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Indian vultures: victims of an infectious disease epidemic?

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Abstract

During the 1990s, populations of two species of griffon vulture, the Indian white-backed *Gyps bengalensis* and the long-billed *Gyps indicus*, declined by more than 90% throughout India. These declines are continuing and are due to abnormally high rates of both nesting failure and adult, juvenile and nestling mortality. Affected birds exhibit signs of illness (neck drooping syndrome) for approximately 30 days prior to death. Epidemiological observations are most consistent with an infectious cause of this morbidity and mortality. To investigate the cause of these declines, 28 vulture carcases, including adults and juveniles of both species, were examined in detail. Significant post-mortem findings included visceral gout, enteritis, vasculitis and gliosis. Although we have not yet been able to identify the causative agent of the declines, the results of our pathological studies are most consistent with those for an infectious, probably viral, aetiology. We examine hypotheses for the cause of the declines and, based on our epidemiological and pathological findings, we show infectious disease to be the most tenable of these.

INTRODUCTION

During the 1990s, two of India's commonest griffon vultures, the Indian white-backed *Gyps bengalensis* and the long-billed *Gyps indicus*, have declined by more than 90% (Prakash *et al*., 2003). Between the 1996 and 2000 evaluations of threat by the International Union for the Conservation of Nature and Natural Resources (IUCN), the status of both species rapidly deteriorated, from common and not threatened in 1996 to the most serious level of threat, 'Critically Endangered', in 2000 (BirdLife International, 2000). Such a rapid deterioration in the classification of two previously common species is extremely worrying and has wider ramifications for the security of other species, regardless of their threat classification. It is, therefore, of great importance to determine the mechanism(s) driving these rapid declines of *Gyps* vulture populations and to ensure that lessons are learned from these investigations, such as the employment of preventative or early-warning systems for related and other species.

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The *Gyps* vulture declines were first noted by a study at the Keoladeo National Park (KNP) World Heritage Site in Rajasthan, which recorded 96% and 97% declines in the numbers of white-backed and long-billed vultures respectively between 1987/88 and 1998/99 (Prakash, 1999). Prakash (1999) also noticed abnormally high morbidity and mortality rates of the declining species, with sick vultures appearing lethargic with drooping necks (Fig. 1). Prakash (1999) reported that once a vulture was seen to be sick, it invariably died, with affected birds following the same clinical course of disease lasting for approximately 30 days before death. During this period, sick birds continued to feed and fly, but both the degree of lethargy and the periods of neck drooping behaviour progressively increased (V. Prakash, unpubl. data, 1999). Since 1999, white-backed vultures have become extinct in KNP, while long-billed vultures are now only rarely seen (Prakash *et al*., 2003). The long-billed vulture probably comprises two distinct species, the Indian vulture *G. indicus* and the slender-billed vulture *Gyps tenuirostris* (Rasmussen & Parry, 2000), but it is treated as one species here as the two forms were not differentiated during fieldwork.

Further to the findings at KNP, population surveys of vultures were conducted across north, central, west and

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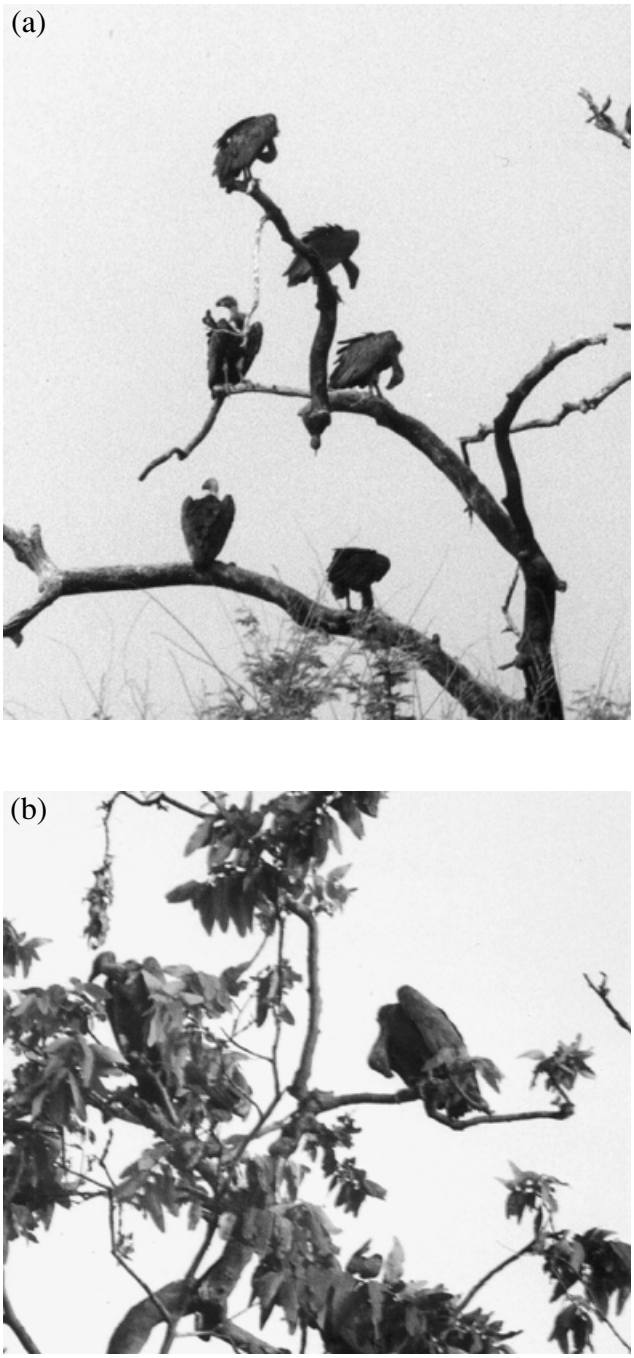

