

Assessing the health risks of reintroduction: The example of the Amur leopard, *Panthera pardus orientalis*

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Abstract

Translocation of wildlife as a means of reintroducing or reinforcing threatened populations is an important conservation tool but carries health risks for the translocated animals and their progeny, as well as wildlife, domestic animals and humans in the release area. Disease risk analyses (DRA) are used to identify, prioritize and design mitigation strategies to address these threats. Here, we use a DRA undertaken for Amur leopards (*Panthera pardus orientalis*) to illustrate how specific methodology can optimize mitigation strategy design. A literature review identified a total of 98 infectious hazards and 28 non-infectious hazards. Separate analyses were undertaken for disease risks in leopards from hazards of *source origin* (captive zoo collections and the transit pathway to the Russian Far East), or of *destination origin* (in breeding enclosures and wider release areas); and for disease risks in other wildlife, domesticated species or humans, similarly from hazards of *source or destination origin*. Hazards were assessed and ranked as priority 1, priority 2, priority 3 or low priority in each of the defined scenarios. In addition, we undertook a generic assessment of stress on individual leopards. We use three examples to illustrate the process: *Chlamydomphila felis*, canine distemper virus (CDV) and feline immunodeficiency virus (FIV). We found that many potentially expensive screening procedures could be performed prior to export of leopards, putting the onus of responsibility onto the zoo sector, for which access to diagnostic testing facilities is likely to be optimal. We discuss how our methods highlighted significant data gaps relating to pathogen prevalence in the Russian Far East and likely future unpredictability, in particular with respect to CDV. There was emphasis at all stages on record keeping, meticulous planning, design, staff training and enclosure management, which are relatively financially inexpensive. Actions to minimize stress featured at all time points in the strategy and also focussed on planning, design and management.

KEYWORDS

Amur leopard, disease risk analysis, *Panthera pardus orientalis*, re-introduction

1 | INTRODUCTION

All wildlife translocation programmes carry potential health risks for the translocated individuals, as well as for other wild and domesticated animal species, and for humans in the area receiving translocated animals. Translocations may be part of re-introduction or population reinforcement programmes and may involve captive-to-wild or wild-to-wild animal movements. Regardless of the precise nature of the programme, each time a wild animal is moved from one location to another, the health risks to that individual, its conspecifics, other species (wild and domesticated), humans and the wider environment should be taken into consideration (Jakob-Hoff et al., 2014).

Such health risks generally equate to disease (defined as a disruption of physiological homeostasis) arising from the host response to an infectious or non-infectious agent (Blaustein et al., 2012). Disease risks may arise from the following: direct translocation of infectious agents accompanying the wildlife species of interest; exposure of the translocated wildlife species to novel infectious and non-infectious agents at or during transport to the destination; alterations in host response in association with translocation stress; and ecological changes associated with the presence or increase in numbers of the wildlife species being translocated.

Furthermore, factors that modify host immune responses may have a role in determining the outcome following exposure to infectious or non-infectious agents, both at an individual level and a population level. Such factors might include naturally occurring stressors (to which hosts are more likely to be well-adapted) and anthropogenic factors, such as habitat degradation and loss, human encroachment, the impact of invasive species and climate change (Blaustein et al., 2012). Predicting the scope and scale of these exposure risks is made considerably more challenging in the light of the dynamic and complex interaction between these stressors. As our ability to incorporate greater complexity of individual perceived population threats into epidemiological models has advanced over time, so our interest in disease as a driver of population declines and extinctions has increased (MacPhee & Greenwood, 2013). Indeed, it has been postulated that the contribution of disease to free-living wildlife population declines and even extinctions may have been historically underestimated (MacPhee & Greenwood, 2013; Pedersen, Jones, Nunn, & Altizer, 2007).

Following on from well-established risk-based frameworks developed for mitigating health impacts of trade in domesticated animals and associated products, international guidelines are now available for the analysis of disease risk in association with wildlife translocations (Jakob-Hoff et al., 2014). Disease risk analysis (DRA) is now well accepted as a fundamental and necessary component

of wildlife translocations, although perhaps still overlooked in many. Unintended disease outcomes have occurred historically in association with translocations, such as the introduction of *Batrachochytrium dendrobatidis* into Mallorca following release of captive-bred Mallorcan midwife toads (*Alytes muletensis*) (Walker et al., 2008). As more DRAs are undertaken so the value of learning from others' experiences increases. To that end, we wish to present our own experience from undertaking a DRA for the Amur leopard (*Panthera pardus orientalis*) re-introduction programme in the Russian Far East (RFE), thereby assisting others in their work, and promoting consistency in approach.

The Amur leopard is one of the most endangered taxa of large cat, classified by the International Union for the Conservation of Nature (IUCN) as 'critically endangered' (Stein et al., 2016). Remaining free-living Amur leopards number up to 90 individuals, predominantly in southwest Primorski Krai in the RFE, with some individuals present in the transboundary area in northeast China (Feng et al., 2017; Vitkalova & Shevtsova, 2016). Major threats to survival of the species in the wild include poaching of leopards and their prey species; habitat loss, degradation and fragmentation; and dwindling genetic diversity (Stein et al., 2016; Uphyrkina, Miquelle, Quigley, Driscoll, & O'Brien, 2002). A programme to establish a second population in a nearby protected area has been proposed which involves the importation of adult captive Amur leopards from selected zoological collections in Russia and/or Europe into purpose-built breeding facilities within or near a release area in former habitat in south-east Primorski Krai, with offspring considered for release. An alternative or complementary approach would be to use subadult leopards bred in zoos specifically for the reintroduction programme, moved to the RFE and further reared and acclimatized prior to release. In both scenarios, imported leopards would be maintained in fenced enclosures with natural outdoor areas and indoor breeding den facilities where applicable, and have access to live prey introduced particularly during the offspring rearing period. Offspring will be considered for release at about 15–18 months of age (Spitzen et al., 2012).

