

Role of veterinary diclofenac in decline of vulture populations in South Asia

Richard Cuthbert^{1*}, Vibhu Prakash², Chris Bowden¹, Devojit Das², Rhys Green³, Yadvendradev Jhala⁴, Debbie Pain^{1**}, Kalu Ram Senach², Nita Shah² and Mark A. Taggart⁵
Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2DL, UK.

Abstract

Gyps vulture populations declined by more than 98% across the Indian subcontinent in last one decade. Post-mortem examination showed that the majority of vultures found dead in the wild had a lethal accumulation of uric acid crystals in the tissues (visceral gout), due to kidney damage that prevented the elimination of this excretory product. Diclofenac, a non-steroidal anti-inflammatory drug potentially nephrotoxic to birds, has become a widely used veterinary medicine and was identified as the cause of the decline; toxicity testing of diclofenac in four species of *Gyps* vulture caused dose-dependent mortality (LD₅₀ of 0.225 mg kg⁻¹ vulture body weight) and identical clinical signs (visceral gout) to those found in carcasses of wild birds. In carcass dumps, detectable diclofenac in 10.1% of 1848 domestic ungulate carcasses was noticed which was the important and sole cause of vulture population decline. Others causes like food availability; loss of breeding habitat and disease did not have sufficient evidence. It is now imperative that diclofenac is effectively removed from the veterinary market in South Asia as an alternative drug, meloxicam is available.

Keywords: Conservation, Diclofenac, *Gyps*, NSAIDs, Vulture population.

Three resident species of vulture, the Oriental white-backed vulture (*Gyps bengalensis*) [OWBV], long-billed vulture (*G indicus*) [LBV] and slender-billed vulture (*G tenuirostris*) [SBV], which together used to number tens of millions, have declined by more than 98% since the early 1990s (Prakash *et al.*, 2007), and are now all listed as Critically Endangered. It had a major impact on the ecological balance and absence of vultures as scavengers has resulted in increasing populations of feral dogs (ILC, 2003), with

packs of several hundred animals now common at carcass dumps and there is an increased risk of dog bites and the spread of rabies. Increases in putrefying carcasses and changes in the scavenger populations also have associated disease risks for wildlife, livestock and humans, including the spread of anthrax, plague, TB and brucellosis (Anderson *et al.*, 2005). This paper reviews the research and results that have led to the conclusion that the non-steroidal anti-inflammatory drug (NSAID) diclofenac is the main cause of vulture declines in South Asia.

Materials and Methods

Carcasses of dead vultures were collected from across India, Pakistan and Nepal by visiting known breeding colonies and roosting sites and necropsied by experienced veterinarians and research biologists. Tissue samples from fresh carcasses (<24 hr) were tested for the presence of arsenic, cadmium, copper, iron, lead, manganese, mercury, molybdenum, zinc, organophosphate, carbamate, organochlorine pesticides and polychlorinated biphenyl. Virus isolation in cell

¹Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, UK

²Bombay Natural History Society, Hornbill House, S.B. Singh Road, Mumbai-400 023, India

³Conservation Science Group, Department of Zoology, University of Cambridge CB2 3EJ, UK

⁴Wildlife Institute of India, Post Bag # 18, Chandrabani, Dehradun-248 001, Uttarakhand, India

⁵School of Biological Sciences, Department of Plant and Soil Science, University of Aberdeen, AB24 3UU, UK

*Author for correspondence, email: Richard.Cuthbert@rspb.org.uk

** Present Address: Wildfowl and Wetlands Trust, Slimbridge, Gloucestershire GL2 7BT, UK

[†]Present Address: Institute de Investigación en Recursos Cinegéticos, IREC (CSIC, UCLM, JCCM), Ronda de Toledo s/n, 13071 Ciudad Real, Spain.

