Avian malaria and decline of White-backed vulture population

Poharkar *et al.*[1] have reported the presence of a single genotype of avian *Plasmodium* spp. AP70 in 14 White-backed vultures in Central India. This result and the treatment of ill vultures with an antimalarial drug, as well as the lack of diclofenac residues implied that malaria could be an additional cause for the decline in vulture population.

While the result is interesting, the interpretation offered would seem most unlikely. Avian haematozoan parasites in the genera *Plasmodium* and *Haemoproteus* are widespread and cosmopolitan (except for Antarctica) and have been detected in many avian species[2]. These parasites have been reported from eastern and southern Asia using microscopy-based[3] and molecular-based (cytochrome *b* gene)[4] methods in a broad spectrum of avian species. The fact that vultures have been detected with parasites does not imply that these parasites are pathogenic to the vultures. *Plasmodium* spp. tend to be less host specific than *Haemoproteus*[5,6]. Ishtiaq *et al.*[4] have reported three lineages identical to LaC1 (EF552403)[1] and AP70 (AY714203)[6] from three host species in Myanmar: LIN 3A (EF380115; 533 bp) in common woodshrike (*Tephrodornis pondicerianus*), LIN 3B (EF380116; 255 bp) in wryneck (*Jynx torquilla*) and LIN 3C (EF380117; 256bp) in red-breasted flycatcher (*Ficedula parva*). It is quite possible that the reported parasite lineage (AP70) could have other avian hosts as reservoirs that probably allow for higher abundance of the lineage that 'spills' into vultures or vice-versa. This can only be confirmed by extensive screening of other avian hosts in the region.

Poharkar *et al.*[1] have begun an intriguing debate in parasitology that warrants further clarification on the host–parasite interactions and their coevolution. Avian haematozoan parasites have been endemic to India (Asia) for millions of years. This is unlike isolated island communities (e.g. Hawaii and Bermuda[7]) that have been deprived of the benefits of host–parasite coevolution, such that they often lack sufficient immune defence and are thus highly susceptible to infection from novel parasites that could also cause mortality. The Hawaiian islands have been a classic example where introduction of exotic avifauna and introduced mosquito species *Culex quinquefasciatus*, brought the single genotype of *Plasmodium relictum capistronae* which was deadly to the native endemic birds. Subsequently, Beadell *et al.*[6] showed that *P. relictum* is a globally distributed strain, found in over 60 bird species across five continents as well as in oceanic islands, but it rarely causes mortality in birds on continents. Hence, birds in India have co-evolved with haematozoan parasites and developed immunity to parasite lineages from long exposure. While constant exposure to malarial parasites can elicit a protective immune response as has been observed in humans, naturally acquired immunity is lost if the individual is not constantly exposed to the parasites. Given the prevalence and distribution of malarial parasites in India, it is highly unlikely that vultures have weak immune defences towards one particular lineage of *Plasmodium* species (AP70) that could lead to significant mortality and a decline in population. There are also some surprising features in the report. The authors have failed to explain the presence of identical lineages from all vulture samples and do not offer any insights as to why this lineage matched with that infecting mountain thornbill in Australia and Papua New Guinea, but not with any existing malarial lineage reported from Asia. What measures were taken to make sure that contamination could not have been the cause of retrieval of this single lineage from all birds? By ignoring the existing literature from Asia, the authors have missed the opportunity to discuss the phylogenetic relationships between the lineage found in multiple hosts and wide-ranging distribution. Much of the article has been written as if this is the first evidence of avian malaria parasites from India.

Based on small sub-unit and large sub-unit rRNA genes, the authors have highlighted a 96% similarity in the 180 bp sequence of the avian and mammalian *Plasmodium falciparum*. It is important to note here that this method underestimates the diversity that could be found using a full-length or bigger sequence of the gene. When asking questions about the evolutionary relationship between organisms, it is desirable to have data from longer sequences because they contain more phylogenetic informative sites.

Although these results are potentially important, I believe that they are undermined by several methodological and ecological limitations of the study. The lesions described are not typical of malaria and are consistent with natural and experimental diclofenac poisoning in vultures. Malaria is not known to cause gout, but extensive visceral gout has been found in a majority of wild vultures found dead during the population decline[8,9]. Hence, the agent causing the decline should also cause visceral gout. Microscopic examination of blood smears from 14 vultures revealed the presence of intra-erythrocytic and exoerythrocytic *Plasmodium* schizonts. The species of the family Plasmodidae sometimes show both exo erythrocytic and erythrocytic phases. Hence, an increase in parasitemia could be associated either with the activation of exoerythrocytic merogony (relapse) or with the resumption of erythrocytic merogony (recrudence)[2]. It is difficult to distinguish relapses from recrudescences in birds naturally infected with malaria parasites without using special experimental tests, because the intensity of chronic parasitaemia is often low. A relapse can occur in certain seasons; for example, parasite load is known to increase in spring–summer owing to the increased abundance of flying vectors at that time[10,11] and because higher levels of sexual steroid hormones are circulating in the bird's blood, which in general depress the immune system and allow parasites to survive[12,13]. Poharkar *et al.*[1] have failed to consider seasonal effects and their relationship with parasite load detected in other studies, which could have been a reason for schizont phase of parasites in the peripheral blood.

