Rapid population declines and mortality clusters in three Oriental white-backed vulture *Gyps bengalensis* colonies in Pakistan due to diclofenac poisoning

Martin Gilbert, Richard T. Watson, Munir Z. Virani, J. Lindsay Oaks, Shakeel Ahmed, Muhammad Jamshed Iqbal Chaudhry, Muhammad Arshad, Shahid Mahmood, Ahmad Ali and Aleem A. Khan

Abstract The population declines affecting Asian *Gyps* vultures are among the most rapid and geographically widespread recorded for any species. This paper describes the rates and patterns of mortality and population change over 4 years at three Oriental white-backed vulture *Gyps bengalensis* colonies in Pakistan: Dholewala (initially 421 pairs), Toawala (initially 445 pairs) and Changa Manga (initially 758 pairs). Vulture mortality led to the extirpation of two of these colonies (Changa Manga and Dholewala) in 3 years, and a decline of 54.3% in the third. Visceral gout, indicative of diclofenac poisoning, was the largest single cause of death in vultures examined. Annual adult mortality from diclofenac poisoning was significantly positively correlated with annual population declines at each colony indicating a direct causal relationship. Visceral gout occurred in temporal and spatial clusters suggesting multiple point sources of diclofenac exposure. The spatial and temporal distribution of dead vultures and approximate time since death were used to estimate minimum rates at which colonies encountered carcasses with sufficient diclofenac to cause mortality of 1.26–1.88 carcasses per colony per month. By estimating total carcass consumption at each colony, the percentage of carcasses contaminated with diclofenac was calculated as 1.41–3.02%, exceeding the minimum required to have caused the observed population decline. With populations declining by approximately 50% annually, the long term survival of *Gyps* vultures in South Asia will require the removal of diclofenac from vulture food and establishment of captive populations for future restoration once the environment is free from contamination.

Keywords Diclofenac, *Gyps bengalensis*, mortality cluster, Pakistan, population decline, visceral gout, vulture.

Introduction

Gyps vultures in South Asia were considered among the most common large raptors in the world in the mid 1980s (Houston, 1985) but by 2000 three species (Oriental white-backed vulture *Gyps bengalensis*, long-billed vulture *Gyps indicus*, and slender-billed vulture *Gyps tenuirostris*) were categorized as Critically Endangered as a result of rapid population declines (BirdLife International, 2001; IUCN, 2006). These were

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noticed in the mid 1990s and the first numerical evidence was recorded at Keoladeo National Park in India, where numbers of breeding vultures in 1996/1997 were found to be less than half compared to a decade earlier and declined to zero by 1999/2000 (Prakash, 1999). These steep local declines drew attention to a wider problem affecting vulture populations across the Indian subcontinent (Prakash *et al.*, 2003). The proximate cause of the decline was identified as being due to high rates of mortality from renal failure (Gilbert *et al.*, 2002) and renal failure was found to be due to the toxic effect of diclofenac residues in livestock carcasses (Oaks *et al.*, 2004). Correlation between renal failure and diclofenac residues in vultures was documented across the subcontinent (Oaks *et al.*, 2004; Shultz *et al.*, 2004) and modelling showed that very low rates of diclofenac contamination (between 1:130 and 1:760) of livestock carcasses could cause the observed rates of vulture population decline (Green *et al.*, 2004).

Here we provide evidence that death from diclofenac poisoning was the cause of rapid decline in vulture populations at the three largest Oriental white-backed

vulture breeding colonies known in 2000. We provide quantitative measures of rates, patterns, and causes of vulture mortality and breeding population decline over 3 years from 2000 to 2003/2004 that resulted in the extirpation of two colonies and decline by 54.3% of the third. Based upon field observations we estimate the rate of exposure of vultures to carcasses that contain sufficient diclofenac to cause mortality, and compare these results with estimates from population models of the proportion of contaminated carcasses needed to cause the observed rates of decline (Green et al., 2004).

Study area

The studies were at three locations in Punjab province, Pakistan, which held the largest known breeding populations of Oriental white-backed vulture in 2000 (Fig. 1). Dholewala, in Layyah and Muzaffargargh districts, is a linear plantation of sheesham *Dalbergia sisoo* and *Acacia* spp. trees stabilizing an elevated flood defence that runs north from Taunsa Barrage, parallel to the eastern bank of the Indus River. Changa Manga is a forest plantation in Kasur district, eastern Punjab province, with a wide diversity of tree species of variable age, offering a mosaic of suitable vulture nesting habitat. Toawala, north-east of Multan, is a linear plantation of sheesham and *Acacia* spp. lining both banks of the Shujabad Canal, crossing areas of Muzaffargargh, Multan and Khanewal districts. There are three seasons in the southern Punjab: a hot and dry summer (April to June), a hot and wet monsoon (July to

September) and a cool and dry winter (October to March), with variation in mean monthly temperatures from a minimum of 5.6℃ in January rising to a maximum of 42.3℃ in June (Pakistan Meteorological Department, Lahore, unpubl. data). Mean annual rainfall in Multan is 195 mm and is mainly concentrated in the monsoon months, with less regular winter rains occurring during January-March. Vulture courtship and nest building were noted in the study areas as early as August, with the majority of eggs laid in November. All clutches observed consisted of a single egg. Hatching occurred in late December and January with fledging from mid March to mid May.

