Health Assessment of White-rumped Vultures (*Gyps bengalensis*) from Changa Manga, Lahore: A Case Study

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Abstract

Vultures are considered as an important indicator of a healthy environment, and they play a vital ecological role as scavengers. The present study was designed to estimate the health of critically endangered *Gyps bengalensis* in Changa Manga, Lahore, using a technique that does not require drawing blood. Fecal matter of seven pairs were examined and analyzed for protozoan or helminth infections. Gross examination involved checking consistency and color of feces, presence of blood in feces and gross parasites. Obtained samples had different colors and consistency but no blood was found in them. Pair 1 was infested with oocytes, but in pairs 5 and 6 only males were infested, while the female was infested in pair 7. Pair 2, 3, and 4 was completely healthy. Gram staining was also performed to assess the presence or absence of bacteria culture in digestive tracks of *Gyps bengalensis*. All pairs were infected with either gram positive or negative except Pair 5 which was negative for all gram staining. The present work introduced a technique to assess the health of vultures without drawing blood samples, a process that causes disturbances to their life cycle.

Keywords: Changa Manga, Gram Staining, *Gyps bengalensis*, Parasites

1. Introduction

Pakistan has a diverse biodiversity and landscape richness. About 670 species of birds have been recorded from Pakistan, including migratory species. Vultures are scavenging birds and play an important role in maintaining the environment. They mostly eat dead or sick animals, and thus recycle the environment[1, 2]. There are two main groups of vultures, i.e. Old World vultures and New World vultures. The New World vulture belongs to Family Cathartidae, Order Accipitriformes. Seven species are found in temperate and warm areas of the Americas. New world vultures have a good sense of smell. The Old World vultures are found in Europe, Africa and Asia. They belong to family Accipitridae and order Accipitriformes [1, 3].

There are 23 species of vultures in the world and from that nine species are critically endangered, four species are near threatened, and three are endangered [4]. Nine species of vultures are found in Asia. They are WRV (white rumped vulture; *Gyps bengalensis*), CV (cinereous vulture; *Aegypius monachus*), HV (himalayan vulture; *Gyps himalayensis*), RHV (red-headed vulture; *Sarcogyps calvus*), EV (Egyptian vulture; *Neophron percnopterus*), GV (griffon vulture; *Gyps fulvus*), SBV (slender-billed vulture; *Gyps tenuirostris*), BV (bearded vulture; *Gypaetus barbatus*) and IV (Indian vulture; *Gyps indicus*) [5]. In Pakistan eight species of vultures are found in Pakistan except Slender billed vulture the rest of eight species found in Pakistan.

White rumped vultures (WRV) are medium sized vultures. The adults are about 75-85 cm tall, with a wingspan of 180-210 cm. The weight ranges from 3.5-7.5 kg. The sexes are approximately equal in size. Adults are darker than juveniles, a white neck ruff, and with blackish plumage [6]. White vulture lays eggs twice in a year but egg hatching ratio is once a year [7]. Their name is derived from the white patch of feathers on the lower back and upper tail. WRV are endemic to south Asia, and critically endangered in Pakistan. The population of WRV has declined by over 95% in the early 1990s [8]. The cause of its rapid decline is the veterinary use of non-steroidal anti-inflammatory drug (NSAID) diclofenac. Diclofenac is used in domesticated animals as a pain killer [9]. This decline has resulted in a decrease of WRV population to 0.1 % of its original size. [10-12]. The loss of vulture's population caused

major secondary impacts including increasing numbers of feral dogs that exploit vultures' food sources. Gyps Vulture Restoration project, run by WWF-Pakistan, has the center piece of this project established at Changa Manga in the province of Punjab of Pakistan, located at 80 km southwest of Punjab [13]. The present study was designed to estimate the health of critically endangered WRV in Change Manga, Lahore, and a technique that does not require withdrawing blood.

1. Materials and Methods

2.1. Chemicals and Reagents

The chemicals which were used are absolute alcohol, acid alcohol, 3% malachite green and Kinyoun's carbol fuchsin. The acid alcohol was prepared 10 ml of sulfuric acid with 90 ml of absolute alcohol and stored at room temperature. Three percentage of malachite green was prepared by dissolving 3 g of malachite green in 100 ml of distilled water.

2.2. Study Population

The study population comprised of white rumped vultures from Change Manga, Lahore (Fig. 1). . Fourteen samples were collected of 7 pairs that are 7 males and 7 females. Due to limited equipment and chemicals gross examination of feces and feathers, gram staining of stool sample and fecal float method were performed.

