemper vaccination, and whether dogs were taken to visit forested areas. Once questionnaires were completed, householders were asked for their consent to allow the collection of biological samples from their dogs. Where informed written consent was given, dogs were manually restrained, and blood was drawn from the cephalic vein. Serum was collected and stored in the same manner as samples from mesocarnivores. Target sample sizes for each study area were calculated using the ‘pwr’ package in R (Champely 2015). Sample sizes of 170 unvaccinated dogs in each area were selected using a two-tailed test to detect differences in seroprevalence of 15%, with an expected seroprevalence of 50% (which maximizes the sample size required to meet the desired difference, power and significance levels), at a power of 80% and a 95% significance level.

Antibody titres were measured using virus neutralization at the Washington Animal Disease Diagnostic Laboratory (WADDL) at Washington State University (tigers and other large-bodied wild carnivores), and by the Veterinary Diagnostic Services at the University of Glasgow (mesocarnivores and domestic dogs). The methods used in both laboratories

were based on Appel and Robson (1973). Briefly, in Glasgow, a 1:4 starting dilution was prepared consisting of 30 µl of serum and 90 µl Dulbecco's modified Eagle's medium with 5% fetal calf serum (DMEM 5%). Four fold serial dilutions of each serum sample were prepared in a 96 well flat bottom plate from 1:16 to 1:16,384, consisting of 25 µl dilute serum and 75 µl diluent DMEM 5% in each well. Titration series were prepared four times, such that each sample was tested in quadruplicate. The Bissell strain[2] of CDV was then added to each serum dilution (100 $TCID_{50}$/75 µl) (Bussell and Karzon 1965). A serum control was prepared using a positive serum sample of known titre, and was titrated in the same manner as test sera. A virus control was prepared, with 75 µl of virus placed in the first well, and titrating two-fold dilutions thereafter. Dilutions were incubated in the dark for 1 hr at room temperature, followed by 1 hr at 4 Celsius. African green monkey derived Vero cells were added to each well in 50 µl volumes ($2x10^5$ cells/ml), and plates were incubated at 37 Celsius in a 5% CO_2 humidified incubator for 72 hr. The four replicate dilutions were then examined microscopically for cytopathic effect, with the endpoint of each determined as the highest dilution at which cytopathy was inhibited. Final antibody titres were calculated using the Spearman-Karber method (Hamilton et al. 1977).

Minor differences in the virus neutralization technique performed at WADDL included the preparation of single titrations of serum at two-fold dilutions from 1:4 to 1:512, and the use of the Onderstepoort strain of CDV at 1,000 $TCID_{50}$. Cytotoxic serum samples were identified by performing additional titrations without virus, and assessed for cytopathicity.

Serum samples with antibody titres equal to, or exceeding a cutoff titre of 1:16 were considered to be positive to reduce numbers of false positives due to non-specific neutralization at lower titres. Samples with a measurable titre lower than 1:16 were therefore considered to be inconclusive. While some dogs with vaccine-derived antibody titres as low as 1:8 may survive experimental challenge (Jensen et al. 2015), a higher titre of 1:64 was used as a more reliable indicator of presumed protective immunity (following the recommendations of Bohm et al. 2004, and Jensen et al. 2015). Only serum neutralization titres from animals greater than four months old were included in data analyses, to exclude possible maternally derived antibodies. Prevalence was considered to be the number of animals testing positive, divided by the total number of animals tested. The R package "prevalence" (Devleesschauwer, Brecht Torgerson et al. 2014) was used to

[2] A vero cell adapted clone of the Onderstepoort strain of CDV

calculate 95% binomial confidence intervals for all seroprevalence estimates. Chi-square goodness to fit tests, and Fisher exact tests were used to measure differences in seroprevalence in different study areas using R.

Before constructing multivariable models, subsets of cleaned data were prepared from the raw data, which excluded outcome variable entries where information on the appropriate explanatory variables was incomplete. Data were tabulated for cleaned and raw data subsets, to identify any changes in the distribution of observations for each explanatory variable that may have occurred during the cleaning process (Appendices IXX, and XX). No significant differences were found between raw and cleaned sets of data for either domestic dogs or tigers based on chi-square contingency tests.

Multivariate generalized binary logistic regression models were prepared to identify explanatory variables that were significantly associated with the outcome variable CDV exposure (0 or 1) in unvaccinated domestic dogs, and in tigers. Explanatory variables used for dog models included age, gender, whether owners reported taking the dog to the forest, study area, community type, residence type, number of people (adults and children within residence), presence of children, cats, poultry, and livestock, whether the dog was a guard, a hunting dog, or a companion animal, and origin. Explanatory variables used for tiger models included age, gender, study area, human population density, and whether tigers were research or conflict animals. To estimate human population density, the locations of sampled tigers were plotted within a geographical information system (QGIS 2.6.0-Brighton), over a 10 km^2 tessellated polygon prepared using the national census statistics 2010 (Russian Federal State Statistics Service. 2011). Tiles were classified based on human population density as ‘negligible’ (0 people per km^2), ‘low’ (>0 to 1.0 people per km^2), ‘moderate’ (>1.0 to 10), or ‘high’ (>10 people per km^2). Further details of explanatory variable categories for domestic dogs and tigers are provided in Tables 5.1 and 5.2 respectively. Models were prepared using a forward selection process, and AIC values were used to assess model quality. The final model was identified when addition of explanatory variables did not reduce AIC values further. Odds ratios were estimated as a measure of association between explanatory and outcome variables expressed within 95% confidence limits.

Table 5.1. Explanatory variables used to assess the outcome variable exposure in unvaccinated dogs using multivariate generalized binary logistic regression models.

Explanatory variable	Variable type	Levels
Study Area	Categorical	Southwest*
		Lazovskii
		SABZ
Community Type	Categorical	Village*
		Town
		Large town
Residence type	Categorical	Apartment*
		Cottage
People in house	Numeric	Number of people
Children in house	Categorical	No*
		Yes
Cat owner	Categorical	No*
		Yes
Poultry owner	Categorical	No*
		Yes
Livestock owner	Categorical	No*
		Yes
Age	Numeric	Age in months
Gender	Categorical	Female*
		Male
Forest visits	Categorical	No*
		Yes
Guard dog	Categorical	Non-guard*
		Guard
Hunting dog	Categorical	Non-hunter*
		Hunter
Companion dog	Categorical	Non-companion*
		Companion
Source	Categorical	Local*
		Non-local

* Indicates reference level for categorical variables.

Table 5.2. Explanatory variables used to assess the outcome variable exposure in tigers using multivariate generalized binary logistic regression models.

Explanatory variable	Variable type	Levels
Age	Numeric	Age in months
Gender	Categorical	Female*
		Male
Study Area	Categorical	Southwest*
		Lazovskii
		SABZ
		Non-Study Area
Animal category	Categorical	Research*
		Conflict
Sampling period	Categorical	Before 2000*
		2000 onward
Human density	Ordered factor	Negligible*
		Low
		Moderate

* Indicates reference level for categorical variables.

The force of infection (λ), or number of new infections per unit time was estimated by fitting a catalytic model to the seroprevalence data for unvaccinated dogs, stratified by their age (*a*) in years. Assuming that CDV is endemic in the dog population, the number of susceptible dogs (*S*) declines over time according to the formula:

$$\frac{dS}{da} = -\lambda S$$

This equation can be integrated to give the formula:

$$S = S_0 e^{-\lambda a}$$

Therefore, the proportion of dogs that remain susceptible to CDV by age *a* is given by:

$$p_\lambda(a) = e^{-\lambda a}$$

The number of seropositive dogs n_{pos} in each age category *a* represents the number of dogs surviving infection, and can be used to estimate the number of infected animals n_{inf} for a given CDV mortality ratio *M* (defined as the proportion of infected dogs that die):

$$n_{inf} = \frac{n_{pos}}{(1 - M)}$$

If the number of seronegative dogs is n_{neg} then the contribution of each age category to the model likelihood is given by:

$$p_\lambda(a)^{n_{inf}} \, (1 - p_\lambda(a))^{n_{neg}}$$

Corresponding to a log likelihood of:

$$\log\left(p_\lambda(a)^{n_{inf}}\right) + \log\left(\left(1 - p_\lambda(a)\right)^{n_{neg}}\right)$$

$$= n_{inf} \log\left(p_\lambda(a)\right) + \, n_{neg} \log\left(1 - p_\lambda(a)\right)$$

The sum of the log likelihood values for each age category then gives the log likelihood for the catalytic model.

A saturated model was then constructed using the age stratified serological data only, with the likelihood contribution of each age category *a* given by:

$$n_{inf} \log(p_{inf}) + n_{neg} \log(1 - p_{pos})$$

The sum of the log likelihood values for each age category then gives the log likelihood for the saturated model. The deviance between the two models is equal to -2 multiplied by the difference between the log likelihoods of the catalytic model and the saturated model. Alternate catalytic and saturated models were prepared using R based on a low mortality rate of 0.2, and a high mortality rate of 0.4 (Greene and Appel 2006). Models were run for a range of values of λ, with the best fit value minimizing the deviance between the catalytic and saturated models. Estimates of 95% confidence intervals for λ were obtained based on the values that corresponded to the optimal deviance minus 3.84 (χ^2 value for one degree of freedom at the p=0.05 level).

Best fit estimates of λ were then used to estimate R_e using the estimated lifespan (L, mean age at death) of dogs in the population of 3.39 years (estimated in Chapter 3), using the formula for R_e under the assumption of an exponential age distributions: (Vynnycky and White 2010).

$$R_e = 1 + \lambda L$$

Assuming that CDV infections did not occur seasonally, mean prevalence *P* was estimated based on λ values for low and high mortality assuming a mean period of infection *I* of 21 days.

$$P = \lambda \frac{I}{365}$$

Results

Serum samples were tested from a total of 233 individual wild carnivores of 12 species, including 67 tigers (Table 5.3, Appendices XXI and XXII). Of these, CDV neutralizing antibodies were detected in eight species, although sample size was limited to four or fewer individuals for the species from which antibodies were not detected (for reference,

29 animals would need to be sampled in order to have a 95% probability of detecting at least one positive individual in a population of infinite size, with a true seroprevalence of 10%, based on the hypergeometric distribution provided by Cannon and Roe 1982). Seroprevalence was greatest in raccoon dogs (34.3%, CI: 19.7-53.3%, n=35), followed by tigers (29.9%, CI: 19.6-42.4%, n=67). Of the 20 seropositive tigers, 16 were found to have presumed protective titres (Figure 5.2A), whereas presumed protective titres were only found in five of 16 mesocarnivores (Figure 5.2B). Inconclusive titres were particularly evident among Asian badgers, with five samples registering an antibody titre of 1:11. Had a cutoff of 1:11 been used (rather than 1:16), seroprevalence among badgers would have been considerably higher, at 16.3% (CI: 7.3-31.3%, n=43). The impact on seroprevalence estimates would have been much lower in other wildlife species, with only one, or two additional animals being considered seropositive had a lower threshold been used (Appendix XXV). Serum samples from three tigers that were later found to be infected with CDV at the time of sampling (PT79, PT56 and PT61[3]) were all found to have a titre of 1:128. Both PT56 and PT61 exhibited neurological signs at the time of sampling, and were subsequently euthanized or died from their infections respectively, while PT79 survived for at least four months before disappearing (Chapter 4). The basic generalized logistic regression model was found to be a better predictor of exposure in tigers than any of those that included explanatory variables (Appendix XXVI).

Table 5.3. Results of virus neutralization analyses against canine distemper virus (CDV) for serum samples collected from wild carnivores in the Russian Far East between 1992 and 2014. Neutralizing antibody titres of 1:16 or higher were considered positive. Seroprevalence is given as the number of positive samples expressed as a percentage of sample size, with lower and upper 95% binomial confidence intervals (CI).

Species	Positive	Sample size	Seroprev. (%)	Lower CI (%)	Upper CI (%)
Amur tiger *	20	67	29.9	19.6	42.4
Far Eastern leopard †	2	10	20.0	3.5	55.8
Eurasian lynx *	1	7	14.3	0.8	58.0
Leopard cat	2	16	12.5	2.2	39.6
Asiatic black bear *	1	25	4.0	0.2	22.3
Brown bear *	2	20	10.0	1.8	33.1
Raccoon dog †	12	35	34.3	19.7	52.3
Red fox †	0	4	0.0	0.0	60.4
Sable †	0	2	0.0	0.0	80.2
Siberian weasel †	0	2	0.0	0.0	80.2
American mink †	0	2	0.0	0.0	80.2
Asian badger †	2	43	4.7	0.8	17.1

* Samples tested in Washington State University against CDV Onderstepoort strain. † Samples tested in the University of Glasgow using CDV Bussell strain.

[3] PT61 is referred to as Pt 2004 in Seimon et al. 2013.

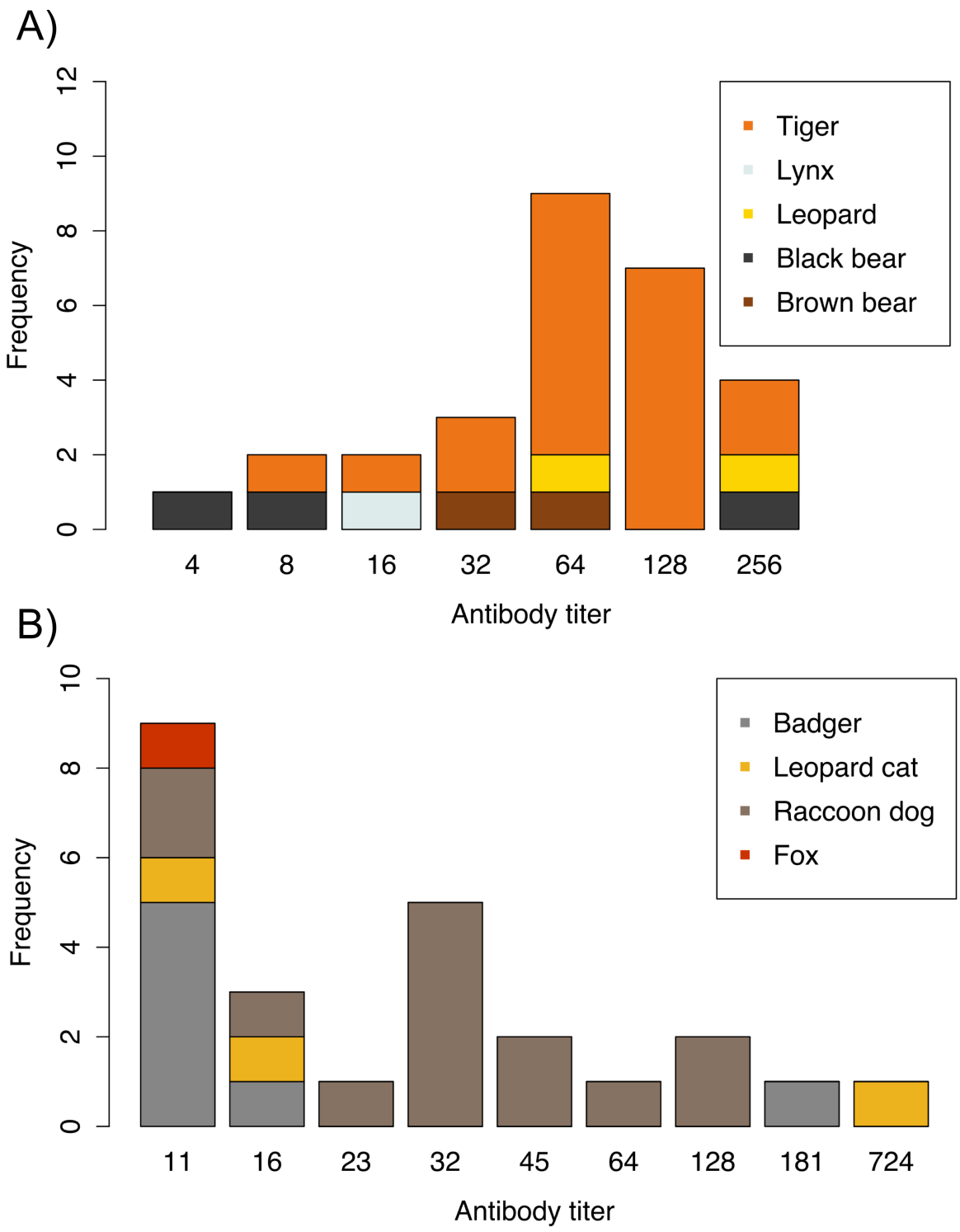

Figure 5.2. Distribution of canine distemper virus (CDV) neutralizing antibody titres in A) large carnivores measured in Washington State University using CDV Onderstepoort strain, and B) mesocarnivores measured in the University of Glasgow using CDV Bussell strain.

Antibodies to CDV were detected in wild carnivores from nine locations across Primorskii Krai and southern Khabarovskii Krai (Figure 5.3), including tigers in eight locations. Seropositive mesocarnivores were found in all three primary study areas, and no

significant difference was found in the seroprevalence recorded at the three sites using Fisher's exact test (p=0.465).

Only three wild carnivores sampled prior to the year 2000 were found to have antibodies to CDV (Table 5.4, Figures 5.4 and 5.5). These included two female leopards sampled in Southwest Primorskii during June 1993 and August 1994 (with titres of 1:64 and 1:256 respectively, and estimated to be three and four years old), and a male brown bear captured in SABZ during July 1993 (with a tire of 1:32, and estimated to be nine years old). None of the 19 tigers sampled prior to 2000 were found to have antibodies to CDV. The first cases of exposure in tigers were detected in a six year old male sampled in SABZ during October 2000, and a two year old male captured during a conflict mitigation incident in December 2000 in the district of Krasnoarmeyskii (N46.45°, E135.84°). Antibodies to CDV were detected more frequently in animals sampled after 2000, particularly in tigers, with 20 seropositive individuals detected between 2000 and 2014 (n=56).

Table 5.4. Summary of virus neutralization results against canine distemper virus (CDV) from large carnivores sampled in the Russian Far East from 1992 to 1999, and from 2000 to 2011. For animals sampled on more than one occasion, only the most recent sample in each period is used. Samples were analysed by Washington State University using the CDV Onderstepoort strain.

	Animals sampled before 2000				Animals sampled in 2000 and later			
Species	**+ve**	**n**	**%**	**95% CI**	**+ve**	**n**	**%**	**95% CI**
Amur tiger	0	19	0.0	0 - 20.9	20	56	35.7	23.7 - 49.7
Far Eastern leopard	2	6	33.3	6.0 - 75.9	0	4	0.0	0.0 - 60.4
Eurasian lynx	0	0	-	-	1	6	16.7	0.9 - 63.5
Asiatic black bear	0	9	0.0	0.0 - 37.1	1	17	5.9	0.3 - 30.8
Brown bear	1	13	7.7	0.4 - 37.9	1	8	12.5	0.7 - 53.3
Total	**3**	**47**	6.4	**1.7 - 18.6**	**23**	**91**	**25.3**	**17.0 - 35.7**

Survey effort was most consistent and intensive in SABZ between 1992 and 2011, providing some indication of the temporal distribution of CDV exposure among large carnivores in the protected area (Figure 5.5B). The seropositive nine year old brown bear (UA007) sampled during July 1993 confirms the presence of CDV during the late 1980s or

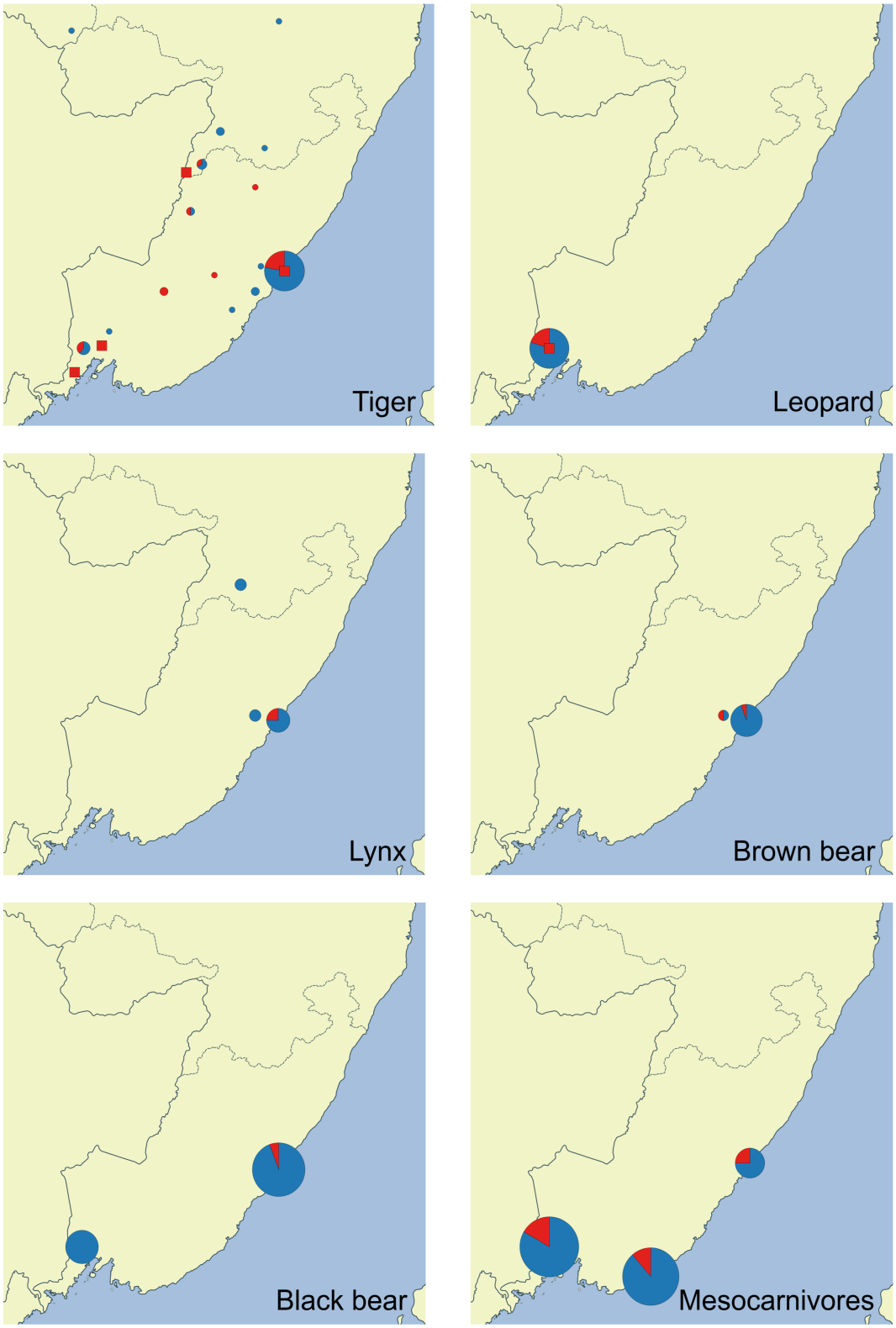

Figure 5.3. Locations in the Russian Far East from which serum was collected from Amur tigers, Far Eastern leopard, Eurasian lynx, brown bear, Asiatic black bear and mesocarnivore species between 1992 and 2014. Positive samples with canine distemper virus (CDV) neutralising antibodies (red) and negative samples (blue) from the same location are indicated as pie charts that are scaled based on sample size. Confirmed cases of CDV affecting tigers and leopards are indicated by red squares.

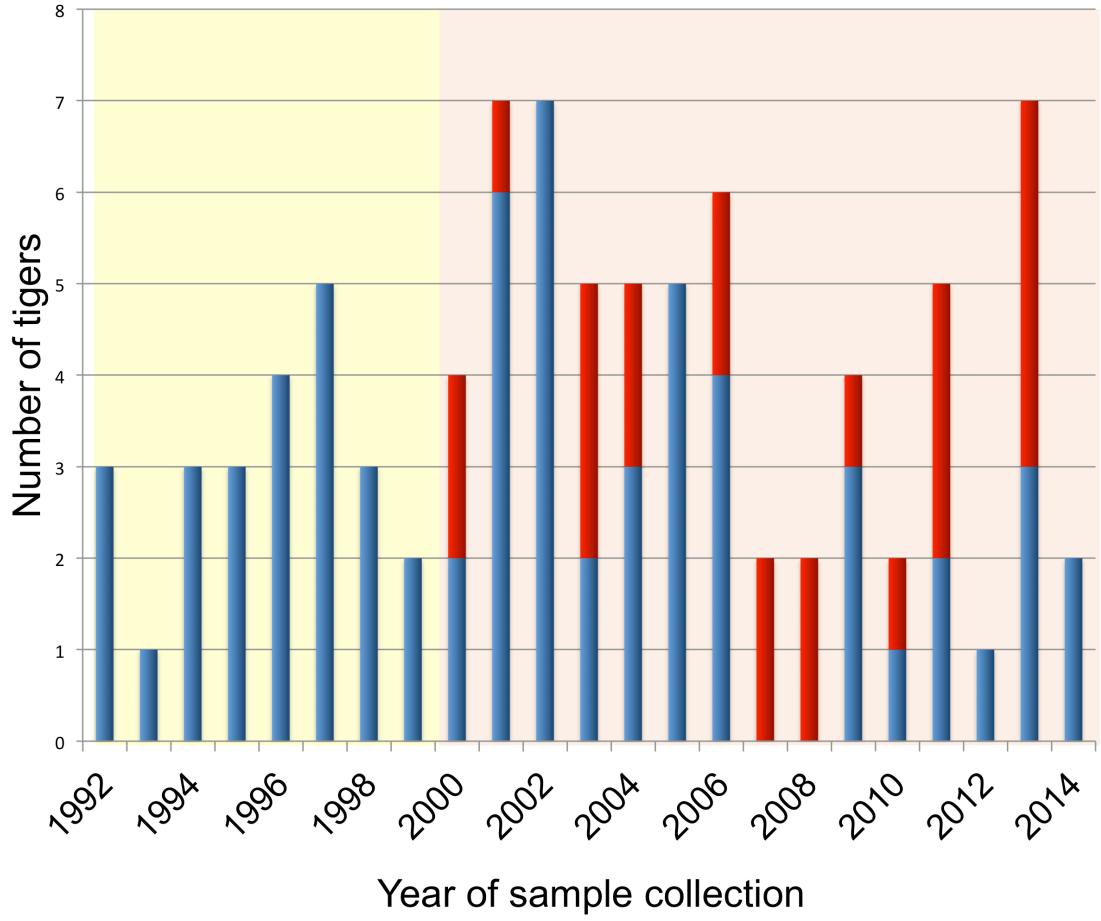

Figure 5.4. Temporal distribution of serum samples collected from Amur tigers in the Russian Far East between 1992 and 2014. Samples found to be negative for neutralizing antibodies to canine distemper virus (below a cut-off titre of 1:16) are represented in blue, and positive samples in red. Background shading indicates sampling periods before 2000 (yellow) and following 2000 (orange).

early 1990s. However, no further exposure was detected in any large carnivores in SABZ sampled between 1992 and 1999 (including 17 tigers, Appendix XX). Table 5.5 summarizes the serology status of 32 tigers sampled in SABZ between 2000 and 2011. Based on the age of tigers at the time of sampling, it is evident that CDV exposure occurred during 1996-2000 (PT40), 2001-2004 (PT63), 2006 (siblings PT79 and PT81), 2007-8 (siblings PT88 and PT89) and 2010 (PT56). The detection of antibodies in several older tigers could have occurred during a longer time period, and are therefore less informative. Notably, PT56, the mother of PT88 and PT89 appears to have been exposed later than her two dependent cubs (that were still in contact with her at the time of their sampling in May 2008), as she was infected at the time of her death in June 2010[4].

[4] The longest period of CDV infection recorded in a captive tiger was 16 months (Blythe et al. 1983), but this animal exhibited severe clinical disease that would not have been compatible with survival in the wild.

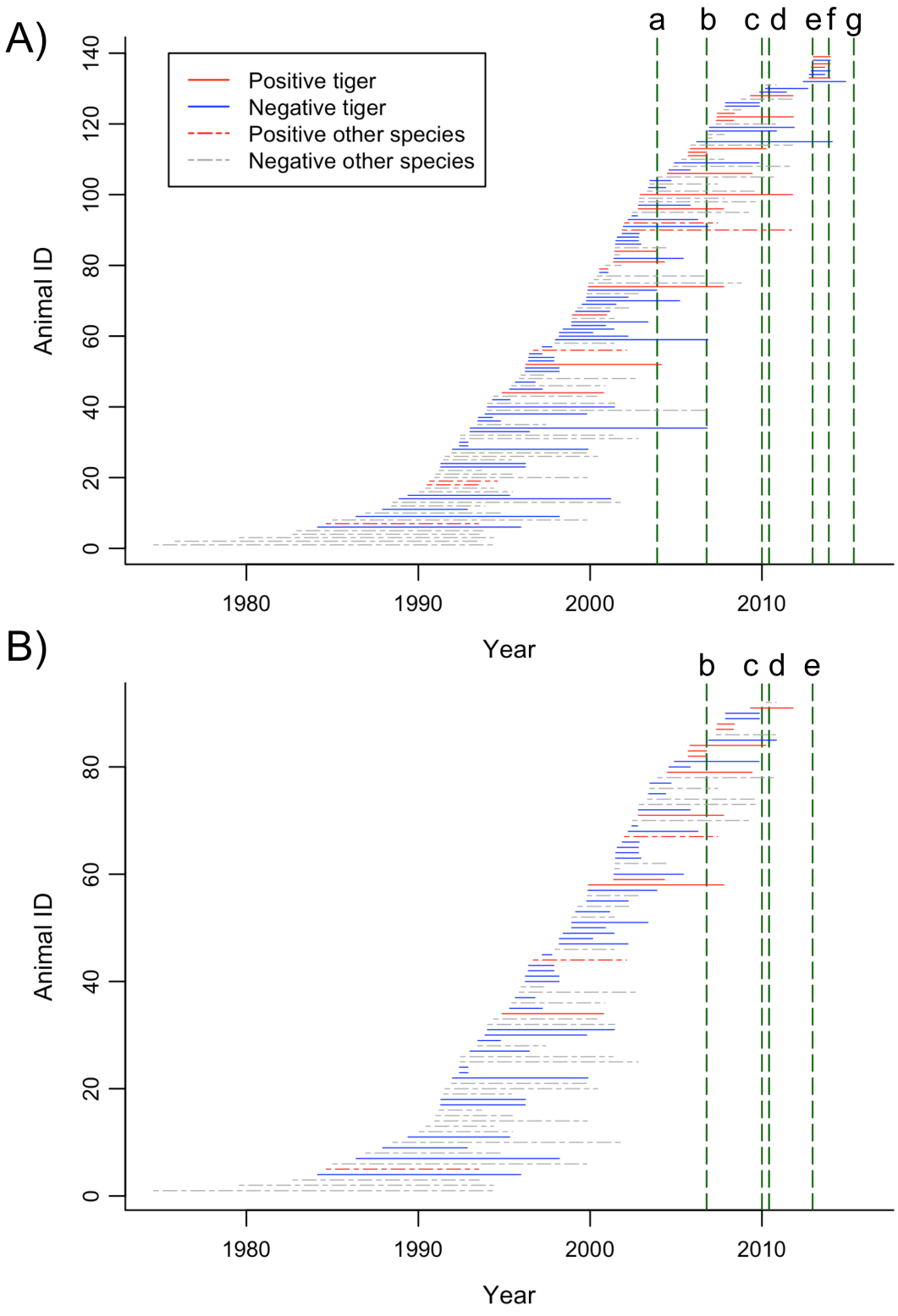

Figure 5.5. Temporal representation of canine distemper virus serology data for large carnivores sampled in A) the whole Russian Far East, and B) Sikhote-Alin Biosphere Zapovednik (SABZ) only. Each horizontal bar represents an individual animal, extending from birthdate (or the date of last sample collection), to the date on which sample was collected. Solid bars indicate tigers, dashed bars indicate other large carnivore species. Samples with a titre of 1:16 or greater are coloured red, negative samples are coloured blue (tigers) or grey (other species). Vertical green dashed lines indicate confirmed clinical cases: a) PT61 –Khabarovskii Krai, 2003, b) PT79 – SABZ, 2006, c) PT90 – SABZ, 2010, d) PT56 – SABZ, 2010, e) wolf – SABZ, 2013, f) tiger, Khasanskii, 2013, g) leopard – Khasanskii, 2015.

Table 5.5. A summary of serology results for all tigers sampled in Sikhote-Alin Biosphere Zapovednik from 2000-2011. Seropositive samples are highlighted in red.

Animal ID	Collection date	Age (months)	Titre	Notes
PT1	17-Mar-02	132	<8*	
PT20	26-Feb-00	168	<4	
PT35	20-Mar-02	108	<16*	
PT35	09-Apr-06	156	<16*	
PT37	20-Nov-03	144	<4	
PT40	15-Oct-00	72	128	Exposed during 1996-2000
PT41	22-Nov-00	24	<4	
PT47	18-Feb-01	24	<4	
PT49	22-May-01	36	<8*	
PT49	05-Jun-05	90	<4	
PT50	01-Jun-01	90	<4	
PT54	06-Oct-02	4	<4	
PT55	24-Oct-02	15	<4	
PT55	07-Oct-07	72	128	Exposed during 2002-7
PT56	24-Oct-02	16	<8*	
PT56	28-Oct-05	52	<4	
PT56	24-Mar-10	108	128	Confirmed with CDV June 2010
PT57	07-Nov-02	12	<4	
PT58	15-Dec-02	18	<4	
PT60	15-May-03	54	<4	
PT63	30-Apr-04	36	64	Exposed during 2001-04
PT64	24-May-04	12	<4	
PT67	16-Aug-04	1	<16*	
PT69	17-Sep-04	15	<8*	
PT74	11-Oct-05	1	<4	
PT75	28-Oct-05	15	<4	
PT79	13-Oct-06	13	128	Infected at time of sampling
PT81	13-Oct-06	13	256	Likely infected at time of sampling
PT85	14-Oct-07	96	32	Exposed during 1999-2007
PT88	03-May-08	12	32	Exposed during 2007-8
PT89	23-May-08	12	128	Exposed during 2007-8
PT94	04-Jun-09	60	64	Exposed during 2004-9
PT95	01-Nov-09	60	<16*	
PT96	07-Nov-09	24	<16*	
PT97	07-Nov-09	24	<16*	
PT100	05-Nov-10	48	<16*	
PT114	21-Oct-11	30	256	Exposed during 2010-11

* Denotes samples where cytotoxicity prevented an assessment of neutralization at low titres. Figure denotes the lowest titre well that could be assessed.

Outside SABZ, seropositive tiger cubs were detected in two locations, providing further information on the time and location of infections. During November 2012, three six month old cubs that were assumed to have been orphaned, were captured near the village of Andreevka, Yakovlevskii district (N44.56°, E133.52°). Samples from two of these three cubs were found to be seropositive (Borya and Kuzya, Appendix XX). During August 2013, two female cubs were captured near the village of Svetlogorie, Pozharskii district

(N46.86°, E134.46°). Although both cubs were assumed to have been orphaned, as neither was old enough to be independent, it is possible that they were not siblings, as one was estimated to be 11 months old at capture, and the other approximately nine months old. The younger of the two was found to be seropositive (PT125), while the other was seronegative (PT124, Appendix XX).

The period from which mesocarnivore samples were available was less extensive than for large carnivore species (Figure 5.6). Samples from Southwest Primorskii were primarily collected during 2007 and 2008, of which eight of 46 were found to be seropositive. Samples were collected during two periods in Lazovskii. During 2008 and 2009, 13 mesocarnivores were sampled, of which all were negative (CI: 0.0-28.3%). However, during 2013, five of 31 animals were found to be seropositive (16.1%, CI: 6.1-34.5%). Of ten samples collected in SABZ during 2014, three were positive (33.3%, CI: 8.1-64.6%), while single samples collected during 2005 and 2011 were both negative. Full details of the species sampled at each location are provided in Appendix XXI.

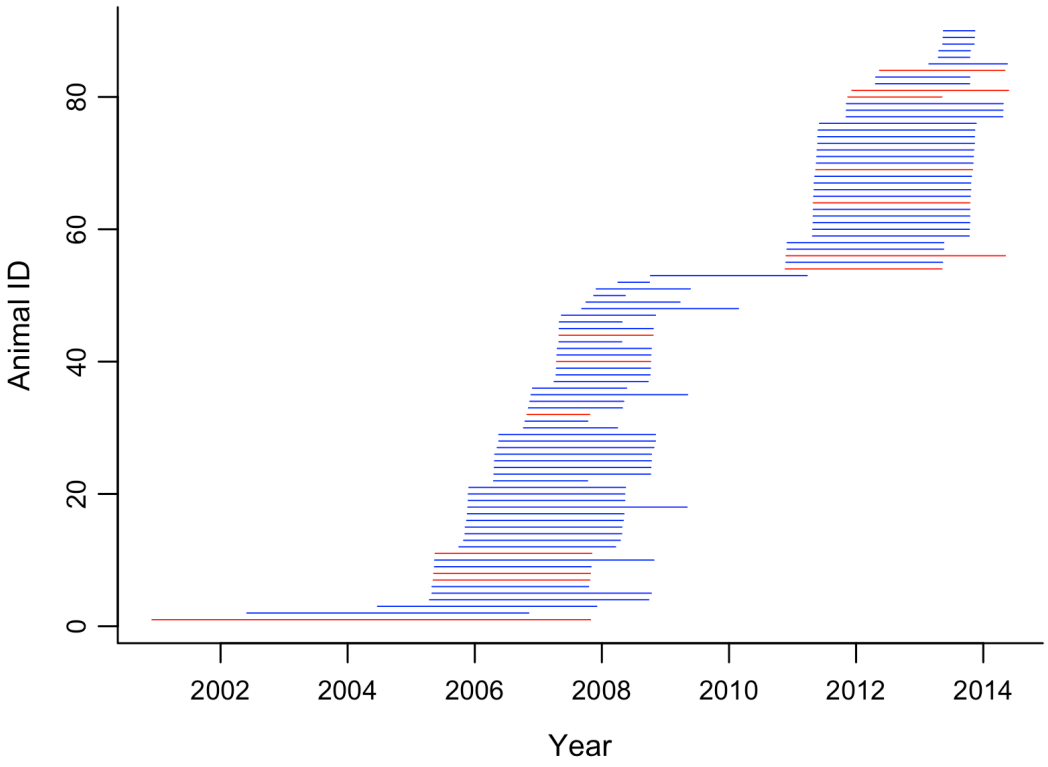

Figure 5.6. Temporal representation of canine distemper virus serology data for mesocarnivores sampled between 2006 and 2014. Each horizontal bar represents an individual animal, extending from birthdate to the date on which sample was collected. Samples with a titre of 1:16 or greater are coloured red, negative samples are coloured blue.

Samples were collected from 616 domestic dogs, of which owners reported that 464 had not been vaccinated against CDV at any time in their lives (Appendices XXIII and XXIV). A total of 182 dogs were sampled in Southwest Primorskii (in 28 settlements), 166 in Lazovskii (in seven settlements), and 113 in SABZ (in three settlements). Seroprevalence was lowest in Southwest Primorskii, where dog densities were highest, and were highest in SABZ where dog densities were lowest (Table 5.6). These differences were found to be significant (chi-square = 23.918, $p<0.01$, df = 2). Overall 27.2% of unvaccinated dogs were found to be seropositive (CI: 23.2 - 31.5%), and 8.6% (CI: 6.3 - 11.6%) had titres considered protective.

Table 5.6. Results of virus neutralization analyses against canine distemper virus (CDV) for serum samples collected from unvaccinated dogs in the study sites Southwest Primorskii, Lazovskii and Sikhote-Alin Biosphere Zapovednik (SABZ). Neutralizing antibody titres of 1:16 or higher were considered positive. Seroprevalence is given as the number of positive samples expressed as a percentage of sample size, with lower and upper 95% binomial confidence intervals (CI). Samples were tested in the University of Glasgow using CDV Bussell strain.

Study area	Positive	Sample size	Seroprev. (%)	Lower CI (%)	Upper CI (%)
Southwest Primorye	29	182	15.9	11.1	22.3
Lazovskii	49	166	29.5	22.8	37.2
SABZ	48	116	41.4	32.4	50.9

The detection of antibodies in young dogs that exceed the age at which maternal antibodies have waned (approximately four months), provide a valuable indicator of recent outbreaks. Of the young dogs between the ages of four and twelve months, 20.2% (CI: 13.6 - 28.7%) were found to have antibodies of 1:16 or higher. Antibodies were detected in young unvaccinated dogs in all three study areas, indicative of widespread infections (Figure 5.7). Young dogs were sampled in 21 settlements, of which 8 showed evidence of recent exposure. However, low sample sizes (fewer than five dogs) may have reduced detectability in 13 of the settlements where no evidence of recent exposure was detected. Recent exposure were found in fewer settlements in Southwest Primorskii (2/12), than Lazovskii (3/6), with recent exposure found in all three settlements sampled in SABZ. Furthermore, the village of Ternei in SABZ was sampled during both 2012 and 2014, and evidence of recent exposure was found during both visits.

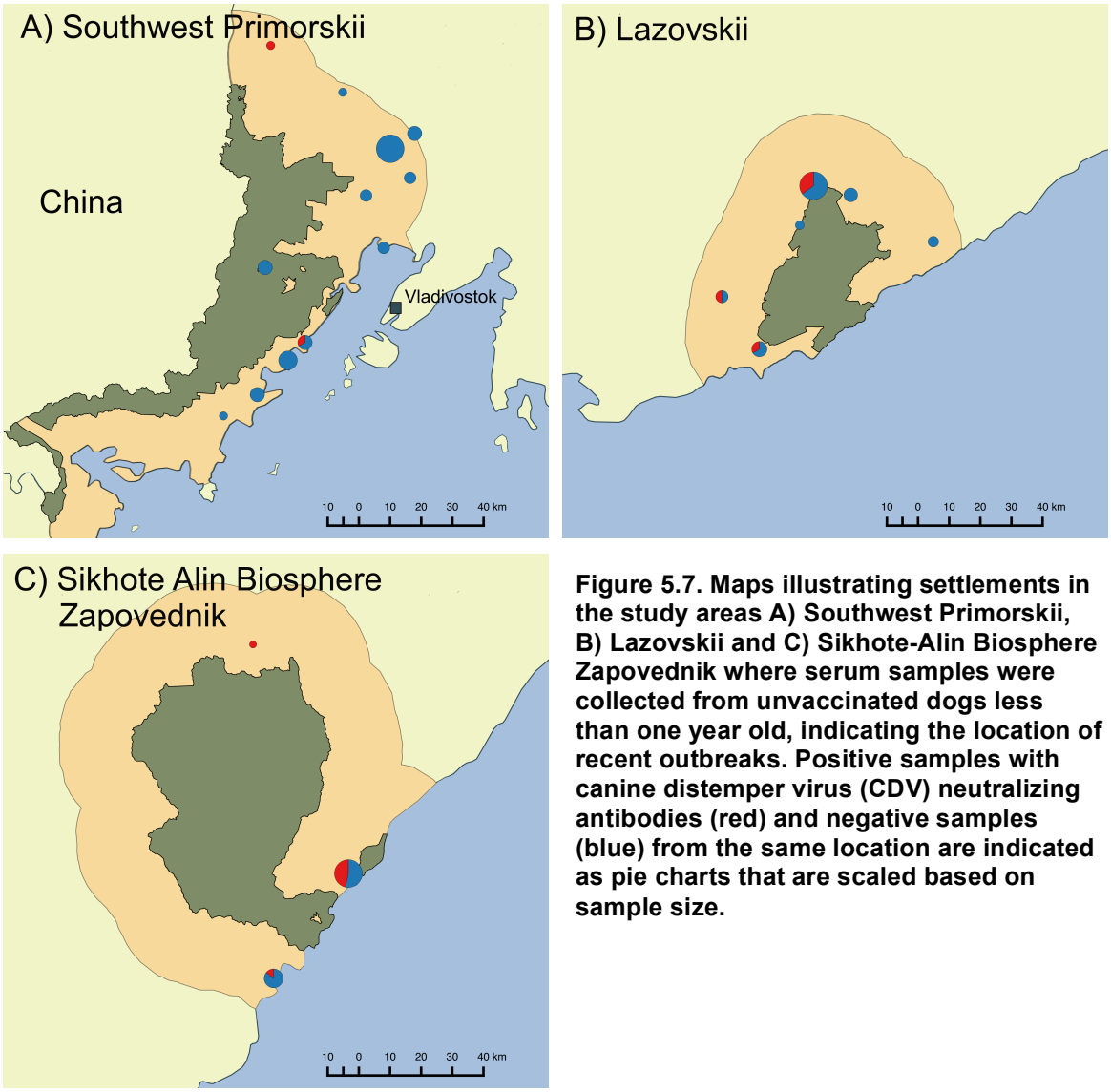

Figure 5.7. Maps illustrating settlements in the study areas A) Southwest Primorskii, B) Lazovskii and C) Sikhote-Alin Biosphere Zapovednik where serum samples were collected from unvaccinated dogs less than one year old, indicating the location of recent outbreaks. Positive samples with canine distemper virus (CDV) neutralizing antibodies (red) and negative samples (blue) from the same location are indicated as pie charts that are scaled based on sample size.