Our objectives here are to describe the methodology we used to assess a complex range of possible disease outcomes, present the results of our approach and suggest proportionate and achievable mitigation strategies. At first glance, the scale of the task in identifying and prioritizing potential health threats appears overwhelming, involving as it does potential impacts on the leopards, as well as all other species that might be affected. However, the process was rendered manageable by applying a systematic approach to risk analysis. We provide a discussion of how the methodology used was central to development of mitigation strategies and suggest that themes that emerged from our analyses are likely to be common to many, if not all, wildlife translocations.

FIGURE 1 Schematic outline of the DRA process for re-introduction of the Amur leopard (*Panthera pardus orientalis*) into the Russian Far East (adapted from Jakob-Hoff et al., 2014) [Colour figure can be viewed at wileyonlinelibrary.com]

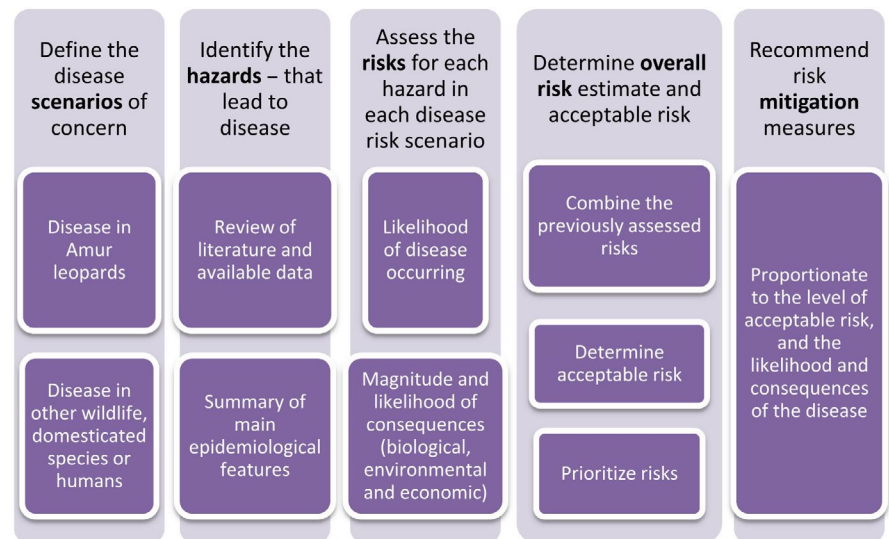


TABLE 1 'Disease risk scenario-hazard combinations' in the DRA for the Amur leopard re-introduction into the Russian Far East

Description	Location	Type of hazard	Shorthand term used
Disease risk scenario 1 (disease in Amur leopards) DRS1	Captive collections (source hazard)	Infectious	DRS1_ISH
		Non-infectious	DRS1_NSH
	Captive enclosure in RFE (destination hazard)	Infectious	DRS1c_IDH
		Non-infectious	DRS1c_NDH
	Wider release zone RFE (destination hazard)	Infectious	DRS1r_IDH
		Non-infectious	DRS1r_NDH
Disease risk scenario 2 (disease occurring in other wildlife, domesticated species or humans) DRS2	Captive collections (source hazard)	Infectious	DRS2_ISH
	Wider release zone RFE (destination hazard)	Non-infectious	DRS2_NDH

2 | MATERIALS AND METHODS

2.1 | Disease risk analysis

The methods used were based on guidance in the IUCN/OIE Manual of Procedures for Wildlife Disease Risk Analysis (Jakob-Hoff et al., 2014), are summarized schematically in Figure 1 and described in more detail below.

The initial stage was to define the disease risk scenarios which may arise: firstly, disease occurring in Amur leopards (applies to imported leopards and their progeny—categorized as disease risk scenario 1, DRS1) and secondly, disease occurring in other wildlife, domesticated species or humans in the vicinity (categorized as disease risk scenario 2, DRS2). A list of hazards (infectious and non-infectious) was then compiled for each disease risk scenario based on a systematic literature review covering disease in *Panthera* spp. Literature searches were performed using keywords ('panthera pathogen'; 'panthera parasite'; 'panthera disease'; 'amur leopard'; and 'panthera pardus altaica') entered into online databases 'PubMed' and 'Web of Knowledge'.

Hazards were classified either as 'source hazards' (infectious and non-infectious in imported leopards), or 'destination hazards' (infectious and non-infectious) occurring in two distinct locations:

the captive enclosure and in the wider release zone. Some hazards could be considered both as source hazards and as destination hazards, in which case they were considered separately according to the context in which they occurred. This categorization enabled us to identify eight 'disease risk scenario-hazard' combinations as shown in Table 1.

