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Isolation and identification of helminth parasitic eggs having zoonotic potency from sewage-fed pond sediments

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Abstract

Helminth parasitic zoonoses are well recognized and causing important public health problems both in developed and developing country like India. Fish borne zoonoses are common in particular places where eating of raw or undercooked fishes occur. Sediment samples from sewage-fed pond were collected to analyze the presence of helminth eggs throughout the study period from December 2014 to August 2015. Zoonotically important helminth eggs were isolated from sediment samples by the modified Zinc Sulphate flotation technique previously described by Dada and Lindquist (1979). Slides are observed under compound microscope. Morphology and morphometry of the helminth eggs were done and documented using Toup View softwere.

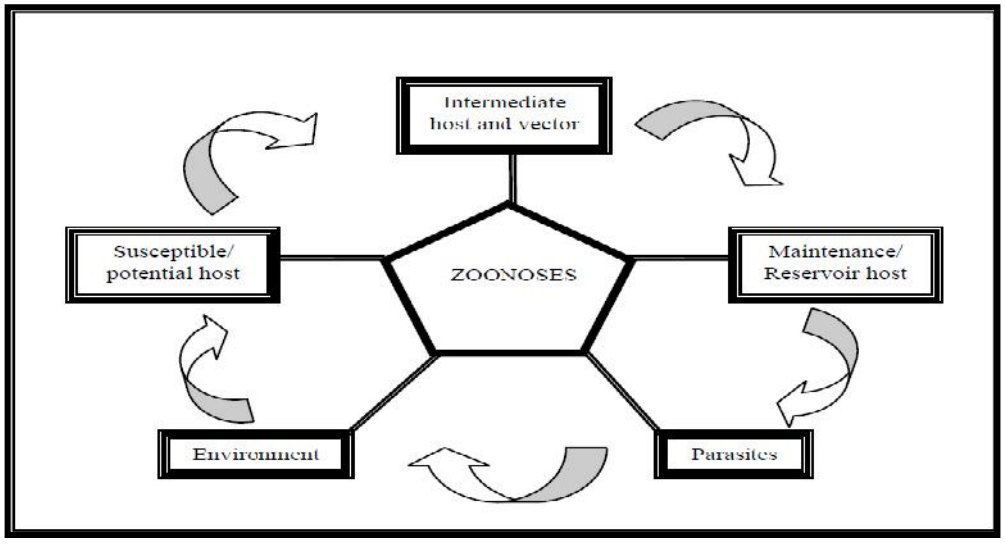
Keywords: Zoonotic diseases, Helminth parasites , Fishes, Sewage, Flotation Technique.

