

Asian Vulture Crisis

Loss of an Ecosystem Service

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Goals

By exploring this classic “whodunnit” story from the history of conservation and One Health, students will learn about the close linkages between human, animal, and environmental systems via the ecosystems they all share, and the consequences felt when a keystone species is decimated.

Through in class discussion, students will explore and apply the precautionary principle, the power of the scientific method, and basic epidemiological principles such as Koch’s Postulates; as well as gain an understanding of the collaborative work necessary to successfully achieve scientific breakthroughs.

Students will gain an appreciation for the complexities applying scientific findings through political and policy collaboration and leadership, and of the scope of change that is possible when successful.

Learning Objectives

Students will:

- Demonstrate understanding of the concepts of keystone species and ecosystem services, and their interaction with systems thinking
- Explain how an environmentally persistent chemical that is “harmless” to humans could still affect human health & wellbeing, directly & indirectly, using diclofenac as one example One Health principles (psychosocial, medical, cultural, ecological, etc.)
- Discuss how the process of the scientific method can produce accurate and inaccurate assessments under the pressures of reality
- Define and describe the importance of Koch’s Postulates to the Asian vulture crisis
- Assess the impact of the disappearance of vultures on humans, animals and the environment and the unintended consequences that result from it.
- Relate the importance of habit and behavior to the success or failure of a diclofenac ban
- Describe how vultures in Asia are affected by NSAIDs, and the implications of this discovery for worldwide veterinary and human use of these medications
- Explain the precautionary principle and the challenges of applying it
- Discuss successes and failures of attempted solutions, brainstorm next steps

Introduction

Vignette

It was the late 1990s, and the international raptor and bird conservation communities were nervous. Rumors of large numbers of vultures dying in India were becoming persistent. India was home to over 40 million vultures, and was one of the only places in the world where vulture species were well respected by the general public. Vibhu Prakash and a team from the Bombay Natural History Society (BNHS) set out to investigate these rumors through repetitive transect surveys in and around national parks. By 1999 it was clear that something dramatic was happening. Prakash's team documented, a 95+% decrease in the populations of all the three griffon vulture species, or species of the *Gyps* family, found in India (Prakash 1999). Their report further described large numbers of dead and apparently ill vultures found hanging in trees, collapsed under roosts, and perching with their necks drooping below their feet in weak exhaustion. Even a population of more than 40 million birds could not sustain such precipitous declines without risking extinction.

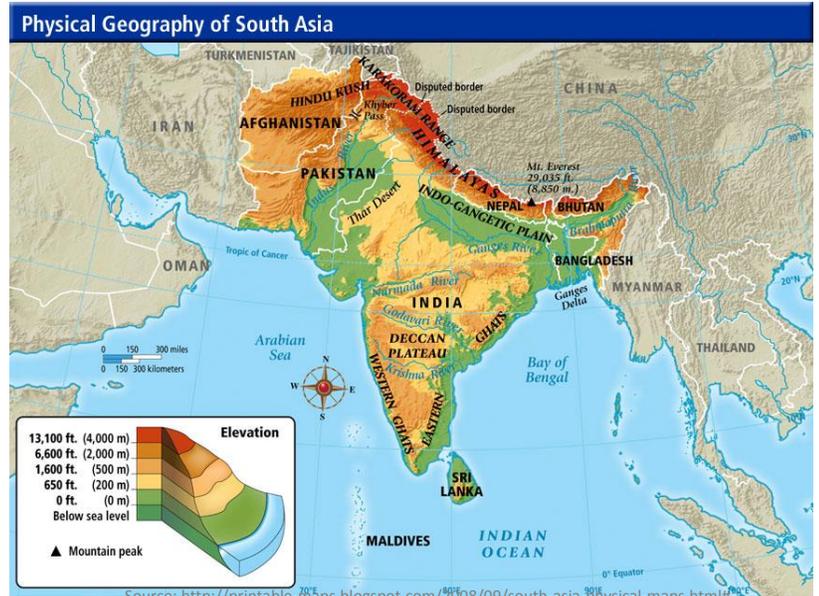
Prakash's survey work found no obvious cause of the population crash – neither food shortages nor habitat changes were evident and the animals seemed ill. Vultures, however, are strongly resistant to many infectious diseases. What could be causing such widespread mortality? As the raptor conservation community gathered to brainstorm with specialists coming in from the United Kingdom, the United States of America, India and the subcontinent, it became clear that this problem might not be confined to India. A team from Pakistan and Nepal also had anecdotal reports of fewer than usual sightings of vultures. Prakash and his team at BNHS joined with branches of the Indian Forest Department, the Royal Society for the Protection of Birds, the Zoological Society of London, and the National Birds of Prey Trust to investigate the cause of the decline. To find out if Pakistani vultures were similarly affected, and if so whether the cause were the same as in India, another team led by Lindsay Oaks, veterinary diagnostician from Washington State University, supported by The Peregrine Fund, joined forces with the Ornithological Society of Pakistan. If the vultures did completely disappear, which seemed possible, they would leave their place in the food web as highly efficient scavengers empty. They would also leave tens of millions of livestock carcasses, and uncounted numbers of wildlife carcasses, uneaten in the hot Indian sun.

By 2007, India had lost between 96.8 and 99.9% of its vulture populations (Prakash et al. 2007), one of the fastest declines of a bird species recorded in human history. Since 2007, vultures have been lost at rates between 30-70% annually ((SAVE) 2016). Their absence has affected many sectors, including human health, the fertilizer production industry, water provision, and waste management. All told, losing vultures has cost India an estimated 34 billion dollars in damages ((RSBP) 2013).

Background

India

India is a vast country stretching across 3,287,263 kilometers square, encompassing mountain ranges, plains, major rivers, plateaus, and even a desert. Bounded on the north by the giant Himalayas, it also contains four other mountain ranges, the Satpura, Vindhya, Eastern Ghats and Western Ghats. The climate is governed by monsoons, which interact with the mountains and the Thar desert to create India's wide variety of weather and ecosystems. Pakistan and Bangladesh are similarly affected by the Himalayas, and Nepal and Bhutan are dominated by the enormous mountain chain.



