A Cautionary Tale: Diclofenac and Its Profound Impact on Vultures

Ngaio L Richards, Working Dogs for Conservation, Bozeman, MT, United States; University of Florida, Gainesville, FL, United States
Martin Gilbert, Cornell University, Ithaca, NY, United States
Mark Taggart, University of the Highlands and Islands, Inverness, United Kingdom
Vinny Naidoo, University of Pretoria, Pretoria, South Africa

Glossary

Acidotic Too highly acidic, containing too much acid.
Analgesic Having the property of reducing pain.
Anthropogenic Arising from human activities.
Antipyretic Having the property of reducing fever.
Autolysis Self-degradation of the tissues or cells by the body's own enzymes.
Enteritis Inflammation of the intestines—particularly the small intestine.
Excipient An inert substance (in principle), formulated with a medication's active ingredient, provides complimentary functionality such as stability, absorption, or increased solubility, among others.
Gliosis Eruptive growth of cells in response to a trauma to the brain.
Histopathology Examination of tissue, under a microscope, often by a pathologist, to assess the possible role of disease.
HPLC-MS High-performance liquid chromatography and mass spectroscopy, a powerful and highly sensitive analytical method often used to analyze samples for contaminant residues.
Lesion A damaged area of an organ or tissue (e.g., an ulcer) resulting from a disease or injury.
Moribund Approaching the point of death, depressed demeanor.
Necropsy Dissection and analysis of a deceased animal, usually to determine cause of death.
Nephrotoxic/ant Harmful to the kidneys.
Osmolarity The concentration of a solution.
Pathogen Disease-causing entity, usually a virus, bacteria or microorganism.
Pharmacokinetic Relating to the fate of pharmaceuticals in and through the body.
Renal cortex The outer part of the kidneys, where ultrafiltration takes place.
Starlicide© DRC-1339 (3-chloro-4-methylbenzenamine), a chemical avicide developed in the United States, known to impair renal function.
Surrogate A species whose sensitivity and susceptibility to a toxicant of concern mirrors that of one (generally) accorded a higher protective status, present in lesser abundance or less able to withstand conditions of captivity.
Uric acid A compound composed of nitrogen, carbon, hydrogen, and oxygen—generated by the breakdown of purines found in food.
Visceral gout Precipitation of uric acid crystals onto the surface of the visceral organs.

Foreword

In the late 1990s, something unthinkable happened: birds present in such abundance that they were taken completely for granted—and largely regarded as a nuisance—began hurtling toward extinction. Once considered the most populous large raptors on earth, three species of vultures on the Asian subcontinent came within a breath of vanishing in less than a decade. As an increasing number were found dead or dying and the skies emptied, several lines of inquiry were employed to identify the cause of the mortality. Soon after, healthy birds were captured from the wild, to initiate a captive breeding program, until the environment

was deemed sufficiently safe for their release. Today, some of these populations are very slowly recovering, though they remain precariously low, whereas in certain parts of the subcontinent, they have been lost entirely. Other species since found to be vulnerable remain at risk. Here, we describe the research that identified the driving force behind this precipitous decline, ponder lessons learned throughout the process of discovery, and conclude briefly—but significantly—with a plea for reason where the very strong warnings issued following this unparalleled loss are still not being heard.

Introduction: The First Alarm

Vultures on the Indian subcontinent once benefited immensely from the Hindu culture and traditions which hold the cow as sacred and highly prized as a living, working animal. Religious prohibitions dictate that upon death, the bodies of cows (and other livestock) cannot be consumed. Both factors ultimately led to a wide availability of carcasses which drew in scavenging birds and allowed their populations to soar. By the 1980s, vulture presence was prodigious—with some species estimated to number the tens of millions (Fig. 1).

Somewhat ironically, a frequent fate of common species is that their ubiquity causes them to be neglected and overlooked—not solely by the public, but also by scientists. Hence, a sudden drop in vulture numbers could well have gone unnoticed for quite some time. However, the birds had several human admirers and allies. One of these was Dr. Vibhu Prakash—an Indian biologist with the Bombay Natural History Society—who had been undertaking long-term monitoring of vultures in the Keoladeo National Park in Rajasthan, India. During the 1987/88 breeding season, he counted 353 nesting pairs of Oriental white-backed vultures (OWBVs; *Gyps bengalensis*). When this nest survey was repeated in 1999/2000, only 20 nesting pairs were found, and no active nests were observed in this or the subsequent breeding season. In parallel, numbers of long-billed vultures (LBVs; *Gyps indicus*) visiting the National Park had plunged—from 816 in 1985/86—to 25 in 1998/99, and only a single LBV was seen during the 1999/2000 nesting season. Dr. Prakash was finding dead OWBVs in all age classes—including adult birds—which indicated that mortality, rather than poor hatchability, recruitment, or geographic shift of nesting sites, was responsible for the decline. Clearly something was awry.

