
	J	P	E	0	9	5	4	Operator: Xiao Xiuli		Dispatch: 12.08.04	PE: Penny Baker
Journal Name		Manuscript No.		Proofreader: Chen Xiaoming				No. of Pages: 8		Copy-editor: Eva Fairnell	

Journal of Applied  
Ecology 2004  
41, 793–800

# Diclofenac poisoning as a cause of vulture population declines across the Indian subcontinent

RHYS E. GREEN\*†, IAN NEWTON‡, SUSANNE SHULTZ\*§,  
ANDREW A. CUNNINGHAM§, MARTIN GILBERT¶, DEBORAH J. PAIN\*  
and VIBHU PRAKASH\*\*

\*Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire SG19 2 DL, UK; †Conservation Biology Group, Department of Zoology, University of Cambridge, Cambridge CB2 3EJ, UK; ‡Centre for Ecology and Hydrology, Monks Wood Research Station, Abbots Ripton, Huntingdon, Cambridgeshire PE28 2LS, UK; §Institute of Zoology, Zoological Society of London, Regent's Park, London NW1 4RY, UK; ¶The Peregrine Fund, 5668 West Flying Hawk Lane, Boise, ID 83709, USA; and \*\*Bombay Natural History Society, Hornbill House, SB Singh Road, Mumbai–400 023, India

## Summary

1. Rapid population declines of the vultures *Gyps bengalensis*, *Gyps indicus* and *Gyps tenuirostris* have recently been observed in India and Pakistan, continuing at least up to 2003. Surveys indicate annual rates of decline of 22–50% for *G. bengalensis* and *G. indicus* during 2000–03. Previous studies in Pakistan have shown that the non-steroidal anti-inflammatory drug diclofenac causes renal failure and is lethal to *G. bengalensis* when it feeds on the carcass of a domestic animal that received a normal veterinary dose shortly before death. In Pakistan, diclofenac poisoning was found to be by far the most frequent cause of death.

2. A simulation model of vulture demography, described in this paper, demonstrated that the observed rates of population decline could be caused by contamination with a lethal level of diclofenac in a small proportion (between 1 : 130 and 1 : 760) of ungulate carcasses available to vultures.

3. Proportions of adult and subadult vultures found dead or dying in the wild that had signs of diclofenac poisoning were similar to the proportions of deaths expected from the model if the observed population decline was due entirely to diclofenac poisoning. The proportion of the excess mortality required to cause the observed population declines that could be attributable to diclofenac was estimated to be between 71% and 100%, depending on model assumptions. However, across all or most of the plausible range of assumed values for adult survival, the upper 95% confidence limit for the proportion of excess mortality due to diclofenac was 100%. Hence, available data are consistent with diclofenac poisoning being at least the major cause, and possibly the only cause, of rapid population declines of *Gyps* vultures across the Indian subcontinent.

4. *Synthesis and applications.* We recommend that urgent action is taken in the range states of the three currently threatened vulture species to prevent the exposure of vultures to livestock carcasses contaminated with diclofenac. Research is also needed to identify alternative drugs that are effective in livestock and safe for vultures. Efforts should also be made to raise awareness, among veterinarians, pharmacists, livestock owners and the general public, of the problem of diclofenac contamination and the availability of safe alternatives. Captive holding and breeding of vultures until diclofenac is controlled is recommended as a precaution to ensure the long-term survival of the threatened species and to provide a stock of birds for future reintroduction programmes.

*Key-words:* demography, non-steroidal anti-inflammatory drugs, simulation model

*Journal of Applied Ecology* (2004) **41**, 793–800

## Introduction

Since the 1990s vulture populations across the Indian subcontinent have collapsed (Gilbert *et al.* 2002; Prakash *et al.* 2003; The Peregrine Fund 2004). At least three species have been affected: the oriental white-backed vulture [OWBV; *Gyps bengalensis* (Gmelin)], long-billed vulture [LBV; *Gyps indicus* (Scopoli)] and slender-billed vulture (*Gyps tenuirostris* Rasmussen & Parry). Populations have declined by more than 95% within about 10 years (Prakash *et al.* 2003; The Peregrine Fund 2004), leading the IUCN–World Conservation Union to list all three species as critically endangered (Hilton-Taylor 2000). Much evidence indicates that the decline of OWBV in Pakistan has been caused by toxic effects of diclofenac, a non-steroidal anti-inflammatory drug used widely on cattle *Bos indicus* L. and water buffalo *Bubalus bubalis* (L.) in the Indian subcontinent to reduce inflammation caused by trauma and infectious disease (Oaks *et al.* 2004). Diclofenac poisoning of wild vultures is thought to occur when they feed on carcasses of treated livestock. Diclofenac causes renal disease in OWBV. In experiments, captive vultures died after feeding on tissues of domestic animals that had received a normal veterinary dose of the drug a few hours before death. At post-mortem examination, these birds showed extensive visceral gout: deposits of uric acid on and within internal organs due to kidney failure (Oaks *et al.* 2004).