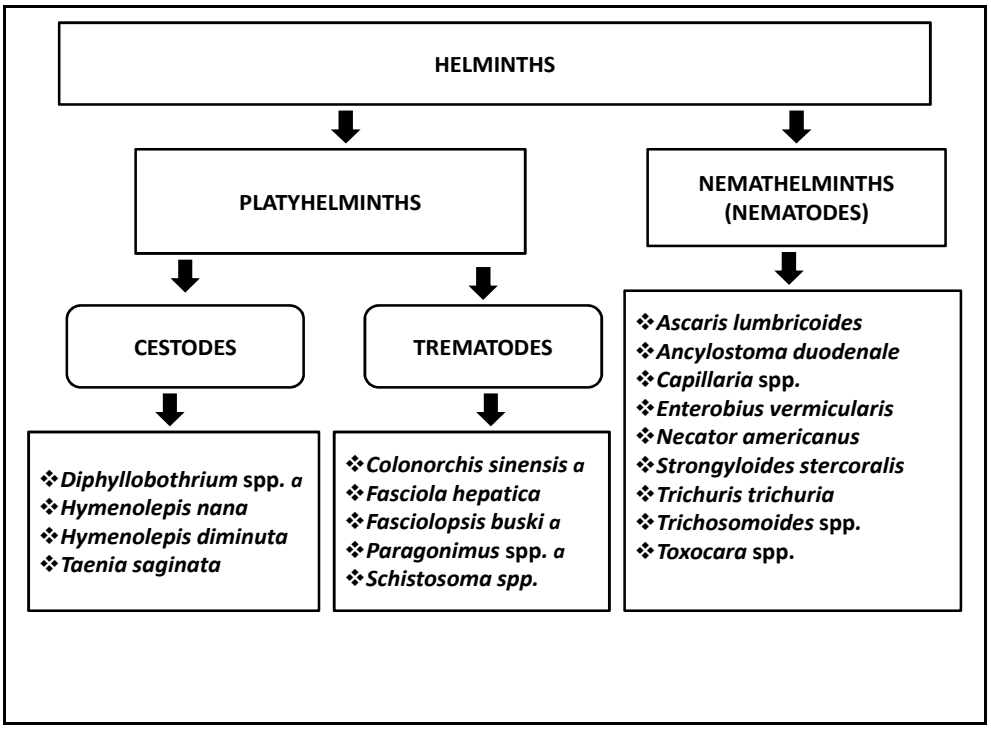
Introduction

Zoonoses are the diseases which are naturally transmitted between animals and humans. Parasitic zoonoses are well recognized and causing important public health problems both in developing and developed countries. A wide spectrum of helminthes infections is transmitted to humans by contamination of food and water. Food borne parasitic infections have recently been identified as an important public health problem with considerable economic impact in terms of morbidity, loss of productivity and healthcare costs. Poor sanitation and traditional methods of food preparation have accelerated the spread of food borne helminth infection (Phan *et al*., 2010). The human behavioral patterns including habits, customs, traditions and socio economic practices are closely related to such infections. The World Health Organization (WHO) has estimated that the number of people currently infected with fishborne trematodes exceeds 18 million and many more are at risk (WHO, 1995). More than 50 species of helminth parasites from fish and shell fish are known to cause disease in man. Most are rare and involve only slight to moderate injury but some pose serious potential health risk. Most of the organs of fish are affected by these parasites. Infestation of fishes by these parasite in natural waters do not manifest any serious disease outbreak but infestation in confined waters very often cause serious disease outbreaks. In many cases these parasites in association with other parasitic groups cause serious damage to the host fishes. Larvae of this group parasitize various fish species causing their growth retardation and mortality. Some helminth parasites are used as biological tags to monitor fish migration routes and fish stock composition (Mackenzie, 1983) and also used as indicators of environmental quality.

Zoonotic parasitic diseases are transmitted to humans by ingesting environmentally robust transmissive stages (spores, cysts, ova, larval and encysted stages).Humans can be final, intermediate or paratenic (maintenance) or accidental hosts. While the transmissive stages of some of these parasites can be transmitted directly (e.g. by animal-human contact or through contact with contaminated faeces, soil and garbage) they can also be transmitted through contaminated water and food. Eating raw, undercooked, cured, smoked, salted, pickled or air-dried fish can increase the risk of contracting foodborne parasitic zoonoses in human, especially when the preservation treatment is inadequate.

All of the important fish-borne zoonotic parasites (FZPs) are helminths, the majority of which are trematodes. There are strong linkages of FZPs to poverty, pollution and population growth. For example, in some areas, shortage of fuel in poor homes may permit only partial cooking of raw fish. Overcrowding in endemic areas lead to deterioration of the environment and increased pollution of surface waters with sewage, night-soil, and animal excreta. Development can also create increased risks; for example, dams create reservoirs highly favorable for snail vectors, as well as fish, which are the major transmitters of fish-borne trematodes (Omar, 2014).





a - Reported only in wastewater and sludge from some regions of Asia.

Helminth classification and genera found in wastewater and sludge (Jimenez, 2007)

Helminth ova characteristics

An important characteristic of helminth ova is that they are covered by 3-4 layers. The 1-2 outer layers are formed with muco-polysacharides and proteins. The middle layers consist of chitinous and serve to give structure and mechanical resistance to eggs. Finally, the inner layer is composed of lipids and proteins and is useful to protect eggs from desiccation, strong acid and bases, oxidants and reductive agents as well as detergent and proteolytic compounds. Thus the combination of all these layers is responsible for making eggs very resistant to several environmental conditions. Helminth ova of concern in the sanitary field measure between 20-80 µm with a density of 1.06-1.15 and are gelatinous which makes them very sticky. All these properties determine helminth ova's behavior during wastewater and sludge treatment. First, it is very difficult to inactivate them unless the temperature is raised above 40°C or moisture is reduced to below 5%. But details about the contact time under these conditions and other related environmental factors are generally not well-defined for every type of helminth ova genus or for high ova contents.

