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DICLOFENAC LEVELS IN LIVESTOCK CARCASSES IN INDIA BEFORE THE 2006 "BAN"[1]

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Three species of *Gyps* vulture, once common across the Indian subcontinent, have declined by more than 97% in India since 1992, and are now on the verge of global extinction. The decline is due to contamination of their food with diclofenac, a non-steroidal anti-inflammatory drug (NSAID) commonly used as a painkiller for livestock in India. On May 11, 2006, the Drug Controller General (India) ordered the withdrawal of all licences granted for the manufacture of diclofenac for veterinary use in India within three months. To monitor the effectiveness of this ban in protecting *Gyps* vultures it is vital to verify levels of diclofenac in livestock carcasses in India both before and after the ban, to determine whether diclofenac use is being reduced. In this study, we collected liver tissue samples from 1,848 livestock carcasses at 63 carcass dumps and four slaughterhouses across 12 Indian states during the period May 2004 to June 2005. The diclofenac levels were quantified using liquid chromatography-electrospray ionization mass spectrometry, with a limit of quantification (LOQ) of 10 µg/kg and limit of detection (LOD) of 4 µg/kg. Across the 12 states, diclofenac residues were found in 10.1% of livestock carcasses sampled: a prevalence of contamination more than sufficient to cause widespread mortality of vultures. There were significant differences in the prevalence of diclofenac between states and sites, and between the species and age-classes of animals, with cattle having a higher prevalence of diclofenac than any other species, and with higher levels of contamination in female animals. In addition, our sampling revealed differences in the daily intake rate of carcasses between sites, with an overall average of 7.47 ±0.58 animals per day across the 63 carcass dumps, and a maximum of >50 animals per day at Ludhiana (Punjab). Despite the large number of carcasses available, *Gyps* vultures were only sighted for three days out of the total 169 days of survey time spent at carcass dumps. The large number of carcasses and low numbers of vultures demonstrate that food availability is not an important factor affecting vulture populations in India. Repeated surveys, following the methods detailed in this study, are now vital to monitor and assess the impact of diclofenac levels in livestock carcasses available to vultures.

Key words: Diclofenac, livestock carcasses, conservation, *Gyps* vultures, India

INTRODUCTION

Veterinary use of the non-steroidal anti-inflammatory drug (NSAID) diclofenac is the main cause of the catastrophic decline in populations of three *Gyps* species of vulture, *Gyps bengalensis* (Oriental White-backed vulture), *Gyps indicus* (Long-billed vulture) and *Gyps tenuirostris* (Slender-billed vulture) endemic to South Asia (Green *et al*. 2004, 2006; Oaks *et al*. 2004; Shultz *et al*. 2004). Their populations in India have declined by more than 97% since 1992 (Prakash *et al*. 2003; Green *et al*. 2004), with numbers of Oriental White-backed vultures decreasing by more than 99.9% from 1992 to 2007 (Prakash *et al*. 2007). These population declines have left all three species of vulture at a high risk of global extinction and led to them being listed as Critically Endangered by the IUCN (World Conservation Union) (IUCN 2007). Population declines continue at rates of 16% to 44% per year (Green *et al*. 2004; Prakash *et al*. 2007).

Diclofenac is a widely available NSAID across the Indian subcontinent, where it is used as an antipyretic, anti-inflammatory and/or analgesic for livestock treatment. Vultures are exposed to the drug when they consume carcasses of livestock that were treated with diclofenac shortly before death. *Gyps* given therapuetic doses of diclofenac, or fed diclofenac-contaminated tissue, die within days from kidney failure with clinical signs of extensive visceral gout (the formation of uric acid crystals on/within tissue) (Oaks *et al*. 2004; Swan *et al*. 2006).

Modelling has shown that the observed rate of population decline could be caused by contamination of a very small proportion (0.13% to 0.75%) of ungulate carcasses available to vultures with a lethal level of diclofenac (Green *et al*. 2004). Until recently there have been no data available

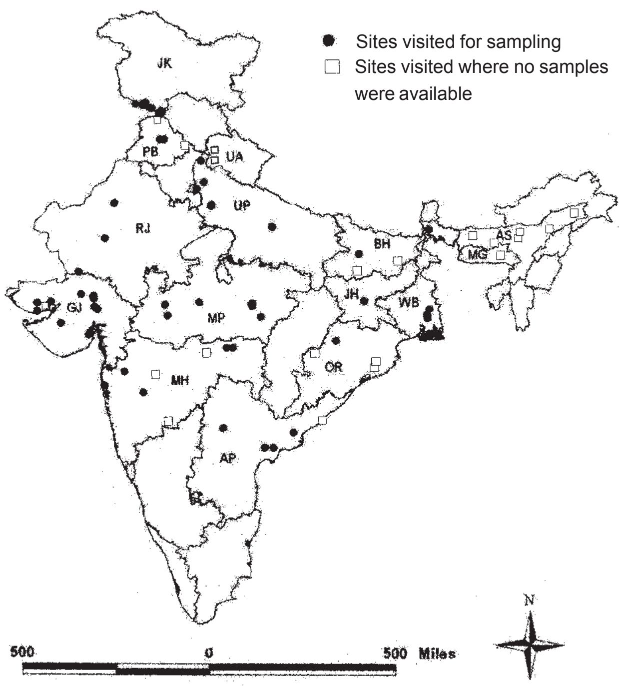

Fig. 1: Sites visited for sampling

on the residue levels and prevalence of diclofenac in ungulate carcasses available to vultures across India. Following the publication of a very small data set (Taggart *et al.* 2007a), Taggart *et al.* (2007b) further reported on the analysis of 1,848 liver samples collected from livestock carcasses from 12 states in India. This analysis revealed that the overall prevalence of detectable diclofenac (>10 µg/kg) across all states was 10.1% and varied significantly between states, with up to 22.3% prevalence determined in the state of Bihar.

On May 11, 2006, the Drug Controller General (India) ordered the withdrawal of all licences granted for the manufacture of diclofenac for veterinary use in India within 3 months of this date (Kumar 2006). Though this was a very positive and significant step in terms of acting to halt the rapid decline of *Gyps* vultures in India, it may in reality take considerable time and effort to effectively remove all existing stocks of veterinary formulations of the drug and to prevent the use of the drug derived from other sources (Taggart *et al.* 2007b). Before the ban, diclofenac was probably one of the most (if not the most) commonly administered and cheapest NSAIDs used in veterinary medicine in India. Industry sources estimate that some 10 million domesticated animals are treated annually with diclofenac (MoEF 2006). Therefore, substantial stocks probably still exist, and demand is likely to continue at a high level.