cultures was undertaken to look for the presence of avian influenza, infectious bronchitis, West Nile virus, avian malaria and novel pathogens. Analysis for residues of NSAIDs was undertaken by high performance liquid chromatography mass spectrometry (HPLC-MS) for acetaminophen, diclofenac, flunixin, ibuprofen, phenylbutazone, oxyphe-nylbutazone, indomethacin, ketoprofen, mefenamic acid, salicylic acid, tolmetin and naproxen. Tests on the toxicity of diclofenac were undertaken for four vulture species including OWBV, the African White-backed Vulture (*Gyps africanus*) {AWBV}, the Eurasian Griffon Vulture (*Gyps fulvus*) {EGV} and Cape Griffon Vulture (*Gyps coprotheres*) {CGV} with additional data available from a fifth species the Himalayan Griffon Vulture (*Gyps himalayensis*) {HGV}. Birds were either administered diclofenac by oral gavage (Table 1) or fed tissues from animals that were treated with diclofenac (once daily injections @ 2.5 mg/kg b wt for 3 days). Behaviour, time to death and blood biochemistry were recorded for all birds and full necropsies and histopathology was undertaken (Oaks *et al.*, 2004a; Swan *et al.*, 2006a). Control animals were present in all toxicity testing experiments (except CGV) and were administered water by oral gavage or fed tissues from untreated animals.

A diclofenac dose to toxicity curve and LD₅₀ value were estimated through probit analysis (Oaks *et al.*, 2004a). Population modelling, using a plausible range of values (from other *Gyps* species) for vulture demographic parameters and feeding frequency (2 to 4 days) were used to estimate the rate of vulture population decline expected to be caused by a specified proportion of livestock carcasses that contained a lethal dose of diclofenac (Green *et al.*, 2007). To estimate levels of diclofenac contamination, liver samples from domestic ungulates at 67 carcass dumps were collected from across Andhra Pradesh, Bihar, Gujarat, Jammu and Kashmir, Jharkhand, Madhya Pradesh, Maharashtra,

Orissa, Punjab, Rajasthan, Uttar Pradesh, and West Bengal and tested through HPLC-ESI/MS (high performance liquid chromatography-electrospray ionisation mass spectrometry) with limits of quantification (LOQ) and limits of detection (LOD) equivalent to 10 µg/kg and 4 µg/kg, respectively (Taggart *et al.*, 2007).

Results and Discussion

Survey teams in India recorded 87-100% decline in vulture despite the presence of large numbers of carcasses available at this and many other carcass dumps. Very large numbers of feral dogs were recorded at carcass dumps (ca 1200 dogs at one site; Prakash *et al.*, 2003), and it has been proposed that the decline in vultures is due to the increase in dogs (Chhangani and Mohnot, 2004). However, vultures and dogs have always coexisted at carcass dumps in India and in over 8 yr of observations, no instances of predation by dogs on vultures was recorded. Dog numbers increased only after the collapse in vulture numbers as there were 18 million dogs in 1987 and 29 million in 2003 (ILC, 2003). Hence, the data indicate that the increase in feral dog numbers is a consequence, not a cause, of the loss of vultures. Observations of vulture breeding colonies indicated that in some areas there had been localized losses of nesting sites, with felling of nesting trees and quarrying of some cliffs, as also reported around Jodhpur, Rajasthan (Chhangani and Mohnot, 2004). However, OWBV and SBV exploit a large range of tree species for nesting and across India the availability of large suitable trees far exceeds the number of nesting vultures. Similarly, observations of LBV colonies on cliffs indicate the presence of many suitable nesting sites but very low numbers of vultures (e.g. intact cliffs at Bayana, Rajasthan that formerly supported thousands of breeding LBV had 10 breeding pairs in 2007/08). These observations indicate that depletion of food resources, competition with dogs or loss of nesting habitat, are not responsible for the decline in vulture numbers.

Table 1. Summary of diclofenac toxicity testing for Oriental White-backed Vulture [OWBV], African White-backed Vulture [AWBV], Eurasian Griffon Vulture [EGV], Himalayan Griffon Vulture [HGV] and Cape Griffon Vulture [CGV], indicating diclofenac dose (mg kg⁻¹ vulture body weight), route of administration, sample size of birds dosed, the number that died and percent mortality. Clinical signs of extensive visceral gout were recorded in all mortalities following exposure to diclofenac.

