



Reports on *Tetrameres fissipina* from the Naturally Infested Indigenous Chicken in Phek District of Nagaland

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ABSTRACT

Background: Phek district, with its varied topography and climate, serves as a significant area for the study of animal diseases that may be influenced by environmental factors. The district shares international borders with Myanmar, which makes it a key area for monitoring and controlling animal diseases.

Methods: Morphological identification of the parasites was conducted, followed by PCR amplification of 18S rDNA to confirm the identity of the parasite. Gross and histopathological studies were performed to observe the effects of the infection on the chickens.

Result: Mature female *Tetrameres* are spherical, dark blood-red in colour and have four longitudinal furrows. The males are small, white and filiform, without spines on the cuticle and tail. Based on the morphological characteristics vis-a-vis PCR amplification and subsequent sequencing, the species was identified as *Tetrameres fissipina*. The gland of the proventriculus exhibited dilation, with degeneration and desquamation of the glandular cells with numerous nematode parasites, parasite eggs, bacterial colonies and mixed inflammatory cells (heterophils and lymphocytes). In the ventriculus (gizzard), there was mild lymphoplasmacytic and heterophilic inflammation that expanded the lamina propria (connective tissue layer). Liver and kidney showed significant inflammatory changes. There were lesions in the small intestine, including catarrhal inflammation, edema and localized necrosis. Mild histopathological changes in lungs was noted. This report signifies the first occurrence of *Tetrameres fissipina* in Nagaland.

Key words: Indigenous chicken, Necropsy, Phek, Pox, *Tetrameres*.

INTRODUCTION

Poultry production is an integral part of smallholder agriculture in the developing world and has a multidimensional contribution to the livelihood of both rural and urban households. Its contribution spans economic and social to cultural and environmental benefits (Gueye, 2000; Akinola and Essien, 2011; Birhanu *et al.*, 2022, Birhanu *et al.*, 2023). In Nagaland, backyard poultry farming covers about 97.56% of total poultry population and mostly consists of desi (60.27%) birds (Singh *et al.*, 2018). The local or desi chicken population therefore, serves as a crucial source of readily available animal protein for rural households in Naga villages, along with other livestock such as pigs, rabbits, ducks, mithun and tho-tho (indigenous hill cattle).

Phek district in Nagaland features diverse terrain and has a moderately warm climate, with average temperatures around 27°C, without exceeding 32°C. Winters are cold with the temperature dropping to 0°C in the coldest months of January and February (Borkotoky *et al.*, 2024). Looking at the geo-climatic condition, north eastern region is quite conducive for growth and propagation of parasites (Chamuah *et al.*, 2019a). Harsh and varied climatic conditions, combined with limited access to poultry vaccines and medicines, significantly increase the challenges posed by poultry diseases in the state. The humid tropical climatic conditions largely favour faster propagation and development of larval stages of helminth parasites (Matta and Ahluwalia 1981; Malhotra 1983; Kulkarni *et al.*, 2001). Further endoparasitic infection produces ill effects, immuno-suppression and predisposes

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the animals for various potential pathogens (Chamuah *et al.*, 2019) that adversely affect the successful poultry farming. It is, therefore, important to monitor and manage gastrointestinal helminthic infections in backyard free range birds to safeguard the health and productivity of these birds and the well-being of the rural communities they support.

Tetrameres is a dimorphic genus of spirurid nematode that infects domestic poultry such as chicken, duck, turkey, pigeon, quail, guinea fowl, pheasant and wild birds, almost exclusively parasitising the proventriculus (Megha *et al.*, 2022). Mature females are almost spherical, dark blood-red in colour, while males are small, white and filiform and found free in lumen (Kamil *et al.*, 2011). Bird infested with *Tetrameres* spp. manifest reduced body weight, delayed

maturity, decreased egg production, emaciation, diarrhoea and anemia (Ramaswamy and Sundaram, 1983). Birds become infected through the oral route when exposed to intermediate hosts, such as crustaceans, grasshoppers and cockroaches. Various studies highlight the presence of *Tetrameres* infections in poultry populations in different regions, emphasizing the significance of understanding and addressing this parasite's impact on poultry health. The present communication, reports finding of exceptionally high incidence of *Tetrameres* spp. in local chicken in Phek district of Nagaland.