A high proportion (85%) of OWBV found dead and dying in Pakistan had extensive gout. All kidney samples analysed from birds with gout contained residues of diclofenac, whereas none of the samples from birds without gout did so (Oaks *et al.* 2004). These findings led Oaks *et al.* (2004) to propose that that diclofenac was the cause of the OWBV population decline in Pakistan and possibly elsewhere in the subcontinent, wherever diclofenac is widely used in the treatment of livestock. Recent post-mortem and diclofenac data collected from dead or dying OWBV and LBV in India and Nepal also show a high proportion with gout, and the same perfect association of gout with diclofenac in both species (Shultz *et al.* 2004) as found for OWBV in Pakistan. However, neither of these studies established whether diclofenac poisoning is sufficient, on its own, to cause the very rapid rates of population decline observed across the subcontinent. In this study, we used a simulation model of vulture demography and foraging ecology to estimate the proportion of vulture deaths caused by diclofenac that would be expected, at observed rates of vulture population decline, if diclofenac was the only cause of the declines. We then compared the modelling results with post-mortem data from Pakistan, India and Nepal. We also used the model, with post-mortem and population survey data, to estimate the proportion of excess mortality, beyond that expected in a stable population, that is caused by diclofenac.

## Methods

### COLLECTION OF VULTURE CARCASSES, POST-MORTEM EXAMINATIONS AND DICLOFENAC ANALYSES

OWBV and LBV were collected dead or dying (maximum period between collection and death, 3 days) from six Indian states (Assam, Gujarat, Haryana, Madhya Pradesh, Maharashtra and Rajasthan) and Nepal between August 2000 and February 2004. The birds were collected opportunistically in states where permits had been issued. Although we cannot be sure they formed a representative sample, we have no reason to believe they were biased with regard to cause of death. Data were collected from five subadult (> 1 year but not adult) and eight adult OWBV and four subadult and six adult LBV, with age classes distinguished from plumage characteristics. Post-mortem examinations for visceral gout and analyses of kidney and/or liver for diclofenac were carried out as described elsewhere (Cunningham *et al.* 2003; Shultz *et al.* 2004). The presence/absence of both gout and diclofenac residues was determined for 15 birds, gout only for seven birds and diclofenac only for one bird.

Areas in and around breeding colonies of OWBV in the Punjab province of Pakistan were searched regularly for dead and dying vultures between 2000 and 2002. Carcasses of 82 subadult and 177 adult OWBV were recovered in good enough condition for determination of presence or absence of gout. Post-mortem examinations were carried out as described elsewhere (Gilbert *et al.* 2002; Oaks *et al.* 2004).

We excluded data for juveniles (< 1 year old) because dead samples included many collected from around breeding sites that probably included a high proportion of young that died soon after fledging. The causes of their deaths may not be typical of all juvenile deaths throughout the year.

### POPULATION TRENDS

Population trends of OWBV and LBV in India were measured using 397 counts made in 2000, 2002 and 2003 along 155 road transects distributed widely across northern India. Methods and transects are described in Prakash *et al.* (2003), although the network of transects they reported was subsequently expanded considerably. Every transect included was surveyed in at least 2 years (93, 155 and 149 transects, totalling 11 183, 18 978 and 18 553 km in road length, surveyed in 2000, 2002 and 2003, respectively, were included in this study). To allow for transects with missing data in 1 year, log-linear Poisson regression models were fitted, with transect modelled as a factor and years elapsed since 2000 as a covariate. The rate of population change,  $\lambda$ , was calculated as  $e^b$ , where  $b$  is the regression coefficient for elapsed years. The significance of the difference in  $b$  between OWBV and LBV was tested by a likelihood

ratio test, comparing (i) a model with transect, species and years elapsed as main effects and the transect–species interaction with (ii) a model with these effects plus the species–years elapsed interaction. Overdispersion was allowed for using the ratio of residual deviance to residual degrees of freedom (9.09; Crawley 1993). *Gyps tenuirostris* has only been separated taxonomically from LBV recently (Rasmussen & Parry 2001) and the two species were counted separately only in 2002 and 2003. However, the proportion of *G. tenuirostris* in the counted sample of both species combined in these 2 years was very small (1.7%, 23/1373), so we analysed data for the two species together as if they represented LBV alone.

The population trend of OWBV in the Punjab province of Pakistan was measured using counts of active nests in the 2000–01, 2001–02, 2002–03 and 2003–04 breeding seasons, at 16 localities widely distributed across the study area and thought to include most of the breeding population (The Peregrine Fund 2004, map A). All localities were surveyed in 2000–01 and 2003–04 and six and four of these localities were also surveyed in 2001–02 and 2002–03, respectively. Estimation of rate of population change,  $\lambda$ , was carried out as described above for road transect counts.

#### DEMOGRAPHIC MODEL

Using assumptions about vulture demographic rates and foraging ecology based on scientific literature and expert opinion, it is possible to estimate the rate of population change and proportion of vultures killed by diclofenac in relation to the proportion of ungulate carcasses that contain a lethal level of diclofenac. We assume that a constant proportion,  $C$ , of the carcasses available to a vulture on each day of the year carries sufficient diclofenac to kill it if it were to feed. If the vulture selects carcasses at random with respect to their level of contamination, the expected annual probability per year,  $S_D$ , of it escaping being killed by diclofenac would be  $(1 - C)^{365/F}$ , where  $F$  is the average interval in days between feeding sessions.  $F$  has not been estimated from field data, but is likely to be in the range 2–4 days (D. Houston, personal communication). Further evidence of the plausibility of this range is that *Gyps coprotheres* (Forster) can eat a single meal capable of supporting its energetic requirements for about 3 days (S. Piper, personal communication). Assuming an annual survival rate of adult vultures,  $S_O$ , before the population decline, the overall annual survival rate,  $S$ , of adults is  $S_O \times S_D$ . We know of no estimates of  $S_O$  for OWBV or LBV, but  $S_O = 0.987$  for *Gyps fulvus* (Hablizl) in France (Sarrazin *et al.* 1994). This high estimate is for an increasing, reintroduced population that was being managed using supplementary feeding and protection to enhance its viability. Supplementary feeding is known to enhance the survival rate of at least immature *G. coprotheres* (Piper, Boshoff & Scott 1999). Adult survival rates in the range 0.90–0.97 are more typical for large-bodied, long-lived bird species, including large raptors, many

