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Short communication

Diclofenac is toxic to the Steppe Eagle *Aquila nipalensis*: widening the diversity of raptors threatened by NSAID misuse in South Asia

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Summary

Three Critically Endangered *Gyps* vultures endemic to South Asia continue to decline due to the use of diclofenac to treat livestock. High nephrotoxicity of diclofenac to *Gyps* vultures, leading to death, has been established by experiment and observation, in four out of five *Gyps* vulture species which occur in South Asia. Declines have also been observed in South Asia's four other non-*Gyps* vulture species, but to date there has been no evidence about the importance of diclofenac as a potential cause. Neither is there any evidence on the toxicity of diclofenac to the Accipitridae other than vultures. In this study, gross and microscopic lesions and diclofenac tissue levels in Steppe Eagles *Aquila nipalensis* found at a cattle carcass dump in Rajasthan, India, show evidence of the toxicity of diclofenac for this species. These findings suggest the possibility that diclofenac is toxic to other accipitrid raptors and is therefore a potential threat to much wider range of scavenging species in South Asia.

Introduction

Three species of *Gyps* vultures endemic to South Asia have undergone catastrophic population declines since the mid-1990s (Gilbert *et al.* 2006, Prakash *et al.* 2007, Chaudhary *et al.* 2012) and are now listed as 'Critically Endangered' (IUCN 2011). A wide range of evidence has established that veterinary use of diclofenac, a non-steroidal anti-inflammatory drug (NSAID), is the main cause of these population declines (Green *et al.* 2004, Oaks *et al.* 2004, Shultz *et al.* 2004). Vultures are exposed to diclofenac when they feed upon carcasses of domestic ungulates treated with this drug shortly before death. Birds consuming a toxic dose die within 1–2 days of exposure from renal failure and have clinical signs of extensive visceral gout, characteristic of NSAID toxicity (Oaks *et al.* 2004, Swan *et al.* 2006), along with diclofenac residues in their kidney and liver tissues (Oaks *et al.* 2004, Shultz *et al.* 2004). To date the toxic effects of diclofenac has been determined in five species of *Gyps* vultures (Oaks *et al.* 2004, Swan *et al.* 2006, Naidoo *et al.* 2009, Das *et al.* 2011) and all eight species in this genus are likely to be susceptible (Johnson *et al.* 2006). Concerns have been raised about the potential impact of diclofenac on other scavenging species in the region, including the Red-headed Vulture *Sarcogyps calvus* and Egyptian Vulture *Neophron percnopterus* that have undergone rapid declines in India over the same period as the *Gyps*

vultures (Cuthbert *et al.* 2006). However, testing of diclofenac on other species thus far has found no evidence for a high sensitivity to this drug to species outside the *Gyps* genus. This includes testing on Turkey Vultures *Cathartes aura* and Pied Crows *Corvus alba* which were unaffected by diclofenac even at very high doses (Rattner *et al.* 2008, Naidoo *et al.* 2011) and domestic chickens *Gallus gallus domesticus* which were only susceptible at doses 50–100 times larger than estimated for the White-rumped Vulture *Gyps bengalensis* (Naidoo *et al.* 2007). Notably, besides *Gyps* species, no other species of raptor from the family Accipitridae has been tested. If other carrion-eater species were found to be sensitive to diclofenac then a much wider range of species could potentially be affected by veterinary misuse of diclofenac in South Asia.

Methods

On 13 February 2012, carcasses of dead raptors were noticed at the Jorbeer cattle carcass dump (latitude N27°57'59.4", longitude E73°22'35.7"), situated 10 km from Bikaner city, Rajasthan, India (Sharma and Gopi Sunder 2009). An investigation into the causes of death was carried out at the site on 17 February 2012 by expert veterinarians who undertook necropsy examinations of two Steppe Eagles *Aquila nipalensis*. All gross lesions were recorded and morbid materials were collected from kidneys, liver, heart, spleen and joints and fixed immediately in vials containing 10% neutral buffered formalin (NBF) for subsequent histopathological examination. Each tissue (0.5 cm thickness) was processed by conventional techniques to obtain 4 µm thick paraffin embedded sections (Luna 1972). Staining was done with routine haematoxylin and eosin (HE) stain and/or with De Galantha stain for demonstration of urate crystals in the sections. Sections were then evaluated for pathological changes associated with nephrotoxicity. Images of representative and consistent changes were captured using a trinocular microscope with attached camera (Leica Microsystems, Wetzlar, Germany). Additional tissue samples from liver, kidney and muscle from one Steppe Eagle were frozen upon collection and brought to the laboratory in cold conditions and were analysed for diclofenac using an indirect competitive ELISA, following procedures detailed in Saini *et al.* (2012).

