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THE PREVALENCE OF HELMINTH PARASITES IN AMPHIBIA FROM SAMASPUR BIRD SANCTUARY, A RAMSAR SITE IN UTTAR PRADESH, INDIA

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Amphibian hosts from the Samaspur Bird Sanctuary were investigated for the study of helminth parasites. A total of four species of hosts were studied and revealed the infection of five species of helminth parasites. In the study area, *Euphlyctis cyanophlyctis* harboured four species namely, *Cosmocerca* sp., *Rhabdias* sp., *Ganeo tigrinum* and *Pleurogenoides gastroporus* with prevalence of 0.33% each and intensity of 1.33, 1.66, 0.33 and 2 respectively. While, *Duttaphrynus stomaticus* was infected by a single species, *Cosmocerca kalesari* with prevalence of 1%, intensity 4-6 and abundance of 4.66. No infection was found in *Polypedates maculatus* and *Hoplobatrachus tigerinus*. This is the first study on the helminth parasites in Amphibian hosts from Samaspur Bird Sanctuary, a Ramsar site in Uttar Pradesh.

Keywords: *Euphlyctis cyanophlyctis; Duttaphrynus stomaticus; Cosmocerca kalesari; Ganeo tigrinum and Pleurogenoides gastroporus.*

ABBREVIATIONS

mm=millimeter; µm=micrometer; Prevalence=the percentage of host individuals infected in a sample of host species examined; Intensity=the number of individuals of a particular species in each infected host; Abundance=the mean number of individuals of a particular species per host examined.

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1. INTRODUCTION

Helminth parasites are studied under two Phylum namely, Platyhelminthes for flat-worms and Nematoda for roundworms. The Samaspur Bird Sanctuary, in the Raebareli district of Uttar Pradesh, is a perpetual lowland marsh characteristic of the Indo-Gangetic Plains. Its six connected lakes are heavily relevant on monsoon rains. Annual counts regularly find more than 75,000 birds present, with over 250 resident and migrant species documented. It was declared as Ramsar wetland in 2019, Ramsar sites are wetland region of international importance for the conservation of biodiversity of the area [1].

To the best of our knowledge no work on helminth parasites of Samaspur Bird Sanctuary has been reported yet but several works has been reported from the state. Few notable works from the state are, Mehra and Negi [2], reported few new species from amphibians; Other workers include Srivastava [3,4]; Mukherjee and Ghosh [5,6]; Hafeezullah and Dutta [7]; Srivastava and coworkers [8, 9]; Gupta et al. [10] etc. Similar type of works has been reported from different parts of country (Devi and Rao [11]; Kumari and Madhavi [12]; Tandon et al. [13]; Gupta et al. [10]; Chandra and Gupta [14], Rizvi and Bhutia [15]).

2. MATERIALS AND METHODS

Study Area: Samaspur Bird Sanctuary situated in Raebareli District of Uttar Pradesh has an area of 800 Hect. There are five connected lakes namely Samaspur, Mamani, Gorwa Hasanpur, Hakganj and Rohnia. The sixth lake Bissaiya is close by but not connected to the main water body. It also forms a part of the sanctuary. These lakes and surrounding marshlands with rich concentration of water plants provide ideal habitat and nesting grounds for water birds.

Collection of Hosts and Parasites: Amphibian hosts were collected from Samaspur Bird Sanctuary and preserved in 100% ethanol. The body cavity of each host was opened by a longitudinal incision and the gastrointestinal tract was removed. The esophagus, stomach, small intestine, and large intestine were examined separately for helminths. Each nematode was removed, hot fixed in 4% formalin, dehydrated in glycerin alcohol, identified from wax-sealed slides. The trematodes were removed, fixed in AFA (Alcohol: Formaldehyde: Acetic acid), after fixation they were preserved in 70% alcohol, stained using Borax carmine stain and were then dehydrated by passing through graded alcohol series (70%-100%). After clearing in clove oil, they were mounted in DPX mounting medium and identified from permanent slides. All the helminths were subsequently photomicrographed and measurements were taken by microscope (BX51-DIC). The Nematode identification was done following "CIH Keys to the Nematode Parasites of Vertebrates" Eds. Anderson et al. [16]. Identification of Trematodes was done following "CIH Keys to the Keys to the Trematoda." Volume I, Eds. Gibson et al. [17], Volume II Eds. Jones et al. [18] and Volume III. Eds. Bray et al. [19]. Voucher specimens of hosts were deposited in Zoological Survey of India, Northern Regional Centre, Dehradun and parasites were deposited in the collections of the Zoological Survey of India, Kolkata.