If *Gyps* vultures are to survive in India, the Indian veterinary market should be strictly monitored to ensure the complete removal of diclofenac. The collection of liver tissue samples from livestock carcasses, available countrywide, and their analysis for the detection of diclofenac residues is a reliable technique to test whether or not this drug is being used for veterinary treatment in India. Therefore, relevant pre-ban data is required to assess the effectiveness of the ban in the future. Taggart *et al.* (2007b) published data on overall and state-wide residual concentration levels and the prevalence of diclofenac; however, site-specific details were beyond the scope of that report and remain unspecified. Such data is of critical importance for assessing the effectiveness of the ban at local and regional scales. Here, we present site-specific data using the 1,848 liver samples utilised by Taggart *et al.* (2007b) and examine variations in the data by sites within states. We also note differences between livestock species, sex, type of death, category of collection site and age.

METHODS

Field Sampling of Livers from Domestic Ungulate Carcasses

Liver samples from cattle (n_t = 893), buffalo (n_t = 861), sheep (n_t = 48), goat (n_t = 39), horse (n_t = 6) and camel (n_t = 1) were collected from 67 sites in 12 states of India (Fig. 1) between May 2004 and July 2005. The states from which samples were collected were Andhra Pradesh (AP, n_t = 161), Bihar (BH, n_t = 121), Gujarat (GJ, n_t = 65), Jammu and Kashmir (JK, n_t = 77), Jharkhand (JH, n_t = 54), Madhya Pradesh (MP, n_t = 195), Maharashtra (MH, n_t = 194), Orissa (OR, n_t = 52), Punjab (PB, n_t = 76), Rajasthan (RJ, n_t = 310), Uttar Pradesh (UP, n_t = 449) and West Bengal (WB, n_t = 94). Three more states, Assam (AS), Meghalaya (MG) and Uttarakhand (UA), were also visited (for 20 days in all), but samples could not be collected because sites in these states were either not receiving any livestock carcasses at the time that we visited, or, we could not obtain permission to take samples from the carcasses, or, carcasses were not being skinned and left for scavengers because local authorities had switched to carcass burial as a disposal method.

Samples were collected from carcasses at Municipal Corporation Carcass Dumps (MCCD, n_t = 1,068), Co-operative Carcass Dumps (CCD, n_t = 28), Animal Charity Carcass Dumps (ACCD, n_t = 32), Private Carcass Dumps (PCD, n_t = 448) and Slaughterhouses (SH, n_t = 272). MCCDs are managed by the municipalities of certain cities to dump/process carcasses, whereas CCDs are owned by co-operative societies involved in the business of carcass processing (for leather and bone). PCDs are owned by independent skinners or small contractors, while ACCDs are owned by animal charities. Slaughterhouses were included in the survey because a substantial amount of waste (the offal) is disposed of on-site and is available to vultures. The sites visited were simply those encountered during fieldwork visits for which it was possible to obtain access and permission to gather samples. Consequently, they were not necessarily a representative sample of all locations at which livestock carcasses were available to vultures across India, however; we did not consciously select sites based on any criteria that were likely to lead to an atypical prevalence of diclofenac-treated animals.

For every dead animal that arrived at the site, the liver was initially removed from the carcass by local skinners working at these sites; we then removed three tissue subsamples of 3-4 gm each from three different regions of the whole liver using a surgical scalpel. These three subsamples were then bulked together into one watertight 25 ml polypropylene sample container, and further sealed with a tape and individually labelled. The scalpel, gloves and a marble cutting stone (upon which subsamples were excised from the bulk liver) were thoroughly cleaned after sampling each liver to reduce the risk of cross-contamination. Batches of ten samples were then placed into labelled ziplock bags and stored on ice in a portable refrigerator. Subsequently, all samples were transferred to a freezer and stored at -20 °C until extraction.

At all sites, except Ludhiana in the state of Punjab, every carcass that arrived during our visit was sampled, regardless of species, age or condition. There is therefore no bias with respect to the species, age or condition of the dead animals sampled at 66 of the 67 sites visited. At Ludhiana, >50 carcasses arrived at the site each day and it was not possible to sample every carcass. Samples at this site (n_t = 61) were taken predominantly from young prime adults and mature adults. We stayed at sampling sites during the day time to record the total number of carcasses arriving there and spent an average of 3.4 days (range 1-21 days) at each of the 63 carcass dumps and 3.3 days (range 1-4 days) at each of the slaughterhouses.

Diclofenac Extraction and Measurement

Full details of the extraction and measurement techniques used can be found in Taggart *et al*. (2007b). However, briefly, diclofenac was extracted from 0.5 gm of liver tissue using 2 ml of HPLC grade acetonitrile, and an Ultra Turrax IKA T8 hand held homogeniser. Mixtures were centrifuged at 1,000 g for 5 minutes, the supernatant filtered and then stored in crimp-top LC vials at -20 °C until analysis. Diclofenac levels were determined by liquid chromatography-electrospray ionisation mass spectrometry (LC-ESI/MS) using an Agilent 1100 series instrument (1946D). The instrument was calibrated using standards ranging from 5 to 1,000 µg/kg in diclofenac concentration, generated using diclofenac sodium salt (Sigma-Aldrich, D6899). The limit of quantification (LOQ) for this technique (back calculated to wet tissue concentration) was found to be 10 µg/kg, and the limit of detection (LOD) was 4 µg/kg.

Statistics

We used chi-square tests to compare the diclofenac prevalence levels between sites within each state, and restricted this analysis to sites where >6 livestock carcasses were sampled. Chi-square tests between two groups utilised the Yates correction for continuity. Tests of variation among sites in daily carcass intake rates were made using one-way ANOVA analysis. All statistical tests were two-tailed with significance set at $p < 0.05$. Because of the large number of comparisons (for each state; between sites, site type, age, gender and species) we did not perform post-hoc tests after the initial chi-squared test or ANOVA, but we report if there were differences between groups and present either the mean value (if there is no difference) or the highest intake rates or prevalence rate if there were significant differences. The individual prevalence and intake rates are presented for each site and state in Tables 1 and 2. A detailed statistical analysis of the influence of gender, species, dump-type and age for the nationwide results is presented in Taggart *et al.* (2007b).

RESULTS

A total of 1,848 samples were collected from 67 sites across India, of which 63 sites were carcass dumps processing animals that died naturally, and four were slaughterhouses. Overall, the percentage of livestock carcasses found positive for diclofenac was 10.1% (n_t = 1,848). There was no significant difference in diclofenac prevalence for animals collected in urban areas as opposed to rural areas (urban diclofenac prevalence (d_p) = 10.3% (n_t = 1,718); rural d_p = 6.9% (n_t = 130); $\chi^2_1 = 1.06$, $p = 0.30$). Excluding slaughterhouses, carcass arrival rate varied significantly between sites (one-way ANOVA $F_{62,148} = 12.864$, $p < 0.001$), ranging from 40.8 ±6.3 to 0.3 ±0.3 carcasses per day, with an overall average of 7.5 ±0.6 (n_d = 211). Arrival rates also varied significantly between site type ($F_{66,157} = 9.978$, $p < 0.001$), with average rates varying in the order SH (20.9 ±4.5) > MCCD (14.6 ±1.2) > ACCD (5.3 ±2.5) > PCD (3.6 ±0.3) > CCD (3.5 ±0.8).