The economy of India is driven by a variety of industries including textiles, telecommunications, biotechnology, information technology, chemicals, food processing, steel, transport equipment, mining, cement, petroleum, machinery, and software in addition to traditional agricultural livelihoods. The breadbasket of India is in the Ganges River Valley, but various types of agriculture are practiced across the country (Heitzmann et al 1996), and there are over 105 million buffalo, over 70 million cows, over 140 million goats, over 71 million sheep, and over 11 million pigs in the country ((APEDA) 2015). The human population in 2011 was 1,210,193,422 (india.gov.in), and has continued to grow quickly. The combination of this population growth and the liberalization (?) of import/export regulations, has fueled the quick growth of India's economy. Socioeconomic challenges have been to some extent reduced by this growth, but poverty and income inequality remain to varying extents (UNDP 2016, Transparency International 2011, UNICEF 2003).

Basic Socioeconomic and Demographic Indicators - India

INDICATOR		YEAR
UN Human Development Index ranking	0.609	2014
Population (thousands)	1,311,050.53	2015
Urban population (% in urban agglomerations > 1 million)	14	2015
Poverty gap (% living under USD 3.10 per day, 2011 PPP)	18.5	2011
Gini index	35.1	2011
GDP per capita in PPP (2011 international dollar)	5,497	2014
GDP per capita (constant 2015 USD)	1,581.60	2015
Adult Literacy Rate (% 15 years and over, 1995-2004) (total, female, male)	72, 63, 81	2015

Sources: World Bank, UNDP Retrieved Nov 8 2016

India's cultural landscape is as varied as its physical geography. The Indian government recognizes 22 languages which come from four language families. Several religions are practiced. Hinduism is one of the most popular, but there are also Muslims, Christians, Parsee, Buddhists, and more. The mixture of this wide variety of peoples and beliefs has sometimes meant that the political process in this, the world's most populous democracy, is slow to act on any given issue.

Ramayana

In the epic Ramayana, a sacred poetic text that has had huge influence on Indian culture through its central place in Hinduism, a demi-god in the form of a vulture named Jatayu plays a key heroic and sacrificial role in an attempt to save a main female protagonist, Sita, from abduction by demons (Dallapiccola 2002). The central role of Ramayana in the cultures of the subcontinent is in its allegorical narrative of the ideal wife, husband, son, etc. The sympathetic portrayal of a vulture as a loyal martyr in the epic has contributed to the respected position that vultures enjoy in the subcontinent. Versions of the epic are also important in Buddhism and Jain, two other religions practiced in India. Ramayana also has an important place in the cultures of many nearby countries, and versions have been translated into many languages (Dallapiccola 2002).

Vultures in India

As common and largely respected scavengers, vultures were once plentiful in India. In the 1990s, their populations were large and healthy. One species, *G. bengalensis*, was even considered the most plentiful bird in the world, with an estimated population (of all combined species of 100 to 160 million ((SAVE) 2016) (see Appendix for image of healthy *G. bengalensis*). There are eight species of vulture in India, but half are from one family: *Gyps*.

Vulture Species in India (IUCN 2016)	IUCN Status 2016
<i>Aegypius monachus</i> (Cinereous Vulture)	Near Threatened
<i>Gypaetus barbatus</i> (Bearded Vulture)	Near Threatened
<i>Gyps bengalensis</i> (White-rumped Vulture)	Critically Endangered
<i>Gyps fulvus</i> (Griffon Vulture)	Least Concern
<i>Gyps indicus</i> (Indian/ Long-billed Vulture)	Critically Endangered
<i>Gyps tenuirostris</i> (Slender-billed Vulture)	Critically Endangered
<i>Neophron percnopterus</i> (Egyptian Vulture)	Endangered
<i>Sarcogyps calvus</i> (Red-headed Vulture)	Critically Endangered

Vulture Ecology

Vultures are obligate scavengers, meaning they do not prey upon other species, instead waiting for injured or ill animals to die. They may eat either fresh or putrid carrion and often cover vast distances in their search for food, leading to very large home ranges. Communal roosting sites are common. Many Indian species nest in colonies on cliffs ledges or caves, though *G. tenuirostris* and *Sarcogyps calvus* prefer to nest alone, and in trees. Vultures which are more tolerant of human habitation may also nest on buildings or electrical pylons (IUCN 2016). Most

vultures eat the soft tissue of large mammals, but *G. barbatus* is also known for breaking open bones and eating them. If opportunity or necessity arises, some species will also eat much smaller carrion, from turtles to large insects, but all typically rely on large mammals to fuel a successful brood of young. Vultures often feed in large groups, or wakes, and some species are specialized to allow for more efficient feeding in mixed species wakes. For example, one species might be more proficient at breaking through the hide, where another can reach deeper into the carcass. *G. bengalensis* and *G. indicus* are one such pair.

As in many other ecosystems worldwide, vultures provide a valuable ecosystem service consuming dead wildlife (a form of waste management) and helping to recycle nutrients back into soils. As such, they are sometimes referred to as keystone species, or a species that is integral to the function of the smaller systems that make up an ecosystem. This was equally true of the human ecosystems on the subcontinent. For centuries, Indians largely depended upon vultures for safe and effective disposal of animal carcasses. The sight of a wake of hundreds of vultures scavenging at slaughterhouses or in rubbish dumps was, prior to the vulture decline, very common in. The estimated 100 million plus vultures living in India in the 1990s consumed approximately 20 million tons of carrion each year ((SAVE) 2016). Further, an entire system of carcass disposal and recycling was anchored by the efficacy of vulture disposal was widely used. One industry would skin the cows, buffalos, and other livestock and dispose of the carcass. Then the vultures would clean the bones, and the bones would be collected as a key ingredient for fertilizer manufacture. Disposal of human remains by vultures was once also common in some communities on the subcontinent.