Overall the seroprevalence of dogs that visited the forest was 34.2% (CI: 26.8 to 42.4%, n=152), compared to 25.0% (CI: 19.4 to 31.6%, n=208) in dogs that did not. This difference was not found to be significant at the 95% confidence level (chi-squared = 3.1922, df = 1, p = 0.074). Similarly, forest visits approached significance as a predictor of exposure in the univariate generalized binary logistic regression model (odds ratio 1.64, CI: 0.95-2.84, p= 0.07). The univariate model for 'community size' failed to converge, and so the categories 'town' and 'large town' were combined (as the latter were represented by only six dogs). The final multivariate model (Appendix XXVII) indicated that exposure was greater for unvaccinated dogs from SABZ (odds ratio 8.73, CI: 2.84-32.63, $p < 0.01$), and Lazovskii (odds ratio 3.76, CI: 1.44-10.51, $p< 0.01$) in comparison to Southwest Primorskii, and increased with the age (in months) of dogs sampled (odds ratio 1.01, CI: 1.00-1.02, $p< 0.01$).

A total of 152 samples were collected from dogs whose owners reported that they had been vaccinated, of which only 62 (40.8%, CI:30.0–49.1%) were found to be seropositive, and only 32 (21.1%, CI:15.0–28.6%) were considered protective.

Estimates of the force of infection λ were 0.114 (±0.019) cases per dog per year with a low CVD mortality of 0.2, versus 0.142 (±0.020) cases per dog per year with a high CDV mortality of 0.4. Assuming a mean infectious period of 21 days (Greene and Appel 2006), this equates to a mean prevalence of 0.54% to 0.68%. Estimates for R_e were 1.39 (±0.068) with a low mortality of 0.2, and 1.48 (±0.063) with a high mortality of 0.4.

Discussion

The findings of this study confirm that CDV exposure is widespread in Amur tigers, with infections occurring across much of their current range in the Russian Far East. Although no antibodies were detected in tigers until after 2000, seropositive cases in other large carnivores in two sites during the early 1990s confirm that CDV has been present in the region for some time, and is a basis for rejecting the hypothesis that a regional absence of CDV could explain the lack of antibodies in tigers sampled prior to 2000. However, the exposure of young tigers in the intensively monitored population in SABZ indicate that infections have occurred regularly since 2000, with at least five discrete exposure events occurring in the years since. Although clinically affected tigers have primarily been detected close to human population centers, along transport corridors, and in well-monitored SABZ, additional cases may be going undetected in remote areas, where human activity is limited. For instance, CDV antibodies were detected in one tiger (PT43), in sparsely populated Krasnoarmeyskii district in northern Primorskii (Figure 5.3 Appendix XX), where detection of mortality would be unlikely. While the case fatality ratio of infected tigers is unknown, levels of infection, and mortality are likely to be greater than has been indicated by the handful of confirmed clinical cases, augmenting calls to consider management interventions.

The high seroprevalence found in tigers sampled after 2000 suggests that a proportion of tigers are surviving CDV infection. Of the 20 seropositive tigers, active CDV infections were only confirmed in three tigresses (PT61, PT79 and PT56, Chapters 4 and 5, Table 5.5 and Appendix II), with the remaining animals either representing undiagnosed cases, or

(more likely) those that had survived prior infection. While this indicates that mortality of CDV infected tigers falls below 100%, the actual mortality rate is open to speculation, as tigers which die from infection may be under-represented in the sample set. However, the scale of this unseen mortality cannot be determined from the examination of seroprevalence data alone. Antibodies to CDV remain detectable for extended periods, and may last for the remainder of the animal's life (Greene and Appel 2006). Therefore, a high seroprevalence could be achieved despite a high mortality rate, if the rate of exposure were also high. Conversely high seroprevalence could also occur in long-lived animals like tigers where exposures were infrequent, but mortality rate was very low. The implications that these mortality rate uncertainties may have on population extinction have been examined using a population viability model (Gilbert et al. 2014), with the outcome being relatively insensitive to changes in mortality rate. For instance, according to one infection scenario, in which mortality rate was increased from 30% to 50%, while all other factors remained constant, resulted in a comparatively minor increase in fifty year extinction probability of 34.0% to 38.5%.

The intensively monitored population of tigers in SABZ provides an opportunity to examine the evidence for tiger-to-tiger transmission. The availability of samples from three female tigers, occupying neighbouring territories in SABZ represent the best example of this (Table 5.5). The older female, PT35 held a territory bordering Lake Blagodatnoye from approximately 1995, and gave birth to a daughter, PT56 in 2001 to which she succeeded a portion of her territory (Figure 5.8). A neighbouring tigress, PT55 was born in a territory immediately to the northwest in 2001, which she occupied until 2009. Serum collected from PT35 in 1999, 2002 and April 2006, was negative for CDV antibodies on each occasion. However, in October 2006 samples collected from two of her 13 month old cubs, PT79 and PT81 were both seropositive (no samples were collected from the third cub, PT80). This finding, together with the detection of a CDV sequence in a blood clot collected from PT79 (Chapter 4), confirms exposure (at least of two of the cubs) during 2006. During 2007, PT35 disappeared (although at approximately 14 years of age, this may have been due to senescence), and her three cubs were thought to have been killed by poachers (with last records of PT79 in February 2007, PT80 in November 2007 and PT81 in February 2008). In the neighbouring territory, two 12 month old cubs belonging to PT56 (PT88 and PT89) were both found to have antibodies to CDV in May 2008. Fewer samples are available from the third tigress, PT55 but it is evident that she was exposed to CDV sometime between 2002 and October 2007 (Table 5.5).

Clearly these results indicate a period during which tigers were exposed to CDV in SABZ between 2006 and 2008. However, it is also worth noting that several of the tigers in this area do not appear to have been exposed at that time. These include PT90, the territorial male tiger whose range overlapped that of PT35, PT55 and PT56. Although no samples were available from this tiger, it is unlikely that he was infected earlier than 2009, as CDV sequences were detected in his tissues at the time of his death on 1 January 2010, when he was shot after killing a fisherman (Chapter 4, and Appendix I). Likewise, PT56 was found to be seronegative in 2002 and 2005, but was seropositive in March 2010, and infection was confirmed at the time of her death on 1 June 2010 (Chapters 4, Appendix I and Seimon et al. 2013). Radiotelemetry locations indicate close proximity between PT90 and PT56 during December 2009 (D. G. Miquelle pers. comm. 2012), and with her litter born during May 2010, it is likely that PT56 was infected by PT90 during a mating encounter in the last days of his life (Appendix I). It is unclear why PT56 did not contract the virus from her cubs during 2007 and/or 2008 (considering that the 12 month old cubs were seropositive when sampled in May 2008). A possible interpretation is that CDV is not

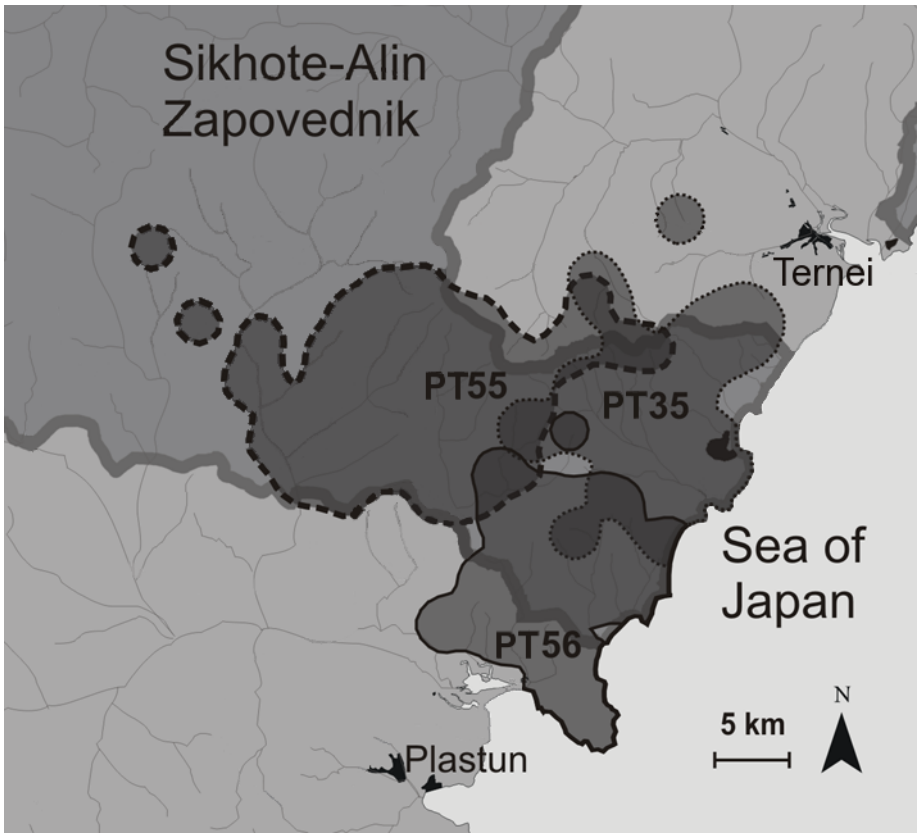

Figure 5.8. The home ranges of tigresses PT35, PT55 and PT56 based on 95% fixed kernel contours using telemetry locations collected during 2004-6. Adapted from Goodrich et al. 2010.

readily transmitted between tigers, and PT56 avoided contracting the virus from her dependent cubs. An alternative explanation is that the course of infection could be substantially longer in tigers than previously assumed, with animals infected for periods of up to three years before succumbing to infection (as in the case of PT56). This latter explanation cannot be excluded from the Russian data, but is unlikely based on the reports of CDV infection in captive tigers which indicate a course of infection lasting from a few days to several months (Gould and Fenner 1983, Appel et al. 1994, Zenker et al. 2001, Konjević et al. 2011, Nagao et al. 2012). The only exception in the literature was a case involving a captive Bengal tiger (*P. tigris tigris*) that exhibited severe and progressive neurological signs for a period of 16 months, however the severity of disease in this tigress would not have been compatible with survival in the wild (Blythe et al. 1983).

This sequence of exposures in this well studied group of tigers raises a number of observations and questions that have important implications for the epidemiology of CDV in the species. Analysis of telemetry data from PT35 and PT56 indicate that on no occasion during 2006 and 2007 were these tigers within 1 km of each other (J. M. Goodrich pers. comm. 2016), suggesting that the exposure of their respective litters of dependent cubs were unlikely to have been contracted from each other. Assuming that PT56 was not infected for the unprecedented period of three years before her death, then she did not contract the virus from her cubs, despite prolonged periods of close contact. Likewise, the infection of PT90 was unlikely to have occurred before 2009, suggesting that he did not contract infection from any of the breeding females within his territory. It would then follow that at least four separate inter-specific transmission events were involved in the exposure of these tigers (i.e. 1. PT79/PT80/PT81; 2. PT88/PT89; 3. PT55; 4. PT90/PT56). Furthermore, intra-specific transmission between tigers is not inevitable, even when there is prolonged contact such as that between a mother and dependent cubs (i.e. PT56 and her cubs PT88 and PT89). Taken together, this suggests that transmission from other species may occur fairly regularly, and may be comparatively more important than tiger-to-tiger transmission for facilitating exposure across a population. This is despite the suggestion based on PT56 that infectious period may be longer in some tiger cases than is typical in other species (e.g. up to 60-90 days in dogs, Greene and Appel 2006; and 9-32 days in ferrets, Ludlow et al. 2012). Long infectious periods could contribute to intra-specific transmission, in a species where contacts are infrequent, but may only have been important in the tiger-to-tiger transmission that occurred between PT90 and PT56.

The identification of reservoir populations has important implications for management decisions, and the findings of this study support the involvement of wildlife in CDV maintenance. The phylogenetic analysis of CDV sequences obtained from mesocarnivores clustered together with those obtained from tigers and other large carnivores, supporting their possible role as sources of infection (Chapter 4). Mesocarnivore sequences from four geographic locations were also well distributed across the Russian wildlife lineage, suggesting long chains of transmission that would be expected in a maintenance population (Chapter 4, Viana et al. 2014). Serological findings add to this picture, with exposure detected among archived samples from Southwest Primorskii collected during 2007-8, and among samples collected during the present study in Lazovskii and SABZ in 2013 and 2014. Furthermore, CDV sequences were detected in wildlife in the vicinity of SABZ in all three seasons from which fur trapper samples were collected (Chapter 4). This contrasts with the situation in other parts of the world, where CDV infections appear to occur in cyclical waves, with epizootics occurring during discrete periods of time, interspersed by several years during which the virus falls below readily detectable levels (Roscoe 1993, Alexander and Appel 1994, Williams 2001). Long term monitoring would be required to confirm temporal trends, but current data suggests that Russian forests support sufficient numbers of susceptible wildlife to maintain CDV at detectable levels over a period of at least three years.

Molecular data (Chapter 4) and serological data presented here suggest that several wildlife species such as sable and raccoon dogs may be involved in CDV transmission, while the role of others is less certain. While the samples obtained from fur trappers were biased toward sable, the number of viruses detected from this species suggests an important role in the circulation of CDV (Chapter 4). Unfortunately, the live capture of sable in cage traps is difficult, and therefore very few serum samples were available to investigate patterns of exposure in this species further. Among other mesocarnivore species, seroprevalence was highest among raccoon dogs, with seropositive individuals detected in all three study areas. Similar seroprevalence levels have also been found in this species in Japan (13.2%, n=317, Suzuki et al.2015, and 20.1%, n=19, Kameo et al.2012), and the Republic of Korea (44.1%, n=102, Cha et al.2012), with a further Korean study finding a markedly higher seroprevalence (89.4%, n=94, Yang et al.2013). It is unclear why there should be higher seroprevalence found in the Korean studies, than neighbouring countries. The two studies measured seroprevalence in different locations (Chonbuk province in Cha et al.2012, and the provinces of Gyeonggi-do and Gangwon-do in Yang et al.2013), and the disparity in

seroprevalence may have reflected variation in raccoon dog density, or other factors such as the age distribution of animals surveyed. High rates of turnover in other raccoon dog populations may favour the maintenance of pathogens, with between 68.4% and 77.1% of raccoon dogs in Japan and Finland found to be less than a year old (Obara 1983, Helle and Kauhala 1993). If the age structure of raccoon dogs in Primorskii were similar, then annual production of immunologically naïve juveniles would contribute to the maintenance of CDV in wild mesocarnivores.

The relatively low seroprevalence detected in Asian badgers is difficult to interpret. Only two of 43 badgers were considered positive, but inconclusive titres in a further five could suggest a higher level of exposure (e.g. if CDV infection elicited a lower humoral response in badgers than other species, which is currently unknown and requires further investigation). Even discounting inconclusive titres, a low seroprevalence need not indicate a lack of exposure, if case fatality of badgers were higher than in other species. The detection of CDV sequences in a dead badger from Southwest Primorskii (Chapter 4) confirmed that at least some animals succumb to infection. Infections with CDV are common in the closely related Eurasian badger *M. meles* in Europe (Van Moll et al. 1995, Monne et al. 2011, Nouvellet et al. 2013), although most reports refer to mortalities occurring during known outbreaks. Serological surveys conducted in Japan found 8.7% of closely related Japanese badgers *M. anakuma* carried antibodies to CDV (CI: 1.2 - 28.0%, n=23, Suzuki et al.2015), which is roughly comparable to the 4.7% (CI: 0.4-16.3%, n=43) measured during this study. While the role of Asian badgers in the circulation of CDV in Primorskii remains unclear, it is possible that their contribution may be greater than suggested by serological findings alone. This highlights the importance of combining multiple sources of data (such as the molecular findings from Chapter 4, and the serological data reported here), as well as the need for further research.

One common species that is notably under-represented in both molecular and serological surveys was the red fox. This species has been widely sampled in Europe, with seroprevalence measured between 4.4% and 18.7% (Amundson and Yuill 1981, Truyen et al. 1998, Sobrino et al. 2008, Akerstedt et al. 2010), and virus detected in several countries (Monne et al. 2011, Denzin et al. 2012, Trebbien et al. 2014). Techniques used in the present study are unsuitable for the capture of foxes, and further work would be required to assess their role in the epidemiology of CDV in Primorskii. In other parts of the world, red

foxes have adapted to urban environments, potentially increasing the opportunity for contact and CDV transmission with domestic dogs. Similar increases in the zoonotic transmission of the tapeworm *Echinococcus multilocularis* have been proposed as foxes become more urbanized, however there is currently little evidence to support this (Deplazes et al. 2004, Bradley et al. 2007). However, the commercial value of red fox pelts in Russia has limited their encroachment on populated areas in the Primorskii, with raccoon dogs being a more familiar inhabitant on the periphery of human settlements.

The pattern of exposure recorded in unvaccinated domestic dogs was unexpected, with progressively higher seroprevalence found in more remote and less populated study areas. This contrasts with the epidemiology of the closely related measles virus, which is maintained in large urban centers, where human host populations exceed a critical community size sufficient to maintain the pathogen, and give rise to waves of sporadic outbreaks that spread outward through smaller more remote communities (Bartlett 1957, Grenfell et al. 2001). This pattern of infections would lead to high seroprevalence in dense urban populations, with exposure less likely in more remote and less populated areas. Serological surveys of dogs along an urban-rural gradient in two areas of Chile have followed this pattern, with CDV seroprevalence in urban areas of 61-76% comparing to 34-47% in rural communities (Acosta-Jamett et al. 2011, 2015). In the present study, seroprevalence was lowest among dogs in Southwest Primorskii, located adjacent to large urban centers and supporting more dogs than Lazovskii and SABZ. Furthermore, recent outbreaks (based on the presence of antibodies in unvaccinated dogs aged 4-12 months) were evident in all communities and study periods in SABZ, compared to just 2/12 communities surveyed in Southwest Primorskii, suggesting a greater frequency of outbreaks in remote areas.

One factor that might explain this pattern of exposure might be a disparity in veterinary care. If fewer dogs in remote areas were vaccinated against CDV, then outbreaks might be larger, resulting in higher seroprevalence among survivors. This pattern is indeed evident, with 39.3% of dogs in Southwest Primorskii receiving a CDV vaccination at least once in their lives, compared to 23.9% in Lazovskii and 26.4% in SABZ (Chapter 3). However, the serological survey also raised questions about the effectiveness of vaccination in the settlements visited, with only 40.8% of vaccinated dogs found to be seropositive (n=152), and only 21.1% with presumed protective titres. Other studies have reported antibody titres

of 1:16 or higher in 60-92% of vaccinated dogs, suggesting that levels of protection are unusually low in Primorskii (Olson et al. 1988, 1996, 1997, McCaw et al. 1998, Bohm et al. 2004, Ottiger et al. 2006, Schoder et al. 2006). Given that vaccination programmes aim to achieve a coverage of $1-1/R_0$, in order to keep the proportion of susceptible animals below the threshold that allows a pathogen to proliferate, it is possible to estimate the adequacy of existing vaccine provision in the study areas. Even if titres of 1:16 were to be considered protective, this level of vaccination and vaccine response would only be sufficient to control CDV if R_e were between 1.11 in Lazovskii and 1.19 in Southwest Primorskii. Estimates of R_e based on age-matched seroprevalence in this study were between 1.39 (±0.068) and 1.48 (±0.063), suggesting that vaccination in each of the study areas would be insufficient to control CDV.

An alternative explanation for the high seroprevalence found in dogs in remote areas might be a greater likelihood of exposure through contact with wildlife. One of the highest seroprevalence levels was recorded in the small village of Taejnoye in SABZ, located approximately three hours by road from the town of Ternei, and the most remote settlement surveyed. This forest community contained just 32 dogs in 2014, none of which were reported to visit other settlements (Chapter 3). Maintenance of CDV is not possible in such a small population, and opportunities for introduction from larger population centers are limited. However, four of five unvaccinated dogs that were sampled in the settlement were found to be seropositive, including one young dog of 12 months, which had been born in the village. One distinctive feature of Taejnoye was the high proportion of dogs that were taken to the forest (reported for 18 of 30 dogs for which owners provided information). Based on ownership surveys (Chapter 3), the proportion of dogs with forest access was greater in SABZ (54.6%,), than Lazovskii (40.4%) and Southwest Primorskii (37.1%). Seroprevalence of dogs with access to the forest (34.2%) was greater than those without (25.0%). This difference was not found to be significant at a 95% confidence level ($p = 0.074$), but only by a small margin, and may have been confirmed with a larger sample size. Also it is likely that dogs that contract CDV from wildlife in the forest could act as a source of infection for dogs that do not leave the confines of human settlements. Of the four seropositive dogs detected in Taeojnoye, three were reported to visit the forest, and the fourth shared a house with a dog that was reported to do so (although no samples were collected from this animal).

Although contact with wildlife may increase the exposure of domestic dogs, the detection of Asia 4 clade viruses in Vladivostok in 2016 suggest the circulation of CDV independent from wildlife (Chapter 4). These Asia 4 clade viruses were distantly related to those found in wildlife, and were likely to have been imported from tropical Asia (Chapter 4). While the Arctic-like clade found in wildlife, was not detected in domestic dogs, patterns of seroprevalence suggest that some dogs are exposed to these viruses through contact with wildlife, particularly in more remote rural settlements such as those in SABZ. The low levels of seroprevalence detected in dogs in the more densely populated study area of Southwest Primorskii, adjacent to large urban centers could be due to the circulation of dog-specific strains that are distinct from those found in wildlife, transmission of Arctic-like viruses from wildlife sources, or a combination of the two. Based on current data, it is not possible to determine whether dogs are contributing to the maintenance of multiple CDV strains in Primorskii, or whether exposure relates to introduction from other species, or regions.

While CDV infections have become particularly evident among tigers, they are not the only large carnivore species to have been exposed to the virus. The finding of two seropositive Far Eastern leopards is of particular importance, given the ‘Critically Endangered’ status of the subspecies, and the fatal infection recorded during 2015 (Chapter 4). The only previous indication of CDV exposure in a wild leopard involves a single seropositive animal from Kenya (Kock et al. 1998), although several fatal infections have been recorded during two separate outbreaks in captivity (Appel et al. 1994). With the entire population of Far Eastern leopards limited to fewer than 60 individuals in a single isolated subpopulation (Stein et al. 2016), CDV could represent an important threat to the survival of the subspecies. This adds to the justification for establishing one or more insurance populations of leopards in other locations (Goncharuk et al. 2015), and heightens the need to manage CDV in the Southwest Primorskii region itself.

Conclusion

This study has confirmed that exposure of tigers to CDV is widespread, and has increased since 2000. The presence of CDV antibodies in wildlife in 1993-4 indicates that recent tiger infections are not purely related to an expansion of CDV distribution. Exposure of mesocarnivores in all study areas augments phylogenetic evidence showing closely related viruses in tigers and other wild carnivores, and supports the case for a wildlife reservoir of infection. Growth of mesocarnivore populations, and possibly elevated hunting pressure might explain the observed increase in tiger exposure. The role of dogs in CDV maintenance remains unresolved. Exposure in all study areas confirmed that domestic infections are common, but higher seroprevalence in remote areas may suggest transmission from wildlife. The apparent prominence of wildlife in CDV maintenance indicates that management strategies directed at domestic dogs would be unsuccessful in preventing infection of tigers. Given the difficulty of controlling CDV in a wildlife reservoir, management strategies that focus on tiger populations may have greatest chance of success.

Author contribution

The author conceived and developed the overall study design, and secured primary sources of funding for the project. The author also obtained ethical approval for the study from the Institutional Care and Use Committee of the Wildlife Conservation Society (WCS, Appendix III). Permission to collect samples from dogs was secured from the State Veterinary Inspection by the author and colleagues with this Institute of Biology and Soil Sciences (IBSS, Appendix IV). Permission to collect samples from wildlife was secured from the Ministry of Natural Resources and Environment by WCS. Archived samples were collected by WCS, IBSS, National Cancer Institute (NCI) and the Zoological Society of London (ZSL). Additional contemporary wildlife samples were collected by the author, and veterinarians with ZSL and IBSS. Veterinary and Endangered Species permits to enable the export of samples were obtained by the author, with the assistance of staff with IBSS, ZSL, WCS and the University of Glasgow (UoG). Virus neutralization analyses were performed by the Veterinary Services Laboratory at UoG, and by the Washington Animal Disease Diagnostics Laboratory at Washington State University. The author performed all data entry, analysis and interpretation of results.

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Chapter 6 A review of potential strategies for managing canine distemper virus as a threat to Amur tiger populations in the Russian Far East

When measured against the declines that have driven global tiger distribution to just 7% of its historic range, the population of Amur tigers (*Panthera tigris altaica*) in the Russian Far East has fared comparatively well (Sanderson et al. 2006, Miquelle et al. 2010b). After surviving a bottleneck during the 1940s, where human pressures reduced numbers of Amur tigers to as few as 20-30 individuals, the population climbed steadily to 331 to 393 adult and subadult tigers by 2005 (Miquelle et al. 2007, 2010b). However, recent surveys indicate that numbers have plateaued, and may now be in decline once again (Miquelle et al. 2010a). In the face of these conditions, it is essential that wildlife managers are equipped with sufficient information to prioritize conservation measures accordingly.

While poaching and human-tiger conflict remain the primary threats facing Amur tigers (Goodrich et al. 2008), recent cases of canine distemper virus (CDV) represent a new and additive cause of mortality (Quigley et al. 2010, Seimon et al. 2013, Robinson et al. 2015). Simulations that include CDV epidemiology in a population viability analysis have shown that even conservative levels of CDV infection increases the potential for tigers to decline to extinction, an effect that disproportionally affects small and isolated populations (Gilbert et al. 2014). The objective of this PhD was to generate data on the epidemiology of CDV in the Russian Far East, to inform management recommendations appropriate to local conditions. This chapter will review the findings of this work, and use the salient features of host demography, CDV exposure and virus phylogeny, to prioritize mitigation strategies that could address the impact of CDV on the Amur tiger population.

Synthesis of study findings

Canine distemper virus infections in tigers in Primorskii Krai

Based on the results of serological and molecular based surveys it is evident that there has been widespread exposure of Amur tigers to CDV since the year 2000 (Chapters 4 and 5). Prior to this study CDV infections had been confirmed in one tigress in 2003 and two more

individuals in 2010 (Quigley et al. 2010, Seimon et al. 2013), with antibodies detected in six tigers (including the clinically affected tigress from 2003, Goodrich et al. 2012). The results of molecular analyses described in Chapter 4 identified CDV infection in a further three tigers (in 2006, 2010 and 2013), and detected the first case of infection in a Far Eastern leopard (*P. pardus orientalis*) in 2015. Serological surveys described in Chapter 5 detected antibodies in a further 14 tigers between 2000 and 2014, equating to an overall seroprevalence of 35.7% recorded since 2000 (CI: 23.7-49.7%, n=56). Exposure has occurred regularly in the intensively monitored population in the Sikhote-Alin Biosphere Zapovednik, with the detection of infected tigers (Chapter 4), and seropositive young tigers (Chapter 5) indicating that transmissions have occurred during at least five discrete periods between the late 1990s and 2011. Although cases of infection have only been recorded in tigers near human population centers, or well-monitored areas, seropositive tigers in remote locations suggest that further undetected mortality is likely (Chapter 5).

The lack of antibodies to CDV detected in the 19 tigers sampled between 1992 and 1999 (CI: 0.0-20.9%), could reflect a type II sampling error (where the presence of exposure in the wider tiger population was not detected due to a relatively small sample size), or indicate that tigers were not exposed to the virus during that period. The significant increase in exposure following 2000 could be explained by the acquisition of adaptive mutations to the CDV genome that enhance transmissibility to tigers, or environmental factors, such as changes in viral distribution, or increased transmission from other host species. The detection of antibodies in two Far Eastern leopards and a brown bear (*Ursus arctos*) in two locations in 1993-4 indicated the presence of CDV in areas occupied by tigers prior to 2000, confirming that any increase in tiger exposure was not due to an expansion of viral distribution (Chapter 5). Several mutations in the CDV genome have been proposed as promoting infection in non-canid hosts (Nikolin et al. 2012), but none of these were detected in the CDV sequences obtained from tigers in this study. However, the existence of other as yet unidentified adaptive mutations cannot be discounted. The potential for an increase in transmission from other host species will be discussed in more detail below, in the sections titled ‘CDV infections in domestic dogs in Primorskii Krai’ and ‘CDV infections in mesocarnivores in Primorskii Krai’.

The presence of antibodies in approximately one third of the tigers sampled after 2000 indicates that tigers are exposed to CDV on a regular basis, and also that a proportion are

surviving infection. Of the 20 seropositive tigers, three were found to be infected at the time of sampling (PT61, PT79 and PT56, Chapters 4 and 5, Table 5.5 and Appendix II), with the virus implicated in the death of at least two of these animals (PT61 and PT56, Chapter 4). The remaining seropositive tigers showed no signs of sickness, and many of them survived for years following sample collection. Published results from outbreaks in captive tigers are insufficient to estimate levels of morbidity and mortality, but it is clear that a proportion of clinically affected tigers in some of these outbreaks have survived infection (Appel et al. 1994, Zenker et al. 2001, Ngao et al. 2001). Comparable levels of exposure have been recorded in African lions, without apparent sickness or increased mortality (Munson et al. 2008, Alexander et al. 2010). In dogs, 25% to 75% of CDV infections are thought to be subclinical, and mortality of up to 50% has been estimated for dogs that develop disease (Appel 1987, Greene and Appel 2006). While the actual mortality rate among tigers in Primorskii remains unknown, model simulations indicated that this uncertainty should have little impact on the extinction probability of affected populations (Gilbert et al. 2014).

The diagnosis of CDV in more subtle cases during the present study, including animals involved in human-tiger conflict also suggests that infections may be under-reported. The introduction of CDV testing as part of a routine health screen whenever tigers are handled would therefore provide further information on CDV infections, and a means of assessing the role of the virus in human-tiger conflict. Additional factors may also influence the outcome of infections. During two outbreaks among lions in Tanzania, co-infection with *Babesia* spp. was proposed as a contributor to high mortality (Munson et al. 2008). No signs of coinfections were noted during review of histological samples from four of the Primorskii tigers where CDV infection has now been confirmed (Seimon et al. 2012, Chapter 4). However, only a limited set of tissues were available in several of these cases, and so the potential for co-infection with other pathogens cannot be excluded. Mortalities in CDV infected tigers and leopards were only confirmed in 2003, 2010 and 2015 (although CDV may also have contributed to the deaths of tigers infected in 2006 and 2013). The contribution of coinfections to these mortalities warrants further study, and in future cases would be aided by the collection of representative sets of tissues as a routine part of mortality investigations.

The maintenance of a pathogen in a population that is as small and sparsely distributed as the Amur tiger is only possible where infectious period is long, as this increases the potential for transmission during infrequent intra-specific contact events. Little is known about the infectious period of CDV in tigers, however in dogs viral shedding of 60 to 90 days has been recorded, although is usually shorter than this (Greene and Appel 2006). The period of infection in ferrets varies with viral strain, with shedding evident for 9-32 days depending on the virus used (Ludlow et al. 2012). While the present study was not designed to describe the infectious period of CDV in tigers, the sequence of events in the case of PT56 suggested that infections in wild tigers may be as long as six months (Chapter 5, Appendices I and II). While this should favour tiger-to-tiger transmission, this appears to have been less important for exposure across a population than transmission from other species, based on serological data from one well-studied population of tigers in Sikhote Alin Biosphere Zapovednik (Chapter 5). Even if long infectious periods were to promote tiger-to-tiger transmissions, the fragmented social structure of tigers would make the maintenance of CDV in tigers unlikely. In these circumstances, infections are more likely to arise through transmission from more abundant host species, that act as a reservoir of infection (Woodroffe 1999, Haydon et al. 2002a). Identifying the species that contribute to the reservoir, and act as sources of infection for tigers is therefore critical to the design of rational control strategies.

Canine distemper virus infections in domestic dogs in Primorskii Krai

The study found little evidence to suggest that CDV is being maintained in populations of domestic dogs in Primorskii krai, but this cannot be ruled out unequivocally. In numerical terms, the large population of dogs in Primorskii could promote the maintenance of CDV, particularly given the rapid reproduction, and low rates of vaccination coverage revealed through demographic surveys (Chapter 3). However, this would be countered by the limited movement of dogs between settlements, and restrictions on roaming behaviour within communities, which would limit opportunity for disease transmission (Chapter 3). The detection of CDV neutralizing antibodies in unvaccinated dogs in many communities reported in Chapter 5 (including young dogs <1 year old) provides evidence of widespread circulation. Higher seroprevalence and outbreak frequency among dogs in remote and less densely populated areas, and dogs with access to the forest suggests possible transmission from wildlife. However, this is not incompatible with dogs maintaining CDV in their own

right, and the detection of Asia 4 clade viruses (which were distantly related to those found in tigers), suggests this could be occurring (Chapter 4).

The apparent increase in CDV exposure of tigers in the years following 2000 may provide further indication of potential maintenance populations. Assuming that tigers are contracting CDV through direct contact with infected hosts (e.g. through predation), an increase in the frequency of exposure would require either an increase in tiger contact with the source population(s), an increase in CDV incidence in source species, or both. Numbers of domestic dogs have changed little in the decade preceding this study (Chapter 3), and it is unlikely that dog populations were considerably lower during the 1990s when tiger exposure was limited or absent. The only source of historic information on CDV infections in Primorskii dogs was collected in 2004, in locations that correspond to the three study areas referred to in the present survey (Quigley et al. unpublished, Goodrich et al. 2012, Table 6.1). Although sample sizes were small, seroprevalence is much higher than recorded in the present study, at 62.5% in Southwest Primorskii (CI: 25.9-89.8%, n=8), 73.3% in Lazovskii (CI: 44.8-91.1%, n=15), and 42.9% in SABZ (CI: 22.6-65.6%, n=21). Although incidence in dogs remains unknown during the 1990s, these findings do not support an increasing trend in CDV circulation. With no evidence for increasing CDV incidence, or population size, it is unlikely that changes in the dog population or their CDV infections are linked to the increasing CDV exposure observed in tigers.

Canine distemper virus infections in mesocarnivores in Primorskii Krai

The serological and molecular data gathered during this study implicates wild mesocarnivores as potential sources of infection for tigers in the Russian Far East (Chapters 4 and 5). Viruses from tigers shared a recent common ancestor to those detected in wild mesocarnivores, indicating that transmission was likely between their populations (Chapter 4). The finding that CDV sequences from mesocarnivores were spatially well mixed, rather than forming local sub-lineages, indicates that CDV is circulating widely, and suggests long chains of transmission that would be consistent with a maintenance population (Chapter 4, Viana et al 2014). Exposure and infections were detected in a range of mesocarnivore species suggesting a complex multi-host dimension to CDV maintenance

Table 6.1. Immunofluorescent antibody titres (IFA) measured against canine distemper virus for dogs sampled in Primorskii in 2004. Source: Quigley *et al.* unpublished.

Dog ID	Location	IFA result	Positive titre	Vaccinated	Age (months)	Sex
KD1	Southwest	Negative	-	Unknown	Unknown	Unknown
KD2	Southwest	Negative	-	Unknown	Unknown	Unknown
KD3	Southwest	Negative	-	Unknown	Unknown	Unknown
KD4	Southwest	Positive	1:50	Unknown	Unknown	Unknown
KD5	Southwest	Positive	1:250	Unknown	Unknown	Unknown
KD6	Southwest	Positive	1:6250	Unknown	Unknown	Unknown
KD7	Southwest	Positive	1:50	Unknown	Unknown	Unknown
KD8	Southwest	Positive	1:50	Unknown	Unknown	Unknown
LD1	Lazovskii	Negative	-	No	6	Male
LD2	Lazovskii	Positive	1:50	No	7	Male
LD3	Lazovskii	Negative	-	No	12	Male
LD4	Lazovskii	Positive	1:50	No	18	Male
LD5	Lazovskii	Negative	-	No	24	Male
LD6	Lazovskii	Positive	1:50	No	30	Male
LD7	Lazovskii	Negative	-	No	36	Male
LD8	Lazovskii	Positive	1:50	No	36	Female
LD9	Lazovskii	Positive	1:50	No	36	Male
LD10	Lazovskii	Positive	1:50	No	48	Female
LD12	Lazovskii	Positive	1:6250	No	60	Male
LD13	Lazovskii	Positive	1:250	No	84	Female
LD14	Lazovskii	Positive	1:50	No	96	Male
LD15	Lazovskii	Positive	1:6250	No	168	Male
LD16	Lazovskii	Positive	1:250	No	180	Male
TD1	SABZ	Negative	-	No	1.8	Female
TD2	SABZ	Positive	1:250	No	4	Male
TD3	SABZ	Negative	-	No	5	Female
TD4	SABZ	Negative	-	No	7	Male
TD5	SABZ	Negative	-	No	9	Female
TD6	SABZ	Negative	-	No	12	Male
TD7	SABZ	Negative	-	No	12	Male
TD8	SABZ	Negative	-	No	12	Male
TD9	SABZ	Positive	1:1250	No	12	Male
TD10	SABZ	Positive	1:50	No	12	Female
TD11	SABZ	Negative	-	No	24	Male
TD12	SABZ	Negative	-	No	24	Male
TD13	SABZ	Positive	1:250	No	24	Male
TD14	SABZ	Negative	-	No	36	Male
TD15	SABZ	Positive	1:6250	No	48	Male
TD16	SABZ	Positive	1:6250	No	48	Female
TD20	SABZ	Positive	1:6250	No	72	Male
TD21	SABZ	Positive	1:6250	No	84	Male
TD22	SABZ	Negative	-	No	120	Male
TD23	SABZ	Positive	1:1250	No	144	Male
TD25	SABZ	Negative	-	No	156	Female

in wildlife, with exposure of raccoon dogs (*Nyctereutes procyonides*, Chapter 5), and virus in sable (*Martes zibellina*, Chapter 4) implicating these species as part of the CDV reservoir. Although virus was detected in one dead Asian badger (*Meles leucurus*, Chapter 4), serological findings were unable to confirm their involvement in the reservoir, due to the difficulty in assessing the specificity of low antibody titers. Under sampling of abundant red foxes (*Vulpes vulpes*) also prevented conclusions being drawn for this species. Further research would be needed to confirm the involvement of Asian badgers and red foxes in the CDV reservoir in Primorskii krai.

Long-term datasets suggest that at least some populations of wild carnivores have been increasing in the Russian Far East, which could explain the increase in tiger infections. Populations of fur-bearing species are monitored during an annual winter track count that is conducted using standardized methods (Dronova and Shestakov 2005). These figures indicate that the sable is the most abundant wild carnivore species nationally, with a population increasing from an estimated 599,900 in 1982 to 1,286,640 in 2014 (Dronova and Shestakov, 2005, Tsentrohotkontrol pers. comm. 2016, Figure 6.1). In the Russian Far East, sable were estimated to number 220,000 in 2000, and their populations are believed to be stable, or possibly increasing in the region (Dronova and Shestakov 2005). While the validity of these approaches to estimate absolute population size is open to question, the standardized approach supports their value as an index of population size, and suggests that numbers of sable are increasing.

In recent decades, some of the most influential drivers of wildlife abundance were triggered by the sudden socioeconomic and institutional changes that followed the collapse of the Soviet Union in 1991 (Wells and Williams 1998, Baumann et al. 2012, Prishchepov et al. 2012, Bragina et al. 2015, Sieber et al. 2015). The near total disintegration of central government, and transition from socialism to a market-driven economy had major repercussions on wildlife populations, which responded to the changes in a variety of ways (Prishchepov et al. 2012, Bragina et al. 2015). Initially, severe economic hardship, and erosion of wildlife law enforcement brought an increase in poaching pressure, leading to sudden declines in many large-bodied mammal species (Wells and Williams 1998, Bragina et al. 2015). However, the transition also brought opportunities, particularly in the abandonment of former state-run farmland collectives, which over time began to revert to habitat types more suitable for many wild species (Baumann et al. 2012, Bragina et al.

2015, Sieber et al. 2015). The beneficial effects of habitat reversion can be deferred for several years (Bragina et al. 2015), and so any impacts on CDV dynamics may take time to become apparent. While the effect of these habitat changes on mesocarnivore populations remains unknown, it is possible that they may have contributed to the increasing numbers evident in winter track counts nationally, and potentially in the RFE.

Changes in hunting pressure following the collapse of the Soviet Union may also have increased the potential of the sable population to act as CDV maintenance hosts. In areas where sable are hunted, juveniles (< one year old) are disproportionately represented in the population, as hunting reduces median life expectancy (Monakhov 2011). For instance, in two Russian populations subject to hunting, juveniles represented 62.7% and 75.6% of all animals in the population (Monakhov 2011). By altering the age structure in this way, hunting may act to reduce herd immunity, with the recruitment of immunologically naïve juveniles each spring creating a highly susceptible population. Modelling has predicted that these mechanisms can increase the prevalence of directly transmitted density dependent pathogens in culled populations (Choisy and Rohani 2006, Bolzoni and De Leo 2013), and may also contribute to increased virulence (Bolzoni and De Leo 2013). Thus, subjecting a growing population of sable to an increase in hunting pressure could lead to more explosive outbreaks, and increase the inter-species transmission of CDV.

Fluctuating demand for sable fur led to a decline in hunting pressure during the 1990s, but an increase in the years since. Following the dissolution of the Soviet Union in 1991, state support for the fur trade ceased (Dronova and Shestakov 2005). This combined with a decline in the international fur market led to a marked reduction in the value of pelts. As a consequence, the annual harvest of fur-bearing species fell considerably below the annual quota for much of the 1990s (Dronova and Shestakov 2005). By the late 1990s, demand began to increase with the emergence of new domestic and international markets, and hunting pressure returned to former levels, and has increased steadily in the years since. These trends in hunting pressure are evident using a proxy based on the number of sable pelts sold through the St Petersburg auction each year, expressed as a proportion of the estimated national population (Figure 6.1B). At the end of the Soviet era, in 1990 sales of sable pelts were equivalent to 21.0% of the national population. These figures fell below 10% from 1992 through 1994 as the market collapsed, then began to recover in the latter half of the decade. By 2001, hunting pressure was back to Soviet levels (20.9%), and

increased steadily for the remainder of the decade. By 2012, sales of sable pelts passed the half million mark, equivalent to 42.3% of the national population. A year later this rose further to 50.1%, before dropping back to 40.4% in 2014. Although speculative, the growing populations of mesocarnivores, and the potential for hunting to amplify CDV prevalence represent a feasible mechanism for the increased incidence of CDV in tigers, and strengthen the case for a wildlife reservoir in which the sable population plays an important role.