Materials and Methods

The present study on helminth parasites having zoonotic potency were carried out for a period of nine months between December, 2014 and August, 2015. The samples were collected from Bamanghata Bheri of East Kolkata Wetland, West Bengal, India.

Sediment sampling for helminthes egg analysis

Soil samples were collected from the top 0 – 30 cm layer. Most microorganisms are found in this zone and the conditions here are usually different from the profiles beneath as reported by Assadian *et al.* (2005) and Ogunmwonyi *et al.* (2008). Sample beds from the different ponds of Bamanghata Bheri were randomly chosen and samples collected from soil depths (i.e. 0 – 30 cm), using a 60 mm diameter soil auger and sterile spatulas. Composite sampling is reported by Williams and Gray as a strategy (1973) to keep the error of estimates at reasonable limits. However US EPA (2005) mentions the United States' *National Environment Protection (Assessment of Site Contamination) Measure 1999* (NEPM), which specifies that composite sampling should not be used for sitespecific health and ecological risk assessments. Discrete sampling was therefore adopted in this study, following some of the reasons suggested by the US EPA (2005) guideline on sampling. The samples collected from the different ponds were placed in plastic receptacles and immediately transported to the laboratory for analysis of helminths egg having zoonotic potency. Samples were placed in another polythene bag to retain humidity, and were kept refrigerated (4^{o}C) until used within 24 hours. Sampling was done monthly over a period of 9 months from December 2014 to August 2015. A total number of 18 soil samples were taken over the study period for isolation and identification of helminth eggs having zoonotic potency.

Helminth ova isolation procedure from soil

The samples were analyzed by the modified zinc sulphate ($ZnSO_4$) flotation technique previously described (Dada and Lindquist, 1979; Giacometti et al., 2000) with little modification. Briefly, 100 g of the pond sediment's sample was placed in a cup and stirred with 100 ml water and sieved to remove larger particles. The homogenized solution was placed into sedimentation cups, filled with 500 ml water and left overnight. After the supernatant was decanted, 20 g of the sediment was resuspended with 30 ml water, placed in two 50 ml centrifuge tubes and centrifuged at 1500 rpm for 5 min. After centrifugations supernatant removed then the sediment was re-suspended in 15 ml saturated $ZnSO_4$ solution for centrifugation at 1500 rpm for 5 min. Soon after centrifugation tubes was filled by $ZnSO_4$ upto brim and the cover slip was superimposed. After 30 minutes cover slip was removed and placed on a microscopic slide for examination the helminth eggs under the microscope.

Results

Identification of helminth parasites having zoonotic potency

Eggs of zoonotically important helminth parasites were isolated from the sediment sample of Bamanghata Bheries and identified based on their specific morphological features, observed under microscope. The life stages of some of those helminthes were also isolated from Indian Major Carps and freshwater snail and identified based on their definite morphological features. The observations and the micrometry are given in Table-1.

Table No.1 : Description of morphological features and characteristics of different life stages of helminth parasites observed

Sl. No.	Description of Parasite	Availability of Parasite		Important Characteristics	Measurement			
		Host	Other		Picture no.	Length (µm)	Width (µm)	Figure no.
1.	**Trematodes**	Freshwater snail, apple snail.	Sediment	Small ovoid and yellow brown in colour. Operculated at one end, small knob at opposite end.	A	28.752	16.284	1
	Clonorchis sp.				B	28.864	13.185	1
					H	29.53	17.63	1
2.	*Clonorchis* sp. metacercariae	*Labeo rohita*	–	Elliptical and have nearly equal sized oral and ventral suckers, brownish pigment granules and o shaped excretory bladder.	C	152.25	135.54	1
3.	*Clonorchis* sp. raedia	Apple snail	_	Raedia contains a mouth on the top of the head, a thick walled pharynx, short gut and many developing cercariae.	D	517.32	89.25	1
4	*Paragonimus* sp.	_	Sediment	Large, thick shelled, ovoid and elongated in shape. Flattened operculum.	E	72.516	37.891	1
					F	47.03	33.59	1
					G	101.685	47.099	1
					X	57.99	31.23	2