3. RESULTS AND DISCUSSION

A total of eight amphibian host specimens were studied belonging to four species (table 1) namely, *Euphlyctis cyanophlyctis* (Schneider, 1799), *Duttaphrynus stomaticus* (Lutken, 1864), *Polypedates maculatus* (Gray, 1830) and *Hoplobatrachus tigerinus* (Daudin, 1803). Dissection of eight host specimens revealed the parasitic infection in six specimens with 30 helminth specimens belonging to five species (table 1). Of these, three belongs to Phylum Nematoda and two belongs to Phylum Plathylminthes. Maximum helminths richness is observed in *E. cyanophlyctis* with 4 helminth species (16 specimens) i.e., *Cosmocerca* sp., *Rhabdias* sp., *Ganeo tigrinum* Mehra & Negi, 1928 and *Pleurogenoides gastroporus* (Lühe 1901), Travassos 1921. Whereas, *Duttaphrynus stomaticus* (Lutken, 1864) was found to be infected by a single nematode species, *Cosmocerca kalesari* Rizvi, Bursey & Bhutia, 2011(14 helminth specimens). *Polypedates maculatus* (Gray, 1830) and *Hoplobatrachus tigerinus* (Daudin, 1803) showed no helminthic infection. Further it is observed that *E. cyanophlyctis* was infected by *Cosmocerca* sp., *Rhabdias* sp., *Ganeo tigrinum* and *Pleurogenoides gastroporus* with prevalence of 0.33% each and intensity of 1.33, 1.66, 0.33 and 2 respectively. While, *D. stomaticus* was infected by *Cosmocerca kalesari* with prevalence of 1%, intensity 4-6 and abundance of 4.66 (Table 1).

Nematodes: 2 genera, 3 species

Genus ***Cosmocerca*** Diesing, 1861

Cosmocerca kalesari Rizvi, Bursey & Bhutia, 2011 (Fig. 1 A, B)

Material Examined: Uttar Pradesh, Samaspur BS, Samaspur lake, Raebareli district, 03.12.2015, 14 specimens, from the intestine of *Duttaphrynus stomaticus*, coll. A. N. Rizvi, Reg. No. WN-1665.

Description: General morphology: Small, stout nematodes. Prominent sexual dimorphism, males almost one-third length of females. Lateral alae present in both males and females. Cuticle transversely striated. Minute somatic papillae present in both sexes, starting from esophageal region and reaching tail region. Mouth with three prominent lips, dorsal lip with two sessile papillae, each ventrolateral lip with one ventral, sessile papilla and one lateral amphid. Esophagus with indistinct buccal cavity, short pharynx, cylindrical corpus and valved bulb. Excretory pore anterior to esophageal bulb.

Male (based on 2 male specimens): Body 1.40-1.41 mm long, 0.15-0.19 mm wide, lateral alae beginning at level of nerve ring and continuous up to tail region, it is narrow at the anterior region, broadens near the tail region. Esophagus 0.30-0.31 mm long. Nerve ring at 0.11-0.12 mm from anterior end. Tail, conical 0.12-0.14 mm long with pointed tip. Heavily sclerotized gubernaculum, 104-108 µm long. Spicules, 98-101 µm, equal and lightly sclerotized. Five pairs of preanal plectanes. Each plectanes with 11-12 sclerotized rosette projections. 9 pairs of caudal papillae also present, one large mammaliform adanal pair and 8 postanal pairs, of which, 1st pair is large mammaliform, 5 pairs medium sized mammaliform ventral in position and 2 pairs simple papillae dorso-lateral, located at the tail region just before the narrowing of the tail.