Gyps Species	Dose mg kg ⁻¹	Route	No. Dosed	No. Died	% Mortality
OWBV	2.5	Oral gavage	2	2	100
OWBV	0.25	Oral gavage	2	1	50
OWBV	0.007 to 0.940	Fed treated tissue	20	13	65
AWBV	0.8	Oral gavage	2	2	100
EGV	0.8	Oral gavage	3	3	100
HGV	3.8	IM injection	1	1	100
CGV	0.8	Oral gavage	2	2	100

Table 2. Results from vulture carcasses collected in the wild in Pakistan (Oaks *et al.*, 2004a), India and Nepal (Shultz *et al.*, 2004) for both Oriental White-backed Vulture [OWBV] and Long-billed vulture [LBV] indicating the number of carcasses found with and without visceral gout at necropsy, the prevalence of visceral gout, the number of fresh carcasses testing for diclofenac, and the prevalence of diclofenac residues.

Presence or absence of Gout	No. of Carcasses	% Gout	No. tested for Diclofenac	% Diclofenac	Country
OWBV with gout	220	-	25	100	-
OWBV no gout	39	85	13	0	Pakistan
OWBV with gout	10	-	9	100	-
OWBV no gout	3	77	2	0	India and Nepal
LBV with gout	8	-	5	100	-
LBV no gout	4	67	2	0	India

The most consistent finding during vulture necropsies was the presence of extensive visceral gout (an accumulation of uric acid crystals in the tissues), and renal failure and gout were considered the proximate cause of death. Visceral gout was recorded in a high proportion of birds from across India and Nepal: 77% of 13 OWBV and 67% of LBV had gout (Table 2). In Pakistan necropsies of 259 vulture carcasses confirmed this prevalence, with gout indicated extensive organ damage and tubular necrosis within the kidneys. The presence of visceral gout in vulture carcasses collected from across a large expanse of South Asia (Shultz *et al.*, 2004) indicated a common cause for the widespread decline in vultures. Extensive analyses of tissues of dead vultures failed to find significant numbers of birds contaminated at levels likely to have caused death (Oaks *et al.*, 2004a). Novel vulture pathogens including a mycoplasma, virus in a single WBV and a herpes virus in a single LBV were identified

(Oaks *et al.*, 2004b; Cardoso *et al.*, 2005). Avian malaria has also apparently been reported from two OWBV in Maharashtra. However, while vultures (and other wild birds) undoubtedly carry a range of known and novel diseases, there is nothing to suggest that these played a significant part in the declines, as the prevalence of disease was only detected in a very small proportion of birds. Vultures are likely to have carried these diseases for a long time and prior to the declines, and there is no evidence to suggest these pathogens were associated with or caused visceral gout; the clinical finding linked with 84% of deaths (n=284 birds).

Domestic ungulate carcasses from the most common food source for vultures in South Asia and the team working in Pakistan hypothesized that contamination of food sources may be responsible for the decline. Total 74 veterinarians and veterinary pharmacies were surveyed to identify livestock drugs on sale that were potentially nephrotoxic

and capable of being absorbed orally. NSAIDs fitted these criteria, with reports of nephrotoxicity and renal disease associated with NSAID use in both mammals and birds (Oaks *et al.*, 2004a). The only NSAID in widespread use was diclofenac, which became available for veterinary use in India in 1989-90 and widely used from the mid 1990s; the timing of which coincides with the estimated start of the vulture declines in 1995 or earlier (Cuthbert *et al.*, 2006). Kidney tissues from fresh OWBV carcasses revealed diclofenac residues in birds that died with visceral gout. No other NSAIDs were detected (Oaks *et al.*, 2004a). Toxicity testing undertaken on vultures, indicated that of 11 birds dosed by oral gavage, 10 died 48-68 hr after dosing. The only vulture to survive was a OWBV dosed at 0.25 mg/kg.