Methods

Surveys, study period and effort

At Dholewala and Toawala intensive transects were visited at least once weekly, and non-intensive transects at least once monthly. At Dholewala vulture nests were scattered irregularly along 23.8 km of which 5.2 km were selected as an intensive transect and the remaining 18.6 km as non-intensive. The colony at Toawala was 18.7 km long, with 6.4 km designated as an intensive transect and 12.3 km as non-intensive. At Dholewala and Toawala smaller numbers of vulture nests were located in surrounding areas and these were monitored less regularly and provided only incidental observations. At Changa Manga vulture nest trees did not follow linear terrain features and therefore data were
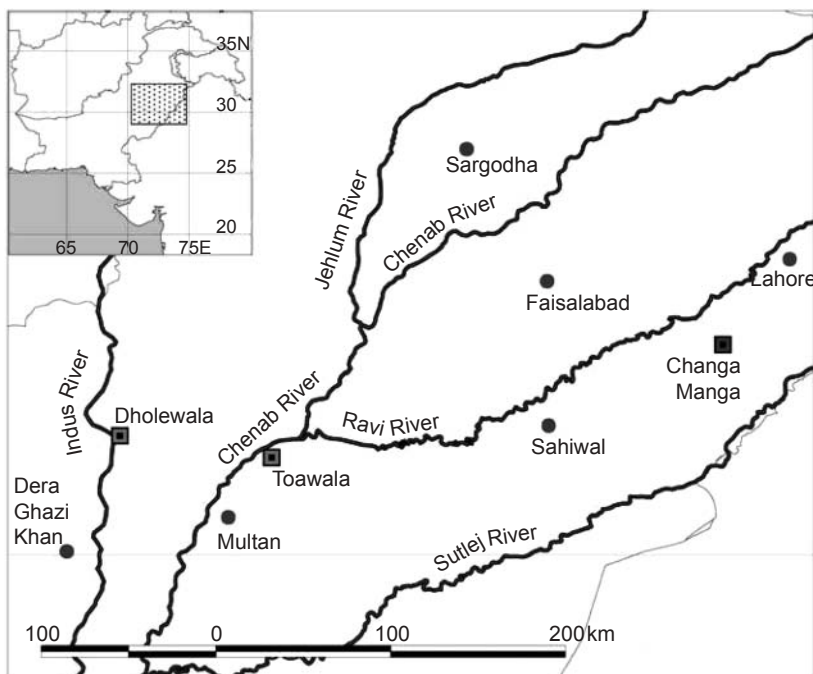

Fig. 1 Punjab Province, Pakistan, showing the three study areas at Dholewala, Toawala and Changa Manga, and major towns and rivers. The inset indicates the location of the main figure.

collected in a plot defined by the highest nesting density at the beginning of the study in 2000, rather than from linear transects. The location of transects and study plots remained the same throughout the study.

Fieldwork commenced in December 2000 at all sites and continued until 27 August 2003 at Dholewala and Changa Manga, and 31 May 2004 at Toawala. Single visits to Changa Manga were made on 18 and 19 November 2003, and to Dholewala on 22 and 24 November 2003 and 21 February 2004 to assess nest status. Regular visits were made throughout the study but no visits were made from 13 August to 24 October 2001 at Dholewala, 20 July to 24 October 2001 at Toawala and 11 July to 20 October 2001 at Changa Manga, when vultures were not breeding. Mean monthly study effort at each site was 14.0 $\pm$ SD 4.2 days at Dholewala, 17.3 $\pm$ SD 4.3 days at Toawala and 14.8 $\pm$ SD 4.1 days at Changa Manga. Study effort was increased at Toawala during the 2003/2004 breeding season with daily visits to the site from 27 November 2003 to 31 May 2004.

Nest status

All nests in intensive and non-intensive transects and plots were located at the start of each season, and their status monitored until the outcome of all nesting attempts had been determined. Nests where eggs were laid were defined as being active, indicated by direct observation of an egg or nestling, or where an adult was observed in a posture consistent with incubation or brooding (Postupalsky, 1974). We could not use occupied nests (Postupalsky, 1974) as the basis of breeding population size because it was impossible to distinguish an occupied nest from an unoccupied nest being used as a roost platform by a vulture from an adjacent nest in the same or nearby tree. As studies in the 2000/2001 season did not commence until incubation was underway the number of active nests at laying was estimated using the observed daily failure rate as described by Gilbert *et al.* (2002). Breeding success in each colony was defined as the ratio of the number of successfully fledged young to the total number of active nests where an egg was laid. Young were considered successfully fledged when observed leaving the nest, or more commonly when activity at the nest ceased on or after the first observation of a fledgling in the colony. Observations of dead chicks, unhatched eggs or cessation of activity prior to the first fledging indicated nest failure.

Vulture counts

A monthly census of vultures in intensive and non-intensive transects was carried out in Dholewala from November 2001 to August 2003, and in Toawala from November 2001 to May 2004. Surveys were carried out by two observers who traversed the length of the colony by motorbike at 10 km h^{-1}, stopping to record vultures observed. It was not practical to traverse the whole study area at Dholewala and Toawala in a single evening, and thus surveys began in intensive transects within 2 hours of sunset, with remaining areas surveyed within 2 hours of sunrise the following day. A census of vultures roosting in intensive plots at Changa Manga was carried out by a single observer on foot on a weekly basis between June 2001 and August 2003. Age class of all vultures encountered was assessed using plumage characters as juvenile (<1 year), subadult (>1 year but not adult) or adult.

