Case Report

Tetrameres Fissispina Infection in Ducks from Bandipora Area of Kashmir Valley

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ABSTRACT

During an investigation of a severe and fatal outbreak of Duck Viral Enteritis, *Tetramers fissispina* was detected for the first time in flocks of local ducks reared on the banks of Wular Lake in the Bandipora area of Kashmir Valley. The disease outbreak was characterized by 100% morbidity and massive mortality of 45% in a short span of 10 days. Post-mortem examination of 75 birds revealed nodular structure in the wall of proventriculi of all the ducks, visible both from the mucosal and serosal surfaces. Examination of proventricular washings under light- and stereo- microscope revealed presence of small, slender nematodes resembling male *Tetrameres fissispina* parasite. Females of the parasite were recovered following dissection of the proventricular nodules revealing the characteristic morphology of the species. These were cherry red in colour resembling structure of gram seed. Either one or two female parasites were present per nodule, and were occasionally associated with males. Embryonated eggs of the parasite were found in the intestinal contents. The finding suggested that the parasite induces significant damage of proventriculus in ducks and might have contributed to the mortality.

Keywords: Tetrameres, ducks, outbreak, Kashmir

INTRODUCTION

Tetrameres spp., (Nematoda: Spiruridae) are parasites of poultry and wild birds prevalent throughout the world (1, 2, 3, 4, 5). Members of this genus exhibit strong sexual dimorphism. Mature females are almost spherical, dark blood-red in colour, and have four longitudinal furrows which correspond to the medium and lateral lines. *Tetrameres* are found buried in the mucus glands of the proventriculus. The males are small, white and filiform, with or without spines on the cuticle and tail (6, 7). The male parasite is usually free in the lumen of the proventriculus, though it may be present in the glands in association with the female for copulation. The life cycle is indirect involving an arthropod intermediate host. Eggs once passed with droppings hatch and are swallowed by intermediate hosts which are water crustaceans, *Daphnia pulex*, and *Gammarus pulex*. The final host acquires the infection following ingestion of the infected intermediate host. Immediately following ingestion, the male and female parasites migrate to the proventriculus where they embed in the glands. After copulation males migrate to the lumen and die (7).

Representatives of the genus *Tetrameres* have been known to parasitize about 300 species of domestic and wild birds belonging to at least 20 orders (8). Four species have been reported commonly in poultry, *T. Americana*, *T. crami*, T. *fissispina*, and *T. pattersoni* (9). *T. fissispina* is found principally in wild ducks and geese and other water fowl in various parts of the world, but also, occurs in domestic duck, pigeon, fowl, turkey, guinea fowl, and quails in Europe, Asia and the Far East. The male is 3 to 6 mm in length, and its median and lateral lines are each provided with a longitudinal row of spines. The female is 2.5 to 3 mm in length by 1 to 2 mm wide. The eggs are thick shelled, measure 48 to 56 µm by 26 to 30 µm, and contain an embryo when passed in the faeces.

The larvae hatch from the eggs and develop in the body cavity of the crustacean to the infective stage. Sixteen to twenty four hours after infection 3rd stage larvae are found deep in the lumen of the fundal glands of the proventriculus, without evidence of any pathological changes at this stage. Five days after infection dilation of the gland lumen occurs and 4th stage larvae, which measure 1.65 mm in length, are found. At 10 days marked sexual dimorphism is evident; in some glands males and females are found together, but in others only females are seen, the males being present in the mucus membrane. By the twelfth day the young females have undergone considerable increases in size and by the 18th day only adult females are found in the fundal glands (10).

Pathogenicity of the parasite varies with the species, the host resistance and the parasite load. It results in anaemia and emaciation due to sucking of blood. Migration of the young parasites into the proventricular glands causes marked irritation and inflammation, which may cause the death of the birds (6).

A severe and fatal outbreak of *T. fissispina* infection in chicken has been reported from Nigeria which was associated with sudden and massive mortality (11). The present article describes *Tetramers fissipina* associated with a severe and fatal outbreak of duck viral enteritis in the flocks of local ducks reared on banks of Wular Lake in the Bandipora area of Kashmir Valley.

MATERIAL AND METHODS

A total of 75 dead ducks from different flocks, on banks of Wular Lake in the Bandipora area of Kashmir Valley, were necropsied during a severe and sudden outbreak of Duck Viral Enteritis. Clinical investigations were performed. Morbidity, mortality and post-mortem lesions were recorded. Representative tissue materials and the parasites were collected for detailed laboratory examinations.

Parasitological Examination

Contents from proventriculi were washed into a petri-dishes using sterile normal saline and examined using light-and stereo- microscopes. The proventricular nodules were dissected to recover the parasites which were identified using the helminthological keys (6).