of which, like *Gyps*, raise a maximum of one young per year (Newton 1979; Ricklefs 2000; Sæther & Bakke 2000). Hence, we use 0.90, 0.95, 0.97 and 0.99 to cover a plausible range of values of  $S_O$  for *Gyps* vultures in the Indian subcontinent, although we consider 0.90–0.97 spans the most likely range.

By an argument similar to that used for adults, the probability of an immature vulture escaping death by diclofenac poisoning between fledging and breeding at  $B$  years of age is  $(1 - C)^{365 \times B/F}$ . We assume that breeding can only be successful if both parents survive for the whole of the breeding season, which has duration  $M$  days. The probability of both members of a breeding pair escaping death by diclofenac poisoning during a breeding attempt is  $(1 - C)^{2 \times M/F}$ . Vulture chicks might also be killed if they are fed diclofenac-contaminated tissues, but at least one of their parents might then also be expected to die, so we ignore that effect. We use values of  $B = 5$  years and  $M = 160$  days from the literature for *G. fulvus* (Cramp & Simmons 1980). In the stable population of adults assumed to exist before the decline began, annual recruitment of new adults in year  $i$  per breeding adult alive in year  $i - B$  would need to be  $(1 - S_O)$  to replace losses of adults from causes other than diclofenac poisoning. Combining the effects of diclofenac on breeding success and pre-reproductive survival, and assuming no density-dependence in demographic rates, the number of young adults,  $R$ , recruiting to the breeding population per adult alive in the year of their birth is given by  $R = (1 - S_O) \times (1 - C)^{2 \times M/F + 365 \times B/F}$ . The adult population,  $N_t$ , in year  $t$  is  $N_t = N_{t-1} \times S + N_{t-B} \times R$ .

For this model, the geometric rate of population change,  $\lambda$ , for the adult population is then given by  $\lambda = S + R \times \lambda^{1-B}$ . Given an observed value of  $\lambda$  from population surveys and assumed values of other parameters,  $C$  is calculated numerically.

The modelled proportion,  $K_{Dad}$ , of dead adults killed by diclofenac is  $\log(S_D)/\log(S_D \times S_O)$ . We also have data on causes of death of subadults, but the proportion of dead subadults killed by diclofenac can only be modelled if the annual survival rate of this age group in the absence of diclofenac is specified. The annual survival rate of immature (first 3 years of life) *G. fulvus* in France was 0.858, 13% lower than the annual survival of adults in the same population (Sarrazin *et al.* 1994). We therefore assume that the annual survival of subadult OWBV and LBV in the absence of diclofenac is  $0.87 \times S_O$ . The modelled proportion  $K_{Dsubad}$  of dead subadult vultures killed by diclofenac can then be written as  $\log(S_D)/\log(0.87 \times S_D \times S_O)$ .

We evaluate the fit of the model to the observed proportions of adult and subadult vultures with signs of diclofenac poisoning in each of the three country  $\times$  species examples by calculating the difference in deviance under the fitted model and the full model and comparing it with  $\chi^2$ , with  $3 \times 2 = 6$  degrees of freedom (Collett 1991). We also calculate the Pearson correlation coefficient between observed and modelled proportions,

weighted by sample size. A one-tailed *t*-test of the statistical significance of this correlation is considered appropriate because prior information indicates that only a positive relationship is meaningful.

ESTIMATING THE PROPORTION OF EXCESS DEATHS CAUSED BY DICLOFENAC

We expand the model to include a new hypothetical unknown mortality factor, different from diclofenac poisoning, that was not operating before the decline. This is to evaluate the possible contribution of mortality factors, other than diclofenac, that have been suggested previously (Cunningham *et al.* 2003). We model the effects of this factor in the same way to those of diclofenac by assuming that full-grown vultures have a daily probability *U* of dying from it and an annual probability  $S_U = (1 - U)^{365}$  of escaping death caused by this factor. Under this model, the method described above to calculate *C*, and hence  $S_D$ , for a given  $\lambda$ , instead gives the expected value  $E(S_U \times S_D)$  of the product  $S_U \times S_D$ . The expressions for  $K_{Dad}$  and  $K_{Dsubad}$  become  $\log(S_D)/\log(S_U \times S_D \times S_O)$  and  $\log(S_D)/\log(0.87 \times S_U \times S_D \times S_O)$ , respectively. We use observed numbers of dead or dying adult vultures recorded with ( $N_{Dad}$ ) and without ( $N_{NDad}$ ) visceral gout and/or diclofenac contamination and equivalent numbers,  $N_{Dsubad}$  and  $N_{NDsubad}$ , of subadults to estimate  $S_U$  by a maximum-likelihood approach in which we obtain numerically the value of  $S_U$  that maximizes  $N_{Dad} \times \log[\log(E(S_U \times S_D)/S_U)/\log(E(S_U \times S_D) \times S_O)] + N_{NDad} \times \log[1 - \log(E(S_U \times S_D)/S_U)/\log(E(S_U \times S_D) \times S_O)] + N_{Dsubad} \times \log[\log(E(S_U \times S_D)/S_U)/\log(0.87 \times E(S_U \times S_D) \times S_O)] + N_{NDsubad} \times \log[1 - \log(E(S_U \times S_D)/S_U)/\log(0.87 \times E(S_U \times S_D) \times S_O)]$ .