The Parsee

An important, though waning, religion in India is a descendant of Zoroastrianism, practiced by the Parsee. The religion was practiced across the subcontinent and all the way into Iran. The city of Mumbai benefited from the creative industries of the Parsee community, a role the community filled there for centuries (Dallapiccola 2002). That community has recently dwindled, but the Towers of Silence used in sky burials, a culturally important after-death purification rite, still stand. In Zoroastrianism, Towers of Silence are places where the human body, considered to be quickly possessed by demons after death, is purified through consumption by vultures, allowing any remains to be disposed of without defiling any of the elements, an important value in the religion (Dallapiccola 2002). Also, vultures are considered to have a “mystic eye,” which is believed to assist in the soul’s cosmic transition after death during these purification rites. These burial rites are similar in function to the sky burials also practiced in Nepal. Both depend on large numbers of vultures being present to dispose of bodies quickly, before they truly decompose. Though some other Towers are now abandoned due to the encroachment of municipalities as they grew, the Tower in Mumbai is in a protective green space of 54 acres within the city itself, and was under regular use up till the 1990s when the vultures began to disappear.

Losing the Vultures

In late 1990s, Dr. Vibhu Prakash of the Bombay Natural Historical Society (BNHS) noted large numbers of dead and dying vultures during a BNHS survey of raptor populations in the Keoladeo National Park, Bharatpur, Rajasthan (See Appendix for pictures of vultures). Previously, the team had surveyed the raptor population in the mid 1980s, and the early 1990s. They surveyed again in the late 1990s amidst rumors of vultures disappearing. During the 1998 survey, they found both sick and dead vultures. The ill vultures were lethargic, with necks drooping down past their feet or perch. Prakash's team concluded that, in the space of a decade, the populations of three species of vulture had declined by more than 95%, or were no longer found at all (Prakash 1999). These three species, the oriental white-backed or white-rumped vulture (*G. bengalensis*), the long-billed vulture (*G. indicus*), and the slender-billed vulture (*G. tenuirostris*) are all from the *Gyps* family.

This population crash publicized in Prakash's 1999 report and further anecdotes and rumors of vulture population declines across the subcontinent, from Pakistan to Nepal, mobilized researchers to quickly call a meeting of locally involved vulture and wildlife health experts. In Mumbai in 1999, under the direction of Asad Rahmani (director of the BNHS), a conference was held to discuss and consider what the causes might be (Risebrough 2004). At the meeting, anecdotal evidence from Pakistan and Nepal established that vulture populations there might also be at risk. The "rapidity and ubiquity of... declines" seemed to suggest a toxic contaminant or infectious disease (Pain et al. 2003), but no consensus or plan was formed until another, larger meeting was held the following September, 2000. This meeting had support from India's Ministry of Environment and Forests and the Royal Society for the Protection of Birds (RSPB). It was attended by Indian and international scientists, conservationists and conservation groups such as The Peregrine Fund, and Indian governmental representatives. (Pain et al. 2008). Though no causes were agreed upon at the time, researchers were galvanized to organize themselves by the discussion, and plans for investigations into the cause of the population decline were made.

Out of the conference, two research groups formed. The first focused on Indian vultures, and was led by BNHS and Dr. Prakash, and was initially made up of the Royal Society for the Protection of Birds, the Zoological Society of London, the National Birds of Prey Trust, and the Forest Department of the state government of Haryana. Other national and international organizations later joined on. The other, second, group was organized by The Peregrine Fund (TPF), which named it the Asian Vulture Crisis Project in 2000 (Oaks et al. 2004), and included Washington State University and the Ornithological Society of Pakistan. Under the leadership of Lindsay Oaks, a veterinary diagnostician with expertise in wildlife health, they would focus on Pakistan. Both groups also collaborated with Nepal (Pain et al. 2008).

Population declines in birds typically involve reductions in either the proportion of adults breeding, reproductive success, or the survival rate. These reductions are usually caused by changes in external factors like availability of nesting sites, food supply, disease, or

predation/persecution from humans (Gilbert et al. 2002). To understand the vulture decline, the two research groups would need to investigate each of these possible causes and find evidence to show which one or ones were responsible. The types of evidence that could be used would range from data on epidemiology, pathology, to population demographics, qualitative observations, and ecological factors.

In 2000, the three endemic Gyps species were placed on the IUCN 'Critically Endangered' list, and subsequently on Schedule 1 of India's Wildlife Protection Act (1972). These listings, though necessary and meant to protect the vultures, initially slowed the BNHS's work by hampering the permitting process for carcass collection, sampling, shipping, and testing. As a result, researchers in India struggled to get timely permits. Many sampling opportunities were lost as carcasses rotted or were eaten by other scavengers (Pain et al. 2008). Once they got the samples, other laws prevented them from sending them away for testing. The team continued to negotiate with officials, and analyzed what data they could. Still the BHNS team managed to gather 28 carcasses for analysis, some of which were collected as sick, live birds which subsequently died and some of which were carcasses collected in as un-decomposed condition as possible. Meanwhile vulture populations continued to dwindle alarmingly.