Pathological Examination

Representative tissue samples were collected in 10% buffered formal saline for histopathological examination. The samples were processed using routine paraffin embedding technique; 5 µm sections were cut and stained with Harri's haematoxylin and eosin (H&E) staining technique (12). Duplicate sections were stained with combined Alcian Blue PAS technique for acid and neutral mucopolysaccharide (13).

RESULTS

Clinical Picture

Investigation of the flocks revealed approximately 100% morbidity. Owners of the flocks reported massive mortality (45%) in a short span of 10 days. The outbreak was characterized by observation of sudden deaths. Ailing birds showed inappetence, ruffled feathers, dullness, watery diarrhoea, and disinclination to move. They stood head down with drooping outstretched wings. Nasal discharges, tremors in the head and neck, and extension of the neck were also observed. Paralysis was observed in terminal stage of the disease (Fig 1).

Figure 1. Sick paralysed duck infected with *Tetrameres fissispina*,

Figure 2. Proventriculus showing nodules formed by *Tetrameres fissispina*

Necropsy examination

Post-mortem examination revealed wide spread haemorrhages, ranging from petechiae to suffusions, on the viscera including lungs, heart, liver, pancreas, spleen, kidneys, serosa of gut, and mesentery. Trachea showed congestion and slight haemorrhages. Nodular structures were visible in the wall of proventriculus and were evident both from the mucosal and serosal surfaces. The number of the nodules per proventriculus varied from three to seven and ranged in size from 1 to 2 cm (Fig. 2).

Parasitological examination

Examination of proventricular washings under light- and stereo- microscope revealed the presence of small, slender nematodes, with spines, which were identified as male *T. fissispina*. Embryonated eggs of the parasite were found in the intestinal contents.

Dissection of the proventricular nodules revealed the presence of cherry red coloured parasites resembling the structure of a gram seed. On the basis of morphological features these were identified as female of *Tetrameres*. Either one or two female parasites were present per nodule, and occasionally were associated with males (Fig. 3 & 4).

Figure 3. Proventricular nodule dissected to reveal two cherry coloured female *Tetrameres fissispina*,

Figure 4. Dissected proventricular nodule revealing male and female *Tetrameres fissispina* in copulatory position.

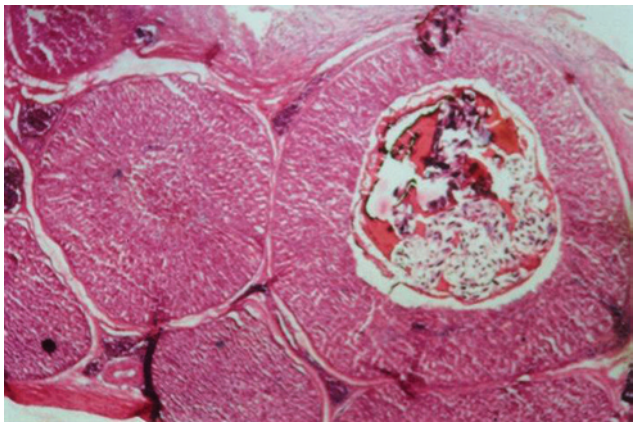

Figure 5. Photomicrograph of proventricular section showing female *Tetrameres fissispina*, in a gland. (H&E x 40)

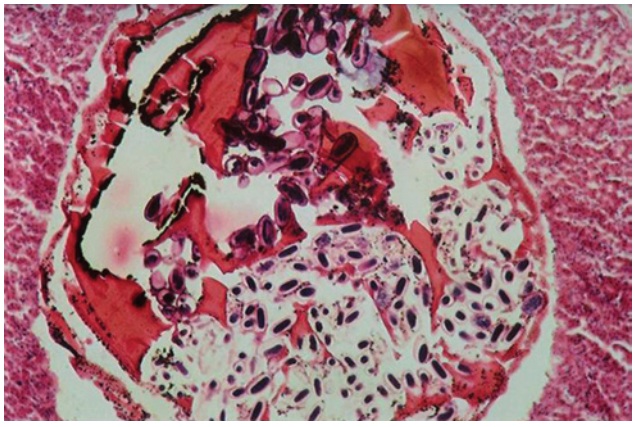

Figure 6. Higher magnification of Fig 5 showing embryonated eggs in female *Tetrameres fissispina*, in a gland. (H&E x 100).

Histopathology

Histopathological examination of proventriculus revealed dilation of the gland lumen, atrophy, degeneration and desquamation of the glandular cells (Fig. 5). Sections of the parasite containing embryonated eggs were observed in the glandular lumen (Fig. 6).