Fig. 1. Indian white-backed vultures showing neck drooping syndrome (NDS) in Ranthambhore National Park, Rajasthan, July 2000 (a) and in the centre of Delhi, September 2000 (b). Sick birds appear to be lethargic with intermittent, often prolonged, periods of neck drooping, in which their heads often drop vertically to the level of their feet. When disturbed, birds with NDS appear temporarily alert.

east India between April and June 2000, repeating similar surveys conducted in 1991 and 1993 (Prakash *et al*., 2003). Comparison of survey results showed nation-wide population declines of a similar degree to those seen in KNP for white-backed and long-billed vultures, while other (non-*Gyps*) species of vulture were apparently either unaffected or had declined by a much lesser degree (Rahmani & Prakash, 2000; Prakash *et al*., 2003). Similarly, other scavengers, e.g. rats, pigs, feral dogs, crows (*Corvus* spp.), kites (Accipitridae) and egrets (*Egretta* spp.), showed no evidence of being adversely affected by the agent causing the declines of *Gyps* vultures. In all regions surveyed in 2000, the extent of the decline was similar for both *Gyps* species and declines were equally severe within and outside protected areas. Also, in all areas surveyed, sick (lethargic with intermittent, prolonged, periods of neck drooping) and dead *Gyps* vultures were observed, as in KNP, suggesting that the same factor was causing the declines throughout India. In some areas, such as Kashipur in northern India, all ($n = 20$) white-backed vultures observed had this neck drooping syndrome (NDS) (V. Prakash, unpubl. obs.). Monitoring of roosting and breeding colonies of long-billed and white-backed vultures in Rajasthan and Maharashtra has shown that, once birds start to be seen with NDS, the disease – which is invariably fatal – gradually spreads throughout the colony over a period of several years (authors' unpubl. obs.).

Recently, abnormally high mortality rates of *Gyps* species and signs of NDS have been reported from the neighbouring countries of Nepal and Pakistan (Rahmani & Prakash, 2000; Virani *et al*., 2001). In Pakistan, disease appears to be spreading in *Gyps* spp. vulture populations westwards from the Indian border, with an easterly, and annual, trend of increasing prevalence of NDS, increasing mortality and increasing rates of population declines (Rahmani & Prakash, 2000; Virani *et al*., 2001; The Peregrine Fund, 2002). In addition, there is some evidence that two further species of *Gyps* vulture, *G. fulvus* and *G. himalayensis*, which have small breeding and large overwintering populations in India (Ali & Ripley, 1983), may have been affected with the same disease syndrome (Prakash *et al*., 2003).

Toxicological testing of tissues from a small number of *G. indicus* and *G. bengalensis* in India failed to show evidence of poisoning with organophosphate, organochlorine or carbamate pesticides (Rahmani & Prakash, 2000; authors' unpubl. data). More extensive toxicological analyses conducted in the USA on tissues from similarly affected *G. bengalensis* from Pakistan were negative for significant concentrations of contaminants (Oaks *et al*., 2001). In this paper, we present the gross and histological findings resulting from detailed pathological examinations of affected vultures from India.

METHODS AND MATERIALS

For meaningful pathological studies, fresh carcases are required, as post-mortem changes obscure evidence of disease processes and hinder the observation or recovery of pathogens and other causative agents. During the present study, freshly dead *Gyps* vultures were very difficult to obtain from the field owing to a combination of the low remaining population densities and high ambient temperatures causing rapid decomposition. Many of the birds included in this study, therefore, were collected while sick and held in captivity until death (see Table 1). Vultures were included in this study only if a

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Table 1. Details of vultures examined post-mortem with a summary of the main lesions found

Vulture reference	Species[1]	Age[2]	Sex[3]	Weight (kg)[4]	State[5]	Status when found[6]	Carcase condition	Body condition[4]	Thymus present[7]	Bursa fabricius present[7]	Lesion[7]					
											Visceral gout	Enteritis	Vasculitis	Gliosis	CNS degeneration	Purkinje cell loss
1	WB	AD	M	NR	Raj	sick	fresh	thin	0	0	0	1	1	0	0	0
4	WB	JUV	F	NR	Raj	sick	fresh	normal	1	1	0	1	0	0	1	0
5	LB	JUV	M	4.8	Raj	sick	fresh	normal	1	1	0	1	1	0	0	1
6	LB	AD	F	NR	Raj	dead	autolyzed	NR	0	0	1	0	NE	NE	NE	NE
7	LB	SUB	F	NR	Raj	dead	autolyzed	NR	0	0	0	1	1	0	0	0
8	LB	SUB	M	NR	Raj	sick	autolyzed	NR	0	0	1	0	1	NE	NE	NE
9	WB	SUB	F	NR	Raj	dead	frozen	NR	0	0	1	0	0	NE	NE	NE
10	LB	AD	M	4.9	Raj	dead	frozen	thin	0	0	1	0	0	NE	NE	NE
11	LB	AD	M	4.8	Raj	dead	frozen	thin	0	0	1	0	NE	NE	NE	NE
12	WB	AD	F	5.0	MP	sick	fresh	normal	0	0	0	1	0	0	1	1
13	WB	JUV	M	4.9	MP	sick	fresh	normal	1	1	0	0	1	0	0	NE
14	LB	JUV	F	4.3	MP	sick	fresh	thin	1	1	0	1	1	0	0	1
15	WB	JUV	M	5.2	MP	sick	fresh	thin	1	0	0	1	1	0	0	1
16	WB	JUV	F	4.1	MP	sick	fresh	NR	1	0	0	0	1	1	1	1
17	WB	JUV	F	4.3	MP	sick	fresh	NR	1	1	0	1	1	0	0	1
18	LB	AD	F	5.4	MP	sick	fresh	NR	0	0	0	0	1	0	1	1
19	WB	AD	F	4.3	Raj	sick	fresh	normal	0	0	0	1	1	0	1	1
20	WB	AD	M	4.0	Raj	sick	fresh	NR	0	0	0	1	0	1	0	1
21	LB	SUB	U	5.0	Mah	dead	autolyzed	NR	0	0	1	0	NE	NE	NE	NE
22	LB	JUV	M	4.2	Raj	dead	autolyzed	NR	0	1	1	0	NE	NE	NE	NE
23	HG	JUV	M	7.5	UP	sick	fresh	fat	1	1	0	1	1	0	0	1
24	LB	AD	M	5.3	Raj	dead	autolyzed	NR	0	0	0	0	NE	NE	NE	NE
25	LB	AD	U	4.6	Raj	sick	autolyzed	thin	0	0	0	0	NE	NE	NE	NE
26	LB	JUV	F	3.9	Raj	sick	fresh	normal	1	1	0	1	1	1	1	1
27	LB	SUB	F	3.9	Raj	sick	fresh	thin	0	0	0	0	1	1	1	NE
28	LB	JUV	F	4.6	Raj	sick	fresh	normal	0	1	0	1	1	1	1	NE
29	LB	JUV	F	4.3	Mah	sick	fresh	normal	0	1	0	1	1	1	1	1
30	LB	JUV	F	4.6	Raj	sick	fresh	normal	1	1	0	1	1	1	1	1