Tables 1 and 2 show the breakdown of carcass intake by site within each state, as well as name of the site, type and geographical coordinates to enable repeated surveys to be undertaken in the future. As none of the samples collected in Orissa were positive for diclofenac (all samples were taken at a slaughterhouse), this state is not considered further in comparative analysis of prevalence by species, gender, death type, dump type and age group.

In Andhra Pradesh, two of four sites sampled were positive for diclofenac, and d_p varied significantly between these two sites ($\chi^2_1 = 6.00, p < 0.05$; 2.6% at Hyderabad and 28.6% in Gudiwada). There was no significant difference in the arrival rate of dead animals between these sites ($F_{3,14} = 2.917, p = 0.071$) with an overall average of 8.9 ±1.4 animals per day for the state. By species, diclofenac was detected in buffalo, cattle and horse carcasses, and the d_p varied significantly between them (Table 2). The d_p was not significantly different between the genders, age groups or the site types.

In Bihar, Patna was the only site sampled, which received an average of 5.8 ±0.5 carcasses per day, the d_p was 22.3%, and differed significantly by gender and age, but not by species.

In Gujarat, of 12 sites sampled, five were positive for diclofenac, but the d_p was not significantly different amongst them ($\chi^2_4 = 3.94, p = 0.41$). There were significant differences among sites in the arrival rate of carcasses ($F_{11,6} = 14.911$, $p = 0.002$), with the highest numbers arriving at the Dabala Panjarapole site, and an overall mean of 3.6 ±1 carcasses per day for the state (Table 1). Diclofenac was recorded in cattle and buffalo, with no significant differences noted in d_p by species, gender or site type, but, there were differences with age.

In Jammu and Kashmir, of eight sites sampled only two were positive for diclofenac, and the d_p was not significantly different between them ($\chi^2_1 = 0.20, p = 0.66$). There was no significant difference among sites in the arrival rate of carcasses ($F_{7,16} = 1.193, p = 0.361$), with an overall average of 3.4 ±0.6 for the state. The R.S. Pura site received the maximum number of carcasses per day, but was not significantly different in this respect to the other sites. By species, diclofenac was found in cattle and buffalo, but the d_p was not significantly different between these, and it did not differ by gender or age.

In Jharkhand, two sites were sampled, one of which, the Kantatoli slaughterhouse site, was positive for diclofenac. Samples collected at the Harmu Road site were all from animals that died naturally but none were positive for diclofenac. However, this site only received an average of 1.5 ±0.5 carcasses per day, so only three animals were sampled. Diclofenac was found in cattle and buffalo, and there were no significant differences in d_p by species, gender, site type or age.

In Madhya Pradesh, of the eight sites sampled five were positive for diclofenac, but the d_p did not differ significantly between them ($\chi^2_4 = 4.48, p = 0.35$). There was a significant difference in daily arrival rate among sites ($F_{7,19} = 7.682, p < 0.001$), with the highest number arriving at Bhopal (15 ±2.7). The d_p differed significantly by gender and age, but not by species or site.

In Maharashtra, of eight sites sampled only two were positive for diclofenac, and the d_p did not vary significantly between them ($\chi^2_1 = 2.81, p = 0.09$). There was a significant difference among sites in daily arrival rate ($F_{7,10} = 13.696$, $p < 0.001$), which was highest at the Mumbai site (40.8 ±6.3). The d_p varied significantly by age but not by gender, species or site type.

In Punjab, of the seven sites sampled three were positive for diclofenac, but the d_p did not vary significantly between them ($\chi^2_2 = 0.30, p = 0.86$). There was a significant difference among sites in daily carcass arrival rate ($F_{6,8} = 6.349, p = 0.010$), with the highest rate occurring at Ludhiana (15.3 ±3.1 animals sampled per day, with >50 arriving each day). The d_p did not differ significantly by species, gender, site type or age.

In Rajasthan, of the three sites sampled two were positive for diclofenac, but the d_p did not vary significantly between them ($\chi^2_1 = 2.93, p = 0.09$). The arrival rate was highest in Jodhpur (22.7 ±2.4) and was significantly different to the other two sites visited ($F_{2,15} = 18.52, p < 0.001$). The d_p varied significantly by age, but not by gender, species or site type.

In Uttar Pradesh, eight out of nine sites sampled were positive for diclofenac, and the d_p varied significantly among them ($\chi^2_7 = 26.01, p < 0.001$). There was a significant difference in the daily arrival rate at sites taking animals that died naturally ($F_{6,24} = 23.89, p < 0.001$), with the highest rates at Ghaziabad (19.7 ±1.7 carcasses per day). The d_p varied significantly by species, gender and site type, and among age groups.

In West Bengal, two of the four sites sampled were positive for diclofenac, but the d_p did not vary significantly between them ($\chi^2_1 = 0.45, p = 0.50$). There was no significant difference among sites in daily arrival rates ($F_{3,16} = 2.939, p = 0.065$), with an overall average of 4.7 ±0.7 for the state. The d_p varied significantly by age but not by gender, species or site type (Table 2).

Table 1: Prevalence and concentration of diclofenac in liver samples from domestic ungulate carcasses sampled in 12 states of India