Fig. 1 A wide availability of livestock carcasses on the Asian subcontinent drew in scavenging birds and allowed their populations to soar. By the 1980s, some species of vultures numbered the tens of millions. Photo credit: Rishad Naoroji.

Further Investigation Reveals Visceral Gout as the Predominant Postmortem Finding

Early on, investigations into the mortality observed in India established a characteristic postmortem finding among dead vultures: *visceral gout*, a condition caused by the impairment of kidney function. All being well, the avian kidney removes *uric acid* from the blood. However, if kidney function is impeded, uric acid can build up to lethal levels and precipitate out, producing instead a chalky deposit on the surface of organs. The cause of this observed renal failure was not immediately apparent.

Further research was conducted in India to gather more data, with emphasis on fresh carcasses to avoid the myriad problems associated with *autolysis*. A diagnostic investigation was performed on 28 vultures—comprising both OWBVs and LBVs. Both *enteritis* and *gliosis*—inflammation of the intestine and brain, respectively, were noted, though both were reported to be mild. The authors of this line of inquiry (Cunningham et al., 2003) proposed that an infectious disease was likely the cause of vulture mortality, with visceral gout being secondary to dehydration. However, fewer than 30% of the vultures assessed presented visceral gout, which had characterized the overall mortality in OWBVs and LBVs. And, of those that did have visceral gout, none showed signs of either enteritis or gliosis. Therefore, further work was necessary to unearth the cause of the kidney failure and visceral gout.

Investigations in Pakistan

During late 2000, in Pakistan, a team led by US-based nongovernmental organization The Peregrine Fund (TPF), and their partners the Ornithological Society of Pakistan, joined efforts to identify the cause of the vulture declines. The task of leading the investigation was entrusted to Dr. Lindsay Oaks, a veterinarian and avid falconer who was working at the Department of Veterinary Microbiology at Washington State University. Oaks and fellow veterinarian Martin Gilbert (also with TPF) trained a team of local zoology students to begin monitoring vulture colonies, collect epidemiological data, and necropsy dead vultures. Together, this collaborative team monitored 17 OWBV breeding colonies across Pakistan's Punjab Province, with students stationed at the three largest colonies throughout the year. Over time, this intensive approach yielded a large number of vulture carcasses found shortly after death, thereby enabling the collection of high quality tissue samples for analysis.

The levels of mortality the team observed were staggering. Breeding populations declined from 2292 breeding pairs in 2000/01, to 308 by 2003/04, with 8 of the 17 colonies declining to extinction within 3 years. As in India, visceral gout was the predominant finding during *necropsy*, with a thick coating of chalky white urate precipitates covering the organs of 85% of 259 vulture carcasses examined. Samples were analyzed for infectious and noninfectious agents capable of causing the associated degree of kidney failure. Tests for avian influenza and infectious bronchitis, both recognized as renal *pathogens* in poultry, were negative, and no other potential infectious agents were seen under ultra-high magnification (i.e., electron microscopy). Further analyses for heavy metals—also known to be *nephrotoxic*—were negative in gout cases, as were tests for various pesticide classes (organophosphate, OP; carbamate, CM; and organochlorine, OC) known to kill birds of prey.

Pathological Investigations

In 2001, a team of pathologists led by Dr. Carol Meteyer of the USGS National Wildlife Health Center examined tissues from 55 OWBVs with visceral gout. These samples were procured by the Pakistani team. In all cases, the *lesions* in the kidneys were severe, with vast fields of cells in one area of the *renal cortex* having died suddenly, and without inflammation, ruling out chronic renal gout that would be expected in dehydration. Visceral gout is an "end stage" manifestation of renal failure, whether the result of dehydration caused by an infectious disease (previously discussed), or of primary renal failure (as seen here). As such, this shift may seem subtle, but in fact these two hypotheses contrast sharply, each showing different pathologies (chronic versus acute gout, respectively) and lead down very different paths of investigation.