Annual mortality estimates from counts of dead vultures (M_{DV})

Dead vultures were located opportunistically at all sites throughout the study and intensive transects were searched at least twice weekly. The ages of dead vultures were assessed and time since death estimated as 0–1, 2–7, 8–30 or >31 days based on decomposition rates. Necropsy examinations were carried out on all birds where decomposition was not too advanced, using the methods described in Gilbert *et al.* (2002). Annual mortality rates were estimated to compare between seasons and sites based on the number of dead vultures of breeding age found within a predefined area of the colony (intensive transects) and observation period (OP) during the breeding season, using annual mortality based on dead vultures, $M_{DV} = 1 - (1 - (DV/(2AN)))^{(365/OP)}$, where mortality measured during OP (in days) was calculated as the proportion of dead adult vultures (DV) located in intensive transects relative to the number of breeding birds (active nests, AN, multiplied by two) present in the intensive transects at the beginning of OP. Mortality during OP was converted to survival during OP by subtracting from 1. Survival during the observation period was standardized to an annual rate for purposes of comparison between sites or years by raising to the power 365/OP, because survival combines multiplicatively. Annual survival was then converted to annual mortality by subtracting from 1.

The observation period began on 1 October or the first visit of the season where monitoring began later than 1 October, and ended on the last fledging date in the colony. Only those adult vultures dying within the observation period were included in the analysis, thus all 2–7 day birds located within the first week, all 8–30 day birds located within the first month and all >31 day birds were assumed to have died before the observation period and were excluded. Estimates of

annual mortality from counts of dead vultures were probably affected by two variables of unknown magnitude: (1) breeding vultures that died in a location away from the colony or not visible to observers were not counted and would reduce mortality; (2) non-breeding adult vultures found dead within the colony were counted and would increase mortality. We made the assumption that these variables were spatially and temporally consistent for comparisons of mortality between years at the same site or between sites.

Breeding population declines (dN_POP)

Population decline in each breeding colony was calculated as the annual decline in number of breeding pairs represented by maximum number of active nests, using $dN_{POP} = (f_{x+1} - f_x)/f_x$, where f was the number of breeding birds at active nests (2AN), f_x was the number of breeding birds in year x and f_{x+1} was the number of birds in the following year.

Temporal and spatial patterns of mortality

Study effort at Toawala was increased during the 2003/2004 breeding season to define the temporal and spatial distribution of vulture mortality more accurately. From 27 November 2003 to 31 May 2004 daily mortality surveys were made along a 42.5 km length of canal (including all nests in intensive, non-intensive and peripheral transects). Surveys were carried out using motorbikes and all accessible vulture carcasses were removed to prevent double counting. All dead vultures that were in a suitable condition were assessed for visceral gout. Temporal and spatial clusters of mortality were identified using the space-time scan technique (Kulldorf et al., 1998). This method scans for clusters of mortality using a temporal and spatial window continually varying in size that is moved across the entire study area and period. The null hypothesis is that the number of dead vultures in any given area and time period will not differ significantly from a Poisson distribution. This technique controls for variations in population density across the study area. The distribution of vultures across the study colony was assumed to mirror the distribution of active nests throughout the study period (27 November 2003 to 31 May 2004). The linear study colony (comprising intensive, non-intensive and peripheral areas) was subdivided into 1.2 km sections, and the breeding population determined as 2AN. Cases were defined as dead or sick vultures located within these sections during daily surveys. The age of vultures was considered to be a covariate as rates of visceral gout increased with age and therefore

analysis was limited to adults and subadults. Analyses were run using SaTScan 4.0 (Kulldorf, 2003), using 9,999 Monte Carlo replications.

Estimating the number of contaminated carcasses

Given the findings of Oaks et al. (2004) it is reasonable to assume that all vultures recorded with visceral gout had ingested lethal doses of diclofenac 1–6 days prior to death. As post mortem interval was classified as 0–1 or 2–7 days it was possible to determine a risk period during which all cases of visceral gout had been exposed. For instance, all birds estimated to have died within the previous day would have been exposed within the previous 7 days. All dead birds considered to have died within the previous week would have been exposed at some point during the previous 2 weeks. If we assume that any overlapping risk periods represent a single exposure event and that necropsy examinations were performed on at least one bird from each exposure event, it is possible to make a conservative estimate of the number of contaminated carcasses encountered at each colony during the study.

The availability of diclofenac at each colony was estimated by expressing the minimum number of contaminated carcasses measured as a proportion of the estimated number of carcasses consumed. To facilitate comparison, minimum numbers of contaminated carcasses were estimated based on necropsies performed on dead vultures found in intensive and non-intensive transects at Dholewala and Toawala, and intensive plots only at Changa Manga. Carcass consumption at each colony can be estimated by multiplying daily food requirements of individual vultures by the sum of the number of vultures present each day (vulture days) during the period when necropsy examinations were performed. If we assume that annual counts of active nests in areas where necropsies were performed (AN) were made on 19 December each year, we can calculate the number of vultures present each day by assuming that numbers of breeding vultures (f) declined exponentially, as vultures present on day D = vultures present on the previous day D_{-1} $((f_x/f_{x+1})^{(1/365)})$, where f was the number of breeding birds at active nests (2AN), f_x was the number of breeding birds in year x and f_{x+1} was the number of birds in the following year.

Houston (1976) calculated that African white-backed vultures Gyps africanus (which are similar in size to Oriental white-backed vultures) require 0.315 kg of tissue to satisfy daily maintenance requirements. This can be used to estimate carcass requirements if we assume a mean carcass weight of 100 kg (equivalent to a yearling buffalo), of which 65% is available to foraging vultures (based on Mundy et al., 1983). This method

underestimates carcass consumption, as it assumes that vultures consumed the minimum number of carcasses required to meet daily maintenance, and ignores the additional energy requirements of reproduction and non-breeding vultures.