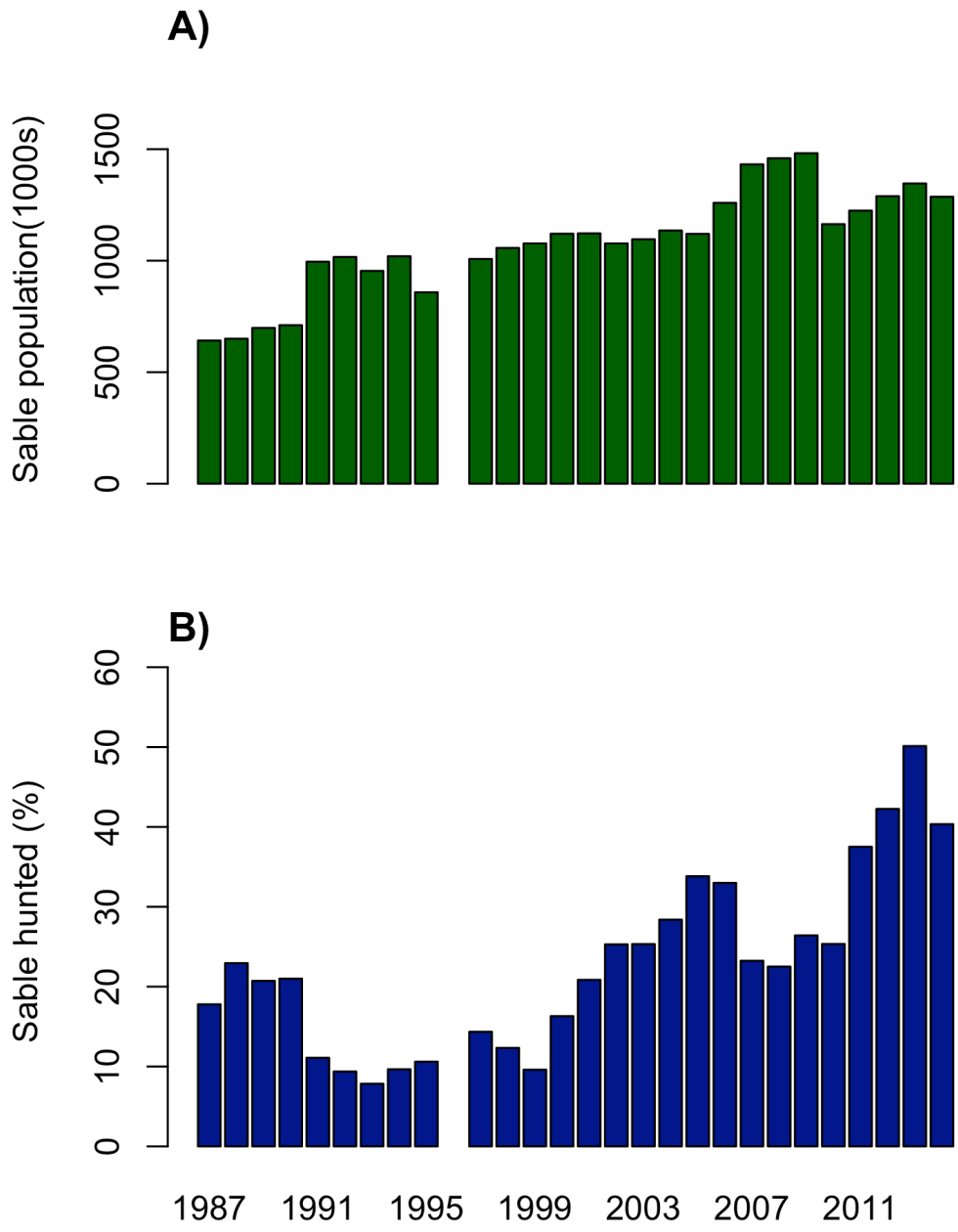

Figure 6.1. National figures relating to sable numbers in the Russian Federation, 1987-2014, including: A) Estimated national population of sable based on annual winter tracking counts; B) Annual percentage of sable hunted, based on the number of wild sable pelts sold through the St. Petersburg auction, expressed as a percentage of the national sable population. Winter tracking counts were unavailable for 1996. Source: Tsentrohotkontrol.

Canine distemper virus management options

Although further research could clarify the epidemiology of CDV in domestic dog populations, the apparent maintenance of the virus in wild carnivores represents the most important conclusion of this study. Even the successful control of CDV in the domestic dog population would not prevent exposure of wild tigers, while in the presence of a wildlife reservoir. This finding has considerable influence on the selection of management strategies that was the ultimate objective of the study.

Small populations are more vulnerable to stochastic effects including infectious disease outbreaks that increase the potential for extinction (Woodroffe and Ginsberg 1998, Haydon et al. 2002b, De Castro and Bolker 2005, Gilbert et al. 2014). Model simulations have predicted that isolated populations of Amur tigers numbering 25 individuals were 1.65 times more likely to decline to extinction within fifty years in the presence of CDV than control populations of equivalent starting size that were unexposed to CDV (Gilbert et al. 2014). This contrasts with the situation in large populations that are able to withstand CDV outbreaks, with no impact on fifty year extinction probability above an estimated founder population threshold of 208 individual tigers (Gilbert et al. 2014).

Given that the remaining population of Amur tigers is distributed in two subpopulations of unequal size, strategies to manage the impact of CDV should focus on the small, but strategically important subpopulation in Southwest Primorskii (Sorokin et al. 2016). Comprising as few as 10-20 individuals, this population has a high conservation value, as it acts as a source for recolonizing areas of northeastern China, where tigers have recently been extirpated (Henry et al. 2009, Miquelle et al. 2010b, Hebblewhite et al. 2012). The Southwest Primorskii region also supports the remaining wild population of the critically endangered Far Eastern leopard, which elevates the conservation importance of the region further. The detection of CDV cases in both species in Southwest Primorskii during this study emphasizes the vulnerability of these populations, and is an indicator that measures to mitigate the impact of CDV should be considered.

The primary objective of managing CDV in Amur tigers is to reduce or eliminate the impact of the virus on population viability. Fundamentally, there are two main approaches

to achieving this: 1) measures to mitigate the impact of outbreaks when they occur, and 2) active control strategies to reduce the incidence of CDV infections in the tiger population.

Impact mitigation measures

One approach that could reduce the impact of future CDV outbreaks on the extinction potential of tigers in Southwest Primorskii is the introduction of measures to increase the ability of tigers to move to and from the main Sikhote-Alin population. Measures to improve connectivity between fragmented populations can benefit overall survival through a 'rescue effect' mediated through dispersal across the metapopulation, even at modest rates of immigration (Brier 1993, Gilbert et al. 1998). While some have cautioned that improved connectivity in a metapopulation could enable the dissemination of an emerging infectious disease (Hess 1996), modelling approaches have indicated that this is unlikely in wild situations, where infections spillover from an abundant reservoir, even when the force of infection is low (Gog et al. 2002, McCallum and Dobson 2002). Re-establishing connectivity with the Sikhote-Alin Mountains would enable immigration from tigers that could offset any future declines, and provide a corridor for leopards to recolonize former parts of their range.

At the narrowest point, just a few hundred meters separates the disjunct tiger populations, which are bisected by the main Vladivostok/Khabarovsk highway (Figure 6.2). In Europe and North America, highway overpasses and underpasses have been used to facilitate dispersal of wildlife, including carnivores such as cougars (*Puma concolor*) (Foster and Humphrey 1995, Clevenger and Waltho 2000, Gloyne and Clevenger 2001, Corlatti et al. 2009). The placement, and design of structures requires careful consideration, and acceptance by wildlife can be enhanced through habitat modification on either side of the crossing (Clevenger and Waltho 2000, Gloyne and Clevenger 2001). While the construction of crossings may require a large capital investment at the outset of the project, it would have the advantage of remaining effective as long as habitat continuity was maintained. If successful, this one action could help buffer the tigers and leopards in Southwest Primorskii from the impacts of CDV outbreaks, while also maintaining the genetic diversity of these isolated populations.

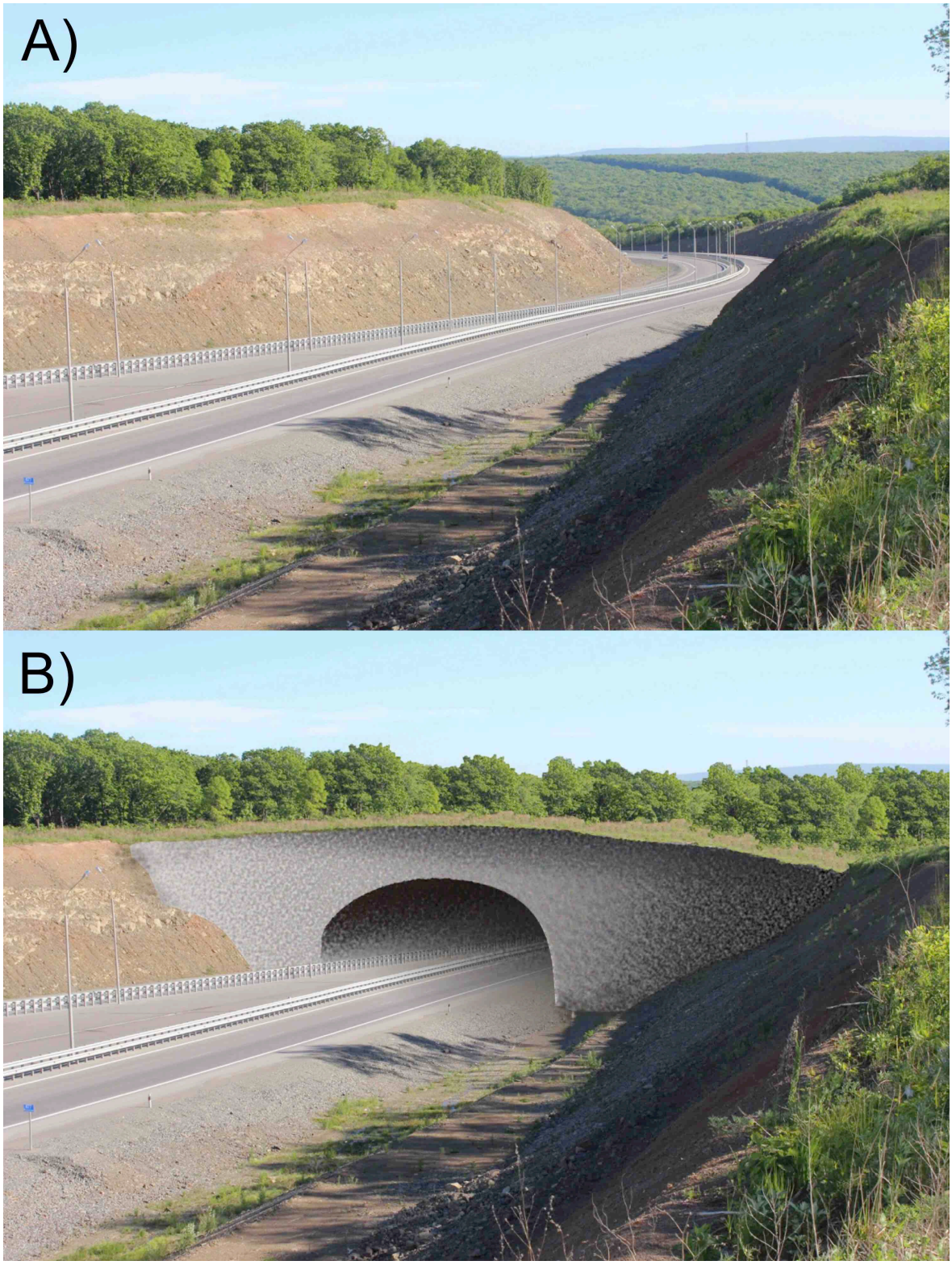

Figure 6.2. A) The Vladivostok-Khabarovsk highway close to the village of Razdol'noye. This highway represents an important barrier to tiger movement from the main Sikhote-Alin population (occupying forest on the left), with the strategically important Southwest Primorskii population (occupying forest on the right); B) A representation of a highway overpass that could effectively link the two populations, and reduce the potential for extinction of the Southwest Primorskii population through outbreaks of canine distemper virus, or other stochastic factors.

Canine distemper virus control measures

In the context of reservoir systems, control strategies have been defined with reference to a target population, the intended beneficiary of the intervention strategy, in this case the tiger (Haydon et al. 2002a, Viana et al. 2014). Available control strategies fall into three broad categories: i) reduce transmission within the reservoir, ii) minimize the opportunity for transmission between the reservoir and the target population, or iii) reduce transmission within the target population itself (Woodroffe 1999, Haydon et al. 2002a, Laurenson et al. 2005). Only the first of these strategies seeks to reduce or eliminate the pathogen in the environment, while the latter aims to reduce the impact on the target population directly.

Several factors conspire against the management of CDV in a wildlife reservoir. Across the tiger range, collective populations of susceptible mesocarnivores are likely to comprise several hundred thousand individuals (Chapters 3 and 5). The prospect of reducing transmission in a reservoir of this size, particularly across a rugged and often inaccessible landscape of approximately 155,000 km^2 is remote. Even if it were possible to vaccinate sufficient numbers of mesocarnivores over an area of this size, the continuity with the vast taiga forests extending north and west across Siberia would act as a continued source of viral reintroduction (as suggested by the recent common ancestor shared between sequences from Primorskii, Alaska and Europe, Chapter 4). Even if such widespread coverage were possible, the rapid turnover of mesocarnivore populations would necessitate the continued delivery of vaccines on an indefinite basis. Further confounding factors are the lack of orally deliverable vaccines, and safety concerns in some species (in which vaccine strains are capable of invoking clinical disease and mortality, Halbrooks et al. 1981, McCormick 1983, Montali et al. 1987), which together render reservoir control strategies unfeasible.

Measures to prevent contact between tigers and a wildlife reservoir are also unfeasible. Theoretically, transmission from a domestic reservoir could be limited by fencing tiger habitat, and restricting activities such as hunting, where dogs enter the forest (Laurenson et al. 2005, Belsare and Gompper 2015). But with only 7% of Amur tiger range falling within protected areas (Miquelle et al. 2005), and the cultural and economic importance of forest ecosystems to local communities, such measures would be unworkable and unacceptable, even if dogs were important reservoirs of the virus. For these reasons, managers are left

with one feasible strategy, vaccination of the tigers themselves, to protect the target population.

Overview of wildlife vaccination

There are few examples of vaccination being used for the long-term control of infectious disease in wildlife, with products more commonly used as an emergency response in the face of an outbreak (Table 6.2). The notable exception to this is fox rabies, which has been eliminated across western Europe through the widespread delivery of orally available vaccines (Mähl et al. 2014, Müller et al. 2015). However, these successes have yet to be replicated in the elimination of skunk and raccoon rabies in North America, illustrating the challenges in eliminating infectious disease from wildlife (Slate et al. 2005). The species composition of the rabies reservoir differs in North America, and some key populations (particularly skunks) have proven challenging to vaccinate using oral baits (Slate et al. 2005). Due to the financial costs involved, large-scale elimination programmes are only considered for the control of serious zoonotic pathogens (e.g. rabies), or potentially for pathogens that exert a heavy economic impact (e.g. *Mycobacterium bovis*). For this reason, most other wildlife vaccination programmes have been smaller in scale, and many are experimental (Table 6.2).

The use of vaccines in conservation management has largely focused on targeting the threatened species of interest, rather than attempting to eliminate the pathogen from the reservoir (with a few notable exceptions such as the vaccination of domestic dogs against rabies as an approach to limit exposure of Ethiopian wolves, Laurenson et al. 1998, which was ultimately unsuccessful in preventing outbreaks in the wolves Randall et al. 2004). As a result, sources of infection often remain unaffected, and the objective is to increase the capacity of the threatened population to withstand outbreaks, rather than eliminating infections entirely (Haydon et al. 2006, Prager et al. 2011). A clear disadvantage of this approach is the need to continue vaccinating in perpetuity, at least while transmission from the reservoir continues, or until the threatened population reaches a size where outbreaks no longer impact population viability.

Table 6.2. Examples of vaccines use to control infectious disease in wildlife populations in the field. Delivery route is indicated as oral (O), intramuscular injection (IM), subcutaneous injection (SC), or unspecified parenteral route (PAR). Circumstances of vaccine use are summarised as outbreak response (OUTBRK), long-term control (CONTR), prophylaxis of released animals (RELEASE), field trial (TRIAL), or in development (DEV). Interventions reported by authors as unsuccessful are denoted by †.

Target species		Pathogen	Country /Region	Delivery route	Situation	Source
Mountain gorilla	*Gorilla beringei beringei*	Measles	Rwanda	IM	OUTBRK	(Hastings et al. 1991)
Black-footed ferret	*Mustela nigripes*	Canine distemper virus	USA	SC	CONTR	(U.S. Fish and Wildlife Service 2013)
Island fox	*Urocyon littoralis*	Canine distemper virus	USA	IM, O	OUTBRK	(Vickers et al. 2004, Timm et al. 2009)
Red wolf	*Canis rufus*	Canine parvovirus, canine distemper virus	USA	IM	RELEASE	(Harrenstein et al. 1997)
Reservoir species	-	Rabies	Europe	O	CONTR	Reviewed in (Mähl et al. 2014, Müller et al. 2015)
Reservoir species	-	Rabies	North America	O	CONTR	Reviewed in (Slate et al. 2005)
African wild dog	*Lycaon pitus*	Rabies	Tanzania, South Africa	IM	OUTBRK, RELEASE	(Gascoyne et al. 1993, Hofmeyr et al. 2004) †
Ethiopian wolf	*Canis simensis*	Rabies	Ethiopia	IM	OUTBRK	(Randall et al. 2006, Knobel et al. 2008)
European rabbit	*Oryctolagus cuniculus*	Viral haemorrhagic disease, myxomatosis	Spain	SC	TRIAL	(Calvete et al. 2004)
Wild boar	*Sus scrofa*	Classical swine fever	Germany	O	OUTBRK	Reviewed in (Moennig 2015)
Florida puma	*Puma concolor coryi*	Feline leukaemia virus	USA	IM	OUTBRK	(Cunningham et al. 2008)
Cheetah	*Acinonyx jubatus*	Anthrax	Namibia	PAR	TRIAL	(Turnbull et al. 2004)
Black rhinoceros	*Diceros bicornis*	Anthrax	Namibia	IM	CONTR	(Turnbull et al. 2004)

Target species		Pathogen	Country /Region	Delivery route	Situation	Source
Indian one-horned rhinoceros	*Rhinoceros unicornis*	Anthrax	India	IM	OUTBRK	(Pandit and Sinha 2006)
Black rhinoceros, white rhinoceros, roan antelope, kudu, waterbuck, African buffalo hippopotamus	*Diceros bicornis, Ceratotherium simum, Hippotragus equinus, Tragelaphus strepsiceros, Kobus ellipsiprymnus, Hippopotamus amphibius*	Anthrax	Zimbabwe	IM	OUTBRK	(Clegg et al. 2007)
Prairie dog spp. and other rodents	*Cynomys spp.*	Plague	United States	O	TRIAL	(Tripp et al. 2015)
Black-footed ferret	*Mustela nigripes*	Plague	United States	SC	CONTR	(Rocke et al. 2008, U.S. Fish and Wildlife Service 2013)
Common brushtail possum	*Trichosurus vulpecula*	Mycobacterium bovis	New Zealand	O	TRIAL	(Tompkins et al. 2009)
Eurasian badger	*Meles meles*	Mycobacterium bovis	United Kingdom	IM	TRIAL	(Chambers et al. 2011)
Eurasian badger	*Meles meles*	Mycobacterium bovis	Ireland	O	TRIAL	(Corner et al. 2009, Aznar et al. 2011)
Wild boar	*Sus scrofa*	Mycobacterium bovis	Spain	O	DEV	(Beltrán-Beck et al. 2012)
Bighorn sheep	*Ovis canadensis*	Pasturella multocida, P. trehalosi, Mannheimia haemolytica	United States	IM	OUTBRK	(Cassirer et al. 2001) †
Koala	*Phascolarctos cinereus*	Chlamydia pecorum	Australia	SC	TRIAL	(Waugh et al. 2016)
Tasmanian devil	*Sarcophilus harrisii*	Tasmanian devil facial tumour disease	Australia	SC	DEV	(Kreiss et al. 2014)

Risks of intervention

As with any interventionist approach to wildlife management, there is a risk that vaccination programmes could have unintended and detrimental consequences for the animals involved (Woodroffe 2001, Laurenson et al. 2005, Cleaveland et al. 2007, Cleaveland 2009). These risks must be recognised, and measures taken to minimize their impact. The safety and efficacy of vaccine products for target species can often be assessed in a captive setting, enabling the development of protocols that minimize the risks to individuals, and ensuring the desired levels of immunity are achieved. Vaccine delivery methods must also take account of risks to non-target species, particularly when live vaccines that may be virulent in some taxa (Halbrooks et al. 1981, McCormick 1983, Montali et al. 1987, Sutherland-Smith et al. 1997). In cases where products must be delivered by the parenteral route, all measures must be taken to minimize risk of injury when animals are captured or when vaccine is delivered by remote injection. Anticipating these risks in advance, and adopting mitigation strategies can reduce the threat to target and non-target populations substantially.

It is also important not to underestimate the wider implications of a vaccination programme that is perceived to have failed. Such negative perceptions, be they from other researchers, wildlife management authorities or the general public could jeopardise the prospect of future vaccination programmes, or even other interventionist research, such as placement of telemetry collars (Woodroffe 2001, Cleaveland et al. 2007).

As a cautionary example, during the early 1990s the immunization of African wild dogs (AWDs, *Lycaon pictus*) against rabies in the Serengeti received high profile criticism, following the subsequent extinction of remaining packs (Burrows 1991, Creel 1992, Heinsohn 1992, Macdonald 1992, Burrows et al. 1994, Dye 1996). Critics proposed that immunosuppression related to the stress of capture may have activated latent rabies infections, and drew a direct link between the vaccination programme and the extinction of AWDs in the park (Burrows 1991, Burrows et al. 1994). Although these claims lacked support (Creel 1992, Macdonald 1992, Dye 1996, Woodroffe 2001), the negative perceptions led to a culture of risk-aversion among wildlife managers in many AWD range states and elsewhere (Cleaveland et al. 2007). Securing permission for wildlife captures

became increasingly difficult in many areas, and there has been a marked resistance to further wildlife vaccination initiatives.

Lessons can be learned from the experiences in Serengeti that would greatly reduce the potential for similar situations arising in the future. Preparatory discussions with local stakeholders, exploring the likely consequences of inaction, and both positive and negative outcomes of intervention would help to achieve realistic *a priori* expectations. By incorporating vaccinated and non-vaccinated animals in programme design provides a means for assessing the impact of intervention on survival, and could also gain valuable insights into the epidemiology of the pathogen (Viana et al. 2014). Careful monitoring prior to, during and following the intervention, establishes health baselines, and increases the body of data available to determine the cause of any unforeseen outcomes. By incorporating local support at the outset, and maximizing opportunities to learn from the intervention, vaccination programmes stand the best chance of reaching conservation objectives, and encouraging successful outcomes in the future.

Other risks inherent in wildlife vaccination include the consequences of withdrawal of coverage in event that the programme is interrupted due to financial constraints or other factors (Woodroffe 1999). In the case of the tigers, the objective of vaccination would be to mitigate the population effects of CDV infection in the tiger population, rather achieve a local elimination in the reservoir. Tigers would continue to be exposed to CDV as there would be no interruption in transmission from the reservoir population, but as long as vaccine delivery continued, inoculated tigers would be protected from natural challenge for the remainder of their lives. A cessation in vaccine delivery would lead to a gradual decline in the herd immunity of the tiger population, as vaccinated individuals died from other causes and were not replaced. Assuming no changes had occurred in the reservoir population, herd immunity would decline over time to pre-vaccination levels, with immune tigers represented solely by those surviving natural CDV challenge. In cases where vaccination is targeted to a reservoir population, the impact of vaccine withdrawal can be more problematic, particularly for vaccines that do not provide lifelong immunity (Woodroffe 1999). These situations can lead to a large proportion of a population simultaneously becoming susceptible; creating conditions that can lead to large outbreaks. This situation is exacerbated further if vaccination led to an increase in the size of a reservoir population, thus increasing host density and rate of contact.

Available canine distemper virus vaccine products

Vaccination has been the mainstay of CDV control since the introduction of formaldehyde-inactivated products during the 1920s (Chappuis 1995, Bresalier and Worboys 2013). The virus is represented by a single antigenic subtype, and while some concerns have been raised about the risk posed by antigenic drift (Blixenkrone-Møller et al. 1993, Gemma et al. 1996, Iwatsuki et al. 1997), vaccine strains are generally thought to infer protection against all contemporary wild-type strains (Greene and Appel 2006). Humoral, cytokine and cell-mediated immune responses contribute to protection (Appel et al. 1982), but most studies use the humoral response to measure vaccine-derived immunity. There are several classes of CDV vaccines, each with distinct advantages and disadvantages:

Modified live vaccines (MLVs) – These vaccines are widely used for the immunization of domestic dogs, and several Russian and international brands are available in the RFE. Derived from North American strains collected prior to the 1950s, MLVs have been attenuated through serial passages in live animals or cell culture (principally of canine or avian origin). Although MLV structure differs from contemporary strains (70.1-93.4% identity at the amino acid level of the external haemagglutinin protein), they continue to invoke a strong and long-lasting humoral immune response in dogs. Canine adapted strains (e.g. Rockborn) induce a 'sterile immunity', where viral replication does not occur following subsequent challenge, but vaccine-induced clinical infection are possible. Clinical disease is rarely associated with avian-adapted strains (e.g. Onderstepoort), but lead to a less potent "non-sterile" immunity. Both canine and avian-derived strains have been associated with clinical disease among some non-domestic carnivores such as red panda (*Ailurus fulgens*), black-footed ferret (*Mustela nigripes*) and others (Bush et al. 1976, Carpenter et al. 1976, Halbrooks et al. 1981, Kazacos et al. 1981, McCormick 1983, Thomas-Baker 1985, McInnes et al. 1992). However, recent trials in captive tigers have demonstrated a strong humoral response to Onderstepoort-based vaccines, without clinical complications (Sadler et al. 2016), replicating the results of a more limited trial in lions (*P. leo*) (Kock et al. 1998).

Recombinant vaccines – Vaccines based on a non-replicating viral vector expressing CDV haemagglutinin (HA) and fusion (F) genes have been used to invoke a "non-sterile" immunity in inoculated animals (Pardo et al. 1997, Schultz 2006). This has been achieved

experimentally with vectors based on canine adenovirus 2, and a vaccinia vector carrying measles HA and F glycoproteins (Taylor et al. 1991, Fischer et al. 2002). The principal advantage of recombinant vaccines is their safety, as they are unable to induce clinical CDV infections in sensitive species, and several canarypox-vectored products are marketed for this purpose. The main disadvantage of recombinant products is the short duration of immunity, with booster doses recommended after one year. However, a recent trial of a canarypox vectored vaccine in six tigers did not produce measurable antibodies by 26 days post inoculation, and only two tigers had measurable antibodies by day 66 (1:16 and 1:32) (Sadler et al. 2016). Based on these findings, and the short duration of immunity in other species, current recombinant products are unlikely to produce desired levels of immunity in free-ranging tigers, where revaccination is unlikely.

Experimental vaccines – The potential for virulence of attenuated vaccines in some species, and low levels of immunity of recombinant products have encouraged the development of new experimental vaccines.

1. Rationally attenuated vaccines based on a wild-type CDV virion replicate less efficiently in the host, due to the insertion of sequences into the open reading frame of the large (L) gene (Silin et al. 2007). These vaccines maintain the structure of external glycoproteins, which stimulate both a humoral and cell-mediated immune response. Desired levels of attenuation are achieved by manipulating the length of inserted sequences, so that replication is sufficient to stimulate immunity, while minimizing virulence. Other methods of attenuation could include the selective depletion of N-linked glycosylation sites on the haemagglutinin protein (Sawatsky and von Messling 2010).

2. A chimera vaccine combining the exterior glycoproteins from a wild-type CDV with the interior proteins of a measles vaccine strain has been used experimentally in ferrets (Rouxel et al. 2009). The vaccine replicated within the host without inducing clinical disease, and conferred protection from subsequent challenge with virulent CDV. The attenuation of the chimera was attributed to pre-existing attenuating mutations in the measles vaccine, and the inability of measles V and C proteins to modify the immune response in non-natural hosts.

3. Finally, plasmids containing DNA encoding for CDV haemagglutinin, fusion and nucleoprotein genes has been used successfully in protecting mink (*Mustela vison*) from challenge with wild-type CDV (Sixt et al. 1998). The ability to confer immunity without using whole viruses removes any potential for virulence in susceptible species. However, the need for repeated delivery to achieve protective immunity remains a significant drawback (Nielsen et al. 2012).

Commercial CDV vaccines are generally delivered through parenteral routes, which would require the capture of tigers, or remote delivery via a dart. Experimental delivery of an avian-adapted MLV, rationally attenuated and chimeric vaccines via the nasal route have invoked humoral responses (Chappuis and Terre 1973, Silin et al. 2007, Rouxel et al. 2009). This raises the possibility of delivering vaccines via scent marking sites, that are regularly visited by tigers (David Smith et al. 1989), and are therefore one of the few predictable aspects of the species movements. To date, the delivery of CDV vaccines via the oral route has received little attention, but so far results have not been encouraging (Chappuis and Terre 1973). However, there have been considerable advances in the development of oral vaccines against other pathogens, and therefore further research is warranted.

Use of canine distemper virus vaccines in endangered species

Although CDV vaccines are widely used for immunizing non-domestic carnivores in captivity (Deem et al. 2000), their use has been limited in the conservation of carnivores in the wild. Although contemporary CDV vaccines have shown high safety and immunogenicity in a range of domestic and wild species (Goodrich et al. 1994, Greene and Appel 2006, Sadler et al. 2016), the virulence of older-generation modified live vaccine strains in some non-domestic species contributes to concerns about vaccinating highly endangered wild animals. Such reactions can be difficult to predict, as exemplified by the deaths of four of six endangered black-footed ferrets (*Mustela nigripes*) that were inoculated with a modified live vaccine of avian origin, that had been used safely for the vaccination of domestic ferrets (*M. putorius*) (Carpenter et al. 1976). The only alternative vaccine available at the time, an inactivated product, induced a less protective immunity that was too short-lived to have been of practical value in free-ranging animals (Williams

et al. 1996). In this case, the predicament facing conservationists was clear, as the last remaining colony of black-footed ferrets ultimately declined below sustainable levels during a CDV outbreak, and required an emergency captive intervention (Thorne and Williams 1988).

Delivery of vaccines to free-ranging carnivores presents another important challenge. Unlike the orally available vaccines used for the control of sylvatic rabies, contemporary CDV vaccines are designed for parenteral administration. The use of a recombinant canarypox vectored product, for the vaccination of Santa Catalina Island foxes (*Urocyon littoralis catalinae*) may have contributed to the control of a CDV outbreak, but required that individual foxes be captured, and the vaccine be delivered by intramuscular injection (Vickers et al. 2004, Timm et al. 2009). Similarly, a modified live vaccine has recently been used to inoculate free-ranging Ethiopian wolves (*Canis simensis*) against CDV (C. Gordon *pers. comm.* 2015). Such methods are both labour intensive, and therefore financially expensive, and risk stress and injury for the animals that are captured.

Even with relatively accessible species, it takes time to achieve meaningful population immunity (Vickers et al. 2004), and so the approach may be more be appropriate for low coverage vaccination strategies, that aim to limit the impact of outbreaks at the population level (Vial et al. 2006, Haydon et al. 2006, Gordon et al. 2015). The availability of effective oral products could reduce costs, and the risks involved in capture. Oral delivery of a recombinant vaccine produced serum antibody titers in Island foxes in an experimental setting (Vickers et al. 2004), however, oral administration of the same vaccine was unable to stimulate a measurable humoral response in African wild dogs (Connolly et al. 2013). This disparity may have been dose related, and so further work is warranted (Connolly et al. 2013). Development of a reliable oral delivery system could have important implications for CDV management in the field, particularly for cryptic species such as Amur tigers that are rarely observed and challenging to capture.

Features of a desirable vaccine

It is evident that none of the existing CDV vaccines meet all of the criteria that would be optimal for use in free-ranging endangered carnivores. Methods of remote delivery that do

not require the capture or even visualization of target animals would be particularly useful for rarely observed species such as the Amur tiger. Due to the absence of economic incentives to develop vaccines specifically for endangered species, it will be necessary to evaluate existing and emerging products for conservation uses. For this reason, the following framework is proposed to enable the assessment of existing and new technologies, which could be adapted for the vaccination of endangered species:

1. *Safe in target species* – A key pre-requisite, which should preferably be evaluated in a captive population of the same species, or if necessary a closely related model species.

2. *Safe in non-target species* – Relevant to non-parenteral delivery methods, where there is no assurance that vaccine will be delivered exclusively to the target species.

3. *Induce protective immunity in target species* – Requires an assessment of humoral and cell-mediated response in captive animals of the same species, or a closely related model species. The presence of antibodies in target species may imply immunity, but for ethical reasons challenge studies are unlikely to be possible to confirm this. Therefore, it may be necessary to infer immunity in endangered hosts based on response of related species in challenge studies.

4. *Protection with limited delivery* – Opportunities to provide multiple doses may be limited in many free-ranging species, therefore products should ideally invoke protection through a single dose.

5. *Effective delivery to induction site* – Of relevance to non-parenteral delivery, a vaccine must prime appropriate induction tissues to stimulate protection against natural infection (e.g. Peyer's patches, oral or nasal mucosa). This can be promoted through use of formulations that:

    a. Prolong contact – Such as the use of viscous compounds for increasing contact with oral mucosa (Fry et al. 2012)

    b. Abrasive adjuvants – Use of abrasive materials to scarify oral mucosa to increase vaccine uptake and absorption (Edmonds et al. 2001).

c. Resist digestion – For products requiring contact with Peyer's patch induction sites, suspension of active ingredients in a lipid matrix that permits transit through the stomach (Aldwell et al. 2003).

6. *Environmental stability* – Non-parenteral preparations must maximize environmental stability, either through use of more stable recombinant products, or make use of packages to reduce degradation.

7. *Accommodate behaviour of target species* – Predictable behaviour may provide opportunities for vaccine delivery, such as oral or nasal delivery at trees used for scent marking. Strategies might also reduce exposure of non-targets, such as seasonal baiting of carcasses when other species are hibernating.

8. *Cost effective* – Uptake of non-parenteral products is likely to be low, and so cheaper products can be distributed in greater numbers.

Critical questions

Before vaccination could proceed, several key questions must be addressed.

1. Are current vaccines safe and effective in target species? – While experimental products may ultimately prove to be safer and more efficacious, any control programme in the near term must rely on contemporary vaccines that are already being marketed. As both modified live and recombinant vaccines are designed for parenteral administration, chosen products should be capable of inducing protective and ideally long-lasting immunity with a single dose, as revaccination may be difficult in many cases. Vaccines could be given to tigers that are handled for rehabilitation, or translocation, and potentially also for free-ranging animals captured as part of the control programme.

   A recent trial by Sadler et al. (2016) compared humoral response to a recombinant product given to eight captive tigers on days 0 and 39, yet only two had measurable titers of CDV-neutralizing antibodies by day 66. A further eight tigers were given a MLV, and were found to have measurable antibodies at least 171 days later. Vaccine safety was assessed by dosing an additional 41 tigers with the recombinant vaccine, and 38 tigers with the MLV, and no adverse effects were observed. Based on the strong

humoral immunity induced by a single dose, and apparent safety in a relatively large cohort, suggests that the MLV may be most appropriate for use in free-ranging tigers.

Currently the MLV product is unavailable in monovalent form, and the product tested included a canine parvovirus 2 component. This raises concerns for use in pregnant females, as parvovirus is associated with cerebellar hypoplasia in young born to infected cats (Kilham et al. 1967). This could be addressed through further safety testing of the bivalent product, or cooperation from the manufacturer to produce batches of monovalent CDV vaccine. Additional testing to determine the longevity of the humoral and cell mediated immunity following a single dose would also be desirable. Further testing in leopards would be required if vaccination were to extend to this species.

2. How many tigers should be vaccinated? – Capture of free-ranging tigers is a time-consuming and labour intensive activity, therefore it would be important to know if meaningful vaccine coverage is achievable. The existing population viability model should be adapted to assess the impact of practical vaccination strategies on population extinction risk. Low coverage vaccination strategies have been modelled for use in social canids (Vial et al. 2006, Haydon et al. 2006, Gordon et al. 2015), but their effectiveness should be verified for the distinct life history and ecology of the more solitary tiger. The population viability model should also be modified to reflect the biology of the Far Eastern leopard, if vaccination is to be considered for this species.

3. Can vaccines be delivered orally? – The availability of an oral CDV vaccine could greatly facilitate the immunization of a free-ranging tiger population. Assuming that tigers would accept bait, laid for example in a carcass placed within their territory, then it could provide a mechanism for reaching more tigers, including those in remote areas, and potentially enable the delivery of booster doses. Few data have been published on the oral availability of CDV vaccines, and much of what has is contradictory (Chappuis and Terre 1973, Vickers et al. 2004, Connolly et al. 2013).

While initial entry of CDV is dependent on cells bearing the CD150 receptor (e.g. dendritic cells, macrophages and B and T lymphocytes), the identity of initial target cells is unknown (Ludlow et al. 2014). An oral CDV vaccine would need access to the same receptors used by the wild-type virus to stimulate an immune response. The process of attenuation of vaccine strains may have reduced their ability to bind to receptors in the oral mucosa, which could explain the lack of success in previous oral vaccination trials (Connolly et al. 2013). Therefore, confirming the viability of oral infection by exposing the oral mucosa of a susceptible surrogate species to wild-type CDV would be a valuable first step to assess the potential of an oral route of vaccine delivery. Confirmation that the oral mucosa is a suitable induction site could lead to further work to identify adjuvants to increase vaccine contact, and promote the stimulation of an immune response. Additional considerations would include measures to address environmental stability, and the potential for virulence in non-target species.

The circumstances facing Amur tigers in the Russian Far East are not unique, as many other small populations of threatened carnivores are susceptible to CDV worldwide. Of the 261 extant species of carnivore assessed by the International Union for Conservation of Nature (IUCN) in 2015, 25.7% are currently classified as threatened (vulnerable, endangered or critically endangered, IUCN 2015). A review of the IUCN Redlist database in 2006 found that CDV was the pathogen that most commonly contributed to the threat status of any mammal species (Pedersen et al. 2007). Outbreaks have already caused the near extinction of the black-footed ferret and Santa Catalina Island fox (Thorne and Williams 1988, Timm et al. 2009), and led to sustained declines in lion populations in the Ngorogoro Crater, Tanzania (Kissui and Packer 2004). Just as in Russia, management of CDV in threatened species is complicated wherever wildlife makes a significant contribution to CDV maintenance, and where control is warranted, vaccination of threatened populations should be considered. Further fragmentation of carnivore populations that are unable to adapt to an increasingly human-dominated landscape, will increase their vulnerability to CDV outbreaks. Consequently, the issues highlighted by CDV in Amur tigers are likely to occur more frequently, increasing the urgency for developing vaccine technologies and CDV control strategies for use in threatened species worldwide.

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Appendices

Appendix I. Canine distemper virus as a threat to wild tigers in Russia and across their range. Integrative Zoology 10:329–343

The following paper summarizing the background of canine distemper virus in wild tigers was published in Integrative Zoology:

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ORIGINAL ARTICLE

Canine distemper virus as a threat to wild tigers in Russia and across their range

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Abstract

Canine distemper virus (CDV) has recently been identified in populations of wild tigers in Russia and India. Tiger populations are generally too small to maintain CDV for long periods, but are at risk of infections arising from more abundant susceptible hosts that constitute a reservoir of infection. Because CDV is an additive mortality factor, it could represent a significant threat to small, isolated tiger populations. In Russia, CDV was associated with the deaths of tigers in 2004 and 2010, and was coincident with a localized decline of tigers in Sikhote-Alin Biosphere Zapovednik (from 25 tigers in 2008 to 9 in 2012). Habitat continuity with surrounding areas likely played an important role in promoting an ongoing recovery. We recommend steps be taken to assess the presence and the impact of CDV in all tiger range states, but should not detract focus away from the primary threats to tigers, which include habitat loss and fragmentation, poaching and retaliatory killing. Research priorities include: (i) recognition and diagnosis of clinical cases of CDV in tigers when they occur; and (ii) collection of baseline data on the health of wild tigers. CDV infection of individual tigers need not imply a conservation threat, and modeling should complement disease surveillance and targeted research to assess the potential impact to tiger populations across the range of ecosystems, population densities and climate extremes occupied by tigers. Describing the role of domestic and wild carnivores as contributors to a local CDV reservoir is an important precursor to considering control measures.

Key words: canine distemper virus, conservation threat, extinction, *Panthera tigris altaica*, population decline

INTRODUCTION

Global populations of tigers, *Panthera tigris* (Linnaeus, 1758), are at an all time low, with numbers of reproductive females in the wild dropping below 1000 individuals (Walston *et al*. 2010). Pressure from agriculture,

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industry and urbanization has fragmented tiger habitat, such that remaining populations occupy less than 7% of their former range and more than half of the world's tigers are confined to habitat islands containing 25 or fewer individuals (Sanderson *et al*. 2006; Walston *et al*. 2010). Even in suitable habitat, tigers face a variety of threats, including competition with humans for prey resources, direct poaching to meet the demand for their body parts and retaliation due to conflicts with humans (Walston *et al*. 2010; Chundawat *et al*. 2011). While these anthropogenic factors are the main drivers of declining tiger numbers (Robinson *et al*. 2015), these depleted populations face new pressures associated with stochastic processes that have the potential to drive small, isolated populations to extinction. While inbreeding depression is well recognized as a threat to small populations (Kenney *et al*. 2014), disease agents (pathogens) can also be important drivers of stochastic extinction in carnivore populations (Thorne & Williams 1988; Timm *et al*. 2009); however, their potential impact on free-ranging tigers has received little research attention. In Russia, canine distemper virus (CDV) has recently been recognized as a cause of death in Amur tigers, *Panthera tigris altaica* Temminck, 1844 (Quigley *et al*. 2010; Seimon *et al*. 2013), and could pose a potential extinction threat, particularly to small populations (Gilbert *et al*. 2014). Recent reports have also confirmed cases of CDV in wild tigers in India, indicating that the threat may extend to tigers in other regions as well (ProMED 2014). The objectives of the present paper are: first, to assess our current understanding of the status and impact of CDV on Amur tigers; second, to consider the potential impact of CDV to tigers across their range; and third, to outline steps needed to assess and monitor the threat of CDV to tiger populations both in Russia and elsewhere across their range.

BIOLOGY OF CANINE DISTEMPER VIRUS

Canine distemper is caused by a paramyxovirus with a single-stranded RNA genome within the Morbillivirus genus, which has a near worldwide distribution (Williams 2001; Green & Appel 2006). Transmission of CDV primarily occurs through the respiratory tract during close contact with an infected individual, but quantities of the virus are also shed in the urine and feces. The virus generally enters the body via the respiratory tract by infecting alveolar macrophages, and then spreads rapidly throughout the lymphatic system (Ludlow *et al*. 2014). Infection of lymphatic cells, particularly T and B lymphocytes, and the severity of the resulting immunosuppression dictates the outcome of the disease (Green & Appel 2006). By the second week of infection the virus spreads to epithelial cells, resulting in respiratory and gastrointestinal signs as well as viral shedding in the urine (Ludlow *et al*. 2014). The virus also enters the brain by crossing the blood–brain barrier, or migrating along the olfactory nerve (Ludlow *et al*. 2014). Many animals die during the initial stages of the disease, but a proportion of the survivors may relapse some time later, with a progression of neurological signs (including behavioral changes, muscle twitching and seizures) as replication continues in the brain. Dogs may continue to shed the virus for up to 60 days (Green & Appel 2006), but captive tigers have been reported to shed the virus in urine for at least 150 days (V. Keahey 2014, pers. comm.), although this was based on the results of molecular testing (reverse transcription polymerase chain reaction [RT-PCR]), and therefore the presence of viable virus cannot be confirmed. Pathological lesions consistent with CDV infection were still present in a captive tiger with progressive neurological disease 18 months after initial exposure (Blythe *et al*. 1983), and may be analogous to 'old dog syndrome' described in domestic dogs (Green & Appel 2006).

