



Prevalence and pathology of *Heterakis gallinarum* mono-infection and co-infection with *Histomonas meleagridis* in free roaming domestic chicken of Kashmir Valley

¹ Sheikh Tanveer Salam, ² Showkat Ahmad Wani, ³ Pervaiz Ahmad Dar, ⁴ Fayaz Ahmad Ahangar

^{1,3} Department of Zoology, AS. College Srinagar, Jammu and Kashmir, India

² Department of Zoology, SP College, Srinagar, Jammu and Kashmir, India

⁴ Department of Zoology, Women's College, MA. Road Srinagar, Jammu and Kashmir, India

Abstract

A two year study was carried out from January 2012 to December 2013 on prevalence and pathology of mono infection of nematode *Heterakis gallinarum* and its co-infection with a protozoan parasite *Histomonas meleagridis*. For the study a sample size of 478 domestic chicken (*Gallus g. domesticus* L., 1758), 243 males and 235 females, weighing between 1 - 2.5 kgs collected from the different localities covering almost entire Kashmir valley were selected. The study revealed that prevalence rate of *Heterakis gallinarum* for 1st Year of study (2012) was 3.43% (8/233) and for the 2nd Year (2013) it was 5.3 % (13/245) giving an overall prevalence rate for two year study (Jan 2012 to Dec 2013) to be 4.39% (21/478). Range of intensity of worms was found to be 02 – 55 with Mean intensity of infection observed to be 20 ± 2.1 . Faecal Egg Count was found to be very low i.e. <40 eggs per gram of faeces. On the other hand only two cases of *Heterakis gallinarum* co-infection with *Histomonas meleagridis* were reported, one in September 2012 and other in August 2013 giving overall prevalence of co-infection to be only 0.42% (2/478) and mean intensity of infection of *Heterakis gallinarum* during co-infection with *Histomonas meleagridis* was comparatively lower (07 ± 2). Gross lesions were seen on the ceca and liver only during co-infection of *Heterakis gallinarum* with *Histomonas meleagridis* and not during mono-infection of *Heterakis gallinarum*. Histological findings in the caeca during mono-infection revealed presence of adult worms in the lumen of intestine along with cellular debris and the infected tissue revealed intense chronic diffuse inflammatory processes with mononuclear and polymorphonuclear (heterophils) leucocyte infiltrations extending up to submucosa. During co-infection, histological studies of the infected livers revealed extensive infiltration of lymphocytes, mononuclear cells and scarce heterophils. Hepatocytes towards the periphery of the lesions contained punched out ovoid bodies which stained positive and became visible with PAS (Periodic Acid Schiff) stain. Microscopic examination of cecal lesions during co-infection showed hyperemia, ulceration and infiltration of heterophils, lymphocytes and macrophages extending from mucosa to the muscular layer. There was sloughing off of the epithelium and lumen was packed with fibrin, red blood cells and tissue debris.

Keywords: prevalence, pathology, *Heterakis gallinarum*, *Histomonas meleagridis*, domestic chicken, Kashmir Valley

Introduction

Domestic chicken are very susceptible to parasitic infections because they are freely roaming in the backyard in search of food, thereby exposing themselves to various helminth larvae and eggs. One such common helminth infection in chicken is of the nematode *Heterakis gallinarum* because backyard chicken frequently feed on earthworms which act as intermediate hosts for this nematode. *Heterakis gallinarum* infection in chicken is usually subclinical but it may function as a vector for *Histomonas meleagridis* (black head) which induces severe pathological lesions in the gut and liver leading to high mortality rates in susceptible hosts (Gibbs, 1962, Springer *et al.*, 1969; Lund & Chute, 1974) [6, 18, 12]. The present study was designed to have an idea about the prevalence of the nematode *Heterakis gallinarum* in free ranging chicken of Kashmir Valley and to study the extent of pathology caused by it to the caecum and liver in both mono infection and coinfection with *Histomonas meleagridis* to design some strategy in future to curb this fatal association of these two parasites which causes great economic losses to our

backyard poultry industry.

Materials and methods

The present two year study was carried from January 2012 to December 2013 and for the study a sample size of 478 domestic chicken (*Gallus g. domesticus* L., 1758), 243 males and 235 females, weighing between 1 - 2.5 kg were selected. Chicken were collected from the different localities covering almost entire Kashmir valley. Individual clinical evaluation and euthanization was carried out according to Zander *et al.* (1997) [23]. Nematodes were collected, rinsed in normal saline (0.85%), fixed in hot 70% alcohol and then counted using a stereoscopic microscope. The nematodes were cleared in lactophenol, mounted in glycerine jelly, photographed and identified following Vicente *et al.* (1995) [21].