The TPF team in Pakistan was starting from square one, and needed to establish whether vulture populations there were equally affected, as they suspected they might be. However, they wanted to move on quickly to finding the cause as well. The team leader, Dr. Oaks called in a young wildlife veterinarian at TPF named Martin Gilbert from field work in Madagascar to lead the team on the ground in Pakistan. Working together with local university students from B.Z. Multan University and the University of Agriculture at Faisalabad, Dr. Gilbert and Dr. Oaks helped design and conduct careful surveying and sampling techniques and protocols. The university students were valuable assets of passionate person-power, but they largely had to be trained to conduct any ecological, clinical, or pathological observations and sampling. Dr. Gilbert coordinated this training and sampling. Further, as in India, samples would have to be sent out for testing. Fortunately, Pakistan's laws did not prevent permitting for this. However, finding a way to transport the samples proved challenging until an agreement was finally reached with British Airways. Shipping the samples was necessary because Oaks wanted the samples to be examined in a double-blind study by a few highly respected pathologists abroad, to make sure than any results found and conclusions drawn would be accurate. A mistake could cost the vulture populations valuable time. Very quickly it became clear that Pakistani populations of vultures in the *Gyps* family were in an earlier stage of a crash like that observed in India (Oaks et al. 2001). Further, most of the birds they found appeared to have died of severe renal and visceral gout (Gilbert et al. 2002). The whole TPF team was very worried, as Dr. Gilbert described, "It was desperate and depressing... we knew how they were dying, but [had] no idea what was causing it."

Dissection of the 28 sampled birds by the BNHS team revealed that many, but not all, were suffering from severe renal and visceral gout at the time of death. Visceral gout can be caused by a diet abnormally high in protein, primary renal failure, or dehydration amongst other causes. Roughly one third of the 28 birds were in good body condition at the time of death, one

third were in poor condition, and one third were too decomposed to determine body condition (Cunningham et al. 2003). With roughly half of the birds whose body condition had been recorded showing poor body condition, a sign of chronic disease, the BHNS team inferred that the gout they were observing could be a secondary symptom, caused by dehydration due to diarrhea, of a primary infection or toxicity due to contamination (Cunningham et al. 2003). From the beginning, there was suspicion of possible organophosphate/ organochlorine (pesticide) poisoning (Prakash 1999) but tests for these on Pakistani birds failed to reveal any significant toxicity (Oaks et al. 2001). Further, the pattern of population decline was so ubiquitous and widespread that point source contamination with pesticides seemed unlikely. The epidemiological evidence could fit either a widely disseminated contaminant or a quickly spreading infectious disease but, again, there had been no evidence of toxicity in Pakistani birds. Then, the BNHS team found signs of glial cell inflammation that could indicate viral infection in some of their 28 samples (Cunningham et al. 2003). The combination of this epidemiological and pathological data lead the BHNS team to point to a viral infectious disease as their prime suspect (Cunningham et al. 2003, Pain et al. 2003, Prakash et al. 2003), and they redoubled their efforts to find the suspected viral pathogen. This diagnosis turned out to be wrong.

In 2002, the cause of the declines remained a mystery (Pain et al. 2008). All the teams hoped conservation efforts could be started quickly, but without knowing what they were protecting the vultures from, they feared their efforts would be futile.

Contamination

Back in Pakistan, Dr. Gilbert's students collected 259 vulture carcasses, all of which were necropsied. 219 of those had clearly visible urate deposits on internal organs, indicating visceral gout. 42 birds were recently deceased (less than 24 hours), and samples from those 42 were sent out for a battery of tests. The tests looked for pesticide contamination, histopathological examination for clinical signs (of an unknown infectious disease or other cause), and attempted to isolate viruses from the most gout-affected organs. Through this process, no viruses were isolated, nor were any clinically meaningful levels of pesticides detected, and the only consistent clinical or pathological findings were severe renal and visceral gout (Oaks et al. 2004). One pathologist at the National Wildlife Center in the USA, Dr. Carol Meteyer, provided a new insight. After looking at the primary literature around avian gout secondary to dehydration, she noted that her samples were missing a key pathological finding that should have been present. For gout to have been caused by dehydration, there should have been inflammation "ascending" the ureters (Meteyer et al. 2005). Instead, under pathological examination her samples looked like somethings else: acute gout due to tissue necrosis in the microstructures of the kidneys. Specifically, the histopathology was comparable to the effects of gentamycin toxicity in snakes, which Meteyer had seen before (Meteyer 2016, personal communication). This careful scientific process of "standing on the shoulders of giants" combined with meticulous examination provided the TPF team a new question to investigate. Where might Pakistani vultures be ingesting a nephrotoxic chemical that they hadn't already tested for?

Gilbert and Oaks formed a new hypothesis: vultures might be affected by residual pharmaceutical products or agents contaminating livestock carcasses they had consumed. By surveying local Pakistani veterinarians and pharmacists, the team identified just one pharmaceutical agent that fit the bill. It had to be potentially toxic to the kidneys, absorbed orally, and widely used. Diclofenac, a non-steroidal anti-inflammatory drug (NSAID) was the only one that ticked all the boxes. It had also only recently become available for veterinary use; interviews with veterinarians revealed the drug had become available in India in 1994, and in 1998 in Pakistan. This roughly matched the timeline of the vulture declines in both countries (Pain et al. 2008, Cuthbert et al. 2014, Cuthbert et al. 2016). It was available across the subcontinent, and due to its high effectiveness and low price, it was quickly adopted for wide use in both human and veterinary medicine. It continues to be used worldwide in human medicine to treat pain from arthritis to migraines. In humans, it is considered safe, though as with many NSAIDs, it does have nephrotoxic properties in patients with NSAIDs sensitivities and/or kidney disease ((FDA) 2016). Diclofenac has powerful analgesic and anti-inflammatory as well as anti-pyretic properties, and its introduction into the veterinary medicine box had been welcomed as a generally safe and highly effective drug. Surveys with the veterinarians in both Pakistan and India revealed that many clinicians and farmers would often use diclofenac to effectively increase the working lifespan of draft livestock, and would continue to give it until the day the animal died.

Oaks' team decided to test for diclofenac in all their tissue samples, and compare those samples in the gout syndrome group to the "other" group (samples from vultures whose demise was known to be from injury, carbamate poisoning, etc.). It turned out to be a eureka moment. Diclofenac was present in all vultures with the gout syndrome and none of the others, a perfect result which was confirmed to have high statistical significance (Oaks et al. 2004). When the results came in, Dr. Gilbert's partner asked him if he'd "seen a ghost" as he had gone completely blank reading the message – and perhaps he had in a sense. Pakistani vulture samples were speaking through the tests, revealing their killer.

