ORIGINAL ARTICLE

Mallophaga Species on Long-billed Vultures (*Gyps indicus*) in Bundelkhand Region of India and Remarkable Defence Mechanisms of Vultures Against Them

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Abstract

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Scavenging avian species play an important ecological role in many regions and it is important to understand the adverse effect of ecto and endo parasites for conservational purposes of the endangered bird species. During the study duration (2007-2011) in Bundelkhand Region, total 9 vultures (8 dead and one live) were examined for ectoparasites. Only two vultures i.e. one dead adult (VA4) and one live juvenile (VJ) were found to have ectoparasites. The 3 Mallophaga collected from vulture adult 4 (VA4) were identified through various identifying key and scientific grey literature as female of Laemobothrion maximum belonging to the family Laemobothriidae. The 2 Mallophaga collected from Vulture Juvenile (VJ) were identified as male of Colpocephalum polonum belonging to family Menoponidae. The infestation was very low and insignificant to cause any adverse effect on vulture health. By observing the remarkable behaviour and morphological features of vultures it was concluded that vultures had a number of defence mechanisms. The behaviour such as preening, sunning and adding green material to nests, along with morphological features like melanin in feathers and beak overhang clearly shows that good management by the vultures has significant positive impact in controlling detrimental effects of ectoparasites.

Key words: Vultures, Mallophaga, Laemobothriidae, Menoponidae, Defence mechanism.

1. Introduction

Scavenging avian species play an important ecological role in many regions (Mundy et al., 1992). For the conservation of ecologically important vulture species, it is imperative to study all the aspects associated with them such as their status, breeding biology, feeding habits, general behavioural aspects, causes of mortalities (pathology as well as parasitology) and threats to the last surviving population. There are 9 species of vultures found in India (Ali et al., 1995), King vulture (Sarcogyps calvus), Cinereous vulture (Aegypius monachus), Griffon vulture (Gyps fulvus), Himalayan Griffon vulture (Gyps himalayensis), Long-billed vulture (Gyps indicus), Slender-billed vulture (Gyps tenuirostris), White-backed vulture (Gyps bengalensis), Egyptian vulture (Neophron percnopterus), Bearded vulture (Gypaetus barbatus). Bundelkhand Region has 7 vulture species. Slender-billed vulture and Bearded vulture are absent in Bundelkhand Region. The parasites commonly found in aves include protozoans, helminths and arthropods. Ectoparasites may reside in the skin or feathers of the birds for diverse time periods, and are damaging to them. Lice (singular: louse) belong to either the sucking louse order (Anoplura) or the chewing or biting louse order (Mallophaga). About 460 species of sucking lice have been reported and 3,000 species of chewing lice. A particular species of louse is also restricted to one part of the body of one kind of host. Host morphology also plays a role in occurrence of ectoparasites. Among species of birds, the abundance component of diversity is correlated with host body size and bill morphology (Clayton and Walther, 1997). Abiotic factors such as humidity can also influence louse distribution. Birds living in arid environments tend to have fewer lice than similar birds in humid environments (Moyer et al., 2002b). Feeding activity of the ectoparasites may result in significant blood loss, secondary infestations, pruritus, and excoriation and in some cases premature death (Berriatua et al., 2001). In some cases, infected animals may alternate to self-wounding, particularly

when ectoparasites are present in high densities (Berriatua et al., 2001).

On the basis of present state of knowledge it is evident that so far no systematic study has been done on vulture species in Bundelkhand Region of India. Since vultures were common and had large population no preliminary surveys and research has ever been done in the selected study area. This study is a part of parasitological investigation undertaken in the Bundelkhand Region. The aim here is to present the overall view of Mallophaga species on the vultures.