The relevant epidemiological information for each hazard identified from our literature search was summarized to facilitate the risk assessment process. In addition, to assist with risk assessment of disease arising specifically in captive source leopards, a database was created compiling all available veterinary data relating to Amur leopards, living and dead, in the European Endangered species Programme (EEP¹) from which leopards for the reintroduction programme will be sourced. The database, hereafter referred to as the Amur leopard Veterinary Database or ALVDB, was initiated by and is maintained by Dr John Lewis. Details of all laboratory tests conducted on each leopard are included in the database and original laboratory reports embedded wherever possible. Of particular value was the identification of causes of mortality and results of disease screening tests conducted on EEP leopards. Cats for inclusion in the reintroduction programme will be chosen from the EEP programme based on both their genetic importance and health status.

The outcome of the risk assessments was an overall risk estimate—a combination of the likelihood of disease occurring in the species of concern (leopards, other wildlife, domesticated species or humans), and the consequences (likelihood and magnitude from biological, environmental and economic perspectives) of each disease. The likelihood of disease occurring was further broken down into two distinct elements, the *release assessment* of a hazard and the *exposure assessment*. Release assessment refers to the risk of an agent being released out of its host resulting in either environmental contamination or direct transmission, depending on the characteristics of the agent. In terms of our DRA, release assessment was not applicable to non-infectious hazards. Exposure assessment refers to the likelihood that the individuals being translocated or their progeny might be exposed to a hazard. Consequence estimates considered likelihood and magnitude of potential effects of each hazard on individual leopards (e.g. morbidity, reproduction and mortality); on leopard population dynamics; on other wildlife populations particularly other sympatric felids (e.g. Amur tigers, *Panthera tigris altaica*); on domesticated animals; on humans; and on ecosystem balance. The overall risk estimates combined release (where applicable), exposure and consequence assessments, as described in the IUCN manual (Jakob-Hoff et al., 2014).

Risk assessments were undertaken independently by a panel of three of the authors (Lewis, Tomlinson & Gilbert), between whom there is expertise in veterinary wildlife field work, disease epidemiology and conditions in the RFE. Three qualitative categories were used for each assessment, namely low, medium and high. Assessments, necessarily subjective, were made based on prior experience, clinical knowledge, data from the ALVDB and the hazard-based epidemiological information from the literature search (see Appendix S2). Where discrepancies occurred between the three assessors, these were discussed within the panel until resolution was reached. In some cases, information was insufficient to completely assess a hazard (e.g. unknown pathogen status in the release area). In these cases, assumptions were made based on agreed likelihood and recorded on a case by case basis, noting the limitations on our assessments. These records highlight priorities in need of future research.

For each hazard in each 'disease risk scenario-hazard' combination, we also determined the highest level of risk that might be deemed acceptable ('acceptable risk'). Each risk estimate was then evaluated in the light of the acceptable risk to enable us to reach a decision as to whether mitigation was advisable or unnecessary—a prioritization process advised by the IUCN Guidelines (Jakob-Hoff et al., 2014). In addition, we decided to further prioritize

our hazards using values assigned to the overall risk estimate, the acceptable risk, the consequences assessment and the mitigation category, respectively, due to the overwhelming number of hazards for which mitigation was considered advisable. Four priority categories were created: priority 1, priority 2, priority 3 and low priority. Hazards for which the acceptable risk was lower than the risk estimate were the highest 'priority 1' category; hazards for which the acceptable risk and the risk estimate were equivalent and the consequences were high, were priority 2; remaining hazards for which mitigation was categorized as 'advisory' were considered priority 3; and all remaining hazards were considered low priority (Table 2).

In addition to our hazard specific assessments, we undertook a 'stand-alone' review of the effects of translocation stress on leopards in the programme. Acute stress is highly likely for any animals involved in a translocation programme, whether wild-caught or captive (Dickens, Delehanty, & Romero, 2010). In addition to acute stress, we specifically considered the effects of chronic stress, arising from a persistent stressor, or multiple acute stressors.

An acute stress response in an individual animal is a natural and appropriate reaction to a perceived stressor (e.g. escape from predation), and one that is essential for survival in the wild. The acute stress response is a combination of the 'fast fight or flight' (or 'fight, flight or freeze') response, mediated by the sympathetic nervous system; and the slower glucocorticoid-mediated response. Its purpose is to functionally divert physiological resources away from non-essential processes such as reproductive physiology and behaviour, and immune system maintenance (Dickens et al., 2010), in order to survive, escape or avoid the perceived stressor. Return to a normal physiological state via negative feedback loops occurs once the acute stressor ends. However, in the event of an unusually severe stressor, excessive catecholamine stimulation may affect cardiac function, and where an individual is prevented from escape, physical trauma may occur.

If the stressor persists or multiple acute stressors occur in series or in parallel, the response can become dysregulated, 'mal-adaptive' and potentially detrimental—a state of 'chronic stress'. Such dysregulation can have negative effects on the immune system, reproductive behaviour and adaptive behaviour. For these reasons, we considered both severe acute stress and chronic stress as potentially detrimental and reviewed the risks to leopards accordingly.

Three hazards, *Chlamydomydia felis*, canine distemper virus (CDV) and feline immunodeficiency virus (FIV), were selected

Categorization criteria	Priority level
Acceptable risk < risk estimate	1
Acceptable risk = risk estimate AND consequence assessment HIGH	2
All other hazards for which mitigation was categorized as 'advisable'	3
All remaining hazards	Low

TABLE 2 Hazard prioritization criteria for each 'disease risk scenario-hazard' combination in the DRA for Amur leopard reintroduction into the Russian Far East

retrospectively to demonstrate how this process was applied. These hazards were selected to illustrate in differing ways how our method of assessing risks in different 'disease risk scenario-hazard' combinations affected the individual assessments and mitigation strategies.