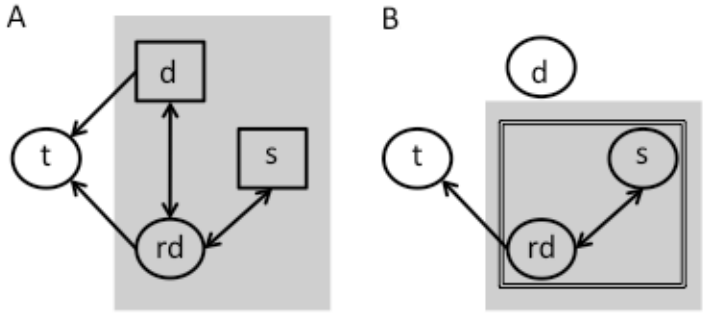
Most families within the order Carnivora are susceptible to CDV infection (Deem *et al*. 2000). However, the severity of clinical disease varies widely, being largely subclinical in some species (e.g. in domestic cats), while causing severe systemic disease leading to high mortality in others (e.g. ferrets) (Williams 2001). Clinical infections and mortality have been recorded in a number of felids, but to date all published reports have been within the genera of *Panthera* (including lion, tiger, leopard, *Panthera pardus*, *s*now leopard, *Panthera uncia*, and jaguar, *Panthera onca* [Appel *et al*. 1994]) and *Lynx* (including Canadian lynx, *Lynx canadensis*, Iberian lynx, *Lynx pardinus*, and bobcat, *Lynx rufus*) (Daoust *et al*. 2009; Meli *et al*. 2010). Antibodies to CDV without clinical disease or mortality have been reported in a number of other cat species (including puma, *Puma concolor*, cheetah, *Acinonyx jubatus*, Geoffroy's cat, *Leopardus geoffroyi*, and ocelot, *Leopardus pardalis* [Biek *et al*. 2002; Munson *et al*. 2004; Fiorello *et al*. 2007; Dales Nava *et al*. 2008; Thalwitzer *et al*. 2010; Uhart *et al*. 2012]), suggesting that susceptibility may vary within the Felidae. This is supported by the low competence of domestic cats as CDV hosts during experimental studies (Appel *et al.* 1974), and relates to differences in the

structure of the cellular receptor (CD-150 or signaling lymphocyte activation molecule [SLAM]) used by CDV to enter lymphoid cells (Ohishi *et al*. 2014). Clinical infections and mortality have also been recorded in other taxa, including rodents (Origgi *et al*. 2013), nonhuman primates (Yoshikawa *et al*. 1989; Sun *et al*. 2010) and peccaries (Appel *et al*. 1991).

The multi-host nature of CDV represents a particular threat to endangered populations in situations where they coexist with more abundant susceptible hosts, which can act as a reservoir of infection (see Fig. 1). The fortunes of many single-host pathogens are density-dependent, where a decline in host density (e.g. through infection-related mortality) leads to a reduced

Taken in isolation, populations of endangered species, such as tigers, are generally too small and at too low a density to maintain canine distemper virus (CDV) in the long term. These populations fall below a critical community size (CCS), beneath which a pathogen is unable to persist due to a depletion of susceptible hosts over time (Bartlett 1960). Multi-host pathogens, such as CDV, may represent a persistent threat to small populations, through regular spillover transmission from a pathogen reservoir. In the face of such complexity, a framework proposed by Haydon *et al*. (2002) for describing the constituents of a reservoir system provides a useful basis for understanding its functional dynamics. This defines a reservoir as one or more epidemiologically connected populations in which the pathogen can be permanently maintained and from which infection is transmitted to the defined target species (e.g. tigers). Individual populations that exceed the CCS, and can, therefore, maintain infection indefinitely are termed maintenance populations, although several non-maintenance populations could act synergistically to form a maintenance community. Finally, a source population is that which transmits infection directly to the target, and may either be a maintenance population, or be connected to the maintenance population as a transmission link to the target.

The structure and constituent populations within a CDV reservoir are likely to vary across the global tiger range depending on the diversity, density and demography of susceptible host species. In Russia, reservoir candidates include domestic dogs and abundant wild carnivores, including sable (*Martes zibellina*), red fox (*Vulpes vulpes*), raccoon dog (*Canis lupus familiaris*) and Eurasian badger (*Meles meles*). Two simplistic representations of possible reservoir structures in Russia are illustrated in diagrams A and B.



Populations can either be maintenance populations (squares) or non-maintenance populations (circles). Transmission of CDV occurs in the direction indicated by the arrows. In A, dogs (d) and sable (s) exceed the CCS and are maintenance populations, while only raccoon dogs (rd) and dogs act as source populations of CDV infection for tigers (t). In this case all 3 populations contribute to the reservoir (indicated in grey), and control measures would need to target both transmission from dogs and raccoon dogs to tigers. In B, no individual population exceeds the CCS, but transmission between raccoon dogs and sable is such that the 2 populations can form a maintenance community (represented by the black frame). In this case raccoon dogs represent the only source of infection for tigers, and control measures would need to target either one or both of the populations contributing to the maintenance community, +/or the transmission of virus from raccoon dogs to tigers. Clearly, these are just examples, and many other possible combinations exist. However, successful control of CDV requires management of infection in maintenance populations or communities and/or their transmission linkages with the tiger population.

Figure 1 Defining the canine distemper virus reservoir.

opportunity for infection. By contrast, more cosmopolitan multi-host pathogens may continue to infect rare host species in areas where a reservoir continues to act as a source of the virus, even as the endangered population declines. Outbreaks of CDV have been implicated in population declines and near extinction of several wildlife species, including the African wild dog, *Lycaon pictus* (Fanshawe *et al*. 1991), the Santa Catalina Island fox, *Urocyon littoralis catalinae* (Timm *et al*. 2009), and the black-footed ferret, *Mustela nigripes* (Thorne & Williams 1988).

Even in susceptible species, the epidemiology of CDV can be complex. For instance, CDV has been implicated in local population declines of lions and African wild dog in several areas in East Africa (Fanshawe *et al*. 1991; Roelke-Parker *et al*. 1996). However, in southern Africa, populations of these species have remained stable, despite high levels of CDV exposure (Alexander *et al*. 2010). Alexander *et al.* propose that habitat heterogeneity in southern regions led to a more complex host population structure, limiting the spread of outbreaks and enabling recolonization from surrounding areas in the wake of local extinctions. However, even in the more homogeneous grassland environments of East Africa, CDV-induced losses are not inevitable, with multiple waves of CDV exposure evident in the serology profiles of the lion populations without coincident sickness or population impact (Munson *et al*. 2008; Viana *et al.* 2015). Overt outbreaks among the lions of Serengeti in 1994 and Ngorogoro in 2001 were attributed to climatic patterns resulting in high vector numbers, with mortality from CDV associated with *Babesia* infection loads (Munson *et al*. 2008). The involvement of viral co-infections has been implicated in other cases of CDV mortality (Fix *et al*. 1989; Burtscher & Url 2007; Origgi *et al*. 2013), and, therefore, it is important to consider these, or other physiological stressors as a precursor to disease. In spite of this, apparently uncomplicated CDV infections have led to mortality in captive tigers in North America, Europe and Asia, and so it appears that clinical outcome is not always dependent on co-infections (Appel *et al*. 1994; Nagao *et al*. 2012; Seimon *et al*. 2013). This may be due to variation in the virulence of different CDV strains, although it should be noted that genetically diverse strains have caused mortality in *Panthera* species without apparent co-infections (including viruses from the Arctic-like, North America-2 and Asia-1 clades) (Appel *et al*. 1994; Nagao *et al*. 2012; Seimon *et al*. 2013).

CANINE DISTEMPER VIRUS IN AMUR TIGERS

Comparatively more is known about the health of wild tigers in Russia than any other range country, as samples are routinely collected whenever live or dead tigers are handled. Serum collected from tigers immobilized during the placement of telemetry collars and in response to tiger–human conflict situations provides a baseline for assessing pathogen exposure (Goodrich *et al*. 2012; Naydenko *et al*. 2012). No CDV antibodies were detected in 27 tigers sampled from 1992 to 1999, suggesting that tigers at this time were not exposed to the virus (Goodrich *et al*. 2012). However, Goodrich *et al*. (2012) report antibodies to CDV in 6 of 13 tigers captured between 2000 and 2004, suggesting the introduction of CDV into this population during the early 2000s. In November 2003, a tigress captured in the village of Pokrovka, Khabarovskii Krai (46.69°N, 134.03°E) was taken into care but died five weeks later (Quigley *et al*. 2010). Although ambulatory at the time of capture, this tigress was non-responsive to stimuli and unafraid of humans. She was later confirmed as the first case of CDV in a wild tiger (Seimon *et al*. 2013).

Further cases of CDV in Amur tigers were confirmed in 2010. These included a 3–4-year-old male captured near the village of Aleksayevka, Primorskii Krai (43.56°N, 132.00°E) during February 2010, and an 8.5-year-old tigress who entered the village of Ternei, Primorskii Krai (45.04°N, 136.78°E) and was shot on 1 June 2010 (Seimon *et al*. 2013). A third case in 2010 has recently been confirmed based on sequences obtained from archived tissues and involved an adult male tiger that was shot close to Ternei in January 2010 (Gilbert *et al.* 2014, unpubl. data). All of these animals displayed neurological signs and were unafraid of humans. Video footage of a tiger behaving in this characteristic manner was taken along the Vladivostok-Khabarovsk highway between the towns of Vyazemski and Bikin, Khabarovskii Krai during the spring of 2010 (http://tinyurl.com/las2yt7). Although this animal later died in care, no samples were available for analysis; therefore, CDV could not be confirmed in this case.

CANINE DISTEMPER VIRUS IN SIKHOTE-ALIN BIOSPHERE ZAPOVEDNIK

One of the most closely monitored populations of Amur tigers inhabits the Sikhote Alin Biosphere Zapovednik (SABZ) in Primorskii Krai. The reserve is of sufficient size to hold territories for 11 breeding females (assuming a territory of 384 km^2, with average overlap of 11% between adjacent female territories) and 4 breeding males (assuming a territory of 1160 km^2, with average overlap of 14% between adjacent male territories), and lies within a wider matrix of suitable habitat that enables tigers to disperse to and from surrounding areas. This protected area limits access, allowing only rangers and researchers, such that tigers in core areas may rarely, if ever, encounter humans. However, four villages (inhabited by between 67 and 5350 people in 2010) and a small number of isolated dwellings lie outside the protected area and represent a source of contact for tigers with territories along the reserve boundary, as well as individuals without territories that may move more widely through the landscape.

One of the confirmed CDV cases in 2010, the 8.5-year-old tigress known as T02 (referred to as Pt 2010-3 in Seimon *et al*. 2013), held a territory along the southern border of SABZ (Figs 2a and 3). This tigress had been captured in 2002 and 2005 as part of a telemetry study, yet no CDV antibodies were detected from routine samples. She was subsequently recaptured on 24 March 2010, by which time CDV antibodies were circulating (with a virus neutralization titre of 1:256 measured at the Washington Animal Disease Diagnostic Laboratory, Pullman, WA, USA). In view of subsequent events, and the strong protective immunity that develops in animals that survive infection, it is likely that T02 was already infected by March 2010.

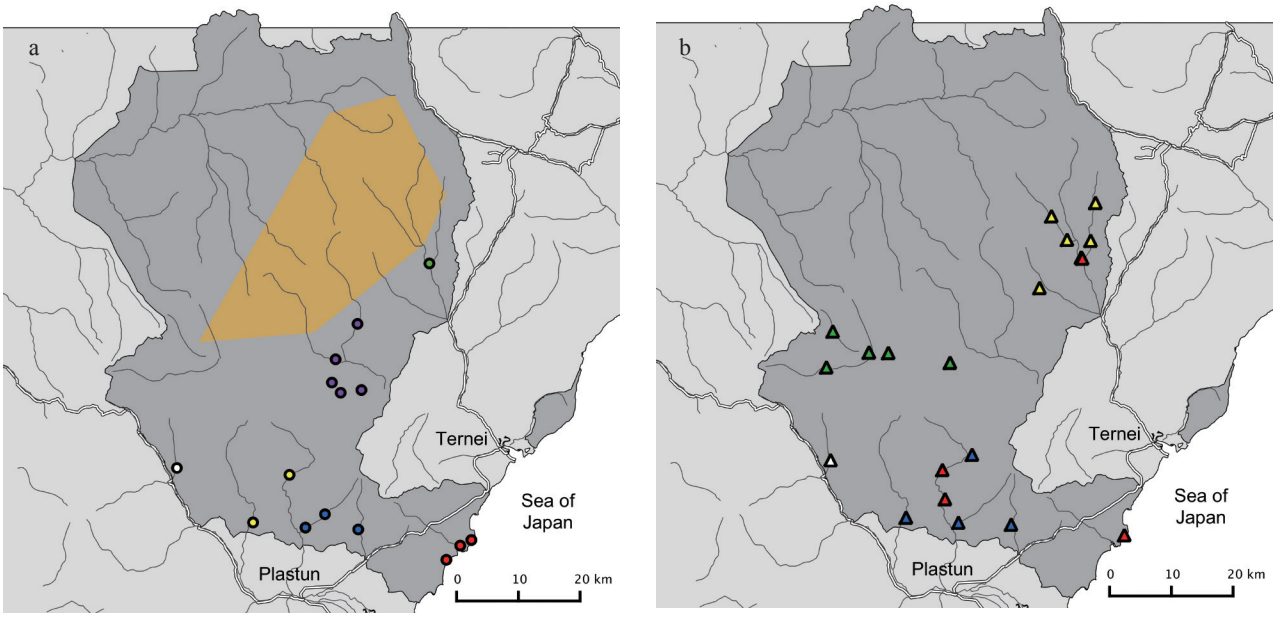


Figure 2 (a) Locations of resident female tigers in Sikhote-Alin Biosphere Zapovednik (dark grey). Map includes rivers (black lines) and roads (double lines). Tigresses are illustrated by circles, and include T02 (red), T5 (blue), T6 (yellow), T7 (green), T14 (purple) and T21 (white). Locations refer to camera trap captures made during 2009 and 2010, with the exception of T14, where captures from 2007 and 2008 are used (as this tiger was not photographed in 2009 or 2010). The home range of a further tigress (T47) is represented using a minimum convex polygon (orange), based on telemetry positions obtained during November and December 2009. (b) Locations of resident male tigers in Sikhote-Alin Biosphere Zapovednik (dark grey). The map includes rivers (black lines) and roads (double lines). Tigers are illustrated by triangles, and include T10 (green), T15 (yellow), T16 (red), T19 (white) and T27 (blue). Locations refer to camera trap captures made during 2009 and 2010, with the exception of T10, T15 and T19, where captures from 2007 and 2008 are used (as these tigers were not photographed in 2009 or 2010).

Tiger ID number	Estimated year of birth	Sex	Status	Date of last record	2006	2007	2008	2009	2010	2011	2012	Outcome
T02	2001	F	R	1 June 2010								Mortality (CDV confirmed)
T03	~1992	F	R	2007								Mortality (poached)
T04	~1998	M	R	2007								Mortality (poached)
T05	2001	F	R	27 October 2009								Mortality (unexplained)†
T06	2004	F	R	1 November 2009								Disappeared (unexplained)
T07	UNK	F	R	6 November 2009								Disappeared (unexplained)
T08	2006	F	DC	2008								Dispersed to North SABZ
T09/PT85	UNK	M	R	6 December 2007								Mortality (unexplained)
T10	UNK	M	R	2007								Disappeared (unexplained)
T14	UNK	F	R	Alive 2013								Alive (circa 2013)
T15	UNK	M	R	2007								Disappeared (unexplained)
T16/PT90	~1999	M	R	January 2010								Mortality (CDV confirmed)
T17/PT80	2005	F	R	16 November 2007								Mortality (poached)
T18/PT89	2006	M	DC	30 July 2008	*							Disappeared (dispersed?)
T19	UNK	M	R	February 2011	>							Mortality (natural)‡
T20	2006	F	DC	8 December 2008	*							Disappeared (dispersed?)
T21	UNK	F	R	13 April 2011		>						Disappeared (unexplained)
T25/PT88	2006	M	DC	22 September 2008	*							Emigrated from SABZ
T26/PT35	1993	F	R	2007								Disappeared (unexplained)§
T27	UNK	M	R	Alive 2013			>					Alive (circa 2013)
T29/PT96	2008	M	DC	17 January 2010			*					Disappeared (dispersed?)
T05 Cub A	2008	UNK	DC	2009			*					Disappeared (unexplained)
T05 Cub B	2008	UNK	DC	2009			*					Disappeared (unexplained)
T47/PT97	2008	F	R	11 December 2009			*					Mortality (unexplained)†
T30	UNK	M	R	Alive 2013					>			Alive (circa 2013)
T32/PT100	2006/07	M	R	December 2011				>				Mortality (poached)
T02 Cub A	2010	F	DC	May 2010				*				Mortality (CDV related)
T02 Cub B	2010	F	DC	May 2010				*				Mortality (CDV related)
T02 Cub C	2010	F	DC	May 2010				*				Mortality (CDV related)
T33	2010/11	F	DC	December 2011					*			Disappeared (dispersed?)
T34	2010/11	M	DC	December 2011					*			Disappeared (dispersed?)
T21 Cub A	2010	UNK	DC	2011					*			Mortality (natural)¶
T21 Cub B	2010	UNK	DC	2011					*			Mortality (natural)¶
T35/PT114	2009	F	R	Alive 2013					>			Alive (circa 2013)
T35 Cub A	2012	UNK	DC	Alive 2013							*	Alive (circa 2013)
T35 Cub B	2012	UNK	DC	Alive 2013							*	Alive (circa 2013)
T35 Cub C	2012	UNK	DC	Alive 2013							*	Alive (circa 2013)
PT95	2004	M	UNK	8 November 2009			>					Disappeared (dispersed?)††

Figure 3 A summary of camera trap captures of tigers in the central and southern regions of Sikhote Alin Biosphere Zapovednik (SABZ) between 2006 and 2013. Details of individual tigers include identity code, estimated year of birth, sex (F = female, M = male, UNK = unknown), status (R = resident, DC = dependent cub), the date and circumstances of last sightings. Identifiers with the prefix T refers to tigers recorded by camera trap, and the prefix PT refers to tigers fitted with radio collars. Both systems are used here to facilitate comparison with other publications. Transient tigers (recorded in only a single year) are excluded, as outcome could not be determined. Annual status of each tiger is indicated for animals captured at least once (dark green), not captured and presumed absent (yellow), not captured but subsequently confirmed (light green), or not surveyed for (grey). The timing of births are indicated by blue asterisks, and confirmed tiger deaths are indicated by cells outlined in red. Additional notes on the circumstances of tiger deaths and disappearances are provided as footnotes. The arrival of immigrants is indicated using blue arrows. (†Scavenged/predated by large carnivore. ‡Killed by another tiger. §Likely old age [14 years]. ¶Killed by T19. ††Possible transient. CDV, canine distemper virus.

Antibodies to CDV appear after 10 to 20 days post-infection in dogs (Green & Appel 2006), which if comparable in tigers would suggest an infection lasting at least 80 to 90 days in this tigress. By 1 May, T02 localized her movements, and (as was later confirmed) gave birth to a litter of three cubs. Although T02 had proven to be a typically attentive mother when raising her three prior litters, on this occasion her behavior was unusual, leaving the den for several days at a time before finally abandoning her cubs entirely on 17 May. She was subsequently observed at a nearby military outpost, before entering Ternei, where she was shot on 1 June 2010 to prevent injury to local residents. The presence of CDV was confirmed in brain tissue collected from T02, by sequencing of amplified gene products, and demonstration of consistent pathology (Seimon *et al* 2013). All three of her cubs consequently died. Evidence of CDV was not found in samples collected from 1 of those cubs, although decomposition may have hampered test sensitivity.

A recent re-examination of tissues collected from another SABZ tiger, T16 (referred to as Pt 2010-1 in Seimon *et al*. 2013) has confirmed that he was infected with CDV at the time of death (Gilbert *et al.* 2014, unpubl. data, Fig. 3). This tiger was an 11-year-old male, who occupied a territory that encompassed that of T02. On 31 December 2009, T16 approached and killed a local fisherman close to a group of houses 10 km west of Ternei. In common with other CDV cases, T16 displayed an unusual lack of fear, remaining in the open until he was shot and killed the following day. T16 was recorded in association with T02 in the fall of 2009. Assuming that T16 had sired the litter of T02, then mating must have occurred just a few days prior to his death (given a gestation period of 98–111 days [Wack 2003]). In captive tigers mortality from CDV usually occurs within days or weeks of developing clinical signs (Gould & Fenner 1983; Appel *et al*. 1994; Konjevic *et al*. 2011; Nagao *et al*. 2012; V. Keahey, pers. comm.), but the length of the refractive period (before clinical disease is evident) remains unknown, and a delayed onset may be possible (Blythe *et al*. 1983). Therefore, it is conceivable that T02 contracted her infection through contact with T16.

The deaths of T16, T02 and her 3 cubs coincided with a period of heavy losses for the SABZ tiger population (Fig. 3). Population estimates for the whole of SABZ based on snow tracking data indicated a decline from 25 tigers in 2008 to 9 by 2012 (Fig. 4). Camera trap surveys carried out in the central and southern sections of SABZ provide a more detailed account of the numbers and movements of a subset of the reserve's tigers, and highlight a similar decline (Fig. 3 [Soutyrina *et al*. 2013]). The population of 15 tigers identified on camera traps in 2008 (representing a minimum population) had declined to 7 identified by the start of 2011 (Table 1). Determining the cause of death in cryptic spe-

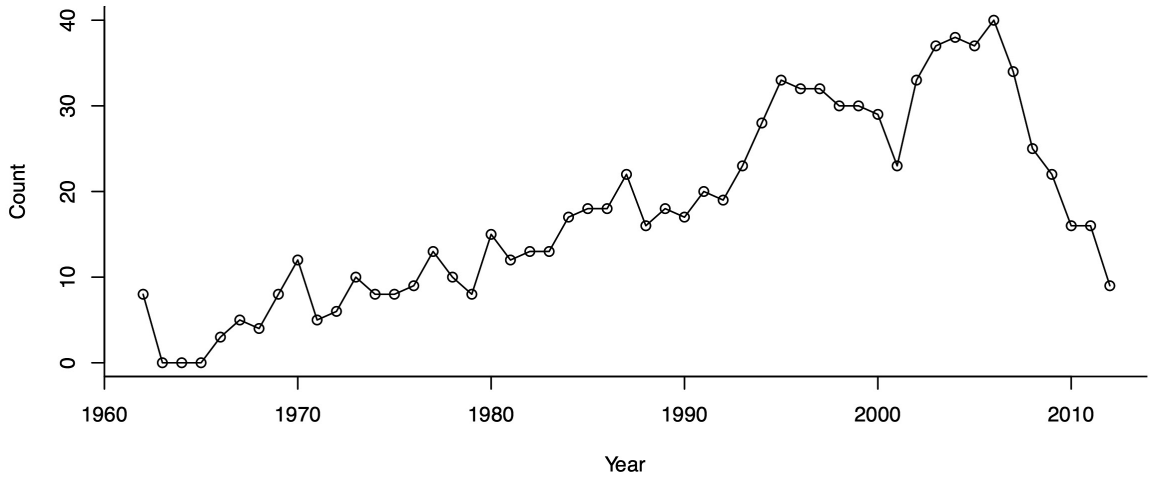


Figure 4 Annual tiger population estimates based on snow track surveys of Sikhote-Alin Biosphere Zapovednik from 1962 to 2012. Surveys are conducted annually from December through February along transects throughout the entire reserve.

Table 1 Population demographics of tigers in Sikhote-Alin Biosphere Zapovednik based on camera trapping surveys of central and southern regions of the reserve during 2006–2013. Numbers represent minimum estimates based on individual identifications of all tigers captured during camera trap surveys

Year	Tigers at year start (minimum)	Immigrants	Births	Deaths	Disappear	Emigrants	Transient
2006	?	0	1	?	?	?	4
2007	14	1	3	3	0	0	0
2008	15	2	2	2	3–5	2	0
2009	10–12[†]	1	2	3	0–2	0	1
2010	10	1	5	6	3	0	0
2011	7	2	2	3	0	0	1
2012	8	1	3	1	3	0	1
2013	8	4	8	?	?	?	0

"Deaths" refers to confirmed mortalities (e.g. where a body was recovered, or intelligence indicated a poaching incident), "Disappear" indicates absence of tigers where cause is unknown. [†]Two tigers (T10 and T15) disappeared some time during the years 2008 and 2009. However, no camera traps were set within their territories during this period to confirm the timing of their disappearance. For this reason a range of values is used to express the minimum number of tigers present at the start of 2009.

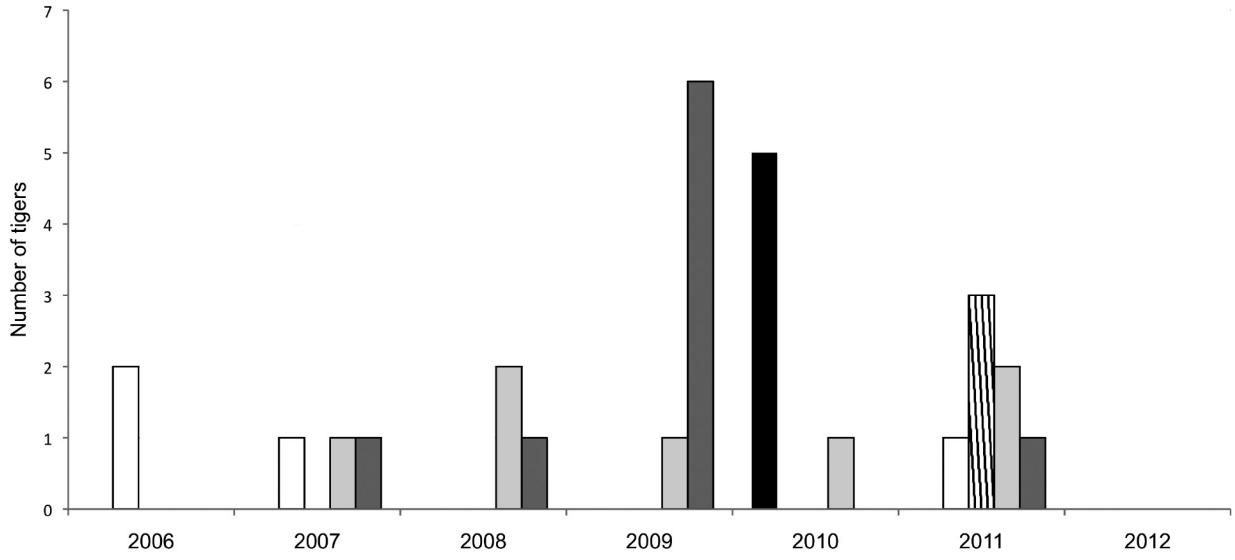


Figure 5 Annual tiger mortality and disappearances in Sikhote-Alin Biosphere Zapovednik between 2006 and 2012 attributed to poaching (white), canine distemper virus (CDV)-related (black), dispersal (confirmed or suspected based on disappearance at an age appropriate for dispersal [light gray]), natural (confirmed mortalities unrelated to humans and excluding CDV-related [cross hatch] and unexplained [dark grey]).

cies like tigers can be challenging, particularly in remote areas such as SABZ. However, the unexplained death or disappearance of 6 tigers during 2009 was unusual (Fig. 5), and it is possible that several of these may have been related to CDV infections that were undetected.

During late 2009 a resident adult tigress (T05, Fig. 3) was found dead, with no sign of her litter of 2 or more dependent cubs (although at approximately 1.5 years of age these tigers may have dispersed beyond the study area). The body of a second younger tigress (T47) was

found several months later (Fig. 3). Both of these carcasses had been eaten by a bear or other large carnivore, although it was unclear whether this was the result of predation or scavenging. Consequently, the cause of death in these cases is open to speculation, and it is likely that a young newly independent tigress such as T47 could have succumbed to any of a number of possible dangers. However, T05 shared a territory with T16, the likely father of her cubs (Fig. 2), as did another resident adult tigress (T07) that was last recorded by camera trap on 6 November 2009 (Figs 2 and 3). A neighboring tigress (T06) also disappeared in late 2009, with the last camera trap record on 1 November 2009 (Fig. 3). Mortalities unrelated to CDV continued in early 2011, with the death of 2 dependent cubs attributed to infanticide carried out by an adult male (T19), who also succumbed soon after to injuries sustained in a fight with another tiger (Fig. 3). In May 2011, the carcass of a female with enlarged nipples (which suggested she was still nursing a litter) was found with 2 bullet wounds (This tiger was not captured or recorded on camera traps, and so is not included in Table 1).

These results suggest that there were multiple causes of death occurring in the SABZ population within a small (18-month) timeframe (late 2009–early 2011) (Fig. 3). CDV was not solely responsible for the dramatic declines in SABZ tigers during 2009 and 2010, but in a worse case scenario (including the unknown causes that might have been disease-related) it is possible that as many as 6 adults/subadults succumbed to the virus, and at least 1 litter of 3 was lost because their mother was diseased. This additive mortality factor (Robinson *et al*. 2015) demonstrates the vulnerability of small tiger populations to stochastic events. The SABZ population has benefited from the continuity of habitat, which has enabled immigration of tigers from surrounding areas, and with continued protection and successful reproduction, recovery is already occurring (a minimum of 20 tigers were recorded by winter 2014). For smaller tiger populations, or those that are more isolated, the likelihood of withstanding additive losses similar to those occurring in SABZ during 2009 and 2010 would be considerably lower.

The close monitoring of tigers in SABZ enables a detailed reconstruction of individual life histories of the tiger population during the period that CDV was circulating in 2009 and 2010. However, even in this intensively monitored sub-population we are limited to best-guess estimates of the impact that CDV had on the tiger population in the reserve. Beyond SABZ, tigers with confirmed and suspected cases of CDV in 2010 occurred in disparate locations 300–500-km apart, near human habitation and in distant corners of the Amur tiger's range. It is unknown whether the proximity of these tigers to human habitation increased opportunities to contract CDV, or merely the chance that cases would be reported. However, with the majority of Amur tigers occupying vast, largely uninhabited areas, it is possible that other tigers may have succumbed to CDV during the 2009–2010 period without detection. Yet with so many uncertainties relating to the epidemiology of CDV across the Amur tiger range, there is a limit to the inferences that can be drawn from the SABZ outbreak, and the extent to which the overall Amur tiger population may have been affected. Under these circumstances, population modeling can be a valuable tool to explore the key determinants that influence the impact of CDV on tiger populations. Recent models have shown that even modest levels of tiger contact with a CDV reservoir will impact population growth, and that small and isolated tiger populations are disproportionately impacted (Gilbert *et al*. 2014). Refinements of models such as this require a more detailed understanding of reservoir composition and dynamics, if they are to provide further insights into the threat to a particular tiger population.

UNDERSTANDING RESERVOIR STRUCTURE

An understanding of local reservoir structure is a critical first step to begin assessing the impact that a multi-host pathogen will have on a population, and is an important precursor to the design of management practices. Defining a reservoir is complex, but a framework proposed by Haydon *et al*. (2002, and summarized in Fig. 1) provides a useful means of conceptualizing alternative structures. All populations of tigers share habitat with a number of susceptible species that could contribute to the local CDV reservoir as maintenance or non-maintenance hosts depending on their susceptibility, population size, turnover and frequency of effective contacts. More abundant susceptible hosts are likely to have a greater contribution to CDV maintenance, and in the context of the Russian Far East this is likely to include domestic dogs, and small or medium-bodied wild carnivores, particularly raccoon dogs, *Nyctereutes procyonoides*, red foxes, *Vulpes vulpes*, Eurasian badgers, *Meles meles*, and sable, *Martes zibellina*. Tigers prey on each of these host species, providing a likely route for CDV transmission (Miquelle *et al*. 1996; Ludlow *et al*.

2014). Underscoring this potential route of exposure, rangers in SABZ reported mortalities of red foxes and raccoon dogs from an unidentified disease in both 2009 and 2010. Although tigers are largely solitary, they do interact regularly, albeit infrequently, providing a potential mechanism for tiger-to-tiger transmission (Goodrich *et al*. 2010). Aside from contact between mother and cubs, intra-specific contact is likely to be greatest between territorial tigers of the opposite sex, and in Russia these contacts occur around 1 or 2 times per month (Goodrich *et al*. 2010). In other tiger populations where tigers occupy smaller home ranges, and occur in higher densities, these interactions are likely to be more frequent, potentially increasing the rate of tiger-to-tiger transmission.

In the Russian Far East domestic dogs occur at comparatively low densities compared to other parts of the world. Due to harsh climatic conditions, feral dog populations are almost non-existent, with most animals relying on provisioning by humans for survival. Based on 2010 census data there were almost 2 million people in Primorskii Krai, of which more than 75% resided in urban centers and were, therefore, unlikely to come into contact with tigers. The remaining population is sparsely distributed across the landscape, at mean densities as low as 2.83 people/km^2. Based on preliminary estimates of human : dog ratios, this would equate to a mean density of approximately 5.10 dogs/km^2, dramatically lower than the dog density of 719 dogs/km^2 recorded in Maharashtra, India (Belsare & Gompper 2013), suggesting that the contribution of domestic dogs to the CDV reservoir may be much more important in other parts of the tiger range.

OTHER FACTORS POTENTIALLY INFLUENCING CANINE DISTEMPER VIRUS ECOLOGY IN RUSSIA

Due to the relative fragility of CDV virion to environmental conditions (e.g. heat, desiccation and ultraviolet radiation), transmission is typically thought to require close contact between infected individuals (Green & Appel 2006). However, considering the extreme cold of the Russian winter, viability could be prolonged, with the virus persisting for extended periods outside the host, raising the potential for indirect modes of transmission. Most local carnivore species will scavenge from carcasses in the forest, including tiger kills. Several carnivores, particularly canids, are known to scent mark, urinate or defecate on or around food (Goszczynski 1990), and as CDV is shed in both feces and urine, and as the virus has a half life of 9–11 days at 4°C (Appel 1987), contaminated carcasses could remain infectious for an extended period. A similar mechanism could facilitate indirect transmission between tigers, through use of urine scent marks on trees and landmarks that are regularly visited by territorial tigers of both sexes, as well as non-territory holders that may be passing through.

Although other carnivore species represent the most likely source of CDV infection for tigers, it should be noted that the virus has been associated with infections in non-carnivores, including Artiodactyls (Appel *et al*. 1991; Noon *et al*. 2003; Kameo *et al*. 2012). An outbreak of CDV in collared peccaries, *Tayassu tajacu*, in Arizona was associated with high mortality (Appel *et al*. 1991), and the virus was found to be common and enzootic in the population (Noon *et al*. 2003). While such a severe clinical syndrome has not been recorded in other ungulates, viraemia has been demonstrated in domestic pigs following experimental exposure (Appel *et al.* 1974), and antibodies to CDV (indicating prior exposure) were found in 11/41 wild boar, *Sus scrofa*, and 2/5 sika deer, *Cervus nippon*, tested in Japan (Kameo *et al*. 2012). Amur tigers prey on boar or deer with far greater frequency than carnivore species. While these ungulates may be unlikely contributors to a reservoir, they could enhance effective contact between tigers and the reservoir. A potential scenario could arise if wild boar were to contract subclinical infections when scavenging the carcasses of infected carnivores, and transmit the virus when subsequently predated by a tiger. Such a scenario remains unsubstantiated, but worthy of study.

POTENTIAL CONTROL MEASURES

Options for managing the impact of CDV infections on tiger populations will depend on the structure of the local reservoir, the mechanism of viral maintenance and the source of infection for the tigers. Intervention strategies for managing disease in wildlife are often expensive, and so it is important that control measures are weighed against the risk that CDV represents to the tiger population, are kept proportional and are achievable (Woodroffe 1999). In principal, potential management strategies could be directed at the control of disease in the target tiger population, at blocking transmission between the target and source population, or at the maintenance population(s) that contribute to the virus reservoir (Haydon *et al*. 2002). Each of these strategies requires progressively more understanding of the reservoir structure to ensure confidence of success.

Strategies directed at target populations could theoretically include treatment of infected individuals or immunization (Woodroffe 1999). At present, antiviral therapies are of limited use in treating CDV, although the development of pharmaceuticals that block the RNA polymerase enzymes utilized during CDV replication could lead to applications in the treatment of affected individuals (Krumm *et al*. 2014). This is unlikely to represent a solution in the Russian context, where there is a low probability of encountering infected tigers, but could be considered in higher density populations that can be monitored more closely.

Contemporary vaccines fall into two main categories: modified live vaccines (MLV [grown on canine or avian kidney cell lines]); and recombinant vaccines that use a canarypox vector to present CDV antigens to the immune system. Each of these has innate advantages and disadvantages. While MLVs can induce a strong and long-lasting immunity in many species, older MLVs (particularly those derived from canine cell culture such as Rockborn or Snyder Hill strains) can cause sickness and death in select taxa (McCormick 1983; Montali *et al*. 1983). New generation MLVs have been used successfully in a limited trial in lions (Kock *et al*. 1998), and offer potential for use in tigers. However, it would be important to verify their safety and immunogenicity in captive tigers before their use was proposed in a wild population. Recombinant vaccines are safer, but produce a less pronounced immune response that requires multiple doses to induce life-long immunity. One major disadvantage of both vaccine classes is that they are only available in injectable form, presenting a major challenge for delivery to most free-ranging tigers.

Strategies to block transmission from the reservoir to tiger populations are limited, particularly if wildlife constitute an important source of infection. Measures to reduce dog predation, such as preventing access to tiger habitat, could be beneficial in theory, but are unlikely to be socially acceptable where licensed hunters extensively use dogs, as they do in the Russian Far East.

Attempts to control CDV in the reservoir require a detailed understanding of maintenance host identity. Potential strategies include measures to reduce the density of maintenance populations, or to increase their immune status. Vaccination has been very effective in controlling CDV among domestic dogs in many developed countries, but may be less successful where a large proportion of the dog population is free-roaming and cannot be restrained (Belsare 2013). Strategies that target unvaccinated puppies might be more successful, as older dogs are more likely to have encountered the virus, and may be less important to CDV circulation (Belsare 2013). Reduction of dog populations through responsible ownership combined with vaccination of puppies might have the greatest chance of success. However, in situations where wildlife are important contributors to CDV, maintenance control will be extremely difficult, as the lack of an oral vaccine, and low efficacy and ethical issues associated with wildlife population control prohibit management of CDV in a wild reservoir (Woodroffe 1999).

CRITICAL STEPS NEEDED TO ASSESS AND MONITOR THE THREAT OF CANINE DISTEMPER VIRUS

As CDV is known from all countries where tigers occur, the virus represents a potential threat to wild tigers throughout their range. While the diversity of CDV susceptible hosts may vary across tiger range countries, abundant populations of domestic dogs and/or wild carnivores, acting alone or in concert, could represent a CDV reservoir, and source of infection for tigers. Wildlife managers and veterinarians in tiger range countries should be encouraged to introduce the following measures as a first step to assess the risk that CDV represents for their tiger populations:

1. Recognize and diagnose clinical cases of CDV in tigers when they occur: CDV should be considered among the differential diagnoses for any tiger that displays behavioral or neurological abnormalities. Previous cases of CDV in tigers have presented with some or all of the following: fearlessness, sensory deficits (e.g. blindness), ataxia and or muscular tremors, as well as general poor body condition. Behavioral changes, particularly loss of fear, may predispose animals to situations of human–tiger conflict. Suspected cases can be confirmed through detection of genetic sequences specific to CDV (e.g. using RT-PCR or equivalent techniques). Post mortem samples such as brain tissue has the greatest diagnostic value during the later stages of infection when infected tigers are likely to present, but other samples that may facilitate diagnosis include lymph node, lung, spleen, bladder, urine and whole blood (or fractionated blood containing leucocytes such as buffy coat). Confirmation of ante mortem cases can be more challenging, as virus may no longer be detectable in the respiratory tract or circulatory system by the time animals present. In such cases detection of virus in conjunctival or respiratory swabs, whole blood or urine would be diagnostic,

but negative results need not imply an absence of CDV infection.

2. Collect baseline data on the health of wild tigers: Every effort should be made to take full advantage of opportunities to collect samples from live or dead tigers. Collection of at least minimal sample sets including serum should take place whenever tigers are handled (whether healthy or sick), and post mortem examinations should be performed (including collection of brain tissue) whenever carcasses are found. Samples need not be analyzed immediately, particularly where laboratory resources are limited, but should be archived in secure facilities and be clearly labeled, sufficient to link material to corresponding sampling data. Appropriate storage includes freezing at or below −20 °C (for serum, fresh tissue and samples stored in media for maintaining nucleic acid such as RNA later) or maintaining at room temperature (for tissues fixed in 10% formalin). It should be emphasized that these are minimal sample sets that would be sufficient to detect antibodies to CDV (indicating prior exposure) or diagnose active CDV infections. More comprehensive sets of diagnostic samples would enable a more extensive assessment of tiger health. However, it is recognized that those involved in the handling of live or dead tigers often face a variety of constraints, including access to supplies and cold storage facilities, expertise and available time. Therefore, we encourage wildlife managers to adapt protocols, and ensure adequate supplies are available to take full advantage of sampling opportunities in their circumstances.

In the event that CDV is detected in tigers in other areas, further research would be required to assess the risk that this represents at a population level. This could include epidemiological modeling, and research directed at the reservoir to determine species composition and dynamics of CDV circulation. Such research would be vital to assessing the need for any intervention, and to identify control strategies that might be appropriate. However, ultimately, due to the problems inherent in the available control methods, and the limitations of the virus itself to spread, the most viable management strategy would be to maintain tigers in large and inter-connected populations that are able to withstand CDV outbreaks should they occur. This recommendation, of course, is in concordance with existing conservation strategies for most wildlife populations.

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Appendix II. Confirmed and suspected cases of canine distemper virus in tigers and other large felids in the Russian Far East

PT61/Pt 2004, 'Morka':

In November 2003, a tigress captured in the village of Pokrovka, Khabarovskii Krai (46.69°N, 134.03°E, Figure II) was taken into care but died 5 weeks later (Quigley et al.2010). Although ambulatory at the time of capture, this tigress was non-responsive to stimuli and unafraid of humans. She was later confirmed as the first case of CDV in a wild tiger (Seimon et al.2013). As part of the current project, further RNA was extracted from formalin-fixed paraffin embedded (FFPE) brain tissue from PT61/Pt 2004, and leading to the reconstruction of approximately 99% of the full CDV genome using Illumina methods (Chapter 4). The sequence obtained from this tiger has been submitted to GenBank (KX774415).

PT90/Pt 2010-1/T16, 'Ivan':

This tiger was an 11-year-old male, who occupied a territory in Sikhote-Alin Biosphere Zapovednik (SABZ) that encompassed that of PT56 (see below). On 31 December 2009, PT90 approached and killed a local fisherman close to a group of houses 10 km west of the village of Ternei. In common with other CDV cases, PT90 displayed an unusual lack of fear, remaining in the open until he was shot and killed the following day. Although no brain tissue was collected following his death, approximately 3.6% of the CDV genome was sequenced from a (FFPE) sample of lymph node using Illumina sequencing (Chapter 4). The detection of CDV in this case raises questions about the possible role that CDV might have in precipitating incidents of human-tiger conflict.