Results

Nest status

Numbers of active nests declined in all colonies, with greatest losses in Changa Manga and Dholewala, which were extirpated during the study (Table 1). At Changa Manga active nest counts fell from 198 at egg-laying in the 2000/2001 breeding season (extrapolated from an actual count of 177 in January 2001) to zero by the 2003/2004 breeding season. A survey of the whole forest in 2001 located 758 active nests. A 2-day survey of the whole forest in November 2003 failed to locate any vultures or active nests. A comparable decline was also recorded in Dholewala where numbers of active nests across the intensive and non-intensive transects declined from 421 (projected from an actual count of 412 in December 2000) to only two by November 2003.

By 21 February 2004 both of these nesting attempts had failed. By contrast, declines at Toawala were less severe, although numbers of active nests fell by 54.3% in 3 years from 445 active nests (projected from 418 in December 2000) to 203 in 2003/2004. Breeding success generally declined in successive years at all colonies during the study (Table 1). There was no significant difference in breeding success between colonies during 2000/2001 ($\chi^2 = 3.493$, df = 2, P = 0.174). However, a greater proportion of active nests produced fledglings at Toawala during subsequent breeding seasons. This difference was significant during 2001/2002 ($\chi^2 = 52.953$, df = 2, P <0.001), and not significant at 95% during 2002/2003, when only four of nine active nests produced fledglings at Changa Manga, ($\chi^2 = 5.515$, df = 2, P = 0.063).

Vulture counts

Counts of adult and subadult vultures declined at each site throughout the study, although there was a small peak in numbers in about December each year and at each site (Fig. 2a-c). Quadratic non-linear curves gave an acceptable fit to the data, and regression equations were solved for combined adult and subadult populations on

Table 1 Annual number, breeding status and outcome of Oriental white-backed vulture nests at Changa Manga, Dholewala, and Toawala colonies. See text for details of survey methods.

Study colony, survey method	Breeding season	Total active nests	Success	Fail	Outcome unknown	Breeding success[3]
Changa Manga, intensive	2000/2001	198[1]	112	64	1	0.57
	2001/2002	49	21	28	0	0.43
	2002/2003	9	4	5	0	0.44
	2003/2004	0				
Dholewala, intensive	2000/2001	246[1]	153	84	3	0.62
	2001/2002	151	53	96	2	0.35
	2002/2003	31	12	19	0	0.39
	2003/2004	0				
Dholewala, intensive & non-intensive	2000/2001	421[1]	Not recorded	Not recorded	Not recorded	
	2001/2002	337	125	210	2	0.37
	2002/2003	101	44	57	0	0.44
	2003/2004	2	0	2	0	0.00
Toawala, intensive	2000/2001	230[1]	124	82	19	0.54
	2001/2002	205	148	54	3	0.72
	2002/2003	152	92	60	0	0.61
	2003/2004	108	52	56	0	0.48
Toawala, intensive & non-intensive	2000/2001	445[1]	256	124	38	0.58
	2001/2002	393	265	125	3	0.67
	2002/2003	298	170	128	0	0.57
	2003/2004	203	81	122	0	0.40
Total[2]	2000/2001	889	521	272	42	0.59
	2001/2002	779	411	363	5	0.53
	2002/2003	408	218	190	0	0.53
	2003/2004	205	81	124	0	0.40

[1]Active nest estimates at the point of laying in the 2000/2001 breeding season were extrapolated using known failure rates from active nest counts obtained in December 2000 and January 2001.
[2]Calculated using maximum numbers
[3]The ratio of the number of successfully fledged young to the total number of active nests where an egg was laid
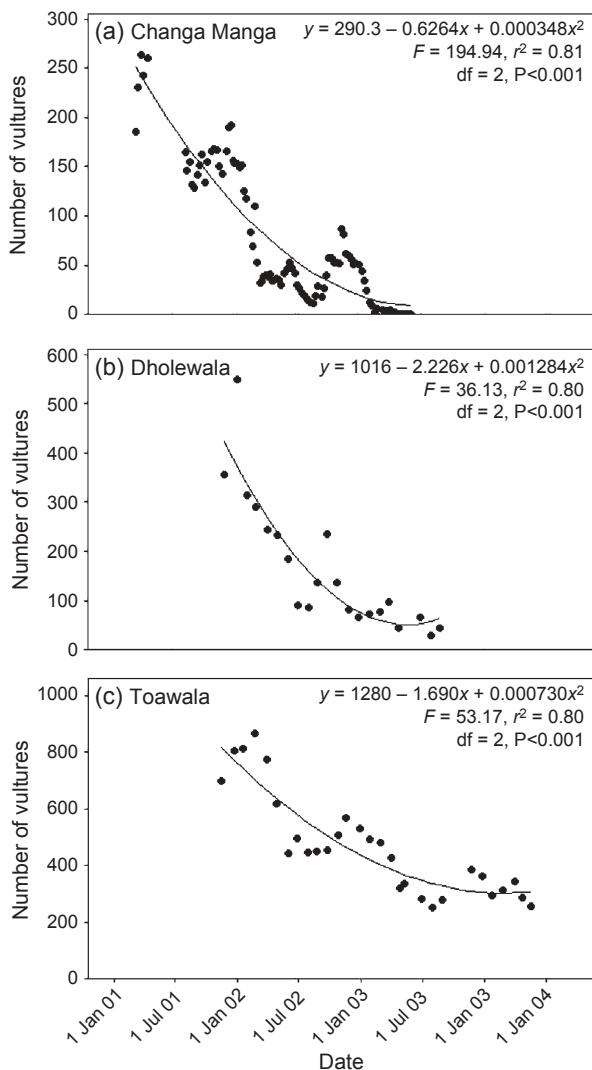
Fig. 2 Combined roost counts of adult and subadult Oriental white-backed vultures made weekly at Changa Manga (a), and monthly at Dholewala (b) and Toawala (c) colonies. Lines of best fit are given as quadratic equations with regression statistics. For the purposes of the quadratic equations, dates were expressed numerically with 1 January 2001 equating to day 1.