Results and Discussion

Necropsy examinations indicated the presence of extensive visceral gout in the carcasses of both Steppe Eagles (Figure S1A in the online Supplementary Materials). This finding was supported by histological examinations of kidney tissue sections, which demonstrated renal tubular nephrosis and the deposition of urate crystals (Figure S1B). The synovial membrane of the knee joints also showed uric acid crystals, which were formed into numerous large discs with needle-thin peripheral radiations, and occasional inflammatory mononuclear cell foci (Figure S1B). These results were suggestive of diclofenac toxicity, as they are the same clinical signs (extensive visceral gout) and same histopathology (lesions in the kidneys, liver and spleen with extensive uric acid crystal deposition) as reported for diclofenac toxicity in *Gyps* vultures (Oaks *et al.* 2004, Swan *et al.* 2006, Daset *et al.* 2011). Testing of kidney tissue samples of one Steppe Eagle with indirect competitive ELISA indicated this bird contained residues of diclofenac, with diclofenac levels estimated at 0.051 µg g⁻¹ in this bird's kidney tissues. This residue level of diclofenac in the Steppe Eagle is comparable with those found within kidney and liver tissues of wild *Gyps* vultures found dead with extensive visceral gout, where diclofenac residues ranged from 0.051–0.643 µg g⁻¹ in Pakistan (Oaks *et al.* 2004) and 0.004–0.160 µg g⁻¹ for vultures from India and Nepal (Shultz *et al.* 2004).

Our findings of extensive visceral gout in the carcasses of two Steppe Eagles and the co-occurrence of gout with diclofenac residues in the one bird tested are the first case of diclofenac related mortality outside the *Gyps* genus. While this result is only correlative, there is a perfect positive association between gout and diclofenac in *Gyps* vultures: with all birds dying from gout having diclofenac residues; and birds dying without gout containing no residues (Oaks *et al.* 2004, Shultz *et al.* 2004). Consequently, the finding of gout and diclofenac in Steppe Eagles is given added credibility by the 100% association in *Gyps* vultures. Further experimental testing conclusively

established that oral dosing of *Gyps* vultures with diclofenac and feeding them carcasses of animals dosed with diclofenac will result in mortality and the same clinical signs of visceral gout and diclofenac residue levels (Oaks *et al.* 2004, Swan *et al.* 2006). A similar study is recommended for verifying the toxicity of diclofenac to Steppe Eagles. If such results are borne out, then the finding of diclofenac toxicity in this species has important implications for wintering populations of this species in South Asia and also considerably widens the potential diversity of raptor species, mainly facultative scavengers, that may be affected by diclofenac and other toxic NSAIDs used for veterinary purposes, such as ketoprofen (Naidoo *et al.* 2010). Steppe Eagles wintering in India are seen in large numbers at cattle carcass dumps (Sharma and Gopi Sunder 2009) and frequently scavenge livestock carcasses (Naoroji 2006), thereby being exposed to diclofenac residues. Other species of *Aquila* eagles are also known to frequently scavenge at carcass dumps in the region including the Tawny Eagle *A. rapax*, Eastern Imperial Eagle *A. heliaca* and Indian Spotted Eagle *A. hastata* (Sharma and Gopi Sunder 2009). For these species, scavenging is particularly important during the winter (Naoroji 2006). If the finding of diclofenac sensitivity in the Steppe Eagle is confirmed then two phylogenetically distinct genera with the Accipitridae family (the *Gyps* vultures and *Aquila* eagles) will be affected by this compound, and potentially other species and genera within the Accipitridae that are known to scavenge on livestock could also be vulnerable. These include the Red-headed, Egyptian, and Bearded Vulture *Gypaetus barbatus* where there is also evidence for rapid population declines in the region (Cuthbert *et al.* 2006, Acharya *et al.* 2010).

In summary, this study reports the first incidence of diclofenac-related mortality to species outside the *Gyps* genus of vultures and we recommend that further experimental testing is undertaken to establish the sensitivity of Steppe Eagles and other *Aquila* species, and to understand the wider threat of veterinary NSAIDs to scavenging raptors within South Asia.

Supplementary Material

The supplementary materials for this article can be found at journals.cambridge.org/bci

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