Chlamydomphila felis is a Gram-negative obligate intracellular bacterium, which is ubiquitous and common in domestic cats. Exposure to *C. felis* has been documented in free-living wildlife such as European wild cats (*Felis silvestris silvestris*) (Millán & Rodríguez, 2009) and Iberian lynx (*Lynx pardinus*) (Millán et al., 2009), but no reports of exposure or disease in *Panthera* spp. were found in our literature review.

Canine distemper is caused by a morbillivirus with a wide carnivore host range (Fröhlich, 2012). Canine distemper virus associated mortality has been recorded in canid, mustelid, felid, ursid, phocid, otariid, viverrid, ailurid and procyonid species (Barrett, Wohlsein, Bidewell, & Rowell, 2004; Deem, Spelman, Yates, & Montali, 2000). Infection has also been recorded in non-carnivores including primates, rodents and ungulates (Martinez-Gutierrez & Ruiz-Saenz, 2016). A fatal case of CDV has been diagnosed in one free-ranging Amur leopard in 2015 (Sulikhan et al., 2018). Canine distemper virus has been responsible for mortality events in several other species, including free-ranging lions (*Panthera leo*) (Roelke-Parker et al., 1996), red foxes (*Vulpes vulpes*), badgers (*Meles meles*) (Origgi et al., 2012) and African wild dogs (*Lycaon pictus*) (Goller et al., 2010). Canine distemper virus mortality has also been confirmed in free-ranging Amur tigers in the RFE (see Gilbert et al., 2014) and in tigers (*Panthera tigris*) in India (ProMED, 2013). In addition, evidence of CDV infection has been detected in captive *Panthera* species in North America (Appel et al., 1994), Europe (Myers, Zurbriggen, Lutz, & Popischil, 1997), India (Ramanathan, Malik, & Prasad, 2007) and Japan (Nagao et al., 2012). The impact of CDV on small vulnerable free-ranging populations can be very serious (Gilbert et al., 2014) making it a significant hazard for any species conservation programme. Data from the RFE reveal serological evidence of exposure to CDV in domestic dogs (Goncharuk, Kerley, Naidenko, & Rozhnov, 2012), free-ranging Amur tigers (Goodrich, Lewis, & Quigley, 2012; Goodrich, Quigley, et al., 2012) and free-ranging Amur leopards (Goodrich, Lewis, et al., 2012; Sulikhan et al., 2018). The epidemiological picture of CDV dynamics in the RFE is currently incomplete, but a large number of wild and/or domestic carnivores could be contributing to the local CDV reservoir. The potential severity of an outbreak in leopards led us to select CDV as an illustrative hazard.

Our third illustrative hazard was FIV, a lentivirus that replicates in T cells, resulting in T-cell-CD4 depletion in domestic cats (Reperant & Osterhaus, 2012). Geographical clustering of genetically differing subtypes, or clades (A–E), is a feature of FIV (Hosie et al., 2009; O'Brien et al., 2012). Although difficult to predict, clades A, B and D are likely in Amur leopard range (Hosie et al., 2009). Wild felid species appear to carry their own host-adapted FIV viruses, with low rates of transmission between species (O'Brien et al., 2012). Species-specific FIV viruses have been detected in

lions (Brown, Yuhki, Packer, & O'Brien, 1994), pumas (*Puma concolor*) (Carpenter et al., 1996) and Pallas' cat (*Otocolobus manul*) (Brown et al., 2010). However, there is evidence of cross-transmission between species, notably between puma and bobcat (*Lynx rufus*) in North America (Franklin et al., 2007); between domestic cats and Tsushima cat (*Prionailurus bengalensis euptilura*) in Japan (Nishimura et al., 1999); and between domestic cats and guinea (*Leopardus guigna*) in Chile (Mora, Napolitano, Ortega, & Poulin, 2015). There are several explanations for the low rates of inter-species transmission, but it would seem likely that increased human encroachment into wildlife habitat can only increase the risk of spill-over of FIV from domestic cats to free-ranging wild felid species (VandeWoude, Troyer, & Poss, 2010). It is probable that the full epidemiological picture has not been elucidated and is likely to change over time with cross-species transmission events and the evolution of new lentiviruses (Lee et al., 2017).

Previously, FIV infection in wild felids was considered asymptomatic, but studies of free-ranging lions suggest that view is oversimplistic (O'Brien et al., 2012; Roelke et al., 2009). Depletion of CD4 cells has been observed in association with FIV infection in both free-ranging lions and pumas (Roelke et al., 2006), and it has been suggested that infection with different strains of FIV in Serengeti lions may be associated with differing survival rates following CDV infection (Troyer et al., 2011). Feline immunodeficiency virus is thus considered a potential threat to felid species in both captive and free-ranging populations, and the many data gaps relating to species dependent pathogenicity, host specificity and prevalence led to its selection as the third illustrative hazard.

2.2 | Mitigation strategy

We designed a chronological detailed mitigation strategy, from pre-export of leopards, through to post-release of leopards or offspring, linking specific hazards to specific measures to illustrate the purpose of the measure. We used our hazard prioritization to inform the mitigation measures, thereby ensuring a thorough, but proportionate, realistic and achievable approach.