Prevalence was calculated as a percentage of the host population infected at a point in time (Thrusfield, 1995) [20]. Mean intensity was calculated as number of parasites per infested bird.

Faecal examination was carried out following Mc Master's

flotation technique. Worm eggs were identified using the keys described by Thienpont *et al.* (1986)^[19]. Faecal egg counts (FECs) were undertaken within 24 hours by a modification of the McMaster technique with a sensitivity of 50 eggs per gram of faeces (Thienpont *et al.*, 1986)^[19].

Impression smears from the liver and scrapings from the caeca and intestine were stained with Giemsa and Gram's stain.

For pathological studies, fragments of the parasitized caecae and liver fixed in formalin and then routinely processed (Bancroft and Gamble, 2002)^[2] for paraffin embedding. 5µm sections were cut and stained with hematoxylin and eosin (H&E). Mc Manus periodic acid Schiff (PAS) stain was used to demonstrate protozoan inclusions in the sections. Grocott's stain was used to differentiate with fungal elements. Micrographs were obtained using digital microscope model BX60F-3, Olympus Optical Co. Ltd. (Tokyo, Japan), fitted with the Olympus camera model DP12.

Results and Discussion

Prevalence

Heterakis gallinarum is characterized by the presence of oesophageal bulb (Fig 3) and its eggs are more barrel shaped (Fig 4). Prevalence rate for 1st Year of study (2012) was found to be 3.43% (8/233) and that for the 2nd Year (2013) was found to be 5.3 % (13/245) giving an overall prevalence rate for two year study (Jan 2012 to Dec 2013) to be 4.39% (21/478). Reported prevalence rates in chicken range from 10.2% to 72.5% in Europe (Kokozidou and Zafeires, 1996; Permin *et al.*, 1999)^[11, 15], 1 to 84% in the USA (Waters *et al.*, 1994) and 17.28% to 78.8% in Africa (Permin *et al.* 1997)^[16] but low prevalence in the present study can be attributed to either better adaptability of the nematode to chicks or to the more resistance of chicks which could be either innate or due to better nutrition. *Heterakis gallinarum* worm burden was slightly higher in backyard chickens with poor body conditions (Jansson *et al.*, 2004)^[7].

Range of intensity of worms was found to be 02 – 55 with Mean intensity of infection observed to be 20 ± 2.1 . Faecal Egg Count was found to be very low i.e. <40 eggs per gram of faeces

Only two cases of *Heterakis gallinarum* co-infection with *Histomonas meleagridis* were reported, one in September 2012 and other in August 2013 giving overall prevalence of coinfection only 0.42% (2/478) and mean intensity of infection of *Heterakis gallinarum* during coinfection with *Histomonas meleagridis* was comparatively lower (07 ± 2) which could be attributed to the fact that the establishment of Histomonads in the host could hinder the survival and establishment of the nematode.

Prevalence of nematode infection and Mean intensity of infection was found to be high during Summer and Autumn seasons of the study (Figs 1,2) which can be attributed to decreased resistance of chicks to infection and increased availability of intermediate host (Earthworms) due to high temperature and more rainfall in Summer and autumn (especially August – September).

Pathology

▪ Monoinfection

No gross lesions were seen in the caeca and liver during mono-infection of *Heterakis gallinarum*. However histological findings in the caeca revealed presence of adult worms in the lumen of intestine along with cellular debris. Although some workers have reported larvae penetrating the epithelium of cecum (Sang-Ik Park and Sung-Shik Shin, 2010) but in the present study sections with larvae could not be recovered. The infected tissue revealed intense chronic diffuse inflammatory processes with mononuclear and polymorphonuclear (heterophils) leucocyte infiltrations extending up to submucosa. T.S of the infected caeca showed mucosal erosion with parasites and cellular debris (Fig 5) as observed by other workers (Kaushik and Sharma Deorani, 1969; Riddel and Gajadhar, 1988; Khan *et al.*, 1994; Choudury and Das, 1993)^[18, 17, 10, 3]. However in the present study during mono-infection, *Heterakis gallinarum* has been found to show less severe pathological changes as reported by other workers (Lund and Chute, 1973; Anna Schwarz *et al.*, 2011)^[12, 1]. Toluidine blue staining of the infected sections clearly reveals the presence of mast cells.