1. WB = Indian white-backed vulture; LB = long-billed vulture, HG = Himalayan griffon; 2. AD = adult, SUB = subadult, JUV = juvenile; 3. M = male, F = female; 4. Thin = no or depleted (scanty, dark orange) fat reserves found, normal = yellow intra-coelomic and subcutaneous fat present, fat = large fat deposits, including fat enveloping viscera, NR = not recorded; 5. Raj = Rajasthan, MP = Madhya Pradesh, Mah = Maharashtra, UP = Uttar Pradesh; 6. sick = seen to exhibit neck drooping syndrome (see text); 7. 0 = not present, 1 = present, NE = not established.

necropsy was performed before decomposition was any more advanced than the early stages of autolysis.

Between February 2000 and June 2001, 28 wild vultures (17 *G. indicus*, 10 *G. bengalensis* and one *G. himalayensis*) were examined post-mortem. These birds had been collected from four Indian states: Rajasthan (19 vultures), Madhya Pradesh (7), Uttar Pradesh (1) and Maharashtra (1). The birds were classified as juvenile (> 4 months, <12 months), subadult (>12 months, <36 months) or adult (>36 months), as indicated by their plumage (Alström, 1997). Details of the birds examined are presented in Table 1.

Sick birds were captured by employing the traditional Indian method of bird trapping using a 'snake trap'. Briefly, this involved an extendable bamboo pole, up to 20 m long, with a split end coated with gum (a mixture of *Ficus bengalensis* latex and mustard oil). The sticky end was slowly advanced towards the target vulture until within 1 m of the bird, when the pole was thrust forward to adhere to the feathers. If successful, the terminal metre of the pole would break off, still stuck to the vulture, and the compromised bird could easily be caught.

PATHOLOGICAL EXAMINATION

Each carcase was subjected to a necropsy using a standard protocol, which included external, followed by internal, systematic visual examination. The organs were observed *in situ* before being removed. Tissue samples were collected for a variety of tests. The samples were taken and handled in such a way as to preclude damage or cross-contamination between organ systems. Samples were preserved (1) frozen for virus isolation, toxicological and other future examinations, (2) fixed in neutral buffered 10% formalin for histopathological examination, or (3) fixed in 2.5% glutaraldehyde for electron microscopical examination (EM). Tissues taken routinely for histopathological examination included lung, trachea, liver, kidney, heart, spleen, crop, oesophagus, three levels of small intestine (including duodenum), rectum, gonad, pancreas, thyroid and brain. If present, sections of the bursa of Fabricius, the thymus and any overtly diseased tissues were also taken for microscopical examination. These tissues were processed using routine methods, embedded in paraffin wax, sectioned (5 μm) and stained with haematoxylin and eosin. Histopathological examination of the brain was limited to a single transverse section each of the cerebrum and cerebellum. The results of EM and toxicological analyses from these samples have yet to be completed and will be published later.

Bacteriological and mycological cultures were conducted, using routine methods, on a selection of major organs (liver, spleen, kidney, intestine, heart, lung) from vulture carcases considered fresh at the time of examination (see Table 1). Tissues for bacteriological examination were plated on to MacConkey's agar and blood agar and incubated aerobically and anaerobically at 37°C for 24 and 48 hours. For mycology, tissues were plated on to Sabauraud's agar and incubated at 37°C for up to 7 days. Organisms grown were identified using colony and microscopic morphology, Gram's staining characteristics and biochemical fermentation profiles.

Any macro-parasites found at post-mortem examination were fixed in 70% ethanol prior to identification.

The condition of certain carcases when examined (e.g. frozen as opposed to fresh) precluded a full spectrum of histological analyses on all birds. Exact sample sizes used for each examination are given in the results.

STATISTICAL ANALYSES

Possible associations between observations made during the course of this study were tested using the Pearson chi-squared test for contingency (Kirkwood, 1988). As the numbers involved were small, the analyses were repeated using Fisher's exact test for small sample sizes (Kirkwood, 1988).