Case history and observation

Twenty local poultry pullets from Gidemi village (25°37'9"N latitude, 94°0'22.0"E longitude; 5300 feet MSL), located in the Pfutsero block of the Phek district, were presented to the Division of Animal Science, Krishi Vigyan Kendra-Phek for post-mortem examination. The birds were reared in free range backyard system, unvaccinated and received occasional supplementary grains (maize and rice) as feed. The farmers reported regular death of chicken pullets exhibiting decreased activity levels, gradual emaciation, distended crop, coughing, poor feather quality, occasional diarrhoea, torticollis and paralysis before their death. No such symptoms were reported in adult birds. Pock lesions were frequently reported in both diseased and apparently healthy birds. Upon gross examination, majority of the birds displayed pock lesions around the eye and beak, although no pock lesions were observed in the shanks. The birds appeared emaciated and the paleness observed in their combs, wattles and legs indicated chronic anemia. A detailed post-mortem examination was conducted, during which representative tissue samples and parasites were collected for laboratory examinations.

MATERIALS AND METHODS

Morphological identification

Adult parasites were collected from naturally infected poultry during-postmortem at Division of Animal Science, Krishi Vigyan Kendra-Phek. The recovered parasites were washed with normal distill water to remove dirt and debris, then placed in 10% langhans lactophenol solution, observed under a microscope and identified according to keys based on species characteristics provided by Soulsby (2012). The adult parasites were preserved in 70% alcohol for DNA extraction and stored at -20°C until further use.

Molecular identification

Total genomic DNA was extracted from individual adult helminth parasites using a commercial genomic DNA extraction kit according to the manufacturer's instructions (G-Sure Tissue Kit, GCC Biotech, India) and DNA concentration was measured using spectrophotometer (Nanodrop 2000, Thermoscientific, USA). Genomic DNA was diluted to a working concentration of 100 ng/μL and 1 μL of this dilution was used as a template in a 25 μL of

reaction mixture. The PCR was performed with 18SrDNA with the help of primer F: 5'ATACCAACCAGCGTTCCGTT3' and R: 5'TTCTCGAAACGGCTCAGTCC3'. The PCR condition included initial denaturation at 95°C for 5 mins, 30 cycles of 94°C for 30 secs, 50°C for 30 secs (Annealing temperature), 72°C for 45 secs and final extension at 72°C for 10 mins. The resulting amplicons were confirmed by gel electrophoresis using 1.0% agarose-TBE gel and subsequently sequenced from University of Delhi, Delhi. The sequence analysis and similarity searches were performed with the basic local alignment search tool using default matrix. For phylogenetic analyses, multiple sequence alignment of the resulting sequences with already existing databases in GenBank was carried out using ClustalW of MEGA 6.01 software (<http://www.Megasoftware.net/mega6.01.html>). The evolutionary history was inferred using the Neighbor-Joining method as per Saitou N. and Nei M. (1987) with 1000 bootstrap values.

Pathological study

At time of post-mortem examination, visible gross lesions were recorded and representative tissue samples were collected in 10% formalin solution. The tissues were properly processed and embedded in paraffin wax. Paraffin sections were cut 4-5 μm thick and stained with Hematoxylin and Eosin (H and E) by method as described by Luna (1968). Histological alteration of all sections was examined using light microscope and photographed. The grossly visible parasites were collected from different organs and identified based on the morphological characteristics (Soulsby, 2012).

RESULTS AND DISCUSSION

The necropsy results and subsequent analysis revealed several notable observations in the examined birds. The birds were emaciated, anaemic, with a poor body condition (Fig 1) and their feathers appeared dull and unkempt. Among the cases studied, 20% exhibited a diphtheric membrane in the trachea. A majority of the cases (65%) demonstrated petechial haemorrhages in the kidneys and liver. Mild to moderate congestion was observed in the lungs, spleen and pancreas. All birds showed catarrhal inflammation in the small intestine. All pox infected birds (Fig 2) had concurrent infections with *Tetrameres* spp.