▪ Co infection

During co-infection of *Heterakis gallinarum* and *Histomonas meleagridis* gross lesions in the caeca and liver were found typical to those described by earlier workers (McDougald LR, 2003, 2005)^[14]. Livers were found to be enlarged brilliant greenish with numerous round to oval, circumscribed lesions ranging from 0.5 to 1.5 cm in diameter and extending deep into the parenchyma (Fig 6). Cortes *et al* (2004)^[4] have recorded 9 out of 10 chickens to have oval, circumscribed liver lesions with white to pale yellow depressed centers and a dark gray to dark green periphery. Histological studies of the infected livers revealed extensive infiltration of lymphocytes, mononuclear cells and scarce heterophills (Fig 7). Hepatocytes towards the periphery of the lesions contained punched out ovoid bodies which stained positive and became visible with PAS (Periodic Acid Schiff) stain (Fig 8). No fungal elements could be demonstrated with Grocott's stain. Differential staining with PAS and Grocott have been recommended by Kemp and Reid (1966)^[9] to differentiate between *Histomonas* and fungal infections. Thrombosis and necrotic lesions around the surrounding blood vessels in the liver indicate a vascular spread. McDougald, L.R (2005)^[14] reviewed that infections from caecal lesions spread via blood to the liver, kidneys, spleen and lungs but no lesions were reported in kidneys, spleen and lungs in the present study.

Microscopic examination of cecal lesions showed hyperemia, ulceration and infiltration of heterophils, lymphocytes and macrophages extending from mucosa to the muscular layer. There was sloughing off of the epithelium and lumen was packed with fibrin, red blood cells and tissue debris (Fig 9). These observations are in line with the observations of Anna Schwarz *et al.*, 2011^[1] who observed formation of fibrinous to fibrinohaemorrhagic exudates in the 14 out of 15 of dual infected birds and 6 out of 15 birds showed focal hepatic necrotic areas.

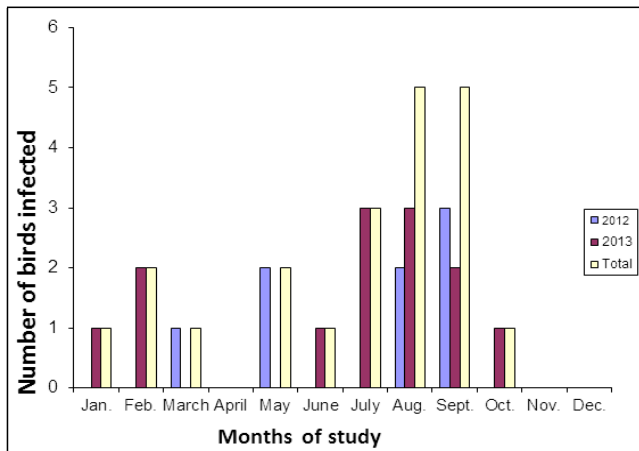


Fig 1: Monthwise prevalence of *Heterakis gallinarum* in domestic fowl

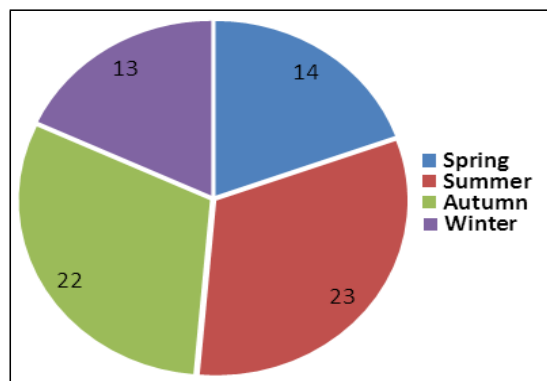


Fig 2: Season Wise Mean Intensity of infection of *Heterakis gallinarum*

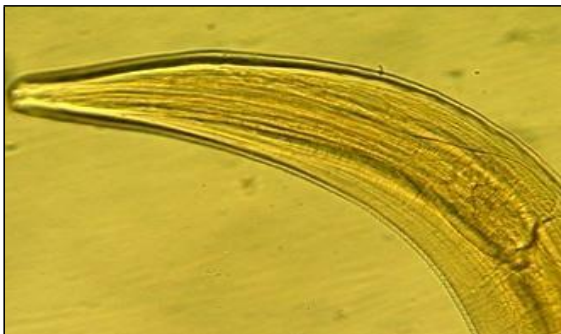


Fig 3: *Heterakis gallinarum* recovered from the domestic fowl showing anterior end revealing mouth, lips and oesophageal bulb.