1 January each year. Vulture counts declined by 80.1% at Dholewala, 77.8% at Changa Manga and 42.7% at Toawala during 2002, and were similar to the decline in number of active nests between the 2001/2002 and 2002/2003 breeding seasons of 70.0, 81.6 and 24.2% at each site respectively. In 2003 vulture counts at Toawala declined by 29.9% compared with a reduction in active nests of 31.8% over the same period between the 2002/2003 and 2003/2004 breeding seasons. We assumed that vultures counted included both breeding and non-breeding birds and declining counts demonstrate a reduction in the total population comparable to, and concurrent with, the decline in breeding population.

Annual mortality estimated from counts of dead vultures (M_{DV})

During the study 1,729 dead vultures were located in Punjab province, of which 1,518 (87.8%) were collected at our three study sites. Annual mortality (M_{DV}) based on counts of dead vultures increased in successive years at each of the colonies during the study (Table 2) with increases of 0.10–0.26 at Changa Manga and 0.10–0.60 at Dholewala from 2000/2001 to 2002/2003. Mortality rates were consistently lower at Toawala, increasing from 0.02 in 2000/2001 to 0.14 in 2003/2004. Diclofenac residues have been found in all cases of visceral gout in vultures examined in South Asia (Oaks et al., 2004; Shultz et al., 2004) and therefore we assumed that all cases of visceral gout in this study were due to diclofenac poisoning. The proportion of dead adult vultures examined in a defined area during the observation period and found with visceral gout was used to estimate annual mortality due to diclofenac in that area (MD_{DV}). Sufficient numbers of necropsy examinations to determine the annual proportion of adult mortality due to diclofenac were performed on dead adult vultures only in combined intensive and non-intensive transects at Dholewala and Toawala (Table 2). Annual mortality due to diclofenac (MD_{DV}) increased from 0.08 to 0.31 at Dholewala over 2000/2002–2002/2003 and 0.01 to 0.14 at Toawala. Only five necropsy examinations were performed on adult vultures at Changa Manga, of which four (80%) had visceral gout. Assuming that the proportion of dead adults with visceral gout did not change during the study at Changa Manga, estimates of annual mortality due to diclofenac increased from 0.08 to 0.20 over 2000/2001–2002/2003.

Breeding population decline rate (dN_{POP})

The rate of breeding population decline (dN_{POP}, Table 2) in each colony between successive years was already high in Changa Manga when studies began (0.75), with lower rates estimated at Dholewala (0.39), and Toawala (0.11). The rate of breeding population decline increased at Dholewala during the second year of the study (0.79 in 2001/2002), similar to that of Changa Manga the previous year. This rate increased progressively at all sites in each year, reaching 1.0 at Changa Manga and Dholewala in the year those colonies were extirpated, but did not exceed 0.29 in Toawala. If we assume that mortality was the only population parameter contributing to the breeding population decline, or at least the contribution of immigration, emigration and recruitment to the breeding population was relatively negligible in comparison to mortality, then the rate of decline in breeding population size between successive

Table 2 Summary of information collected on Oriental white-backed vulture colonies at Changa Manga, Dholewala, Toawala, and the combined population, for four breeding seasons, from 2000/2001 to 2003/2004.

Study colony, survey method	Breeding season (year)	Adults with gout	Adults without gout	Annual prevalence of gout	OP[1]	DV[2]	AN[3]	f[4]	M_{DV}[5]	MD_{DV}[6]	dN_{POP}[7]
Changa Manga, intensive[8]	2000/2001 (0)	4	0	1.00	139	14	178	396[9]	0.10	0.08	0.75[9]
	2001/2002 (1)	0	1	0.00	227	15	49	98	0.23	0.19	0.82
	2002/2003 (2)	0	0		226	3	9	18	0.26	0.20	1.00
	2003/2004 (3)						0	0			
Dholewala, intensive	2000/2001 (0)				166	23	237	492[9]	0.10		0.39[9]
	2001/2002 (1)				209	32	151	302	0.18		0.79
	2002/2003 (2)				203	25	31	62	0.60		1.00
	2003/2004 (3)						0	0			
Dholewala, non-intensive	2000/2001 (0)	12	3	0.80	166	40	412	842	0.10	0.08	0.20
	2001/2002 (1)	22	4	0.85	209	64	337	674	0.16	0.14	0.70
	2002/2003 (2)	23	1	0.96	203	39	101	202	0.32	0.31	0.98
	2003/2004 (3)						2	4			
Toawala, intensive	2000/2001 (0)				152	4	206	460	0.02		0.11[9]
	2001/2002 (1)				218	28	205	410	0.11		0.26
	2002/2003 (2)				212	23	152	304	0.13		0.29
	2003/2004 (3)				226	19	108	216	0.14		
Toawala, intensive & non-intensive	2000/2001 (0)	1	1	0.50	152	9	418	890	0.03	0.01	0.12[9]
	2001/2002 (1)	16	2	0.89	218	40	393	786	0.08	0.07	0.24
	2002/2003 (2)	13	1	0.93	212	35	298	596	0.10	0.09	0.32
	2003/2004 (3)	20	0	1.00	226	35	203	406	0.14	0.14	
Total for all sites[10]	2000/2001 (0)	17	4	0.81				2,128			0.27
	2001/2002 (1)	38	6	0.86				1,558			0.48
	2002/2003 (2)	36	2	0.95				816			0.50
	2003/2004 (3)	20	0	1.00				410			