Further examination of the proventriculus (glandular stomach) uncovered the presence of *Tetrameres* spp., a blood-red nematode parasite (Fig 3), from the *Spiruridae* family, embedded within the proventricular glands (Fig 4). These parasites were visible from both the serosal and mucosal surfaces of the proventriculus. The proventricular nodules contained tarry red-colored parasites, identified as female *Tetrameres*, with each nodule typically housing one or occasionally two females. Additionally, small, slender male *Tetrameres* were observed in association with some of the females within the nodules. Morphologically, mature female *Tetrameres* were spherical, dark blood-red

and characterized by four longitudinal furrows, while males are smaller, white and filiform, without spines on the cuticle and tail. Species identification as *Tetrameres fissipina* was



Fig 1: *Tetrameres* infested chicken pullet with concurrent fowl pox infection.



Fig 2: Severe muscle wasting, resulting in a gaunt or skeletal appearance.



Fig 3: Luminal surface of proventriculus showing engorged *Tetrameres* parasites embedded in the musosa.

confirmed through PCR amplification of 18S rDNA and sequencing (Fig 5). The sequence (Accession number: MN305802.1) of the present investigation is 100% similar with the Canadian isolate (EF180077.1) and 98.86% similarity with Kashmir isolate (Accession number: OM920823.1) in the present investigation (Fig 6). It is worth noting that *T. fissipina* infestation has been reported in ducks from the Bandipora area of the Kashmir Valley (Kamil *et al.*, 2011), domestic indigenous fowl in Nigeria (Kamani *et al.*, 2010; Biu and Haddabi, 2005), as well as in local poultry in Selangor, Malaysia (Lee *et al.*, 1986).

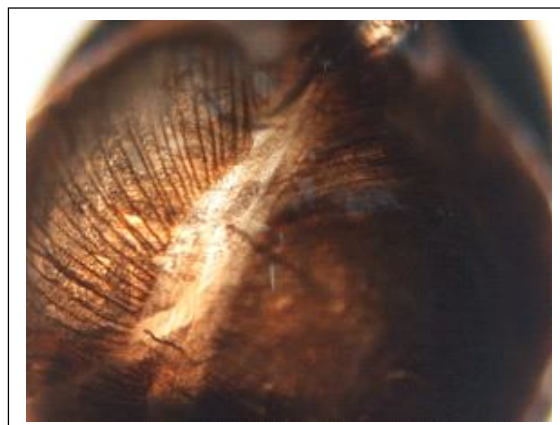


Fig 4: Photomicrograph showing a female *Tetrameres* parasite 4x.

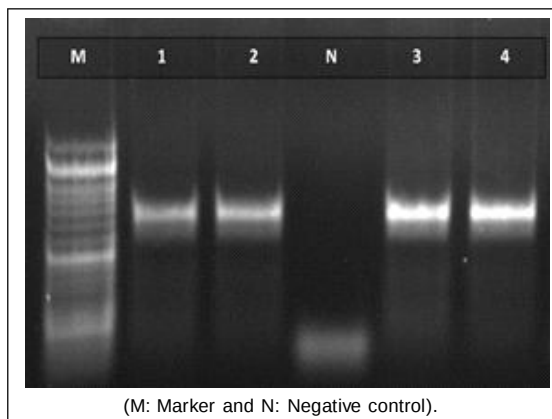


Fig 5: Amplified *Tetrameres fissipina* 18SrDNA (721 bp).

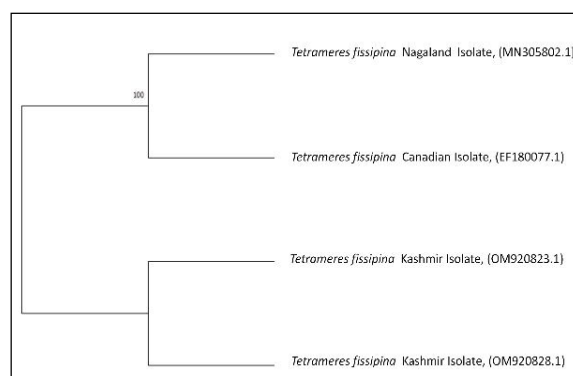


Fig 6: Phylogenetic tree of *T. fissipina*.