Noteworthy in itself—there has been confusion in the literature regarding dehydration as a primary cause of visceral gout, stemming from an often (but erroneously) cited study (Siller, 1981). This may have contributed to the initial conclusion that enteritis and secondary dehydration were the cause of death in the vultures. In fact Siller himself actually remarked that at the time of his article there was poor documentation of *histopathology*, or changes to tissue, in cases of birds that have died from water deprivation and dehydration. A subsequent report by Julian (1982) described the pathology associated with dehydration as chronic "ascending" renal pathology (i.e., from the bottom to the top). Indeed, severe chronic dehydration causes the formation of dry urates that block the main drainage conduits of the kidney. This obstruction and "back up" of urate material begins in the ureters where the renal damage is first seen, and damage and pathology progresses in an "ascending" pattern to the other areas of kidney, eventually progressing to the cortex of the kidneys. Meteyer and her group noted that the renal pathology in OWBVs *began* in the cortex, with severe acute necrosis and death of a specific group of kidney cells lining the proximal convoluted tubules, and known for their role in eliminating certain classes of toxins from the body. Above all, the pathology observed, and associated with the mass mortality, was deemed not consistent with dehydration, but rather with a powerful primary *nephrotoxicant*—the identity of which was not immediately clear.

Interpreting the Findings

In late 2001, Oaks and Meteyer sat huddled at a coffee table under a stairwell at the back of a conference poster session and brainstormed useful leads. Similar kidney failure had been seen in snakes given the antibiotic gentamicin during the early days of veterinary care of reptiles, and in birds that had ingested an avicide developed in the United States to kill invasive starlings. But Starlicide©, the avicide in question, was not, according to Oaks, used in Pakistan. Another reported cause of clinical renal failure in birds and mammals was due to the administration of nonsteroidal antiinflammatory drugs (NSAIDs). Meteyer proposed that the renal pathology in vultures pointed to an *anthropogenic* toxicant that targeted the kidneys, and perhaps a pharmaceutical drug. She then apologized for not having any other ideas that would be more pertinent to vulture declines. But Oaks took their discussion a step further, applying solid epidemiological principles based on the ecology of OWBVs in Pakistan to further shed light on what had so far proven to be tantalizingly out of reach.

Epidemiology: The Bigger Picture

Back in Pakistan, a picture was emerging of the mortality taking place within the vulture colonies. The OWBVs nested communally in large colonies located in forest plantations which often extended for miles along irrigation canals where trees were planted to stabilize their banks. Vultures were dying at all sites, with mortality occurring throughout the year, and with visceral gout found in all age classes of dead birds examined. Every day—from the winter chill through the blistering heat of the Punjabi summers—the field team conducted surveys, traversing colonies each morning and recording the location of all dead birds recovered. It became clear that mortality was occurring in clusters, with many dead vultures often found in a particular area in a short space of time. The researchers also noted that birds in adjacent nests frequently left roosts together, following their neighbors to scour the landscape for their primary food source: dead livestock. This observation chimed with the musings of Oaks and Meteyer back in the United States. Were the birds being exposed to a nephrotoxicant while feeding in groups at domestic livestock carcasses which then claimed them quickly, after they returned to the colony to roost in the evening? (Fig. 2).

To identify candidate toxicant products, Lindsay Oaks developed a simple questionnaire through which the student team could collect information on the chemicals and pharmaceuticals that were being used on local livestock. He hypothesized that the

Fig. 2 Hundreds of dead vultures were observed, but many, like these, were found in various forms of decomposition, which made it difficult to ascertain the cause of mortality via necropsy. Photo credit: Aditya Roy.

mystery chemical would be (1) widely available; (2) likely a recent introduction to the market; (3) able to pass through the stomach acid unchanged; and, most critically, (4) a known nephrotoxicant.

The students visited many veterinarians and pharmacies across the Punjab gathering information on a large number of products sold for use in livestock. Oaks then collated the results and used his hypotheses to filter the list of possible products. Only one product met all four criteria: DICLOFENAC, an NSAID commonly used in human and animal medicine. The drug had been introduced to the veterinary market during the 1990s, and, by 2003, was being used daily by most veterinarians within the country.

Pursuing the Hypothesis via Analytical Chemistry

Armed with this information, Oaks performed new analyses on the archived tissues from the surveillance study at the nesting colonies. Kidney samples from 23 vultures with renal failure/gout and 13 vultures without (control birds known to have died of other causes like trauma and lead poisoning, and which presented no renal pathology) were tested for diclofenac analytically, via high-performance liquid chromatography (HPLC-MS). All of the renal failure cases were positive for diclofenac, while none of the nonrenal failure cases had diclofenac residues—a 100% correlation.

