e - ISSN - 2348 - 2168
Print ISSN - 2348 - 215X

Acta Biomedica Scientia

Journal homepage: www.mcmed.us/journal/abs

STUDIES ON MORPHOLOGY AND HISTO-PATHOLOGY OF DIGENETIC TREMATODES INFECTING CERTAIN VERTEBRATES OF GOVINDGARH LAKE

Ramyash Sahu

Department of Zoology, R.K Shukla Collage, Beohari, Shahdol, Madhya Pradesh, India.

Article Info

Received 29/05/2016
Revised 16/06/2016
Accepted 19/07/2016

Keywords :-
Pesticide, Contaminant, Sub-lethal.

ABSTRACT

Increasing recognition of interactions between disease and aquatic pollution, few studies have explored how these stressors act together on aquatic vertebrates at environmentally relevant contaminant concentrations. The combined effects of trematode infection, and glyphosate formulation exposure at an environmentally relevant sub-lethal concentration, were not predictable from their individual effects because we observed a synergistic effect on fish survival but no effect of glyphosate formulation alone. We are aware of a single study reporting increased virulence of micro-parasitic infections in aquatic micro-crustaceans after simultaneous exposure to the pesticide carbaryl. Studies on vertebrates have shown synergistic increases in infection rates in the presence of contaminants, but impacts on host survival were either insignificant or not reported.

INTRODUCTION

Animal species are host to a wide range of parasites and, equally, parasites can target a range of viable hosts: multi-host–multi-parasite systems are the norm [1,2], with potential consequences for the structure and diversity of host–parasite communities [2]. From a host perspective, multiple infections occur when conspecific strains or parasite species co-infect a single host [3,4] and, though coexisting parasites can act independently of one another, they may interact synergistically (by cooperating in extracting host resources, for example [5,6] or antagonistically, by inhibiting each other's growth or even preventing the establishment of weaker competitors Competing parasites can alter one another's distributions, affecting their fitness, population size and, ultimately, leading to changes in the richness and abundance of parasite communities [7].

Corresponding Author

Ramyash Sahu
Email: - shivaniawasthi786@gmail.com

In turn, these can have significant impacts on epidemiology [8–9], with major repercussions for disease control in humans [10,11] and other animal hosts [12]. Freshwater fish are common hosts for trematode worms whose infective cercariae are released by aquatic snails, the first intermediate host, before encysting as metacercariae in the body or organs of fish, the second intermediate host [13]. Infection can cause fish skeletal malformations and mortality, with effects particularly acute in juvenile stages. The *Telogaster opisthorchis* infection caused spinal malformations and increased mortality in juvenile *Galaxias anomalus*, affecting population dynamics in the field. *G. anomalus*, a threatened non-migratory freshwater fish [14], is a common second intermediate host for metacercariae of *T. opisthorchis*; infection prevalence and intensity range 33–100 and 2·6–370, respectively; [15]. *G. anomalus* become infected after the release of cercariae by the snail *Potomopyrgus antipodarum*. Completion of the parasite life-cycle requires transmission to the definitive host (a freshwater eel) by predation on infected *G. anomalus*. For parasites with complex life-cycles, toxicants may modulate such disease impacts in several ways: (a) by direct

immunosuppression of the host, (b) by changing transmission probabilities of infective stages from alternative hosts, (c) by changing the survival probability of alternative hosts and (d) by directly influencing survival or infectivity of the disease-causing agent. Conversely, infection may weaken host susceptibility to toxins and metal contaminants [16].

TREMATODE TRANSITIONS

A remarkable physiological aspect of trematode life cycles is the sequence of totally different habitats in which the various stages must survive, with physiological adjustments that must often be made extremely rapidly. As an egg passes from the vertebrate, it must be able to withstand the rigors of the external environment in fresh water or seawater, if only for a period of hours, before it can reach haven in the mollusc. There conditions are quite different from both the water and the vertebrate. The trematode's physiological capacities must again be readjusted on escape from the intermediate host and again on reaching the second intermediate or definitive host. Environmental change may be somewhat less dramatic if the second intermediate host is a vertebrate, but often it is an invertebrate.