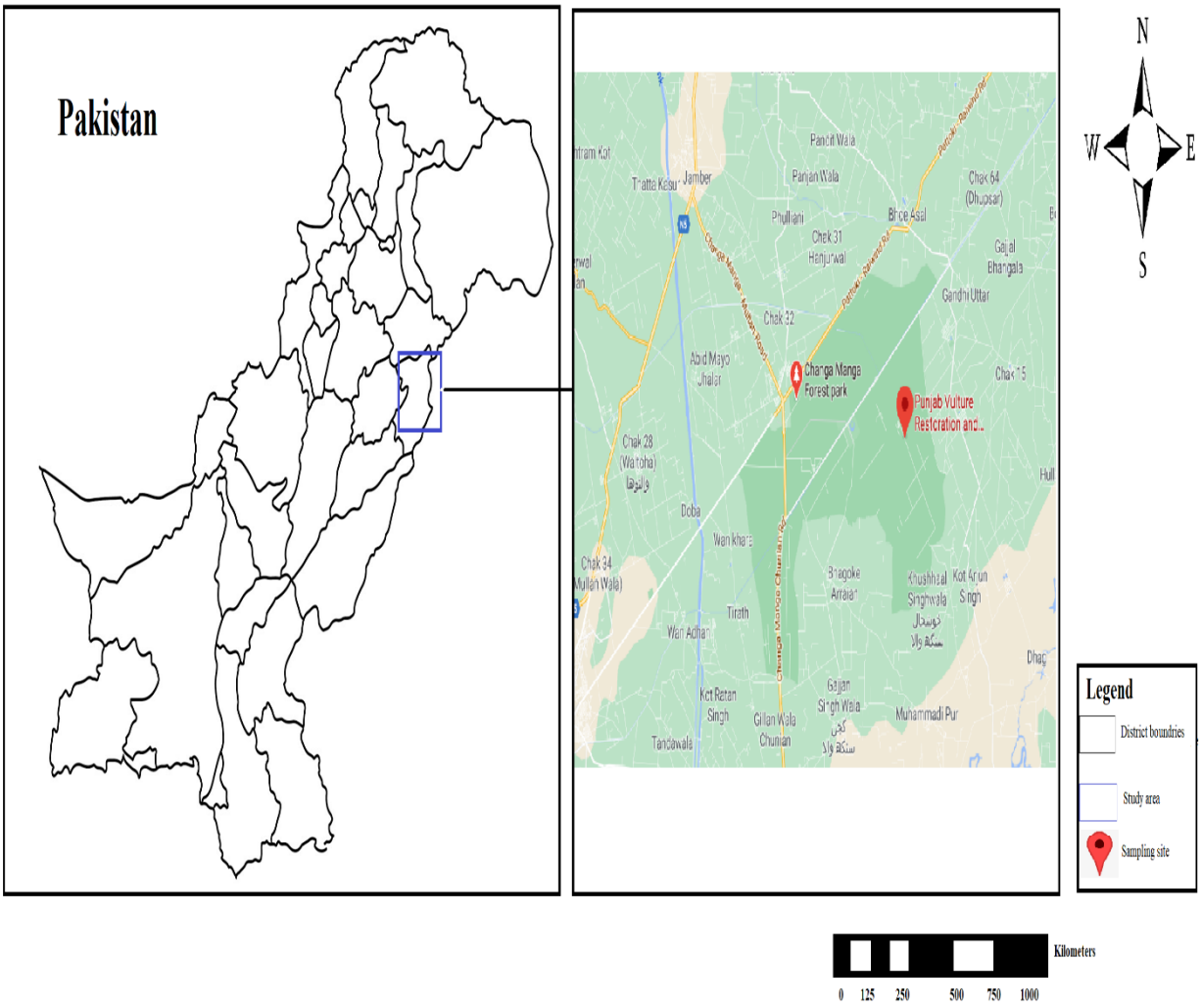

Fig 1. Map of study area and location of sampling sites at Changa Manga, Pakistan.

2.3. Fecal Collection

Fecal sample were collected without any contamination by spreading a polyvinyl sheet in the cage. Samples were collected in a clean container, by the lid of bottles [14]. They were transported to Virtual University of Pakistan, Lahore to check for endoparasites.

2.4. Fecal Sample Preparation

Different chemical tests were performed with fecal samples. A fecal smear was prepared to check bacteria and other microorganisms which is indicative of disease in the digestive tract. A small amount of fecal sample was examined under a microscope at 10× and 40× power. For oocytes of coccidian species (*Cyclospora* and *Cyctoisospora*) a smear was prepared and dried at 60 °C, fixed with absolute alcohol for 30 seconds, stained with carbol fuchsin for one minute, and washed with distilled

water. The slide was then de-stained with acid alcohol for 2 minutes, washed, and dried on a slide warmer at 60 °C. This was observed under 40× or higher magnification. Fecal matters of 7 pairs were examined and analyzed for protozoan or helminthes infection. The gross examination involved consistency of feces, color of feces, presence of blood in feces and gross parasites.