2. Study Area

The study was carried out in an area of two States of India, Uttar Pradesh and Madhya Pradesh known as Bundelkhand (Fig 1). The Bundelkhand region has an area of around 70,000 sq. km. It lies between $23^{\circ}-26'$ N and $78^{\circ}-82'$ E. The region stretches over districts of Southern Uttar Pradesh and Northern Madhya Pradesh (Bundelkhand Vikas Nidhi 1990-1991; M.P. Bundelkhand Development Authority 2007). The principal rivers are the Sindh, Betwa, Ken, Bagahin, Tons, Pahuj, Dhasan and Chambal. Bundelkhand is a hot and semi-humid region. Average rain fall is 600 - 700 mm but concentrated only during July - August. Minimum temperature varies from around 4°C to 12°C. The temperature during summer goes up to 48°C.



Fig 1: Map of Bundelkhand Region. Source:http://www.thehindu.com/multimedia/archive/0 1810/Contours_of_Bundel

3. Methodology

First ticks and lice were searched with unaided eyes. Smaller parasites (like feather mites) were searched on flight and tail feathers. For skin and scale mites scraps were collected. The dead bird was dusted with insecticidal powders and was then placed in a confined area over a collecting surface (white cloth or paper) for a fixed period. The plumage of the bird was thoroughly brushed for the collection of ectoparasites. The feathers, head, neck, body, legs and cloaca were raised and thoroughly examined with magnifying hand lens for ectoparasites. Attached ectoparasites, which could not be removed by brushing, were gently dislodged with a pair of thumb forceps and their positions noted (Clayton and Walther, 1997). Ectoparasites were collected manually or with tweezers and stored into a labelled vial with 70% alcohol. Ectoparasites were cleared in 10% KOH (potassium hydroxide), washed and dehydrated in ascending grades of alcohol, cleaned in xylene for permanent mounts in DPX. The identification of the ectoparasites was done using the Biological Phase Contrast Inverted Microscope and the bibliography including identifying key and scientific papers for Laemobothrion maximum by Nelson and Price (1965), Lakshminarayana (1970), Perez (1995), Dik (2007) and Colpocephalum polonum by Price and Beer (1963), Dik (2006; 2011).

4. Result and Discussion

The 9 samples (8 dead and one live) were examined (Table 1) for ectoparasites and only two vultures i.e. one dead adult vulture (VA4) and one live juvenile vulture (VJ) were found to have Mallophaga Species. The 3 lice collected from Vulture adult 4 (VA4) were identified as female of *Laemobothrion maximum* belonging to the family Laemobothridae; whilst the 2 lice collected from VJ were identified as male of *Colpocephalum polonum* belonging to family Menoponidae. Both the families come in the Order Mallophaga, Suborder Amblycera and family Amblyceran.



Fig 2: Triangular head of *Colpocephalum polonum*. a: anterior subocular setae, s: subocular seta, c: subocular comb row, d: dorsolateral margin of head

S. No.	Name	Date	Species/Stage	No.	Species
1	VA1 Standard	2 nd March, 2008	G. indicus/Adult	0	-
2	VA2	10 th March, 2008	G. indicus/ Adult	0	-
3	VA4	28 th February, 2009	G. indicus/Adult	3	Laemobothrion maximum
4	VA6	16 th March 2009	G. indicus/Adult	0	-
5	VJ7	13 th May, 2009	G. indicus/Juvenile	0	-
6	VJ	7 th June, 2009	G. indicus/Juvenile	2	Colpocephalum polonum
7	VA9	17 th February, 2010	G. indicus/Adult	0	-
8	VJ10	15 th June, 2010	G. indicus/Juvenile	0	-
9	VA11	6 th December, 2010	G. indicus/Adult	0	-

Table 1: Details of the samples examined for ectoparasites.