Histopathological examination of the proventriculus revealed gland lumen dilation (Fig 7), atrophy, degeneration and desquamation of the glandular cells (Fig 8). Numerous nematode parasites, eggs, bacterial colonies and mixed inflammatory cells (heterophils and lymphocytes) with associated necrosis were observed in the proventriculus (Fig 9,10). Mild lymphoplasmacytic and heterophilic inflammation in the ventriculus and multifocal cystic spaces (parasite tracts) within the koilin of the ventriculus were noted. Mild to moderate congestion of hepatic blood vessels was observed, contributing to the overall picture of hepatic stress and compromised blood flow. In liver parenchyma evidence of significant inflammatory response characterized by the presence of mixed inflammatory cells, including heterophils and lymphocytes were noted. In some cases, there was early evidence of fibrosis, which manifested as increased collagen deposition in the liver parenchyma. This fibrotic change was indicative of chronic inflammatory responses and ongoing tissue damage. There was significant inflammatory infiltration in the renal interstitium. This inflammation primarily involved heterophils and lymphocytes, resulting in diffuse or focal areas of interstitial nephritis. Lungs showed mild inflammation, congestion, edema and localized haemorrhage. The lesion in small intestine including catarrhal inflammation, epithelial damage, inflammatory infiltration, edema and localized necrosis. This report marks the first identification of *Tetrameres* species in the Phek district of Nagaland.

The reports of *T. fissipina* from the local chicken of the Phek district in Nagaland mark the first documented occurrence of this parasite species in the area. The identification of *T. fissipina* in the local chicken population was achieved through PCR and subsequent sequencing. This molecular analysis provided a high degree of accuracy in confirming the presence of *T. fissipina* and allowed for the detection of genetic variations within the parasite population. The reports of *T. fissipina* in the local chicken population of the Phek district provides important information for understanding the prevalence and impact

of this parasite species in the region. By obtaining a molecular-based identification, potential confusion or misidentification based solely on morphological characteristics can be minimized. This accurate

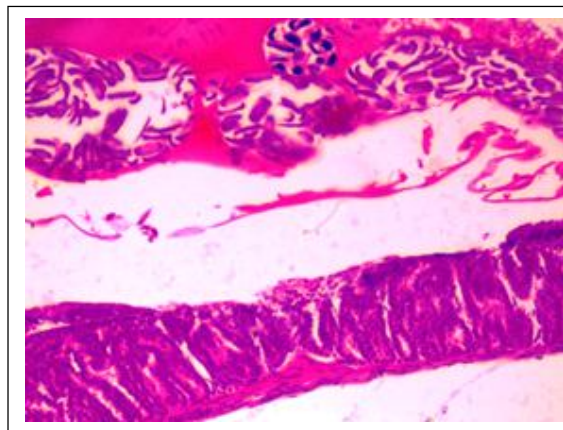


Fig 8: Pressure atrophy, degeneration and desquamation of the glandular mucosa of proventriculus H and E 40X.

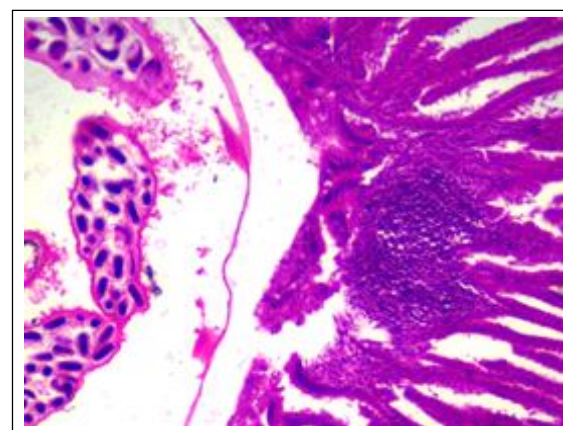


Fig 9: Parasite eggs, bacterial colonies and mixed inflammatory cells (heterophils and lymphocytes) with associated necrosis.

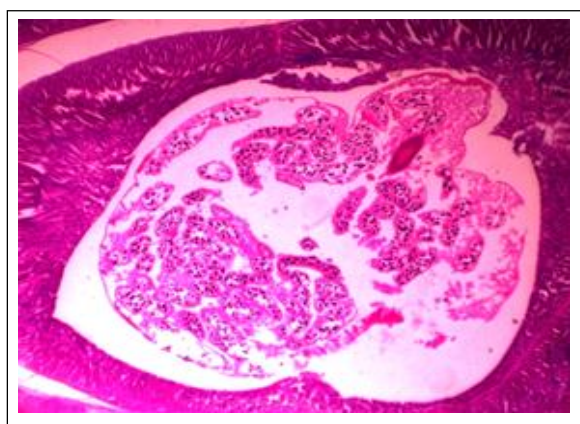


Fig 7: Dilation of the gland in the proventriculus due to the presence of adult tetrameres. H and E 40X.