The  $E(S_U \times S_D)$  are obtained from the values of *C* calculated using the demographic model for a given vulture population as described above. The modelled proportion of excess deaths, beyond those expected from mortality at the pre-decline level, that are caused by diclofenac rather than the unknown factor, is then given

by  $1 - \log(S_U)/\log(E(S_U \times S_D))$ . This proportion is constrained not to exceed 1. We obtain 95% confidence limits as the bounds of the central 9500 estimates from 10 000 bootstrap samples taken from the observations of circumstances of death of adult and subadult vultures.

Results

OBSERVED RATES OF POPULATION DECLINE 2000–03

We restricted our analyses of population trend to the period 2000–03 because this was the period in which we knew the causes of death in vultures. In India, numbers of OWBV counted per kilometre of transect declined from 0.098 in 2000, through 0.060 in 2002, to 0.012 in 2003. The rate of population change,  $\lambda$ , averaged over this period by Poisson regression, was 0.520 (a 48% decline per year). For LBV, the encounter rates in the 3 years were 0.066, 0.051 and 0.022 birds transect km<sup>-1</sup> and  $\lambda$  was 0.775 (a 22% decline per year). The rate of decline was significantly slower for LBV than OWBV ( $\chi^2 = 22.44$ , 1 degree of freedom,  $P < 0.001$ ). Surveys of active OWBV nests in the Punjab province of Pakistan (The Peregrine Fund 2004) showed a decline from 2292 to 251 between the 2000–01 and 2003–04 breeding seasons and  $\lambda$  was 0.500 (a 50% decline per year).

LEVELS OF DICLOFENAC CONTAMINATION REQUIRED TO CAUSE POPULATION DECLINES

We used the demographic model (see Methods) to estimate the rate of population change that would result if a vulture population that was stable before the introduction of diclofenac was then exposed to contaminated carcasses. We calculated the rate of population change for a specified proportion, *C*, of the ungulate carcasses available to vultures that was contaminated with a lethal level of diclofenac. An example of the model output is given in Fig. 1 for an adult survival rate before the

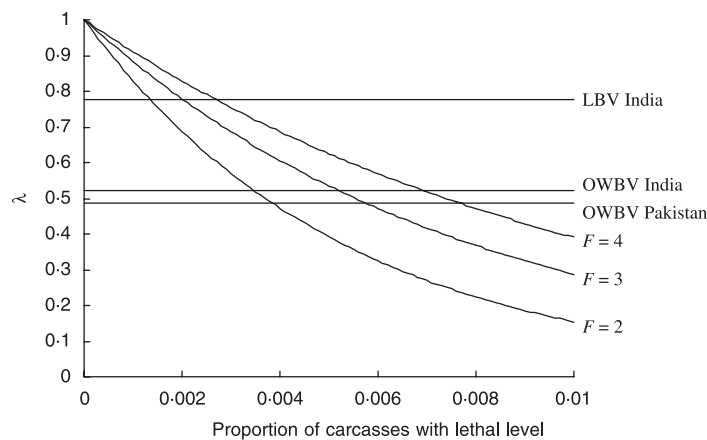


Fig. 1. Modelled relationship of the rate of population change,  $\lambda$ , to the proportion of ungulate carcasses contaminated with a lethal level of diclofenac for three assumed values of the feeding interval, *F*. Adult survival rate before the population decline and in the absence of diclofenac is assumed to be 0.95. Horizontal lines show values of  $\lambda$  estimated from population survey data for 2000–03.

**Table 1.** Modelled percentages of ungulate carcasses with lethal levels of diclofenac required to cause population declines at rates observed for OWBV and LBV in India and Pakistan in 2000–03. For each population, results are given for four values of the adult survival rate in the absence of diclofenac,  $S_o$ , and three values of the feeding interval in days,  $F$ 

	$F$	Percentage of carcasses with lethal level			
		$S_o = 0.90$	$S_o = 0.95$	$S_o = 0.97$	$S_o = 0.99$
LBV India	2	0.132	0.135	0.137	0.138
	3	0.198	0.202	0.205	0.208
	4	0.263	0.271	0.273	0.277
OWBV India	2	0.339	0.347	0.349	0.350
	3	0.508	0.521	0.526	0.533
	4	0.677	0.693	0.699	0.700
OWBV Pakistan	2	0.360	0.368	0.372	0.376
	3	0.538	0.551	0.558	0.564
	4	0.730	0.734	0.743	0.751