4.1 Colpocephalum polonum

The Colpocephalum polonum, was reported only a few times and first time in *Gyps indicus*. The two males characteristics were studied (Eichler and Zlotorzycka, 1971) in this work as follows:

- a. Dark Preoculer and occipital area.
- b. Occipital nodi were unified by noticeable occipital and temporal carinae.
- c. Triangular head was expanded behind the eyes (Fig 2).
- d. The maxillary palp was four-segmented; the labial palp was one-segmented, and had five distal setae.
- e. The antennae were four segmented with two adjacent sensilla on the terminal segment.
- f. Combs of tiny spiniform setae constrained to venter of femora III and abdominal sternite III.
- g. Absence of ventral sclerotized processes on Head.
- h. Ventral sclerotized processes arised near palpal bases.
- i. Terminal antennal segment without any distinct splitting up.
- j. Prosternum with simply center setae.
- k. Only two medium-length setae with spacious gap anterior to the comb row on the latero-ventral head margin.
- 1. Male genitalia with genital sclerite of characteristic shape, had an ovoid basal segment, with or without a pair of projections lateral to that (Fig 3).

The head and thorax of both sexes of *Colpocephalum* provided a few significant characters for species separation. The shape of the head and thoracic segments was consistently similar. There was usually only a shallow preocular indentation, but some specimens indicated a faint slit. The abdomen often demonstrated a pronounced sexual dimorphism.Basic divisions of the males were established according to the shape of the genital sclerite as well as that of the penis. Metasternal plate had 13 setae. Further abdominal differences involved the presence or absence of anterior

setae on tergite IX, the numbers and lengths of the tergocentral and anteior setae on tergites I-VIII, and the lengths of the post-spiracular setae. No consistent characters for species recognition were found in the sternal chaetotaxy. Also, the males had all abdominal segments essentially of the same length and always had undivided abdominal tergites (Fig 4). Cephalic length: 0.34-0.37 mm; Cephalic width: 0.58-0.62 mm; Thoracic length: 0.51-0.54 mm; Thoracic width: 0.49-0.52 mm; Abdominal Length: 1.05-1.07 mm; Abdominal width: 0.84-0.86 mm; Total length: 1.90-1.98 mm (Table 2). The morphogenic characters of *C. polonum* had not been described in detail, but were said to resemble *C. turbinatum* and *C. qermanum* (Dik, 2011)



Fig 3: Male genitalia of Colpocephalum polonium.

Clay (1951) described the morphological characters of *Colpocephalum heterosoma* (Piaget, 1880) and *Colpocephalum salimalii* (Clay, 1951) in detail, and reported resemble of these species to each other. This investigation indicated that the characters of male genitalia of *C. salimalii* were quite different from

C. heterosoma and there were some differences in the shape of the metathorax and abdomen and the protergal, mesotergal and abdominal chaetotaxy. In addition, Price and Beer (1963) explained the morphological characters and identification key of the Colpocephalum species found on Ciconiiformes. Price and Beer (1963) reported that Colpocephalum nanum occurred on goshawk in addition to the other prey birds. Eichler and Zlotorzycka (1971) identified that the lice specimens were collected from goshawks as C. polonum (=Neocolpocephalum polonum). The morphologic characters of C. polonum were not described by Eichler and Zlotozycka (1971) in detail, however, they pointed out that this species resembles to *C. turbinatum* and *C. germanum*.



Fig 4: *Colpocephalum polonum* (Male). [pp: pleural plates; ta: transverse abdominal bands]

4.2 Laemobothrion maximum

Three females were studied (Scopoli, 1763) in this work. The flattened head of the Mallophaga, which was relatively large for the body, was horizontal in position with the labrum forward. The mandibles were the most conspicuous portion of the mouth parts; the maxillae and the labium were small and less imperfect. In the jaws, the hypopharynx was an important appendage with some curious features (Fig 5). The characteristic feature of hypopharynx was a conspicuous cup-shaped sclerite on the frontal hypopharyngeal facade just ahead of the mouth. The sitophore was attached with the lingual sclerites by a branched duct-like filament, the arms of which pass through the front surface of the hypopharynx. The mandibles had the typical biting type of arrangement. They were strongly toothed, and were hinged to the head by the common ball and socket articulations. The jaws lied in a plane parallel with the under surface of the head with their articulations dorsal and ventral. The maxillae were better developed, each having a short, segmented palp. The labium was simple, consisting of a broad plate in the under wall of the head bearing two or four small terminal lobes.



Fig 5: Head of Laemobothrion maximum.