Although the adjustments must be extensive, the nature of these physiological adjustments made by trematodes during their life cycles has been little investigated, the most studied trematodes in this respect being Schistosoma spp. Penetration of the definitive host is a hazardous phase of the life cycle of schistosomes, and it requires an enormous amount of energy. The hazards include a combination of the dramatic changes in the physical environment, in the physical and chemical nature of the host skin through which the schistosome must penetrate, and in host defense mechanisms. Depending on the host species, losses at this barrier may be as high as 50%, and the glycogen content of the newly penetrated schistosomules (schistosomule is the name given the young developing worm) is only 6% of that found in cercariae. Among the most severe physical conditions the organism must survive is the sequence of changes in ambient osmotic pressure.

The osmotic pressure of fresh water is considerably below that in the snail, and that in the vertebrate is twice as great as in the mollusc. Assuming that the osmotic pressure of the cercarial tissues approximates that in the snail, the trematode must avoid taking up water after it leaves the snail and avoid a serious water loss after it penetrates the vertebrate. Aside from the possible role of the osmoregulatory organs (protonephridia), there appear to be major changes in the character and probably permeability of the cercarial surface. The cercarial surface is coated with a fibrillar layer, or glycocalyx, which is lost on penetration of the vertebrate, and with it is lost the ability to survive in fresh water; 90% of schistosomules recovered from mouse skin 30 minutes after penetration die rapidly if returned to fresh water. Chemical changes in the tegument occur after penetration: The schistosomule surface is much less easily dissolved by a number of chemical reagents, including 8 M urea, than is that of the cercaria. Antigenic epitopes on the tegument are changed as well. When cercariae are incubated in immune serum, a thick envelope called the CHR (cercarienhullenreaktion) forms around them, but schistosomules do not give this reaction. In several cases cercarial attraction to the next host is mediated by substances different from those that stimulate actual penetration [17]. Schistosome cercariae are apparently attracted to host skin by the amino acid arginine, whereas the most important stimulus for actual penetration is the skin lipid film, specifically essential fatty acids, such as linoleic and linolenic acids, and certain nonessential fatty acids. Human skin surface lipid applied to the walls of their glass container will cause cercariae to attempt to penetrate it, lose their tails, evacuate their preacetabular glands, and become intolerant to water. The presence of the penetration-stimulating substances causes loss of osmotic protection and a reduction of the CHR, even in cercariae free in the water [18]. Successful penetration and transformation have been correlated with cercarial production of eicosanoids, such as leukotrienes and prostaglandins (fatty acid derivatives with potent pharmacological activity) [19]. These eicosanoids may enable the schistosomules to evade the host defenses by inhibiting superoxide production by neutrophils [20].

MATERIALS AND METHODS

Description of the research Site :- The Govind Garh lake is one of the unique water body in India and located in south of Rewa (M.P) at a distance of 20 K.M from Rewa, with longitude 24º20 25" and latitude 81º 15'20". The lake is connected with weather Rewa –Shahdol and Satna –Sidhi Rode. The lake was formed by impounding of small Nalla originating from Kaimore Hill. With view to storing rain water the Maharaja of Rewa at that time built bandh across the Nalla to form a tank in 1985.The Lake is a big water body and rich flora and fauna General:- (1) Collection of Parasites:- A large number of vertebrate hosts ranging from fishes, Amphibian and reptiles from different parts of Govind garh lake Rewa (M.P).

Collection of Parasites :- A number large vertebrate hosts ranging from different parts of Govind garh lake Rewa (M.P).Collection and then were cut in Saline water and thorough examination of not only the Alimentary canal but whole of the body was made for Trematodes Parasites. For very small worms scraping of the gut contents was first applied and the whole collection was kept in the common Salts with several decantation worms were separated from others matters.Large worms were picked up one by one the help of either brush or forceps or needles.

2- Killing and fixation:- The Trematode Parasites thus obtained from the hosts were cleaned thoroughly by saline water or ordinary tap water. Collected Trematodes were killed and fixed quickly by suitable reagents on the type of parasites. Trematodes were always fixed under pressure of the cover glass.

Trematodes were stained by:- 1-Ehrlichs hematoxylin 2-Gowers carmine. 3- Semichons carmine stain.

1-Ehrlichs hematoxylin:-
Solution A;- Hematoxylin -2gm , Ethanol absolute -100ml.
Solution B :- Glycerol -100 ml, Distilled water -100 ml ,Acetic acid -10 ml , Aluminium potassium sulphate -3mg.
Solution A and B were mixed and allowed to ripen before use in bright day light for several weeks.