However, to prove diclofenac the cause of the decline would require fulfilling Koch's Postulates (see Appendix). These were desperate times – despite feeling sure they had solved the mystery and daily finding more and more dead vultures, Gilbert recalled the pressure the team felt to complete the scientific process so their discovery could be acted on before it was too late. So, the team tested their hypothesis experimentally by feeding captured, unreleasable vultures with meat laced with high and low doses of diclofenac. Three of the four fed diclofenac died, with signs before and after death identical to field observations of stricken vultures. The fourth showed clinical signs of kidney damage, but recovered. No control vulture showed clinical signs before or after necropsy. Further tests were done by feeding vultures with carcasses from animals treated with typical clinical doses of diclofenac prior to slaughter and, again, the vultures became ill in a dose-dependent way. Several died following the dosing, and those showed extensive kidney damage and gout upon necropsy (Oaks et al. 2004, Meteyer et al. 2005).

Having fulfilled Koch's Postulates, these findings were presented in May 2003 at a conference ahead of publication. This transparency on the part of the TPF team allowed other researchers to test and validate their theory. Quickly following up on this, the BHNS team found convincing evidence of diclofenac poisoning in samples from wild, dead vultures collected across the northern half of India and Nepal (Shultz et al. 2004).

Still, what if there were other undiscovered factors in the population decline? Such unknowns could hamper or nullify conservation efforts based on diclofenac. This was one basis of skepticism in the scientific community to the suggestion of diclofenac as a lone culprit (Proffitt and Bagla 2004). Other questions included whether vultures could really get lethal doses of diclofenac from the available livestock carcasses? Could it really be so very toxic to vultures when it was largely safe in other species? So, in addition to conducting careful pathological studies to investigate diclofenac's strong toxicity in *G. bengalensis* (Meteyer et al. 2005), researchers used simulation modelling to study population effects. These models were based on literature, expert opinion, and the previous dose-response experiments done by the team in Pakistan.

The models calculated what dose of diclofenac in livestock in what percentage of carcasses would be needed to produce the observed decline in vulture populations. It was found that <1% of carcasses would have to be contaminated to produce the rates of decline observed in real life from 2000-2003 (Green et al. 2004). They also used the model to determine what proportion of dead vultures would display visceral gout if diclofenac were the sole cause of the decline. The calculated proportion was also consistent with field observations from the period. This work led researchers to conclude that diclofenac poisoning was the major and likely only cause of vulture declines in the subcontinent. This final data helped build adequate scientific consensus to pave the way for conservation and policy efforts. And none too late, for it was becoming clear that without some action, the vulture species endemic to India would indeed become extinct: their populations at that time were mere hundreds, and were approximately halving each year (Proffitt and Bagla 2004). In early 2004, conservation groups issued a report as a call to action (Gulati et al. 2004).

Environmental ramifications

While the scientific detective work was developing, the ramifications of the vulture disappearance to the human and natural ecosystems were being felt across the subcontinent. The disappearance of vultures meant a large food source, carcasses left unconsumed, was now available for other scavengers. The potential human health impacts revolved around three things in addition to cultural impacts: water and sanitation concerns, dog bites, and rat populations.

The water and sanitation concerns were largely concerning whether failed carcass disposal could affect water quality or represent a source of disease, particularly anthrax contamination in the environment. Vultures are not affected by anthrax due to their specialized digestive systems, and their speedy consumption of carcasses reduces the opportunity for anthrax

bacteria to sporulate and contaminate the environment (Pain et al. 2003). Without their presence, carcasses could sit for days before being completely consumed by scavengers, leaving ample time for anthrax to contaminate surrounding areas. Indeed, a rise in anthrax cases did occur after the vulture decline, and they may have been linked (Mudur 2001). Other bacteria and insects from rotting carcasses could also potentially contaminate the areas near disposal sites, including any adjacent water sources.

Other concerns were based on the feral dog and rat populations, which had begun taking over carcass dumps in the absence of vultures (Pain et al. 2003, Prakash et al. 2003). With increased food availability, dog and rat numbers would increase. In fact, increases in dog populations after the decline in vulture populations were reported ((SAVE) 2016). Increased numbers of feral dogs and rats represented potential problems in rabies and bubonic plague control (Pain et al. 2003). Rabies control has been an ongoing problem in the sub-continent, and most human rabies cases are the result of dog bites, often from feral dogs (Kole 2014). The increased population of feral dogs was very concerning to those in the rabies control effort (Markandya et al. 2008). The increasing rat population was the precipitating concern for potential increasing risk of bubonic plague, since rats are a known reservoir for the disease ((CDC) 2015). These human health risks amplified the importance of India's vultures to the human ecosystem, in addition to their role in the larger ecosystem.

For the Parsee, there were deeply concerning cultural implications. In the Towers of Silence, the dead had begun literally piling up as there were no scavengers eating them. The bodies were rotting away slowly, leading to the contamination of the towers themselves, the ground, and the air – many of the elements that the Parsee hold sacred. This outcome was precisely the one the Towers of Silence were meant to prevent, but without the vultures playing their role, the desired post-death purification was impossible. The Parsee community had to decide: should they wait and hope the vultures would return, or support their own colony of the birds, or abandon their traditional death rites and opt for the “next best thing,” cremation? This important cultural group added their voices to those calling for a solution.

The loss of the vulture role in carcass disposal also had economic impacts. The need for a new disposal method would mean local governments or others would have to cover the costs of designing and implementing systems for carcass disposal. It also meant a reduction in the profitability of the bone-based fertilizer industry in India (Markandya et al. 2008). Without the free service provided by the vultures, bone cleaning was far more labor and time intensive. This cut into the thin margins between profits and loss that bone collectors and fertilizer producers were already operating under, damaging a widespread traditional livelihood strategy. As is observed in many places, the people most vulnerable to changing ecosystem services are those who are most dependent upon them for their livelihoods, often the rural poor. During the height of the Asian vulture crisis, the effects loss of the vultures freely provided ecosystem service of carcass removal similarly landed between issues of environmental justice and health equity, as not all populations were equally effected (Van Dooren 2010). When the price tags for these impacts were added together, the estimated cost of the vultures disappearance to the Indian government was \$34 million ((RSBP) 2013).