State (sampling date)	Site name	Geographic location (GPS reading) (decimal degrees)	Rural (R) / Urban (U)	Dump type	Sampling days (n_d)	Daily carcass Arrival rate	Samples collected (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentration µg/kg
Andhra Pradesh (12.04.05 to 26.04.05)	Gandepalli	N 17.1366 & E 81.9645	R	PCD	1	2	2	0	n/a	n/a
	Gudiwada	**N 16.4301 & E 80.9977**	**R**	**PCD**	**2**	**3.5**	**7**	**28.6**	**229**	**195 - 269**
	Hyderabad	N 17.3508 & E 78.5823	U	MCD	14	10.8	151	2.6	44.5	24 - 80
	Vijaywada	N 16.4270 & E 80.5727	U	PCD	1	1	1	0	n/a	n/a
Bihar (25.05.05 to 14.06.05)	**Patna**	**N 25.5817 & E 85.1152**	**U**	**PCD**	**21**	**5.8**	**121**	**22.3**	**258.8**	**15 - 3,582**
Gujarat (25.05.04 to 06.06.04)	Ahmedabad	N 22.9760 & E 72.5620	U	MCCD	4	2.3	9	0	n/a	n/a
	Bhachau	N 23.3283 & E 70.3469	R	MCCD	1	10	10	10	1,150	1,150
	Bhavnagar	N 21.7813 & E 72.1327	U	MCCD	2	2.5	5	0	n/a	n/a
	Bhuj	N 23.2655 & E 69.6960	U	MCCD	2	2	4	25	1,695	1,695
	Dabala panjarapole	N 23.0937 & E 72.4114	R	ACCD	1	16	16	0	n/a	n/a
	Linch panjarapole	N 23.4951 & E 72.3846	R	ACCD	1	1	1	0	n/a	n/a
	Mahesana panjarapole	N 23.6106 & E 72.3694	U	ACCD	2	2.5	5	0	n/a	n/a
	Nagalpur	N 23.5790 & E 72.3606	R	PCD	2	1	2	50	743	743
	Paragpur panjarapole	N 22.8910 & E 69.6905	R	ACCD	1	1	1	100	1,186	1,186
	Rajkot	N 22.3313 & E 70.8639	U	MCCD	1	2	2	0	n/a	n/a
	Rajkot panjarapole	**(N 22.3114 & E 70.8131)**	**U**	**ACCD**	**1**	**9**	**9**	**22.2**	**523.2**	**86 - 3,183**
	Roadside (Mehsana - Bhuj)	N 23.7059 & E 71.7930	R	PCD	1	1	1	0	n/a	n/a
Jammu & Kashmir (20.01.05 to 04.02.05)	Gandhinagar	N 32.7180 & E 74.8543	U	PCD	8	4.8	38	5.3	26.5	16 - 44
	Karan Baug	**N 32.6725 & E 74.8314**	**U**	**PCD**	**5**	**3**	**15**	**6.7**	**100**	**100**
	Kujwani	N 32.6705 & E 74.3767	U	PCD	3	1.3	4	0	n/a	n/a
	Miran shahib	N 32.6322 & E 74.8030	R	PCD	1	2	2	0	n/a	n/a
	R. S. Pura	N 32.6228 & E 74.7093	U	PCD	1	6	6	0	n/a	n/a
	Sambha	N 32.4640 & E 75.1732	U	PCD	1	1	1	0	n/a	n/a
	Sohanjan village	N 32.6915 & E 74.7334	R	PCD	3	3.3	10	0	n/a	n/a
	Vijaypur	N 32.5054 & E 75.0731	R	PCD	1	1	1	0	n/a	n/a
Jharkhand (07.06.05 to 10.06.05)	Harmu Road (Ranchi)	(N 23.3632 & E 85.3491)	U	PCD	2	1.5	3	0	n/a	n/a
	Kantatoli (Ranchi)	**(N 23.3632 & E 85.3491)**	**U**	**SH**	**4**	**12.8**	**51**	**3.9**	**107**	**105 - 109**

Table 1: Prevalence and concentration of diclofenac in liver samples from domestic ungulate carcasses sampled in 12 states of India (*contd.*)

State (sampling date)	Site name	Geographic location (GPS reading) (decimal degrees)	Rural (R) / Urban (U)	Dump type	Sampling days (n_d)	Daily carcass Arrival rate	Samples collected (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentration µg/kg
Madhya Pradesh (13.02.05 to 04.03.05)	Bhaneka	N 22.6188 & E 80.3912	R	PCD	1	1	1	0	n/a	n/a
	Bhopal	N 23.2991 & E 77.4359	U	MCCD	6	15.3	92	8.7	227	14 - 1,071
	Indore	**N 22.6744 & E 75.9267**	**U**	**MCCD**	**7**	**9.4**	**66**	**16.7**	**199.7**	**17 - 2,156**
	Jabalpur	N 23.2111 & E 79.9558	U	PCD	3	1.7	5	20	211	211
	Pariyat	N 23.2496 & E 79.9735	R	PCD	1	12	12	0	n/a	n/a
	Saliwala	N 23.1081 & E 79.9946	R	PCD	1	2	2	50	2,020	2,020
	Mixi Road (Ujjain)	N 23.1933 & E 75.8033	U	PCD	4	2.5	10	10	28	28
	Somvaria (Ujjain)	N 23.1952 & E 75.7688	U	PCD	4	1.8	7	0	n/a	n/a
Maharashtra (09.09.04 to 12.09.04)	Ahmednagar	N 19.0282 & E 74.7422	U	MCCD	1	1	1	0	n/a	n/a
(03.03.05 to 12.03.05)	Bajargoan	N 21.1360 & E 78.7684	R	PCD	1	2	2	0	n/a	n/a
	Mumbai	**N 19.3432 & E 72.8956**	**U**	**MCCD**	**4**	40.8	163	6.1	196.2	15 - 4,135
	Bhandewali (Nagpur)	(N 21.1525 & E 79.0678)	U	PCD	2	0.5	1	100	1,835	1,835
	Daroga (Nagpur)	(N 21.1525 & E 79.0678)	U	PCD	2	1	2	0	n/a	n/a
	Dairy Farm (Nashik)	N 19.9798 & E 73.7846	U	PCD	3	0.3	1	0	n/a	n/a
	Wada Naka (Nashik)	N 19.9604 & E 73.7929	U	PCD	3	7.3	21	0	n/a	n/a
	Nandwar Naka (Nashik)	N 20.0135 & E 73.8545	U	PCD	2	1	2	0	n/a	n/a
Orissa (13.06.05 to 16.06.05)	**Sambalpur**	**N 21.4685 & E 83.9929**	**U**	**SH**	**4**	**13**	**52**	**0**	**n/a**	**n/a**
Punjab (01.02.05 to 08.02.05)	Jalandhar	N 31.0091 & E 75.5640	U	PCD	1	2	2	0	n/a	n/a
	Khanikui	N 32.1665 & E 75.5538	R	PCD	2	1.5	3	0	n/a	n/a
	Kuthed	N 32.3047 & E 75.6893	R	PCD	3	0.3	1	0	n/a	n/a
	Ludhiana*	**N 30.9870 & E 75.7745**	**U**	**MCCD**	**4**	**15.3**	**61**	**16.4**	**457.3**	**34 - 1,879**
	Pathankot	N 32.2534 & E 75.6425	U	PCD	2	2	4	25	1,228	1,228
	Sujanpur	N 32.3194 & E 75.6125	R	PCD	2	2	4	25	69	69
	Thariyal	N 32.3429 & E 75.6086	R	PCD	3	0.3	1	0	n/a	n/a
Rajasthan (28.06.04 to 18.07.04)	Bikaner	N 27.9676 & E 73.3755	U	MCCD	8	18.8	150	12.7	507.5	15 - 13,723
	Jodhpur	**N 26.3131 & E 72.9065**	**U**	**MCCD**	**7**	**22.7**	**159**	**21.4**	**614.1**	**13 - 4,102**
	Sanchore	N 24.7400 & E 71.6500	R	PCD	3	0.3	1	0	n/a	n/a