Pt 2010-2:

During February 2010, a 3–4-year-old male tiger was captured near the village of Aleksayevka, Primorskii Krai (43.56°N, 132.00°E). This tiger was moderately poor body

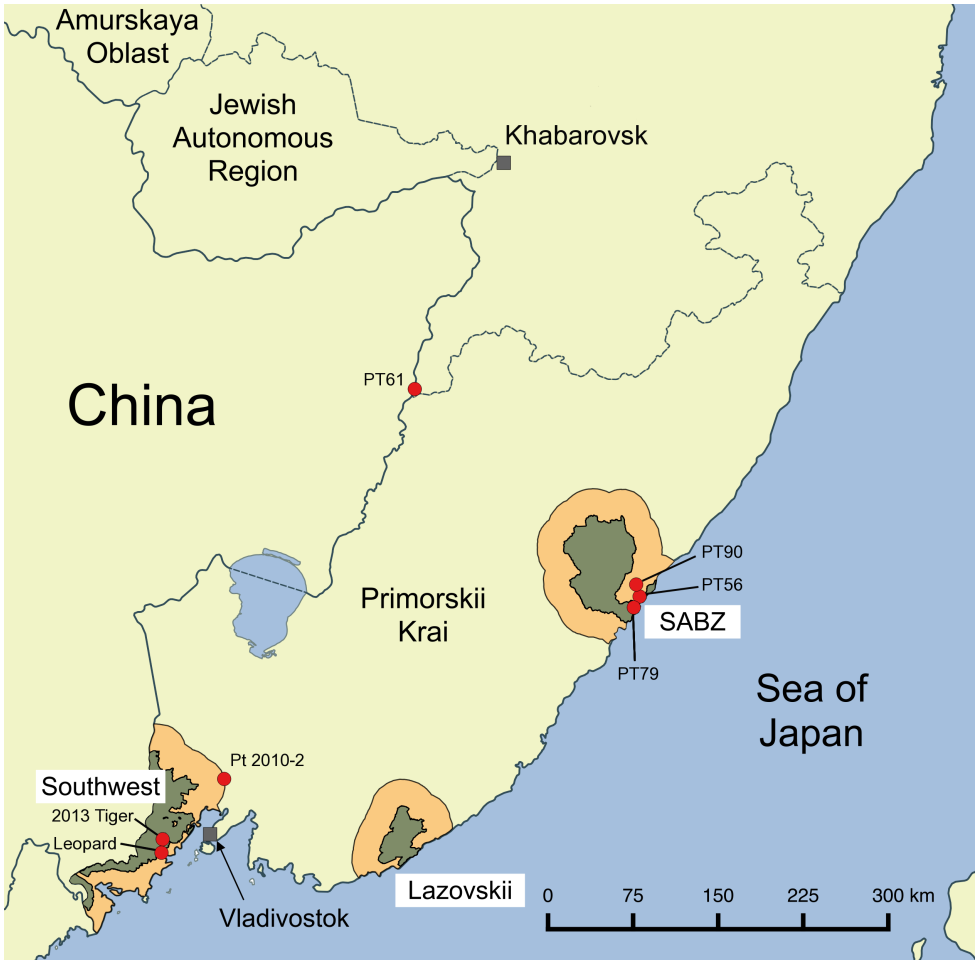

Figure II.I. Map illustrating the location of clinical cases of canine distemper virus in *Panthera* spp. In Primorskii Krai, Russia.

condition when first encountered, and exhibited a lack of fear of vehicles and people. A video of the tiger's capture is available here: http://tinyurl.com/h7yvljb. Following capture, the tiger's condition deteriorated, he became unresponsive to stimuli and subsequently died while in care. A 114 bp fragment of the CDV P-gene was sequenced from this tiger, confirming the diagnosis (Seimon et al.2013).

PT56/Pt 2010-3/T02, 'Galia':

This 8.5-year-old tigress held a territory along the southern border of SABZ. The tigress had been captured in 2002 and 2005 as part of a telemetry study, yet no CDV antibodies were detected from routine samples. She was subsequently recaptured on 24 March 2010, by which time CDV antibodies were circulating (with a virus neutralization titre of 1:256 measured at the Washington Animal Disease Diagnostic Laboratory, Pullman, WA, USA).

In view of subsequent events, and the strong protective immunity that develops in animals that survive infection, it is likely that PT56 was already infected by March 2010. Antibodies to CDV appear after 10 to 20 days post-infection in dogs (Greene & Appel, 2006), which if comparable in tigers would suggest an infection lasting at least 80 to 90 days in this tigress. By 1 May, PT56 localized her movements, and (as was later confirmed) gave birth to a litter of three cubs. Although PT56 had proven to be a typically attentive mother when raising her three prior litters, on this occasion her behaviour was unusual, leaving the den for several days at a time before finally abandoning her cubs entirely on 17 May. She was subsequently observed at a nearby military outpost, before entering the village of Ternei, where she was shot on 1 June 2010 to prevent injury to local residents. The presence of CDV was confirmed in brain tissue (yielding a 278 bp fragment of the HA-gene, GenBank accession number KC579362), and demonstration of consistent pathology (Seimon et al.2013). All 3 of her cubs consequently died. Evidence of CDV was not found in samples collected from one of those cubs, although decomposition may have hampered test sensitivity.

PT56 was recorded in association with PT90 in the autumn of 2009. Assuming that PT90 had sired the litter of cubs born to PT56 during May, then mating must have occurred just a few days prior to his death (given a gestation period of 98–111 days [Wack, 2003]). In captive tigers mortality from CDV usually occurs within days or weeks of developing clinical signs (Appel et al.1994; Gould and Fenner, 1983; Konjević et al.2011; Nagao et al.2012, V. Keahey, personal communication 2014), but the length of the refractive period (before clinical disease is evident) remains unknown, and a delayed onset may be possible (Blythe et al.1983). Therefore, it is conceivable that PT56 contracted her infection through contact with PT90.

Khabarovsk highway tiger:

Video footage of a tiger behaving in a similar dissociative manner to confirmed cases of CDV in tigers was taken along the Vladivostok-Khabarovsk highway between the towns of Vyazemski and Bikin, Khabarovskii Krai during the spring of 2010 (http://tinyurl.com/las2yt7) Although this animal later died in care, no samples were available for analysis; therefore, CDV could not be confirmed in this instance.

PT79

This case was confirmed during the present study, based on RT-PCR analysis of an archived blood sample collected 2006 in SABZ (Chapter 4). The HA-gene sequence from this tiger has been submitted to GenBank, with the accession number KX708720. The tiger was one of three female 13 month old dependent cubs, and was sampled in SABZ on 13 October 2006. Her sample was selected for RNA extraction based on the presence of CDV neutralizing antibodies in serum (titre 1:128). Another sibling (PT80) was also found to have a CDV antibody titre of 1:256, but no virus could be detected in her serum. A whole blood sample from the third sibling (PT81) tested weakly positive for a 114 bp fragment of the P-gene (with a cycle threshold value of 37.5 using primers CDVF4 and CDVR3). This sample had been preserved in Queen's lysis buffer (Seutin et al.1991), and attempts to amplify longer fragments were unsuccessful. None of the three sisters exhibited the dissociative behaviour that has characterized prior CDV cases in Russian tigers. However, during late 2006 all three cubs were frequently observed close to a main road, and appeared unconcerned by people and vehicles. Attempts at hazing the siblings were largely unsuccessful, and all three eventually disappeared, and were assumed to have been killed by poachers (with last known locations recorded on 24 February 2007 for PT79, 20 November 2007 for PT80 and 26 February 2008 for PT81). Their mother, PT35 also disappeared during 2007, however at 14 years of age, her disappearance could have been due to natural senescence.

Khasanskii Tiger 2013:

This case was diagnosed during the present study based on RT-PCR analysis of frozen brain tissue. The HA-gene sequence from this tiger has been submitted to GenBank, with the accession number KX708726. The case involved a young male of approximately two years of age, which was found dead on 22 November 2013, in the district of Khasanskii in Southwest Primorskii (N43.06245 E131.35773). The tiger had sustained a gunshot wound, fracturing a foreleg, and died while trying to negotiate a river. Had CDV not been detected in this case, the tiger's death would have been attributed entirely to events related to the gunshot wound. Like PT90, this case raises the possibility that CDV may be contributing to incidents of human-tiger conflict. Although the tiger had been shot, the recovery of his body suggests that this was not a poaching incident.

Far Eastern leopard 2015:

On 8 May 2015, a young female leopard of approximately two years of age was found along a road close to the village of Bamburovo in Khasanskii district in Southwest Primorskii (N42.95539 E131.35150). The leopard was in poor body condition, and unresponsive to her surroundings. Despite several weeks of supportive care, the leopard's condition deteriorated, with progressive ataxia, anorexia and seizures, and on 25 May the leopard was euthanized. This case constitutes the first report of CDV in this critically endangered leopard subspecies. Preliminary sequencing of frozen brain tissue has produced an 528 bp fragment of the HA-gene. Further work is in progress to obtain the full HA-gene sequence, and a manuscript is in preparation.

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Appendix III. Wildlife Conservation Society Institutional Care and Use Committee approval letter

WILDLIFE CONSERVATION SOCIETY

July 16, 2012

Drs. Dale Miquelle & Martin Gilbert
Wildlife Conservation Society

Dear Dale & Martin:

Your research proposal **12:07: Understanding and managing canine distemper virus as a disease threat to Siberian tigers"** was submitted to the Wildlife Conservation Society's (WCS) Institutional Animal Care and Use Committee (IACUC) for review.

Under the policies and procedures of the WCS IACUC, research involving vertebrates in both the animal collections and performed on free-ranging animals on WCS facility properties is defined as those procedures, including animal handling, that go beyond routine husbandry or veterinary care to address scientific hypotheses. All such research is required to be reviewed and approved by the WCS IACUC prior to initiation of the study.

Even though based on these criteria WCS IACUC approval is not required for this study to be performed, I am pleased to inform you that the committee has reviewed and approved your research proposal.

It is the policy of WCS to comply with all applicable laws and regulations of the United States, including international treaties to which the United States is a signatory, and all applicable laws and regulations of countries in which WCS conducts its work.

Thank you for your submission. We look forward to receiving updates and follow up reports for this project prior to each subsequent WCS IACUC meeting, and a final report upon completion of the study. If you have any questions, or I can be of further assistance, please contact me.

Sincerely,

Paul P. Calle

Paul P. Calle, VMD, Dipl ACZM
Chief Veterinarian
Director, Zoological Health
Chair, WCS Institutional Animal Care and Use Committee
Wildlife Conservation Society
718-220-7100
pcalle@wcs.org

Appendix IV. State Veterinary Inspection, Primorskii Krai approval letters

Letters of support permitting the collection of health data and samples from domestic dogs in Primorskii Krai, 2012-2014.

УПРАВЛЕНИЕ ВЕТЕРИНАРИИ ПРИМОРСКОГО КРАЯ

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ИНН/КПП 2540149496/254001001

25.04.2012 № 43-13/448
На № 16147/346 от 12.04.2012

Директору БПИ ДВО РАН,
академику

Ю.Н. Журавлеву

Проспект 100-летия
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край, 690022

О согласовании исследований

Уважаемый Юрий Николаевич!

Управление ветеринарии Приморского края не возражает в сборе биоматериала (крови, кала) от домашних собак и кошек в поселениях Надеждинского и Хасанского районов для исследования на наличие чумы плотоядных и других заболеваний.

Главные ветеринарные врачи указанных районов будут предупреждены о проведении данной работы с 15 мая по 30 июня 2012 года. Сотрудникам института Уфыркиной О.В. и Сулихан Н.С. необходимо иметь документ, удостоверяющий личность. Полученные результаты исследований просим предоставить в управление ветеринарии Приморского края.

Кроме того, Вами указано, что у нескольких особей амурского тигра из природной популяции была достоверно диагностирована чума плотоядных. Просим направить в наш адрес экспертизу лабораторных исследований, подтверждающих данный факт.

С уважением,
начальник управления В.А. Волков

А.А.Уманец
41 14 37

26 04 253 12

ГОСУДАРСТВЕННАЯ ВЕТЕРИНАРНАЯ ИНСПЕКЦИЯ ПРИМОРСКОГО КРАЯ
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ИНН/КПП 2540149496/254001001

_____ № _____
На № 16147/228 от 10.04.2013

Директору БПИ ДВО РАН
Академику

Ю.Н. Журавлеву

О согласовании исследований

Уважаемый Юрий Николаевич!

Государственная ветеринарная инспекция Приморского края не возражает в сборе биоматериала (крови, мазков со слизистой носа, кала) от домашних собак и кошек в поселениях Тернейского, Лазовского, Надеждинского и Хасанского районов для исследования на наличие чумы плотоядных и других заболеваний.

Главные ветеринарные врачи указанных районов будут предупреждены о проведении данной работы с 15 апреля по 15 июля 2013 года. Сотруднику института Сулихан Н.С. необходимо иметь документ, удостоверяющий личность. Полученные результаты исследований просим предоставить в госветинспекцию Приморского края.

С уважением,
и.о. руковрдителя инспекции А.А.Уманец

Л.Г. Ковеза
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Федеральное государственное бюджетное учреждение науки Биолого-почвенный институт Дальневосточного отделения Российской академии наук (БПИ ДВО РАН)
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Согласовано.
20.06.13.
Кузин Д.Ю.

№ 16147/442
от 19.06.2013г.

И.о. главного государственного
ветеринарного инспектора
Приморского Края
Г-ну Кузину Д. Ю.

Уважаемый Дмитрий Юрьевич!

БПИ ДВО РАН просит способствовать сбору биологического материала (крови, мазков со слизистой носа, кала) от домашних собак и кошек в поселениях Уссурийского городского округа и Надеждинского района Приморского края. В 2013 году мы планируем продолжить этап сбора образцов для проведения исследований на наличие инфекционных заболеваний, являющихся потенциально опасными для популяций Амурского тигра и Дальневосточного леопарда. Обработка полученного биологического материала запланирована на 2014 – 2015 годы.

Сбор материала будет проводиться сотрудницей Биолого-почвенного института м.н.с. Сулихан Н. С., ветеринарным врачом Гончарук М. С. и помощниками (3 человека), в период с 21 июня по 25 июня 2013 года. С каждого поселка планируется взять образцы от 25 животных.

Список запланированных поселков:

Барановский
Николо Львовское
Кроуновка

Директор, академик Ю. Н. Журавлев

Appendix V. Demography of owned cats in Primorskii Krai

Introduction

Like dogs, domestic cats are also commonly kept in Primorskii Krai. While domestic cats show a low susceptibility to CDV, and are unable to transmit infections (Appel et al.1974), they do share a range of other pathogens with tigers. Since co-infections are known to play a role in the clinical course of CDV infections in *Panthera* spp. (Munson et al.2008; Silinski et al.2003; Fix et al.1989), it is possible that domestic cats may influence the outcome of tiger infections in other ways. The main objective of the household surveys described in Chapter 3 was to investigate the ownership patterns of domestic dogs that impact CDV maintenance, and opportunities for transmission with wildlife. However, the surveys also represented an opportunity to collect baseline information on domestic cat ownership in Primorskii Krai as a precursor to wider assessments of domestic cat health.

Domestic cats show a greater degree of independence than dogs, and are able to survive without relying on people for provisioning. While this study concentrated on owned cats, it is acknowledged that this overlooks a population of unknown size that is able to survive with limited or no support from people.

Methods

Data were collected in conjunction with household questionnaire surveys described in Chapter 3 (full questionnaires are provided in Appendices V, VII and VIII). A more limited set of data was obtained for individual cats, including age, sex, whether cats were neutered, reproductive history of females, vaccination history and whether cats were allowed to roam outside. Owners were also asked about details of any dogs and cats that died within the previous 12 months, including their age, sex and cause of death. Total numbers of cats in each settlement, study area and the whole of Primorskii were estimated using the methods described for dogs (Chapter 3).

Results

Median human to cat ratios were calculated as 2.04 in villages (SD=1.69 (n=22), 2.30 in towns (SD=1.43 (n=3), 6.19 in the only large town surveyed (n=1), and 3.87 in the city of Ussuriysk. This equated to an estimated population of 4,032 cats, (CI: 3,439-4,742) in the vicinity of SABZ, 5,917 cats, (CI: 5,159-6,880) around Lazovskii, and 20,777 cats, (CI: 19,183-22,494) in Southwest Primorskii (V.I). However, it should be noted that these figures only represent owned cats, and an unknown number of feral cats may survive without human provisioning. A total of 55.9%, residences surveyed were cat owned households (COHH, n=2,576), representing 28.4% of apartments and 21.6% of cottages. The mean number of cats per COHH was (SD=1.11, n=1,442). The majority of owned cats had free access to roam out of doors (90.89 %, n=2,383).

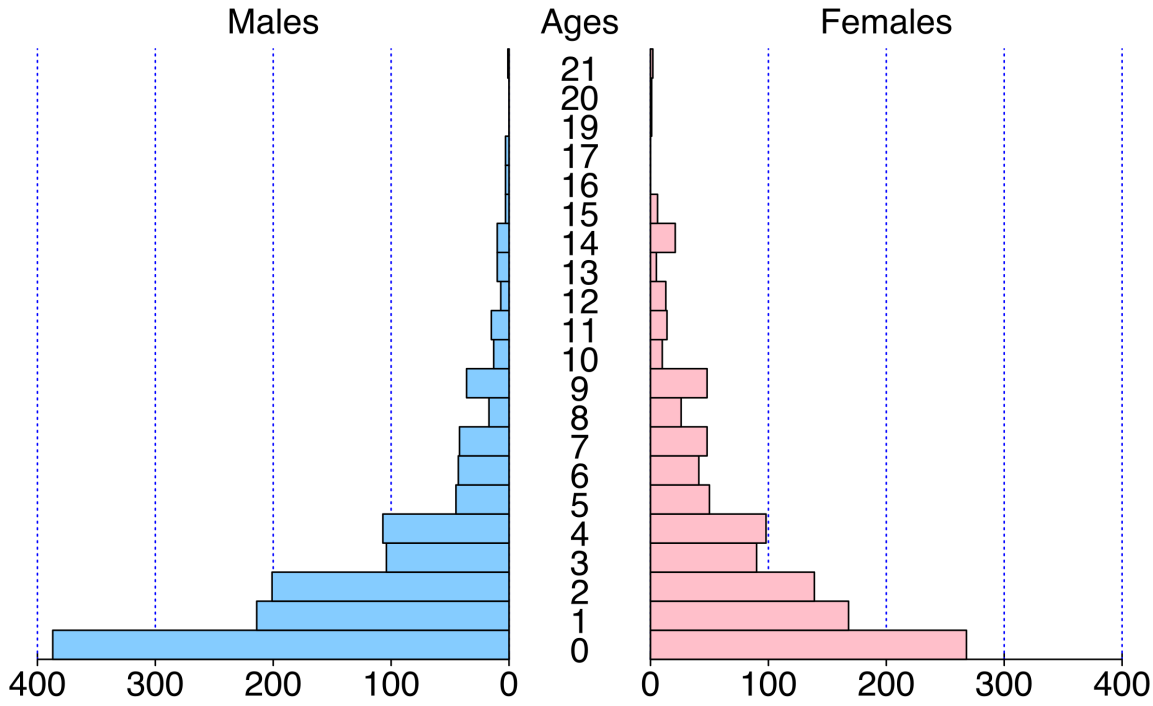

Figure V.I. An age pyramid illustrating the frequency distribution of cats at yearly age intervals, generated using the R package 'pyramid' (Nakazawa, 2014).

The mean age of male cats was estimated to be 3.57 years (SD=3.4, n=1,261), and was 4.22 years for female cats (SD=3.82, n=1,049). The expected lifespan of cats at birth (mean age at death) was 2.6 years, and cats surviving to one year of age have a mean life expectancy of 5.57 years. The sex ratio of male:female cats was skewed toward males at 1.21:1, although this was less marked than the skewed sex ratio recorded in the dog

population (Figure V.I). Only 6.1% of males (n=1,282), and 5.3% of females (n=1,067) had been sterilized. Owners reported the breeding status for 1,045 queens, of which 885 were considered of breeding age (taken to be 7 months or older). There were 599 queens who bred in the last year, representing 67.7% of the female population of breeding age. These queens had a total of 1,061 litters. Mean litter size was 3.6 kittens (SD=1.3, n=605). When extrapolated over the whole population of females in the survey, this equated to approximately 3,879 kittens in the previous year, which equated to a per capita breeding rate of 1.59 kittens per cat per year.

There were 233 cats reported to have died in the previous year. This equated to a per capita mortality rate of 0.1 cats per cat per year, which given the productivity estimates is likely to grossly underestimate true mortality. A possible explanation for this is the reclusive nature of cats at the end of life, as owners reported that 32.2% of cats that were assumed to have died in the previous year had either disappeared, or died of unknown causes (n=233). Other common causes of death were road accident (20.6%), anthropogenic causes (12.0%), senescence (11.6%), and sickness (10.7%).

Information on vaccination history was provided for 2,386 cats, of which owners claimed that 14.4% had received vaccinations. However, owners were unable to provide details of products given to 52.8% of vaccinated cats, and based on their responses many of these may have been other medications such as antibiotics. Of remaining treated cats, 39.9% had received vaccinations for rabies, 2.9% for feline respiratory complex (containing feline viral rhinotracheitis, feline calicivirus and *Chlamydophila felis*) and 4.4% received medications other than vaccines.

Discussion

Numbers of owned cats were comparable to the number of dogs in each of the three study areas. Cat ownership was notably higher than dog ownership in apartments, an effect that led to higher estimates than dogs at the province level. Considering the propensity of cats for independent survival, it is possible that additional feral populations would push this total even higher. Although cats are not susceptible to CDV, several features of their ecology in Primorskii may promote the maintenance of other pathogens to which tigers

may be susceptible. With little attempt at sterilization, a high proportion of female cats are reproductive, and produce more than three times the number of young compared to that of dogs. Although first year mortality will exceed that for dogs, such productivity of naïve and susceptible kittens will promote the transmission of acute infections. Cats are also afforded limited access to veterinary care in comparison to dogs, with low vaccination coverage doing little to hinder pathogen transmission at the population level. Almost all cats are permitted to roam at will beyond the confines of the household, leading to a greater potential for mixing compared to that of dogs. However, the typical home range size of owned domestic cats is relatively small (<2 Ha [Horn et al.2011]), which may limit their opportunity for direct contact with wildlife.

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Appendix VI. Household questionnaire (pet-owning households)

Simple Household Survey Form (Pet-owning)

Interviewer name: ФИО опрашивающего:		**Day**: День:		**Month**: Месяц:		**Year**: Год:		**Time**: Время:	

Household data: Общая информация о доме и тех, кто в нем проживает:

Household ID: ID дома: ____ **UTM**: (**Northing**) (Северная) ____ (**Easting**) (Восточная) ____

Address: Адрес: ____ **Settlement**: Населенный пункт: ____

Residence type: Вид жилья: **Cottage**: Частный дом: ☐ **Apartment**: Квартира: ☐ **Other**: Другое: ☐ **Specify**: Особенности: ____

People in household: Количество человек в доме: **Adult (≥18y)**: Взрослые (≥18лет): ☐ **Child (<18y)**: Дети (<18лет): ☐

☐ **Number of small animals**: Количество животных: **Dogs**: Собак: ☐ **Cats**: Кошек: ☐ **Number of dogs 10 yrs ago**: Количество собак 10 лет назад: ☐

☐ **Number of livestock at property**: Кол-во с/х животных во дворе: **Cattle**: К.р.с.: ☐ **Pigs**: Свиньи: ☐ **Chickens**: Куры: ☐ **Other**: Другое:________ ☐ ________ ☐

Number of livestock elsewhere: Кол-во с/х животных в округе: **Cattle**: К.р.с.: ☐ **Pigs**: Свиньи: ☐ **Chickens**: Куры: ☐ **Other**: Другое:________ ☐ ________ ☐

Do you see neighbor's dogs roaming in the area?: Как часто Вы видите свободно гуляющих соседских собак?: **Always**: Всегда: ☐ **Sometimes**: Иногда: ☐ **Never**: Никогда: ☐ **Unknown**: Не знаю: ☐

Do you see un-owned (feral) dogs roaming in the area?: Как часто Вы видите неизвестных собак рядом с Вашим домом?: **Always**: Всегда: ☐ **Sometimes**: Иногда: ☐ **Never**: Никогда: ☐ **Unknown**: Не знаю: ☐

☐ Individual dog data: Данные о собаке:

Please complete one column for each animal, (tick appropriate boxes)

Пожалуйста, вносите данные о каждой собаке в отдельную колонку

Animal ID (if needed) ID животного (если треб.)	____	____	____	____
Samples collected? (if yes then enter Animal ID) Были ли взяты образцы? (если да, то присвойте животному ID)	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐ **Faeces**: Кал: ☐	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐ **Faeces**: Кал: ☐	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐ **Faeces**: Кал: ☐	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐ **Faeces**: Кал: ☐
Hunting breed? Охотничья ли порода?	**Yes**: Да: ☐ **No**: Нет: ☐	**Yes**: Да: ☐ **No**: Нет: ☐	**Yes**: Да: ☐ **No**: Нет: ☐	**Yes**: Да: ☐ **No**: Нет: ☐
Age (Tick and specify) Возраст (отметить и ниже написать подробно)	**Ad. (>3 mo.)**: Взр. (>3 мес.): ☐ **Juv. (<3 mo.)**: Мол. (< 3 мес.): ☐ ____ ☐ **Yrs**/Лет ☐ **Mo**/Мес	**Ad. (>3 mo.)**: Взр. (>3 мес.): ☐ **Juv. (<3 mo.)**: Мол. (< 3 мес.): ☐ ____ ☐ **Yrs**/Лет ☐ **Mo**/Мес	**Ad. (>3 mo.)**: Взр. (>3 мес.): ☐ **Juv. (<3 mo.)**: Мол. (< 3 мес.): ☐ ____ ☐ **Yrs**/Лет ☐ **Mo**/Мес	**Ad. (>3 mo.)**: Взр. (>3 мес.): ☐ **Juv. (<3 mo.)**: Мол. (< 3 мес.): ☐ ____ ☐ **Yrs**/Лет ☐ **Mo**/Мес
Gender Пол	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐
Spayed/castrated? Стерилизована/ кастрирован?	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐

Current reproductive status? Текущий репродуктивный статус?	**Pregnant**: Беременна: **Lactating**: Кормящая: **Unknown**: Неизвестно:	**Pregnant**: Беременна: **Lactating**: Кормящая: **Unknown**: Неизвестно:	**Pregnant**: Беременна: **Lactating**: Кормящая: **Unknown**: Неизвестно:	**Pregnant**: Беременна: **Lactating**: Кормящая: **Unknown**: Неизвестно:
No. of litters in last year? Кол-во пометов в год?				
No. pups in last litter? Кол-во щенков в последнем помете?				
Outcome of last litter? (indicate no. pups per category) Что произошло со щенками из последнего помета? (отметьте количество щенков в каждой категории)	**Alive (present):** Живут в доме: **Given away**: Отдали: **Died (accident):** Гибель(случайно): **Died (sickness):** Гибель(болезнь): **Euthanized:** Эвтаназия: **Unknown**: Неизвестно: **Other**: Другое: **Specify**: Подробности:	**Alive (present):** Живут в доме: **Given away**: Отдали: **Died (accident):** Гибель(случайно): **Died (sickness):** Гибель(болезнь): **Euthanized:** Эвтаназия: **Unknown**: Неизвестно: **Other**: Другое: **Specify**: Подробности:	**Alive (present):** Живут в доме: **Given away:** Отдали: **Died (accident):** Гибель(случайно): **Died (sickness):** Гибель(болезнь): **Euthanized:** Эвтаназия: **Unknown**: Неизвестно: **Other**: Другое: **Specify**: Подробности:	**Alive (present):** Живут в доме: **Given away:** Отдали: **Died (accident):** Гибель(случайно): **Died (sickness):** Гибель(болезнь): **Euthanized:** Эвтаназия: **Unknown**: Неизвестно: **Other**: Другое: **Specify**: Подробности:
Source of dog? Откуда была взята собака?	**Local**: Местная: **Non-local:** Привезенная: **Specify**: Подробности: **Month**: Месяц: / **Year**: Год:	**Local**: Местная: **Non-local:** Привезенная: **Specify**: Подробности: **Month**: Месяц: / **Year**: Год:	**Local**: Местная: **Non-local:** Привезенная: **Specify**: Подробности: **Month**: Месяц: / **Year**: Год:	**Local**: Местная: **Non-local:** Привезенная: **Specify**: Подробности: **Month**: Месяц: / **Year**: Год:
Reason for ownership? (tick all that apply) С какой целью заведена собака? (отметить все, что подходит)	**Pet**: Питомец: **Guard**: Охрана: **Hunting**: Охота: **Other**: Другое: **Specify**: Подробности:	**Pet**: Питомец: **Guard**: Охрана: **Hunting**: Охота: **Other**: Другое: **Specify**: Подробности:	**Pet**: Питомец: **Guard**: Охрана: **Hunting**: Охота: **Other**: Другое: **Specify**: Подробности:	**Pet**: Питомец: **Guard**: Охрана: **Hunting**: Охота: **Other**: Другое: **Specify**: Подробности:
Is dog taken to forest? Вы берете собаку в лес?	**Yes**: Да: **No**: Нет:	**Yes**: Да: **No**: Нет:	**Yes**: Да: **No**: Нет:	**Yes**: Да: **No**: Нет:
How often does dog travel beyond the settlement? Как часто собака уходит/вывозится в другие населенные пункты (в среднем)?	**At least weekly:** Min раз в неделю: **At least monthly**: Min раз в месяц: **At least annually:** Min ежегодно: **Rarely**: Реже: **Never**: Никогда:	**At least weekly**: Min раз в неделю: **At least monthly**: Min раз в месяц: **At least annually**: Min ежегодно: **Rarely**: Реже: **Never**: Никогда:	**At least weekly**: Min раз в неделю: **At least monthly**: Min раз в месяц: **At least annually**: Min ежегодно: **Rarely**: Реже: **Never**: Никогда:	**At least weekly**: Min раз в неделю: **At least monthly**: Min раз в месяц: **At least annually**: Min ежегодно: **Rarely**: Реже: **Never**: Никогда:
Most distant place the dog visited? Самое отдаленное место, посещаемое собакой?	**Specify**: Название:	**Specify**: Название:	**Specify**: Название:	**Specify**: Название:

Household ID:
ID дома:

How much time is the dog unconfined? (includes escaping) Как долго собака находится за пределами двора без присмотра? (в том числе **срывается с привязи**)	**All day:** Весь день: ☐ **Part of day:** Часть дня: ☐ **Sometimes/Rare:** Иногда/Редко: ☐ **Never:** Никогда: ☐ **Notes:** Примечание: ____	**All day:** Весь день: ☐ **Part of day:** Часть дня: ☐ **Sometimes/Rare:** Иногда/Редко: ☐ **Never:** Никогда: ☐ **Notes:** Примечание: ____	**All day:** Весь день: ☐ **Part of day:** Часть дня: ☐ **Sometimes/Rare:** Иногда/Редко: ☐ **Never:** Никогда: ☐ **Notes:** Примечание: ____	**All day:** Весь день: ☐ **Part of day:** Часть дня: ☐ **Sometimes/Rare:** Иногда/Редко: ☐ **Never:** Никогда: ☐ **Notes:** Примечание: ____
Vaccinated against? (tick all that apply) Против каких заболеваний вакцинирована? (отметить все подходящие)	**Month / Year** Месяц / Год **Rabies:** Бешенство: ☐ __/__ **Distemper:** Чума: ☐ __/__ **Other:** Другое: ☐ __/__ **Specify:** Подробности: ____	**Month / Year** Месяц / Год **Rabies:** Бешенство: ☐ __/__ **Distemper:** Чума: ☐ __/__ **Other:** Другое: ☐ __/__ **Specify:** Подробности: ____	**Month / Year** Месяц / Год **Rabies:** Бешенство: ☐ __/__ **Distemper:** Чума: ☐ __/__ **Other:** Другое: ☐ __/__ **Specify:** Подробности: ____	**Month / Year** Месяц / Год **Rabies:** Бешенство: ☐ __/__ **Distemper:** Чума: ☐ __/__ **Other:** Другое: ☐ __/__ **Specify:** Подробности: ____
Reason(s) for not vaccinating? (tick all that apply) Почему Вы не вакцинируете собак? (отметить все подходящие)	**Not aware of:** Я не знаю: **Purpose:** Зачем: ☐ **Yearly need:** Нужно ежегодно: ☐ **Location:** Где это делают: ☐ **Other reason:** Другие причины: **Too distant:** Слишком далеко: ☐ **Too expensive:** Слишком дорого: ☐ **No time:** Нет времени: ☐ **Can't handle:** Не могу удержать собаку: ☐ **Other:** Другое: ☐ **Specify:** Подробности: ____	**Not aware of:** Я не знаю: **Purpose:** Зачем: ☐ **Yearly need:** Нужно ежегодно: ☐ **Location:** Где это делают: ☐ **Other reason:** Другие причины: **Too distant:** Слишком далеко: ☐ **Too expensive:** Слишком дорого: ☐ **No time:** Нет времени: ☐ **Can't handle:** Не могу удержать собаку: ☐ **Other:** Другое: ☐ **Specify:** Подробности: ____	**Not aware of:** Я не знаю: **Purpose:** Зачем: ☐ **Yearly need:** Нужно ежегодно: ☐ **Location:** Где это делают: ☐ **Other reason:** Другие причины: **Too distant:** Слишком далеко: ☐ **Too expensive:** Слишком дорого: ☐ **No time:** Нет времени: ☐ **Can't handle:** Не могу удержать собаку: ☐ **Other:** Другое: ☐ **Specify:** Подробности: ____	**Not aware of:** Я не знаю: **Purpose:** Зачем: ☐ **Yearly need:** Нужно ежегодно: ☐ **Location:** Где это делают: ☐ **Other reason:** Другие причины: **Too distant:** Слишком далеко: ☐ **Too expensive:** Слишком дорого: ☐ **No time:** Нет времени: ☐ **Can't handle:** Не могу удержать собаку: ☐ **Other:** Другое: ☐ **Specify:** Подробности: ____
Bite history? Кусала ли собака человека?	**Positive:** Имело место: ☐ **Negative:** Не было: ☐ **Unknown:** Неизвестно: ☐	**Positive:** Имело место: ☐ **Negative:** Не было: ☐ **Unknown:** Неизвестно: ☐	**Positive:** Имело место: ☐ **Negative:** Не было: ☐ **Unknown:** Неизвестно: ☐	**Positive:** Имело место: ☐ **Negative:** Не было: ☐ **Unknown:** Неизвестно: ☐
Bite victim(s)? (give numbers) Кого кусала собака? (отметить **количество**)	**Self:** Опрашиваемого: ☐ **Family:** Домочадцев: ☐ **Other:** Прочих: ☐	**Self:** Опрашиваемого: ☐ **Family:** Домочадцев: ☐ **Other:** Прочих: ☐	**Self:** Опрашиваемого: ☐ **Family:** Домочадцев: ☐ **Other:** Прочих: ☐	**Self:** Опрашиваемого: ☐ **Family:** Домочадцев: ☐ **Other:** Прочих: ☐

Notes:
Примечание:

Dead dog data:
Данные о мертвых собаках:

Please complete one column for each animal dying in previous year (tick appropriate boxes) Пожалуйста, вносите данные о каждой собаке, погибшей в предыд. году, в отдельную колонку

Age (Tick and specify) Возраст (отметить и ниже написать подробно)	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол. (<3 мес.): ☐ ☐ **Yrs/**Лет **Mo/**Мес	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол. (<3 мес.): ☐ ☐ **Yrs/**Лет **Mo/**Мес	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол. (<3 мес.): ☐ ☐ **Yrs/**Лет **Mo/**Мес	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол. (<3 мес.): ☐ ☐ **Yrs/**Лет **Mo/**Мес
Gender Пол	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐
Cause of death Причина смерти	**Road accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____	**Accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____	**Accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____	**Accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____
Bite history? Кусала ли собака человека?	**Positive**: Имело место: ☐ **Negative**: Не было: ☐ **Unknown**: Неизвестно: ☐	**Positive**: Имело место: ☐ **Negative**: Не было: ☐ **Unknown**: Неизвестно: ☐	**Positive**: Имело место: ☐ **Negative**: Не было: ☐ **Unknown**: Неизвестно: ☐	**Positive**: Имело место: ☐ **Negative**: Не было: ☐ **Unknown**: Неизвестно: ☐
Bite victim(s)? (give numbers) Кого кусала собака? (отметить **количество**)	**Self**: Опрашиваемого: ☐ **Family**: Домочадцев: ☐ **Other**: Прочих: ☐	**Self**: Опрашиваемого: ☐ **Family**: Домочадцев: ☐ **Other**: Прочих: ☐	**Self**: Опрашиваемого: ☐ **Family**: Домочадцев: ☐ **Other**: Прочих: ☐	**Self**: Опрашиваемого: ☐ **Family**: Домочадцев: ☐ **Other**: Прочих: ☐

Notes:
Примечание:

Household ID:
ID дома :

Individual cat data: Данные о кошке:

Please complete one column for each animal, (tick appropriate boxes)

Пожалуйста, вносите данные о каждой кошке в отдельную колонку

Animal ID (if needed) ID животного (если треб.)	___	___	___	___
Samples collected? (if yes then enter Animal ID) Были ли взяты образцы? (если да, то присвойте животному ID)	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐	**Serum**: Сыворотка: ☐ **Nasal swab**: Мазок из носа: ☐ **Blood slide**: Мазок крови: ☐ **FTA card**: FTA карточка: ☐
Age (Tick and specify) Возраст (отметить и ниже написать подробно)	**Ad. (>3 mo)**: Взр.(>3 мес.): ☐ **Juv. (<3 mo.)**: Мол.(<3 мес.): ☐ ☐ ☐ **Yrs/**Лет ☐ **Mo/**Мес	**Ad. (>3 mo)**: Взр.(>3 мес.): ☐ **Juv. (<3 mo.)**: Мол.(<3 мес.): ☐ ☐ ☐ **Yrs/**Лет ☐ **Mo/**Мес	**Ad. (>3 mo)**: Взр.(>3 мес.): ☐ **Juv. (<3 mo.)**: Мол.(<3 мес.): ☐ ☐ ☐ **Yrs/**Лет ☐ **Mo/**Мес	**Ad. (>3 mo)**: Взр.(>3 мес.): ☐ **Juv. (<3 mo.)**: Мол.(<3 мес.): ☐ ☐ ☐ **Yrs/**Лет ☐ **Mo/**Мес
Gender Пол	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐
Spayed/castrated? Стерилизована/ кастрирован?	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐	**Yes**: Да: ☐ **No**: Нет: ☐ **Unknown**: Неизвестно: ☐
Current reproductive status? Текущий репродуктивный статус?	**Yes** Да / **No** Нет / ? **Pregnant**: Беременна: ☐ ☐ ☐ **Lactating**: Кормящая: ☐ ☐ ☐	**Yes** Да / **No** Нет / ? **Pregnant**: Беременна: ☐ ☐ ☐ **Lactating**: Кормящая: ☐ ☐ ☐	**Yes** Да / **No** Нет / ? **Pregnant**: Беременна: ☐ ☐ ☐ **Lactating**: Кормящая: ☐ ☐ ☐	**Yes** Да / **No** Нет / ? **Pregnant**: Беременна: ☐ ☐ ☐ **Lactating**: Кормящая: ☐ ☐ ☐
No. of litters in last year? Кол-во пометов в прошлом году?	___	___	___	___
No. kittens in last litter? Кол-во котят в прошлом помете?	___	___	___	___
Does the cat spend time outside? Выходит ли на улицу?	**Yes**: Да: ☐ **No**: Нет: ☐	**Yes**: Да: ☐ **No**: Нет: ☐	**Yes**: Да: ☐ **No**: Нет: ☐	**Yes**: Да: ☐ **No**: Нет: ☐
Is the cat vaccinated? Есть ли вакцинация?	**Yes**: Да: ☐ **No**: Нет: ☐ **Specify**: Подробности: ___	**Yes**: Да: ☐ **No**: Нет: ☐ **Specify**: Подробности: ___	**Yes**: Да: ☐ **No**: Нет: ☐ **Specify**: Подробности: ___	**Yes**: Да: ☐ **No**: Нет: ☐ **Specify**: Подробности: ___
Last vaccine date? Дата последн. вакц-и?	**Month**: Месяц: / **Year**: Год:	**Month**: Месяц: / **Year**: Год:	**Month**: Месяц: / **Year**: Год:	**Month**: Месяц: / **Year**: Год:

Dead cat data: **Данные о погибших:**	**Please complete one column for each animal dying in previous year (tick appropriate boxes)** Пожалуйста, вносите данные о каждой кошке, погибшей в предыд. году, в отдельную колонку			
Age **(Tick and specify)** Возраст (отметить и ниже написать подробно)	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол.(<3 мес.): ☐ ____ ☐ **Yrs/**Лет ☐ **Mo/**Мес	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол.(<3 мес.): ☐ ____ ☐ **Yrs/**Лет ☐ **Mo/**Мес	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол.(<3 мес.): ☐ ____ ☐ **Yrs/**Лет ☐ **Mo/**Мес	**Ad. (>3 mo):** Взр. (>3 мес.): ☐ **Juv. (<3 mo.):** Мол.(<3 мес.): ☐ ____ ☐ **Yrs/**Лет ☐ **Mo/**Мес
Gender Пол	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐	**Male**: Самец: ☐ **Female**: Самка: ☐ **Unknown**: Неизвестно: ☐
Cause of death Причина смерти	**Road accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____	**Road accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____	**Road accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____	**Road accident**: Автоавария: ☐ **Sickness**: Болезнь: ☐ **Other**: Другое: ☐ **Specify**: Подробности: ____

Notes:
Примечание:

Appendix VII. Household questionnaire (non-pet-owning households)

Simple Household Survey Form (No Pets)

Interviewer name: ФИО опрашивающего: | **Day**: День: | **Month**: Месяц: | **Year**: Год: | **Time**: Время:

Household data: Общая информация о доме и тех, кто в нем проживает:

Household ID: ID дома: | **UTM**: (**Northing**) (Северная) | (**Easting**) (Восточная)

Address: Адрес: | **Settlement**: Населенный пункт:

Residence type: Вид жилья: | **Cottage**: Частный дом: | **Apartment**: Квартира: | **Other**: Другое: | **Specify**: Особенности:

People in household: Количество человек в доме: | **Adult (≥18y)**: Взрослые (≥18лет): | **Child (<18y)**: Дети (<18лет):

Number of small animals: Количество животных: | **Dogs**: Собак: | **Cats**: Кошек: | **Number of dogs 10 yrs ago**: Количество собак 10 лет назад:

Number of livestock at property: Кол-во с/х животных во дворе: | **Cattle**: К.р.с.: | **Pigs**: Свиньи: | **Chickens**: Куры: | **Other**: Другое:_____

Number of livestock elsewhere: Кол-во с/х животных в округе: | **Cattle**: К.р.с.: | **Pigs**: Свиньи: | **Chickens**: Куры: | **Other**: Другое:_____

Do you see neighbor's dogs roaming in the area?:
Как часто Вы видите соседских собак, гуляющих без присмотра?:
Always: Всегда:
Sometimes: Иногда:
Never: Никогда:
Unknown: Не знаю:

Do you see un-owned (feral) dogs roaming in the area?:
Как часто Вы видите неизвестных собак рядом с Вашим домом?:
Always: Всегда:
Sometimes: Иногда:
Never: Никогда:
Unknown: Не знаю:

Number of dead dogs in the last year:
Количество собак, погибших в прошлом году:

Age Возраст	**Gender** Пол	**Cause of death** Причина смерти
Yrs/Лет **Mo/**Мес	**Male**: Самец: **Female**: Самка: **Unknown**: Неизвестно:	
Yrs/Лет **Mo/**Мес	**Male**: Самец: **Female**: Самка: **Unknown**: Неизвестно:	

Number of dead cats in the last year:
Количество кошек, погибших в прошлом году:

Age Возраст	**Gender** Пол	**Cause of death** Причина смерти
Yrs/Лет **Mo/**Мес	**Male**: Самец: **Female**: Самка: **Unknown**: Неизвестно:	
Yrs/Лет **Mo/**Мес	**Male**: Самец: **Female**: Самка: **Unknown**: Неизвестно:	

Notes:
Примечание:

Appendix VIII. Urban questionnaire survey

Urban Pet Survey Questionnaire

Interviewer name: ФИО опрашивающего:		**Day**: День:		**Month**: Месяц:		**Year**: Год:		**Time**: Время:	
UTM: (**Northing**) (Северная)		(**Easting**) (Восточная)				**Sheet number**: Номер листа:			

Settlement: пункт:		**Residence type**: Вид жилья:		**People in household**: Количество человек В доме:		**No. of small animals**: Количество животных:		**Number of dogs 10 yrs ago**: Количество собак 10 лет назад:
Ussuriysk: Уссурийск:	**Other**: Другое:	**Cottage**: Частный дом:	**Apartment**: Квартира:	**Adult**: Взрослые:	**Child**: Дети:	**Dogs**: Собак:	**Cats**: Кошек:	

Appendix IX. Effect of data cleaning on dog ownership explanatory variables

Table summarizing the effect of data cleaning on the distribution of explanatory variables used in multivariate binomial generalized linear models. Full data sets were cleaned to remove dogs with incomplete sets of explanatory data, to produce an edited dataset.

