

SEVERE NODULAR TYPHLITIS IN A CAPTIVE BLYTH'S TRAGOPAN (*TRAGOPAN BLYTHII*) ASSOCIATED WITH *HETERAKIS ISOLONCHE* LINSTOW, 1906 FROM KOHIMA ZOOLOGICAL PARK, NAGALAND, INDIA

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ABSTRACT

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The paper describes clinical sign, gross and histopathological changes in the caecum of a male Blyth's tragopan (*Tragopan blythii*) from the Kohima Zoological Park of Nagaland. The bird had concomitant infection of coccidia and caecal nematodes. Coccidian infection was confirmed by ante-mortem coprological examination and treated. Clinically the bird exhibited inappetance for 6 days, thereafter died without revealing further clinical signs. Necropsy revealed petechiae in subcutaneous tissues and musculature. Ileum and caecum had granulomatous lesions. Caecal wall was thickened and greatly enlarged. The mucosal surface was distorted with necrotic materials almost filling the lumen. Large number of *Heterakis isolonche* were isolated from the caecal lumen found free and also embedded in caecal nodular mass. Histopathologically, the caecal mucosa including villi were completely lost and replaced by multiple granulomatous lesions. Each granuloma was consisted of cross or longitudinal sections of adult parasites at the centre with massive fibroblastic proliferation, surrounded by a zone of cellular infiltrations with macrophages, lymphocytes, epithelioid and giant cells, finally merging into a connective tissue capsule. In some granulomas, only remnants of degenerated parasites with necrotic tissues could be noticed at the centre; often the granulomas were associated with the formation of leiomyoma. At places, haemosiderin pigments were engulfed by macrophages and giant cells. Authors concluded the caecal lesions as severe nodular typhlitis induced by natural *H. isolonche* infection.

Key words: *Heterakis isolonche*, Blyth's tragopan, *Tragopan blythii*, captive, Nagaland

Introduction

Birds under the family Phasianidae are heavy-bodied and nests on the ground. They can run fast. Though they have fast and strong flight ability, in crisis they prefer to escape by running than flight. Their strong feet are used to scratch the ground for exploration of food below the decomposed leaves at the forest floor. Their food consists of plant seeds, often fruits and small invertebrates. Tragopans are expressively colorful pheasants of the Phasianidae family. Blyth's tragopan (*Tragopan blythii*) is an endemic wild pheasant species (Fig. 1 and Fig. 2) of the eastern Himalaya and the north east of India. Their natural home range extends from Bhutan, through Arunachal Pradesh, Nagaland, Mizoram and Manipur in India and Myanmar to the southeast Tibet and northwest Yunnan in China (Choudhury, 1997; Fuller and Garsen, 2000; Choudhury, 2001; Lalthanazara *et al.*, 2011; Ramesh Kumar *et al.*, 2012 and Sailo *et al.*, 2013). The size of male and female bird is 65-70 cm and 58 cm, respectively (Grimmett *et al.*, 2015). The current status of wild population is obscure. It is categorized as vulnerable species by International Union for Conservation of Nature and Natural Resources (IUCN) (Fuller and Garsen, 2000). According to legal provisions, it is protected by the Indian Wildlife (Protection) Act 1972 under Schedule I, Part III. *T. blythii* represents the state bird of Nagaland. In the northeast India, the prime threat factor for *T. blythii* assessed by IUCN is deforestation consequent to shifting cultivation (Fuller and Garsen, 2000). In addition, fuel wood collection, commercial timbering has lead to fragmentation of habitat, lack of enforcement regulations, and hunting for food has added to the decline of wild population. Fuller and Garsen (2000) has enumerated the global captive population of *T. blythii* including the private and public ownership from both published and unpublished sources to be 50-100 (International Studbook). There is paucity of information on the parasitic diseases of *T.*

blythii though, helminthic infections are reported from other pheasant species at different point of time from various countries like India, America, Germany, Brazil, Canada and Britain (Griner *et al.*, 1977). For the rescue, rehabilitation, captive breeding facilities and exhibits where the free ranged *T. blythii* are kept, information on helminthic infection is very essential to undertake appropriate husbandry and management practices for such small population of species. We describe below the chance occurrence of a case of severe nodular typhlitis revealed at post-mortem examination of a male *T. blythii* carcass received from Kohima Zoological Garden, Nagaland, India during the month of August, 2006.

Materials and Methods

Source of materials

A male Blyth's tragopan (*Tragopan blythii*) was reared in captivity at the Kohima Zoological Park, Nagaland. The bird clinically exhibited inappetance and coprological examination revealed presence of coccidian oocysts for which anticoccidial treatment was provided. On the sixth day after initial expression of the clinical sign, the bird died without showing any further sign. The carcass was dispatched by the Park authority to the Department of Pathology, College of Veterinary Science, Khanapara for conducting post-mortem (PM) examination. During PM examination a few number of nematode parasites found in the caecal lumen and loosely embedded in nodular masses were collected, fixed and preserved in 10% formalin and sent for taxonomic identification to the Department of Parasitology, College of Veterinary Science, Khanapara. Representative tissue samples were fixed and preserved in 10% formalin solution. Samples were processed through routine paraffin embedding technique and sections were cut at 5 µm thicknesses and stained with Meyer's haematoxylin and eosin stain (Luna, 1968). Stained sections were examined

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under a compound microscope and changes were recorded.