5	*Metagonimus* sp.	–	Sediment	Eggs are very small and have a smooth hard shell. Ovoid shape and has a very slight opercular shoulder.	P	18.424	11.274	2
6	*Dicrocoelium* sp.	–	Sediment	Eggs are oval and dark brown in colour. Typically operculated and small.	K	34.758	25.912	1
7	*Fasciola* sp.	–	Sediment	Inconspicuous operculum and un embryonated. Shell irregularity seen at the opercular end.	Q	122.156	72.979	2
8	*Fasciolopsis* sp.	–	Sediment	Oval shaped and slight yellow in colour. Thinner shell with an operculum encloses an ovam.	R	142.612	97.130	2
9	**Nematodes** *Enterobius* sp.	–	Sediment	Eggs are flattened asymmetrically on one side. Shape is ovoid.	J	58.75	26.30	1
10	Hookworm egg	–	Sediment	Oval in shape and shell is thin and colourless. It consist of segmented 4-8 cells.	L	58.242	29.550	1
11	*Capillaria* sp.	–	Sediment	Bipolar plug present and thick shell. Eggs contain a single cell.	O	33.942	15.540	2
12	*Ascaris* sp. (Decorticated egg)	–	Sediment	Both fertilized and unfertilized eggs lack of albuminous coats.	S V	61.298 40.176	57.298 42.609	2 2
13	*Ascaris* sp. (Fertilized egg)	–	Sediment	Broad, oval in shape and thick shell. Chitinous layer, fertilizing membrane and mammilated albuminous coat present.	T U	40.176 41.37	22.226 29.32	2 2
14	*Ascaris* sp. (Unfertilized egg)	–	Sediment	Longer and slender than a fertilized egg. Lack of albuminous coat.	W	62.849	29.284	2
15	**Cestodes** *Hymenolepis* sp.	–	Sediment	Spherical, yellowish brown in colour and contain 6 hooked embryos.	I	53.730	53.730	1
16	*Taenia* sp.	–	Sediment	Thick and prismatic appearing cell wall. It contains 6 hooked embryos and the onchosphere.	M N	32.52 49.34	32.52 49.34	2 2