Strategies for Solution

To protect and nurture the remaining vultures' populations, diclofenac needed to be entirely removed from their food supply – research showed that contamination of even 1% of carcasses would mean a continued decline (Green et al. 2004). This would require political and legal changes, as well as effective enforcement of any new laws. Enacting the desired ban would be a huge challenge, though. A study by Taggart et al. in 2007 observed that 10% of samples from carcasses of domesticated ungulates were contaminated by diclofenac. By extrapolating from these numbers, Pain et al. (2008) estimate that well over 5 million courses of treatment of diclofenac were given to livestock in India annually. Curbing use of such a popular drug would be a huge challenge.

However, the local extirpation of vulture populations was already occurring, and *G. bengalensis* was on the brink of extinction. Future genetic diversity of India's vulture species was also a concern. A well-demonstrated principle in conservation genetics shows small populations tend to lose genetic diversity (a vital component of long term species health, adaptation, and survival) and once gone cannot be recovered (Bouzat 2010). The most affected species in India were becoming increasingly vulnerable to such losses. It was hoped that captive breeding programs could shore up populations and prevent such an irreversible loss of genetic diversity and associated species fitness.

Two international meetings were held in 2004, to further build consensus and momentum for needed changes. First was the Vulture Summit in Kathmandu, put on by The Peregrine Fund and Bird Conservation Nepal, followed by the International South Asian Recovery Plan Workshop organized by the Bombay Natural History Society research and conservation group (ISARPW 2004). Representatives from the several South Asian governments, NGOs, and experts attended the latter to build joint recommendations. The recommendations were:

- (1) that government authorities in all range states [nations within the home range of the vultures of concern] introduce legislation or regulations to prevent all veterinary uses of diclofenac that pose a risk to vultures,
- (2) that captive populations of all three affected *Gyps* species be established immediately in South Asia, for the purposes of conservation breeding and subsequent reintroduction to a diclofenac-free environment.

A vulture research and conservation center had been founded in Pinjore in 2001, as a collaboration between BHNS and the Haryana Forest Department. Facilities for breeding captive populations were established in 2004, by converting existing facilities designed to care for and release vultures at in Pinjore, and plans were made to have more built in West Bengal and Assam by 2008, with hopes of others to follow in the region. Chris Bowden, a British scientist with expertise species recovery working for the partnering organization of the Royal Society for the Protection of Birds, was assigned to the Asian vulture crisis. He would help

coordinate and manage the organizations working to protect wild vultures and establish the needed breeding populations, per the recommendations from the Vulture Summit. He and his colleagues at the various partnering organizations from India and abroad would oversee applying the scientific discoveries to reality.

Another conservation strategy was establishing Vulture Safe Zones, sometimes accompanied with vulture “restaurants.” The safe zones were areas where diclofenac was excluded through intensive effort and public education. Establishing feeding areas for vultures, the “restaurants,” with carcasses determined to be diclofenac free was the key argument behind this idea. However, the typically enormous range (100s of kilometers) vultures regularly travelled to forage for food meant that a well-stocked restaurant and a large safe zone might be insufficient to protect a large population. Until diclofenac use was fully phased out, vulture populations seemed likely to continue their decline.

In 2005, in response to the recommendations of the Vulture Summit and the advocacy of Bowden, Dr. Prakash (the scientist who had lead the original reports of the population crash in the late 1990s), and their colleagues, the Indian government announced its intention to ban veterinary use of diclofenac and off-label use of human diclofenac (Swan et al. 2006) in the near future. The next year, August 2006, manufacture of diclofenac for veterinary use was formally banned by the Drug Controller General of India, and human labels were to be marked with “not for veterinary use (Prakash et al. 2007, International 2016). Nepal and Pakistan followed suit with bans of their own. Bowden and his colleagues had to consider what their strategy of choice for phasing out diclofenac would be: education or strict law enforcement or a combination of the two. However, the drug was so popular that the conservationists feared that enforcing the ban would be nearly impossible. After all, it would still be available for human use, and sold in local pharmacies. There would be little to stop farmers or veterinarians from purchasing diclofenac labeled for human use and giving it to their livestock instead.

A suitable substitute for diclofenac in veterinary use would greatly assist diclofenac phase out, given its apparent importance to agricultural communities. Researchers now turned to identifying a potential substitute. By surveying veterinarians at zoos and wildlife rehabilitation centers around the world, meloxicam was identified as a potential alternative. It had been used in hundreds of instances with various raptor species successfully. It was subsequently tested and found to be safe (Swan et al. 2006). It was of special interest because it was already produced for veterinary use in both oral and injectable form, and licensed for use in India. Having cleared these hurdles, it seemed ready for production scale up and marketing. However, it was more expensive than diclofenac. Farmers and veterinarians were unlikely to substitute a more expensive drug for the affordable diclofenac. It was sold cheaply in human form, and, sold in large, convenient multiuse bottles. It was clear that the remaining vultures in the wild might not be safe from diclofenac for years if veterinary habits didn’t change. To save the remaining vultures, conservation programs needed to be scaled up quickly.

Successfully breeding the three species of highest concern, the *G. bengalensis*, *G. tenuirostris*, and *G. indicus*, was a high priority for collaborating partners. Dr. Prakash lead the breeding

program. In 2008-2009 the center at Pinjore, now called the Vulture Conservation Breeding Centre, successfully hatched and fledged chicks of two species (*G. bengalensis* and *tenuirostris*) in their vulture breeding facilities. Even more importantly, they hoped to successfully hand rear chicks hatched from eggs removed from the captive vultures' nests, which would stimulate a second egg laying and allow one pair of vultures to effectively double the number of chicks they could produce each breeding cycle. Collectively, these breeding programs aimed to help the vulture species avoid a detrimental bottleneck in genetic diversity, and build up a population that could be released into the wild once if it ever became safe for them.