2- Gowers carmine stain: - 10 mg of carmine was boiled in 100 ml of 45% glacial acetic acid, cooled and filtered. The filter paper was removed carefully and dried for the preparation of the stain as follows:-
Acidified Carmine -1 mg, Potash Alum -10 mg, Distilled Water -200 mg.

The ingredients were mixed dissolved by the aid of heat. When completely dissolved Solution was cooled filtered and crystal of thymol was added to prevent mould growth.

3- Semichons carmine stain: - Acetic Acid -50 ml, Distilled water - 50 mg, Carmine powder –in excess. Staining procedures of the trematodes.

ECOLOGY ;-
Water Sample:- Samples of water was collected twice a month from five sampling sites of the lake during the period September 2005 to August 2007. The time of water sample collection was more or less fixed through out the period of investigation. Dissolved O_2 content and total alkalinity content of water was analyzed on the spot but for the rest of chemical analysis. The water sample were collected in a Winchester Bottle of 200 ml capacity in which few drops of chloroforms was added. The determination of PH, Temperature and turbidity was done on the spot.

Temperature;- The temperature of surface water was almost daily recorded at the water sampling time with a mercury thermometer having a graduation of 0º to 50 ºc.The thermometer was dipped directly in water and was kept for 2 minutes and then the temperature was recorded.
Hydrogen ions concentration: - The PH of water was determined on the spot with the help of PH paper. Since the PH meter was not available hence the determination of the PH was done with the help of PH paper.

Intensity;- It is the quotiened from the number of the Helminthes parasite divided by number of infected host. Intensity = NO, of parasite obtained / No of infected host.

Density:- It is the concentration of the helminthes parasite in terms of parasite per unit space. (Single host)
Density = No. of parasite collected / No of host examined.
Relative density: - It is the concentration of one individual Helminthes burden in relation to the total helminthes burden and is expressed in terms of the percentage.

HEMATOLOGY;-
Estimation of hemoglobin:- Hemoglobin concentration (in gm/ 100 ml of blood) was estimated by Sahlis Acid – Haematin method as described by dormady and Devenport

Determination of absolute values:- From the R.B.C count hemoglobin content of blood and packed cell volume certain absolute values can be calculated which are of much help in the hematological diagnosis.

The absolute values which have been calculated are as follows:-
(a)Mean corpuscular volume (M.C.V):- This is the average volume of a single red cell expressed in cubic microns. It is calculated as follows:-
MCV = Volume of packed cell in 1 liter of blood / Red cells in million / c.m.m of blood (cubic microns)

(b)Mean corpuscular hemoglobin (M.C.H):- It is the average amount of hemoglobin contained in a single cell and is expressed in micrograms. It is calculated as follows:- MCH = Hemoglobin in grams per liter of blood / Red cell in millions per c.m.m of blood.(C)M.C.H.C (Mean corpuscular hemoglobin concentration)

It indicates the percentage of red cell with hemoglobin. This is calculated as below:-
M.C.H.C = Hemoglobin in gm per 100 ml of blood ×100 /Volume of packed cell in per ml of blood

OBSERVATIONS

Author has collected about 51 Trematode parasite of this species from the intestine of locally available 18 fishes of punctatus during month of October 2005-2006.This parasite was identified as E. multicaecum.

Diagnosis:- The body is aspinose , elongated and thick with rounded ends. Measuring 7.63× 2.2244. The suckers are well developed located in the anterior 3rd of the body. The oral sucker is small highly developed sub terminal and measures 0.2324 × 0.3154.The ventral sucker is larger strongly muscular and measures 1.1786 × 0.45. The Esophagus measuring is 0.1494 × 0.316. The intestinal Caeca is Gorged with blakish food contents slightly protrude interiorly before turning back wards in the forms of shoulder at the side of esophagus.They are long and extend up to the posterior end of the body where they end

blindly. Behind the posterior level of ventral sucker the intestinal caeca have long lateral diverticula usually 8 on the left side and 11 on the right side. These Diverticula gradually increase in the size towards the hind ends of the body but some anterior, middle or posterior diverticula are quite short. Gonads are well developed.