The average length of the reported females was 9.81 mm (Table 3). Cephalic length: 1.60-1.61 mm; Cephalic width:1.75-1.82 mm; Thoracic length:1.99-2.14 mm; metathoracic width: 2.00-2.02 mm; Abdomen Length: 6.05-6.30 mm; Abdomen width: 2.60-2.98 mm; Total length: 9.64-9.91 mm. The head narrowed in anterior. The body was divided into head; thorax and abdomen (Fig 6). Temples were not very big in width. There were four long setae on each side of the temple. Each of the temples covered rather major portion of the surface and were limited internally by the dorsal occipital band; temporal lobes (Fig 7). The femur and the tarsus were the first and last joints of the legs respectively. Sitophore sclerite of hypopharynx had two large holes, with an obvious U-shaped stricter. Lacking large un-pigmented areas along the median line of abdominal tergites; there were prominent lateral preoculer swellings in front of eyes. The palps had four segments. Maxillary palps were four-segmented.

There were four or five setae in antero-lateral prosternal plate. Meso-metasternal plate had small setae on either side of latero-anterior portion. Femur II had four setae in proximodorsal portion. Proximodorsal aspect of femur II was with 4 or less stout spiniform setae. There was no pigmentation in the subvulvar region. It had two long and three short setae on both sides (Dik, 2007). The last abdominal segment had fringe of fine hairs (Fig 8 A-B).

Morishita *et al.* (2001) observed two *Laemobothrion* species on raptor species. Only 4 species of *Laemobothrion* infest raptors worldwide, but

each species has a large number of hosts (Nelson and Price, 1965). They observed *Laemobothrion maximum* on the free-living Swainson's hawk. More than 35 hosts were documented for this louse, most in the family Accipitridae; *Buteo* species were its most common hosts (Nelson and Price, 1965).



Fig 6: Female of Laemobothrion maximum. ac: conspicuous lateral bulbous capsule in laemobothrion or groove or furrow on the ventral surface of the head for head for reception of antenna in Amblyceron Mallophaga, bound on each side by the facial ridge; pf: prothorax; the first segment of the thorax, bearing the anterior pair of legs (foot jaws); tt: pterothorax; meso and meta-thorax fused together; tp: tergal plates; transverse bands; the dorsal pigmented sclerites of the abdominal segments, lying between the lateral or pleural plates; these may be entire, median or confined on the lateral or submedian region of the abdominal segments; tu: tarsus, the last joint of the legs; tl: temples; lateral plates, each of which covers rather major portion of the surface and is limited internally by the dorsal occipital band; temporal lobes; ol: ocular slit; cl: claw; fe: femur, femora; the first conspicuous long joint of the legs, nearly always the strongest of all, preceded by the coxa and the very small connecting joint between these two called the trochanter and followed by tibia; thigh.

From the study area, the prevalence of ectoparasites was found to be insignificant. Various defensive mechanisms exist to minimize the detrimental impact. These mechanisms include morphological barriers, immune responses, and behavioural defences. At the level of host populations, the host density raises the parasite transmission (Grenfell and Dobson, 1995), i.e. densely populated host specie should have more parasites than those with dispersed populations. The host density has been established as a significant interpreter of parasite richness by a number of studies (Morand and Poulin, 1998) and abundance (Arneberg et al., 1998, Tella et al., 1999). As Gregory (1997) has argued, it is conceivable that small-bodied species will have higher

parasite abundance, since such species tend to have denser populations, which improves the efficiency of parasite transmission. Although vultures are colonial, they maintain their territories particularly their nest site. The population is low and the distance between nests is enough to neglect the parasite transmission among the species (Fig 9). The blue circles in Fig 9 show the nests of vultures in a colony that are about 6-10 metres apart from each other.