At necropsy, all dead birds had extensive visceral gout and renal damage (tubular necrosis); the same clinical signs as recorded in necropsies of wild vultures. In addition, a HGV clinically treated with diclofenac died 48-56 hr after dosing, with clinical signs of visceral gout. To test if vultures could consume a lethal dose from treated livestock, buffalo and goats were treated with a veterinary course of diclofenac, slaughtered 4 hr after the last dose and fed to 20 OWBVs (Oaks *et al.*, 2004a). Thirteen of these vultures died, all with extensive visceral gout. All non-treated control birds survived and necropsies of 5 controls did not reveal visceral gout or signs of renal damage. Toxicity to vultures was dose dependent, with an estimated LD₅₀ of 0.225 mg/kg vulture body weight (this LD₅₀ is estimated after the omission of an outlier from the data set, a vulture that apparently received a very low dose of diclofenac but died with gout: inclusion of the outlier gives a lower LD₅₀ of 0.098 mg/kg b wt; Swan *et al.*, 2006b). The LD₅₀ value indicates that the median lethal quantity of diclofenac that needs to be ingested over a period of a few days is around 1.069 mg. There was no indication that dose-dependence was different for diclofenac administered directly by gavage or by ingestion of tissue from treated ungulates.

Since its introduction in 1989-90, diclofenac is one of the most widely used veterinary drugs and industry sources estimated that 10 million animals are treated annually in India (MOEF, 2006). In the cattle that die within 1-2 days of treatment, there is sufficient diclofenac to kill at least 10% of vultures feeding from the carcass (Green *et al.*, 2006). Vulture population modelling with annual adult survival rates set over the range of 0.90-0.97, annual subadult survival from 0-3 yr of 0.77-0.84, first breeding at age 5 yr, annual breeding, and a feeding frequency of 2 to 4 days, indicates that only 1:140 to 1:295 of ungulate carcasses need to contain a lethal diclofenac dose in order for the population to decline at the same rate as OWBV were seen to decline in India (48% a year; Green *et al.*, 2004). That such low levels of contamination (0.34 to 0.70%) can have such a major impact on the population is surprising until it is considered that an individual vulture with an average feeding frequency of three days will feed on ca. 120 occasions in the year, and each time will face the same risk of consuming a toxic dose; for example, if 0.5% of carcasses contain a toxic dose, then after 120 feeds a vulture has a 45% chance of death (death rate = $1 - (1 - 0.005)^{120} = 0.45$).

Analysis of liver samples of ungulates by LC-ESI/MS revealed that 10.1% of livestock carcasses had detectable levels of diclofenac. Diclofenac was found in cattle, water buffaloes, goats and horses (Taggart *et al.*, 2007), with highest levels of prevalence in cattle (14.7%) and higher levels in female (13.5%) versus male animals (4.5%). These results indicate substantial contamination of India's vultures principal food source with a compound (diclofenac) of known toxicity to *Gyps* vultures. For a vulture consuming 1.02 kg of liver (the mass of tissue sufficient to support a OWBV energetic requirements for 3 days; Swan *et al.*, 2006a) the measured levels of diclofenac indicates that 3.2% of carcasses would provide a diclofenac dose in excess of the LD₅₀; a percentage greater than

the proportion of toxic carcasses (0.34 to 0.70%) that were needed to cause the observed rate of decline.

From information on cattle residue levels in different tissues following dosing with diclofenac (liver, kidney, intestine, muscle and fat; Taggart *et al.*, 2006) and the average mass of edible tissues in cattle carcasses, it is possible to estimate the average diclofenac concentration across all edible tissues. These calculations indicate that, averaged across all edible tissues, diclofenac levels are 37.6% of the level measured in liver samples (Green *et al.*, 2007).

Based on the measured diclofenac levels in liver samples from 1848 ungulate carcasses, the estimated diclofenac concentration in all edible tissues, the fitted diclofenac dose to toxicity curve, and the vulture population model, diclofenac levels measured across India in 2004-05 were sufficient to cause populations of OWBV to decline at rates of 80-83% a year, a rate not significantly different to the observed decline rate of 48% a year. The observed diclofenac levels and modeled decline rate demonstrate that diclofenac is the main cause of vulture declines in India and the results are most consistent with diclofenac being the only causal agent responsible for the decline in vulture numbers. The safe alternative drugs such as meloxicam should be used. Until the environment is free from diclofenac, vulture conservation breeding centres, as established by BNHS and Indian Central Zoo Authority, are critical for preventing the extinction of India's critically endangered vultures.

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