Table 1: Prevalence and concentration of diclofenac in liver samples from domestic ungulate carcasses sampled in 12 states of India (*contd.*)

State (sampling date)	Site name	Geographic location (GPS reading) (decimal degrees)	Rural (R) / Urban (U)	Dump type	Sampling days (n_d)	Daily carcass Arrival rate	Samples collected (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentration µg/kg
Uttar Pradesh (27.07.04 to 04.09.04) (27.06.05 to 05.07.05)	Baksi ka talab (Lucknow)	(N 26.8642 & E 80.9319)	U	PCD	4	2	8	12.5	1,107	1,107
	Dubagga (Lucknow)	(N 26.8642 & E 80.9319)	U	PCD	6	6.3	38	7.9	248.2	48 - 2,631
	Ghaziabad	N 28.6393 & E 77.3357	U	MCCD	3	19.7	59	3.4	156.2	18 - 1,356
	Madia (Lucknow)	(N 26.8642 & E 80.9319)	R	PCD	2	3	6	16.7	2,320	2,320
	Meeruth	N 28.9502 & E 77.6826	U	MCCD	7	19.1	134	14.2	697.4	34 - 6,524
	Nivari carcass dump (Aligarh)	**N 27.8735 & E 78.0323**	**U**	**PCD**	**5**	**4.6**	**23**	**26.1**	**185.4**	**28 - 1,855**
	Nivari Slaughterhouse (Aligarh)	N 27.8601 & E 78.0189	R	SH	1	19	19	0	n/a	n/a
	Purvi Din Kheda (Lucknow)	(N 26.8642 & E 80.9319)	R	PCD	4	3	12	8.3	2,948	2,948
	Saharanpur	N 29.9828 & E 77.5422	U	SH	4	37.5	150	1.3	12	11 - 13
West Bengal (28.04.05 to 10.05.05)	Barakpur	N 22.7480 & E 88.3632	R	CCD	3	4.3	13	0	n/a	n/a
	Kalyani	**N 22.9742 & E 88.4850**	**U**	**CCD**	**5**	**3**	**15**	**20**	**815.5**	**459 - 1,976**
	Kolkata	N 22.5608 & E 88.3865	U	PCD	10	6.4	64	9.4	370.9	63 - 2,038
	Siliguri	N 26.7456 & E 88.4563	U	MCCD	2	1	2	0	n/a	n/a

Abbreviations: d_p = prevalence of diclofenac, n_t = total number of samples, n_d = total number of sampling days, n/a = not applicable, GPS = Global Positioning System

Note: Rows highlighted in bold show where the d_p is highest (where sites have >6 samples),

GPS readings in brackets show the location of the city/village but not the exact dump site, whereas those without brackets are specific to dumps.

* An average of 15.3 carcasses were sampled each day, however >50 animals per day were arriving at the site

Geometric mean concentrations were calculated only from those samples with detectable residues

MCCD: Municipal Corporation Carcass Dumps; CCD: Co-operative Carcass Dumps; ACCD: Animal Charity Carcass Dumps; PCD: Private Carcass Dumps; SH: Slaughterhouses

Table 2: Prevalence d_p (%) and concentration of diclofenac in liver samples of domestic ungulate carcasses in 12 states of India, by species, gender, type of death, type of site and age

	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg
	Andhra Pradesh				**Bihar**				**Gujarat**				**Jammu and Kashmir**			
	Species: $\chi^2_2 = 8.546$, $p < 0.05$				Species: $\chi^2_1 = 1.712$, $p = 0.191$				Species: $\chi^2_1 = 1.708$, $p = 0.191$				Species: $\chi^2_1 = 0.194$, $p = 0.662$			
Buffalo	**41**	**9.8**	**101.8**	**28-269**	11	0	n/a	n/a	**18**	**11.1**	**252.8**	**86-743**	48	2.1	100	100
Cattle	53	1.9	80	80	**110**	**24.5**	**258.8**	**15-3,582**	39	10.3	1,647	1,150-3,183	**29**	**6.9**	**26.5**	**16-44**
Goat	28	0	n/a	n/a	0	n/a	n/a	n/a	1	0	n/a	n/a	0	n/a	n/a	n/a
Sheep	37	0	n/a	n/a	0	n/a	n/a	n/a	7	0	n/a	n/a	0	n/a	n/a	n/a
Camel	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
Horse	2	50	24	24	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
	Gender: $\chi^2_1 = 0.511$, $p = 0.475$				Gender: $\chi^2_1 = 6.147$, $p < 0.05$				Gender: $\chi^2_1 = 3.726$, $p = 0.054$				Gender: $\chi^2_1 = 1.091$, $p = 0.296$			
Female	**128**	**4.7**	**76.8**	**24-269**	**83**	**30.1**	**274.1**	**15-3,582**	**34**	**17.6**	**881.8**	**86-3,183**	**41**	**7.3**	**41.3**	**16-100**
Male	32	0	n/a	n/a	38	5.3	126	27-588	31	0	n/a	n/a	36	0	n/a	n/a
Unsexed	1	0	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
	Type of death: n/a				Type of death: n/a				Type of death: n/a				Type of death: n/a			
Natural	**161**	**3.7**	**76.8**	**24-269**	**121**	**22.3**	**258.8**	**15-3,582**	**65**	**9.2**	**881.8**	**86-3,183**	77	**3.9**	**41.3**	**16-100**
Slaughtered	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
	Site type: $\chi^2_1 = 3.636$, $p = 0.057$				Site type: n/a				Site type: $\chi^2_2 = 2.102$, $p = 0.349$				Site type: n/a			
ACCD	0	n/a	n/a	n/a	0	n/a	n/a	n/a	**32**	**9.4**	**687.3**	**86-3,183**	0	n/a	n/a	n/a
CCD	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	0	n/a	n/a	0	n/a	n/a	n/a
MCCD	151	2.6	44.5	24-80	0	n/a	n/a	n/a	30	6.7	1,396.2	1,150-1,695	0	n/a	n/a	n/a
PCD	**10**	**20**	**229**	**195-269**	**121**	**22.3**	**258.8**	**15-3,582**	3	33.3	743	743	**77**	**3.9**	**41.3**	**16-100**
SH	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	0	n/a	n/a
	Age: $\chi^2_2 = 3.806$, $p = 0.149$				Age: $\chi^2_2 = 11.196$, $p < 0.01$				Age: $\chi^2_1 = 4.118$, $p < 0.05$				Age: $\chi^2_3 = 7.542$, $p = 0.056$			
IN	15	0	n/a	n/a	47	6.4	388.6	27-3,354	0	n/a	n/a	n/a	42	0	n/a	n/a
IM	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	13	0	n/a	n/a
YPA	75	1.3	24	24	**9**	**55.5**	**170.8**	**59-588**	42	2.4	1,150	1,150	8	12.5	100	0.100
OA	**71**	**7.0**	**97**	**28-269**	65	29.2	270.7	15-3,582	**23**	**21.7**	**836.2**	**86-3,183**	**14**	**14.3**	**26.5**	**16-44**

