

Short communication

Diclofenac is toxic to the Himalayan Vulture *Gyps himalayensis*

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Introduction

Veterinary use of the non-steroidal anti-inflammatory drug (NSAID) diclofenac has been shown to be the major cause of the collapse of populations of three *Gyps* vulture species endemic to South Asia. The White-rumped Vulture *Gyps bengalensis*, Indian Vulture *G. indicus* and Slender-billed Vulture *G. tenuirostris*, have declined by more than 98% in the Indian subcontinent since the early 1990s, and are now all listed as 'Critically Endangered' (IUCN 2004). *Gyps* vultures are exposed to diclofenac through consuming the contaminated carcasses of livestock that have been treated with the drug shortly before death and die from kidney failure, with clinical signs of extensive visceral gout and renal damage. These clinical signs and diclofenac residues have been found in carcasses of wild *G. bengalensis* and *G. indicus*, and in *G. bengalensis* either dosed with diclofenac orally or given tissues from diclofenac treated livestock. Research on White-backed Vultures *G. africanus*, Eurasian Griffons *G. fulvus* and Cape Vultures *G. coprotheres* has established that these three species are about as sensitive to diclofenac as *G. bengalensis*, with birds dying with the same clinical signs of visceral gout and characteristic renal damage. This experimental testing has established that diclofenac is toxic to four species of vultures in the genus *Gyps*, but information on the toxicity of diclofenac to other members of the genus is lacking.

Methods

On 20 November 2005, an injured Himalayan Vulture *G. himalayensis* was found by a member of the public on the outskirts of Kullu village, Himachal Pradesh, India. The bird was captured by villagers and taken on the same day to a state veterinarian of the local forest department. On examination the bird was found to have a broken leg and, in order to treat the associated swelling and pain, the bird was administered an intramuscular injection of 1 ml of an injectable veterinary formulation of diclofenac (Volvaren, diclofenac 25 mg/ml). The mass of the bird (recorded at death) was 6.5 kg indicating that the bird received a diclofenac dose of around 3.8 mg kg⁻¹ vulture body weight. Staff at the Bombay Natural History Society/Haryana State Vulture Conservation Breeding Centre (VCBC) were informed of the bird's capture on the following day and the vulture was transferred to the VCBC arriving at 18h00 on 23 November 2005. The bird was alive and dehydrated on arrival and was immediately given rehydration fluids (100 ml subcutaneous injection of 'Ringer lactate') and, to treat pain, an intravenous injection of the NSAID meloxicam at a dose rate of 2 mg kg⁻¹ (Melonex, Intas Pharmaceuticals Ltd, Ahmedabad). Following death, liver and kidney tissue samples were collected, and tissue residue analysis was undertaken at the University of Aberdeen, UK using a validated HPLC-MS-MS methodology (Oaks *et al.* 2004, Shultz *et al.* 2004). Concentrations of diclofenac are expressed per unit wet weight.

Results and Discussion

The bird died at 20h15 on 23 November 2005, two hours after arrival at the VCBC and 48–56 hours after dosing with diclofenac. Post-mortem examination revealed visceral gout: the same clinical sign observed in other *Gyps* vultures following diclofenac toxicity (Oaks *et al.* 2004, Swan *et al.* 2006a). With the exception of a fracture of the left femur, no other injuries or clinical signs could be found, and the bird was in good condition. Analysis of tissue samples confirmed that the bird was contaminated with diclofenac, with residue levels of 0.087 mg kg⁻¹ and 0.034 mg kg⁻¹ in the kidney and liver tissues, respectively.

The result of this case indicates that diclofenac is toxic to the Himalayan Vulture, adding a fifth species to the list of *Gyps* vultures known to be killed by this compound. The timing of death c. two days after receiving a high dose of diclofenac, the clinical signs of visceral gout, and the absence of any injuries likely to cause the bird's immediate death, are consistent with diclofenac being the cause of death of this individual and closely match the clinical signs of toxicity of diclofenac in other *Gyps* vultures. The evidence for diclofenac toxicity is complicated by the administering of fluids and the NSAID meloxicam two hours before death. However, the meloxicam treatment is very unlikely to have contributed to the death of this vulture for the following reasons. (a) Extensive safety testing has established the safety of meloxicam to other *Gyps* vultures, with 40 birds from three *Gyps* species (*africanus*, *bengalensis* and *indicus*) administered meloxicam at 2 mg kg⁻¹ (Swan *et al.* 2006b, Swarup *et al.* 2007). (b) Clinical treatment of birds also indicates the safety of meloxicam, which has been administered to over 700 individuals from 60 species with no reported death or ill affects, including the treatment of seven *Gyps himalayensis* (Cuthbert *et al.* 2006). (c) Meloxicam treatment did not result in any detectable elevation in the concentration of uric acid in the blood plasma of *Gyps africanus*, *G. bengalensis* and *G. indicus* (Swan *et al.* 2006b, Swarup *et al.* 2007). (d) The timing of death just two hours after meloxicam treatment makes it too rapid for this drug to be responsible for the observed clinical signs of visceral gout (Oaks *et al.* 2004, Swan *et al.* 2006a, Naidoo *et al.* 2009). Even diclofenac treatment, which elevates the concentration of uric acid in the blood plasma by a factor of about ten times, 24 hours after treatment, has little effect on plasma uric acid levels within two hours of treatment, indicating little or no effect on kidney function within this time (Swan *et al.* 2006a).

An additional important caveat of this case is that the vulture received a very high dose of diclofenac: a dose rate 17–39 times greater than the estimated median lethal dose for *G. bengalensis* (Swan *et al.* 2006a) and in excess of the likely maximum level of exposure a vulture could potentially receive in the wild (RSPB unpublished data). However, the time to death and clinical signs were consistent with other cases of diclofenac toxicity in *Gyps* vultures with lower doses.

Some scavenging species appear to tolerate high levels of diclofenac, for example the Turkey Vulture *Cathartes aura*, a New World species unrelated to Old World *Gyps* vultures, exhibited no overt signs of toxicity when dosed with diclofenac at concentrations greater than 100 times the estimated median lethal dose reported for *Gyps* vultures (Rattner *et al.* 2008). However, the discovery that diclofenac is toxic to a fifth species of *Gyps* vultures and the phylogenetic position of these five species each forming a sister relationship with one or more of the remaining *Gyps* species (Johnson *et al.* 2006), strongly suggests that diclofenac is likely to be toxic to all *Gyps* vultures. The genus *Gyps* contains eight species, which includes the three resident Asian species, two migratory species (*G. fulvus* and *G. himalayensis*), and three species within Africa (*G. africanus*, *G. coprotheres* and *G. rueppelli*). The winter ranges of *G. fulvus* and *G. himalayensis* extend into areas of India and Nepal (del Hoyo *et al.* 1994) in which veterinary diclofenac is known to be in widespread use. Increasing numbers of juvenile and sub-adult birds from these two species are now seen in northern India (V. Prakash unpubl. data), presumably because of increased feeding opportunities available since the collapse of resident *Gyps* populations. As a consequence of these movements and the toxicity of diclofenac, northern India is likely to be

acting as a major population sink for the pre-breeding cohorts of *G. fulvus* and *G. himalayensis*, and we therefore expect that long-term declines are now occurring, largely undetected, in the breeding populations of these species. Evidence from the Upper Mustang region of Nepal indicates that these declines may already be happening (Acharya *et al.* 2009). Determining the location and monitoring these breeding areas is a priority, although, as is the case for the three resident *Gyps* species, the essential conservation solution is a complete cessation of the veterinary use of diclofenac and other toxic NSAIDs within the Indian subcontinent.

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