decline of  $S_o = 0.95$ . The horizontal lines on this graph cut the modelled curves at values of  $C$  that are sufficient to account fully for the three examples of observed population declines. These critical values of  $C$  vary according to the assumed values of feeding interval  $F$  and  $S_o$  (Table 1) and are more sensitive to the choice of  $F$  than to  $S_o$ . For LBV in India, modelled rates of population decline are consistent with the observed rates if the proportion of ungulate carcasses with a lethal concentration of diclofenac is between 1 : 360 ( $F = 4$ ,  $S_o = 0.99$ ) to 1 : 760 ( $F = 2$ ,  $S_o = 0.90$ ). Critical values of  $C$  are higher for OWBV because of the higher observed rates of population decline. For OWBV in India, from about 1 : 140 ( $F = 4$ ,  $S_o = 0.99$ ) to 1 : 300 ( $F = 2$ ,  $S_o = 0.90$ ) carcasses are required to carry a lethal concentration of diclofenac; the equivalent figures for OWBV in Pakistan are 1 : 130–1 : 280 (Table 1). The proportion of adults that the model indicated would be killed each year by levels of diclofenac contamination required to produce the observed declines was high and varied only slightly with the assumed values of  $S_o$  and  $F$ . For LBV in India about 22% of adults would be expected to die from diclofenac poisoning per year, and for OWBV the expected percentages would be 47% for India and 49% for Pakistan.

#### COMPARISON OF OBSERVED AND EXPECTED PROPORTIONS OF DEATHS ATTRIBUTABLE TO DICLOFENAC POISONING

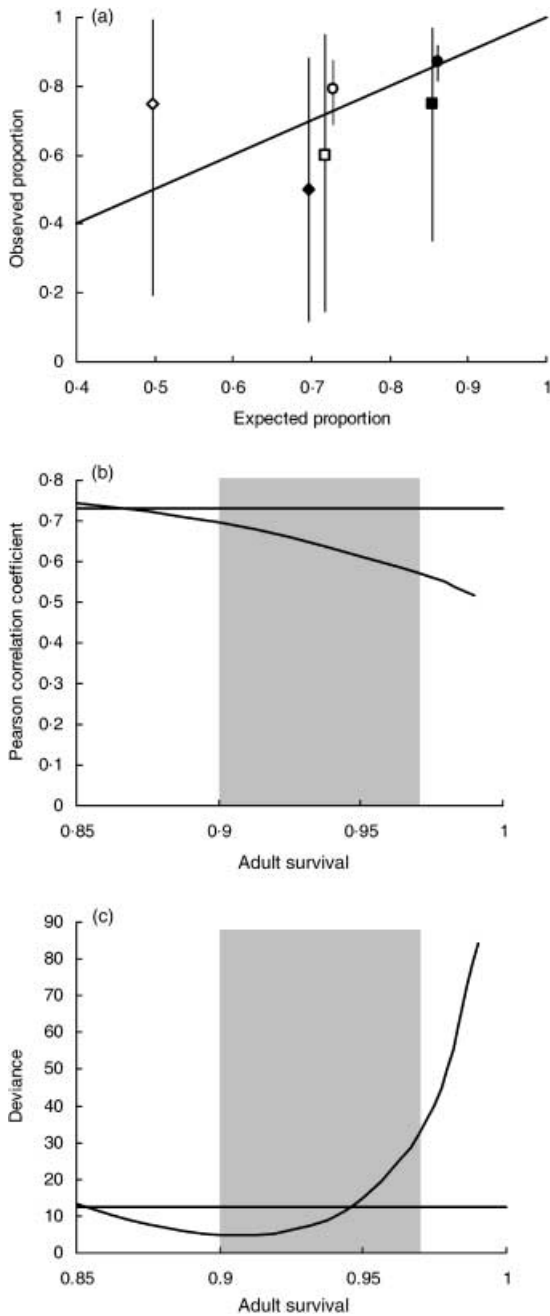
We used the demographic model to calculate the expected proportion of vulture deaths caused by diclofenac when the population was declining at the observed rates, assuming that the decline was caused entirely by diclofenac. The results were not affected by the value of  $F$  used, but were higher if  $S_o$  was high. These expectations from the model were compared with the proportions of vultures found dead or dying in the field that had visceral gout and/or diclofenac contamination. Because the studies of Oaks *et al.* (2004) of OWBV in Pakistan and Shultz *et al.* (2004) of OWBV and LBV in India and Nepal both found a

perfect association between the occurrence of extensive gout and diclofenac residues in the kidneys or liver, we took the proportion of birds with gout, diclofenac residues or both as representing the proportion likely to have been killed by diclofenac poisoning.