The diclofenac detection results are highly biased, with a comparison of three negative vs 16 positive vultures. It is not clear whether or not the 16 vultures in north and north-western states positive for diclofenac were also screened for malarial parasites? There are many points in the communication that need a well-supported reference. For example, the decline in vulture populations in Gadchiroli (p. 553), no use of diclofenac or any other NSAID in that region (p. 556), sick birds being treated but with no controls, and reports elsewhere about sick birds recovering simply through rehydration.

How was the cause of illness ascertained (p. 554)? What are the common malarial parasites in poultry in that region?

Unfortunately, the lack of coherence between the ecology of avian malaria and information about decline in vulture population does not allow one to establish whether or not the results[1] indeed highlight an additional cause for the 99.9% decline of Oriental White-backed vulture populations. However, importantly, the authors endorse earlier findings that diclofenac is the main reason for such declines.

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Response:

In our communication we had provided evidence to the best of our satisfaction that malaria could also cause a decline in vulture population in those regions where diclofenac is not used. We have provided evidence to this effect and are convinced with the study. Nevertheless, Farah Ishtiaq has raised several questions about the presence of a specific malarial parasite and its pathogenicity in the vultures in Central India. We totally agree with her that haemoprotozoan parasites are common and widespread in several avian species. It is also entirely possible that the *Plasmodium* spp. LaC1 reported by us in vultures, or AP70 in mountain thornbill, and the three other identical lineages reported by Ishtiaq *et al.* in three different bird species in Myanmar, indicate that the particular lineage in question is present in several avian hosts. But screening of all other probable avian hosts in the region was not the focus of our study. Following microscopic detection of malarial parasites in the blood smears of the affected vultures, the aim was to identify the particular species infecting vultures. Evolution, phylogenetic relationships with hosts and distribution were never within the scope of our communication.

The first step after microscopic examination was to ascertain through DNA analysis, whether the particular haemoprotozoan was a *Plasmodium* or *Haemoproteus* sp. To do this a small fragment of the mitochondrial rRNA genes is sufficient. Here again we reiterate that this is not a study on evolutionary relationships or phylogeny, but rather a simple identification of the genus of the infective organism. This DNA-based identification was further supported by microscopic identification of the parasite and by the treatment of two live, sick birds with anti-malarial drugs.

Visceral gout in birds, including vultures, is not an exclusive symptom of only diclofenac poisoning, but can be brought about by several causes. Like fever, gout is seen in several conditions and one of the simplest but most potent cause for this condition in flesh-eating birds is starvation/dehydration. A carnivorous bird going off-feed due to illness or lack of food can rapidly develop gout, and it need not be a chronic/debilitating condition leading to loss of body mass or any other external symptom. We have also mentioned that the presence of a large number of mosquitoes around the birds during the nesting season from October to March, led to the investigation for the presence of blood parasites (p. 557). Ishtiaq's observation that this opportunistic infection is due to a compromise of the immune system to allow for reproduction is extremely plausible.

Sixteen vulture tissue samples from the north and northwestern states which were positive for diclofenac residues were not screened for malarial parasites. Due to several constraints discussed by us, we could not screen more than three vulture samples from Gadchiroli which were positive for malaria, for the presence of diclofenac. But we ruled out the possibility of exposure to diclofenac poisoning by several circumstantial evidences.

There are a couple of points towards the end of the correspondence by Ishtiaq which we fail to understand. For example, the statement about treating sick birds with no controls. This would require injecting normal healthy vultures or sick birds only with the vehicle (water). We do not see any reason for injecting water to normal vultures. Also, we had only one sick bird at a time, which we wanted to save rather than experiment upon. Ours was a sincere attempt to treat and save birds of this highly endangered species, with efforts to understand the underlying causes for their decline. Also what we meant by saying, 'Whether these recoveries were due to the anti-malarial drugs or simply due to the availability of sufficient food and water is debatable' (p. 557), is that there is a good chance that the availability of food and water revived the bird sufficiently to fight off the infection on its own, with or without the drugs support. The fact that the infection was there in the first place is not debatable. Again, irrespective of the other malarial parasites present in the other bird species/poultry, this particular lineage (*Plasmodium* spp. LaC1/AP70) was definitely present in the White-backed vultures of Gadchiroli.

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