The eggs were at different stages of development; while most of them presented an acidophilic shell and basophilic larvae, while some were completely acidophilic. The acidophilic shells of some eggs within females stained positively with PAS for neutral mucopolysaccharide while the rest of

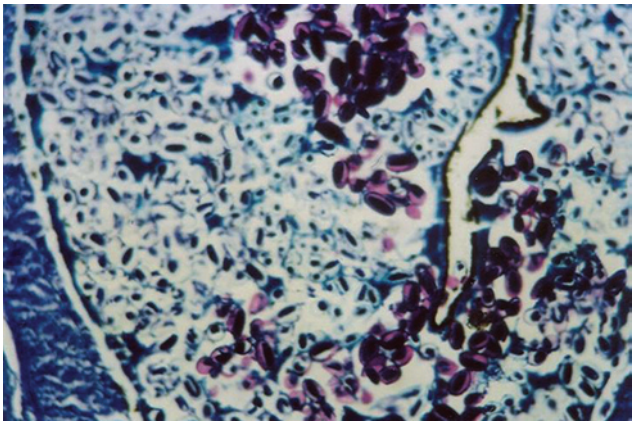

Figure 7. Section of female *Tetrameres fissispina*, in a gland revealing positive neutral mucopolysaccharide in some eggs while the rest are positive for acid mucopolysaccharide. Alcian Blue PAS x 400

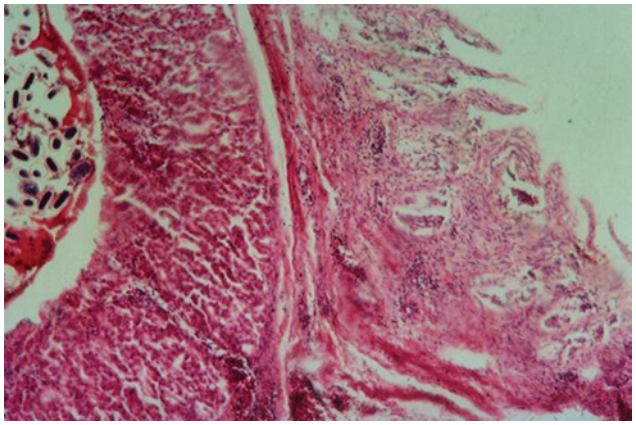

Figure 8. Section of proventriculus infected with *Tetrameres fissispina* showing atrophy of proventricular glands. H&E. x 100

the eggs and tissue were positive for acid mucopolysaccharide (Fig. 7). Cystic changes with flattened and atrophic lining or glandular epithelium were also in evidence (Fig 8). There was peri-glandular oedema and an infiltration of lymphocytes, eosinophils and histiocytes. Fibroplasia was evident in some proventricular glands

DISCUSSION

Wular Lake is the largest freshwater lake in India and lies in the Kashmir Valley, 40 km northwest of Srinagar City in the Northwest of India. With a size of 189 sq. kms, Wular Lake is also one of the largest freshwater lakes in Asia. The lake lies at an altitude of 1,580 meters above sea level. Its maximum depth is 14 meters, it has a length of 16 km and a breadth of 10 km. Wular Lake is a sustainable wintering site for a number of migratory waterfowl species which have been reported to harbor *Tetramers fissispina*. Bandipora district is located at the banks of the lake and the people here raise water birds for ease of access to the water body thereby getting exposed to such type of infections.

In the present report the clinical signs and pathological changes were suggestive of duck viral enteritis which could be the reason for the severe mortality in the outbreak. However, all the birds necropsied revealed varying degrees of involvement with infestation of *Tetrameres fissispina*.

The prevalence of *Tetramres* sp. has been reported as high as 54.5% (14, 15, 16, 17). *Tetrameres grusi* was reported to infect the proventriculous of 82% of Eurasian crane, (*Grus grus*,) in Central Iran (5). A prevalence of 1% has been reported in Desi chickens from Kashmir (18). To the best of our knowledge, so far no report on the occurrence the disease in ducks has been documented in Kashmir valley.

The parasite induced glandular atrophy and inflammatory changes in proventriculus. Most authors consider Tetrameres parasites as not especially pathogenic to birds, but under stressful conditions they may even cause mortalities in poultry (15, 16, 17). Up to 30 specimens of *T fissispina* have been reported in the proventriculous of wild water fowl without there causing any apparent ill effects (19). However, a severe and fatal outbreak of *T. fissispina* causing mortality of 50%, 37%, 40% and 38% in four flocks of local chicken in Nigeria, within 2 weeks has been reported (11). The clinical and pathological picture recorded in the present outbreak, resembled Duck Virus Enteritis, however the contribution of the parasitic infection cannot be ruled out. In serious parasitic infections, the nematode produced vast structural and functional changes, causing organ dysfunction and glandular necrosis, which might lead to the sudden massive deaths as observed in the present case (11).