Female (based on 12 female specimens): Body 2.10-2.95 mm long, 0.22-0.33 mm wide. Somatic papillae very minute at the anterior region and large and prominent near the caudal region and present up to 1/3rd of the tail region. Esophagus 0.291-0.296 mm long. Prodelphic reproductive system. Vulva situated at about 47-51% of body length. The uteri are filled with eggs 98-106 x 49-57 µm in size, eggs containing larvae and free larvae. Tail 0.34-0.36 mm long, conical with a slender posterior part.

Distribution: India: Haryana, Uttarakhand, Punjab and Uttar Pradesh.

Remarks: Length of the spicule (98-101 µm) and gubernaculum (104-108 µm) is larger than the original description by Rizvi et al. [20] (spicule 82-90 µm, gubernaculum 85-95 µm). This is the first report of this species from Uttar Pradesh.

***Cosmocerca* sp.** (Fig. 1 C)

Material Examined: Uttar Pradesh, Samaspur BS, Rohini lake, Raebareli district, 05.12.2015, 5 specimens, from the intestine of *Euphlyctis cyanophlyctis*, coll. A. N. Rizvi, Fc. No. SWLS-2/5.

Description: General morphology: Small, stout nematodes. Prominent sexual dimorphism, lateral alae present. Cuticle transversely striated. Minute somatic papillae present, starting from esophageal region and reaching tail region. Mouth with three prominent lips, dorsal lip with two sessile papillae, each ventrolateral lip with one ventral, sessile papilla and one lateral amphid. Esophagus with indistinct buccal cavity, short pharynx, cylindrical corpus and valved bulb. Excretory pore anterior to esophageal bulb.

Female (based on 4 female specimens): Body 2.20-2.55 mm long, 0.23-0.30 mm wide. Somatic papillae very minute at the anterior region and large and prominent near the caudal region and present up to 1/3rd of the tail region. Esophagus 0.28-0.29 mm long. Prodelphic reproductive system. Vulva situated at about 49-52% of body length. The uteri are filled with eggs 97-104 x 50-59 µm in size, eggs containing larvae. Tail 0.32-0.35 mm long, conical with a slender posterior part.

Remarks: Species identification could not be confirmed as no male specimen was found.

Genus ***Rhabdias*** Stiles & Hassall, 1905

***Rhabdias* sp.** (Fig. 1 D, E)

Material examined: Uttar Pradesh, Samaspur BS, Rohini lake, Raebareli district, 05.12.2015, 5 specimens, from the intestine of *Euphlyctis cyanophlyctis*, coll. A. N. Rizvi, Fc. No. SWLS-3/5.

Description (based on 5 immature hermaphrodite): Body with truncated anterior end and posterior end tapered. Parthenogenetic organism. Body length 1.06-1.37 mm, maximum body width 0.05-0.07 mm. Outer layer of cuticle inflated; oval oral opening, devoid of 6 lips. Clavicular Esophagus, 0.20-0.24 mm in length, Nerve ring 0.094-0.097 mm and excretory pore 91-108 µm from anterior end. Vulva postequatorial, 0.63-0.79 mm from anterior end (59% of the body length); structure of vulva not clearly visible. Reproductive system amphidelphic, ovaries straight, lying along intestine. Uteri wide, thin-walled, eggs not present. Tail 0.07-0.08 mm long, a broad cone tapering to a fine tip.

Remarks: Identification upto species could not be confirmed as no mature specimens were found.