Coincidentally, back in Pakistan, the Oaks-led field team recalled the small group of captive OWBVs they had previously worked with that had died suddenly the year before. Animal custodians had provided consistent care and there had been no evidence of disease, with the exception of visceral gout at death and lesions in the kidney that resembled those seen in the wild OWBVs that had now been shown to contain diclofenac. At the time the cause of death had been a mystery, but the diclofenac results offered a new avenue. The field team recovered buffalo meat from their freezers which had been bought at a local market. This meat had been the final meal for the captive birds. Samples of this meat and of tissues archived from the affected captive vultures were exported for further analysis. All tested positive for diclofenac.

Confirmation and Dissemination

Oaks and Gilbert subsequently documented the exquisite sensitivity of OWBVs to diclofenac using trials on four captive non-releasable individuals. To verify the toxicity of diclofenac to OWBVs, two juvenile vultures were orally administered 2.5 mg/kg of veterinary diclofenac—the standard veterinary dose recommended for mammals—and two were administered 0.25 mg/kg. Within 58 h of administration, both the high dose vultures and one of the low dose birds died with visceral gout and the same histologic lesions as the field cases with diclofenac residues in their tissues. These experiments strongly implicated diclofenac as the ultimate driver behind the rapid population decline in OWBVs seen in Pakistan.

The speed and magnitude at which ongoing declines were occurring made it imperative to publish these findings in order to encourage regional governments to act. However, an initial submission to a high profile journal was promptly rejected, with reviewers dismissing the possibility that a pharmaceutical drug could be responsible for reducing OWBV numbers by $>90\%$ in such a short timeframe. Despite the provision of very solid scientific data, the paradigm shift in thinking appeared to be too great. Publication, and hence dissemination of critical new findings, was thus delayed by several months. Fortunately, the paper was ultimately accepted for publication in the journal *Nature* in early 2004. The report swiftly prompted similar analyses, and findings, for vultures that had died with visceral gout in India, which confirmed that the drug's impact extended well beyond Pakistan, and had also affected the now Critically Endangered LBVs and slender-billed vultures (*Gyps tenuirostris*).

Repercussions to Species, Ecosystems, Human Livelihoods, and Safety

Alongside *Gyps* vultures, several other vulture species feed on dead livestock on the Indian subcontinent (Fig. 3). Worrying parallel declines were soon observed in two other South Asian species: the red-headed vulture (*Sarcogyps calvus*) and the Egyptian vulture (*Neophron percnopterus*). These species have since been upgraded to Critically Endangered and Endangered, respectively, on the IUCN Red List. As vultures declined, a corresponding influx in other scavengers, especially feral dogs, has occurred. Far less efficient than vultures in disposing of livestock carcasses, and often aggressive toward people, the number of bite incidents and rabies transmission has risen accordingly (Fig. 4). What was once an efficient, symbiotic system—in which tanners stripped the hides from dead livestock, vultures picked clean the rest of the carcass then bone collectors retrieved the skeletal matter—has broken down. For people whose livelihoods revolve around livestock carcasses, the vultures have left in their wake an unsafe and even less pleasant working environment. With the culprit (diclofenac) identified—what could or should be used in its stead by veterinarians, farmers, and livestock owners? What action(s) would now be needed to bring a halt to vulture declines and give remaining populations a chance?

Diclofenac: Its Mode of Action and Safety Testing

The chemical name for diclofenac is 2-[2-(2,6-dichloroanilino)phenyl]acetic acid. Commercially, in formulation, it is sometimes referred to (and better known) as Voltaren or Voltarol. NSAIDs are a chemically diverse group of pharmaceuticals that share, to varying degrees, the common pharmacological effects of being *analgesic*, antiinflammatory, and *antipyretic*. While aspirin and ibuprofen remain the two best known NSAIDs in human medicine, many in fact exist. Indeed, the identification of diclofenac as the cause of widespread vulture mortality in Asia raised obvious concerns about other veterinary NSAIDs. A survey of veterinarians and zoos that encompassed nearly 900 captive scavenging birds from 79 species (Cuthbert et al., 2006b) documented mortality of birds of prey, owls, cranes, and storks following treatment with carprofen, flunixin, ibuprofen, or phenylbutazone, with ketoprofen being flagged as a potential concern. Also noteworthy was the fact that 700 of the birds (from 60 species) were administered meloxicam without adverse effect.