3 | RESULTS

3.1 | Hazard specific risk assessments

We identified and assessed risks for 98 infectious hazards (22 bacteria; 7 ectoparasites; 39 endoparasites; 2 fungi; 1 prion; 12 protozoa; and 15 viruses), and 28 non-infectious hazards in each disease risk scenario where applicable (see Appendix S1 for full spreadsheets of the risk levels assigned, and Appendix S2 for hazard specific summary epidemiological information).

Hazards that were categorized as priority 1 for disease arising in leopards from hazard importation into the RFE (hazards of source origin), included the following: the upper respiratory tract

pathogens—feline calici virus (FCV), feline herpes virus (FHV) and *Chlamydomphila felis*; other feline viral infections—feline leukaemia virus (FeLV), feline immunodeficiency virus (FIV), feline parvovirus (FPV) and feline corona virus (FCoV); *Mycobacterium tuberculosis* complex (predominantly *M. bovis*); ticks; brachyuria (short-tail); melanism; infertility (of any origin); trauma; umbilical hernia; and the cardiac abnormalities aortic stenosis, atrial septal defect (ASD) and patent ductus arteriosus (PDA).

The priority 1 hazards in terms of disease arising in any other wildlife species, domesticated species or humans from hazard importation (hazards of source origin) also included FeLV, FIV, *C. felis*, *M. bovis* and ticks, with the addition of rabies, and the zoonotic cestodes, *Echinococcus multilocularis*, *E. granulosus* and *Dipyllobothrium* species. Of particular note in this regard is *M. bovis* in the context of it representing a threat to Amur leopard prey populations.

Hazards that were categorized as priority 1 for disease arising in leopards in the RFE (hazards of destination origin, either in the enclosure or in the wider release area), included the viral infections canine distemper virus (CDV), FeLV and FIV, bacterial infection with *M. tuberculosis* complex (*M. bovis*), non-infectious cardiac abnormalities such as aortic stenosis, ASD and PDA, brachyuria, environmental pollutants (such as industrial pollutants, pesticides and heavy metals), poaching, umbilical hernia, trauma, starvation and leopard-human conflict. Leopard-human conflict was also considered a priority 1 hazard in terms of consequences for humans in and around the release zone. In summary, viral agents were of greatest concern, in particular feline viruses FeLV and FIV, and the multi-host virus CDV.

To illustrate how the risk assessment process was applied and how the results were used to prioritize hazards, the results of each of the risk assessments for our three selected hazards, namely *C. felis*, CDV and FIV, are described below and summarized in Table 3.

For *C. felis*, in all relevant disease risk scenario-hazard combinations, we considered the release and exposure risks as low, and the consequences medium, with an overall medium risk estimate (Table 3). *Chlamydomphila felis* is easily detected in captive leopards, and therefore, the risk of export is largely avoidable. Therefore it was considered less acceptable to export *C. felis* than to have animals exposed to it once in the RFE, resulting in differing levels of acceptable risk for *C. felis* when originating from source than from the destination (Table 3). This difference in acceptable risk affected the prioritization process (Table 2), with *C. felis* being considered a higher priority hazard when originating from source than from the destination.

The release risk of CDV as a source hazard was considered to be low. The likelihood of exposure was considered medium for other leopards in the programme, but low for other wildlife/domesticated species. This was primarily due to the relatively short duration and obvious clinical signs associated with CDV infection, which make detection in a captive collection relatively straightforward. As a destination hazard, release risk was considered to be low in the captive enclosure, but medium in the wider release zone. The exposure risk for other leopards in the programme in both cases was considered medium. In all assessments, the consequence assessments were considered high. These findings resulted in low-risk estimates for both leopards and other wildlife/domesticated animals in terms of CDV as a source hazard; a medium risk estimate for leopards in terms of CDV in the captive enclosure; and a high-risk estimate for leopards in terms of CDV in the wider release zone. In all cases, acceptable risks were considered low. These differing risk estimates affected the prioritization process, such that CDV was considered a priority 1 hazard for leopards in the captive enclosure and the wider release zone. As a source hazard for disease in leopards or other wildlife/domesticated species, CDV was considered a priority 2 hazard.

TABLE 3 Individual risk assessments for three hazards (*C. felis*, CDV and FIV) in association with 'disease risk scenario-hazard' combinations in the DRA for Amur leopard reintroduction into the Russian Far East

Agent	'Disease risk scenario-hazard' combination	Release risk	Exposure risk	Consequence assessment	Risk Estimate	Acceptable risk	Priority level
<i>Chlamydomphila felis</i>	DRS1_ISH	Low	Low	Medium	Medium	Low	1
	DRS1c_IDH	Low	Low	Medium	Medium	Medium	3
	DRS1r_IDH	Low	Low	Medium	Medium	Medium	3
	DRS2_ISH	Low	Low	High	Medium	Low	1
Canine distemper virus (CDV)	DRS1_ISH	Low	Medium	High	Low	Low	2
	DRS1c_IDH	Low	Medium	High	Medium	Low	1
	DRS1r_IDH	Medium	Medium	High	High	Low	1
	DRS2_ISH	Low	Low	High	Low	Low	2
Feline immunodeficiency virus (FIV)	DRS1_ISH	Low	Medium	High	Medium	Low	1
	DRS1c_IDH	Low	Low	High	Medium	Low	1
	DRS1r_IDH	Low	Low	High	Medium	Low	1
	DRS2_ISH	Low	Low	High	Medium	Low	1

Abbreviations: DRS1_ISH, Disease risk scenario 1—infectious source hazard; DRS1c_IDH, Disease risk scenario 1—Infectious destination hazard in the captive enclosures; DRS1r_IDH, Disease risk scenario 1—Infectious destination hazard in the wider release areas; DRS2_ISH, Disease risk scenario 2—infectious source hazard.