Evaluation of parasite species

The nematode parasites were put in lactophenol for 48 hours for clearing. Thereafter temporary mounts were prepared and morphological studies were carried out under a compound microscope using the standard keys of Yamaguti (1985) and compared with the description of Baylis (1936), Yamaguti (1985) and Sood (2006).

Results and Discussion

Evaluation of the parasite

Morphologically all the parasitic nematode species were identical to *Heterakis isolonche* Linstow, 1906 (Fig. 3, Fig. 4, Fig. 5 and Fig. 6) as described by Baylis (1936) and Sood (2006). *H. isolonche* can produce marked pathological lesions in the caecum of pheasants (Soulsby, 2012). The natural infection leading to severe nodular typhlitis in various pheasant species i.e., crimson horned pheasant (*Tragopan satyra*), monal pheasant (*Lophophorus impejanus*), blood pheasant (*Ithaginis cruentus*), crested fireback (*Lophura rufa*), peacock-pheasant (*Polyplectron bicalcaratum*), kalij pheasant (*Gennaeus leucomelano*), silver pheasant (*Gennaeus nychthemerus*) and golden pheasant (*Chrysolophus pictus*) have been recorded from India (Baylis, 1936, Alwar and Lalitha, 1961 and Rao, 1994). Griner *et al.* (1977) quoting the reference of Schwartz (1924) has reviewed the occurrence of parasitic nodular typhlitis in various pheasants in Germany, France, Italy, USA, Brazil, England and Canada. Goldova *et al.* (2006) reported the prevalence of *H. isolonche* (31.7%) in pheasants from Rozhanovce, Slovak Republic based on coprological diagnosis. Recently, Halajian *et al.* (2013) recorded the mixed prevalence of *H. isolonche* and *H. gallinarum* in a small population of pheasant from Iran with a worm burden range of 5-146 and a mean 45. *Heterakis gallinarum* is a widely prevalent caecal nematode of poultry in India (Choudhury *et al.*, 1995). In a systematic study conducted in Assam, Choudhury *et al.* (1995) could record prevalence of *H. gallinarum* (49.03%) and *H. brevispiculum* (0.09%) in local poultry reared under backyard system. Ghose (1987) reported 47% occurrence of heterakiasis in poultry at Dimapur in Nagaland. Hitherto occurrence of *H. isolonche* has not been reported from captive or free ranged *T. blythii*. Therefore, the present finding of the occurrence of *H. isolonche* in *T. blythii* is a new host record from India.

Clinical

Clinical history of the present case revealed concomitant infection with coccidian parasite. From the post-mortem findings it was assumed that the concomitant coccidian infection superimposed by severe heterakiasis in caecum led to general debility, inappetance and death of the pheasant. Pheasants affected with *H. isolonche* may die of diarrhoea, wasting and emaciation (Soulsby, 2012). Rao (1994) also recorded emaciation in a pheasant affected with *H. isolonche*. Halajian *et al.* (2013) examined 10 carcasses from a flock of 50 pheasants which were found to have mixed infections of *H. gallinarum* and *H. isolonche*. The affected birds were weak, emaciated, had depressed appetite, inactive and suffered from diarrhoea. Considering the conservation issues and the population status of *T. blythii*, the clinical signs associated with *H. isolonche* infection needs serious veterinary attention for free-living birds under captivity for proper health management.

Gross and histopathology

During necropsy subcutaneous tissue and musculature of the carcass showed pinpoint haemorrhages. The affected caecum was greatly enlarged with thickened wall (Fig.7). The caecum and ileum showed granulomatous lesions. The mucosal surface was distorted with necrotic materials almost filling the lumen. Numerous *H. isolonche* male and female parasites were found in the lumen and also protruded from the nodules (Fig. 8). There was no lesion corresponding to bacterial infection, or presence of pus or any other helminth parasites. In the present case gross caecal lesions were indicative of severe nodular typhlitis (Fig. 7 and Fig. 8). Complete occlusion of the caecal lumen, pea sized multiple nodule and deposits of necrotic material was recorded in golden pheasant affected with *H. isolonche* (Rao, 1994). Co-infection of *H. gallinarum* and *H. isolonche* in pheasants developed small nodules (Halajian *et al.*, 2013) in caecum. Earlier Balageur *et al.* (1992) reported caecal nodules with sarcomatous appearance in a mixed flock of pheasants affected with *H. isolonche*. Caecal lesions in experimentally induced *H. gallinarum* infection in fowl exhibited conical nodules with necrotic centers (Choudhury and Das, 1993). Griner *et al.* (1977) described the gross lesions in the caecum of a blue eared pheasant infected with *H. isolonche*. The lesions were bilateral, irregularly thick and spongy in nature. Present gross caecal lesions in *T. blythii* were most severe as compared to earlier findings.