RESULTS

A summary of the main findings from the 28 vultures examined is presented in Table 1. The birds were in various states of bodily condition, ranging from fat to thin. Eight vultures had visceral, including renal, gout, characterized by the deposition of uric acid crystals (confirmed by microscopical examination) throughout the kidneys (Fig. 2) and over the surfaces of the heart, liver and spleen. In all cases, the gout was acute (agonal), with no evidence of an inflammatory cell reaction to the presence of the uric acid crystals and concomitant tissue necrosis (Fig. 2). A significantly higher proportion of birds

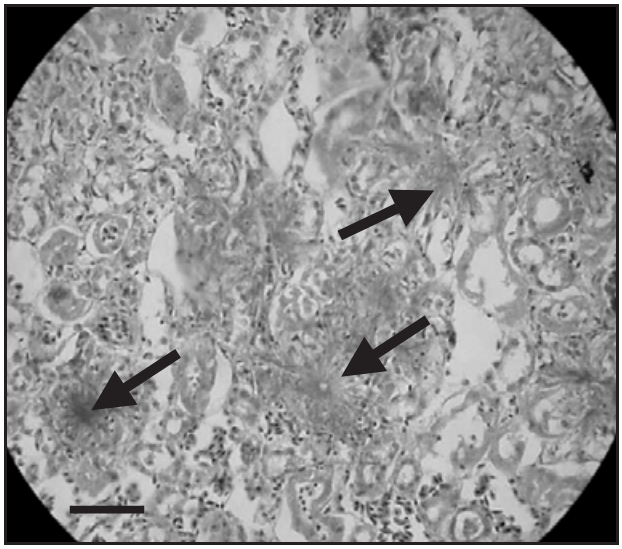

Fig. 2. Light micrograph of the kidney of a long-billed vulture (vulture no. 6) showing acute renal gout. Note the stellate uric acid crystals (arrows) with concomitant tissue necrosis and a lack of an inflammatory response. Although the gout in this bird was almost certainly the proximate cause of death, this was a result of, and not the cause of, the bird's chronic illness. Bar = 77 μm.

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found dead in the wild had visceral gout than was the case with birds that died in captivity ($\chi^2 = 14.9$, $P < 0.001$; Fisher's exact test <0.001). Six of eight birds found dead in the wild had gout, but only one of 19 birds that died in captivity had this lesion.

No gross abnormalities of the central nervous system were detected, but of the 19 vultures for which the brain was examined histologically, seven had areas of gliosis (proliferation of glial cells – inflammatory cells specific to the central nervous system) within the grey matter (Fig. 3, Table 1). In all cases, the gliosis was focal or multi-focal (i.e. not diffuse) and was considered to be low grade in severity because of the generally low degree of inflammatory cell infiltration and the restricted distribution of these lesions (Fig. 3). The forebrains of two of these birds (vulture nos. 27 and 29 – see Table 1) also contained large foci of an apparent astrocytic reaction. In seven vultures (nos. 12, 13, 18, 19, 20, 27 and 28), there was glial cell infiltration around some neurones (satellitosis). Neuronal degeneration, with or without a glial cell response, was seen in the brains of ten vultures (Table 1). Of the 16 vultures for which the cerebellum was examined microscopically, 13 exhibited areas of apparent Purkinje cell depletion, characterized by patchy absence of cells from the Purkinje cell layer of the cerebellum (Table 1).

Inflammation of blood vessel walls, vasculitis, was seen in the brains of five vultures (nos. 23, 27, 28, 29 and 30). Vasculitis was also found in other tissues, most notably in the submucosa of the alimentary tract (Fig. 4). Of the 22 vultures from which a range of tissues was examined histologically, 17 had varying, usually mild, degrees of vasculitis in at least one organ (Table 1). Viscera from the remaining six vultures (nos. 6, 11, 21, 22, 24 and 25) were too autolyzed for useful microscopic examination. As with the gliosis, the vasculitis was focal or multi-focal and was considered to be low grade in severity.

Of the 28 vultures on which a necropsy was performed, the bursa of Fabricius was present in ten (Table 1). In four of these birds (nos. 5, 14, 22 and 30), the bursa was filled with mucus. On microscopic examination, lymphoid depletion and epithelialization of the bursa was seen in five vultures (nos. 4, 5, 13, 14 and 26). The bursal epithelium of four vultures (nos. 13, 14, 16 and 23) contained multiple small cyst-like structures, containing proteinaceous fluid.

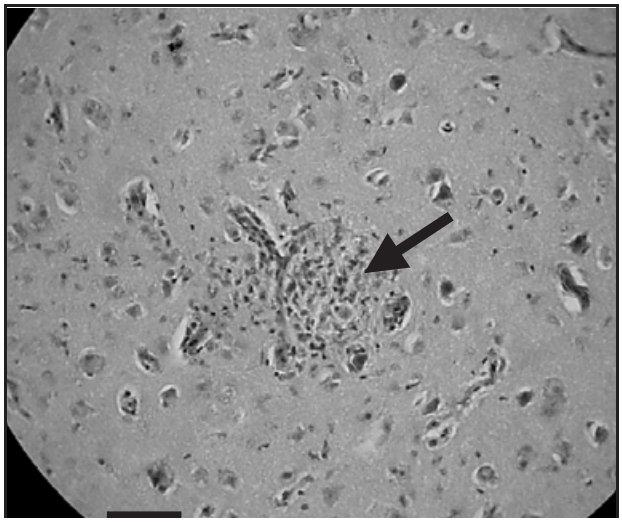

Fig. 3. Light micrograph of an area of gliosis (arrow) in the cerebrum of a long-billed vulture (vulture no. 29). Bar = 77 µm.