Table 2: Prevalence d_p (%) and concentration of diclofenac in liver samples of domestic ungulate carcasses in 12 states in India, by species, gender, type of death, type of site and age (*contd.*)

	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg
	Jharkhand				**Madhya Pradesh**				**Maharashtra**				**Orissa**			
	Species: $\chi^2_1 = 0.105$, $p = 0.746$				Species: $\chi^2_1 = 3.502$, $p = 0.061$				Species: $\chi^2_1 = 0.016$, $p = 0.898$				Species: n/a			
Buffalo	**18**	**5.6**	**105**	**105**	78	5	268.1	179-705	163	5.5	139.9	15-2,353	7	0	n/a	n/a
Cattle	33	3.0	109	109	**117**	**15.4**	**202.5**	**14-2,156**	**23**	**8.7**	**2,754.6**	**1,835-4,135**	45	0	n/a	n/a
Goat	3	0	n/a	n/a	0	n/a	n/a	n/a	3	0	n/a	n/a	0	n/a	n/a	n/a
Sheep	0	n/a	n/a	n/a	0	n/a	n/a	n/a	4	0	n/a	n/a	0	n/a	n/a	n/a
Camel	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
Horse	0	n/a	n/a	n/a	0	n/a	n/a	n/a	1	0	n/a	n/a	0	n/a	n/a	n/a
	Gender: $\chi^2_1 = 0.306$, $p = 0.58$				Gender: $\chi^2_1 = 5.736$, $p < 0.05$				Gender: $\chi^2_1 = 1.896$, $p = 0.168$				Gender: n/a			
Female	24	0	n/a	n/a	**115**	**16.5**	**225.9**	**14-2,020**	**110**	**8.2**	**228.7**	**15-2,353**	27	0	n/a	n/a
Male	**30**	**6.7**	**107**	**105-109**	80	3.8	147.2	20-2,156	84	2.4	301.6	22-4,135	25	0	n/a	n/a
Unsexed	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
	Type of death: n/a				Type of death: n/a				Type of death: n/a				Type of death: n/a			
Natural	3	0	n/a	n/a	**195**	**11.3**	**213.1**	**14-2,156**	**194**	**5.7**	**240.5**	**15-4,135**	0	n/a	n/a	n/a
Slaughtered	**51**	**3.9**	**107**	**105-109**	0	n/a	n/a	n/a	0	n/a	n/a	n/a	52	0	n/a	n/a
	Site type: $\chi^2_1 = 1.44$, $p = 0.23$				Site type: $\chi^2_1 = 0.134$, $p = 0.714$				Site type: $\chi^2_1 = 0.028$, $p = 0.867$				Site type: n/a			
ACCD	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
CCD	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
MCCD	0	n/a	n/a	n/a	**158**	**12**	**210.8**	**14-2,156**	**164**	**6.1**	**196.2**	**15-4,135**	0	n/a	n/a	n/a
PCD	3	0	n/a	n/a	37	8.1	228.5	28-2,020	30	3.3	1,835	1,835	0	n/a	n/a	n/a
SH	**51**	**3.9**	**107**	**105-109**	0	n/a	n/a	n/a	0	n/a	n/a	n/a	52	0	n/a	n/a
	Age: $\chi^2_2 = 1$, $p = 0.606$				Age: $\chi^2_3 = 14.099$, $p < 0.01$				Age: $\chi^2_3 = 17.795$, $p < 0.001$				Age: n/a			
IN	0	n/a	n/a	n/a	78	2.6	221	74-660	132	0.8	22	22	1	0	n/a	n/a
IM	3	0	n/a	n/a	25	8	674.5	211-2,156	8	12.5	4,135	4,135	3	0	n/a	n/a
YPA	15	0	n/a	n/a	77	16.9	299.2	14-2,020	**36**	**16.7**	**423.8**	**18-2,353**	18	0	n/a	n/a
OA	**36**	**5.6**	**107**	**105-109**	**15**	**33.3**	**54.8**	**17-194**	**18**	**16.7**	**66.6**	**15-702**	30	0	n/a	n/a

Table 2: Prevalence d_p (%) and concentration of diclofenac in liver samples of domestic ungulate carcasses in 12 states of India, by species, gender, type of death, type of site and age (*contd.*)