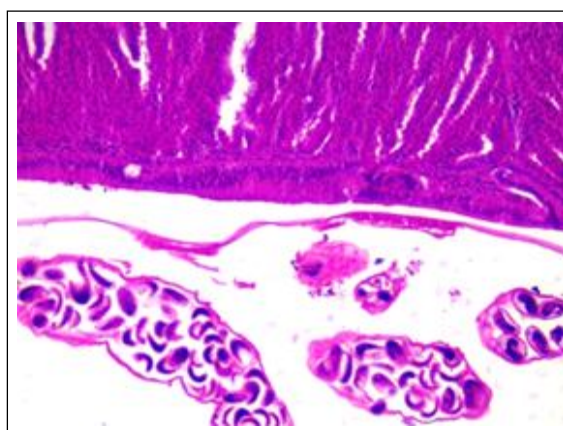


Fig 10: Sections of the parasite containing embryonated eggs (ovoid, nonoperculate, smooth cuticle) at different stages of development.

identification of *T. fissipina* in the local chicken population contributes to better preventive measures and control programs against the parasite. *T. americana* has been reported as a common parasite of free-range chicken in tropical and sub-tropical areas with high prevalence (Fink *et al.*, 2005). Kamani *et al.* (2010) reported *Tetrameres americana* in a semi-intensive flock of local poultry in Katsina State, Nigeria causing mortality of 40% in chicken and 57% in turkeys. A high prevalence of *Tetrameres mohtedai* helminth infection was documented in desi chickens in Cauvery delta region of Tamil Nadu, which causes high morbidity and mortality in desi chickens from Cauvery delta region of Tamil Nadu. Local chicken must have been acquired *Tetrameres* through feeding on the intermediate orthopteran hosts like grasshoppers, locusts or cockroaches, which were picked from the ground due exposure to range situation (Biu and Haddabi, 2005), which upon ingestion developed into adult worms in the proventriculus. *Tetrameres* parasites as not especially pathogenic to birds, but under stressful conditions they may even cause mortalities (Kamil *et al.*, 2011). The adult worm feed on the blood of the host and becomes engorged and gravid. This feeding habit could lead to severe anemia and death in the infested bird as evident in this case. However, pathogenicity of the parasite varies with the species, the host resistance and the parasite load. Migration of the young parasites into the proventricular glands causes marked irritation and inflammation, which may cause the death of the birds (Soulsby, 2012). In the present case, the concomitant infection of pox might have resulted in lowering of resistance and rise in *Tetrameres* load. It is therefore needful to conduct further epidemiological and pathological studies on *Tetrameres* species to establish the pathogenicity and economic importance of the infection in the district. Its correlation with pox needs to be established. Govindan and Annamalai (2019) observed infected proventriculus with areas of hemorrhages across the glandular epithelium, vascular congestion, fibrosis and necrosis of proventricular glandular structures in adult desi backyard chicken in Puducherry analogous to the present findings. *Tetrameres* sp. embedded in the proventricular serosa is thus the most likely cause of death or which may constitute a significant health hazard to the poultry due to scavenging habit in rural areas with little or no access to effective drugs and vaccines as opined by Pallabi *et al.* (2017).

CONCLUSION

The present study conducted morphological vis-à-vis PCR and sequencing to identify *T. fissipina* in local chickens for the first time in Phek district of Nagaland. This sequencing method provides cent percent accuracy in identifying the parasite and allows for the detection of genetic variations within the population. By minimizing ambiguity arising from morphological identification, this genetic identification can contribute to the development of effective preventive

measures for future control programs against the parasite. Histopathological revealed distinct proventricular lesions leading to dysfunction of digestive process and leading to lower immunity and disease incidence. In summary, the study's findings provide valuable data on the identification of *T. fissipina* in local chickens using PCR and subsequent sequencing. This knowledge can aid in implementing targeted control measures to combat the parasite, ensuring the well-being and productivity of the poultry industry in the region.

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Author's contributions

DB, JKC: Involved in investigation, data generation, interpretation and preparing original draft; RD: Laboratory assistance and final editing.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article, funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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