Trematodes: 2 genera, 2 species

Genus ***Ganeo*** Klein, 1905

Ganeo tigrinum Mehra & Negi, 1928 (Fig. 2 A)

(Synonyms: G. attenuatum Srivastava, 1933; *G. gastricus* Srivastava, 1933; *G. kumaonensis* Pande,

1937; *G. linguanensis* Li, 1938; *G. srinagarensis* Kaw, 1950; *G. govinda* Dayal et Gupta, 1953; *G. punjabensis* Gupta, 1954; *G. bufonis* Fotedar, 1959; *G. gazipurensis* Pandey & Chakrabarty, 1968; *G. lucknowensis* Gupta & Jahan, 1976).

Material Examined: Uttar Pradesh, Samaspur BS, Mamoni Lake, Raebareli district, 03.12.2015, 01 specimen, from the intestine of *Euphlyctis cyanophlyctis*, coll. A. N. Rizvi, Reg. No. W.9870/1.

Description: Oval shaped, spinose, anteriorly tapering body, posterior end broadly rounded posteriorly. Rounded, sub-terminal oral sucker and spherical, ventral sucker, which is located in anterior one-third of the body length. Globular pharynx, long oesophagus bifurcated at level of genital pore. Caeca extended beyond vitelline follicles. Testes diagonally tandem, anterior testis pre-acetabular, in median line behind intestinal bifurcation; posterior testis at level with acetabulum, partially covering right caecum. Cirrus sac prominent, situated on the right side. Vesicula seminalis curved antero-lateral to ventral sucker; pars prostatica large, surrounded by prostate gland cells, opening by short ejaculatory duct at base of genital atrium. Pear-shaped, ovary postero-lateral to ventral sucker in position. Vitellaria extending starts from posterior level of acetabulum and extending beyond caecal termination. Uterus filled with numerous eggs, coiled transversely and extending upto posterior end of body. Excretory organ, V-shaped with subterminal excretory pore.

Measurements: Body 2.72 mm long, 0.71 mm wide. Oral sucker, 0.14 x 0.11 mm. Pharynx 0.08-0.9 x 0.06-0.07 mm. Esophagus 0.2-0.25 x 0.02-0.04 mm. Ventral sucker 0.11-0.07. Anterior testis 0.25 x 0.08 mm, posterior testis 0.27 x 0.07 mm. Ovary 0.21 x 0.11 mm.

Distribution: India: Tripura, Meghalaya, West Bengal, Bihar, Uttar Pradesh, Andhra Pradesh, Arunachal Pradesh, Madhya Pradesh, Jammu & Kashmir, Uttarakhand, Haryana and Punjab.

Elsewhere: Bangladesh, China and Vietnam.

Remarks: The measurements were in conformity with earlier descriptions.

Genus ***Pleurogenoides*** Travassos, 1921

Pleurogenoides gastroporus (Lühe 1901), Travassos 1921 (Fig. 2 B)
(Synonyms: *Pleurogenes gastroporus* Lühe, 1901; *P.* (*Pleurogenes*) *gastroporus* (Lühe, 1901) Mehra et Negi, 1928; *P.* (*Pleurogenes*) *gastoporus* var. *equalis* Mehra et Negi; 1928; *Pleurogenes orientalis* Srivastava, 1934, *Pleurogeness awanensis* Gupta, 1954).

Material examined: Uttar Pradesh, Samaspur BS, Mamoni Lake, Raebareli district, 03.12.2015, 14 specimens, from the intestine of *Euphlyctis cyanophlyctis*, coll. A. N. Rizvi, Reg. No. W. 9871/4.

Description: Body elliptical, rounded both ends, spinose in anterior portion of body. Oral sucker sub-terminal, rounded. Ventral sucker equatorial, smaller than oral sucker. pharynx oval, muscular, oesophagus indistinct. Intestinal caeca extending upto anterior margin of testes. Testes round or oval, situated laterally on each side of acetabulum. Cirrus sac well developed, situated on right side of body extending up to anterior margin of ventral sucker. Genital pore situated on left side and at the level of oral sucker. Ovary pre-acetabular, situated in the anterior margin of left testis. Vitellaria present in both side of body, extending from level of oral sucker to anterior margin of testes. Uterus filled with numerous eggs, situated in post-acetabular and post-testicular region.