[1]OP, observation period (days)

[2]DV, number of dead adult vultures collected during OP

[3]AN, active nests

[4]f, number of breeding birds at active nests (2AN)

[5]M_{DV}, annual mortality rates estimated from DV collected during observation periods relative to f

[6]MD_{DV}, estimate of the proportion of annual mortality that can be attributed to diclofenac, calculated using the presence, absence and prevalence of gout in adult vultures examined during observation periods in intensive plots at Changa Manga, and intensive and non-intensive transects at Dholewala and Toawala

[7]dN_{POP}, breeding population decline estimated from the decline in f between successive breeding seasons; it is likely that attrition in each colony was much larger than indicated by M_{DV} because not all dead birds could be located and it is therefore more accurately depicted by dN_{POP}

[8]The number of dead vultures examined in intensive study plots at Changa Manga was insufficient to calculate annual prevalence of visceral gout and therefore MD_{DV} was calculated using mean adult gout prevalence (0.80).

[9]Calculated using corrected number of active nests at the outset of the 2000/2001 season, based on observed rate of nest failure

[10]Calculated using maximum numbers

years (dN_{POP}) approximately equals annual mortality rate. Comparison of annual mortality estimated from counts of dead vultures (M_{DV}, Table 2) during comparable breeding season observation periods shows that dN_{POP}, as a measure of annual mortality, was considerably greater in each colony and year than M_{DV}.

Correlation between annual mortality due to diclofenac (MD_{DV}) and annual rate of breeding population decline (dN_{POP})

Linear regression was used to examine the association between high rates of diclofenac-induced mortality and rates of vulture population decline. To accommodate the binomial distributions of proportional datasets, arcsine transformations were first performed on the square root of MD_{DV} and dN_{POP} (Fig. 3). There was a significant positive correlation between mortality due to diclofenac and annual rate of breeding population decline ($r^2 = 0.74$, df $= 1$, $F = 20.35$, $P = 0.003$), with back-transformation giving a y intercept of <0.001. This relationship also confirms the validity of using mortality based on dead vultures to compare rates between sites and within sites over time. Furthermore, if we assume that diclofenac-related mortality was the only cause of population decline, this relationship demonstrates that
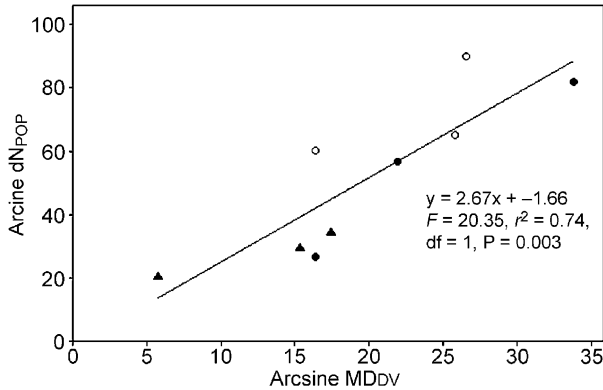
Fig. 3 Linear regression of annual population decline (dN$_{POP}$) with annual mortality from diclofenac based on counts of dead adult vultures (MD$_{DV}$) at Changa Manga (open circles), Dholewala (closed circles) and Toawala (triangles). Arcsine transformations were performed on the square root of dN$_{POP}$ and MD$_{DV}$ to account for binomial distributions of proportional data.

our method of estimating mortality from numbers of dead vultures underestimates actual mortality by a factor of 2.67, which can reasonably be attributed to dead vultures that were not located.

Visceral gout

Necropsy examinations and assessment of visceral gout were performed on 470 dead vultures (Table 3). Visceral gout was found in all age categories, but was significantly more common in adults (87.0%, n = 216) and subadults (80.2%, n = 96) than juveniles (22.9%, n = 131) and nestlings (22.2%, n = 27; χ^2 = 177.286, df = 3, P <0.001). Cases of visceral gout were found in all months of the year, and there was no significant variation in the bimonthly rate of visceral gout in adults and subadults (χ^2 = 7.084, df = 5, P = 0.214). Sex was determined in 256 adult and subadult vultures (n = 312), of which 156 were male and 100 female. Rates of visceral gout were significantly higher in males (89.7%) than females (81.0%; χ^2 = 3.865, df = 1, P <0.05). There was no significant difference in the rates of visceral gout recorded in combined adults and subadults between the three field sites (χ^2 = 2.707, df = 2, P = 0.258). The

Table 3 Age specific incidence and prevalence of visceral gout determined during necropsy examination of Oriental white-backed vultures in Punjab Province, Pakistan.

Age group	Gout	No gout	Total	Prevalence of gout
Adult	188	28	216	0.87
Subadult	77	19	96	0.80
Juvenile	30	101	131	0.23
Nestling	6	21	27	0.22

prevalence of visceral gout increased annually in adult vultures examined during the breeding season in intensive and non-intensive transects at Dholewala (80.0–95.8%) and Toawala (50.0–100%). Numbers of vultures examined in intensive study plots at Changa Manga were insufficient to draw comparisons between years, with gout found in four out of four adults examined in 2000/2001 and in zero of one in 2001/2002. A dead Eurasian griffon *Gyps fulvus* with visceral gout was recovered at Toawala on 20 March 2004, representing the first recorded case of the condition in this species in South Asia.

