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Diagnostic Investigation of Vulture Mortality: the Anti-Inflammatory Drug Diclofenac is associated with Visceral Gout

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Since 2000, high mortality rates in adult White-Backed Vultures (*Gyps bengalensis*) in the Punjab Province of Pakistan have led to significant population declines of these birds (Gilbert *et al.* 2002). The majority of these deaths (~ 85%) have been associated with the clinical syndrome of visceral gout (Gilbert *et al.* 2002, Gilbert unpublished data). Histopathologic investigation has shown that visceral gout is due to acute kidney failure. The renal lesions were primarily characterized as severe, acute tubular necrosis, with minimal inflammatory infiltrates. These lesions are most compatible with an acute toxic etiology.

Toxicologic investigations ruled out toxic levels of heavy metals known to be associated with renal failure in birds, including cadmium, lead and mercury (Furness 1996, Pain 1996, Thompson 1996). Although not classically recognized as nephrotoxic, toxic or deficient levels of other heavy metals, including arsenic, copper, iron, manganese, molybdenum and zinc also were not detected. Similarly, there was no evidence of acute intoxication by organophosphate, carbamate, or organochlorine pesticides. Virus isolation results were negative. A novel mycoplasma bacterium was isolated and characterized (Oaks unpublished data). This organism had an overall prevalence of about 30% in both the renal failure and non-renal failure cases, and thus was not associated with kidney failure. Molecular biology (PCR) studies for avian influenza and infectious bronchitis virus, two viruses

recognized as renal pathogens in poultry (Swayne & Slemons 1994, Ziegler 2002), were negative.

With the exclusion of known causes of renal disease and/or acute death in birds, the studies were directed at novel toxins. Since the primary food source for the vultures in Pakistan are domestic livestock, we hypothesized that veterinary drugs used to treat these livestock may be associated with kidney disease in the vultures. We conducted a survey of 74 veterinarians and veterinary drug retailers in the region and compiled a list of 34 drugs that were commonly used. These identified drugs were then screened for candidates that may be responsible for kidney disease in the vultures by applying the following criteria: known to be nephrotoxic in other birds or mammals, absorbed orally since the presumed route of exposure was ingestion, and to be compatible with the recent and widespread decline of the vultures the drug should also be commonly used and new to the market. One drug, diclofenac, met all these criteria. Diclofenac is a non-steroidal anti-inflammatory drug (Todd & Sorkin 1988) used to treat most types of livestock in the Punjab region of Pakistan, and is known to be nephrotoxic in mammals (Murray & Brater 1993). In addition, other related anti-inflammatory drugs such as indomethacin and flunixin have been shown to cause renal failure and visceral gout in other bird species (Nys & Rzasa 1983, Paul-Murphy & Ludders 2001).

Kidney samples from 23 vultures with renal failure and 13 vultures without renal failure (control birds, most of which were known to die of other causes such as trauma, lead poisoning and intestinal foreign bodies) were tested by high performance liquid chromatography and mass spectroscopy for residues of diclofenac. All of the renal failure cases were positive for diclofenac, while none of the non-renal failure cases had diclofenac residues. To verify the toxicity of diclofenac for White-backed Vultures, two non-releasable 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. Both of the high dose birds and one of the low dose vultures died with visceral gout and the same histological lesions as the field cases within 58 hours post-administration. The 100% correlation between disease and diclofenac residues in the field cases, and the ability to reproduce the disease experimentally, strongly implicates diclofenac as the cause of the visceral gout syndrome in Pakistan.

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