In order to specifically investigate NSAID toxicity toward *Gyps* vultures, it was initially hoped that a common *surrogate* species could be identified and used, given the conservation status of affected vulture species. *Pharmacokinetic* testing was undertaken in chickens and crows, among others, but all to no avail, as none of these species are anything like as sensitive to diclofenac as Old World *Gyps* vultures. Safety testing therefore looked toward the African white-backed vulture (*Gyps africanus*), and later, the Cape griffon vulture (*Gyps coprotheres*) (**Fig. 3**). These species were validated as being of similar sensitivity to diclofenac toxicity before resorting to exposing precious captive vultures in Asia to any other NSAIDs. Validation studies revealed that these two species showed the exact same clinical progression of disease as seen in their Asian counterparts. Further to this, a multiphased testing protocol was established, since these surrogate vulture species are themselves listed as Critically Endangered and Endangered, respectively.

1. The plasma and tissue pharmacokinetics of the NSAID in question is determined in cattle to calculate the dose vultures would be exposed to in the wild.
2. A small number of vultures are exposed to this dose, in pure drug form, to evaluate toxicity—in combination with pharmacokinetic monitoring and clinical chemistry evaluations over time.
3. The safety of the tissue-bound drug is established by treating cattle at double the recommended dose, thought to be a common veterinary practice on the Asian subcontinent, harvesting animal tissue at the point where maximum concentration occurs, and feeding this to a limited number of vultures. If the drug still appears safe, then;
4. a larger number of vultures are exposed; and, finally
5. the safety of the drug is evaluated in an Asian *Gyps* vulture species.

To date, despite a huge amount of research effort, meloxicam remains the only NSAID to have been cleared at all levels of testing. It has therefore been confirmed as a "vulture-safe" alternative, insofar as the concentrations that vultures are likely to encounter in the wild have not been deemed harmful. By contrast, a range of other NSAIDs including ketoprofen, carprofen, flunixin, and phenylbutazone have all raised concerns at the second testing step, either due to signs indicative of depression, worrisome changes in clinical pathology, unacceptably lengthy half-lives, or, unfortunately, death in the dosed birds. Aceclofenac was flagged as a

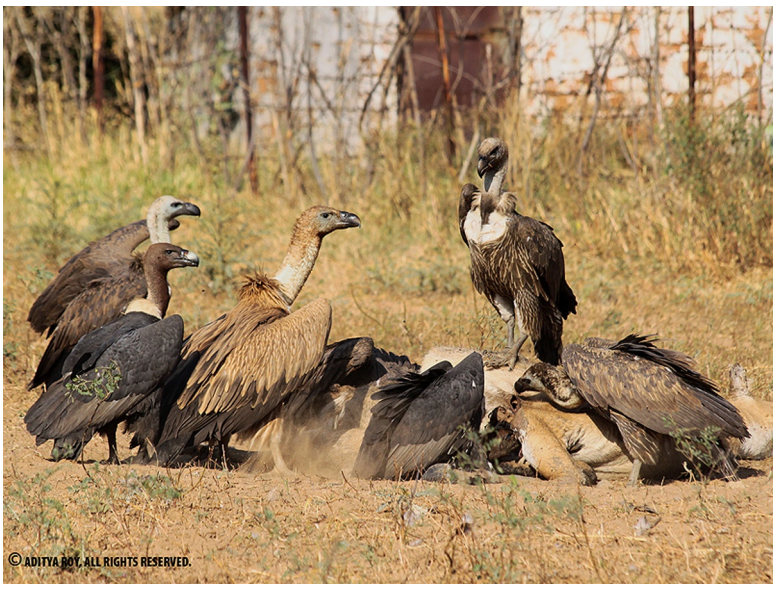

Fig. 3 In a healthy, uncompromised ecosystem, a suite of different vulture species, with different dietary predilections, will assemble to rapidly and efficiently consume a carcass in its entirety. Here white-backed, griffon, and long-billed vultures have gathered to feed on the carcass of a nilgai (an Asian antelope). Photo credit: Aditya Roy.

potential threat in 2012 because it metabolizes into diclofenac in mammals. In 2016, this was conclusively confirmed via experiments in cattle, and it was added to the list of NSAIDs that are unsafe for *Gyps* vultures.

With or without a vulture toxicity component, testing these NSAIDs is very costly in labor and analytical expenses. The price of moving one drug through all five aforementioned steps (though few have made it to Step 5) has been estimated at near US$ 100,000. The process also typically takes 1 to 2 years, allowing for time to obtain ethics and regulatory approvals and for considered analysis and interpretation of all the data obtained. In the case of meloxicam, a team of over 20 people worked diligently across 4 countries (South Africa, Namibia, India, and the United Kingdom) to determine its safety.