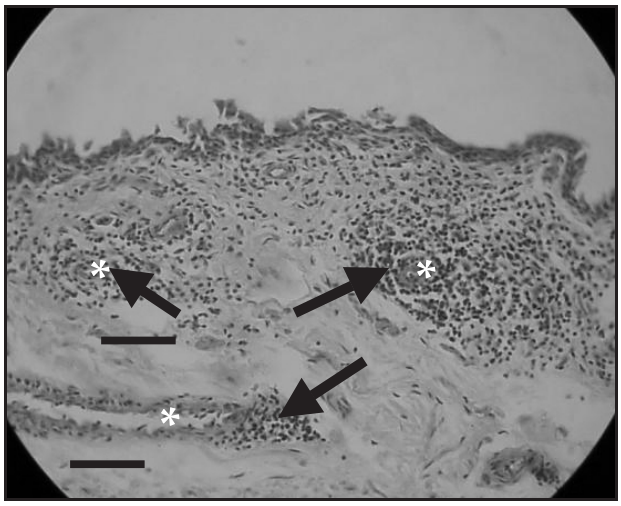

Fig. 4. Light micrograph of the pharynx of a long-billed vulture (vulture no. 8). The walls of several of the blood vessels (*) in the submucosa are infiltrated with inflammatory cells and these also form cuffs (arrows) around some of the affected vessels. Bar = 77 µm.

The thymus was present in ten of the 13 juvenile vultures examined. In most cases it appeared to be normal, but in vulture no. 5 the thymus contained multiple petechial haemorrhages. In addition to the haemorrhages, multiple foci of necrosis and inflammation (heterophil infiltrates) were seen on histological examination of the thymus from this bird. Inflammatory cell (heterophil) infiltration was present also in the thymus of vulture no. 26.

The spleen was examined microscopically for 18 vultures, of which 14 exhibited hyperplasia of the periellipsoidal cuffs, often with varying degrees of lymphoid depletion.

Enteritis was determined grossly (watery gut contents with reddening of the intestinal wall) or microscopically (greater than expected degree of plasma-lymphocytic infiltration within the mucosa) in 15 vultures (Table 1), of which 14 had died in captivity and one had been found dead in the wild. There was, therefore, a significant association between the presence of enteritis and captivity ($\chi^2 = 7.59$, $P < 0.01$; Fisher's exact test = 0.011). Four vultures (nos. 14, 16, 27 and 28) caught sick and kept in captivity until death had haemorrhages within the intestinal mucosa.

All of the vultures examined had some degree of pulmonary anthracosis. In most of the birds this was slight or mild, but of the 18 vultures for which the lungs were examined histologically, five (nos. 8, 17, 18, 19 and 20) were considered to have moderate degrees of anthracosis, while two (nos. 1 and 15) were classified as having severe pulmonary anthracosis.

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Apart from lesions due to visceral gout (see above) in the vultures with this condition, no abnormalities were detected on histopathological examination of the remaining tissues.

The microbiological examinations conducted failed to yield any organisms which were considered significant, with those bacteria isolated (e.g. *Escherichia coli* and *Clostridium* sp. from the intestine) being regarded as part of the normal commensal flora, or as post-mortem invaders.

Many of the vultures examined contained variable, usually low, numbers of parasites. The principal findings were of nematodes in the alimentary tract of 12 birds, cestodes in the small intestine of one vulture, and mild or moderate infestations of feather lice on seven birds. There was no association between the presence of parasites and any lesions found (e.g. there was no association between the presence of enteric parasites and enteritis) and in no case was parasitism regarded as contributory to the cause of death.

DISCUSSION

Populations of two species of *Gyps* vultures in India have declined dramatically over the past decade owing to a combination of abnormally high mortality rates of all age groups and abnormally low reproductive success. The pattern of the mortality (apparent genus-specificity, geographical and temporal spread, lack of biogeographical patchiness) is one that is most consistent with the epidemiology of an infectious disease. The scale and speed of the population declines in India are unprecedented for vulture populations (Pain *et al*., 2003), suggesting that, if an infectious disease is involved, the vultures are immunologically naïve to the disease agent, or that their resistance has been reduced. Immunological naïveté could occur for an introduced pathogen, a pre-existing pathogen that had mutated, or a sympatric pathogen from which the birds had previously been ecologically isolated (Daszak, Cunningham & Hyatt, 2000; Cunningham, Daszak & Rodríguez, 2003).

Although the epidemiology of diseases in wild bird populations is poorly understood, it is known that certain diseases can be transmitted very quickly through naïve populations across large geographical areas (Friend, McLean & Dein, 2001). For example, the density-dependent spread of a novel strain of the common poultry pathogen *Mycoplasma gallisepticum* has caused acute conjunctivitis and population declines in the introduced house finch *Carpodacus mexicanus* across large areas of eastern USA (Hochachka & Dhondt, 2000).

To find the cause of the mortality of the Indian vultures, detailed pathological examinations were conducted on the carcases of 28 affected birds. One of the primary findings of these studies was the occurrence of visceral, including renal, gout, in those vultures found dead in the wild. Similar findings have been reported from studies conducted in Pakistan, where investigations into vulture declines have shown approximately 70–80% of birds examined post-mortem to have visceral gout (Oaks *et al*., 2001; The Peregrine Fund, 2002). Although visceral gout is a dramatic finding, it is a non-specific lesion, which occurs when the poorly soluble uric acid (the normal nitrogenous excretory product of birds) crystallizes out of solution into the body tissues. This is most likely to happen if the blood concentration of uric acid increases because of either primary renal failure or dehydration. In the vultures, no evidence of primary renal disease was found on either gross or microscopic examination of the kidneys. No specific cause was found, either, for enteritis in any of the vultures that had this abnormality. Enteritis may develop in some bird species as a non-specific secondary effect of other illness or a change in environmental conditions (Arnall & Keymer, 1975). An association between poor body condition and the presence of enteritis ($\chi^2 = 5.60$, $P < 0.05$, Fisher's exact test = 0.036), but not between poor body condition and captivity ($\chi^2 = 3.81$, $P > 0.05$, Fisher's exact test = 0.125), indicates that the enteritis seen in the vultures may have been a consequence of chronicity of illness. The higher incidence of enteritis and the lower incidence of gout found in the vultures brought into captivity may be an indicator that the provision of food and water, and perhaps also shade, allowed these birds to survive their illness for longer than would have occurred in the wild. Although the proximate cause of death, it seems that the gout was most likely due to dehydration and that both gout and enteritis are secondary to an underlying disease causing the chronic illness observed in the vultures.