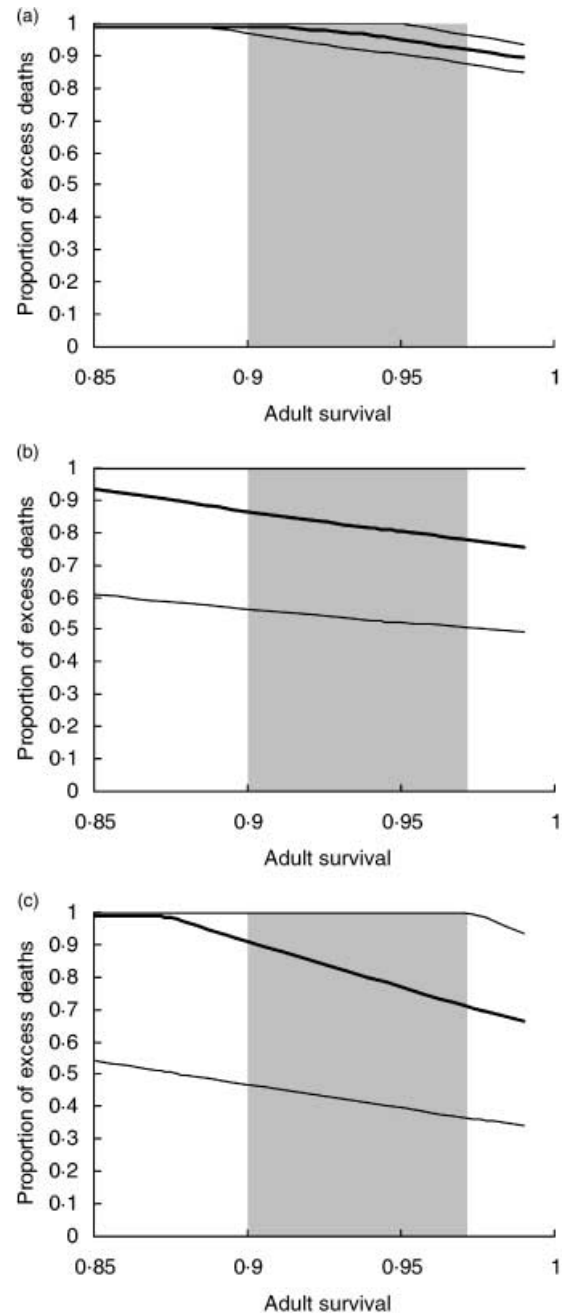
The match between observed and modelled proportions of dead vultures with signs of diclofenac poisoning varied with the assumed value of adult survival. Overall agreement was acceptable (goodness-of-fit  $P > 0.05$ ) provided that  $S_o$  was between 0.85 and 0.95, and was best ( $P = 0.58$ ) when  $S_o = 0.907$  (Fig. 2c). The strength of the correlation, weighted for sample size, across countries, species and age groups ( $n = 6$ ), between the observed and modelled proportions of birds with signs of diclofenac poisoning, declined with increasing  $S_o$  (Fig. 2b). The correlation was statistically significant ( $P < 0.05$ ) only for  $S_o \leq 0.88$ , but remained reasonably high at all values of  $S_o$ . The error bars on the data points in the non-significant ( $r = 0.695$ ,  $P = 0.07$ ) relationship shown in Fig. 2a for  $S_o = 0.90$  illustrate the low precision of the estimates of observed proportions for LBV and OWBV in India and Nepal due to small sample sizes. This, together with the small number ( $n = 6$ ) of country  $\times$  species  $\times$  age classes available, restricts the power of the test.

#### WHAT PROPORTION OF EXCESS MORTALITY CAN BE ACCOUNTED FOR BY DICLOFENAC POISONING?

An extended version of the demographic model postulates the existence of a new, unknown cause of death of full-grown vultures, in addition to diclofenac, and is used to estimate the relative contributions of diclofenac and this hypothetical unknown factor to the excess mortality required to produce the observed population declines (see Methods). Within the most plausible range of  $S_o$  (0.90–0.97), the estimated proportion of excess mortality attributable to diclofenac was high for all three country  $\times$  species examples (Fig. 3). For OWBV in Pakistan, 100% of excess mortality was attributed to diclofenac at  $S_o = 0.90$ , falling to 92% at  $S_o = 0.97$ . For



**Fig. 2.** (a) Correlation between observed proportions of subadult (open symbols) and adult (filled symbols) vultures with extensive visceral gout and/or diclofenac residues and modelled proportions of dead birds killed by diclofenac expected if observed population declines are entirely due to diclofenac poisoning. An adult survival rate of 0.90 before the decline and in the absence of diclofenac is assumed. The line represents identity between observed and modelled values. Each point represents a species  $\times$  country combination; OWBV in India (squares), LBV in India (diamonds), OWBV in Pakistan (circles). Vertical bars are exact binomial 95% confidence intervals (Diem 1962). (b) Correlation coefficient between observed and expected values in relation to the assumed value of adult survival rate. The horizontal line shows the value significant at  $P = 0.05$ . (c) Deviance measure of lack of fit of the model to the data in relation to the assumed value of adult survival rate. Values above the horizontal line show significantly poor fit at  $P > 0.05$ . Shaded areas in (b) and (c) show the most plausible range of adult survival rate (0.90–0.97).



**Fig. 3.** Relationship of the estimated proportion of excess deaths that were caused by diclofenac to the assumed value of adult survival rate before the population decline and in the absence of diclofenac for (a) OWBV in Pakistan, (b) OWBV in India and (c) LBV in India. Thick lines show the maximum-likelihood estimate and thin lines its 95% confidence interval. Shaded areas show the most plausible range of adult survival 0.90–0.97.

OWBV in India the equivalent figures were 87% and 78%, and for LBV in India they were 91% and 71%. The lower 95% confidence limits of these percentages also indicated that, even after allowing for uncertainty, diclofenac was a major cause of excess mortality; the percentages for  $S_o = 0.90$  and  $S_o = 0.97$  were 97% and 86% for OWBV in Pakistan, 56% and 51% for OWBV in India and 47% and 37% for LBV in India. The upper 95% confidence limits included 100% of the excess

mortality being attributable to diclofenac across the whole of the most plausible range of  $S_0$  for OWBV and LBV in India and up to  $S_0 = 0.94$  for OWBV in Pakistan (Fig. 3).