Temporal and spatial mortality clusters

At Toawala 43 dead adult and subadult vultures were collected in 2003/2004. Necropsies were performed on 28 of these and visceral gout was confirmed in all but one, a subadult that was excluded from further analyses. Three temporal and spatial mortality clusters were identified. The most significant of these involved four vultures found within a radius of 2.37 km between 14 and 18 December 2003 (P = 0.0003). Two additional birds were located in other areas of the colony between these dates, and visceral gout was confirmed in all five that could be necropsied. A second cluster occurred between 19 and 21 February 2004 when six dead vultures were found within a radius of 2.39 km (P = 0.0019). An additional dead adult was found outside this area, and all six vultures that were necropsied had visceral gout. A further cluster was identified between 27 and 28 November 2003 when five birds were found in a radius of 3.59 km (P = 0.0224). An additional dead vulture was found outside this area, and both of the birds that could be necropsied had visceral gout. The vultures found during these three clusters constituted 45% of the dead vultures located at Toawala during the 2003/2004 breeding season (n = 42).

Diclofenac contaminated carcass estimate

Necropsy examinations were performed on 158 vultures in intensive and non-intensive transects at Dholewala between 19 December 2000 and 12 May 2003, of which 120 had gout. Based on the temporal pattern of gout cases, estimated time since death, and the assumption that contamination must occur within 1 week of death, we estimated a minimum of 42 contaminated carcasses were responsible for vulture deaths during this period. Considering that no necropsies were performed between 4 June and 28 October 2001, monthly exposure rate was estimated to be 1.75 contaminated carcasses per month over an observation period of 730 days, where 1 month is taken to be 30.42 days (365/12). In Toawala

necropsies were performed on 181 vultures between 3 March 2001 and 4 July 2004. Of these 104 had visceral gout, from which we estimated a minimum of 41 contaminated carcasses. No necropsies were conducted between 19 August and 29 October 2001, and between 30 June and 12 November 2003. Therefore monthly exposure rate at Toawala was 1.23 contaminated carcasses per month over an observation period of 1,012 days. In Changa Manga necropsies were performed on 16 vultures between 13 January 2000 and 10 June 2001, of which nine had visceral gout. We estimated that this represented a minimum of six contaminated carcasses, at a monthly exposure rate of 1.22 contaminated carcasses per month over an observation period of 149 days. Based on the sum of the number of vulture days during the survey period, and assuming a mean carcass weight of 100 kg, vultures required a minimum of 87.2 carcasses per month at Toawala, 66.3 at Dholewala and 40.5 at Changa Manga. Therefore the proportion of contaminated carcasses within the foraging range of each of the study areas was estimated to be 1.41% at Toawala, 2.64% at Dholewala and 3.02% at Changa Manga.

Discussion

The significant positive regression of annual mortality due to diclofenac poisoning (MD_{DV}) against annual breeding population decline (dN_{POP}) shows that diclofenac poisoning was the primary cause of vulture population decline between 2000/2001 and 2003/2004 at the largest known breeding colonies of Oriental white-backed vultures.

Change in animal population size is dependant on four parameters: survival, productivity, immigration and emigration. Reliable estimates of survival and movement in bird populations can only be obtained using capture-recapture methods or radio-telemetry (Nichols *et al.*, 2004), which were beyond the scope of this study. However, assuming that the effects of movement and recruitment (related to productivity) of new breeders to the population were negligible in comparison with mortality during the study, as may be expected in a population that is declining rapidly over most of its range and suffering unusually high rates of mortality, then the annual decline in breeding population at each colony approximates annual mortality rate (Table 2). Estimating mortality rate based on the decline in breeding population size in successive years will produce inflated mortality rate estimates in colonies experiencing net emigration, while underestimating mortality in colonies where there is net immigration or high rates of recruitment. It could be argued, for example, that if the effects of movement between

colonies were not negligible, then lower rates of population decline at Toawala could be explained by vultures deserting Changa Manga and Dholewala in favour of Toawala. However, we have no *a priori* reason to believe this to be true, and two observations suggest that this was unlikely to be the case. Firstly, rapid population decline has been a consistent finding in all Oriental white-backed vulture populations in this and all other studies across the species range since the mid 1990s (Prakash *et al.*, 2003; Gilbert *et al.*, 2004), indicating that movement was of minor importance with respect to mortality. Secondly, estimates of mortality based on numbers of dead vultures (M_{DV}) will be least sensitive to movement effects and yet comparison of M_{DV} with dN_{POP} showed a significant positive linear correlation between them. Comparison of these parameters between colonies and years showed a consistent pattern of annual increase at each site, and were consistently lower at Toawala than the other two sites. Thus, movement of vultures between sites was not measurably important. We believe, therefore, that the difference in rates of breeding population decline between colonies at Changa Manga, Dholewala and Toawala was primarily due to differences in mortality rates caused by different rates of exposure to diclofenac contaminated carcasses within each colony's foraging range.