Pulmonary anthracosis was diagnosed in all of the vultures examined. In most of the birds, the degree of anthracosis was regarded as mild or slight and almost all vultures with moderate or severe anthracosis were adult. Three juveniles, however, were also placed in this latter category and the expected positive association between increasing age and severity of anthracosis was weak ($\chi^2 = 4.92$, $P < 0.05$; Fisher's exact test = 0.036). This is probably a consequence of the high levels of particulate air pollution in India, rather than a factor associated with the birds' demise.

The most significant histopathological findings were those of gliosis and vasculitis. Gliosis is a feature of inflammatory responses in the central nervous system and, in the absence of findings other than vasculitis, is most usually associated with viral disease (Summers, Cummings & de Lahunta, 1995). The prevalence and distribution of vasculitis in the vultures examined suggests that this, too, is most consistent with a response to an infectious agent, although the pattern and nature of the lesions found is unlike that of any known viral disease of raptors (e.g. see Forbes & Simpson, 1997). The degree of inflammation seen was not considered to be severe, as might be expected to occur in an acute infection, but this may indicate a chronic or resolving inflammatory disorder (possibly consistent with the prolonged clinical course of the disease) or infection with an agent that does not provoke a fulminating inflammatory response. The diagnosis of viral infections can be very difficult using histopathology; even the use of specialist techniques such as EM and the polymerase chain reaction can be

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problematic, as the causative agent often has disappeared by the time the bird shows signs of disease (Forbes & Simpson, 1997).

Of the five birds with vasculitis within the brain, all, except vulture no. 23, had concurrent gliosis. An additional 12 vultures had vasculitis outwith the brain. Of the 19 vultures for which the brain was examined histologically, therefore, seven had inflammatory lesions most consistent with a diagnosis of viral encephalitis. To find inflammatory lesions (gliosis, vasculitis or both) in such a large proportion of the brains examined is suggestive that these findings are associated with the disease affecting the vultures. This compares, for example, with similar inflammatory lesions having been detected in the brains of three of 108 captive and free-living wild birds from a variety of taxa examined in the pathology laboratory at the Zoological Society of London between 1989 and 2000. Also, in the current study, only one section each of the cerebrum and cerebellum were examined. The examination of further brain sections from vultures negative for encephalitic lesions, or of brains from more affected vultures, might therefore be expected to result in the observation of an increased frequency of such lesions.

Other histological findings from the brain examinations included satellitosis in seven birds (nos. 12, 13, 18, 19, 20, 27 and 28) and areas of apparent Purkinje cell loss in 13 birds (see Table 1). Whilst it is possible that either or both of these findings are changes associated with disease, it is also possible that they are within the normal range of micro-anatomy for *Gyps* vultures, as there are no reports of the histological features of the brain in these species. Further investigations, including comparison with the brains of *Gyps* vultures from unaffected populations, are required before the significance of these findings is known.

Bursal lymphoid depletion and epithelialization were found in five vultures and epithelial cysts were present in the bursae of Fabricius of four vultures. While these findings may be due to an insult, such as viral infection, it is also possible that they are normal ageing changes for this organ, which becomes involuted as the bird matures. To the authors' knowledge, the normal anatomical changes that occur during involution of the bursa of Fabricius in *Gyps* vultures have not been studied, rendering it difficult to be sure of the significance of the changes observed. Although cystic changes within the lymphoid tissue are recognized normal changes in some avian species during involution (Randall & Reece, 1996), cystic changes within the epithelium are not. As with the satellitosis and Purkinje cell loss in the brain, the bursal changes may be part of a disease process and warrant further investigation.

Hyperplasia of the periellipsoidal cuffs in the spleen was present in 14 of the 18 vultures from which this tissue was examined microscopically. These cuffs comprise B-lymphocytes and antigen-bearing reticular cells (Randall & Reece, 1996) and thus the hyperplasia seen suggests that the birds were mounting an immune response to an antigen at the time of death.

A disease of *Gyps* vultures which causes the same clinical signs (*Gyps*-specific, NDS, high mortality of all age classes) and post-mortem picture (visceral gout, no obvious cause) as in India has spread across at least two international borders (into Pakistan and Nepal), with year-on-year geographic spread being detectable within Pakistan (Rahmani & Prakash, 2000; Virani *et al*., 2001). Nation-wide surveys showed no relationship between density of birds in 1991–93 and subsequent percentage decline (Prakash *et al*., 2003). It therefore appears that, by the time the vulture declines had been recognized in India, it was too late to ascertain the source of the disease and the speed of geographic spread within this country. Although in 2000, >90% population declines of both *G. bengalensis* and *G. indicus* were detected in all regions surveyed, geographic heterogeneity was found for adult to juvenile ratios, which were much greater in West India (at 26:1) than elsewhere in the country (approximately 6:1) (Prakash *et al*., 2003). Whether this is an artefact (e.g. of survey observations, vulture migration or breeding patterns) or a clue to the geographic source of disease (e.g. through differential rates of adult and juvenile mortality) may become clearer following future surveys.