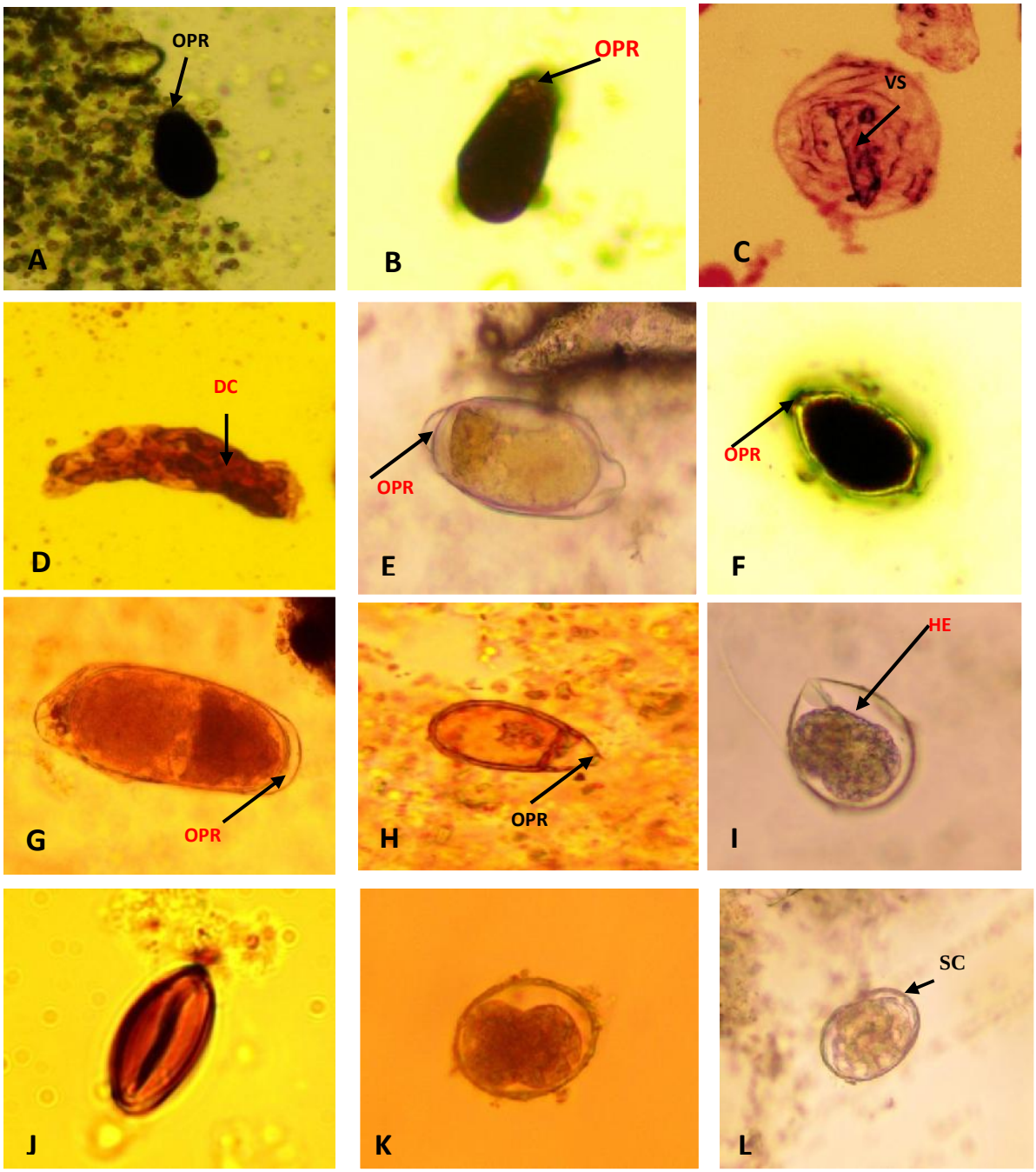


Figure-1: A: *Clonorchis* sp. egg isolated from the intestine of freshwater snail, (Unstained, X 200).**B:** *Clonorchis* sp.egg isolated from the intestine of apple snail, (Unstained, X 200). **C:** Metacercariae of *Clonorchis* sp. isolated from the intestine of Labeo rohita, (Unstained, X 200). **D:** Rediae of *Clonorchis* sp. isolated from the gut of Apple snail *Pila globosa*, (Unstained, X 100). **E:** *Paragonimus* sp. egg isolated from the sediment of East Kolkata Wetland, (Unstained, X 200) **F:** *Paragonimus* sp. egg isolated from the sediment of East Kolkata Wetland, (Unstained, X 200) **G:** *Paragonimus* sp. egg isolated from the sediment of East Kolkata Wetland,(Unstained, X 200). **H:** *Clonorchis* sp. egg isolated from the sediment of East Kolkata Wetland, (Unstained, X200). **I:** *Hymenolepis* sp. Egg isolated from the sediment sample of East Kolkata Wet Land, (Unstained, X 200). **J:** *Enterobius* sp. egg isolated from the sediment sample of East Kolkata WetLand,(Unstained, X 200). **K:** *Dicrocoelium* sp. egg isolated from the sediment sample of East Kolkata WetLand. (Unstained, X 200). **L:** Hookworm egg isolated from the sediment sample of East Kolkata WetLand,(Unstained, X 200).