	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg	Number of sampes (n_t)	Prevalence (d_p) (%)	Geometric mean Concentration µg/kg	Range of Concentrations µg/kg
	Punjab				**Rajasthan**				**Uttar Pradesh**				**West Bengal**			
	Species: χ^2_2 = 2.052, p = 0.358				Species: χ^2_2 = 4.011, p = 0.134				Species: χ^2_1 = 11.968, p < 0.001				Species: χ^2_1 = 0.97, p = 0.324			
Buffalo	21	9.5	145.7	103-206	19	15.8	971.3	409-1,597	396	6.1	382.2	11-6,524	41	4.9	1,650.7	1,337-2,038
Cattle	**53**	**17**	**501.7**	**34-1,879**	**288**	**17**	**583**	**13-13,723**	**51**	**19.6**	**457.7**	**18-5,074**	**52**	**13.5**	**339.3**	**63–1976**
Goat	0	n/a	n/a	n/a	1	100	53	53	2	0	n/a	n/a	1	0	n/a	n/a
Sheep	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
Camel	0	n/a	n/a	n/a	1	0	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
Horse	2	50	793	793	1	0	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
	Gender: χ^2_1 = 0.673, p = 0.412				Gender: χ^2_1 = 0.775, p = 0.379				Gender: χ^2_1 = 5.179, p < 0.05				Gender: χ^2_1 = 2.937, p = 0.086			
Female	**59**	**18.6**	**400.7**	**34-1,879**	19	15.8	480.1	15-4,248	**241**	**10.8**	**512.8**	**18-5,074**	**63**	**14.3**	**482.3**	**63-2,038**
Male	17	5.9	793	793	**288**	**17**	**991.3**	**13-1,3723**	208	4.3	203.8	11-6,524	30	0	n/a	n/a
Unsexed	0	n/a	n/a	n/a	1	0	n/a	n/a	0	n/a	n/a	n/a	1	0	n/a	n/a
	Type of death: n/a				Type of death: n/a				Type of death: n/a				Type of death: n/a			
Natural	**76**	**15.8**	**424.2**	**34-1,879**	**310**	**17.1**	**573.5**	**13-13,723**	**280**	**11.8**	**500.7**	**18-6,524**	**94**	**9.6**	**482.3**	**63-2,038**
Slaughtered	0	n/a	n/a	n/a	0	n/a	n/a	n/a	169	1.2	12	11-13	0	0	n/a	n/a
	Site type: χ^2_1 = 0.009, p = 0.924				Site type: χ^2_1 = 0.635, p = 0.425				Site type: χ^2_2 = 15.85, p < 0.001				Site type: χ^2_2 = 0.232, p = 0.89			
ACCD	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a
CCD	0	n/a	n/a	n/a	0	n/a	n/a	n/a	0	n/a	n/a	n/a	**28**	**10.7**	**815.5**	**459-1,976**
MCCD	**61**	**16.4**	**457.3**	**34-1,879**	**309**	**17.2**	**573.5**	**13-13,723**	193	10.9	604.8	18-6,524	2	0	n/a	n/a
PCD	15	13.3	291.1	69-1,228	1	0	n/a	n/a	**87**	**13.8**	**359.7**	**28-2,948**	64	9.4	370.9	63-2,038
SH	0	n/a	n/a	n/a	0	n/a	n/a	n/a	169	1.2	12	11-13	0	0	n/a	n/a
	Age: χ^2_3 = 2.992, p = 0.393				Age: χ^2_3 = 14.128, p < 0.01				Age: χ^2_1 = 23.31, p < 0.001				Age: χ^2_3 = 7.92, p < 0.05			
IN	6	0	n/a	n/a	145	10.3	1,569.7	13-13,723	217	5.5	782.3	34-6,524	58	3.4	482.9	118-1,976
IM	9	0	n/a	n/a	27	7.4	2,399	1,403-4,102	92	2.2	12	11-13	0	n/a	n/a	n/a
YPA	**34**	**20.6**	**625.3**	**34-1,879**	79	21.5	283.1	45-2,804	**74**	**21.6**	**351.8**	**18-5,074**	17	11.8	687	353-1,337
OA	27	18.5	246.4	69-833	**59**	**32.2**	**419**	**15-4,248**	66	7.6	530.4	48-2,948	**19**	**26.3**	**418.4**	**63-2,038**

Note: The rows highlighted in bold show where d_p is highest in each state/category, whilst discounting prevalences based on n < 6 samples
Geometric mean concentrations were calculated only from those samples with detectable residues. For each state statistical tests indicate results for chi-squared analysis of d_p by species, gender, age and site type. n/a indicates such tests are not applicable.
Abbreviations: IN: Infants; IM: Immatuares; YPA: Young prime adults; OA: Old adults

DISCUSSION

The results reported in this paper present information on the prevalence of diclofenac (d_p) at the site and state level, and are therefore more detailed than those given at the national level in the paper by Taggart *et al.* (2007b). This allows detailed comparisons to be made of the contamination of livestock carcasses that are available to vultures. The overall d_p in livestock carcasses across the country was 10.1%, with levels of contamination varying greatly between sites and ranging from 0% to 28.6% for sites where the number of samples collected was >6 (and between 0% and 100% where n_t was <6) and from 3.7% to 22.3% in the 11 (of 12) states where samples tested positive for diclofenac (Taggart *et al.* 2007b). These d_p levels reveal that a substantial proportion of livestock carcasses available to vultures are now contaminated with diclofenac, and at diclofenac concentrations (averaged across the whole carcass) that will cause appreciable mortality to feeding vultures (Green *et al.* 2006). Detailed modelling that incorporates the observed levels of diclofenac contamination found in this study with the estimated mortality rate of vulture populations reveals that the modelled rate of decline matches the rates of population decline observed across India, and that diclofenac is the only factor needed to explain the observed declines (Green *et al.* 2004, 2007). As a consequence, this survey and these studies highlight the urgent need to effectively prevent the veterinary use of this drug.

The observed variation in d_p detected across India may be related to whether the drug is actively promoted/used by veterinary practitioners in any particular area, which may in turn depend on the predominant livestock species treated and the livestock owners' ability to afford treatment for their animals. However, no significant difference was found between the d_p detected in rural and urban areas, as might have been expected if access to veterinary care (and perhaps wealth) were more limited in rural areas.

The d_p in female animals was found to be significantly higher than in males in three states (Bihar, Madhya Pradesh, Uttar Pradesh), and across the country carcasses of female animals showed higher levels of diclofenac contamination than did males (Taggart *et al.* 2007b). Such a bias in treatment may be because farmers are more acutely conscious of the health of active milking animals since they provide an ongoing income resource. Further, Taggart *et al.* (2007b) suggested that such a trend may be evident because lactating females are commonly given NSAIDs in combination with antibiotics to treat mastitis. By age group, adults (young prime adults (YPA) and old adults (OA)) had a higher d_p than subadults (immatures (IM) and infants (IN)) in eight states, which may again reflect the fact that Indian livestock holders (especially farmers) are more 'concerned' about the health of actively milking animals (i.e. mature animals), and therefore the veterinary care of subadults may be comparatively neglected. Moreover, livestock are probably simply more likely to need veterinary treatment with advancing age, injuries and diseases.

This study and that of Taggart *et al.* (2007b) found that d_p varied significantly between livestock species, with overall levels across the country highest in cattle (14.7%) in comparison with buffalo (6.0%), goats (2.3%) and sheep (0%). The d_p was higher in cattle than in other species in the following states: Bihar, Jammu and Kashmir, Madhya Pradesh, Maharashtra, Punjab, Rajasthan, Uttar Pradesh and West Bengal. Only Andhra Pradesh, Gujarat and Jharkhand reported higher d_p levels in buffalo. Whether the state level differences are a consequence of differing farming practices is unknown, however, the overall higher d_p in cattle is not surprising given the cultural and economic importance of cattle within India.

The d_p also varied in relation to the type of site sampled, with (nationally) lower levels of diclofenac found at slaughterhouses in comparison with carcass dumps. Although we sampled only five charity dumps (ACCDs) in Gujarat, two (Rajkot Panjarapole and Paragpur Panjarapole) of these contained animals that were positive for diclofenac. These ACCDs are particularly interesting as animals that arrive at such sites are probably generally well cared for prior to death, by charity-employed veterinarians. As these charities work for the well-being of all animals, and not just livestock, they may be receptive to information regarding the dangers of diclofenac to vultures. Consequently, it may be beneficial to target such charity-run sites in order to try to protect any residual local vulture populations.