It is likely that NDS, the primary clinical sign of illness in affected vultures, is not pathognomonic for the disease responsible for the vulture declines in South Asia, but more probably is a non-specific indicator of a sick vulture (Prakash *et al*., 2003). The associations of increasingly longer periods of NDS with mortality at an individual level and of an abnormally high prevalence of NDS with mortality at a population level, however, show that this clinical finding is a useful indicator of the presence of the causative agent of the vulture declines in South Asia. Visceral and renal gout was a consistent post-mortem finding from studies of vultures found dead in the wild, both in India (see above; Rahmani & Prakash, 2000) and in Pakistan (Oaks *et al*., 2001). Disease can be caused either by infectious agents (e.g. fungi, bacteria, viruses) or by non-infectious agents (e.g. toxins, malnutrition, trauma). Epidemiological studies (patterns of mortality, clinical illness and gross post-mortem findings) indicate that the most likely cause of the vulture declines in South Asia is a novel infectious disease. The results of our detailed pathological investigations support this hypothesis.

Two alternative hypotheses have been proposed to explain the widespread declines and high levels of mortality of *Gyps* spp. vultures in South Asia: (1) a rapid reduction in food availability, and (2) poisoning, either deliberate or accidental, through exposure to pesticides or other contaminants.

The first of these possible causes has been discounted by the studies of Prakash (1999) and Prakash *et al*. (2003). These workers showed that animal carcases remain abundant throughout India, and that, of several hundred carcases recorded during nation-wide surveys in 2000, less than 5% had attendant vultures. Severe declines in numbers of *Gyps* vultures have been recorded at carcase dumps, where a continuing abundance of meat has, in the absence of vultures, led to huge increases in numbers of mammalian and other avian

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scavengers (Pain *et al*., 2003; Prakash *et al*., 2003; authors' unpubl. obs.).

The second hypothesis (chemical poisoning) could be consistent with the cause of wide-scale bird population declines associated with increased mortality and reduced breeding success. The classic example is DDT, exposure to which resulted in severe population declines of many avian species, including peregrine falcons (*Falco peregrinus*), in the UK and USA (see Ratcliffe, 1967*a*,*b*; Newton *et al*., 1982; Newton, Bogan & Rothery, 1986; Blus, 1996). To explain the mortality of vultures in India on the geographical scale and pattern seen, and with the rapidity apparent in the recent declines, a contaminant would probably have to have been introduced *de novo*, or applied in a novel way to increase its availability to vultures, India-wide, within the last 10–20 years. Furthermore, as the declines have recently spread across at least two international borders (into Pakistan and Nepal), exposure to the causative agent must have similarly increased in distribution. Although pesticide use in India increased dramatically through the 1980s, over the period of *Gyps* vulture declines, pesticide use has apparently decreased by around 40%; although this overall figure incorporates both decreases and increases in certain pesticide groups (Directorate of Economics and Statistics, at http://agricoop.nic.in/statistics/consum1.htm).

Within India, pesticide use varies considerably regionally. In contrast, the declines have occurred to the same degree, and over the same time-scale, throughout the range of *Gyps* spp. in India. Indian vultures feed largely on the flesh of domesticated animals and, as such, they are comparatively unlikely to be exposed to lethal concentrations of pesticides. Also, whilst some non-*Gyps* scavengers may have undergone limited declines, they have not been affected to a similar extent (see Prakash *et al*., 2003). Dead birds, other than *Gyps* spp., are not commonly seen. There is little systematic bird monitoring in India, however, so declines in other species remain a possibility. Toxicological analyses conducted on affected vultures so far (Rahmani & Prakash, 2000; Oaks *et al*., 2001; see above) have failed to detect contaminants at levels associated with toxicity. Consequently, whilst it is not possible to discount the possibility that a contaminant has played some part in the declines, there is currently no evidence to support this hypothesis.

To date, the search for a causative agent for the disease causing the vulture declines in South Asia has been unsuccessful. In the current study, no candidate agent has been found using microbiological or histological examinations of a wide range of tissues, including those from freshly dead birds. Virological studies using a variety of methods, including electron microscopy, egg inoculation, cell culture and molecular techniques, are currently in progress, but, so far, no candidate agent has been identified. Whilst the evidence so far points to an infectious disease, it is important that other investigations, particularly toxicological analyses, are also conducted. Until a causal agent has been identified, its pathogenesis indicated and an epidemiological significance shown, we cannot be certain that an infectious disease is responsible for the declines, or, if it is, what co-factors may be implicated in its emergence.

Disease, and particularly infectious disease, is increasingly being recognized as a major concern to conservation biologists (Daszak *et al*., 2000; Friend *et al*., 2001). In addition to the increased threat of infection to the survival of small or fragmented populations (Cunningham, 1996), disease introduction to naïve populations is a growing threat to biodiversity conservation, including to the conservation of species not classically regarded as being threatened (Cunningham *et al*., 2003). The apparent introduction of the fungal disease chytridiomycosis into pristine rain forests on disparate continents, for example, has led to catastrophic declines of many, and global extinctions of some, amphibian species (Daszak *et al*., 1999). Unlike geostatic threats, such as habitat loss or chemical contamination, infectious disease can spread far from the original point of entry to reach new target species. It is extremely important, therefore, that the causative agent of *Gyps* vulture declines in South Asia is identified as quickly as possible. If infectious disease is responsible for these declines, rapid identification of the causative agent and its mechanism of spread is essential if possible adverse consequences, such as the extinction of Indian *Gyps* vultures or the epidemic spread of disease into European and African *Gyps* species, with likely devastating ecological consequences (see Pain *et al*., 2003), are to be prevented.

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REFERENCES

Ali, S. & Ripley, S. D. (1983). *Handbook of the birds of India and Pakistan: compact edition*. Delhi: Oxford University Press.

Alström, P. (1997). Field identification of Asian *Gyps* vultures. *Oriental Bird Club Bull*. **25**: 32–49.