Table 1. R.B.C number in the blood of *Clarias batrachus* (Male)

S.NO	Uninfected Male	S.NO.	Infected Male
	R.B.C in million / cmm		R.B.C in million / cmm
1	1.6	1	1.25
2	1.7	2	1.35
3	1.8	3	1.45
4	1.9	4	1.5
5	1.6	5	1.1
6	1.5	6	1.15
7	1.6	7	1.35
8	1.6	8	1.25
9	-	9	1.25
Mean	1.6625	Mean	1.2388

Table 2. R.B.C number in the blood of *Clarias batrachus* (Female)

S.NO	Un infected Female	S.NO	infected Female
	R.B.C in million / cmm		R.B.C in million /cmm
1	2.3	1	2.1
2	2.1	2	1,9
3	2.2	3	1.6
4	2.1	4	1.8
5	2.1	5	1.8
Mean	2.16	Mean	1.84

Table 3. Hemoglobin content in the blood of *Clarias batrachus* (Male)

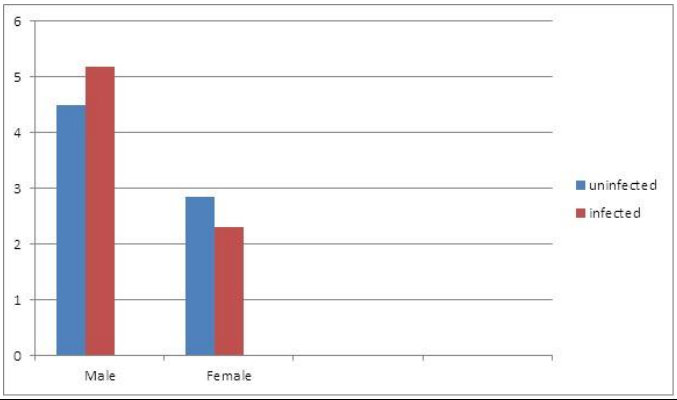
S.N0	Un infected male	S.NO	Infected Male
	Hb/ 100 in gm		Hb/100ml in gm
1	7.25	1	6.34
2	7.12	2	6.21
3	7.05	3	6.32
4	7.62	4	6.41
5	7.53	5	6.81
6	7.72	6	6.52
7	7.84	7	
8	7. 92	8	
9	7.32	9	
Mean	7.4855	Mean	6.435

Table 4. Hemoglobin content in the blood of *Clarias batrachus* (Female)

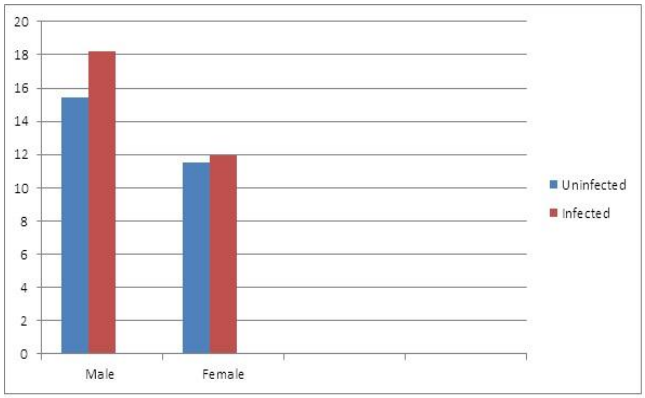
S.N0	Un infected female	S.N0	Infected female
	Hb/ 100 ml in gm		Hb / 100 ml in gm
1	6.1	1	4.0
2	6.0	2	4.4
3	6.2	3	4.2
4	6.1	4	4.5
5	6.0	5	4.1
6	6.5		

7	6.2		
Mean	6.16	Mean	4.24

Graph 1. Colour index value in blood of infection of infected *Clarias batrachus*.



Graph 2. Mean corpuscular volume (MCV) in the blood *Clarias batrachus*.



RESULT AND DISCUSSION

The Helminthes parasite comprises a relatively large group inhabiting practically every organ system in any animal of the globe. Certain species are well known but it is difficult to evaluate the importance of some because their distribution has not yet been accurately determined. Furthermore those parasites believed today to be of relatively minor importance may prove later to need more attention as their number increase or as they become more widely distributed or recorded. A parasite is always under the influence of the 2 types environments viz: - the internal environment in which the host lives. It is the interaction of the influence of these environments and the strategy adopted by the parasite to counter influence that develops the host specificity and host parasite relationship. The survey of the literature revels that majority of vertebrates carry helminthic infection is severe and case hazardous effect leading to the death of hosts but if the rate of infection is not heavy it may inflect internal injury to site where they are attached.