This study gave us an opportunity to visit a large variety of carcass dumping sites across much of India (more than 80 sites in 15 states), and to interact with those involved in the mechanism of livestock carcass processing. Our interaction with skinners suggests that the decline in vulture numbers across India has also had an adverse impact on the profitability of such sites, and on the health and safety of the people processing livestock carcasses. Skinners believed that the decline in vulture numbers meant that the flesh on the carcasses was being less rapidly consumed, and hence sites were producing more unpleasant odours, and acting as an increased hazard to public health for longer, as the carcasses rotted. This had in turn raised public awareness of these sites and therefore municipalities in many cities were under increasing pressure to change the traditional way of disposing of livestock carcasses. In certain areas, i.e., at Siliguri, Guwahati, Shillong, Bogaigoan, Nagoan, Tezpur and Tinsukia in north-eastern India the practice was already being replaced by burial. Hyderabad, Ahmedabad and Jabalpur were also set to follow suit, and in Jodhpur a carcass incineration plant had already been established. We also found that at many of the sites where burial was being used, the carcasses were no longer being skinned, and that this was then having a direct impact on the leather and skinning industry in those areas. Skinners encountered during this work suggested that when vulture numbers were high, they cleaned the bones of the skinned carcasses thoroughly and in a matter of minutes, providing high quality clean bones for market. Without vultures, extra labour was required to remove meat from the bones, and the quality of the final cleaned bone was never as good as when cleaned by vultures. Traces of meat left on bones decayed over time and turned the bones yellow, generating a low-grade product in the bone market. Skinners reported that the price of bone had reduced drastically over recent years.

In total we spent 169 days at 67 different carcass sites, however, in this time we only recorded endangered *Gyps* vultures on three days, and only a very small number of birds were observed at the three sites where they were recorded (i.e. *G. bengalensis* at Dabala Panjarapole ACCD (seven individuals) and Bhachau MCCD (22 individuals) in Gujarat, and two *G. indicus* at Bikaner MCCD in Rajasthan). Older skinners often stated that only a couple of decades earlier, there used to be hundreds of *Gyps* vultures flying around carcass dumps. Unfortunately, if the emerging trend towards livestock burial continues, as it probably will, even eradicating diclofenac will not, on its own, permit wild vulture's numbers to recover to the high levels historically observed in India. Although not currently a significant problem in most areas, a lack of food availability may become an issue in future decades as India continues to rapidly develop its waste management and public health structure and capacity.

In agreement with Prakash *et al*. (2003), our study clearly shows that food scarcity is not a driving force behind the rapid vulture declines being observed in India today. Among the 63 carcass dumps of the 67 sites sampled in this study, the overall mean average carcass intake rate was 7.47 ±0.58 animals per day, with a maximum average intake rate of 41 carcasses per day recorded at Mumbai and more than 50 per day at Ludhiana, Punjab. Despite these high numbers of carcasses, sightings of *Gyps* vultures were extremely infrequent. If the overall mean carcass intake rate (for all 67 sites) of 6.2 animals per day is continued throughout a year, then a typical site would take around 2,270 animals per year, whereas a large site such as at Mumbai may take in nearly 15,000 carcasses (if the daily intake rate of 41 carcasses/day is accurate). Cattle and buffalo formed 95% of the 1,848 carcasses we observed. Since the edible tissue of these species constitutes 75.5% of the total mass (Green *et al*. 2006), and the average mass of the Indian Cattle *Bos indicus* is around 202 kg, a typical carcass dump could provide nearly 3,29,000 kg of edible tissue (edible mass = [2270 x 0.95] x [202 x 0.755]). An individual *Gyps bengalensis* typically requires around 0.341 kg of food per day (Swan *et al*. 2006), which represents 125 kg per year. If all the edible matter at a dump was available to vultures, an average carcass dump could potentially support a vulture population of 2,600 birds, and the Mumbai dump could support over 17,000 birds. These calculations are not entirely realistic, as even in past decades vultures would not have had access to all the edible tissues available, given the presence of other scavengers and decay. However, given the fact that we only visited a small subset of the total number of carcass dumps across India, the availability of carcasses could still easily support a very large national population of vultures, and these calculations demonstrate that lack of food is certainly not, currently, a significant factor for vultures.

Our fieldwork also revealed that Black Kite (*Milus migrans*) and Cattle Egret (*Bubulcus ibis*) are now the most common avian scavengers seen on carcass dumps, with up to 80 Black Kites observed at Bikaner and 300 cattle egrets at Ludhiana MCCDs. Given the toxicity of NSAIDs to a range of scavenging birds (Cuthbert *et al*. 2006a), these observations raise serious concerns about the potential impact of diclofenac contamination on other bird species scavenging at carcass dumps. This is especially so for Egyptian Vultures (*Neophron percnopterus*) and Red-headed Vultures (*Sarcogyps calvus*), which are rapidly decreasing in numbers (Cuthbert *et al*. 2006b). However, there are considerable inter-specific differences in the toxicity of NSAIDs among birds (including diclofenac; Rattner *et al*. 2008); hence not all species present at carcass dumps may be negatively affected. We also noted an abundance of feral dogs (*Canis familiaris*) at the majority of sites visited, and skinners have reported that there has been an increase in the numbers of feral dogs over the last 5 years. Feral dogs were seen at all 67 sites visited for sampling, with a maximum of 88 counted at Rajkot MCCD in Gujarat. Increased numbers of feral dogs may obviously increase the risk of rabies transmission to humans in India, already a very important issue in the country (Sudarshan *et al*. 2007). With very high numbers of feral dogs present at carcass dumps (>1,200 at one site; Prakash *et al*. 2003), competition for feeding resources (either direct and/or interference competition) may also hinder the return of vultures to such feeding areas. Supporting this, observations at the ACCD at

Poladiya Panjarapole in Kutch suggested that vultures either fed upon carcasses in the early morning (between 0500 and 0700 hours), or waited, sitting at a distance from the dump until dusk, when the dumps were free of feral dogs (of which there were around 25 at this site).

In conclusion, this study provides a detailed analysis of the site-specific prevalence of diclofenac in India and of the factors that influence prevalence at a local scale. The data will be of interest in the future, as longer-term assessments are made of the prevalence of diclofenac available to vultures in the environment, and the effectiveness of the diclofenac ban. Assessing the effectiveness of the diclofenac ban should incorporate measurement of diclofenac prevalence across the country (as undertaken in this study) as well as modelling the impact of the measured concentrations upon vulture populations as undertaken by Green *et al*. (2007). This study suggests that diclofenac is particularly heavily used in certain parts of India and an understanding of why this is the case may aid efforts to ensure that an effective ban is implemented. Effective long-term monitoring, utilising this baseline data set, is now imperative in order to assess the effectiveness of the ban, using a re-sampling schedule based on the methods detailed in this study.

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