Histopathological changes observed in proventriculus during the present study were in concordance with those reported earlier (10). The present finding suggested that *T. fissispina* might be widely prevalent in ducks in the Kashmir valley and might have induced severe pathological changes in the organ involved thereby its role in predisposition to other infectious diseases cannot be ruled out.

REFERENCES

1. Bergan, J.F., Radomski, A.A., Pence, D.B. and Rhodes, O.E.: *Tetrameres* (*Petrowimeres*) *striata* in ducks. J. Wildlife Dis. 30:351-358, 1994.
2. Digiani, M.C.: *Tetrameres* (*Gynaecophila*) megaphasmidiata n. Sp. (Nematoda: Tetrameridae), a proventricular parasite of the white-faced ibis *Plegadis chichi* in Agrentina. Syst. Parasitol. 47**:**111-117, 2000.
3. Cremonte, F., Digiani, M. C., Bala, L. O. and Navone, G. T.: *Tetrameres* (*Tetrameres*) megaphasmidiata n. Sp. (Nematoda: Tetrameridae), a parasite of the two-banded plover, *Charadrius falklandicus*, and white-rumped sandpiper *Calidris fuscicollis*, from Patagonia, Argentina. J. Parasitol. 87**:** 148-151, 2001.
4. Fink, M., Permin, A., Magwisha, H.B. and Jensen, K.M.V.: Prevalence of the proventricular nematode *Tetrameres Americana* Cram (1927) in different age groups of chicken in the Morogoro Region, Tanzania. Trop. Anim. Health Prod. 37**:**133-137, 2005.
5. Mowlavi, G.R., Massoud, J., Mobedi, I., Gharagozlou, M. J., Rezaian M. and Solaymani-Mohammadi, S.: *Tetrameres (Tetrameres) grusi* (Shumakovich, 1946) (Nematoda: Tetrameridae) in Eurasian Cranes (*Grus grus*) in Central Iran. J. Wildlife Dis. 42:397-401, 2006.
6. Soulsby, E.J.L.: Helminths arthropods and Protozoa of domesticated animals, 7th ed. Bailliere Tindall, London. 1982.
7. Permin, A. and Hansen, J.W.: Epidemiology, Diagnosis and Control of Parasites, Food and Agricultural Organization of the United Nations. Rome, Italy. 1998.
8. Mollhagen, T.R.: A study of the parasitic nematode genus. Tetrameres. I. The literature, 1835-1985. Spec. Publ. Mus. Tex. Tech. Univ. Texas Tech. University Press (Lubbock, Tex.) 35**:**1–75, 1991.
9. Saif, Y.M., Faldy, A. M., Calnek, B. W., Beard, C. W., Swayne, D. E., Barnes, H.J., McDougald L. R. and Glissin, J. R.: Diseases of poultry 11th ed. Iowa State Press, 937-939, 2003.
10. Cvetajeva, N.P.: Pathomorphologische Ver~inderungeu inl Entenmagen bei experimenteller Tetramerose. Helminthologia (Bratisl.) 2, 143-150, 1960.
11. Kamani, J., Meseko, C. A., Tanko, T. J., Tafarki, A. E., Lazarus, D. D., Peter, J. G. and Oladokun, T.: *Tetrameres fissispina* infection in local chickens in Taraba State, Nigeria, case report. Int. J. Poult. Sci. 7:1216-1218, 2008.
12. Luna, L.G. Manual of histologic staining methods of the Armed forces, Institute of Pathology, 3rd edition New York, McGraw Hill Book Company. 1968.
13. Bancroft, J.D. and Gamble, M.: Theory and Practice of Histological Techniques. 5th edition, Harcourt Publ. Ltd., London. 2002.
14. Oyeka, C. A.: Prevalence of intestinal helminths in poultry farms in Anambra state Nigeria. Bull. Anim. Health Prod. Afr. 37:217-220, 1989.
15. Saidu, L., Abdu, P.A., Umoh, J.U. and Abdullahi, U.S.: Diseases in Nigerian indigenous chickens. Bull. Anim. Health Prod. Afr. 42:19-23, 1994.
16. Fakae, B.B. and Paul-Abiade, C.U.: Rainy season period prevalence of helminths in the domestic fowl (*Gallus gallus*) in Nsukka, eastern Nigeria. Nig. Vet. J. 24:21-27, 2003.
17. Biu, A.A. and Haddabi, I.: An investigation of atetrameres infection among local chickens in Maiduguri. Nig. Vet. J. 26:44-46, 2005.
18. Pandit, B.A., Mir, A.S., Banday, M.A.A. and Shahardar, R.A.: Prevalence of helminth parasites in indigenous fowls of Kashmir valley. Poult. Advisor, XXIV**:** 37-39, 1991.
19. Soulsby, E.J.L.: Textbook of Veterinary Clinical Parasitology. Vol I. Helminths. Blackwell Scientific Publications, Oxford. 1965.