3. Results

The samples were observed and sometimes visible to naked eye and then examined through microscope. All samples had different color and consistency but no blood was found in fecal materials. Pair one was infested with oocytes, but in pair five and six only male was infested, and female was infected in pair 7. Pairs two, three and four were completely healthy (Table 1).

Table 1. Estimation of different parameters of fecal material

Pairs	Gender	Weight (Kg)	Age (Years)	Consistency	Color	Presence of blood	Gross parasite
1	♂	6.8	9	Solid ,sandy	Dark brown, green	No	Visible
	♀	7.3	9.5	Solid	Brown ,green	No	Visible
2	♂	7.5	8	Solid, sandy	Dark brown	No	Not visible
	♀	6.8	8.8	Semi solid	Black	No	Not visible
3	♂	6.7	8.5	Solid	Brown, black	No	Not visible
	♀	7.1	8	Solid, sandy	Brown	No	Not visible
4	♂	6.8	9	Solid	Brown	No	Not visible
	♀	6.7	10	Semi solid	Brown	No	Not visible
5	♂	5.8	11	Solid, sandy	Green	No	Visible
	♀	6.2	10.5	Solid, sandy	Black ,light brown	No	Not visible
6	♂	7.1	9	Solid	Green	No	Visible
	♀	7.2	8.5	Solid	Dark brown	No	Not visible
7	♂	8	11.5	Solid	Black	No	Not visible
	♀	7.9	10	Semi solid	Brown , green	No	Visible

Gram staining was also performed to assess the presence or absence of bacteria culture in digestive tracks of White-rumped Vultures. All the pairs were infected with either gram positive or negative but only pair five was negative for all gram staining (Table 2).

Table 2. Characterization of gram staining from White-rumped Vulture feces

Pairs	Gender	Gram +	Gram -

1	♂	-ve	-ve
	♀	-ve	+ve
2	♂	+ve	-ve
	♀	+ve	-ve
3	♂	-ve	-ve
	♀	-ve	+ve
4	♂	-ve	+ve
	♀	+ve	+ve
5	♂	-ve	-ve
	♀	-ve	-ve
6	♂	-ve	+ve
	♀	+ve	+ve
7	♂	-ve	-ve
	♀	+ve	+ve

-ve; negative and +ve; positive

4. Discussion:

Endoparasites are organisms that live inside an animal's body and harm them [15]. In order to check the health status of the White-rumped Vulture only limited fecal and feather samples were collected as the birds were in breeding season. Gross examination of feces showed result of its consistency, it tells about diarrhea, hard, sandy or soft. Color green can mean parasites, dark brown mean anoxia for about 24 hours and red due to the presence of blood which shows infection in intestines [16].Several studies about intestine parasites had been conducted on wild birds [17-22]. Our study revealed the presence of coccidiosis in fecal samples.[23] also study that coccidiosis are one of the most frequent and common diseases of birds in the world. In almost 30 species of birds, the most common coccidiosis genus was *Cryptosporidium* spp and act as intestinal pathogen [24]. For *Cryptosporidium* genus three species i.e. *C. meleagridis, C. baileyi* and *C. galli* causes renal and respiratory diseases in many bird species [25-27] which results in high mortality [28]. [29] study the Eurasian griffon vultures (*Gyps fulvus*) microflora and digestive tract parasites and concluded that the parasitic findings depend on the feeding habits of the vultures.

The microflora present in predator intestine dependent on its diet [16]. [30] studied that gram negative bacilli especially *E. Coli* of family Enterobacteriaceae were normally found in the intestinal track of whiteback griffon vulture and some other members of family Falconiformes because mostly intestinal flora derived from their prey. The gram staining works on the principle that organism's cell wall determines whether the organism is gram negative or positive. The bacteria which retain crystal violet which is primary stain are called gram positive and which bacteria that decolourized and then counterstained called as gram negative [31]. The bacteria isolated from vultures can spread diseases to other wildlife and human population especially *E. coli* [30, 32]. The presence of bacteria whether its gram

positive /negative show intestinal inflammation or irritation. To detect parasites eggs, a floatation solution that has higher gravity than eggs is used. Fecal flotation is a veterinary test used for the diagnosis of worms and internal parasites. This helps in detection of eggs of mature parasites present in body and passes their egg through host's stool. In this egg floats on the surface and other heavy part of fecal sample sink into bottom [33]. The health status of White-rumped Vultures are also measured by their age, weight and egg hatching ratio [34].

5. Conclusion

The present study revealed that the bacteria found in fecal material considered to be normal in intestine but can evolve into pathogenic status under stress or depressed immune system. The fecal samples indicate the presence of parasites in some pairs and almost all pairs are positive for gram staining.

DECLARATIONS

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- **Conflicts of interest / Competing interests**

  Authors declare no conflict of interest.

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