Variable	Levels	Full dataset distribution	Edited dataset distribution	Difference
Study area	Southwest	50.2% (n=1,414)	50.0% (n=1,397)	0.2%
	Lazovskii	71.4% (n=609)	71.8% (n=592)	-0.4%
	SABZ	61.2% (n=520)	60.2% (n=505)	1.0%
Community type	Village	72.9% (n=1,037)	72.9% (n=1,019)	0.0%
	Town	63.4% (n=943)	63.0% (n=920)	0.4%
	Large town	19.4% (n=563)	18.9% (n=555)	0.4%
Residence type	Apartment	16.9% (n=911)	16.7% (n=900)	0.2%
	Cottage	80.2% (n=1,632)	80.2% (n=1,594)	0.0%
Number of people	1	44.6% (n=498)	44.6% (n=498)	0.0%
	2	57.8% (n=922)	57.8% (n=922)	0.0%
	3	58.6% (n=534)	58.5% (n=533)	0.1%
	4	64.7% (n=334)	64.7% (n=334)	0.0%
	>5	70.0% (n=207)	70.0% (n=207)	0.0%
Children	Present	60.8% (n=816)	60.8% (n=816)	0.0%
	Absent	55.6% (n=1679)	55.5% (n=1,678)	0.0%
Cats	Present	75.6% (n=1429)	75.3% (n=1,399)	0.2%
	Absent	34.4% (n=1,114)	34.2% (n=1,095)	0.2%
Poultry	Present	90.2% (n=673)	90.3% (n=659)	-0.1%
	Absent	45.7% (n=1,869)	45.4% (n=1,835)	0.4%
Livestock	Present	94.6% (n=184)	94.5% (n=182)	0.1%
	Absent	54.6% (n=2,358)	54.3% (n=2,312)	0.3%

Appendix X. Effect of data cleaning on dog origin explanatory variables

Table summarizing the effect of data cleaning on the distribution of explanatory variables used in multivariate binomial generalized linear models. Full data sets were cleaned to remove dogs with incomplete sets of explanatory data, to produce an edited dataset.

Variable	Levels	Full dataset distribution	Edited dataset distribution	Difference
Study area	Southwest	66.2% (n=1,156)	66.2% (n=1,127)	0.0%
	Lazovskii	71.4% (n=696)	71.6% (n=663)	-0.2%
	SABZ	73.8% (n=469)	73.7% (n=433)	0.1%
Community type	Village	65.2% (n=1,293)	65.3% (n=1,246)	-0.1%
	Town	75.1% (n=889)	75.0% (n=844)	0.1%
	Large town	69.8% (n=139)	69.9% (n=133)	-0.1%
Residence type	Apartment	51.5% (n=169)	51.2% (n=162)	0.2%
	Cottage	70.7% (n=2,152)	70.7% (n=2,061)	0.0%
Number of people	1	72.3% (n=332)	72.0% (n=322)	0.2%
	2	67.0% (n=785)	67.0% (n=766)	0.0%
	3	67.7% (n=493)	68.2% (n=484)	-0.4%
	4	68.9% (n=367)	69.8% (n=358)	-0.9%
	>5	72.5% (n=298)	73.4% (n=293)	-0.9%
Children	Present	69.5% (n=865)	70.1% (n=843)	-0.6%
	Absent	687% (n=1,410)	68.8% (n=1,380)	-0.1%
Cats	Owner	70.6% (n=1,821)	70.5% (n=1,748)	0.1%
	Non-owner	64.4% (n=500)	64.8% (n=475)	-0.4%
Poultry	Owner	69.1% (n=1,077)	69.7% (n=1,039)	-0.6%
	Non-owner	69.5% (n=1,244)	68.9% (n=1,184)	0.5%
Livestock	Owner	74.6% (n=398)	75.3% (n=380)	-0.6%
	Non-owner	68.2% (n=1,923)	68.0% (n=1,843)	0.1%
Gender	Female	63.4% (n=715)	63.9% (n=687)	-0.5%
	Male	71.9% (n=1,606)	71.7% (n=1,536)	0.2%
Guard dog	Yes	73.4% (n=1,565)	73.1% (n=1,512)	0.3%
	No	61.3% (n=732)	61.2% (n=711)	0.2%
Hunting dog	Yes	53.3% (n=137)	53.3% (n=135)	0.0%
	No	70.6% (n=2,160)	70.3% (n=2,088)	0.2%
Pet dog	Yes	64.4% (n=873)	64.2% (n=848)	0.2%
	No	72.7% (n=1,424)	72.4% (n=1,375)	0.2%

Appendix XI. Effect of data cleaning on dog vaccination explanatory variables

Table summarizing the effect of data cleaning on the distribution of explanatory variables used in multivariate binomial generalized linear models. Full data sets were cleaned to remove dogs with incomplete sets of explanatory data, to produce an edited dataset.

Variable	Levels	Full dataset distribution	Edited dataset distribution	Difference
Study area	Southwest	13.7% (n=1,109)	13.6% (n=1,079)	0.1%
	Lazovskii	9.4% (n=680)	9.0% (n=635)	0.4%
	SABZ	9.4% (n=465)	10.2% (n=432)	-0.7%
Community type	Village	10.6% (n=1,262)	10.8% (n=1,211)	-0.2%
	Town	11.2% (n=865)	11.2% (n=812)	0.0%
	Large town	22.8% (n=127)	21.1% (n=123)	1.7%
Residence type	Apartment	25.3% (n=150)	23.6% (n=144)	1.7%
	Cottage	10.6% (n=2,104)	10.7% (n=2,002)	-0.1%
Number of people	1	7.7% (n=325)	7.5% (n=318)	0.1%
	2	12.5% (n=766)	12.8% (n=742)	-0.3%
	3	14.8% (n=481)	14.6% (n=466)	0.2%
	4	10.3% (n=349)	10.3% (n=339)	0.0%
	>5	9.0% (n=288)	9.3% (n=281)	-0.2%
Children	Present	10.8% (n=1,377)	11.0% (n=1,342)	-0.1%
	Absent	12.6% (n=832)	12.6% (n=804)	0.1%
Cats	Owner	13.5% (n=480)	13.9% (n=447)	-0.3%
	Non-owner	11.0% (n=1,774)	11.0% (n=1,699)	0.0%
Poultry	Owner	11.9% (n=1,203)	11.9% (n=1,136)	0.0%
	Non-owner	11.1% (n=1,051)	11.2% (n=1,010)	-0.1%
Livestock	Owner	11.4% (n=1,869)	11.5% (n=1,776)	-0.1%
	Non-owner	12.2% (n=385)	11.9% (n=370)	0.3%
Gender	Female	13.0% (n=694)	13.0% (n=663)	0.0%
	Male	10.9% (n=1,560)	10.9% (n=1,483)	0.0%
Source	Local	8.7% (n=1,545)	8.8% (n=1,497)	-0.1%
	Non-local	18.1% (n=669)	17.9% (n=649)	0.2%
Guard dog	Yes	18.4% (n=702)	18.0% (n=683)	0.4%
	No	8.4% (n=1,520)	8.5% (n=1,463)	-0.1%
Hunting dog	Yes	10.9% (n=2,089)	10.9% (n=2,014)	0.0%
	No	21.8% (n=133)	22.0% (n=132)	-0.2%
Companion dog	Yes	10.2% (n=1,388)	10.4% (n=1,335)	-0.2%
	No	13.8% (n=834)	13.4% (n=811)	0.3%

Appendix XII. Effect of data cleaning on dog roaming explanatory variables

Table summarizing the effect of data cleaning on the distribution of explanatory variables used in multivariate binomial generalized linear models. Full data sets were cleaned to remove dogs with incomplete sets of explanatory data, to produce an edited dataset.

Variable	Explanatory levels	Response level	Full dataset distribution	Edited dataset distribution	Diff.
Study area	Southwest	Non-roam	59.0% (n=1,155)	58.4% (n=1,122)	-0.6%
		Non-roam	41.0% (n=1,155)	41.6% (n=1,122)	0.6%
	Lazovskii	Non-roam	77.1% (n=707)	77.4% (n=660)	0.3%
		Non-roam	22.9% (n=707)	22.6% (n=660)	-0.3%
	SABZ	Non-roam	65.3% (n=461)	65.1% (n=427)	0.2%
		Non-roam	34.7% (n=461)	34.9% (n=427)	0.2%
Community type	Village	Non-roam	59.5% (n=1,290)	58.9% (n=1,237)	-0.6%
		Non-roam	40.5% (n=1,290)	41.1% (n=1,237)	0.6%
	Town	Non-roam	72.4% (n=896)	72.3% (n=840)	-0.1%
		Non-roam	27.6% (n=896)	27.7% (n=840)	0.1%
	Large town	Non-roam	81.8% (n=137)	81.8% (n=132)	0.0%
		Non-roam	18.2% (n=137)	18.2% (n=132)	0.0%
Residence type	Apartment	Non-roam	78.71% (n=169)	77.8% (n=162)	-0.9%
		Non-roam	21.29% (n=169)	22.2% (n=162)	0.9%
	Cottage	Non-roam	64.8% (n=2,154)	64.4% (n=2,047)	-0.4%
		Non-roam	35.2% (n=2,154)	35.6% (n=2,047)	0.4%
Number of people	1	Non-roam	63.6% (n=330)	63.2% (n=321)	-0.4%
		Non-roam	36.4% (n=330)	36.8% (n=321)	0.4%
	2	Non-roam	63.7% (n=790)	62.9% (n=761)	-0.8%
		Non-roam	36.3% (n=790)	37.1% (n=761)	0.8%
	3	Non-roam	70.9% (n=494)	71.2% (n=483)	0.3%
		Non-roam	29.1% (n=494)	28.8% (n=483)	-0.3%
	4	Non-roam	61.8% (n=364)	61.9% (n=357)	0.1%
		Non-roam	38.2% (n=364)	38.1% (n=357)	-0.1%
	>5	Non-roam	69.6% (n=299)	68.6% (n=287)	-1.0%
		Non-roam	30.4% (n=299)	31.4% (n=287)	1.0%
Children	Present	Non-roam	67% (n=861)	66.8% (n=835)	-0.2%
		Non-roam	33% (n=861)	33.2% (n=835)	0.2%
	Absent	Non-roam	64.9%	64.5%	-0.4%

			(n=1,416)	(n=1,374)	
		Non-roam	35.1% (n=1,416)	35.5% (n=1,374)	0.4%
Cats	Owner	Non-roam	64.0% (n=1,822)	63.6% (n=1,736)	-0.4%
		Non-roam	36.0% (n=1,822)	36.4% (n=1,736)	0.4%
	Absent	Non-roam	72.3% (n=501)	71.9% (n=473)	-0.4%
		Non-roam	27.7% (n=501)	28.1% (n=473)	0.4%
Poultry	Owner	Non-roam	65.2% (n=1,073)	65.0% (n=1,031)	-0.2%
		Non-roam	34.8% (n=1,073)	35.0% (n=1,031)	0.2%
	Absent	Non-roam	66.2% (n=1,250)	65.7% (n=1,178)	-0.5%
		Non-roam	33.8% (n=1,250)	34.3% (n=1,178)	0.5%
Livestock	Owner	Non-roam	61.7% (n=389)	61.0% (n=377)	-0.7%
		Non-roam	38.3% (n=389)	39.0% (n=377)	0.7%
	Absent	Non-roam	66.6% (n=1,934)	66.3% (n=1,832)	-0.3%
		Non-roam	33.4% (n=1,934)	33.7% (n=1,832)	0.3%
Gender	Male	Non-roam	67.3% (n=713)	66.9% (n=685)	-0.4%
		Non-roam	32.7% (n=713)	33.1% (n=685)	0.4%
	Female	Non-roam	65.1% (n=1,610)	64.7% (n=1,524)	-0.4%
		Non-roam	34.9% (n=1,610)	35.3% (n=1,524)	0.4%
Source	Local	Non-roam	64.6% (n=1,584)	64.3% (n=1,534)	-0.3%
		Non-roam	35.4% (n=1,584)	35.7% (n=1,534)	0.3%
	Non-local	Non-roam	67.8% (n=699)	67.9% (n=675)	0.1%
		Non-roam	32.2% (n=699)	32.1% (n=675)	-0.1%
Guard dog	Yes	Non-roam	65.2% (n=1,573)	65.1% (n=1,502)	-0.1%
		Non-roam	34.8% (n=1,573)	34.9% (n=1,502)	0.1%
	No	Non-roam	66.3% (n=732)	65.9% (n=707)	-0.4%
		Non-roam	33.7% (n=732)	34.1% (n=707)	0.4%
Hunting dog	Yes	Non-roam	70.8% (n=137)	70.4% (n=135)	-0.4%
		Non-roam	29.2% (n=137)	29.6% (n=135)	0.4%
	No	Non-roam	65.2% (n=2,168)	65.0% (n=2,075)	-0.2%
		Non-roam	34.8% (n=2,168)	35.0% (n=2,075)	0.2%
Companion dog	Yes	Non-roam	62.9% (n=874)	62.5% (n=839)	-0.4%
		Non-roam	37.1% (n=874)	37.5% (n=839)	0.4%
	No	Non-roam	67.1% (n=1,431)	67.2% (n=1,370)	0.1%
		Non-roam	32.9% (n=1,431)	32.8% (n=1,370)	-0.1%

Appendix XIII. Selection process for multivariate generalized binary logistic regression models predicting dog ownership based on Akaike information criterion values

Selection process for multivariate generalized binary logistic regression models predicting dog ownership, based on Akaike information criterion (AIC) values. Explanatory variables include study area (SA), community type (CA), children in house (CH), people in house (PL), cat owner (CO), poultry owner (PO), livestock owner (LO), residence type (RT). Settlement was included as a random variable. Models were constructed using a forward selection process using AIC values assess model quality. Lowest AIC values at each stage of model construction are indicated in bold, and final model is highlighted in grey.

Model variables	AIC	ΔAIC
Base	2878.389	693.278
SA	2879.627	694.516
CT	2867.829	682.718
CH	2869.597	684.486
PL	2822.85	637.739
CO	2635.77	450.659
PO	2658.987	473.876
LO	2806.687	621.576
RT	**2386.601**	**201.49**
RT + SA	2385.709	200.598
RT + CT	2384.003	198.892
RT + CH	2367.688	182.577
RT + PL	2333.552	148.441
RT + CO	**2281.289**	**96.178**
RT + PO	2312.834	127.723
RT + LO	2355.767	170.656
RT + CO + SA	2280.623	95.512
RT + CO + CT	2279.155	94.044
RT + CO + CH	2270.779	85.668
RT + CO + PL	2246.669	61.558
RT + CO + PO	**2228.644**	**43.533**
RT + CO + LO	2260.124	75.013
RT + CO + PO + SA	2228.463	43.352
RT + CO + PO + CT	2226.472	41.361
RT + CO + PO + CH	2216.76	31.649
RT + CO + PO + PL	**2198.034**	**12.923**
RT + CO + PO + LO	2216.457	31.346

RT + CO + PO + PL + SA	2197.975	12.864
RT + CO + PO + PL + CT	2195.21	10.099
RT + CO + PO + PL + CH	2199.674	14.563
RT + CO + PO + PL + LO	**2187.272**	**2.161**
RT + CO + PO + PL + LO + SA	2187.628	2.517
RT + CO + PO + PL + LO + CT	**2185.111**	**0**
RT + CO + PO + PL + LO + CH	2189.044	3.933
RT + CO + PO + PL + LO + CT + SA	2186.267	1.156
RT + CO + PO + PL + LO + CT + CH	2186.894	1.783

Appendix XIV. Selection process for multivariate generalized binary logistic regression models predicting dog origin based on Akaike information criterion values

Selection process for multivariate generalized binary logistic regression models predicting dog origin, based on Akaike information criterion (AIC) values. Explanatory variables include study area (SA), gender (GE), community type (CA), children in house (CH), people in house (PL), cat owner (CO), poultry owner (PO), livestock owner (LO), residence type (RT), guard dog (GD), hunting dog (HD), companion dog (CD), and source (SO). Household was included as a random variable. Models were constructed using a forward selection process using AIC values assess model quality. Lowest AIC values at each stage of model construction are indicated in bold, and final model is highlighted in grey.

Model variables	**AIC**	**∆AIC**
Base	2682.611	86.145
SA	2674.446	77.98
GE	2663.394	66.928
CT	2662.925	66.459
CH	2684.493	88.027
PL	2684.29	87.824
CO	2680.672	84.206
PO	2684.497	88.031
LO	2681.163	84.697
RT	2661.959	65.493
GD	**2653.241**	**56.775**
HT	2663.596	67.13
CD	2668.972	72.506
GD + SA	2643.189	46.723
GD + GE	2639.381	42.915
GD + CT	**2627.231**	**30.765**
GD + CH	2655.181	58.715
GD + PL	2655.077	58.611
GD + CO	2653.551	57.085
GD + PO	2655.133	58.667
GD + LO	2653.03	56.564
GD + RT	2645.837	49.371
GD + HT	2642.436	45.97
GD + CD	2654.681	58.215
GD + CT + SA	2631.115	34.649

GD + CT + GE	2614.598	18.132
GD + CT + CH	2629.229	32.763
GD + CT + PL	2629.047	32.581
GD + CT + CO	2626.807	30.341
GD + CT + PO	2629.067	32.601
GD + CT + LO	2625.302	28.836
GD + CT + RT	**2611.304**	**14.838**
GD + CT + HT	2614.271	17.805
GD + CT + CD	2628.432	31.966
GD + CT + RT + SA	**2596.466**	**0**
GD + CT + RT + GE	2599.708	3.242
GD + CT + RT + CH	2610.733	14.267
GD + CT + RT + PL	2612.937	16.471
GD + CT + RT + CO	2613.012	16.546
GD + CT + RT + PO	2613.154	16.688
GD + CT + RT + LO	2613.206	16.74
GD + CT + RT + HT	2613.289	16.823
GD + CT + RT + CD	2614.819	18.353
GD + CT + RT + SA + GE	2603.236	6.77
GD + CT + RT + SA + CH	2616.81	20.344
GD + CT + RT + SA + PL	2616.73	20.264
GD + CT + RT + SA + CO	2616.428	19.962
GD + CT + RT + SA + PO	2616.628	20.162
GD + CT + RT + SA + LO	2614.29	17.824
GD + CT + RT + SA + HT	2599.906	3.44
GD + CT + RT + SA + CD	2616.546	20.08

Appendix XV. Selection process for multivariate generalized binary logistic regression models predicting dog roaming based on Akaike information criterion values

Selection process for multivariate generalized binary logistic regression models predicting dog roaming, based on Akaike information criterion (AIC) values. Explanatory variables include age (AG), study area (SA), gender (GE), community type (CA), children in house (CH), people in house (PL), cat owner (CO), poultry owner (PO), livestock owner (LO), residence type (RT), guard dog (GD), hunting dog (HD), companion dog (CD), and source (SO). Household was included as a random variable. Models were constructed using a forward selection process using AIC values assess model quality. Lowest AIC values at each stage of model construction are indicated in bold, and final model is highlighted in grey.

Model variables	AIC	ΔAIC
Base	2264.436	6.137
AG	2264.522	6.223
GE	2266.436	8.137
SA	2264.639	6.34
CT	2265.134	6.835
CH	2265.8	7.501
PL	2265.685	7.386
CO	2265.845	7.546
PO	2266.325	8.026
LO	2266.439	8.14
RT	2265.855	7.556
GD	2264.777	6.478
HT	2266.205	7.906
CD	**2260.032**	**1.733**
SO	2266.278	7.979
CD + AG	2259.58	1.281
CD + GE	2261.907	3.608
CD + SA	2260.209	1.91
CD + CT	**2259.084**	**0.785**
CD + CH	2261.284	2.985
CD + PL	2261.237	2.938
CD + CO	2260.782	2.483
CD + PO	2262.022	3.723
CD + LO	2262.002	3.703
CD + RT	2259.763	1.464

CD + GD	2261.773	3.474
CD + HT	2262.028	3.729
CD + SO	2261.582	3.283
CD + CT + AG	**2258.408**	**0.109**
CD + CT + GE	2260.968	2.669
CD + CT + SA	2258.889	0.59
CD + CT + CH	2260.506	2.207
CD + CT + PL	2260.335	2.036
CD + CT + CO	2260.476	2.177
CD + CT + PO	2260.754	2.455
CD + CT + LO	2261.084	2.785
CD + CT + RT	2260.53	2.231
CD + CT + GD	2260.988	2.689
CD + CT + HT	2261.084	2.785
CD + CT + SO	2260.363	2.064
CD + CT + AG + GE	2432.884	174.585
CD + CT + AG + SA	**2258.299**	**0**
CD + CT + AG + CH	2260.031	1.732
CD + CT + AG + PL	2259.845	1.546
CD + CT + AG + CO	2259.697	1.398
CD + CT + AG + PO	2260.094	1.795
CD + CT + AG + LO	2260.384	2.085
CD + CT + AG + RT	2259.635	1.336
CD + CT + AG + GD	2260.353	2.054
CD + CT + AG + HT	2260.404	2.105
CD + CT + AG + SO	2259.662	1.363
CD + CT + AG + SA + GE	2291.316	33.017
CD + CT + AG + SA + CH	2263.514	5.215
CD + CT + AG + SA + PL	2280.086	21.787
CD + CT + AG + SA + CO	2372.313	114.014
CD + CT + AG + SA + PO	2264.139	5.84
CD + CT + AG + SA + LO	2260.161	1.862
CD + CT + AG + SA + RT	2334.109	75.81
CD + CT + AG + SA + GD	2292.649	34.35
CD + CT + AG + SA + HT	2262.769	4.47
CD + CT + AG + SA + SO	2271.048	12.749

Appendix XVI. Selection process for multivariate generalized binary logistic regression models predicting dog vaccination based on Akaike information criterion values

Selection process for multivariate generalized binary logistic regression models predicting dog vaccination, based on Akaike information criterion (AIC) values. Explanatory variables include study area (SA), gender (GE), community type (CA), children in house (CH), people in house (PL), cat owner (CO), poultry owner (PO), livestock owner (LO), residence type (RT), guard dog (GD), hunting dog (HD), companion dog (CD), and source (SO). Household was included as a random variable. Models were constructed using a forward selection process using AIC values assess model quality. Lowest AIC values at each stage of model construction are indicated in bold, and final model is highlighted in grey.

Model variables	**AIC**	**∆AIC**
Base	788.9297	8.5174
SA	792.4045	11.9922
GE	790.8916	10.4793
CT	791.764	11.3517
CH	790.9115	10.4992
PL	790.8819	10.4696
CO	790.216	9.8037
PO	790.5131	10.1008
LO	790.8129	10.4006
RT	789.0919	8.6796
GD	784.2839	3.8716
HT	789.8071	9.3948
CD	784.6581	4.2458
SO	**783.9647**	**3.5524**
SO + SA	787.5835	7.1712
SO + GE	785.3715	4.9592
SO + CT	786.651	6.2387
SO + CH	785.934	5.5217
SO + PL	785.9268	5.5145
SO + CO	785.3904	4.9781
SO + PO	785.5706	5.1583
SO + LO	785.8958	5.4835
SO + RT	784.6958	4.2835
SO + GD	**780.4123**	**0**
SO + HT	784.7187	4.3064

SO + CD	780.483	0.0707
SO + GD + SA	783.9979	3.5856
SO + GD + GE	781.8081	1.3958
SO + GD + CT	783.752	3.3397
SO + GD + CH	782.3842	1.9719
SO + GD + PL	782.3896	1.9773
SO + GD + CO	782.1329	1.7206
SO + GD + PO	782.2903	1.878
SO + GD + LO	782.4053	1.993
SO + GD + RT	782.2903	1.878
SO + GD + HT	781.5823	1.17
SO + GD + CD	780.7632	0.3509

Appendix XVII. Canine distemper virus full genomes

Table listing all 51 full canine distemper virus genomes listed retrieved from GenBank

Accession	Epi-data: Clade	Country of origin	Host species
AB474397	Asia 2	Japan	Dog
AB475097	Asia 2	Japan	Dog
AB475099	Asia 2	Japan	Dog
AB476401	Asia 2	Japan	Dog
AB476402	Asia 2	Japan	Dog
AB490670	Asia 2	Japan	Dog
AB490672	Asia 2	Japan	Dog
AB490674	Asia 2	Japan	Dog
AB490676	Asia 2	Japan	Dog
AB490678	Asia 2	Japan	Dog
AB490679	Asia 2	Japan	Dog
AB490680	Asia 2	Japan	Dog
AB490681	Asia 2	Japan	Dog
AB687720	Asia 1	Japan	Crab-eating macaque
AB687721	Asia 1	Japan	Crab-eating macaque
AF014953	America 1	Not applicable	Vaccine
AF164967	America 2	USA	Dog
AF305419	America 1	Not applicable	Vaccine
AF378705	America 1	Not applicable	Vaccine
AY386315	Europe/South America 1	Germany	Dog
AY386316	Europe/South America 1	Germany	Dog
AY443350	America 2	USA	Raccoon
AY445077	America 1	USA	Raccoon
AY466011	America 1	USA	Raccoon
AY542312	America 1	USA	Raccoon
AY649446	America 2	USA	Raccoon
EU716337	America 2	USA	Dog
EU726268	America 1	China	American mink
GU138403	America 1	Not applicable	Vaccine
HM046486	America 1	Kazakhstan	Caspian seal
HM063009	America 1	Kazakhstan	American mink
HM852904	Asia 1	China	Rhesus macaque
JN896331	Asia 1	China	Dog
JN896987	America 1	Not applicable	Vaccine
JX681125	Asia 1	China	Fox sp
KC427278	Asia 1	China	American mink
KF640687	America 3	USA	Dog
KF856711	Asia 1	China	Monkey sp
KF914669	Arctic	Italy	Dog
KJ123771	America 2	USA	Dog
KJ466106	Asia 1	China	Raccoon dog
KJ848781	Asia 1	China	Raccoon dog

KJ994343	Asia 1	China	Raccoon dog
KM926612	America 1	China	European polecat
KP677502	Asia 1	China	Giant panda
KP738610	Asia 1	China	Raccoon dog
KP765763	Asia 1	China	Fox sp
KP765764	Asia 1	China	Fox sp
KP793921	Asia 1	China	Giant panda
KX024708	Arctic	Italy	Eurasian badger
KX024709	Arctic	Italy	Eurasian badger

Appendix XVIII. Arctic-like clade canine distemper viruses

Table listing all haemagglutinin sequences (>1,500 bp) from all 37 canine distemper viruses from the Arctic-like clade retrieved from GenBank. Table includes two unpublished viruses from Arctic foxes, obtained from K. Beckmen, Alaska Department of Fish and Wildlife.

Accession number	Country of origin	Year	Host species	Sequence length
Z47760	Greenland	1988	Dog	1,824
X84998	Russia	1988	Baikal seal	1,824
AF172411	China	1995	Dog	1,824
EF445052	China	2005	Fox sp.	1,824
JQ732170	China	2005	Raccoon dog	1,824
GQ214373	Austria	2003	Dog	1,824
DQ889178	Hungary	2005	Dog	1,824
DQ889184	Hungary	2006	Dog	1,824
DQ889185	Hungary	2006	Dog	1,824
HM443710	Italy	2000	Dog	1,704
HM443713	Italy	2000	Dog	1,668
HM443714	Italy	2000	Dog	1,704
HM443711	Italy	2001	Dog	1,704
HM443721	Italy	2002	Dog	1,704
HM443722	Italy	2002	Dog	1,704
HM443724	Italy	2002	Dog	1,704
DQ226087	Italy	2004	Dog	1,824
HM443715	Italy	2004	Dog	1,704
DQ226088	Italy	2005	Dog	1,824
HM443712	Italy	2005	Dog	1,704
HM443706	Italy	2008	Dog	1,704
KM115533	Italy	2012	Dog	1,824
KF914669	Italy	2013	Dog	1,824
KM115532	Italy	2013	Dog	1,824
KM115534	Italy	2013	Dog	1,824
KM115535	Italy	2013	Dog	1,824
KM115536	Italy	2013	Dog	1,824
KX024708	Italy	2013	Eurasian badger	1,824
KX024709	Italy	2013	Eurasian badger	1,824
KC966928	Italy	2013	Grey wolf	1,824
KR002657	Switzerland	2013	Dog	1,824
KR002658	Switzerland	2013	Dog	1,824
KR002659	Switzerland	2013	Dog	1,824

KR002660	Switzerland	2013	Dog	1,824
KR002661	Switzerland	2013	Dog	1,824
AY964108	USA	2004	Dog	1,824
AY964112	USA	2012	Dog	1,824
[TBD] AF254	USA	2012	Arctic fox	1,824
[TBD] AF207	USA	2014	Arctic fox	1,824

Appendix IXX. Effect of data cleaning on dog serology explanatory variables

Table summarizing the effect of data cleaning on the distribution of explanatory variables used in multivariate binomial generalized linear models. Full data sets were cleaned to remove dogs with incomplete sets of explanatory data, to produce an edited dataset.

Variable	Levels	Full dataset distribution	Edited dataset distribution	Difference
Study area	Southwest	10.2% (n=108)	8.8% (n=102)	1.4%
	Lazovskii	29.7% (n=165)	27.7% (n=155)	2.0%
	SABZ	43.9% (n=107)	48.1% (n=79)	-4.2%
Community type	Village	21.4% (n=182)	18.9% (n=169)	2.5%
	Town	35.4% (n=192)	36.0% (n=161)	-0.6%
	Large town	0.0% (n=6)	0.0% (n=5)	0.0%
Residence type	Apartment	37.5% (n=8)	37.5% (n=8)	0.0%
	Cottage	28.0% (n=372)	26.5% (n=328)	1.4%
Number of people	1	22.2% (n=54)	20.8% (n=48)	1.4%
	2	28.0% (n=125)	27.9% (n=111)	0.1%
	3	30.4% (n=79)	31.1% (n=74)	-0.7%
	4	28.1% (n=57)	28.3% (n=53)	-0.2%
	>5	24.1% (n=54)	22.0% (n=50)	2.1%
Children	Present	24.5% (n=143)	24.6% (n=134)	-0.2%
	Absent	28.8% (n=226)	28.2% (n=202)	0.5%
Cats	Owner	29.2% (n=312)	27.7% (n=278)	1.5%
	Non-owner	23.5% (n=68)	22.4% (n=58)	1.1%
Poultry	Owner	27.4% (n=186)	27.3% (n=172)	0.1%
	Non-owner	28.9% (n=194)	26.2% (n=164)	2.6%
Livestock	Owner	20.8% (n=53)	17.6% (n=51)	3.1%
	Non-owner	29.4% (n=327)	28.4% (n=285)	0.9%
Age	1 year	21.8% (n=101)	21.7% (n=92)	0.0%
	2 years	22.4% (n=67)	22.6% (n=62)	-0.2%
	3 years	22.9% (n=48)	21.4% (n=42)	1.5%
	4 years	19.4% (n=36)	15.2% (n=33)	4.3%
	5 years	26.1% (n=23)	20.0% (n=20)	6.1%
	6 years	35.0% (n=20)	35.0% (n=20)	0.0%
	7 years	57.1% (n=14)	58.3% (n=12)	-1.2%
	8 years	35.0% (n=20)	33.3% (n=15)	1.7%
	9 years	55.6% (n=9)	62.5% (n=8)	-6.9%
	10 years	40.0% (n=20)	31.3% (n=16)	8.8%
	11 years	40.0% (n=5)	40.0% (n=5)	0.0%
	12 years	66.7% (n=6)	80.0% (n=5)	-13.3%
	13 years	50.0% (n=2)	100.0% (n=1)	-50.0%
	14 years	100.0% (n=1)	100.0% (n=1)	0.0%

	15 years	25.0% (n=4)	25.0% (n=4)	0.0%
Gender	Male	28.3% (n=269)	27.4% (n=237)	0.8%
	Female	27.9% (n=111)	25.3% (n=99)	2.7%
Forest visits	Yes	34.2% (n=152)	33.6% (n=146)	0.6%
	No	25.1% (n=207)	21.6% (n=190)	3.5%
Source	Local	26.2% (n=271)	25.7% (n=245)	0.5%
	Non-local	31.7% (n=101)	29.7% (n=91)	2.0%
Guard dog	Yes	25.6% (n=270)	23.5% (n=238)	2.0%
	No	34.0% (n=106)	34.7% (n=98)	-0.7%
Hunting dog	Yes	28.6% (n=14)	36.4% (n=11)	-7.8%
	No	27.9% (n=362)	26.5% (n=325)	1.4%
Pet dog	Yes	30.2% (n=149)	29.2% (n=137)	1.0%
	No	26.4% (n=227)	25.1% (n=199)	1.3%

Appendix XX. Effect of data cleaning on tiger serology explanatory variables

Table summarizing the effect of data cleaning on the distribution of explanatory variables used in multivariate binomial generalized linear models. Full data sets were cleaned to remove tigers with incomplete sets of explanatory data, to produce an edited dataset.

Variable	Levels	Full dataset distribution	Edited dataset distribution	Difference
Age	1 year	24.2% (n=16)	26.7% (n=16)	-2.4%
	2 years	25.8% (n=17)	25.0% (n=15)	0.8%
	3 years	9.1% (n=6)	5.0% (n=3)	4.1%
	4 years	1.5% (n=1)	1.7% (n=1)	-0.2%
	5 years	10.6% (n=7)	11.7% (n=7)	-1.1%
	6 years	4.5% (n=3)	5.0% (n=3)	-0.5%
	7 years	1.5% (n=1)	1.7% (n=1)	-0.2%
	8 years	7.6% (n=5)	8.3% (n=5)	-0.8%
	9 years	4.5% (n=3)	5.0% (n=3)	-0.5%
	11 years	1.5% (n=1)	1.7% (n=1)	-0.2%
	12 years	3.0% (n=2)	3.3% (n=2)	-0.3%
	13 years	3.0% (n=2)	1.7% (n=1)	1.3%
	14 years	3.0% (n=2)	3.3% (n=2)	-0.3%
Gender	Male	57.4% (n=39)	60.0% (n=36)	-2.6%
	Female	42.6% (n=29)	40.0% (n=24)	2.6%
Study area	Southwest	7.4% (n=5)	8.3% (n=5)	-1.0%
	SABZ	64.7% (n=44)	70.0% (n=42)	-5.3%
	Non-study	27.9% (n=19)	21.7% (n=13)	6.3%
Animal category	Conflict	27.9% (n=19)	20.0% (n=12)	7.9%
	Research	72.1% (n=49)	80.0% (n=48)	-7.9%
Human density	Negligible	80.6% (n=50)	81.7% (n=49)	-1.0%
	Low	1.6% (n=1)	0.0% (n=0)	1.6%
	Moderate	17.7% (n=11)	18.3% (n=11)	-0.6%

Appendix XXI. Large carnivore raw serology results

Virus neutralization (VN) titers for large carnivores sampled from 1992 to 2014 in Primorskii Krai study areas Southwest Primorskii (SW), and Sikhote-Alin Biosphere Zapovednik (SZ). Details of individual animals include age (in months, or not recorded NR), and sex (male – M, female – F, or unknown – U). Virus neutralization titres against the Onderstepoort strain of canine distemper virus are reported with negative samples (Neg) reflecting a titre lower than 1:4.

Animal identity	Species	Date	Animal category	Study area	Latitude	Longitude	Age	Sex	VN titre
PT001	Amur tiger	17-Mar-02	Research	SABZ	45.163	136.781	132	F	Neg (1:8)*
PT001	Amur tiger	24-Mar-96	Research	SABZ	45.256	136.761	60	F	Neg (1:4)
PT001	Amur tiger	15-Mar-98	Research	SABZ	45.163	136.781	84	F	Neg (1:4)
PT003	Amur tiger	20-Mar-97	Research	SABZ	44.947	136.106	96	F	Neg (1:16)*
PT003	Amur tiger	27-Apr-95	Research	SABZ	44.941	136.235	72	F	Neg (1:4)
PT003	Amur tiger	11-Oct-97	Research	SABZ	44.955	136.204	96	F	Neg (1:4)
PT004	Amur tiger	08-Nov-92	Research	SABZ	45.350	136.468	60	F	Neg (1:4)
PT008	Amur tiger	22-Nov-92	Research	SABZ	44.926	136.353	6	F	Neg (1:4)
PT009	Amur tiger	22-Nov-92	Research	SABZ	44.926	136.353	6	M	Neg (1:4)
PT014	Amur tiger	25-Apr-93	Research	SABZ	45.340	136.464	NR	F	Neg (1:4)
PT015	Amur tiger	21-Dec-95	Research	SABZ	44.916	136.480	144	F	Neg (1:4)
PT016	Amur tiger	28-Mar-96	Research	SABZ	44.880	136.361	60	M	Neg (1:4)
PT016	Amur tiger	10-Mar-98	Research	SABZ	44.971	136.499	84	M	Neg (1:4)
PT017	Amur tiger	12-Mar-94	Conflict	SABZ	45.112	136.538	NR	M	Neg (1:4)
PT018	Amur tiger	27-Apr-94	Research	Non-study	45.021	135.958	10	M	Neg (1:4)
PT018	Amur tiger	02-May-95	Research	Non-study	44.686	135.681	22	M	Neg (1:4)
PT019	Amur tiger	13-Oct-94	Research	SABZ	45.066	135.954	16	M	Neg (1:4)
PT020	Amur tiger	19-Mar-98	Research	SABZ	44.970	136.190	144	M	Neg (1:4)
PT020	Amur tiger	26-Feb-00	Research	SABZ	44.970	136.428	168	M	Neg (1:4)
PT022	Amur tiger	18-Jun-96	Research	SABZ	44.703	136.048	42	M	Neg (1:4)
PT022	Amur tiger	15-Mar-97	Research	Non-study	44.570	135.810	48	M	Neg (1:4)
PT023	Amur tiger	18-Oct-96	Research	SABZ	45.543	136.505	14	F	Neg (1:4)
PT025	Amur tiger	20-Nov-97	Research	SABZ	44.940	136.244	18	F	Neg (1:4)
PT026	Amur tiger	23-Nov-97	Research	SABZ	44.949	136.206	18	F	Neg (1:4)
PT035	Amur tiger	20-Mar-02	Research	SABZ	44.910	136.351	108	F	Neg (1:16)*
PT035	Amur tiger	09-Apr-06	Research	SABZ	44.915	136.506	156	F	Neg (1:16)*
PT035	Amur tiger	21-Oct-99	Research	SABZ	44.928	136.371	72	F	Neg (1:4)
PT037	Amur tiger	17-Nov-99	Research	SABZ	44.925	136.334	96	F	Neg (1:4)
PT037	Amur tiger	20-Nov-03	Research	SABZ	44.930	136.214	144	F	Neg (1:4)
PT040	Amur tiger	15-Oct-00	Research	SABZ	44.949	136.206	72	M	Pos (1:128)

PT041	Amur tiger	22-Nov-00	Conflict	SABZ	45.042	136.588	24	M	Neg (1:4)
PT043	Amur tiger	09-Dec-00	Conflict	Non-study	46.446	135.837	24	M	Pos (1:256)
PT045	Amur tiger	16-Jan-01	Conflict	Non-study	46.010	134.164	6	M	Neg (1:4)
PT046	Amur tiger	16-Jan-01	Conflict	Non-study	46.010	134.164	6	M	Pos (1:64)
PT047	Amur tiger	18-Feb-01	Conflict	SABZ	44.815	136.106	24	M	Neg (1:4)
PT048	Amur tiger	17-Mar-01	Conflict	Non-study	46.899	134.446	150	M	Neg (1:4)
PT049	Amur tiger	22-May-01	Research	SABZ	44.942	136.316	36	M	Neg (1:4)
PT049	Amur tiger	05-Jun-05	Research	SABZ	44.880	136.361	90	M	Neg (1:8)*
PT050	Amur tiger	01-Jun-01	Research	SABZ	44.926	136.353	90	M	Neg (1:4)
PT051	Amur tiger	04-Jul-01	Conflict	Non-study	49.423	136.536	24	F	Neg (1:4)
PT054	Amur tiger	06-Oct-02	Conflict	SABZ	45.042	136.588	4	F	Neg (1:4)
PT055	Amur tiger	07-Oct-07	Research	SABZ	44.896	136.336	72	F	Pos (1:128)
PT055	Amur tiger	24-Oct-02	Research	SABZ	44.946	136.206	15	F	Neg (1:4)
PT056	Amur tiger	28-Oct-05	Research	SABZ	44.897	136.515	52	F	Neg (1:8)*
PT056	Amur tiger	24-Oct-02	Research	SABZ	44.948	136.326	16	F	Neg (1:4)
PT056	Amur tiger	24-Mar-10	Research	SABZ	44.963	136.490	108	F	Pos (1:128)
PT057	Amur tiger	07-Nov-02	Conflict	SABZ	44.926	136.353	12	M	Neg (1:4)
PT058	Amur tiger	15-Dec-02	Research	SABZ	44.925	136.484	18	M	Neg (1:4)
PT060	Amur tiger	15-May-03	Research	SABZ	45.008	136.497	54	M	Neg (1:4)
PT061	Amur tiger	26-Nov-03	Conflict	Non-study	46.711	134.036	30	F	Pos (1:256)
PT061	Amur tiger	03-Dec-03	Conflict	Non-study	46.711	134.036	30	F	Pos (1:256)
PT061	Amur tiger	14-Dec-03	Conflict	Non-study	46.711	134.036	30	F	Pos (1:128)
PT062	Amur tiger	22-Feb-04	Research	Non-study	44.866	134.783	96	M	Pos (1:16)
PT063	Amur tiger	30-Apr-04	Research	SABZ	44.925	136.334	36	M	Pos (1:64)
PT064	Amur tiger	24-May-04	Research	SABZ	44.936	136.554	12	M	Neg (1:4)
PT067	Amur tiger	16-Aug-04	Research	SABZ	44.799	136.415	1	F	Neg (1:16)*
PT069	Amur tiger	17-Sep-04	Research	SABZ	44.917	136.494	15	M	Neg (1:8)*
PT071	Amur tiger	17-Mar-05	Research	Non-study	43.819	132.172	66	M	Neg (1:4)
PT074	Amur tiger	11-Oct-05	Research	SABZ	45.010	136.583	1	F	Neg (1:4)
PT075	Amur tiger	28-Oct-05	Research	SABZ	44.897	136.518	15	F	Neg (1:4)
PT079	Amur tiger	13-Oct-06	Research	SABZ	44.928	136.558	13	F	Pos (1:128)
PT081	Amur tiger	13-Oct-06	Research	SABZ	44.928	136.559	13	F	Pos (1:256)