Bans and Governmental Action

In 2004, following the discovery that diclofenac was driving declines of OWBVs, researchers met with government officials at a vulture summit in Nepal's capital and signed the Kathmandu Declaration. With this declaration, the governments of India, Nepal, and Pakistan committed to banning the use of diclofenac in veterinary medicine and to implementing vulture breeding centers to help safeguard their long-term survival. In 2006, these same governments withdrew the manufacturing licenses for veterinary diclofenac within their respective countries. In 2008, it was made an offense (punishable by imprisonment) to manufacture, retail, or use diclofenac for veterinary purposes in India. In a further step, the government of India banned the use of multidose vials of diclofenac that had remained on the market but were supposed to be for human use only. At a volume of 50 mL, these vials were an ideal size for continued illegal veterinary use.

However, numerous obstacles remain, chief of which is the drug's low cost relative to other NSAIDs. Its continued availability, and human habit, paired with a perception of the drug's almost miraculous efficacy in the management of pain in cattle, ensures continued stubborn opposition to its withdrawal from veterinary use (as of 2016, 10 years postban). Similarly, concerns have been raised with meloxicam formulations in the region—at least some, appeared to cause minor pain, inflammation, and irritancy on use—which meant, in part, that veterinarians were not switching over to it. Research undertaken to address this showed that the pH and *osmolarity*, or concentration, of the simple off-patent formulations being made in countries such as India were the likely problem (i.e., both were often simply too high). Elevated pH was being used to facilitate dissolution of the meloxicam, in order to obtain the high formulation concentrations required. To help address this, Boehringer Ingelheim, the patent holder for the European meloxicam formulation, made their formulation procedure freely available. This formulation utilized an *excipient* ingredient that permitted formulation at a lower pH/osmolarity, meaning that it did not then cause pain, irritation, etc., upon administration.

Several months past the 2006 ban, residues of diclofenac were detected in roughly 1 in 10 livestock carcasses available to *Gyps* vultures across seven Indian States. Residues of ketoprofen and ibuprofen were detected to a lesser degree but, so, encouragingly, was meloxicam, at the second highest rate of encounter. To date, few regulatory policies have been enacted to restrict the availability or use of other NSAIDs identified as harmful to *Gyps* vultures. An overall decrease in the prevalence of diclofenac residues paired with a corresponding increase in those of meloxicam residues is cause for hope, in conjunction with what appears to be a very gradual reversal in the decline of some *Gyps* species. But complacency, particularly regarding other potentially harmful NSAIDs (and veterinary agents), will never be a viable option. The 2015 ban of ketoprofen in several districts by the south Indian State of Tamil Nadu government, and similar measures by the government of Bangladesh in early 2017, certainly represent hopeful steps in the right direction.

Conclusions

Fundamentally, the virtual extirpation of three species of *Gyps* vultures on the Asian subcontinent in less than a decade highlights with brutal clarity that abundance cannot ultimately shield any species from extinction. An immense amount of research catapulted diclofenac to notoriety as the first pharmaceutical to drive a species onto the Critically Endangered list. Yet, several issues persist in tandem, including: a disregard of the now irrefutable toxicity of diclofenac, and a fixation on the drug, to the exclusion of potential threats from other NSAIDs, related veterinary agents, and poisonings from other causes.

In 2013, the registration of veterinary diclofenac was permitted in Spain, known to be a stronghold for European vultures—including *Gyps fulvus*—the griffon vulture. Part of the rationale for this decision was the unique extent and magnitude of livestock carcass availability on the Asian subcontinent compared to Spain. Notwithstanding the reduced scale, and although there remains resistance to the notion from managers, policy-makers, and regulators alike, the reality is that medicated carcasses are also available to a broad range of scavenging species in Spain, and likely beyond. Since this registration, diclofenac has been found toxic to the emblematic Steppes eagle (*Aquila nipalensis*), in the first incidence of mortality to a species other than *Gyps*. At necropsy, a griffon vulture found near a managed carcass dump in Spain presented (fatal) visceral gout. The absence of diclofenac residues temporarily halted investigations, until an outside member of the research group suggested re-analysis for other NSAIDs—at which point elevated residues of flunixin, with incidental levels of ketoprofen, were detected. This marked the first documented case of wild vulture mortality from exposure to an NSAID other than diclofenac (**Figs. 4** and **5**).