Measurements: Body 0.60-0.91 mm long, 0.46-0.53 mm wide. Oral sucker, 0.16-0.20 x 0.13-0.16 mm. Ventral sucker 0.14-0.18. Anterior testis 0.13-0.14 x 0.11-0.12mm, posterior testis 0.15-0.17 x 0.10-0.12 mm. Ovary 0.10-0.12 x 0.07-0.1 mm. Eggs 0.01-0.02 x 0.01 mm.

Distribution: India: Tripura, Meghalaya, West Bengal, Maharashtra, Haryana, Andhra Pradesh, Arunachal Pradesh, Madhya Pradesh, Uttarakhand and Uttar Pradesh.

Elsewhere: Brazil and Bangladesh.

Remarks: The measurements were in conformity with earlier descriptions.

Till date no studies are available on the helminth parasites in amphibian hosts from Samaspur Bird Sanctuary and hence all the 4 genera reported in this study forms first report from Samaspur Bird Sanctuary. Only four species of amphibian hosts were found in the Sanctuary and among these, *E. cyanophlyctis* was most prevalent host with maximum population and harboured maximum diversity and richness of helminth parasites and this may be because of its aquatic habitat as well as its opportunistic and unselective feeding nature, which favours more parasitic infection [13], [15]. Further among the hosts examined females are mostly affected, as they are having larger body, they feed

more and attain more vermin [21], [15]. Male specimens of *Polypedates maculatus* and *Hoplobatrachus tigerinus* showed no helminthic infection. The Helminth parasite community in amphibian of Samaspur Bird Sanctuary comprised 76.7% of nematodes (23 specimens) and 23.3% of trematodes (7 specimens).

Table 1. Prevalence, intensity and abundance of helminth parasites in Amphibians of Samaspur Bird Sanctuary

Host (No of Example Examined)	Parasite (No. of hosts infected)	Prevalence %	Total no of parasites collected	Intensity	Abundance
Euphlyctis cyanophlyctis (Schneider, 1799) (3)	*Cosmocerca* sp. (1)	0.33	4	4	1.33
	Rhabdias sp. (1)	0.33	5	5	1.66
	Ganeo tigrinum Mehra & Negi, 1928(1)	0.33	1	1	0.33
	Pleurogenoides gastroporus Lühe 1901), Travassos, 1921 (1)	0.33	6	6	2
Duttaphrynus stomaticus (Lutken, 1864) (3)	*Cosmocerca kalesari* Rizvi, Bursey & Bhutia, 2011 (3)	1	14	4- 6	4.66
Polypedates maculatus (Gray, 1830) (1)	No infection found	0	0	0	0
Hoplobatrachus tigerinus (Daudin, 1803) (1)	No infection found	0	0	0	0