Fig 4: Barrel Shaped Egg of *Heterakis gallinarum*



Fig 5: Photomicrograph of caecum of domestic fowl revealing adult *Heterakis gallinarum* in the lumen, Note the mucosal denudation and cellular debris in lumen H&E 40X



Fig 6: Liver of Chicken infected with *Histomonas meleagridis* showing numerous round to oval circumscribed lesions resembling a bull's eye target

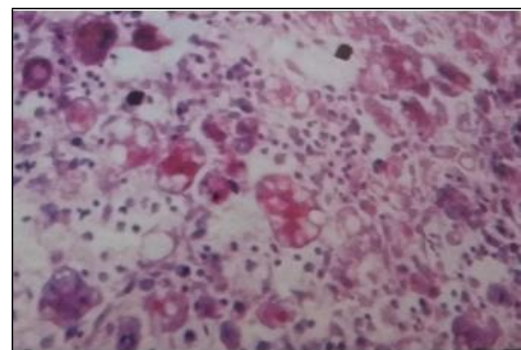


Fig 7: T.S of Liver of Chicken infected with *Histomonas meleagridis* showing punched out vacuolar bodies within hepatocytes (HE x 400)

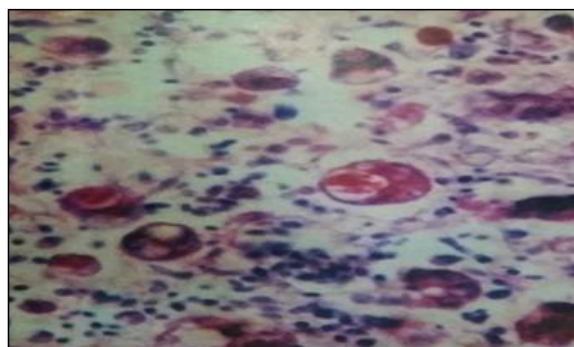


Fig 8: Ovoid bodies representing *Histomonas meleagridis* in the hepatocytes of chicken visible with PAS stain (x400)

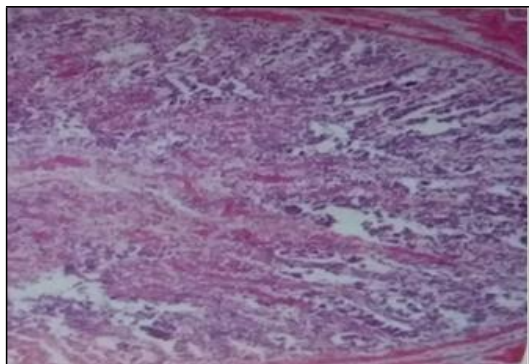


Fig 9: Caeca of chicken coinfected with *Heterakis gallinarum* and *Histomonas meleagridis* showing plugging of lumen with cellular debris, inflammatory cells and fibrin (HE x40)

Conclusion

Heterakis gallinarum is comparatively harmless nematode but with great potential to act as vector for blackhead causing severe pathological changes in the tissues during coinfection with *Histomonas meleagridis* especially in the warm and wet seasons. Investigations are thus advisable to know in detail local immunological responses of the chicken intestine to the mono infection and co infection *Heterakis gallinarum* with *Histomonas meleagridis* to devise the strategies such as recombinant vaccines and dietary immunomodulation to enhance gut immunity rather than relying on antihelminthic drugs which contaminate meat and eggs showing great concerns in public. Possible existence of lateral transmission for *Histomonas meleagridis* without the possible role of *Heterakis gallinarum* ovum vectors needs to be investigated as during the study we come across one dead bird with severe infection of *Histomonas* without any *Heterakis* worm recovered from the cecum.