Arnall, L. & Keymer, I. F. (1975). *Bird diseases: an introduction to clinical diagnosis and treatment of diseases in birds other than poultry*. London: Bailliere Tindall.

BirdLife International (2000). *Threatened birds of the world*. Barcelona: Lynx Ediciones.

Blus, L. J. (1996). DDT, DDD and DDE in birds. In *Environmental contaminants in wildlife: interpreting tissue concentrations*: 49–71. Beyer, N. B., Heinz, G. H. & Redmon-Norwood, A. (Eds). Boca Raton: SETAC, CRC Lewis Publishers.

Cunningham, A. A. (1996). Disease risks of wildlife translocations. *Conserv. Biol*. **10**: 349–353.

Cunningham, A. A., Daszak, P. & Rodríguez, J. P. (2003). Pathogen pollution: defining a parasitological threat to biodiversity conservation. *J. Parasitol*. in press.

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Daszak, P., Berger, L., Cunningham, A. A., Hyatt, A. D., Green, D. E. & Speare, R. (1999). Emerging infectious diseases and amphibian population declines. *Emerg. Infect. Dis.* **5**: 735–748.

Daszak, P., Cunningham, A. A. & Hyatt, A. D. (2000). Emerging infectious diseases of wildlife – threats to biodiversity and human health. *Science* **287**: 443–449.

Forbes, N. A. & Simpson, G. N. (1997). A review of viruses affecting raptors. *Vet. Rec.* **141**: 123–126.

Friend, M., McLean, R. G. & Dein, F. J. (2001). Disease emergence in birds: challenges for the twenty-first century. *Auk* **118**: 290–303.

Hochachka, W. M. & Dhondt, A. A (2000). Density-dependent decline of host abundance resulting from a new infectious disease. *Proc. Natl. Acad. Sci. USA* **97**: 5303–5306.

Kirkwood, B. R. (1988). *Essentials of medical statistics*. Oxford: Blackwell Science.

Newton, I., Bogan, J., Meek, E. & Little, B. (1982). Organochlorine compounds and shell-thinning in British merlins (*Falco columbarius*). *Ibis* **124**: 238–335.

Newton, I., Bogan, J. A. & Rothery, P. (1986). Trends and effects of organochlorine compounds in sparrowhawk eggs. *J. Appl. Ecol.* **23**: 461–478.

Oaks, L., Rideout, B. A., Gilbert. M., Watson, R., Virani, M. & Khan, A. A. (2001). Summary of diagnostic investigation into vulture mortality: Punjab Province, Pakistan, 200–2001. In *Reports from the workshop on Indian **Gyps** vultures*: 133. Katzner, T. & Parry-Jones, J. (Eds). 4th Eurasian Congress on Raptors, Seville, Spain. Estación Biológica Donaña, Raptor Research Foundation. Document can be downloaded from www.vulturedeclines.org. See also: http://www.peregrinefund.org/conserv_vulture_results.html.

Pain, D. J., Cunningham, A. A., Donald, P. F., Duckworth, J. W., Houston, D. C., Katzner, T., Parry-Jones, J., Poole, C., Prakash, V., Round, P. & Timmins, R. (2003). *Gyps* vulture declines in Asia: temperospatial trends, causes and impacts. *Conserv. Biol.* in press.

Prakash, V. (1999). Status of vultures in Keoladeo National Park, Bharatpur, Rajasthan, with special reference to population crash in *Gyps* species. *J. Bombay Nat. Hist. Society*. **96**: 365–378.

Prakash, V., Pain, D. J., Cunningham, A. A., Donald, P. F., Prakash, N., Verma, A., Gargi, R., Sivakumar, S. & Rahmani, A. R. (2003). Catastrophic collapse of Indian white-backed *Gyps bengalensis* and long-billed *Gyps indicus* vulture populations. *Biol. Conserv.* **109**: 381–390.

Rahmani, A. R. & Prakash, V. (Eds). (2000). *A brief report on the International Seminar on the Vulture Situation in India. 18–20 September 2000*. Bombay Natural History Society, Hornbill House, Mumbai, India. 26 pp.

Randall, C. J. & Reece, R. L. (1996). Lymphoid and haematopoietic systems. In *Color atlas of avian histopathology*: 101–124. London: Mosby-Wolfe.

Rasmussen, P. C. & Parry, S. J. (2000). *On the specific distinctness of the Himalayan Long-billed Vulture **Gyps [indicus] tenuirostris***. 118th Stated Meeting of the American Ornithologists' Union, Memorial University of Newfoundland, St. John's, Newfoundland.

Ratcliffe, D. A. (1967*a*). Decrease in eggshell weight in certain birds of prey. *Nature* **215**: 208–210.

Ratcliffe, D. A. (1967*b*). The peregrine situation in Great Britain – 1965–1966. *Bird Study* **14**: 238–246.

Summers, B. A., Cummings, J. F. & de Lahunta, A. (1995). Inflammatory diseases of the central nervous system. In *Veterinary neuropathology*: 95–188. St. Louis: Mosby.

The Peregrine Fund (2002). Asian vulture crisis project: preliminary results for 2nd breeding season, Pakistan and Nepal, 2001–2002 (June 2002). *Falco: The Newsletter of the Middle East Falcon Research Group* **20**: 12.

Virani, M., Gilbert, M., Watson, R., Oaks, L., Benson, P., Khan, A. A. & Baral, H.-S. (2001). Asian vulture crisis project: field results from Pakistan and Nepal for the 2000–2001 field season. In *Reports from the workshop on Indian **Gyps** vultures*: 133. Katzner, T. & Parry-Jones, J. (Eds). 4th Eurasian Congress on Raptors, p 133, Seville, Spain. Estación Biológica Donaña, Raptor Research Foundation. Document can be downloaded from www.vulturedeclines.org.

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