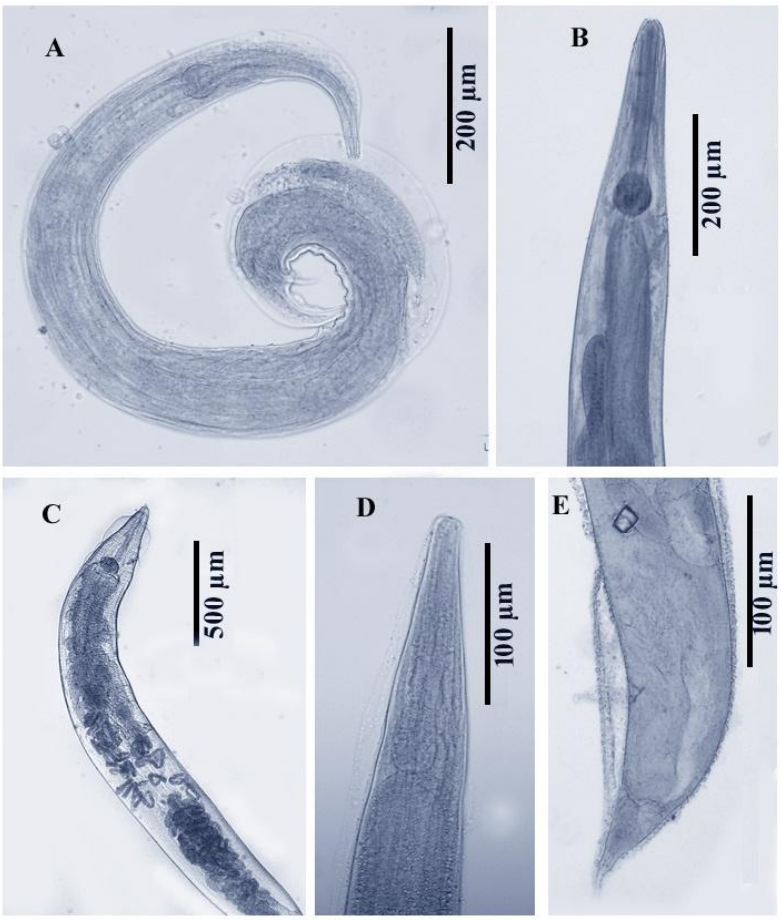

Fig. 1. Photomicrographs of Nematodes; A. *Cosmocerca kalesari*, Male, lateral view; B. *Cosmocerca kalesari*, Female anterior end, lateral view; C. *Cosmocerca* sp., Female, lateral view; D. *Rhabdias* sp., Anterior end, lateral view; E. *Rhabdias* sp. Posterior end lateral view

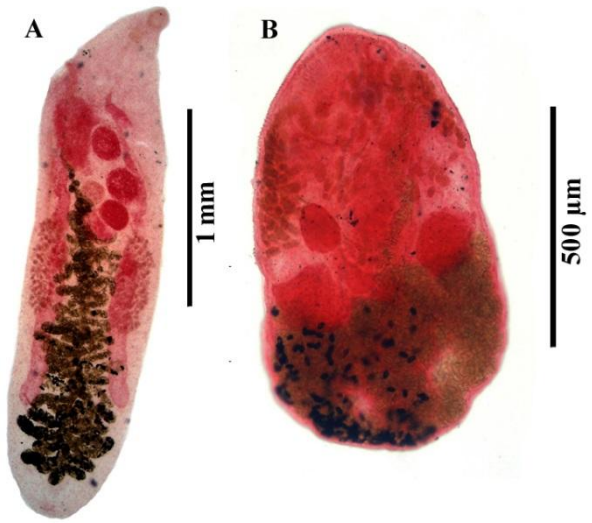

Fig. 2. Photomicrographs of Trematodes; A. *Ganeo tigrinum*, ventral view; B. *Pleurogenoides gastroporus*,ventral view

4. CONCLUSIONS

The Amphibian hosts from the Samaspur Bird Sanctuary were investigated for the helminth parasites in the present study. A total of four species of hosts were studied and revealed the infection of five species of helminth parasites. Maximum helminths were recorded in *Euphlyctis cyanophlyctis* (Schneider, 1799). It was infected by four species namely, *Cosmocerca* sp., *Rhabdias* sp., *Ganeo tigrinum* and *Pleurogenoides gastroporus* with prevalence of 0.33% each and intensity of 1.33, 1.66, 0.33 and 2 respectively. This is the first study on the helminth parasites of Amphibian hosts from Samaspur Bird Sanctuary, a Ramsar site in Uttar Pradesh. Though it is a preliminary study to investigate the helminthic infection of amphibian fauna, but this type of studies has the potential to reveal the amphibian helminthic faunal richness and to contribute towards the baseline information for future studies.

ETHICAL APPROVAL

The Hosts were collected keeping in mind of their local population and with prior permission from Ministry. All the host specimens studied during the study, were submitted at National Zoological Collection (NZC), Zoological Survey of India, Dehradun.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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