Fig 7: Leg of the female of Laemobothrion maximum. fe: femur, femora; the first conspicuous long joint of the legs, nearly always the strongest of all, preceded by the coxa and the very small connecting joint between these two called the trochanter and followed by tibia; thigh. tl: temples; lateral plates, each of which covers rather major portion of the surface and is limited internally by the dorsal occipital band; temporal lobes, tu: tarsus, the last joint of the legs, ol: ocular slit, cl: claw

One defence against parasites is to occupy environments that are relatively free from parasites. Another defence may be to occupy environments too extreme for the survival of the parasites themselves. The nesting sites were mostly in cliffs and monuments that were less favourable for ectoparasites. Moreover, the temperature in Bundelkhand region is usually high with dry conditions. Moyer et al. (2002) showed experimentally that lice cannot survive on birds kept at low relative humidity (<35% RH). Avoidance of parasites also operates on a more local scale. For example, quite a few studies have now shown that birds avoid nesting or roosting in sites that are infested with ectoparasites (Oppliger et al., 1994; Merila and Allandar, 1995; Merino and Potti, 1995; Hart, 1997; Loye and Carroll, 1998; Rytkonon et al., 1998; Thompson, 1999).

Another parameter influencing the parasite -

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Body Part	Colpocephalum polonum-1	Colpocephalum polonum-2	Average dimensions (in mm)
Cephalic length	0.34	0.37	0.35
Cephalic width	0.58	0.62	0.60
Thoracic length	0.51	0.54	0.53
Thoracic width	0.49	0.52	0.51
Abdomen length	1.05	1.07	1.06
Abdomen width	0.84	0.86	0.85
Total length	1.90	1.98	1.94

Table 2: Some dimensions (in mm) of Colpocephalum polonum male

Table 3: Some dimensions (in mm) of Laemobothrion maximum female

Part	Laemobothrion	Laemobothrion	Laemobothrion	Average
	maximum-1	maximum-2	maximum-3	dimensions (mm)
Cephalic length	1.60	1.58	1.61	1.60
Cephalic width	1.75	1.79	1.82	1.79
Thoracic length	1.99	2.14	2.00	2.04
Metathoracic width	2.00	2.02	2.01	2.01
Abdomen length	6.05	6.17	6.30	6.17
Abdomen width	2.60	2.86	2.98	2.81
Total length	9.64	9.89	9.91	9.81



Fig 8A-B: Abdomen of Laemobothrion maximum *F*: fringe of fine hairs on the last abdominal segment

diversity is the host defence. Vultures have tough plumage so that they can support the flight of such gigantic birds. Tough plumage could also deter featherfeeding ectoparasites, analogous to foliage containing cellulose which helps deter feeding by herbivores (Howe and Westley, 1988). The colour of vulture feathers is usually dark brown, brown or black (Fig 10) except that of adult Egyptian vultures. Feathers that contain melanin (the pigment typically responsible for brown, gray or black colours) are known to be more resistant to mechanical abrasion than feathers without this pigment (Burtt, 1986; Bonser, 1995). Studies suggest that melanin may also limit damage by featherfeeding lice (Kose and Moller, 1999). Feathers having melanin are tough and resistant to downgrading, and may also prevent feather feeding ectoparasites. Kose *et al.* (1999) surveyed feather damage in Barn Swallow (*Hirundo rustica*) populations and found significant chewing by the lice the white (melanin-free) spots on the tail feathers.

Avian body maintenance includes grooming, dusting, sunning, and anting (Cotgreave and Clayton, 1994). Preening and scratching are together known as grooming behavior (Cotgreave and Clayton, 1994), that



Fig 9: Sufficient nesting spacing prevents parasite transmission

is crucial for defence against ectoparasites (Marshall, 1981; Hart, 1997). The most common activity seen during the study time was preening. While preening the bird draws its feathers between the bill mandibles and nibble them with the tips of the bill (Clayton *et al.*, 2010). *Gyps indicus* were seen spending hours in the sun, sitting and preening (Fig 11). Sunning and preening combined together have more adverse effect on ectoparasites. Preening is the most common defensive behavior that birds use against ectoparasites.