For FIV as a source hazard, release risks were considered low; exposure risks for other leopards were considered medium, but low for other wildlife/domesticated species. As a destination hazard in the captive enclosures and the wider release zone, both release and exposure risks were considered low. However, in all cases, consequences were considered high. Overall, risk estimates were considered medium for all cases, resulting in FIV being considered a priority 1 hazard (low acceptable risk < medium risk estimate) in all disease risk scenario-hazard combinations.

3.2 | Generic stress assessment

Both acute and chronic stress responses were considered likely to occur in association with transportation and introduction into a new enclosure, despite leopards being born and raised in captivity. Potential consequences of acute stress responses (e.g. attempts to escape) during transportation and following arrival in the RFE included an increased likelihood of physical trauma both during transport and in the enclosures in the RFE. Potential consequences of a chronic stress response in leopards (in association with repeated or prolonged acute stressors) in the enclosures included impaired immune system function, disruption of normal reproductive behaviour and/or disruption of normal behaviour patterns, for example food consumption. Potential consequences of a chronic stress response in leopards post-release included abnormal behaviour (potentially increasing the chances of human conflict or predation), impaired immune system function, disruption of normal reproductive behaviour and impaired hunting ability. It is important to emphasize that detrimental effects of chronic stress may extend beyond the translocation event and affect animals post-release until they have fully adapted to and established themselves in their new environment (Dickens et al., 2010).

3.3 | Mitigation strategy

A chronological strategy was designed to minimize the risks identified by our assessments from pre-export of leopards, transportation to the RFE, activity in the enclosure, pre-release examination of progeny, to activities in the release zone itself. The full strategy is available in Appendix S3.

Specific medicinal products were not recommended due to the wide geographic variation in product availability. It was strongly recommended that the use of particular therapeutic and prophylactic products and diagnostic tests be reviewed (on a regular basis) in more depth, in a separate exercise.

Advised pre-export precautions for each leopard proposed for inclusion in the programme included a review of the animal's history, a review of previous health issues in Amur leopards at the collection, undisturbed observation of the leopard's behaviour, clinical examination under general anaesthesia (including checking microchip identification, recording body weight and echocardiography),

non-specific routine health diagnostic testing, multiple hazard specific diagnostic testing, sample archiving, prophylactic treatment for endo- and ectoparasites, and vaccine administration—all to be conducted 1 month before export. Following this screening process, a 30-day quarantine period paying particular attention to environmental fly control was advised. Of particular note in the protocol was breeding leopard selection based on history and behavioural observations—only selecting proven breeders and those with a temperament consistent with minimizing the risk of conspecific conflict once introduced to potential mates in the RFE breeding enclosure.

Transportation mitigation focussed on minimizing stress, minimizing any mechanical trauma and maximizing biosecurity. We therefore emphasized the importance of travel crate design and the methods for loading and unloading. We advised that training leopards to voluntarily enter transport crates could avoid the need for general anaesthesia and would familiarize individual leopards to the crate. We highlighted the importance of a calm and quiet manner during all procedures. For particularly nervous leopards, we suggested the option of using non-sedative anxiolytic drugs (e.g. buspirone), to minimize stress during transport.

Enclosure management in the RFE focussed mainly on non-invasive techniques, including leopard observation, faecal diagnostic monitoring, post-mortem examination of any vertebrate mortalities, maintaining enclosure biosecurity (excluding domestic dogs, domestic cats and medium/large carnivores), and minimizing infectious and non-infectious hazard exposure from food and water sources. Other therapeutic or diagnostic interventions were advised only where clinically indicated and on a case by case basis, (e.g. in relation to any potential direct or indirect contact with domestic species, especially cats), with the exception of vaccination. Minimizing human contact, utilizing remote camera observation in order to reduce the likelihood of post-release leopard-human conflict, and providing live prey to assess and maximize hunting competence prior to release were also recommended.

A pre-release examination of leopards for release under general anaesthesia was advised for clinical evaluation, fitting of a microchip transponder, recording body weight, non-specific routine diagnostic tests, echocardiography, hazard specific diagnostic testing, sample archiving and vaccine administration. In addition, the fitting of radio-collars to leopards to be released would enable post-release monitoring.

In the release zone, advised measures focussed on good communication with local stakeholders (specifically relating to domestic dog and cat vaccination), opportunistic and proactive surveillance for specified hazards (in leopards and other species, especially wild carnivores), development and maintenance of anti-poaching strategies and finally, although not strictly part of the DRA, monitoring of prey population densities.