PTI339	Amur tiger	26-Oct-06	Research	Southwest	43.498	131.556	168	M	Pos (1:8)
PTI340	Amur tiger	08-Nov-06	Research	Southwest	43.499	131.535	108	F	Neg (1:4)
PTI341	Amur tiger	10-Nov-06	Research	Southwest	43.498	131.556	60	M	Neg (1:8)*
PT085	Amur tiger	14-Oct-07	Research	SABZ	45.023	136.205	96	M	Pos (1:32)
PT088	Amur tiger	03-May-08	Research	SABZ	44.930	136.555	12	M	Pos (1:32)
PT089	Amur tiger	23-May-08	Research	SABZ	44.930	136.557	12	M	Pos (1:128)
PT094	Amur tiger	04-Jun-09	Research	SABZ	44.941	136.192	60	F	Pos (1:64)
PT095	Amur tiger	01-Nov-09	Research	SABZ	45.315	136.479	60	M	Neg (1:16)*
PT096	Amur tiger	07-Nov-09	Research	SABZ	45.318	136.482	24	M	Neg (1:16)*
PT097	Amur tiger	07-Nov-09	Research	SABZ	45.317	136.479	24	F	Neg (1:16)*
PT100	Amur tiger	05-Nov-10	Research	SABZ	44.928	136.206	48	M	Neg (1:16)*
PT100	Amur tiger	17-Nov-11	Research	Non-study	45.022	135.957	60	M	Neg (1:8)*
PT112	Amur tiger	05-Jun-11	Conflict	Non-study	47.279	134.460	19	F	Neg (1:8)*
PT113	Amur tiger	14-Oct-11	Research	Southwest	43.494	131.561	108	M	Pos (1:64)
PT114	Amur tiger	21-Oct-11	Research	SABZ	44.909	136.356	30	F	Pos (1:256)
PT115	Amur tiger	29-Oct-11	Research	Southwest	43.501	131.539	54	M	Pos (1:64)
PT117	Amur tiger	30-Aug-12	Conflict	Non-study	47.146	136.094	30	F	Neg (1:8)*
PT124	Amur tiger	28-Aug-13	Conflict	Non-study	46.858	134.447	11	F	Neg (1:8)*
PT124	Amur tiger	21-Dec-13	Conflict	Non-study	46.858	134.447	13.5	F	Neg (1:8)*
PT125	Amur tiger	29-Aug-13	Conflict	Non-study	46.858	134.447	9.5	F	Pos (1:64)
PT125	Amur tiger	21-Dec-13	Conflict	Non-study	46.858	134.447	15	F	Pos (1:32)
Tga 003	Amur tiger	02-Feb-14	Sick	Non-study	44.563	133.516	96	M	Neg (1:8)*
Tiger Nov-14	Amur tiger	14-Nov-14	Conflict	Non-study	47.450	134.932	30	M	Neg (1:4)
Ustin	Amur tiger	21-Dec-13	Conflict	Non-study	44.240	135.225	12	M	Neg (1:4)
Borya	Amur tiger	21-Dec-13	Conflict	Non-study	44.563	133.516	12	M	Pos (1:128)
Kuzya	Amur tiger	22-Dec-13	Conflict	Non-study	44.563	133.516	12	M	Pos (1:64)
PPO1	Far Eastern leopard	23-Jun-93	Research	Southwest	43.095	131.558	36	F	Pos (1:64)
PPO2	Far Eastern leopard	17-Nov-93	Research	Southwest	43.101	131.556	66	M	Neg (1:4)
PPO3	Far Eastern leopard	21-Apr-94	Research	Southwest	43.512	131.536	NR	M	Neg (1:4)
PPO5	Far Eastern leopard	09-Aug-94	Research	Southwest	43.096	131.558	48	F	Pos (1:256)
PPO7	Far Eastern leopard	26-Apr-96	Research	Southwest	43.030	131.406	NR	U	Neg (1:4)
PPO6	Far Eastern leopard	07-Apr-97	Research	Southwest	43.030	131.406	NR	U	Neg (1:4)

PPA835	Far Eastern leopard	29-Oct-06	Research	Southwest	43.484	131.574	156	M	Neg (1:4)
PPA836	Far Eastern leopard	02-Nov-06	Research	Southwest	43.503	131.537	78	M	Neg (1:8)*
PPA835	Far Eastern leopard	27-Apr-07	Research	Southwest	43.437	131.674	156	M	Neg (1:4)
PPA840	Far Eastern leopard	15-Oct-07	Research	Southwest	43.445	131.598	30	F	Neg (1:4)
PPA836	Far Eastern leopard	18-Oct-07	Research	Southwest	43.451	131.545	90	M	Neg (1:4)
PPA836	Far Eastern leopard	08-Oct-08	Research	Southwest	43.438	131.480	102	M	Neg (1:4)
PPA841	Far Eastern leopard	18-Oct-08	Research	Southwest	43.438	131.480	108	F	Neg (1:4)
LL01	Eurasian lynx	30-Oct-10	Research	SABZ	44.963	136.183	7	F	Neg (1:4)
LL03	Eurasian lynx	16-Mar-01	Research	Non-study	47.379	135.613	12	M	Neg (1:4)
LL05	Eurasian lynx	13-Feb-02	Research	SABZ	44.958	136.511	66	M	Pos (1:16)
LL06	Eurasian lynx	29-Mar-02	Research	SABZ	44.912	136.518	36	M	Neg (1:4)
LL07	Eurasian lynx	23-Nov-05	Research	SABZ	44.917	136.330	NR	M	Neg (1:4)
LL10	Eurasian lynx	31-Oct-10	Research	SABZ	45.015	136.186	84	F	Neg (1:4)
LL11	Eurasian lynx	02-Oct-11	Research	Non-study	45.024	135.960	36	M	Neg (1:4)
UT009	Asiatic Black Bear	19-May-94	Research	SABZ	45.021	135.958	48	M	Neg (1:16)*
UT009	Asiatic Black Bear	27-May-00	Research	SABZ	45.013	135.955	144	M	Neg (1:16)*
UT014	Asiatic Black Bear	03-Jun-95	Research	SABZ	45.023	135.957	48	M	Neg (1:32)*
UT015	Asiatic Black Bear	19-Jun-95	Research	SABZ	45.030	135.958	54	M	Neg (1:32)*
UT016	Asiatic Black Bear	27-Jun-95	Research	SABZ	45.023	135.968	66	M	Neg (1:16)*
UT028	Asiatic Black Bear	19-Apr-97	Research	SABZ	45.043	136.627	16	M	Neg (1:16)*
UT050	Asiatic Black Bear	07-Jun-00	Research	SABZ	45.025	135.957	108	F	Neg (1:16)*
UT053	Asiatic Black Bear	10-Nov-00	Research	SABZ	44.926	136.223	66	F	Neg (1:16)*
UT055	Asiatic Black Bear	29-May-01	Research	SABZ	44.903	136.335	42	M	Neg (1:16)*
UT058	Asiatic Black Bear	08-Jun-01	Research	SABZ	44.906	136.332	90	M	Neg (1:16)*
UT058	Asiatic Black Bear	10-Oct-01	Research	SABZ	44.940	136.104	60	F	Neg (1:16)*
UT060	Asiatic Black Bear	21-Oct-01	Research	Southwest	43.629	132.538	11	F	Neg (1:16)*
UT061	Asiatic Black Bear	29-Oct-01	Research	SABZ	44.939	136.102	162	M	Neg (1:16)*
UT064	Asiatic Black Bear	02-May-01	Research	SABZ	44.936	136.216	108	M	Neg (1:16)*
UT066	Asiatic Black Bear	12-Oct-02	Research	SABZ	44.904	136.333	84	M	Neg (1:16)*
UT067	Asiatic Black Bear	20-Oct-02	Research	SABZ	44.922	136.218	126	M	Neg (1:4)
UT086	Asiatic Black Bear	04-Jun-07	Research	SABZ	44.896	136.344	66	M	Pos (1:256)
UT093	Asiatic Black Bear	03-Jun-07	Research	SABZ	44.902	136.347	48	F	Pos (1:4)

UT098	Asiatic Black Bear	15-May-09	Research	SABZ	45.317	136.481	84	M	Neg (1:16)*
UT106	Asiatic Black Bear	29-Sep-11	Research	Southwest	43.411	131.599	84	F	Neg (1:8)*
UT107	Asiatic Black Bear	30-Oct-11	Research	Southwest	43.470	131.553	NR	F	Neg (1:8)*
UT114	Asiatic Black Bear	10-Aug-93	Research	SABZ	44.939	136.198	NR	M	Neg (1:8)*
UT116	Asiatic Black Bear	09-Sep-93	Research	SABZ	44.890	136.337	NR	M	Neg (1:8)*
UT201	Asiatic Black Bear	09-Aug-93	Research	Southwest	43.341	131.565	216	M	Neg (1:32)*
UT202	Asiatic Black Bear	10-Oct-93	Research	Southwest	43.338	131.566	132	F	Pos (1:8)
UT99	Asiatic Black Bear	30-Sep-09	Research	Southwest	43.411	131.599	NR	Unknown	Neg (1:4)
UTH11	Asiatic Black Bear	18-Oct-07	Research	Southwest	43.457	131.590	60	M	Neg (1:16)*
UA007	Brown Bear	05-Jul-93	Research	SABZ	45.311	136.480	108	M	Pos (1:32)
UA010	Brown Bear	19-May-94	Research	SABZ	45.013	135.954	180	M	Neg (1:16)*
UA029	Brown Bear	29-May-97	Research	SABZ	44.929	136.558	48	F	Neg (1:32)*
UA043	Brown Bear	21-Oct-99	Research	SABZ	44.911	136.330	180	F	Neg (1:16)*
UA045	Brown Bear	31-Oct-99	Research	SABZ	44.917	136.330	96	M	Neg (1:8)*
UA046	Brown Bear	31-Oct-99	Research	SABZ	44.897	136.344	108	F	Neg (1:16)*
UA046	Brown Bear	17-Oct-02	Research	SABZ	44.891	136.336	144	F	Neg (1:8)*
UA056	Brown Bear	31-May-01	Research	SABZ	44.897	136.336	30	F	Neg (1:32)*
UA082	Brown Bear	31-May-04	Research	SABZ	44.930	136.558	36	M	Neg (1:16)*
UA099	Brown Bear	01-Oct-09	Research	SABZ	45.243	136.482	78	M	Neg (1:8)*
UA100	Brown Bear	04-Oct-09	Research	SABZ	45.244	136.478	84	F	Neg (1:8)*
UA101	Brown Bear	15-Oct-10	Research	SABZ	44.980	136.187	42	F	Neg (1:16)*
UA113	Brown Bear	19-Jul-93	Research	SABZ	45.411	136.823	132	M	Neg (1:128)*
UA115	Brown Bear	03-Sep-93	Research	SABZ	45.000	136.171	30	M	Neg (1:16)*
UA117	Brown Bear	10-Sep-93	Research	SABZ	45.042	136.234	NR	M	Neg (1:16)*
UA118	Brown Bear	27-Apr-94	Research	SABZ	45.021	135.959	240	M	Neg (1:8)*
UA121	Brown Bear	31-May-94	Research	SABZ	45.006	135.950	NR	M	Neg (1:8)*
UA122	Brown Bear	05-Jun-94	Research	SABZ	45.008	135.949	NR	F	Neg (1:16)*
UA123	Brown Bear	28-Oct-94	Research	SABZ	45.349	136.444	96	M	Neg (1:16)*
UT104	Brown Bear	23-Sep-11	Research	Non-study	45.026	135.962	120	M	Pos (1:64)
UT105	Brown Bear	12-Oct-11	Research	Non-study	45.006	135.953	72	M	Neg (1:8)*

***Cytotoxicity prevented assessment of neutralization at higher concentrations than the titre indicated.**

Appendix XXII. Mesocarnivore raw serology results

Virus neutralization (VN) titers for mesocarnivores sampled from 2005 to 2014 in Primorskii Krai study areas Southwest Primorskii (SW), Lazovskii (LZ) and Sikhote-Alin Biosphere Zapovednik (SZ). Details of individual animals include age (juvenile – JV, subadult – SA, adult – AD, old – OD, unknown – U), and sex (male – M, female – F, or unknown – U). Virus neutralization titres against the Bussell strain of canine distemper virus are reported with negative samples (Neg) reflecting a titre lower than 1:8.

Animal identity	Species	Date	Study area	Latitude	Longitude	Age	Sex	VN titre
Badger-007	Asian badger	16-Apr-08	LZ	43.011	133.728	AD	M	Neg (1:8)
Badger-010	Asian badger	14-May-08	LZ	42.872	133.792	JV	M	Neg (1:8)
Badger-011	Asian badger	12-Oct-08	LZ	43.384	133.970	AD	F	Neg (1:8)
Badger-033	Asian badger	25-Mar-09	LZ	42.872	133.792	SA	M	Neg (1:8)
Badger-037	Asian badger	08-May-09	LZ	43.011	133.728	AD	F	Neg (1:8)
MM#001	Asian badger	13-Oct-13	LZ	42.872	133.792	AD	F	Neg (1:8)
MM#002	Asian badger	14-Oct-13	LZ	42.864	133.786	SA	M	Neg (1:8)
RU-0009	Asian badger	15-Oct-13	LZ	43.036	134.160	AD	F	Neg (1:8)
MM#003	Asian badger	15-Oct-13	LZ	42.872	133.792	SA	M	Neg (1:8)
MM#004	Asian badger	15-Oct-13	LZ	42.876	133.791	AD	M	Neg (1:8)
RU-0010	Asian badger	18-Oct-13	LZ	43.035	134.155	JV	M	Neg (1:8)
RU-0012	Asian badger	21-Oct-13	LZ	43.034	134.156	AD	M	Neg (1:8)
RU-0013	Asian badger	21-Oct-13	LZ	43.030	134.147	AD	M	Neg (1:8)
MM#005	Asian badger	25-Oct-13	LZ	42.864	133.786	AD	F	Pos (1:11)
RU-0014	Asian badger	05-Nov-13	LZ	43.014	134.127	AD	M	Neg (1:8)
BADGER 2013	Asian badger	24-Jun-13	SZ	45.037	136.632	U	U	Neg (1:8)
RU-0020	Asian badger	23-Apr-14	SZ	44.972	136.554	AD	M	Pos (1:11)
RU-0021	Asian badger	24-Apr-14	SZ	44.977	136.586	AD	M	Neg (1:8)
RU-0022	Asian badger	25-Apr-14	SZ	44.979	136.587	AD	M	Neg (1:8)
RU-0023	Asian badger	05-May-14	SZ	44.966	136.532	U	M	Pos (1:181)
RU-0024	Asian badger	08-May-14	SZ	44.977	136.586	U	M	Pos (1:16)
RU-0025	Asian badger	19-May-14	SZ	44.960	136.528	U	F	Neg (1:8)
RU-0026	Asian badger	19-May-14	SZ	44.943	136.480	U	F	Neg (1:8)
MML44	Asian badger	15-May-07	SW	43.457	131.590	U	U	Pos (1:11)
MML47	Asian badger	11-Oct-07	SW	43.457	131.590	SA	M	Neg (1:8)

MML48	Asian badger	12-Oct-07	SW	43.457	131.590	U	U	Neg (1:8)
MML49	Asian badger	12-Oct-07	SW	43.457	131.590	U	F	Neg (1:4)
MML50	Asian badger	12-Oct-07	SW	43.457	131.590	U	M	Neg (1:8)
MML51	Asian badger	15-Oct-07	SW	43.457	131.590	U	U	Bacteria
MML53	Asian badger	24-Apr-08	SW	43.457	131.590	AD	M	Neg (1:8)
MML54	Asian badger	25-Apr-08	SW	43.457	131.590	AD	F	Neg (1:8)
MML55	Asian badger	28-Apr-08	SW	43.414	131.607	SA	F	Neg (1:8)
MML56	Asian badger	06-May-08	SW	43.414	131.607	SA	F	Neg (1:8)
MML57	Asian badger	07-May-08	SW	43.414	131.607	U	F	Pos (1:11)
MML58	Asian badger	12-May-08	SW	43.414	131.607	AD	M	Pos (1:11)
MML59	Asian badger	12-May-08	SW	43.414	131.607	U	F	Neg (1:8)
MML60	Asian badger	16-May-08	SW	43.414	131.607	AD	F	Neg (1:8)
MML61	Asian badger	04-Oct-08	SW	43.410	131.621	SA	M	Neg (1:8)
MML63	Asian badger	06-Oct-08	SW	43.410	131.621	SA	F	Neg (1:8)
MML62	Asian badger	07-Oct-08	SW	43.410	131.621	AD	F	Neg (1:8)
MML64	Asian badger	11-Oct-08	SW	43.410	131.621	OD	F	Neg (1:8)
MML65	Asian badger	26-Oct-08	SW	43.410	131.621	U	M	Neg (1:8)
MML66	Asian badger	04-Nov-08	SW	43.410	131.621	U	F	Neg (1:8)
RU-0016	Sable	09-Nov-13	LZ	43.015	134.116	JV	M	Neg (1:8)
RU-0017	Sable	13-Nov-13	LZ	43.015	134.116	JV	M	Neg (1:8)
MSB4	Siberian weasel	31-Oct-07	SW	43.457	131.590	AD	F	Neg (1:32)
MSB7	Siberian weasel	28-Nov-07	SW	43.457	131.590	U	U	Neg (1:32)*
Mink-004	American mink	20-Mar-08	LZ	43.015	134.122	AD	M	Neg (1:8)
MVI11	American mink	29-Apr-08	SW	43.414	131.607	U	F	Neg (1:16)
L CAT 009	Leopard cat	05-May-09	LZ	42.873	133.793	OD	M	Neg (1:4)
L CAT #39	Leopard cat	23-May-09	LZ	43.013	133.732	SA	F	Neg (1:4)
RU-0001	Leopard cat	08-May-13	LZ	43.015	134.121	AD	M	Pos (1:724)
RU-0004	Leopard cat	12-May-13	LZ	43.015	134.121	AD	F	Neg (1:8)

RU-0007	Leopard cat	19-May-13	LZ	43.034	134.156	AD	F	Neg (1:8)
RU-0011	Leopard cat	18-Oct-13	LZ	43.018	134.127	AD	M	Neg (1:8)
PB#001	Leopard cat	31-Oct-13	LZ	42.873	133.795	AD	M	Pos (1:16)
RU-0015	Leopard cat	07-Nov-13	LZ	43.015	134.116	AD	M	Neg (1:8)
Pb#003	Leopard cat	11-Nov-13	LZ	42.866	133.782	AD	M	Neg (1:8)
PBE#002	Leopard cat	11-Nov-13	LZ	42.874	133.802	JV	M	Neg (1:8)
PBE197	Leopard cat	07-Nov-06	SW	43.420	131.704	U	M	Neg (1:4)
PBE211	Leopard cat	03-Dec-07	SW	43.409	131.714	OD	M	Neg (1:4)
PBE213	Leopard cat	22-May-08	SW	43.412	131.744	SA	M	Pos (1:11)
PBE214	Leopard cat	22-Oct-08	SW	43.410	131.621	SA	M	Neg (1:8)
PBE215	Leopard cat	04-Nov-08	SW	43.408	131.717	AD	F	Neg (1:8)
L CAT #46	Leopard cat	24-Feb-10	SW	43.455	131.773	AD	U	Neg (1:4)
RaccoonDog-006	Raccoon dog	31-Mar-08	LZ	43.015	134.122	SA	M	Neg (1:8)
RaccoonDog-008	Raccoon dog	03-May-08	LZ	42.873	133.794	AD	F	Neg (1:8)
RaccoonDog-001	Raccoon dog	23-Sep-08	LZ	43.384	133.970	SA	F	Neg (1:8)
RaccoonDog-003	Raccoon dog	27-Sep-08	LZ	43.384	133.970	OD	F	Neg (1:8)
RaccoonDog-004	Raccoon dog	30-Sep-08	LZ	43.384	133.970	JV	F	Neg (1:8)
RU-0002	Raccoon dog	08-May-13	LZ	43.034	134.157	SA	M	Pos (1:45)
RU-0006	Raccoon dog	18-May-13	LZ	43.025	134.136	AD	M	Neg (1:8)
NP#005	Raccoon dog	12-Oct-13	LZ	42.864	133.782	AD	M	Neg (1:8)
RU-0008	Raccoon dog	15-Oct-13	LZ	43.035	134.155	JV	M	Neg (1:8)
NP#007	Raccoon dog	16-Oct-13	LZ	42.875	133.804	AD	M	Neg (1:8)
Np#009	Raccoon dog	16-Oct-13	LZ	42.873	133.791	AD	M	Pos (1:32)
NP#008	Raccoon dog	16-Oct-13	LZ	42.876	133.807	U	U	Pos (1:45)
NP#010	Raccoon dog	03-Nov-13	LZ	42.875	133.762	AD	F	Pos (1:11)
Np#011	Raccoon dog	11-Nov-13	LZ	42.880	133.705	AD	M	Neg (1:8)
RU-0018	Raccoon dog	13-Nov-13	LZ	43.017	134.126	AD	F	Neg (1:8)
R DOG 2005	Raccoon dog	28-May-05	SZ	45.048	136.659	U	U	Neg (1:8)

RU-0027	Raccoon dog	26-May-14	SZ	44.943	136.480	U	F	Pos (1:128)
RU-0028	Raccoon dog	27-May-14	SZ	44.943	136.480	U	F	Neg (1:32)*
NPR5	Raccoon dog	17-Oct-07	SW	43.457	131.590	U	F	Bacteria
NPR6	Raccoon dog	23-Oct-07	SW	43.457	131.590	U	M	Pos (1:32)
NPR7	Raccoon dog	24-Oct-07	SW	43.457	131.590	U	F	Pos (1:32)
NPR8	Raccoon dog	27-Oct-07	SW	43.457	131.590	U	M	Pos (1:64)
NPR9	Raccoon dog	28-Oct-07	SW	43.457	131.590	U	F	Pos (1:32)
NPR10	Raccoon dog	03-Nov-07	SW	43.457	131.590	U	M	Pos (1:128)
NPR12	Raccoon dog	04-Nov-07	SW	43.457	131.590	U	F	Pos (1:32)
NPR13	Raccoon dog	24-Apr-08	SW	43.414	131.607	U	M	Neg (1:8)
NPR14	Raccoon dog	26-Apr-08	SW	43.414	131.607	U	F	Neg (1:64)*
NPR15	Raccoon dog	07-Oct-08	SW	43.410	131.621	SA	F	Pos (1:23)
NPR16	Raccoon dog	09-Oct-08	SW	43.410	131.621	U	M	Pos (1:11)
NPR17	Raccoon dog	09-Oct-08	SW	43.410	131.621	U	M	Neg (1:32)*
NPR18	Raccoon dog	11-Oct-08	SW	43.410	131.621	AD	F	Neg (1:8)
NPR19	Raccoon dog	11-Oct-08	SW	43.410	131.621	SA	F	Neg (1:8)
NPR20	Raccoon dog	21-Oct-08	SW	43.410	131.621	SA	F	Pos (1:16)
NPR21	Raccoon dog	26-Oct-08	SW	43.410	131.621	OD	U	Neg (1:8)
NPR22	Raccoon dog	04-Nov-08	SW	43.410	131.621	SA	M	Neg (1:8)
RU-0019	Red fox	21-Nov-13	LZ	43.379	133.897	AD	F	Neg (1:8)
VULPES 2011	Red fox	26-Mar-11	SZ	45.052	136.651	AD	U	Neg (1:8)
VVU103	Red fox	28-May-08	SW	43.395	131.745	U	U	Neg (1:8)
VVU104	Red fox	29-May-08	SW	43.453	131.753	U	U	Pos (1:11)

Appendix XXIII. Vaccinated dog raw serology results

Virus neutralization (VN) titers for vaccinated dogs sampled from 2012 to 2014 in Primorskii Krai study areas Southwest Primorskii (SW), Lazovskii (LZ) and Sikhote-Alin Biosphere Zapovednik (SZ). Details of individual dogs include age (in months), gender (male – M, female – F, or unknown – U), whether owners reported dogs as guard dogs, pet dogs, hunting dogs or dogs whether they were taken to forested areas (yes – Y, no – N, or unknown – U), source (local – L, non-local – NL, unknown – U). Virus neutralization titres against the Bussell strain of canine distemper virus are reported with negative samples (Neg) reflecting a titre lower than 1:8.

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunter dog	Visits forest	Source	VN titre
Clf # 001	304	SW	Olenevod	24	M	U	U	U	U	U	11
Clf # 002	304	SW	Olenevod	24	M	U	U	U	U	U	Neg
Clf # 050	354	SZ	Plastun	24	F	U	U	U	U	U	11
Clf # 058	413	SZ	Terney	18	F	N	N	Y	U	NL	Neg
Clf # 061	465	SZ	Terney	60	M	N	Y	N	Y	L	Neg
Clf # 063	464	SZ	Terney	174	M	N	N	N	Y	L	64
Clf # 064	464	SZ	Terney	24	F	N	N	N	Y	L	362
Clf # 065	458	SZ	Terney	126	M	N	Y	N	Y	NL	Neg
Clf # 099	387	SW	Slavyanka	60	M	U	U	U	U	U	64
DG-0007	420	SZ	Terney	144	M	Y	Y	N	U	NL	64
DG-0014	425	SZ	Terney	144	M	Y	N	N	U	L	32
DG-0016	427	SZ	Terney	84	M	Y	Y	N	U	L	Neg
DG-0017	428	SZ	Terney	96	M	Y	Y	N	U	L	Neg
DG-0018	429	SZ	Terney	24	M	Y	N	N	U	NL	Neg
DG-0019	430	SZ	Terney	18	M	N	Y	N	U	NL	5,792
DG-0021	432	SZ	Terney	144	F	Y	N	Y	U	L	Neg
DG-0028	439	SZ	Terney	180	M	Y	N	N	N	L	Neg
DG-0029	440	SZ	Terney	96	M	N	N	Y	Y	L	Neg
DG-0030	441	SZ	Terney	54	M	Y	Y	N	Y	L	Neg
DG-0032	443	SZ	Terney	78	M	Y	N	N	Y	L	Neg
DG-0033	444	SZ	Terney	96	F	N	N	Y	Y	NL	Neg
DG-0038	448	SZ	Terney	36	M	Y	N	N	Y	NL	Neg
DG-0043	380	SW	Slavyanka	4	M	N	Y	N	N	L	32
DG-0044	382	SW	Slavyanka	120	M	Y	N	N	Y	L	Neg
DG-0045	382	SW	Slavyanka	120	M	Y	N	N	Y	L	181
DG-0047	383	SW	Slavyanka	120	M	Y	N	N	Y	L	Neg
DG-0050	21	SW	Bezverkhovo	18	M	Y	Y	N	N	L	Neg
DG-0052	25	SW	Bezverkhovo	72	M	N	N	Y	Y	L	32

DG-0053	26	SW	Bezverkhovo	12	M	Y	N	N	N	NL	Neg
DG-0054	26	SW	Bezverkhovo	18	M	Y	N	N	N	NL	Neg
DG-0056	28	SW	Bezverkhovo	84	M	Y	N	N	N	L	Neg
DG-0062	22	SW	Bezverkhovo	60	F	N	Y	N	Y	NL	181
DG-0064	33	SW	Bezverkhovo	18	M	Y	Y	N	Y	NL	Neg
DG-0067	398	SW	Slavyanka	36	M	N	Y	N	N	NL	Neg
DG-0068	397	SW	Slavyanka	156	F	N	Y	N	N	L	Neg
DG-0070	396	SW	Slavyanka	48	M	Y	Y	N	Y	NL	Neg
DG-0071	399	SW	Slavyanka	144	F	N	Y	N	N	L	Neg
DG-0072	400	SW	Slavyanka	48	F	N	Y	N	Y	NL	362
DG-0074	306	SW	Ovchinnikovo	72	F	Y	N	N	Y	NL	Neg
DG-0082	253	SW	Nezhino	24	F	Y	N	N	N	NL	256
DG-0083	254	SW	Nezhino	48	M	Y	N	N	N	NL	11
DG-0087	257	SW	Nezhino	60	F	Y	N	N	Y	NL	724
DG-0091	261	SW	Nezhino	18	F	Y	N	N	N	L	Neg
DG-0095	272	SW	Olenevod	96	M	Y	N	N	Y	L	Neg
DG-0109	285	SW	Olenevod	120	F	Y	N	N	N	NL	Neg
DG-0113	289	SW	Olenevod	36	M	Y	N	N	Y	L	Neg
DG-0117	292	SW	Olenevod	24	M	N	Y	N	N	NL	Neg
DG-0118	293	SW	Olenevod	96	M	Y	Y	N	Y	L	Neg
DG-0123	514	SW	Timofeevka	72	M	Y	N	N	N	L	Neg
DG-0124	514	SW	Timofeevka	120	M	Y	N	N	N	L	Neg
DG-0129	519	SW	Timofeevka	96	M	Y	N	N	Y	L	1,448
DG-0136	60	SW	Devatyy-Val	72	M	N	N	Y	Y	NL	724
DG-0143	65	SW	Devatyy-Val	108	M	N	Y	N	N	NL	23
DG-0144	297	SW	Olenevod	120	M	Y	N	N	N	NL	Neg
DG-0148	301	SW	Olenevod	48	M	Y	N	N	Y	L	Neg
DG-0153	403	SW	Slavyanka	144	M	N	Y	N	N	L	Neg
DG-0154	365	SW	Razanovka	8	M	N	Y	N	Y	L	Neg
DG-0156	368	SW	Razanovka	36	M	Y	Y	N	Y	NL	Neg
DG-0159	369	SW	Razanovka	24	F	N	Y	N	Y	NL	32
DG-0163	358	SW	Poyma	168	F	Y	N	N	Y	NL	Neg
DG-0164	359	SW	Poyma	24	F	N	Y	N	N	NL	Neg
DG-0169	320	SW	Perevoznoye	8	F	Y	N	N	N	L	23
DG-0170	321	SW	Perevoznoye	24	M	Y	N	N	N	L	23
DG-0171	321	SW	Perevoznoye	168	F	Y	N	N	N	L	23
DG-0173	323	SW	Perevoznoye	18	F	Y	Y	N	N	NL	32
DG-0177	10	SW	Baranovskii	18	M	N	Y	N	Y	NL	Neg
DG-0180	13	SW	Baranovskii	96	F	Y	N	N	Y	L	Neg
DG-0184	17	SW	Baranovskii	30	M	Y	N	N	Y	L	Neg
DG-0186	19	SW	Baranovskii	48	F	N	Y	N	Y	NL	45
DG-0191	267	SW	N. Lvovskoe	60	M	N	N	Y	Y	L	11
DG-0195	269	SW	N. Lvovskoe	48	M	Y	N	N	Y	L	Neg
DG-0196	128	SW	Krounovka	72	M	N	N	Y	N	NL	Neg
DG-0203	134	SW	Krounovka	204	F	N	N	Y	N	NL	Neg
DG-0204	135	SW	Krounovka	60	M	Y	Y	N	N	L	Neg
DG-0209	138	SW	Krounovka	4	M	Y	N	N	N	NL	64
DG-0214	144	LZ	Lazo	12	F	Y	N	N	Y	L	Neg
DG-0215	145	LZ	Lazo	36	F	N	Y	N	Y	NL	Neg
DG-0216	146	LZ	Lazo	6	F	Y	N	N	N	L	Neg
DG-0218	148	LZ	Lazo	72	F	Y	N	N	Y	NL	256
DG-0220	150	LZ	Lazo	12	M	N	Y	N	Y	NL	512
DG-0222	152	LZ	Lazo	60	M	N	Y	N	Y	NL	11
DG-0223	152	LZ	Lazo	24	M	N	Y	N	N	L	91

DG-0225	154	LZ	Lazo	36	M	N	Y	N	Y	L	Neg
DG-0227	156	LZ	Lazo	156	F	N	Y	N	N	L	Neg
DG-0228	157	LZ	Lazo	9	M	U	U	U	Y	L	Neg
DG-0230	159	LZ	Lazo	18	M	Y	Y	N	Y	L	45
DG-0231	159	LZ	Lazo	4	M	Y	Y	N	Y	L	Neg
DG-0234	162	LZ	Lazo	72	M	N	Y	N	Y	NL	11
DG-0235	162	LZ	Lazo	24	M	N	Y	N	Y	NL	16
DG-0236	163	LZ	Lazo	24	F	Y	Y	N	N	NL	23
DG-0241	169	LZ	Lazo	24	M	Y	N	N	N	NL	Neg
DG-0242	169	LZ	Lazo	16	M	Y	N	N	Y	NL	32
DG-0246	172	LZ	Lazo	36	M	Y	Y	N	N	L	Neg
DG-0251	177	LZ	Lazo	120	M	Y	N	N	N	L	Neg
DG-0256	179	LZ	Lazo	10	M	Y	N	N	Y	L	724
DG-0257	179	LZ	Lazo	96	F	N	Y	N	Y	L	45
DG-0278	198	LZ	Lazo	24	M	N	N	N	Y	L	Neg
DG-0281	201	LZ	Lazo	12	M	N	Y	N	Y	L	256
DG-0284	203	LZ	Lazo	48	M	Y	N	N	N	L	128
DG-0290	206	LZ	Lazo	7	M	Y	N	N	N	NL	Neg
DG-0294	211	LZ	Lazo	48	M	Y	N	N	N	L	Neg
DG-0296	212	LZ	Lazo	24	M	Y	Y	Y	Y	NL	Neg
DG-0298	214	LZ	Lazo	24	F	Y	N	N	Y	L	128
DG-0302	217	LZ	Lazo	96	M	N	Y	N	N	NL	Neg
DG-0306	220	LZ	Lazo	60	M	Y	N	N	Y	L	Neg
DG-0343	42	LZ	Chis'vodnoye	60	F	Y	Y	N	N	NL	23
DG-0346	44	LZ	Chis'vodnoye	12	M	Y	N	N	N	NL	Neg
DG-0347	45	LZ	Chis'vodnoye	42	M	N	N	N	Y	NL	16
DG-0352	86	LZ	Kievka	36	F	N	Y	N	N	NL	91
DG-0364	96	LZ	Kievka	24	M	N	N	N	N	NL	11
DG-0365	97	LZ	Kievka	96	F	N	Y	N	Y	NL	32
DG-0366	98	LZ	Kievka	10	M	Y	N	N	N	L	128
DG-0367	98	LZ	Kievka	36	F	Y	N	N	N	NL	Neg
DG-0370	102	LZ	Kievka	9	M	N	Y	N	Y	NL	2,896
DG-0374	106	LZ	Kievka	60	M	Y	Y	N	N	NL	23
DG-0376	108	LZ	Kievka	144	F	Y	N	N	N	L	Neg
DG-0377	109	LZ	Kievka	48	M	Y	N	N	N	NL	23
DG-0392	125	LZ	Kishenevka	36	F	Y	N	N	N	L	Neg
DG-0393	121	LZ	Kishenevka	192	M	Y	Y	N	Y	L	Neg
DG-0407	231	LZ	Lazo	36	M	Y	N	N	N	L	Neg
DG-0414	236	LZ	Lazo	36	M	N	Y	N	Y	U	32
DG-0418	239	LZ	Lazo	144	M	Y	N	N	Y	L	1,448
DG-0422	242	LZ	Lazo	132	M	Y	N	N	Y	L	Neg
DG-0426	247	LZ	Lazo	96	F	N	Y	N	Y	NL	Neg
DG-0431	250	LZ	Lazo	120	F	U	U	U	Y	L	512
DG-0432	250	LZ	Lazo	42	F	U	U	U	Y	NL	64
DG-0434	328	SZ	Plastun	96	M	Y	Y	N	N	L	11
DG-0435	328	SZ	Plastun	60	F	Y	Y	N	N	L	16
DG-0436	329	SZ	Plastun	6	M	Y	Y	N	Y	L	11
DG-0441	333	SZ	Plastun	8	M	Y	Y	N	Y	L	11
DG-0448	466	SZ	Terney	18	M	N	N	Y	Y	L	Neg
DG-0449	467	SZ	Terney	24	M	Y	N	N	N	NL	Neg
DG-0454	473	SZ	Terney	24	M	N	N	N	Y	NL	11
DG-0462	339	SZ	Plastun	12	M	Y	Y	N	Y	L	11
DG-0463	339	SZ	Plastun	24	M	Y	Y	N	Y	L	11
DG-0464	339	SZ	Plastun	4	F	Y	Y	N	Y	L	11

DG-0465	339	SZ	Plastun	120	M	Y	Y	N	Y	L	45
DG-0466	339	SZ	Plastun	120	F	Y	Y	N	Y	L	Neg
DG-0467	346	SZ	Plastun	24	F	Y	Y	N	N	L	23
DG-0474	481	SZ	Terney	48	M	N	Y	N	Y	NL	16
DG-0476	478	SZ	Terney	36	M	N	Y	N	Y	L	16
DG-0481	477	SZ	Terney	36	M	Y	N	N	N	L	45
DG-0483	479	SZ	Terney	18	M	N	Y	N	N	NL	256
DG-0484	481	SZ	Terney	72	F	N	Y	N	Y	L	16
DG-0489	485	SZ	Terney	6	F	N	Y	N	N	NL	64
DG-0496	409	SZ	Taejnoye	84	F	Y	N	N	N	NL	64
DG-0499	349	SZ	Plastun	16	M	N	Y	N	Y	L	Neg
DG-0500	351	SZ	Plastun	60	F	N	Y	N	Y	NL	128
DG-0508	352	SZ	Plastun	180	F	N	Y	N	Y	L	128
DG-0509	509	SZ	Terney	72	F	Y	N	N	Y	L	23
DG-0510	509	SZ	Terney	36	M	Y	N	N	Y	U	45
DG-0524	498	SZ	Terney	24	M	N	Y	N	Y	NL	91

Appendix XXIV. Unvaccinated dog raw serology results

Virus neutralization (VN) titers for unvaccinated dogs sampled from 2012 to 2014 in Primorskii Krai study areas Southwest Primorskii (SW), Lazovskii (LZ) and Sikhote-Alin Biosphere Zapovednik (SZ). Details of individual dogs include age (in months), gender (male – M, female – F, or unknown – U), whether owners reported dogs as guard dogs, pet dogs, hunting dogs or dogs whether they were taken to forested areas (yes – Y, no – N, or unknown – U), source (local – L, non-local – NL, unknown – U). Virus neutralization titres against the Bussell strain of canine distemper virus are reported with negative samples (Neg) reflecting a titre lower than 1:8.