CONCLUSION

The present study was held at the A.PS University Rewa (M.P). The R.B.C number in blood of uninfected male (Clarias-Batrachus) mean value was 1.6625. Whereas infected male mean value was 1.2388. The R.B.C number in blood of uninfected Female (*Clarias batrachus*) mean value was 2.16. Whereas the R.B.C number in infected female mane value was 1.84.When tested statically the t-value were significant. The MCV in both sexes were raised in infected host. In case of males the rise from 15.44 (uninfected) to 18.219 (infected).

ACKNOWLEDGEMENT: None

CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

REFERENCES

1. Woolhouse MEJ, Haydon DT, Antia R. (2005). Emerging pathogens: the epidemiology and evolution of species jumps. *Trends Ecol. Evol*, 20, 238–244.
2. Rigaud T, Perrot-Minnot MJ, Brown MJF. (2010). Parasite and host assemblages: embracing the reality will improve our knowledge of parasite transmission and virulence. *Proc. R. Soc. B*, 277, 3693–3702.
3. Cox FEG. (2001). Concomitant infections, parasites and immune responses. *Parasitology*, 122, S23–S38.
4. Read AF, Taylor LH. (2001). The ecology of genetically diverse infections. *Science*, 292, 1099–1102.
5. Griffin A, West S, Buckling A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, 430, 1024–1027.
6. West SA, Buckling A. (2003). Cooperation, virulence and siderophore production in bacterial parasites. *Proc. R. Soc. Lond. B*, 270, 37–44.
7. Poulin R. (2001). Interactions between species and the structure of helminth communities. *Parasitology*, 122, S3–S11.
8. Escribano A, Williams T, Goulson D, Cave RD, Chapman JW, Cabellero P. (2001). Consequences of interspecific competition on the virulence and genetic composition of a nucleopolyhedrovirus in Spodoptera frugiperda larvae parasitized by Chelonus insularis. *Biocontrol Sci. Technol*, 11, 649–662.
9. Telfer S, Lambin X, Birtles R, Beldomenico P, Burthe S, Paterson S, Begon M. (2010). Species interactions in a parasite community drive infection risk in a wildlife population. *Science*, 330, 243–246.
10. Blackwell AD, Martin M, Kaplan H, Gurven M. (2013). Antagonism between two intestinal parasites in humans: the

importance of co-infection for infection risk and recovery dynamics. *Proc. R. Soc. B*, 280, 20131671.

11. Abu-Raddad LJ, Patnaik P, Kublin JG. (2006). Dual infection with HIV and malaria fuels the spread of both diseases in sub-Saharan Africa. *Science* 314, 1603–1606.
12. Lello J, Boag B, Fenton A, Stevenson IR, Hudson PJ. (2004). Competition and mutualism among the gut helminths of a mammalian host. *Nature*, 428, 840–844.
13. Woo P.T.K. (*2*006) Fish Diseases and Disorders, Volume 1: Protozoan and Metazoan Infections, 2nd edn. *CAB International, Cambridge, MA.*
14. New Zealand Department of Conservation. (2005). New Zealand Threat Classification System lists. *Science & Technical Publishing, Wellington, New Zealand.*
15. Kelly DW, Paterson RA, Townsend CR., Poulin R & Tompkins DM. (2009). Has the introduction of brown trout altered disease patterns in native. New Zealand fish? *Freshwater Biology*, 54, 1805–1818.
16. Poulin RP. (1992) Toxic pollution and parasitism in freshwater fish. *Parasitology Today*, 8, 58–61.
17. Haas W and B Haberl. (1997). Host recognition by trematode miracidia and cercariae. In B. Fried and T. K. Graczyk (Eds.), Advances in trematode biology (pp. 197—227). Boca Raton, FL: CRC Press.
18. Haas W and R Schmitt. (1982). Characterization of chemical stimuli for the penetration of Schistosoma mansoni cercariae. II. Conditions and mode of action. *Z. Parasitenkd*, 66, 309-319.
19. Salafsky B and A Fusco. (1987). Eicosanoids as immunomodulators of penetration by schistosome cercariae. *Parasitol. Today*, 3, 279-281.
20. Nevhutalu PA, B Salafsky, W Haas and T Conway. (1993). Schistosoma mansoni and Trichobilharzia ocellata: Comparison of secreted cercarial eicosanoids. *J. Parasitol*, 79, 130-133.