Then, in 2009, there was a break-through on the pharmaceutical side. The company that had originally developed meloxicam, Boehringer Ingelheim, decided to release their patent for the drug in India. This was done out of concern for Asian vultures, and though it would mean competition from generic brands of meloxicam, the company determined that the loss would be minimal and the benefit to vultures and India potentially very high. This patent release allowed the price for meloxicam to come down, making it comparable to diclofenac. For the first time, the possibility of a diclofenac free India seemed potentially possible, though as yet out of reach.

Successes and challenges

In 2010, the Pinjore breeding program successfully hatched and hand raised vulture chicks, greatly increasing the effectiveness of their program. Vulture restaurants and public education about diclofenac had been widely implemented ((SAVE) 2016). From 2010 to the present, progress in protecting the subcontinent's vultures shifted from fast and intense scientific progress to the incremental and complex process of policy adaptation to incorporate continued scientific process, implementation, and behavior change.

The ecological and cultural consequences of the vultures' effective disappearance from the subcontinental ecosystem led to adaptations in carcass disposal and human remains disposal in many communities. Fewer and fewer carcasses were laid out in carcass dumps, and the Parsee community largely shifted to cremation after death. Feral dog populations did surge as they stepped into the vultures' role of prime scavenger ((SAVE) 2016). Transmission rates of rabies were expected to rise when the density of the host population, in this case feral dogs, increased (Begon et al. 2002). However, the connection between the rising populations of feral dogs and rabies has proved difficult to thoroughly investigate. A causal connection between vulture numbers and cases of rabies would be very hard to prove, as India had not consistently tracked the disease throughout the time-period in question. Strategies for estimating mortality and costs on an international scale, using dog bites as a proxy under assumptions of a certain percentage of underreporting have been used (Knobel et al. 2005). However, these are difficult to retroactively reduce to smaller scales. In 2014, the World Health Organization reported that 36% of all rabies cases globally occurred in India alone, or 18-20,000 deaths from rabies annually, approximately 45% of which are in children under 15 years of age (Kole 2014), most of which were infected by bites from feral dogs .

In 2011, Lindsay Oaks – the veterinarian who had lead the TPF Pakistani team that solved the diclofenac mystery – passed away (see Appendix). In some ways, this loss represented the changes the response to the Asian Vulture Crisis would inevitably have to make moving forward. To continue and improve the work in the future, 17 organizations who had been working together on the Asian vulture crisis formally founded the SAVE (Saving Asia’s Vultures from Extinction) Initiative in 2011. Chris Bowden would help manage SAVE’s strategy and progress going forward. They would drive the conservation effort forward through continued advocacy for policy with the Indian and other governments, continue to develop the breeding programs, maintain the Vulture Safe Zones, and keep on top of new challenges and monitor vulture populations through research.

SAVE was particularly concerned about pharmaceutical regulations. Human labeled diclofenac continued to be sold in oversize vials for nearly a decade after veterinary use of diclofenac was banned. The vials were labeled “not for veterinary use,” but the size of the vials enabled and the fact they were available over the counter made it easy to purchase livestock-sized doses of the drug. Then, SAVE had an advocacy breakthrough in 2015, when the Indian government banned production of diclofenac in vials larger than 3mL (SAVE). As that advocacy was bearing fruit, though, it was becoming clear that other NSAIDs might also be dangerous to vultures. As early as 2007 the NSAIDs flunixin and carprofen were shown to be toxic to vultures (Cuthbert et al. 2007). Ketoprofen has also been shown to be detrimental to vultures in the Gyps family (International 2016), and new drugs are in the development pipeline. An example of the threat of new drugs is aceclofenac. It is quickly metabolized into diclofenac within treated animals, leading to carcass contamination comparable to treatment with diclofenac (Galligan et al. 2016).

By using the wealth of research surrounding the effects of diclofenac on the vultures of the Indian subcontinent, the SAVE partners hoped to convince pharmaceutical companies to use the precautionary principle, or the idea that if known risks are high enough they must be considered in decision-making about future related but unknown risks as a “precaution” (see Appendix for more details), in planning their drug testing and licensing projects. The challenge would be to balance the economic goals of the pharmaceutical industry with the risk and benefits to vultures in the subcontinent, and beyond the subcontinent, anywhere *Gyps* vultures have been part of the ecosystems, such as Europe and Africa (Ogada et al. 2016). To proactively manage the risks of environmentally persistent pharmaceuticals would require learning from the Asian vulture crisis and applying the lessons learned holistically (Margalida et al. 2014).

Meanwhile, the SAVE team continued to follow and update the conservation and policy plans laid out through their collaborative work with the 17 founding members. In addition to the pharmaceutical concerns, they would be monitoring the conditions in Indian communities. To successfully detect the impacts of policy change, they would need to know whether diclofenac continued to be present in the environment in harmful quantities. This would dictate when and if captive bred vultures could ever be released in large numbers. Unfortunately, the drug continued to be found in survey after survey of carcasses (Cuthbert et al. 2011, Cuthbert et al. 2016). It was clear that despite the availability of a reasonable alternative in meloxicam,

diclofenac continued to be a popular, though illegal, choice for livestock pain control. They also continued to monitor the wild vulture populations. In 2012, a study looking at the efficacy of diclofenac bans on *G. indicus* vulture populations recorded positive results recorded in Pakistan (Chaudhry et al. 2012), and same in India (Prakash et al. 2012). They both found that the declines in the vulture populations had slowed, but they were continuing to shrink steadily despite the improvement.