To illustrate our approach, the specific mitigation options for the three selected hazards are shown in Table 4. Emphasizing the role of *C. felis* as a priority 1 source hazard, diagnostic (conjunctival PCR) and prophylactic (vaccination) interventions was the focus of the mitigation measures, in order to minimize risks of export to

Agent	'Disease risk scenario-hazard' combination	Mitigation options	Priority level
<i>Chlamydomophila felis</i>	DRS1_ISH	Pre-export PCR conjunctival swab; vaccinate	1
	DRS1c_IDH	Opportunistic screening in enclosure	3
	DRS1r_IDH	Very limited - test wild felids at captures/ <i>post-mortem</i> examinations	3
	DRS2_ISH	Pre-export PCR conjunctival swab; vaccination 1 month prior to export	1
Canine distemper virus (CDV)	DRS1_ISH	Vaccination at least 1 month prior to export	2
	DRS1c_IDH	Biosecurity of facility (domestic and wild). Vaccination	1
	DRS1r_IDH	Vaccination: leopards pre-release, domestic dogs, surveillance of wild/domestic carnivores	1
	DRS2_ISH	Pre-export screening serology, vaccination at least 1 month prior to export	2
Feline immunodeficiency virus (FIV)	DRS1_ISH	Pre-export serology ELISA and Western Blot. PCR for clade A.	1
	DRS1c_IDH	Biosecurity of facility (domestic and wild)	1
	DRS1r_IDH	Raise local awareness regarding domestic cats. Opportunistic surveillance of leopards	1
	DRS2_ISH	Pre-export serology ELISA and Western Blot. PCR for clade A only	1

Abbreviation: PCR, polymerase chain reaction.

TABLE 4 Mitigation options for three selected hazards, *Chlamydomophila felis*, CDV and FIV in association with 'disease risk scenario-hazard combinations' for Amur leopard re-introduction into the RFE

RFE. For CDV, diagnostic and prophylactic interventions were also recommended pre-export, but at a lower priority level than for *C. felis*. However, greater emphasis was placed on mitigation measures in the release enclosures and the wider release zone. Measures included biosecurity of the enclosure; local domestic dog vaccination programmes (based on an assumption that domestic dogs are an important reservoir until proven otherwise); and CDV surveillance in domestic and wild animals in the wider release zone, in order to track exposure risks over time. Encouraging results from safety and efficacy studies of modified live Onderstepoort strain CDV vaccines in *Panthera* spp suggest that vaccination of leopards could also be beneficial (Sadler, Ramsay, McAloose, Rush, & Wilkes, 2016) For FIV

as a priority 1 hazard in all assessments, several mitigation measures were recommended. Diagnostic serology using ELISA and confirmatory Western Blot (using a range of domestic and non-domestic cat FIV antigens) combined with antigen detection using PCR were advised pre-export, with the caveat that available PCR tests currently only detect viruses in clade A (clades A and B are perhaps most likely in Europe—Hosie et al., 2009). In the RFE, enclosure biosecurity measures to exclude domestic cats were considered essential combined with activity to raise awareness in local residents of the potential risk that domestic cats represent. Finally, opportunistic FIV surveillance (serology and antigen detection) in both wild and domestic cats in and around the release area was recommended.

4 | DISCUSSION

A wildlife disease risk analysis is not a purely academic exercise. Its purpose is to assess risks and to then develop measures that proactively minimize risks of disease arising in any species as a consequence of a wildlife translocation. It is therefore essential that any DRA progresses in a logical and thorough manner, to the development of a proportionate, realistic and achievable mitigation strategy.

We have described the methodology used to tackle a complex and potentially overwhelming range of possible disease outcomes arising from an Amur leopard re-introduction programme in the RFE. It was possible to handle the large amounts of data by breaking down analyses into differing categories defined by species affected and origin of hazard. Importantly, this approach ensured that risk estimates for certain hazards were not 'averaged out' where they varied in magnitude depending on the species affected and/or the origin of the hazard. We were also able to link mitigation measures to specific hazards, thereby demonstrating the purpose behind the measures to those responsible for implementing the strategy with the intention of increasing the likelihood of compliance and achievability.

Prioritizing hazards by assessing the risk estimate in the light of the acceptable risk (see Table 2) facilitated formulation of our mitigation strategy to target interventions where they were most needed and avoid potentially over-burdensome and costly measures across the whole programme.

The hazard *C. felis* was considered a higher priority hazard when originating from source than from destination (Table 2). By differentiating between source and destination in this way, much of the burden of diagnostic testing, record keeping and record review is placed on the zoological collections where the leopards originate (see Table 4). These collections are likely to have greater available funding and access to appropriate diagnostic facilities. The principle of 'front-loading' mitigation measures and putting the financial and practical burden on the zoo sector could also be applicable to other captive-to-wild translocation programmes, in particular when applied to infectious agents that are considered more of a threat in captive situations than for free-ranging wildlife.

In contrast, CDV was considered a higher priority as a destination hazard than as a source hazard. The complexity, unpredictability and variability of CDV epidemiology in multi-host ecosystems make CDV a good illustrative hazard. In one study in sub-Saharan Africa, the epidemiology of CDV in free-ranging wild carnivores and domestic dogs was found to be complex and to have changed considerably over a 30-year period (Viana et al., 2015). Initially, domestic dogs may have been the most important reservoir species, but the introduction of dog vaccinations led to a change in the epidemiological picture as a 'meta-reservoir' population became apparent, consisting of several carnivore species and leading to sporadic epizootics in both the domestic dog and wild carnivore populations (Craft, Hawthorne, Packer, & Dobson, 2008; Prager et al., 2012; Viana et al., 2015; Woodroffe et al., 2012). Multi-host pathogens like CDV are able to overcome the density-dependent fade-out that occurs

in simpler single-host pathogen systems (Gilbert et al., 2014). In addition, the role of concurrent infections either contributing to morbidity/mortality in association with CDV immunosuppression or predisposing to CDV mortality is currently unclear. Data from a catastrophic lion mortality event in Tanzania revealed high levels of *Babesia* infection, concomitant with CDV infection, suggesting the possibility of interaction between the two pathogens (Munson et al., 2008).