Fig 10: Vulture feathers have melanin

In the case of a single defence, an ectoparasite can exploit a refuge to escape that defence. For instance, some wing lice are dorsoventrally flattened so that they can slide between the barbs of flight feathers and escape the preening bill. Sunning, however, heats the flight feathers such that lice flee the interbarb refuge and move down the feather towards the body (Moyer, 2002b). Thus lice may not have a refuge from preening in the presence of sunning, and their mortality may increase synergistically. Vultures spend a lot of time in sunning (Fig 12). There are experimental research studies that support the evidence that is consistent with the ectoparasites control hypothesis. Blem and Blem (1993) compared the rate of sunning by non-fumigating controls and they found that fumigated birds sunned less frequently than controls. This implies that the requirement for sunning reduces with fall in ectoparasite population. Additional research is needed to test the impact of sunning on the ectoparasites of live birds.

Different components of bill morphology may be important to different aspects of preening. Bill morphology is an important component of preening efficiency. Numerous anecdotal reports document that wild birds with deformed bills have elevated ectoparasites loads (Rothschild and Clay, 1952; Ash, 1960; Pomeroy, 1962; Ledger, 1969; Marshall, 1981). Controlled experiments, in which bill morphology was dramatically manipulated (Hart, 1997), triggered rapid increases in ectoparasites load. Recent work demonstrates that even subtle features of bill morphology are critical for controlling ectoparasites. The maxillary overhang is the distal portion of the upper mandible (maxilla) that curves over the lower mandible. The negative correlation between length of the overhang and louse load suggests that the overhang is important for controlling lice during preening. Birds with straight mandibles may have trouble damaging lice because the vertical force that can be exerted between the tips of straight mandible is relatively weak (Bock, 1966). Birds with an overhang such as vultures, however, could generate a shearing force sufficient to damage lice (Fig 13). This model assumes that the -



Fig 11: Vultures spend most of their time in preening and sunning



Fig 12: Sunning and preening by Gyps indicus

lower mandible moves forward during preening. Birds may incur a parasite-related cost if their foraging ecology has selected for loss of the bill overhang, which may be compensated by other mechanisms of parasite defence (Moyer and Clayton, 2004).

Green material was commonly seen in the nests constructed by vultures (Fig 14). In Bundelkhand region the commonly used green material was of neem (*Azadirachta indica*), chirol (*Holoptelea integrifoloa*), salai (*Boswellia serrata*), seesham (*Dalbergia sissoo*), dhau (*Anogeissus latifolia*), and teak (*Tectona grandis*). The insertion of green vegetation into nests is thus another behavior to control nest parasites (Clark, 1991; Clayton and Wolfe, 1993; Dumbacher and Pruett-Jones, 1996; Hart, 1997). Clark and Mason (1985) showed that European starlings (*Sturnus vulgaris*) select species of plants that contain volatile chemicals with antibacterial, insecticidal, or miticidal properties. The same authors later showed that nests containing such herbs have lower infestations of blood-sucking mites (Clark and Mason, 1988). More recent research suggests that the addition of herbs to the nest does not

necessarily serve to reduce ectoparasites loads, but may help nestlings cope with the detrimental effects of the ectoparasites.



Fig 13: Beak overhang to combat ectoparasites

5. Conclusion

This study showed that Long-billed vultures had meek external parasite infestation, which had no adverse affect on their health status and did not contribute to decreased population. During the research, it was found out that there were 2 species of Mallophaga among the vultures: Laemobothrion maximum and Colpocephalum polonum. Although they play no role in vulture decline; still the presence of these ectoparasites affects the behavioural aspect. It also contributes to the diversity of ectoparasites (Mallophaga). This study demonstrated that it was not odd for free-living, clinically healthy raptors to harbor one or more species of chewing lice. Vultures had a number of defense mechanisms including morphological features and remarkable behavioral aspects. The behaviour such as preening, sunning and

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adding green material to nests, along with morphological features like melanin in feathers and beak overhang clearly shows that good management by the vultures has significant positive impact in controlling detrimental effects of ectoparasites.



Fig 14: Green twigs in vulture nest

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