### Discussion

Our analyses of population trends show that vulture declines occurred at a rapid rate up to 2003 and indications are that the decline is continuing. The results of Prakash *et al.* (2003) for northern India in the period 1992–2000 indicate maximum values for the annual rate of population change  $\lambda$  of 0.67 for OWBV and 0.73 for LBV (declines of 33% and 27% year<sup>-1</sup>, respectively). The results for this region since 2000 show that the declines have continued at a broadly similar rate ( $\lambda = 0.52$  for OWBV and 0.78 for LBV). A lower value of  $\lambda$  for the more recent period cannot be taken to indicate an acceleration of the rate of decline because the decline may have begun later than 1992. Data collected by The Peregrine Fund (The Peregrine Fund 2004) give a rate of decline for OWBV in Pakistan similar to that for OWBV in India ( $\lambda = 0.50$ ).

The demographic model indicates that a low incidence of contamination of ungulate carcasses available to vultures with lethal levels of diclofenac (0.13–0.75%, depending on model assumptions and vulture population) is sufficient for diclofenac poisoning to be the sole cause of the observed vulture declines. An implication of this result is that it may be difficult to establish, from field surveys of diclofenac levels in ungulate carcasses, that the proportion,  $C$ , contaminated with a lethal concentration is sufficient to account for the decline. To demonstrate that  $C$  was significantly lower than the levels required to cause the observed vulture population declines would require that no carcass with a lethal diclofenac concentration is found in a random sample of 400–2300 carcasses (one-sided  $P$ -value = 0.05; range of carcass numbers includes the extreme values of  $C$  in Table 1). This does not mean that such surveys are not worth doing, but they may require a large number of samples that must be representative of carcasses used by vultures with respect to geographical location, circumstances of ungulate death and disposal, ungulate species and time since death.

Density-dependence of demographic rates would cause the rate of population decline at a given level of contamination to be lower than that predicted by the model, although density-dependence would have to be strong to make much difference to the conclusions. Even in the absence of competition and with all other environmental factors optimal, *Gyps* vultures cannot rear more than one juvenile to independence per year, and the age of first breeding is unlikely to be reduced by more than about 2 years, so there is limited scope for density-dependent effects on these life-history parameters to reduce rates of decline. There is no indication that the rate of population decline has slowed recently, even with populations at less than 1% of their pre-decline

level (for OWBV in India), so it is unlikely that there are strong density-dependent effects in these populations.

Comparison between observed proportions of dead vultures that had symptoms of diclofenac poisoning and modelling results provides a useful test of the hypothesis that diclofenac poisoning is the sole or main cause of the population decline. The validity of the comparison depends upon the assumption that the causes of death in birds found dead or dying were representative of those of all birds. Dead birds were collected during searches by research staff or after reports from the public. Bias with respect to cause of death cannot be ruled out, but we have no reason to believe that it occurred.

The observed proportions of dead adult and sub-adult vultures with visceral gout and/or diclofenac contamination were broadly similar in India and Nepal and closely similar in Pakistan, to the proportions of deaths expected to be caused by diclofenac if the observed rate of population decline was entirely due to diclofenac. Variation among countries, species and age classes was correlated with modelled values. The slower population decline of LBV than OWBV was associated with a lower proportion of dead LBV with gout and/or diclofenac. This might occur because of a lower susceptibility of LBV to diclofenac or preferences for types of livestock or tissue with lower levels of contamination. In addition, LBV nest on cliffs (OWBV nest in trees) and may find more of their food in remote, mountainous areas, where there is a greater proportion of carcasses of domestic livestock untreated with diclofenac and where wild ungulates are also available. The match in the average level of observed and modelled proportions and the strength of the correlation between them was stronger if adult survival rate before the decline was towards the lower end of the most plausible range. Explicit estimates of the proportion of the excess mortality required to cause the population declines that are attributable to diclofenac range between 71% and 100%, depending on vulture population and model assumptions, and confidence limits included 100% over all or most of the plausible range of the assumed value for adult survival. Hence, we conclude that diclofenac poisoning is at least the major cause, and possibly the only cause, of rapid population declines of OWBV and LBV across the Indian subcontinent.

We therefore recommend that urgent action is taken in the main range states of the three currently threatened species, namely Bangladesh, Bhutan, Cambodia, India, Myanmar, Nepal and Pakistan, to prevent the exposure of vultures to livestock carcasses contaminated with diclofenac. This can probably only be achieved by a ban on veterinary use in livestock species likely to be eaten by vultures. We also recommend that, in countries where vultures feed on carcasses of domestic livestock and where diclofenac is not in widespread veterinary use, action is taken to prevent future exposure of vultures to diclofenac. Prevention or restriction of the veterinary use of diclofenac is likely to increase the veterinary use of other non-steroidal anti-inflammatory drugs, so

research is needed to identify alternative drugs that are effective in livestock and safe for vultures. Efforts should also be made to raise awareness of the problem of diclofenac contamination and the availability of safe alternatives among veterinarians, pharmacists, livestock owners and the general public. Diclofenac may not only be a problem for the currently threatened vulture species, but perhaps also for other *Gyps* species and other scavenging birds. Research is needed to monitor better populations of scavenging birds and their causes of death and to assess their level of exposure to diclofenac and other non-steroidal anti-inflammatory drugs.