Across Spain, and Europe, significant human effort and financial resources have long been poured into conserving faltering vulture and scavenger populations. Those that have begun to stabilize should be provided every chance to thrive. Allowing for the

Fig. 4 As vulture numbers plummeted, feral dogs were increasingly emboldened and were increasingly observed at carcasses. Photo credit: Aditya Roy.

use of a proven toxicant when so little is still known about the availability of other NSAIDs in carcasses, particularly given the existence of other harmful factors (e.g., intentional poisoning) is astonishingly reckless at best.

Before diclofenac was released onto the market, the safety testing process failed to flag the hazard that it posed to *Gyps* vultures. Perhaps, to be fair, nobody could have predicted this perfect storm of factors, or foreseen that it would lead to an outcome of such magnitude. But now, though we may not yet know the degree to which other NSAIDs are toxic to various scavengers, or have compiled an exhaustive list of all the vulnerable species, we have seen what can happen if we do not continue to probe at and seek to mitigate every possible source of risk. A veritable army of people continues to advocate tirelessly, and through various means, for vulnerable scavengers. We may yet redeem ourselves as being worthy keepers of a world graced by these splendid birds.

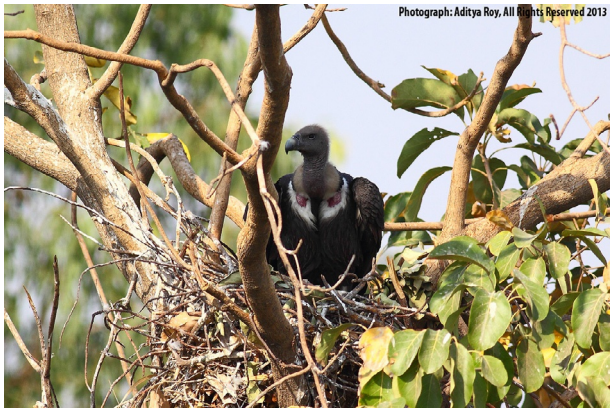

Fig. 5 All those who have fought diligently to restore the safety of the environment hope that one day, in the not too distant future, trees and skies across the Asian subcontinent will once more be replete with vultures. Photo credit: Aditya Roy.

Acknowledgments

This article is dedicated to Dr. Lindsay Oaks. Those fortunate enough to have known and worked alongside this remarkable yet unassuming man will never forget his kindness and professionalism as he strove for collaboration and followed the data along a logical course to reach its conclusion in what proved to be a landmark contribution to the conservation of *Gyps* species and, more widely, to avian ecotoxicology. We also honor the captive, nonreleasable vultures whose sacrifice conclusively established diclofenac as the cause of the renal failure and visceral gout, or, have shown that additional NSAIDs are unsafe for their wild counterparts. We thank Dr. Carol Meteyer profusely for her significant contributions to this article.

Further Reading

Cunningham AA, Prakash V, Pain D, Ghalsasi GR, Wells GAH, Kolte GN, Nighot P, Goudar MS, Kshirsagar S, and Rahmani A (2003) Indian vultures: Victims of an infectious disease epidemic? *Animal Conservation* 6: 189–197.

Cuthbert R, Green RE, Ranade S, Saravanan S, Pain DJ, Prakash V, and Cunningham AA (2006a) Rapid population declines of Egyptian vulture (*Neophron percnopterus*) and red-headed vulture (*Sarcogyps calvus*) in India. *Animal Conservation* 9: 349–354.

Cuthbert R, Parry-Jones J, Green RE, and Pain DJ (2006b) NSAIDs and scavenging birds: potential impacts beyond Asia's critically endangered vultures. *Biological Letters* 3: 90–93.

Cuthbert RJ, Taggart MA, Prakash V, Chakraborty SS, Deori P, Galligan T, Kulkami M, Ranade S, Saini M, Sharma AK, Shringarpure R, and Green RE (2014) Avian scavengers and the threat from veterinary pharmaceuticals. *Philosophical Transactions of the Royal Society B* 369: 20130574.

Galligan TH, Taggart MA, Cuthbert RJ, Svobodova D, Chipangura J, Alderson D, Prakash VM, and Naidoo V (2016) Metabolism of aceclofenac in cattle to vulture-killing diclofenac. *Conservation Biology* 5: 1122–1127.

Gilbert M, Oaks JL, Virani MZ, Watson RT, Ahmed S, Chaudhry MJI, Arshad M, Mahmood S, Ali A, and Khan AA (2004) The status and decline of vultures in the Provinces of Punjab and Sind, Pakistan; a 2003 update. In: Chancellor RD and Meyburg BU (eds.) *Raptors worldwide*. Proceedings of the VI World Conference on Birds of Prey and Owls, Budapest, Hungary, May 18–23, 2003, Budapest: Penti Kft.