The classification of CDV as a priority 1 destination hazard was unsurprising, given its potentially devastating consequences on small populations. However, the real value of our DRA approach was the greater emphasis in highlighting gaps in our understanding in the context of the RFE and how this would influence research priorities and mitigation strategies. The uncertainty over the contribution of domestic dogs and/or wild carnivores to the CDV reservoir in the RFE indicates a need for a conservative response, with mitigation strategies designed to address all possible sources of infection. Epidemiological surveys to tackle these knowledge gaps may facilitate a modified mitigation strategy, tailored more precisely to actual risk.

Traditionally, practice has favoured the use of recombinant CDV vaccines (based on a canarypox vector), due to the possibility of virulence of modified live domestic dog vaccines in some non-domestic carnivores such as red pandas (Bush & Roberts, 1977; Itakura, Nakamura, Nakatsuka, & Goto, 1979). However, to date no cases of vaccine-derived distemper have been recorded in felids. Unfortunately, recombinant products are less immunogenic and require the delivery of annual booster doses, which may be impractical once leopards are released. A recent case of natural CDV infection in a snow leopard vaccinated with a recombinant product is a further note of concern (Chinnadurai, Kinsel, Adkesson, & Terio, 2017). Recent trials of a modified live vaccine based on the Onderstepoort strain of CDV have demonstrated strong antibody responses without clinical side effects in domestic cats and tigers (Ramsay et al., 2016; Sadler et al., 2016). Further trials of these products in leopards are now a priority.

Many conservation translocations will need to address multi-host pathogens like CDV, where epidemiological understanding is incomplete, and transmission occurs within a highly complex, and constantly evolving web of direct and potentially indirect interactions between several species (Haydon, Cleaveland, Taylor, & Laurenson, 2002; Roche & Guégan, 2011). In addition, small isolated populations are particularly vulnerable to stochastic events such as outbreaks of infectious disease (Gilbert et al., 2014). Developing preventive strategies is particularly challenging for such complex pathogens, with serious outcomes and epidemiology that changes over time. In other wildlife translocation scenarios, the challenges of mitigating the effects of multi-host pathogens are likely to be equally complicated by data paucity on species susceptibility, pathogen prevalence, and understanding of current epidemiological patterns. These factors are complicated further when consideration is given to the effects of co-infections, vector-borne pathogens and climate change.

In the case of FIV, we assumed high potential pathogenicity in Amur leopards and other felids regardless of species affected or origin of the hazard, and accordingly focussed mitigation on both source and destination. In the context of other translocation programmes, it is likely that there will be similar infectious agents for which there are considerable data gaps in terms of epidemiology, species susceptibility, pathogenicity and prevalence, prompting a highly cautious and mitigation intensive approach. However, targeting mitigation in the ways outlined in our programme ensures that such intense mitigation measures are not recommended for all hazards at all stages, thereby keeping the mitigation burden in terms of cost and practicality as low as is possible, and increasing the likelihood of compliance with the measures.

In this DRA, we elected to analyse stress and its effects and potential mitigation as a stand-alone exercise, enabling us to focus on minimizing any associated negative effects at targeted stages of the programme. Quantifying the degree of dysregulation of the stress response is challenging, despite a common assumption that elevated glucocorticoid levels are correlated with chronic stress. A recent review of the subject concluded that this was too simplistic and that there was no predictable endocrine measure of chronic stress. Rather, it is the disruption of the response that is central to chronic stress, switching it from a healthy optimal adaptive response to a maladaptive, often harmful response (Dickens & Romero, 2013). Hence, rather than attempting to measure or quantify stress in individual leopards, we considered both acute and chronic stress as inevitable consequences—and thus, our mitigation strategy focussed on minimizing all stressors (number, magnitude and duration) at all stages of the translocation programme. In our mitigation strategy, we focussed on personnel training, equipment design, construction and maintenance, and detailed planning of all stages. Such an approach is unlikely to be prohibitively expensive or burdensome, and the purpose of the mitigation to minimize stress can be conveyed to those involved in the programme, thereby increasing the likelihood of compliance. We therefore consider it advisable that all translocation programmes focus separately in their mitigation strategies on reducing the number, magnitude, duration and severity of all stressors where possible.

In conclusion, a DRA may start out as an in-depth academic desk-based exercise, but for it to achieve its primary purpose, it has to take into account the likelihood, practicality and cost-effectiveness of its recommended mitigation measures. By breaking down a DRA into distinct assessments based on species of concern and location of the hazard, plus analysing translocation stress in isolation, we show how to maximize the likelihood of any mitigation strategy being proportionate, achievable and realistic. In addition, such an approach also directs the targeting of future resources by highlighting significant data gaps.

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CONFLICT OF INTEREST

All authors confirm that there are no conflicts of interest to declare.

ETHICAL APPROVAL

The authors confirm that the ethical policies of the journal, as noted on the journal's author guidelines page, have been adhered to. Sampling of animals within the UK conformed with the Animals (Scientific Procedures) Act 1986 Amendment Regulations (SI 2012/3039). Sampling of animals within other EU member countries conformed with EU Directive 2010/63/EU. Sampling of animals within the Russian Federation complied with the ethical requirements of Wildlife Vets International and those of the Primorskaya State Agricultural Academy, Ussurisk, Russia.

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ENDNOTE

¹ (EEP's are coordinated breeding programmes within responsible zoological collections which are members of the European Association of Zoos and Aquaria).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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