Given that diclofenac is widely used in Pakistan and India (Risebrough 2005) and is produced and distributed by many companies, it may take a considerable time to remove it from the food supply of vultures. In view of this, and the continuing rapid vulture population declines, it is probable that some or all of the three threatened *Gyps* species will soon become extinct in the wild, either completely or over large parts of their range. Therefore, captive holding and breeding of vultures until diclofenac is controlled is recommended as a precaution to ensure the long-term survival of the threatened species and to provide a stock of birds for future reintroduction programmes.

### Acknowledgements

We are grateful to D. Houston, G. Mace, L. Oaks, S. Piper, A.R. Rahmani, P. Rothery and R. Watson for advice and access to unpublished information. We thank Sachin Ranade and S. Saravanan for assistance in the field and R.D. Jakati for his inspirational efforts to conserve vultures in Haryana State. The research in India and Nepal was funded by the UK government's Darwin Initiative for the Survival of Species with additional financial support from the Royal Society for the Protection of Birds and the Zoological Society of London. The research in Pakistan was funded by The Peregrine Fund, Gordon and Betty Moore Foundation, Disney Wildlife Conservation Fund and other contributors.

### References

- Collett, D. (1991) *Modelling Binary Data*. Chapman & Hall, London, UK.
- Cramp, S. & Simmons, K.E.L. (1980) *Handbook of the Birds of Europe, the Middle East and North Africa*. Vol. 2. *Hawks to Bustards*. Oxford University Press, Oxford, UK.

- Crawley, M.J. (1993) *GLIM for Ecologists*. Blackwell Scientific Publications, Oxford, UK.
- Cunningham, A.A., Prakash, V., Pain, D., Ghalsasi, G.R., Wells, G.A.H., Kolte, G.N., Nighot, P., Goudar, M.S., Kshirsagar, S. & Rahmani, A. (2003) Indian vultures: victims of an infectious disease epidemic? *Animal Conservation*, **6**, 189–197.
- Diem, K. (1962) *Documenta Geigy Scientific Tables*. Geigy, Macclesfield, UK.
- Gilbert, M., Virani, M.Z., Watson, R.T., Oaks, J.L., Benson, P.C., Khan, A.A., Ahmed, S., Chaudhry, J., Arshad, M., Mahmood, S. & Shah, Q.A. (2002) Breeding and mortality of oriental white-backed vulture *Gyps bengalensis* in Punjab Province, Pakistan. *Bird Conservation International*, **12**, 311–326.
- Hilton-Taylor, C. (2000) *IUCN Red List of Threatened Species*. IUCN, Gland and Cambridge.
- Newton, I. (1979) *Population Ecology of Raptors*. Poyser, Berkhamsted, UK.
- Oaks, J.L., Gilbert, M., Virani, M.Z., Watson, R.T., Meteyer, C.U., Rideout, B.A., Shivaprasad, H.L., Ahmed, S., Chaudry, M.J.I., Arshad, M., Mahmood, S., Ali, A. & Khan, A.A. (2004) Diclofenac residues as the cause of population decline of vultures in Pakistan. *Nature*, **427**, 630–633.
- Piper, S.E., Boshoff, A.F. & Scott, H.A. (1999) Modelling survival rates in the cape griffon *Gyps coprotheres*, with emphasis on the effects of supplementary feeding. *Bird Study*, **46** (Supplement), S230–S238.
- Prakash, V., Pain, D.J., Cunningham, A.A., Donald, P.F., Prakash, N., Verma, A., Gargi, R., Sivakumar, S. & Rahmani, A.R. (2003) Catastrophic collapse of Indian white-backed *Gyps bengalensis* and long-billed *Gyps indicus* vulture populations. *Biological Conservation*, **109**, 381–390.
- Rasmussen, P.C. & Parry, S.J. (2001) The taxonomic status of the long-billed vulture *Gyps indicus*. *Vulture News*, **44**, 18–21.
- Ricklefs, R.E. (2000) Density-dependence, evolutionary optimisation, and the diversification of avian life histories. *Condor*, **102**, 9–22.
- Risebrough, R.W. (2005) Diclofenac: a new environmental poison in south Asia. *Journal of the Bombay Natural History Society*, **2**, in press.
- Sæther, B.-E. & Bakke, Ø. (2000) Avian life history variation and contribution of demographic traits to the population growth rate. *Ecology*, **81**, 642–653.
- Sarrazin, F., Bagnolini, C., Pinna, J.L., Danchin, E. & Clobert, J. (1994) High survival of Griffon vultures (*Gyps fulvus fulvus*) in a reintroduced population. *Auk*, **111**, 853–862.
- Shultz, S., Baral, H.S., Charman, S., Cunningham, A.A., Das, D., Ghalsasi, G.R., Goudar, M.S., Green, R.E., Jones, A., Nighot, P., Pain, D.J. & Prakash, V. (2004) Diclofenac poisoning is widespread in declining vulture populations across the Indian subcontinent. *Proceedings of the Royal Society of London, B* (Supplement), in press. DOI: 10.1098/rsbl.2004.0223
- The Peregrine Fund (2004) The Peregrine Fund, Boise, ID. <http://www.peregrinefund.org/vulture>.

Received 2 June 2004; final copy received 8 July 2004