References

1. Anna Schwarz, *et al.* Pathobiology of *Heterakis gallinarum* mono-infection and co-infection with *Histomonas meleagridis* in layer chickens. *Avian Pathology*. 2011; 40(3):277-287.
2. Bancroft JD, Gamble M. Theory and Practice of Histological Techniques. 5th ed., Harcourt Publ. Ltd., London, 2002, 125-131.
3. Choudury S, Das MR. Studies on growth rate and pathological changes in chickens experimentally infected with *Heterakis gallinarum*. *J Vet. Parasitol*. 1993; 7:81-85.
4. Cortes PL, Chin RP, Bland MC, Crespo R, Shivaprasad HL. Histomoniasis in the bursa of Fabricius of Chickens. *Avian Dis*. 2004; 48:711-715.
5. Anil Kumar Jha. Histopathological studies of tapeworm *Raillietina tetragona* (Molin, 1858) from the gastro-intestine of indigenous chicken (*Gallus domesticus* L.) farming in Kirtipur, Nepal. *Int J Vet Sci Anim Husbandry* 2019;4(4):01-06.
6. Gibbs BJ. The occurrence of the protozoan parasite *Histomonas meleagridis* in the adults and eggs of the cecal worm *Heterakis gallinae*. *Journal of Protozoology*. 1962; 9:288-293.
7. Jansson D, Fossum O, Engelsen PE, Christensson B, Andersson B, Christensson D. Parasitforekomst hos tamhos I svenska hobbyflockar. *Sven. Veterinartidn*. 2004; 56:11-17.
8. Kaushik RK, Deorani VPS. Studies on tissue responses in primary and subsequent infections with *Heterakis gallinae* in chickens and on the process of formation of caecal nodules. *J Helminthology*. 1969; 43:69-78.
9. Kemp RL, Reid WM. Staining techniques for differential diagnosis of histomoniasis and mycosis in domestic poultry. *Avian Dis*. 1966; 10:357-363.
10. Khan RW, Khan MM, Khan SA. Prevalence and gross pathology of helminth infection in domestic fowls of Hyderabad District. *Proceedings of Parasitology*. 1994; 17:4-7.
11. Kokozidou M, Zafeires P. Prevalence of gastrointestinal parasites in fowls. *Bull. Hell. Vet. Med. Soc*. 1996; 47:99-103
12. Lund EE, Chute AM. The reproductive potential of *Heterakis gallinarum* in various species of galliform birds: implications for survival of *H. gallinarum* and *Histomonas meleagridis* to recent times. *International Journal for Parasitology*. 1974; 4:455-461.
13. McDougald LR. Other protozoan diseases of the intestinal tract – histomoniasis (blackhead). In: *Diseases of Poultry* 11th edn. Saif YM, Barnes HJ, Glisson JR, Fadly AM, McDougald LR, Swayne DE, (Eds.), Ames, Iowa State Press, 2003, 1001-1006.
14. McDougald LR. Blackhead disease (Histomoniasis) in poultry: A critical review. *Avian Dis*. 2005; 49:462-476.
15. Permin A, Bisgaard M, Frandsen F, Pearman M, Kold J, Nansen P. Prevalence of gastrointestinal helminthes in different poultry production systems. *Brit. Poultry Sci*. 1999; 40:439-443.
16. Permin A, Magwisha H, Kassuku AA, Nansen P, Bisgaard M, Frandsen F, *et al.* A cross sectional study of helminthes in rural scavenging poultry in Tanzania in relation to season and climate. *J Helminthol*. 1997; 71:233-240.
17. Riddell C, Gajadhar A. Caecal and hepatic granulomas in chickens associated with *Heterakis gallinarum* infection. *Avian Dis*. 1988; 32:836-838.
18. Springer WT, Johnson J, Reid WM. Transmission of histomoniasis with male *Heterakis gallinarum* (nematoda). *Parasitology*. 1969; 59:401-405.
19. Thienpont D, Rochette F, Vanparijs OFJ. Diagnosing of helminthiasis through coprological examination. 2nd ed. Janssen Research Foundation, Beerse, Belgium, 1986.
20. Thrusfield M. *Veterinary Epidemiology*, 2nd ed, Blackwell Scientific, Oxford, 1995.
21. Vicente JJ, Rodrigues HO, Gones DC, Pinto RM. Nematoides do Brasil. Parte IV: Nematodes de aves. *Revista brasileira de Zoologia*., 12, 1-273.
22. Waters CV, Hall LD, Davidson WR, Rollor EA, Lee KA. Status of commercial and non-commercial chickens as potential source of histomoniasis among wild turkeys. *Wildl. Soc. Bull*. 1994; 22:43-49.
23. Zander DV, Bermudez AJ, Mallinson ET. Principles of disease prevention: Diagnosis and control. In *Diseases of poultry*, 10th ed., B.W. Calnek, H.J. Barnes, C.W. Beard, L.R. McDougald, and Y.M. Saif (eds.). Iowa State University Press, Ames, Iowa, 1997, 3-45.
24. Eshetu Y, Muluamem E, Ibrahim H, Berhanu A, Aberra K. Study of gastro-intestinal scavenging chickens in four rural districts of Amhara region, Ethiopia. *Rev. Sci. Tech*. 2001; 20:791-796.