Abbreviation:- CL- Chitinous layer, YG- Yolk Granule, VM- Vitelline Membrane, AL- Albuminous Layer, OPR- Operculum, SC- Segmented Cell, OP- Oncosphere, BP- Bipolar Plug, OPRS- Opercular Shoulder, OPR- Operculum. OPR- Operculam, VS- Ventral Sucker, DC- Developing Cercariae, HE - Hooked embryo.

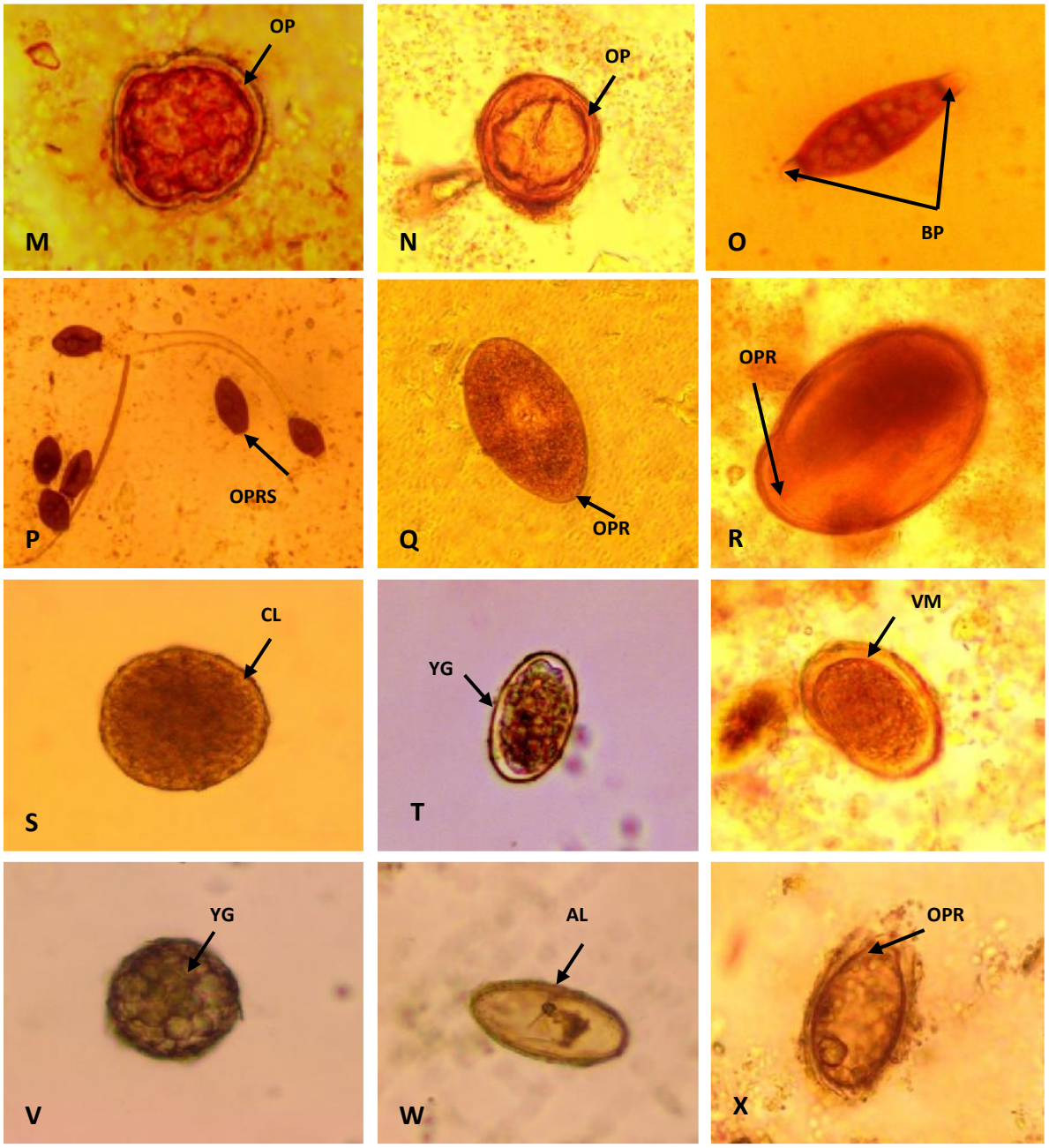


Figure-2: M: *Taenia* sp. egg isolated from the sediment sample of East Kolkata Wetland, Bamanghata (Unstained, X 200). **N:** *Taenia* sp. egg isolated from the sediment sample of East Kolkata Wetland, Nolbon (Unstained, X 200). **O:** *Capillaria* sp. egg isolated from the sediment sample of East Kolkata Wetland (Unstained, X 200). **P:** *Metagonimus* sp. egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 200). **Q:** *Fasciola* sp.egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 100). **R:** *Fasciolopsis* sp.egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 200). **S:** *Ascaris* sp.decorticated fertilized egg isolated from the sediment sample of East Kolkata Wetland,(Unstained, X 200). **T:** *Ascaris* sp.normal fertilized egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 200). **U:** *Ascaris* sp. fertilized egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 200). **V:** *Ascaris* sp. fertilized decorticated egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 200). **W:** *Ascaris* sp. unfertilized egg with isolated from the sediment sample of East Kolkata Wetland,(Unstained, X 200). **X:** *Paragonimus* sp. egg isolated from the sediment sample of East Kolkata Wetland, (Unstained, X 200).

Abbreviation:- CL- Chitinous layer, YG- Yolk Granule, VM- Vitelline Membrane, AL- Albuminous Layer, OPR- Operculum, SC- Segmented Cell, OP- Oncosphere, BP- Bipolar Plug, OPRS- Opercular Shoulder, OPR- Operculum. OPR- Operculam, VS- Ventral Sucker, DC- Developing Cercariae, HE - Hooked embryo

Trematodes

Clonorchis sp. ova, metacercariae and raidea were isolated from the freshwater snail, Rohu fish and apple snail respectively.Other than host trematode ova were also present in sediment of sewage fed pond. *Paragonimus* sp. *Metagonimus* sp. *Dicrocoelium* sp. *Fasciola* sp. *Fasciolopsis* sp. were isolated from the sediment of the sewage fed pond of East Kolkata Wetland.