The first veterinary diclofenac product was registered in Pakistan in 1998 (Drug Control Administration, Ministry of Health, Government of Pakistan, unpubl. data), and vulture mortality due to visceral gout was already evident in November 2000. Comparative rates of mortality at each site suggest that incidence of diclofenac in carcasses was initially highest within the foraging range of Changa Manga in 2000/2001, rapidly increased in Dholewala during 2001/2002 and increased at the lowest rate at Toawala. An expansion in the veterinary diclofenac market in Pakistan during the study could have caused the steady increase in mortality rate recorded. Variation in the geographical availability of a newly introduced drug is not unexpected as marketing effort, veterinary preferences, and economic and distribution factors influence usage patterns. Overall vulture population declines based on the maximum number of active nests counted in each season across all three colonies collectively rose from 0.26 in 2000/2001 to 0.48 in 2001/2002 but increased only slightly in 2002/2003 to 0.50 (Table 2), suggesting that the overall effects of increasing use of diclofenac may have levelled off by the second year of study. Density dependent factors may also contribute to increasing rates of mortality over time as an increasing proportion of birds are exposed to the drug. Oaks *et al.* (2004) showed that diclofenac is not evenly distributed in treated livestock tissues, with higher concentrations found in kidney and liver relative

to muscle. Reduced competition in a declining population may enable a greater proportion of birds to feed on the higher risk visceral tissues preferred by *Gyps* species (Del Hoyo *et al.*, 1994).

Green *et al.* (2004) used a simulation model to predict the proportion of contaminated carcasses that would account for the observed rates of decline. The model predicted contamination rates in Pakistan of between 1:130 and 1:280 carcasses. The proportion of contaminated carcasses estimated in this study was an order of magnitude higher, ranging from 1:33 at Changa Manga to 1:77 at Toawala. It is possible that true carcass consumption rates may have exceeded those calculated, as the method used could not account for numbers and maintenance requirements of non-breeding birds and energy demands of reproduction. The method also assumes that vultures utilize the minimum number of carcasses necessary to satisfy their food requirements, which is unlikely to be the case. To be consistent with the predictions of the model, true numbers of carcasses utilized would need to exceed estimates by a factor of 1.84 to 8.47 times. It seems reasonable that true carcass consumption would fall within this range, and our findings confirm that environmental availability of diclofenac in contaminated carcasses was more than sufficient to cause the observed declines.

The general decline in breeding success recorded at all sites suggests that elevated mortality rates may have reduced productivity, with nest failure following the death of one or both parents. These findings can be contrasted to pre-decline productivity of 82 and 96% recorded in Rajasthan (Sharma, 1970; Prakash, 1999), but is less severe than the total breeding failure reported in Keoladeo in the late 1990s (Prakash, 1999). Breeding success at Toawala was generally higher than elsewhere, but still followed a general negative trend during the study period.

The observation that vulture death and visceral gout occurred in discrete temporal and spatial clusters was an important step in identifying the causative agent. Clusters of mortality indicated a common point of exposure affecting multiple birds in a short period of time. Oriental white-backed vultures typically congregate when they feed, and it seemed reasonable to hypothesize that birds were being exposed together while feeding at carcasses. The spatial pattern of dead birds indicates that vultures from adjacent areas of the colony were foraging together, and a single carcass may contain sufficient diclofenac residues to kill six or more vultures at a time.

The sex ratio of dead adult and subadult vultures was not equal, with 62% of birds examined found to be male. Rates of visceral gout were also lower in females, which, assuming no bias in sampling, may indicate a degree of sexual disparity in the rate of diclofenac exposure. Sexual differences in feeding rate, a division of parental duties at the nest or dominance at carcasses may lead to males encountering diclofenac more frequently. Disproportionate rates of mortality would skew the population sex ratio, reducing the breeding potential of the declining population still further.

Proportionately fewer juveniles were found with visceral gout than adults and subadults. There are two possible explanations for this. Firstly, annual survival is generally lowest during the first year of life, when juvenile vultures experience high rates of mortality related to naiveté (e.g. starvation and collisions). During the first year diclofenac poisoning will therefore constitute a lower proportion of overall mortality than later in life. Secondly, juvenile vultures may be less able to compete for visceral tissues, where diclofenac is more concentrated, and therefore encounter lethal doses less frequently.

The findings of visceral gout in the Eurasian griffon collected in March 2004 represent the first indication of the species' susceptibility to diclofenac toxicity. Subsequent experimental findings have established the toxicity of diclofenac in a captive Eurasian griffon (Swan *et al.*, 2006). Large numbers of juvenile Eurasian griffons winter in western India (Pain *et al.*, 2003). While elevated mortality rates in wintering juveniles would not lead to the rapid declines recorded in resident *Gyps* populations, reduced recruitment could lead to more insidious declines in breeding populations evident over a timescale of decades. Katzner *et al.* (2004) have shown long term declines in populations of Eurasian griffons in Central Asia and the Caucasus, and therefore it is possible that the effects of diclofenac may already extend to these states.

In 2000 the Oriental white-backed vulture breeding sites at Dholewala, Toawala and Changa Manga were the largest known colonies across the species' range. The high rates of mortality that have led to the extirpation of two of these colonies in only 3 years underlines the speed and scale of the decline that has decimated South Asia's vultures. It is clear that continued use of veterinary diclofenac is incompatible with the survival of Oriental white-backed vultures, and unless the drug is withdrawn populations will rapidly decline to extinction wherever it is used. With annual declines in the range of 50% and mortality rate increasing each year, measures to reduce diclofenac contamination are unlikely to occur rapidly enough to prevent the regional extinction of *Gyps* species. Strategies to conserve *Gyps* vultures in South Asia will need to address the use of diclofenac while also taking measures to establish viable captive populations for species' restoration once the environment is free from contamination.

Acknowledgements

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