Discussion Questions

- The scientific method allowed conservationists and researchers to advocate for policy changes, but this process was not necessarily smooth. What challenges were overcome along the way, and which remain?
- The consequences of losing an ecosystem service can be wide reaching. What were the consequences of losing millions of scavengers to the Indian ecosystems and their human inhabitants?
- What are the international implications of using a One Health approach to applying the precautionary principle to management of NSAID contamination in livestock carcasses?

Appendix

1. **Vulture species in India**
2. **Picture of *Gyps bengalensis*, healthy**
3. **Picture of *Gyps bengalensis*, ill**
4. **Koch's Postulates**
5. **Obituary of Dr. J Lindsay Oaks**
6. **Precautionary Principle**
7. **SAVE video summarizing Asian Vulture Crisis**

Appendix 1

Vulture Species in India

Species	IUCN Status	Current Population Estimate	Population Trend	Range
<i>Aegypius monachus</i> (Cinereous Vulture)	Near Threatened	14,000-20,000 mature individuals	decreasing	link
<i>Gypaetus barbatus</i> (Bearded Vulture)	Near Threatened	1,300-6,700 mature individuals	decreasing	link
<i>Gyps bengalensis</i> (White-rumped Vulture)	Critically Endangered	2,500-9,999 mature individuals	decreasing	link
<i>Gyps fulvus</i> (Griffon Vulture)	Least Concern	64,800-68,800 mature individuals	increasing	link
<i>Gyps indicus</i> (Indian/ Long-billed Vulture)	Critically Endangered	30,000 mature individuals	decreasing	link
<i>Gyps tenuirostris</i> (Slender-billed Vulture)	Critically Endangered	<22,000 mature individuals	decreasing	link
<i>Neophron percnopterus</i> (Egyptian Vulture)	Endangered	13,000-41,000 mature individuals	decreasing	link
<i>Sarcogyps calvus</i> (Red-headed Vulture)	Critically Endangered	3,500-15,000 individuals	decreasing	link

Appendix 2

Healthy *Gyps bengalensis*, or White-rumped/ Oriental White-backed Vulture



By (Image: Goran Ekstrom) - Switching Drugs for Livestock May Help Save Critically Endangered Asian Vultures. Gross L, PLoS Biology Vol. 4/3/2006, e61 <http://dx.doi.org/10.1371/journal.pbio.0040061>, CC BY 2.5, <https://commons.wikimedia.org/w/index.php?curid=1441706>



Asian white-backed vulture in flight, dorsal view

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Appendix 3

Sick *Gyps bengalensis* demonstrating the “neck-drooping syndrome” observed in early surveys by Dr. Prakash’s team.



Fig. 1. Indian white-backed vultures showing neck drooping syndrome (NDS) in Ranthambhore National Park, Rajasthan, July 2000 (a) and in the centre of Delhi, September 2000 (b). Sick birds appear to be lethargic with intermittent, often prolonged, periods of neck drooping, in which their heads often drop vertically to the level of their feet. When disturbed, birds with NDS appear temporarily alert.

Source: (Cunningham et al. 2003)

Appendix 4

Koch's Postulates

Four criteria were established by Robert Koch to identify the causative agent of a disease:

1. The microorganism or other pathogen must be **present in all cases of the disease**
2. The pathogen can be isolated from the diseased host and **grown in pure culture**
3. The pathogen from the pure culture must **cause the disease when inoculated into a healthy, susceptible laboratory animal**
4. The pathogen must be **re-isolated** from the new host and **shown to be the same** as the originally inoculated pathogen

Appendix 5

J Lindsay Oaks 1960-2011

February 9, 2011 at 12:31pm

J Lindsay Oaks 1960-2011

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Conservation medicine has lost a champion. Dr J Lindsay Oaks died on 15 Jan 2011 after a short illness.

His leadership in elucidating the role of diclofenac, a veterinary anti-inflammatory drug, in the devastating decline of oriental white-backed and other _Gyps_ vultures on the Indian subcontinent carved his name in the annals of conservation medicine. This work, which in 2004 was published in Nature, provided the insight that led the way in saving that species, once the most abundant bird of prey in the world, from extinction.

Lindsay was a lifelong friend and trusted resource for the Peregrine Fund assisting restoration efforts for falcons and other raptors. He discovered the viral source of high death loss in captive aplomado falcons, and worked to highlight the role of lead in mortality of endangered California condors.

Lindsay had a distinguished career as researcher, teacher, mentor, and microbiologist at the Washington State University, nationally and internationally. Lindsay was born in Texas in 1960 and lived in South America, Israel, and the United Arab Emirates. His field work established him as valued colleague to scientists in Pakistan, Africa, and Asia. Dr Oaks' colleagues and friends from Pullman to the Middle East to Asia mourn his loss along with his family and celebrate both his kindness that touched many, and his extraordinary professional contributions. His life and work will be forever remembered.

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Appendix 6

The Precautionary Principle is defined as follows:

When human activities may lead to morally unacceptable harm that is scientifically plausible but uncertain, actions shall be taken to avoid or diminish that harm. Morally unacceptable harm refers to harm to humans or the environment that is:

- threatening to human life or health, or
- serious and effectively irreversible, or
- inequitable to present or future generations, or
- imposed without adequate consideration of the human rights of those affected.

The judgement of plausibility should be grounded in scientific analysis. Analysis should be ongoing so that chosen actions are subject to review. Uncertainty may apply to, but need not be limited to, causality or the bounds of the possible harm.

Actions are interventions that are undertaken before harm occurs that seek to avoid or diminish the harm. Actions should be chosen that are proportional to the seriousness of the potential harm, with consideration of their positive and negative consequences, and with an assessment of the moral implications of both action and inaction. The choice of action should be the result of a participatory process.

Source: UNESCO - COMEST Report: The Precautionary Principle. 2005.
<http://unesdoc.unesco.org/images/0013/001395/139578e.pdf>

Appendix 7

SAVE video: <https://vimeo.com/19503113>

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