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
Clf # 003	302	SW	Olenevod	4	M	Y	N	N	U	U	Neg
Clf # 004	302	SW	Olenevod	84	M	Y	N	N	U	U	Neg
Clf # 005	303	SW	Olenevod	84	M	Y	N	N	U	U	Neg
Clf # 011	512	SW	Tikhiy	8	M	N	N	Y	U	U	11
Clf # 012	513	SW	Tikhiy	24	F	N	Y	N	U	U	Neg
Clf # 013	520	SW	Venevetinovo	18	F	Y	N	N	U	U	Neg
Clf # 014	521	SW	Venevetinovo	30	M	Y	N	N	U	U	Neg
Clf # 015	263	SW	Nezhino	12	F	Y	N	N	U	U	Neg
Clf # 016	263	SW	Nezhino	84	F	Y	N	N	U	U	Neg
Clf # 018	126	SW	Kravtsovka	9	M	Y	N	N	U	U	2,048
Clf # 019	127	SW	Kravtsovka	36	M	Y	N	N	U	U	Neg
Clf # 021	82	SW	Kazarma-25km	120	M	Y	N	N	U	U	Neg
Clf # 022	83	SW	Kazarma-25km	96	M	Y	N	N	U	U	Neg
Clf # 023	84	SW	Kazarma-25km	108	M	Y	N	N	U	U	Neg
Clf # 024	84	SW	Kazarma-25km	24	M	Y	N	N	U	U	Neg
Clf # 025	85	SW	Kazarma-25km	36	M	Y	N	N	U	U	Neg
Clf # 026	143	SW	Kuchelinovo	6	F	U	U	U	U	U	Neg
Clf # 028	68	SW	Fillipovka	48	F	Y	N	N	U	U	2,048
Clf # 029	68	SW	Fillipovka	18	M	Y	N	N	U	U	Neg
Clf # 030	69	SW	Fillipovka	12	M	Y	N	N	U	U	Neg
Clf # 031	69	SW	Fillipovka	60	F	Y	N	N	U	U	Neg
Clf # 032	70	SW	Fillipovka	48	M	Y	N	N	U	U	Neg
Clf # 033	70	SW	Fillipovka	72	M	Y	N	N	U	U	Neg
Clf # 034	70	SW	Fillipovka	54	F	Y	N	N	U	U	Neg
Clf # 035	71	SW	Fillipovka	36	F	Y	N	N	U	U	11
Clf # 036	522	SW	Zanadvorovka	24	M	Y	N	N	U	U	Neg
Clf # 038	523	SW	Zanadvorovka	60	M	Y	N	N	U	U	181
Clf # 039	524	SW	Zanadvorovka	120	M	Y	N	N	U	U	45

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
Clf # 040	456	SZ	Terney	90	M	N	Y	N	U	NL	Neg
Clf # 041	459	SZ	Terney	120	M	Y	N	N	Y	L	256
Clf # 042	461	SZ	Terney	12	M	Y	N	N	N	NL	Neg
Clf # 043	463	SZ	Terney	18	M	Y	N	N	Y	L	Neg
Clf # 044	455	SZ	Terney	18	F	Y	Y	N	Y	L	Neg
Clf # 045	457	SZ	Terney	30	M	Y	N	N	Y	L	Neg
Clf # 046	353	SZ	Plastun	18	M	N	N	Y	U	U	Neg
Clf # 047	353	SZ	Plastun	72	M	N	N	Y	U	U	Neg
Clf # 048	354	SZ	Plastun	84	M	Y	N	N	U	U	Neg
Clf # 049	354	SZ	Plastun	42	M	Y	N	N	U	U	91
Clf # 051	355	SZ	Plastun	36	F	Y	N	N	U	U	Neg
Clf # 052	356	SZ	Plastun	108	M	Y	N	N	U	U	Neg
Clf # 053	356	SZ	Plastun	72	M	N	Y	N	U	U	Neg
Clf # 054	357	SZ	Plastun	66	M	Y	N	N	U	U	Neg
Clf # 055	357	SZ	Plastun	12	M	Y	N	N	U	U	Neg
Clf # 056	462	SZ	Terney	36	M	Y	N	N	Y	NL	Neg
Clf # 057	460	SZ	Terney	24	F	N	N	Y	Y	NL	Neg
Clf # 059	453	SZ	Terney	30	M	Y	N	N	Y	NL	45
Clf # 060	453	SZ	Terney	11	M	Y	N	N	U	U	Neg
Clf # 062	452	SZ	Terney	18	F	Y	N	N	Y	L	Neg
Clf # 066	5	SW	Barabash	18	M	Y	N	N	U	U	Neg
Clf # 067	6	SW	Barabash	60	M	Y	N	N	U	U	256
Clf # 068	6	SW	Barabash	36	F	Y	N	N	U	U	Neg
Clf # 069	7	SW	Barabash	6	M	Y	N	N	U	U	Neg
Clf # 070	8	SW	Barabash	36	M	Y	N	N	U	U	Neg
Clf # 071	9	SW	Barabash	36	F	Y	N	N	U	U	32
Clf # 072	360	SW	Primorskii	72	M	Y	N	N	U	U	362
Clf # 073	361	SW	Primorskii	48	M	Y	N	N	U	U	128
Clf # 075	362	SW	Primorskii	96	M	Y	N	N	U	U	512
Clf # 076	363	SW	Primorskii	12	M	Y	N	N	U	U	Neg
Clf # 077	364	SW	Primorskii	120	F	Y	N	N	U	U	23
Clf # 078	312	SW	Ovchinnikovo	36	M	Y	N	N	U	U	Neg
Clf # 079	313	SW	Ovchinnikovo	24	F	Y	N	N	U	U	Neg
Clf # 081	315	SW	Ovchinnikovo	38	M	N	N	Y	U	U	Neg
Clf # 082	316	SW	Ovchinnikovo	12	F	Y	N	N	U	U	Neg
Clf # 083	316	SW	Ovchinnikovo	12	M	Y	N	N	U	U	Neg
Clf # 084	316	SW	Ovchinnikovo	120	M	N	Y	N	U	U	32
Clf # 085	1	SW	Bamburovo	36	M	Y	N	N	U	U	Neg
Clf # 086	2	SW	Bamburovo	60	M	Y	N	N	U	U	Neg
Clf # 087	3	SW	Bamburovo	30	M	Y	N	N	U	U	Neg
Clf # 088	3	SW	Bamburovo	48	M	Y	N	N	U	U	Neg
Clf # 089	4	SW	Bamburovo	96	M	Y	N	N	U	U	Neg
Clf # 090	374	SW	Romashka	84	M	Y	N	N	U	U	Neg
Clf # 091	374	SW	Romashka	18	F	Y	N	N	U	U	Neg
Clf # 092	375	SW	Romashka	11	M	Y	N	N	U	U	Neg
Clf # 093	376	SW	Romashka	24	M	N	N	Y	U	U	Neg
Clf # 094	377	SW	Ryazanovka	156	M	N	N	Y	U	U	Neg
Clf # 095	377	SW	Ryazanovka	36	F	N	N	Y	U	U	128
Clf # 096	378	SW	Ryazanovka	96	M	Y	N	N	U	U	Neg

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
Clf # 097	378	SW	Ryazanovka	72	F	Y	N	N	U	U	Neg
Clf # 098	385	SW	Slavyanka	48	M	Y	N	N	U	U	Neg
Clf # 100	388	SW	Slavyanka	9	F	Y	N	N	U	U	Neg
Clf # 101	388	SW	Slavyanka	122	F	Y	N	N	U	U	16
Clf # 102	389	SW	Slavyanka	30	M	Y	N	N	U	U	Neg
Clf # 103	390	SW	Slavyanka	12	M	Y	N	N	U	U	11
Clf # 104	391	SW	Slavyanka	10	M	N	N	Y	U	U	2,896
Clf # 105	392	SW	Slavyanka	96	M	Y	N	N	U	U	Neg
Clf # 106	393	SW	Slavyanka	48	F	Y	N	N	U	U	Neg
Clf # 107	394	SW	Slavyanka	24	M	Y	N	N	U	U	Neg
Clf # 108	386	SW	Slavyanka	14	U	Y	N	N	U	U	Neg
Clf # 109	34	SW	Bezverkhovo	60	M	Y	N	N	U	U	512
Clf # 110	35	SW	Bezverkhovo	5	M	Y	N	N	U	U	Neg
Clf # 111	35	SW	Bezverkhovo	36	M	Y	N	N	U	U	64
Clf # 112	36	SW	Bezverkhovo	12	M	Y	N	N	U	U	Neg
Clf # 113	37	SW	Bezverkhovo	60	M	Y	N	N	U	U	16
DG-0001	454	SZ	Terney	36	F	Y	N	N	N	L	Neg
DG-0002	414	SZ	Terney	36	M	Y	N	Y	U	L	Neg
DG-0003	415	SZ	Terney	12	M	Y	Y	N	U	L	Neg
DG-0004	416	SZ	Terney	48	M	Y	N	N	U	L	Neg
DG-0005	417	SZ	Terney	18	F	N	Y	N	U	L	181
DG-0006	418	SZ	Terney	156	M	N	N	Y	U	L	Neg
DG-0008	419	SZ	Terney	12	F	Y	N	N	U	L	Neg
DG-0009	421	SZ	Terney	84	M	Y	N	N	U	NL	Neg
DG-0010	421	SZ	Terney	108	M	Y	N	N	U	L	Neg
DG-0011	422	SZ	Terney	36	M	Y	N	N	U	L	Neg
DG-0012	423	SZ	Terney	60	M	Y	N	N	U	L	Neg
DG-0013	424	SZ	Terney	96	F	N	Y	N	U	NL	Neg
DG-0015	426	SZ	Terney	12	M	Y	N	N	U	L	Neg
DG-0020	431	SZ	Terney	5	M	Y	N	N	U	L	Neg
DG-0022	433	SZ	Terney	120	F	Y	N	N	U	L	Neg
DG-0023	434	SZ	Terney	12	M	Y	N	N	U	NL	16
DG-0025	437	SZ	Terney	60	F	Y	Y	N	U	NL	45
DG-0026	438	SZ	Terney	12	F	N	Y	N	U	L	Neg
DG-0027	436	SZ	Terney	144	F	N	N	Y	U	NL	Neg
DG-0031	442	SZ	Terney	24	F	Y	N	N	Y	L	Neg
DG-0034	445	SZ	Terney	120	M	N	N	Y	Y	NL	Neg
DG-0037	447	SZ	Terney	36	M	Y	Y	N	Y	NL	Neg
DG-0039	449	SZ	Terney	24	M	N	Y	N	N	NL	Neg
DG-0040	450	SZ	Terney	36	F	N	Y	N	N	NL	Neg
DG-0041	450	SZ	Terney	6	M	N	Y	N	N	L	Neg
DG-0042	451	SZ	Terney	120	M	N	Y	N	Y	L	Neg
DG-0046	383	SW	Slavyanka	9	F	Y	N	N	Y	L	11
DG-0048	384	SW	Slavyanka	72	M	N	Y	N	N	L	Neg
DG-0049	381	SW	Slavyanka	5	M	Y	N	N	N	L	Neg
DG-0051	24	SW	Bezverkhovo	12	F	Y	Y	N	Y	L	Neg
DG-0058	29	SW	Bezverkhovo	4	M	Y	Y	N	Y	L	Neg
DG-0059	30	SW	Bezverkhovo	5	F	Y	N	N	N	NL	Neg
DG-0060	31	SW	Bezverkhovo	12	M	Y	N	N	Y	L	Neg

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0061	32	SW	Bezverkhovo	36	M	Y	N	N	N	L	Neg
DG-0063	23	SW	Bezverkhovo	5	M	Y	N	N	N	L	Neg
DG-0069	395	SW	Slavyanka	30	M	N	Y	N	N	L	Neg
DG-0073	305	SW	Ovchinnikovo	12	M	Y	N	N	N	L	Neg
DG-0075	307	SW	Ovchinnikovo	18	M	Y	N	N	N	L	Neg
DG-0076	308	SW	Ovchinnikovo	6	M	Y	N	N	N	L	Neg
DG-0077	309	SW	Ovchinnikovo	12	M	Y	N	N	N	L	Neg
DG-0078	310	SW	Ovchinnikovo	24	M	Y	N	N	Y	L	Neg
DG-0079	311	SW	Ovchinnikovo	60	F	N	Y	N	N	L	Neg
DG-0080	252	SW	Nezhino	120	M	N	Y	N	Y	L	Neg
DG-0081	252	SW	Nezhino	12	M	N	Y	N	N	L	Neg
DG-0084	255	SW	Nezhino	36	F	Y	N	N	N	NL	Neg
DG-0085	255	SW	Nezhino	18	M	Y	N	N	N	L	Neg
DG-0086	256	SW	Nezhino	24	M	N	Y	N	N	L	32
DG-0088	259	SW	Nezhino	96	M	Y	Y	N	N	L	Neg
DG-0089	259	SW	Nezhino	36	F	Y	Y	N	Y	L	Neg
DG-0090	260	SW	Nezhino	84	F	N	Y	N	Y	L	Neg
DG-0092	261	SW	Nezhino	4	M	Y	N	N	N	NL	Neg
DG-0093	262	SW	Nezhino	48	M	N	Y	N	Y	NL	Neg
DG-0094	258	SW	Nezhino	48	F	Y	Y	N	N	NL	Neg
DG-0096	273	SW	Olenevod	60	M	Y	N	Y	Y	NL	Neg
DG-0097	274	SW	Olenevod	12	M	N	Y	N	N	L	Neg
DG-0098	275	SW	Olenevod	108	F	Y	N	N	Y	NL	Neg
DG-0099	276	SW	Olenevod	12	M	Y	N	N	Y	NL	Neg
DG-0100	276	SW	Olenevod	48	F	Y	N	N	Y	NL	Neg
DG-0101	277	SW	Olenevod	10	M	Y	N	N	N	NL	Neg
DG-0102	278	SW	Olenevod	36	M	Y	N	N	N	L	Neg
DG-0103	279	SW	Olenevod	36	F	Y	N	N	N	L	11
DG-0104	280	SW	Olenevod	15	F	Y	Y	N	N	NL	Neg
DG-0105	281	SW	Olenevod	36	M	Y	Y	N	N	L	Neg
DG-0106	282	SW	Olenevod	120	M	Y	N	N	Y	L	Neg
DG-0108	284	SW	Olenevod	84	F	N	Y	N	N	NL	Neg
DG-0110	286	SW	Olenevod	4	M	Y	N	N	N	NL	Neg
DG-0111	287	SW	Olenevod	12	F	N	Y	N	N	L	Neg
DG-0112	288	SW	Olenevod	48	M	Y	N	N	N	NL	Neg
DG-0114	290	SW	Olenevod	96	M	Y	N	N	Y	L	Neg
DG-0115	290	SW	Olenevod	84	F	Y	N	N	Y	L	Neg
DG-0116	291	SW	Olenevod	60	M	Y	N	N	Y	L	Neg
DG-0119	294	SW	Olenevod	6	M	Y	N	N	Y	L	Neg
DG-0120	295	SW	Olenevod	6	M	Y	N	N	N	L	Neg
DG-0121	296	SW	Olenevod	4	M	N	Y	N	N	NL	Neg
DG-0122	404	SW	Steklozavodsky	48	M	N	Y	N	Y	L	Neg
DG-0125	515	SW	Timofeevka	156	M	Y	N	N	Y	NL	23
DG-0126	516	SW	Timofeevka	12	F	Y	N	N	Y	NL	Neg
DG-0128	518	SW	Timofeevka	12	M	Y	N	N	N	L	Neg
DG-0130	519	SW	Timofeevka	24	M	Y	N	N	N	L	Neg
DG-0131	55	SW	Devatyy-Val	36	F	N	Y	N	N	NL	Neg
DG-0132	56	SW	Devatyy-Val	12	M	N	Y	N	Y	L	Neg
DG-0133	57	SW	Devatyy-Val	18	F	N	Y	N	Y	L	Neg

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0135	59	SW	Devatyy-Val	72	F	N	Y	N	Y	NL	Neg
DG-0137	61	SW	Devatyy-Val	48	F	N	Y	N	N	NL	Neg
DG-0138	62	SW	Devatyy-Val	18	M	N	Y	N	N	L	Neg
DG-0139	63	SW	Devatyy-Val	48	M	N	Y	N	Y	L	Neg
DG-0140	64	SW	Devatyy-Val	6	M	Y	Y	N	N	L	Neg
DG-0141	66	SW	Devatyy-Val	18	M	Y	N	N	Y	U	Neg
DG-0146	299	SW	Olenevod	12	M	N	Y	N	N	L	Neg
DG-0147	299	SW	Olenevod	12	M	N	Y	N	N	L	Neg
DG-0149	300	SW	Olenevod	5	M	U	U	U	U	U	Neg
DG-0150	401	SW	Slavyanka	12	F	Y	Y	N	N	L	Neg
DG-0151	402	SW	Slavyanka	60	F	Y	N	N	N	L	Neg
DG-0152	251	SW	Lebedinoe	36	M	Y	N	N	N	L	32
DG-0155	370	SW	Razanovka	12	M	Y	N	N	Y	L	Neg
DG-0157	366	SW	Razanovka	48	F	Y	N	Y	Y	NL	Neg
DG-0158	367	SW	Razanovka	36	M	Y	N	N	Y	L	11
DG-0160	373	SW	Razanovka	114	M	Y	N	N	Y	NL	Neg
DG-0161	372	SW	Razanovka	36	M	Y	N	N	Y	L	Neg
DG-0162	371	SW	Razanovka	48	F	Y	N	Y	N	NL	Neg
DG-0165	405	SW	Sukhanovka	36	M	Y	N	N	N	L	Neg
DG-0166	317	SW	Perevoznoye	60	F	Y	N	N	Y	L	Neg
DG-0167	318	SW	Perevoznoye	12	M	Y	Y	N	Y	L	16
DG-0168	319	SW	Perevoznoye	12	M	Y	N	N	Y	NL	Neg
DG-0172	322	SW	Perevoznoye	72	M	Y	N	N	N	L	Neg
DG-0174	324	SW	Perevoznoye	48	F	Y	Y	N	Y	L	Neg
DG-0175	325	SW	Perevoznoye	10	M	Y	N	N	N	NL	Neg
DG-0178	11	SW	Baranovskii	6	M	Y	N	N	N	L	11
DG-0179	12	SW	Baranovskii	180	M	Y	Y	N	N	NL	Neg
DG-0181	15	SW	Baranovskii	6	M	Y	Y	N	Y	NL	Neg
DG-0182	14	SW	Baranovskii	24	M	Y	N	N	N	L	Neg
DG-0183	16	SW	Baranovskii	12	M	Y	N	N	N	L	Neg
DG-0185	18	SW	Baranovskii	24	F	N	N	N	N	L	Neg
DG-0187	20	SW	Baranovskii	84	M	N	Y	N	Y	L	32
DG-0188	264	SW	N. Lvovskoe	48	M	N	Y	N	N	NL	32
DG-0190	266	SW	N. Lvovskoe	8	M	Y	N	N	Y	L	32
DG-0192	268	SW	N. Lvovskoe	48	F	Y	N	N	N	L	Neg
DG-0193	270	SW	N. Lvovskoe	48	F	Y	N	N	Y	NL	Neg
DG-0194	271	SW	N. Lvovskoe	24	M	Y	N	N	Y	L	Neg
DG-0197	129	SW	Krounovka	18	M	Y	N	N	N	L	Neg
DG-0198	130	SW	Krounovka	120	M	Y	N	N	N	L	Neg
DG-0199	130	SW	Krounovka	12	F	Y	N	N	N	L	Neg
DG-0200	131	SW	Krounovka	18	M	U	U	U	N	NL	Neg
DG-0201	132	SW	Krounovka	72	F	Y	N	N	N	L	11
DG-0202	133	SW	Krounovka	84	F	Y	N	N	N	L	Neg
DG-0205	136	SW	Krounovka	96	M	Y	N	N	Y	L	Neg
DG-0206	137	SW	Krounovka	84	M	Y	N	N	N	NL	32
DG-0207	137	SW	Krounovka	36	M	Y	N	N	N	NL	32
DG-0210	139	SW	Krounovka	72	F	Y	N	N	Y	L	Neg
DG-0211	140	SW	Krounovka	120	M	Y	N	N	Y	L	Neg
DG-0213	142	SW	Krounovka	24	M	Y	N	N	N	L	Neg

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0217	147	LZ	Lazo	24	M	Y	N	N	Y	L	Neg
DG-0219	149	LZ	Lazo	180	M	Y	N	N	N	NL	Neg
DG-0221	151	LZ	Lazo	12	M	Y	N	N	N	L	Neg
DG-0224	153	LZ	Lazo	36	M	Y	N	N	Y	L	Neg
DG-0226	155	LZ	Lazo	132	F	Y	N	N	N	NL	256
DG-0229	158	LZ	Lazo	6	M	Y	Y	N	N	L	Neg
DG-0232	160	LZ	Lazo	24	F	N	Y	N	Y	NL	23
DG-0233	161	LZ	Lazo	144	F	N	Y	N	Y	L	23
DG-0237	166	LZ	Lazo	24	M	Y	N	N	Y	L	Neg
DG-0239	168	LZ	Lazo	36	M	Y	N	N	N	L	11
DG-0240	165	LZ	Lazo	36	F	Y	N	N	Y	NL	11
DG-0243	170	LZ	Lazo	120	F	Y	N	N	N	L	Neg
DG-0244	171	LZ	Lazo	24	M	Y	N	N	N	L	Neg
DG-0245	172	LZ	Lazo	48	F	Y	Y	N	N	L	Neg
DG-0247	173	LZ	Lazo	5	M	Y	N	N	Y	L	Neg
DG-0248	174	LZ	Lazo	24	M	Y	Y	N	Y	L	Neg
DG-0249	175	LZ	Lazo	4	M	Y	N	N	N	L	Neg
DG-0250	176	LZ	Lazo	36	M	N	Y	N	N	L	Neg
DG-0252	195	LZ	Lazo	36	M	Y	N	N	Y	L	Neg
DG-0253	195	LZ	Lazo	48	M	Y	N	N	Y	L	Neg
DG-0254	178	LZ	Lazo	12	M	Y	Y	N	N	L	Neg
DG-0255	178	LZ	Lazo	12	M	Y	Y	N	N	NL	Neg
DG-0259	181	LZ	Lazo	48	M	Y	Y	N	N	NL	Neg
DG-0260	164	LZ	Lazo	36	M	N	Y	N	N	L	Neg
DG-0261	182	LZ	Lazo	84	M	N	Y	N	Y	L	32
DG-0262	196	LZ	Lazo	144	M	Y	Y	N	Y	L	128
DG-0263	183	LZ	Lazo	12	M	Y	Y	N	Y	L	Neg
DG-0264	184	LZ	Lazo	96	M	Y	N	N	N	L	Neg
DG-0265	185	LZ	Lazo	108	M	N	N	N	N	L	181
DG-0266	186	LZ	Lazo	72	M	Y	Y	N	N	L	Neg
DG-0267	187	LZ	Lazo	72	M	Y	N	N	N	L	Neg
DG-0268	188	LZ	Lazo	18	M	N	Y	N	Y	L	16
DG-0269	189	LZ	Lazo	18	M	Y	N	N	N	L	Neg
DG-0270	189	LZ	Lazo	10	M	Y	N	N	N	L	Neg
DG-0271	190	LZ	Lazo	96	M	Y	N	N	Y	L	Neg
DG-0272	191	LZ	Lazo	120	M	Y	Y	N	Y	L	Neg
DG-0273	192	LZ	Lazo	4	F	N	Y	N	N	L	64
DG-0274	193	LZ	Lazo	24	M	Y	N	N	N	L	Neg
DG-0275	194	LZ	Lazo	12	M	Y	N	N	Y	L	Neg
DG-0276	197	LZ	Lazo	12	F	Y	N	N	N	NL	23
DG-0277	197	LZ	Lazo	84	F	N	Y	N	N	L	91
DG-0279	199	LZ	Lazo	36	M	Y	Y	N	N	L	Neg
DG-0280	200	LZ	Lazo	12	M	N	Y	N	N	L	Neg
DG-0282	202	LZ	Lazo	36	M	Y	N	N	Y	L	Neg
DG-0283	203	LZ	Lazo	6	M	N	Y	N	Y	L	Neg
DG-0285	204	LZ	Lazo	132	F	N	Y	N	N	NL	Neg
DG-0286	205	LZ	Lazo	48	M	N	Y	N	N	L	11
DG-0287	205	LZ	Lazo	10	M	N	Y	N	N	L	45
DG-0288	210	LZ	Lazo	60	M	Y	N	N	N	L	11

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0289	206	LZ	Lazo	4	M	Y	Y	N	N	L	Neg
DG-0291	207	LZ	Lazo	36	M	Y	N	N	N	L	Neg
DG-0292	208	LZ	Lazo	120	M	U	U	U	N	L	256
DG-0293	209	LZ	Lazo	60	M	Y	N	N	Y	L	181
DG-0295	212	LZ	Lazo	24	F	Y	Y	N	Y	L	Neg
DG-0297	213	LZ	Lazo	24	F	Y	N	N	N	NL	Neg
DG-0299	214	LZ	Lazo	24	M	Y	N	N	Y	L	Neg
DG-0300	215	LZ	Lazo	108	M	Y	N	N	N	L	Neg
DG-0301	216	LZ	Lazo	60	M	Y	Y	N	Y	NL	Neg
DG-0303	217	LZ	Lazo	36	F	N	Y	N	N	U	Neg
DG-0304	218	LZ	Lazo	24	M	N	Y	N	Y	L	Neg
DG-0305	219	LZ	Lazo	72	M	Y	Y	N	Y	L	32
DG-0307	221	LZ	Lazo	8	M	Y	N	N	N	L	Neg
DG-0308	222	LZ	Lazo	24	F	Y	N	N	N	L	Neg
DG-0309	47	LZ	Danilchenkovo	60	F	Y	N	N	Y	L	Neg
DG-0310	48	LZ	Danilchenkovo	24	F	Y	Y	N	Y	L	Neg
DG-0311	48	LZ	Danilchenkovo	12	F	Y	Y	N	Y	L	Neg
DG-0312	49	LZ	Danilchenkovo	84	M	N	Y	N	Y	L	Neg
DG-0313	49	LZ	Danilchenkovo	96	F	N	Y	N	Y	NL	Neg
DG-0314	50	LZ	Danilchenkovo	12	M	N	Y	N	N	NL	Neg
DG-0315	51	LZ	Danilchenkovo	48	M	Y	N	N	N	L	Neg
DG-0317	53	LZ	Danilchenkovo	6	U	N	Y	N	N	L	Neg
DG-0318	54	LZ	Danilchenkovo	48	F	Y	N	N	N	L	Neg
DG-0319	72	LZ	Kamenka	72	M	Y	N	N	Y	L	Neg
DG-0320	73	LZ	Kamenka	96	F	N	N	N	Y	L	Neg
DG-0321	74	LZ	Kamenka	108	M	Y	N	N	Y	NL	128
DG-0322	74	LZ	Kamenka	108	M	Y	N	N	Y	NL	512
DG-0323	74	LZ	Kamenka	96	M	Y	N	N	Y	NL	32
DG-0324	74	LZ	Kamenka	60	M	Y	N	N	Y	U	128
DG-0325	75	LZ	Kamenka	24	M	Y	N	N	N	NL	Neg
DG-0326	76	LZ	Kamenka	5	M	N	Y	N	N	L	Neg
DG-0327	76	LZ	Kamenka	5	F	N	Y	N	N	L	Neg
DG-0328	77	LZ	Kamenka	36	M	Y	N	N	N	L	64
DG-0329	81	LZ	Kamenka	18	M	Y	N	N	N	L	Neg
DG-0330	78	LZ	Kamenka	60	F	Y	N	N	Y	L	Neg
DG-0331	78	LZ	Kamenka	24	F	Y	N	N	Y	NL	Neg
DG-0332	79	LZ	Kamenka	24	M	N	Y	N	N	NL	Neg
DG-0333	79	LZ	Kamenka	72	M	N	Y	N	Y	NL	Neg
DG-0334	80	LZ	Kamenka	12	F	Y	N	N	N	L	Neg
DG-0335	80	LZ	Kamenka	12	F	Y	N	N	N	L	Neg
DG-0336	80	LZ	Kamenka	12	M	Y	N	N	N	L	Neg
DG-0337	81	LZ	Kamenka	36	M	Y	N	N	N	L	128
DG-0338	38	LZ	Chistovodnoye	60	M	N	N	Y	Y	NL	2,048
DG-0339	38	LZ	Chistovodnoye	12	M	Y	N	N	N	NL	Neg
DG-0340	39	LZ	Chistovodnoye	96	M	Y	N	N	N	U	32
DG-0341	40	LZ	Chistovodnoye	6	F	Y	N	N	N	NL	32
DG-0342	41	LZ	Chistovodnoye	60	M	Y	N	N	N	NL	23
DG-0344	43	LZ	Chistovodnoye	12	M	N	Y	N	N	L	23
DG-0345	46	LZ	Chistovodnoye	12	M	Y	Y	N	N	NL	Neg

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0348	406	LZ	Svobodnoe	48	M	Y	N	N	N	L	Neg
DG-0349	406	LZ	Svobodnoe	60	F	Y	N	N	N	L	11
DG-0350	407	LZ	Svobodnoe	96	M	Y	Y	N	N	L	11
DG-0351	408	LZ	Svobodnoe	24	M	N	Y	N	N	NL	Neg
DG-0353	87	LZ	Kievka	42	F	N	Y	N	Y	L	11
DG-0354	88	LZ	Kievka	60	M	Y	N	N	Y	NL	Neg
DG-0355	89	LZ	Kievka	6	F	Y	N	N	N	L	11
DG-0356	90	LZ	Kievka	12	M	Y	Y	N	N	L	11
DG-0357	91	LZ	Kievka	60	M	Y	N	N	N	L	Neg
DG-0358	92	LZ	Kievka	36	F	Y	N	N	N	NL	32
DG-0359	93	LZ	Kievka	120	M	N	Y	N	N	L	11
DG-0360	94	LZ	Kievka	12	M	N	Y	N	N	L	16
DG-0361	94	LZ	Kievka	132	M	N	Y	N	N	L	Neg
DG-0362	94	LZ	Kievka	12	M	N	Y	N	N	L	1,024
DG-0363	95	LZ	Kievka	72	M	Y	N	N	N	L	11
DG-0368	100	LZ	Kievka	12	F	Y	N	N	N	NL	Neg
DG-0369	101	LZ	Kievka	84	F	Y	N	N	Y	NL	32
DG-0371	103	LZ	Kievka	24	M	Y	N	N	Y	NL	23
DG-0372	104	LZ	Kievka	24	M	Y	N	N	N	L	Neg
DG-0373	105	LZ	Kievka	5	F	Y	Y	N	N	NL	Neg
DG-0375	107	LZ	Kievka	36	M	N	Y	N	N	NL	91
DG-0378	110	LZ	Kievka	36	F	N	Y	N	N	NL	Neg
DG-0379	111	LZ	Kievka	24	M	N	Y	N	N	NL	Neg
DG-0380	99	LZ	Kievka	24	M	Y	N	N	Y	L	Neg
DG-0381	112	LZ	Kishenevka	24	M	Y	Y	N	N	L	Neg
DG-0382	112	LZ	Kishenevka	12	M	Y	Y	N	N	L	Neg
DG-0383	113	LZ	Kishenevka	120	M	U	U	U	N	NL	23
DG-0384	114	LZ	Kishenevka	120	M	Y	N	N	N	L	32
DG-0385	115	LZ	Kishenevka	72	M	Y	N	N	Y	L	11
DG-0386	116	LZ	Kishenevka	12	F	Y	N	N	Y	NL	Neg
DG-0387	117	LZ	Kishenevka	48	M	Y	N	N	N	L	Neg
DG-0388	118	LZ	Kishenevka	168	M	N	Y	N	Y	L	16
DG-0389	119	LZ	Kishenevka	180	M	Y	Y	N	N	L	Neg
DG-0390	120	LZ	Kishenevka	96	M	Y	Y	N	Y	NL	Neg
DG-0391	125	LZ	Kishenevka	144	M	Y	N	N	N	L	256
DG-0394	122	LZ	Kishenevka	36	M	N	Y	N	N	L	23
DG-0395	122	LZ	Kishenevka	108	M	N	Y	N	N	L	Neg
DG-0396	122	LZ	Kishenevka	72	M	N	Y	N	N	NL	16
DG-0397	123	LZ	Kishenevka	120	M	Y	Y	N	N	NL	91
DG-0398	124	LZ	Kishenevka	60	M	Y	N	N	N	L	11
DG-0399	223	LZ	Lazo	8	M	Y	N	N	N	L	23
DG-0400	224	LZ	Lazo	60	M	Y	N	N	N	NL	16
DG-0401	225	LZ	Lazo	96	M	N	Y	N	Y	L	Neg
DG-0402	226	LZ	Lazo	96	M	N	Y	N	Y	L	91
DG-0403	227	LZ	Lazo	48	M	Y	N	N	Y	L	Neg
DG-0404	228	LZ	Lazo	60	M	Y	N	N	N	NL	Neg
DG-0405	229	LZ	Lazo	30	F	N	Y	N	N	L	11
DG-0406	232	LZ	Lazo	24	M	Y	N	N	N	L	Neg
DG-0408	230	LZ	Lazo	12	F	Y	N	N	Y	L	16

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0409	237	LZ	Lazo	24	M	Y	Y	N	Y	L	Neg
DG-0410	238	LZ	Lazo	48	M	Y	N	N	N	L	64
DG-0411	238	LZ	Lazo	12	M	Y	N	N	N	NL	32
DG-0412	235	LZ	Lazo	30	M	Y	N	N	N	L	Neg
DG-0413	235	LZ	Lazo	120	M	Y	N	N	N	L	32
DG-0415	234	LZ	Lazo	60	M	N	Y	N	Y	L	11
DG-0416	233	LZ	Lazo	18	F	N	Y	N	N	NL	32
DG-0417	239	LZ	Lazo	36	M	Y	N	N	Y	L	Neg
DG-0419	239	LZ	Lazo	24	F	Y	N	N	Y	L	Neg
DG-0420	240	LZ	Lazo	12	F	Y	N	N	N	NL	256
DG-0421	241	LZ	Lazo	48	M	Y	N	N	N	NL	32
DG-0423	243	LZ	Lazo	24	M	Y	Y	N	Y	L	Neg
DG-0424	245	LZ	Lazo	24	M	Y	N	N	N	L	Neg
DG-0425	248	LZ	Lazo	24	M	Y	Y	N	Y	NL	23
DG-0427	246	LZ	Lazo	48	M	N	Y	N	N	L	Neg
DG-0428	249	LZ	Lazo	36	M	Y	N	N	N	L	Neg
DG-0429	249	LZ	Lazo	36	M	Y	N	N	N	L	Neg
DG-0430	244	LZ	Lazo	48	M	Y	N	N	N	L	Neg
DG-0433	327	SZ	Plastun	36	M	Y	N	N	N	L	Neg
DG-0437	330	SZ	Plastun	12	M	Y	N	N	N	NL	Neg
DG-0438	330	SZ	Plastun	144	M	Y	N	N	N	NL	Neg
DG-0439	331	SZ	Plastun	48	F	Y	N	N	N	NL	Neg
DG-0440	332	SZ	Plastun	72	F	N	N	Y	Y	L	Neg
DG-0442	334	SZ	Plastun	36	M	Y	N	N	N	L	Neg
DG-0443	334	SZ	Plastun	12	M	Y	N	N	N	L	Neg
DG-0444	335	SZ	Plastun	132	M	N	N	Y	Y	L	Neg
DG-0445	348	SZ	Plastun	18	M	Y	N	N	N	L	Neg
DG-0446	337	SZ	Plastun	120	M	Y	N	N	N	L	Neg
DG-0447	336	SZ	Plastun	24	F	Y	N	N	N	L	Neg
DG-0450	468	SZ	Terney	24	M	N	Y	N	N	L	Neg
DG-0451	470	SZ	Terney	6	M	Y	N	N	N	L	23
DG-0452	471	SZ	Terney	180	F	N	Y	N	Y	L	16
DG-0453	472	SZ	Terney	24	F	Y	N	N	N	L	Neg
DG-0455	474	SZ	Terney	36	M	Y	N	N	Y	NL	Neg
DG-0456	472	SZ	Terney	10	M	Y	N	N	N	L	Neg
DG-0457	340	SZ	Plastun	12	M	Y	N	N	Y	L	11
DG-0458	341	SZ	Plastun	36	M	N	Y	N	Y	L	45
DG-0459	342	SZ	Plastun	18	M	Y	N	N	N	NL	23
DG-0460	342	SZ	Plastun	5	M	N	Y	N	N	L	11
DG-0461	343	SZ	Plastun	12	M	Y	Y	N	Y	L	Neg
DG-0468	338	SZ	Plastun	12	M	Y	N	N	N	L	23
DG-0469	345	SZ	Plastun	12	F	N	Y	N	N	L	Neg
DG-0470	344	SZ	Plastun	96	M	Y	N	N	N	L	Neg
DG-0471	344	SZ	Plastun	120	F	Y	N	N	N	L	16
DG-0472	347	SZ	Plastun	24	M	Y	N	N	Y	L	16
DG-0473	480	SZ	Terney	96	M	Y	N	N	N	L	256
DG-0475	469	SZ	Terney	96	M	Y	N	N	Y	L	23
DG-0477	474	SZ	Terney	72	M	Y	N	N	Y	L	23
DG-0478	474	SZ	Terney	12	F	Y	N	N	Y	L	16

Animal identity	Household Identity	Study area	Settlement	Age	Gender	Guard dog	Companion	Hunting dog	Visits forest	Source	VN titre
DG-0482	469	SZ	Terney	108	M	Y	N	N	Y	L	32
DG-0485	475	SZ	Terney	8	F	Y	N	N	N	L	45
DG-0486	483	SZ	Terney	8	M	Y	N	N	N	L	23
DG-0487	484	SZ	Terney	24	M	N	Y	N	N	L	Neg
DG-0488	482	SZ	Terney	60	F	N	Y	N	N	L	Neg
DG-0490	486	SZ	Terney	36	M	N	N	Y	Y	L	23
DG-0491	487	SZ	Terney	24	F	Y	N	N	N	L	362
DG-0492	410	SZ	Taejnoye	12	M	Y	Y	Y	Y	L	23
DG-0493	410	SZ	Taejnoye	24	F	Y	Y	Y	Y	L	181
DG-0494	411	SZ	Taejnoye	72	M	N	N	N	Y	L	32
DG-0495	412	SZ	Taejnoye	78	F	N	Y	N	N	U	91
DG-0497	411	SZ	Taejnoye	72	F	N	N	N	Y	L	Neg
DG-0498	350	SZ	Plastun	48	M	N	Y	N	Y	NL	11
DG-0501	494	SZ	Terney	96	F	Y	N	N	N	L	23
DG-0502	491	SZ	Terney	12	M	N	Y	N	Y	L	23
DG-0503	492	SZ	Terney	48	M	Y	N	N	N	L	Neg
DG-0504	489	SZ	Terney	84	M	Y	N	N	Y	L	32
DG-0505	488	SZ	Terney	84	F	Y	N	N	Y	L	45
DG-0506	490	SZ	Terney	96	F	Y	N	N	N	L	181
DG-0507	493	SZ	Terney	24	M	Y	N	N	Y	L	16
DG-0511	507	SZ	Terney	66	M	N	Y	N	Y	L	45
DG-0512	511	SZ	Terney	30	M	Y	Y	N	Y	U	16
DG-0513	505	SZ	Terney	48	F	N	Y	N	Y	L	45
DG-0514	503	SZ	Terney	11	M	N	N	N	Y	L	23
DG-0515	502	SZ	Terney	48	F	N	Y	N	N	NL	23
DG-0517	510	SZ	Terney	48	F	Y	Y	N	Y	L	Neg
DG-0518	510	SZ	Terney	72	F	Y	Y	N	Y	L	16
DG-0519	508	SZ	Terney	108	F	N	Y	N	Y	NL	16
DG-0520	504	SZ	Terney	144	F	Y	N	N	Y	L	16
DG-0521	501	SZ	Terney	24	F	N	Y	N	Y	L	16
DG-0522	501	SZ	Terney	12	M	N	Y	N	Y	NL	Neg
DG-0523	500	SZ	Terney	24	M	Y	N	N	N	L	45
DG-0525	498	SZ	Terney	132	M	N	Y	N	Y	L	16
DG-0526	495	SZ	Terney	72	M	N	Y	N	N	L	16
DG-0527	496	SZ	Terney	48	M	Y	N	N	Y	L	16
DG-0528	499	SZ	Terney	18	M	Y	N	N	N	L	11
DG-0529	506	SZ	Terney	48	M	Y	Y	N	Y	L	16
DG-0530	497	SZ	Terney	120	M	Y	Y	N	Y	L	45
DG-0531	497	SZ	Terney	24	M	Y	Y	N	Y	L	16
Clf # 080	314	SW	Ovchinnikovo	Unk	M	Y	N	N	U	U	362
DG-0142	67	SW	Devatyy-Val	Unk	M	Y	N	N	Y	L	Neg
DG-0189	265	SW	N. Lvovskoe	Unk	M	Y	Y	N	N	L	23
DG-0208	138	SW	Krounovka	Unk	F	Y	N	N	N	L	32
DG-0316	52	LZ	Danilchenkovo	Unk	M	Y	Y	N	N	L	Neg
DG-0024	435	SZ	Terney	3.5	M	N	N	Y	U	L	Neg
DG-0065	379	SW	Slavyanka	2	M	Y	N	N	N	L	Neg
DG-0145	298	SW	Olenevod	3	M	N	Y	N	Y	NL	Neg

Appendix XXV. Table of wildlife serology results based on a 1:8 titre cutoff denoting positive samples

Results of virus neutralization analyses against canine distemper virus (CDV) for serum samples collected from wild carnivores in the Russian Far East between 1992 and 2014. Neutralizing antibody titres of 1:8 or higher were considered positive. Seroprevalence is given as the number of positive samples expressed as a percentage of sample size, with lower and upper 95% binomial confidence intervals (CI).

Species	Positive	Sample size	Seroprev. (%)	Lower CI (%)	Upper CI (%)
Amur tiger *	21	67	31.3	20.9	44.0
Far Eastern leopard *	2	10	20.0	3.5	55.8
Eurasian lynx *	1	7	14.3	0.8	58.0
Leopard cat	3	16	18.8	5.0	46.3
Asiatic black bear *	2	25	8.0	1.4	27.5
Brown bear *	2	20	10.0	1.8	33.1
Raccoon dog †	14	35	40.0	24.4	57.8
Red fox †	1	4	25.0	1.3	78.1
Sable †	0	2	0.0	0.0	80.2
Siberian weasel †	0	2	0.0	0.0	80.2
American mink †	0	2	0.0	0.0	80.2
Asian badger †	7	43	16.3	7.3	31.3

* Samples tested in Washington State University against CDV Onderstepoort strain. † Samples tested in the University of Glasgow using CDV Bussell strain.

Appendix XXVI. Selection process for multivariate generalized binary logistic regression models predicting exposure of tigers to canine distemper virus based on Akaike information criterion values

Selection process for multivariate generalized binary logistic regression models predicting the exposure of tigers to canine distemper virus based on Akaike information criterion (AIC) values. Explanatory variables include aga (AG), gender (GE), study area (SA), animal category (CA) and human density (HU). Models were constructed using a forward selection process using AIC values assess model quality. Lowest AIC values at each stage of model construction are indicated in bold, and final model is highlighted in grey.

Model variables	AIC	ΔAIC
Base	**76.91976**	**0**
AG	78.58473	1.66497
GE	78.80361	1.88385
SA	78.97829	2.05853
CA	78.24981	1.33005
HU	78.78497	1.86521

Appendix XXVII. Selection process for multivariate generalized binary logistic regression models predicting exposure of unvaccinated domestic dogs to canine distemper virus based on Akaike information criterion values

Selection process for multivariate generalized binary logistic regression models predicting the exposure of tigers to canine distemper virus based on Akaike information criterion (AIC) values. Explanatory variables include age (AG), gender (GE), forest visits (FV), study area (SA), community type (CA), children in house (CH), people in house (PL), cat owner (CO), poultry owner (PO), livestock owner (LO), residence type (RT), guard dog (GD), hunting dog (HD), companion dog (CD), and source (SO). Settlement was included as a random variable. Models were constructed using a forward selection process using AIC values assess model quality. Lowest AIC values at each stage of model construction are indicated in bold, and final model is highlighted in grey.

Model variables	AIC	ΔAIC
Base	368.3572	16.0174
AG	**359.7056**	**7.3658**
GE	370.0466	17.7068
FV	367.1778	14.838
SA	360.6175	8.2777
CT	369.5197	17.1799
CH	370.3571	18.0173
PL	369.4637	17.1239
CO	370.1489	17.8091
PO	370.2654	17.9256
LO	368.2278	15.888
RT	368.3453	16.0055
GD	366.8586	14.5188
HT	370.2987	17.9589
CD	369.6611	17.3213
SO	369.1047	16.7649
AG + GE	361.4121	9.0723
AG + FV	360.1592	7.8194
AG + SA	**352.3398**	**0**
AG + CT	360.8004	8.4606
AG + CH	361.635	9.2952
AG + PL	360.314	7.9742
AG + CO	361.3231	8.9833

AG + PO	361.6598	9.32
AG + LO	360.3444	8.0046
AG + RT	360.3317	7.9919
AG + GD	359.2623	6.9225
AG + HT	361.699	9.3592
AG + PT	361.1398	8.8
AG + SO	360.7173	8.3775
AG + SA + GE	354.0126	1.6728
AG + SA + FV	352.817	0.4772
AG + SA + CT	353.7825	1.4427
AG + SA + CH	354.2478	1.908
AG + SA + PL	352.9501	0.6103
AG + SA + CO	354.1682	1.8284
AG + SA + PO	353.8418	1.502
AG + SA + LO	353.2041	0.8643
AG + SA + RT	353.1022	0.7624
AG + SA + GD	352.5913	0.2515
AG + SA + HT	354.3366	1.9968
AG + SA + PT	353.9715	1.6317
AG + SA + SO	353.0115	0.6717

Glossary and abbreviations

Allee effect – A decline in individual fitness at low population size or density, that can result in critical population thresholds below which populations decline to extinction.

CD150 – Cluster of differentiation 150 cell receptor, present on B and T-lymphocytes and dendritic cells. Serves as the primary receptor used by canine distemper virus and other Morbilliviruses during early stages of infection. Also known as the signalling lymphocyte activation molecule (SLAM/F1).

CDV – Canine distemper virus.

CITES – Convention on International Trade in Endangered Species of Wild Fauna and Flora, is a multilateral treaty governing the trade in endangered plants and animals.

Critical community size (CCS) – The host population size, below which a disease cannot persist in the long term.

Enzootic –A state of constant presence of a pathogen within an animal population.

Epizootic – A temporary state of high prevalence of a pathogen in an animal population.

Force of infection – The rate at which susceptible individuals acquire an infectious disease.

Incidence – The number of new cases in a particular time interval.

Maintenance population – Populations of susceptible hosts exceeding the critical community size that permits the long-term persistence of a pathogen.

Maintenance community – Assemblages of several populations or species of susceptible hosts, which collectively exceed the critical community size that permits the long-term persistence of a pathogen.

Metapopulation – In the epidemiological context, metapopulations consist of multiple subpopulations of susceptible hosts that represent patches of 'habitat' for pathogens, and are loosely connected (e.g. through host movement) to enable the dispersal of the infection between patches.

Nectin-4 – Cellular adhesion molecules found on epithelial cells, which are used by canine distemper virus and other Morbilliviruses as receptors for entry into host cells. Also known as poliovirus like receptor 4.

Prevalence – proportion of positive cases in a population at a particular time point.

PVLR – Cellular adhesion molecules found on epithelial cells, which are used by canine distemper virus and other Morbilliviruses as receptors for entry into host cells. Also known as nectin 4.

Reservoir – One or more epidemiologically connected populations or environments in which a pathogen can be permanently maintained and from which infection is transmitted to the target population.

R_0 – The reproductive number of a pathogen. The mean number of secondary infections arising from an infected individual in a freely mixing, fully susceptible population.

R_e – Effective reproductive number. The mean number of secondary infections arising from an infected individual in a freely mixing, partially susceptible population (e.g. where vaccination is practiced).

SIR/D – Susceptible – Infected – Recovered/Dead. A compartmentalised structure used for modelling the epidemiology of short-acting pathogens, for which infections either result in the death, or the recovery of the host, with long term immunity.

SLAM/F1 – Signalling lymphocyte activation molecule cell receptor, present on B and T-lymphocytes and dendritic cells. Serves as the primary receptor used by canine distemper virus and other Morbilliviruses during early stages of infection. Also known as CD150.

Zapovednik – The highest protection status given to protected area reserves in Russia and the former Soviet countries, where public access is totally restricted.