Julian R (1982) Water deprivation as a cause of renal disease in chickens. *Avian Pathology* 11: 615–617.

Meteyer CU, Rideout BR, Gilbert M, Shivaprasad HL, and Oaks JL (2005) Pathology and proposed pathophysiology of diclofenac poisoning in free-living and experimentally exposed oriental whitebacked vultures (*Gyps bengalensis*). *Journal of Wildlife Diseases* 41: 707–716.

Naidoo V, Wolter K, Cromarty D, Diekmann M, Duncan N, Meharg AA, Taggart MA, Venter L, and Cuthbert R (2010) Toxicity of non-steroidal anti-inflammatory drugs to *Gyps* vultures: a new threat from ketoprofen. *Biology Letters* 6: 339–341.

Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJ, Arshad M, Mahmood S, Ali A, and Khan AA (2004) Diclofenac residues as a cause of population decline of white-backed vultures in Pakistan. *Nature* 427: 630–633.

Pain DJ, Bowden CGR, Cunningham AA, Cuthbert R, Das D, Gilbert M, Jakati RD, Jhala Y, Khan AA, Naidoo V, Oaks JL, Parry-Jones J, Prakash V, Rahmani A, Ranade SP, Baral HS, Senacha KR, Saravanan S, Shah N, Swan G, Swarup D, Taggart MA, Watson RT, Virani MZ, Wolter K, and Green RE (2008) The race to prevent the extinction of South Asian vultures. *Bird Conservation International* 18: S30–S48.

Prakash V (1999) Status of vultures in Keoladeo National Park, Bharatpur, Rajasthan with special reference to population crash in *Gyps* species. *Journal of the Bombay Natural History Society* 96: 365–378.

Rattner BA, Whitehead MA, Gasper G, Meteyer CU, Link WA, Taggart MA, Meharg AA, Pattee OH, and Pain DJ (2008) Apparent tolerance of turkey vultures (*Cathartes aura*) to the non-steroidal anti-inflammatory drug diclofenac. *Environmental Toxicology and Chemistry* 27: 2341–2345.

Sharma P (2012) Aceclofenac as a potential threat to critically endangered vultures in India: A review. *Journal of Raptor Research* 46: 314–318.

Sharma AK, Saini M, Singh SD, Prakash V, Das A, Dasan RB, Pandey S, Bohara D, Galligan TH, Green RE, Knopp D, and Cuthbert RJ (2014) Diclofenac is toxic to the Steppe Eagle *Aquila nipalensis*: Widening the diversity of raptors threatened by NSAID misuse in South Asia. *Bird Conservation International* 24: 282–286.

Shultz S, Baral HS, Charman S, Cunningham AA, Das D, Ghalsasi DR, Goudar MS, Green RE, Jones A, Nighot P, Pain DJ, and Prakash V (2004) Diclofenac poisoning is widespread in declining vulture populations across the Indian subcontinent. *Proceedings of the Royal Society of London B* 271(Suppl. 6): S458–S460. http://dx.doi.org/10.1098/rsbl.2004.0223.

Siller WG (1981) Renal pathology of the fowl—A review. *Avian Pathology* 10: 187–262.

Swan G, Naidoo V, Cuthbert R, Green RE, Pain DJ, Swarup D, Prakash V, Taggart M, Bekker L, Das D, Diekmann J, Diekmann M, Killioan E, Meharg A, Patra RC, Saini M, and Wolter K (2006) Removing the threat of diclofenac to critically endangered Asian vultures. *PLoS Biology* 4(3)e66.

Taggart MA, Senacha KR, Green RE, Cuthbert R, Jhala YV, Meharg AA, Mateo R, and Pain DJ (2009) Analysis of nine NSAIDs in ungulate tissues available to critically endangered vultures in India. *Environmental Science & Technology* 43: 4561–4566.
Zorrilla I, Taggart M, Martinez R, and Richards NL (2015) Suspected flunixin poisoning of a wild Eurasian Griffon Vulture from Spain. *Conservation Biology* 29: 587–592.

Relevant Websites

http://www.save-vultures.org/—Saving Asia's Vultures from Extinction—SAVE.
https://www.4vultures.org/—Vulture Conservation Foundation.
https://www.boehringer-ingelheim.com/animal-health-products/corporate-responsibility/saving-vultures-extinction—Boehringer-Ingelheim—Saving Vultures from Extinction.