Nematodes

Enterobius sp. Hookworm egg *Capillaria* sp. *Ascaris* sp. (Decorticated egg), *Ascaris* sp. (Fertilized egg), *Ascaris* sp. (Unfertilized egg) were isolated from the sediment. *Capillaria* sp. can affect fish as well as human. All the specimen were morphologically and morphometrycally identified.

Cestodes

Hymenolepis sp. and *Taenia* sp. ova were isolated from the sediment of the East Kolkata Wetland.Cestodes ova were rarely isolated from the sediment during the study period and identified by the structure and morphometry.

Discussion

Zoonotically important helminth eggs isolated from the sediment of freshwater sewage fed fish farm of East Kolkata Wetland

A large number of zoonotically important helminth parasites from the sediment of sewage fed fish ponds were isolated and identified during the study period listed in Table. The availability of trematodes egg like *clonorchis* sp., *Paragonimus* sp., *Metagonimus* sp. and *Fasciola* sp. were more during the study period. Nematodes eggs like *Ascaris* sp., hookworm eggs, *Capillaria* sp., from sediment samples were very common during the study period. Cestode eggs like *Hymenolepis* sp., and *Taenia* sp. availability in sediment were very rare. It is may be due to the different geographical distribution, and unfavourable climatic condition. Jimenez (2007) classified helminths genera from wastewater and sludge which were similar to the present findings. Sewage of the study area containes huge amount of domestic and leather waste materials which may be the main source of the helminth parasitic egg having zoonotic potency. Before the release of sewage water to fish farm it crosses the long sewage canal where sedimentation of the sewage occurred. Measures also have been taken by the farmer before releases the raw sewage in fish stocking pond. So that the sewage become cleaned before use in sewage fed fish pond and the level of contamination by the zoonotically important helminth parasites decreases. That may be the cause of low prevalence of zoonotically important helminths in IMC and freshwater snails.

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