Histopathologically, the intestinal mucosa including villi structures were completely lost and replaced by multiple granulomatous lesions. Each granuloma was consisted of cross (Fig. 9) or longitudinal sections (Fig.11) of developing parasites with massive fibroblastic proliferation (Fig. 10), surrounded by a zone of cellular reaction of macrophages, lymphocytes, epitheloid cells and giant cells, finally merging in a connective tissue capsule. In certain areas, the dead parasites showed the presence of dystrophic calcification. In others, the granulomas contained remnants of degenerated parasites with necrotic tissues and heterophilic infiltration surrounded by macrophages, epitheloid cells, lymphocytes and giant cells and finally encapsulated by connective tissue capsules (Fig. 12). At places, haemosiderin pigments were also engulfed by macrophages and giant cells. Surrounding the granulomatous lesion there were multiple focal lesions of leiomyoma characterized by spindle-shaped cells with elliptical or vesicular nuclei forming bundles of smooth muscle fibers with development of whorl-like structures. Microscopically, developing stages of *H. isolonche* could be seen at different depths of the caecal wall, however, the lesions did not extend beyond the basement of the epithelium. Halajian *et al.* (2013) recorded *H. isolonche* induced fibrotic granulomatous lesion characterized by mononuclear cell infiltrations and necrosis in the submucosa of caecum in golden pheasants. The cellular involvement and tissue reaction around the cut section of the parasites in the present study were characteristic of chronic granulomatous lesion. The granulomatous response was also seen around dead parasites. Infiltration of heterophils and neutrophils in caecal mucosa of pheasant infected with *H. isolonche* was recorded by Rao (1994). In absence of Van Gieson staining for fibroblastic cells, the routine Meyer's H & E was indicative of profound fibrous tissue proliferations in circumscribed areas. *H. isolonche* infected pheasant's caecal lesions often contained whorl-like pattern formed by pleomorphic spindle shaped cells and absence of cross striations, collagen fibers and elastic fibers led to formation



Fig. 1: A male and Fig. 2: A female Blyth's tragopan in wilderness from Nagaland

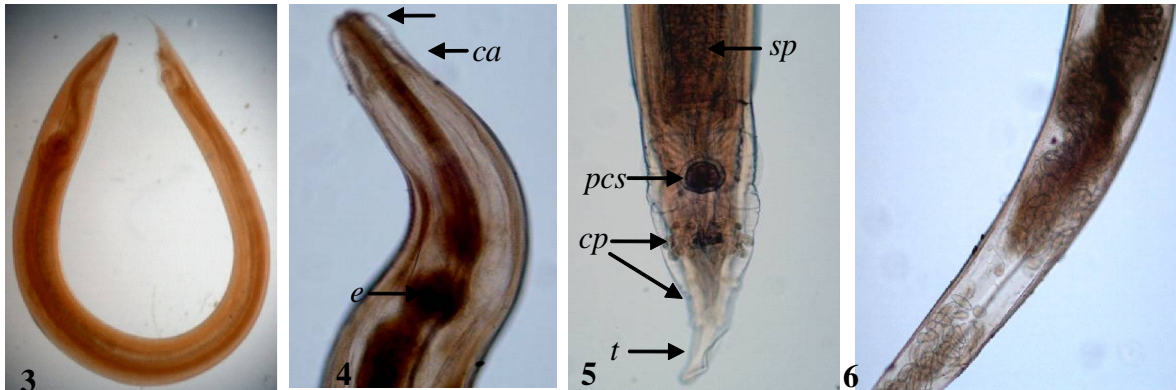


Fig. 3: A male *Heterakis isolonche* recovered from caecal lumen of *T. blythii*; Fig. 4: Anterior end of a male *H. isolonche* showing lip(*l*), narrow cervical alae (*ca*) and bulbous oesophagus (*e*); Fig. 5: Ventral view of caudal end of a male *H. isolonche* showing various structures. *sp*: spicule ; *pcs* : precloacal sucker ; *cp* : caudal papillae ; *t* : elongated tail; Fig. 6: Posterior third of a female *H. isolonche* showing eggs *in utero*

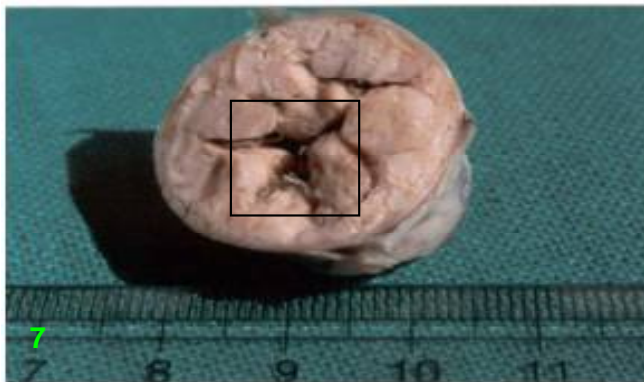


Fig.7: Cross section of a ceca showing thickening of the wall, obliterated lumen and presence of nematode parasites. Scale : Each smallest division is equal to 1 mm.



Fig.8: Inset in Fig. 7 enlarged showing nematodes embedded in the caecal tissues.

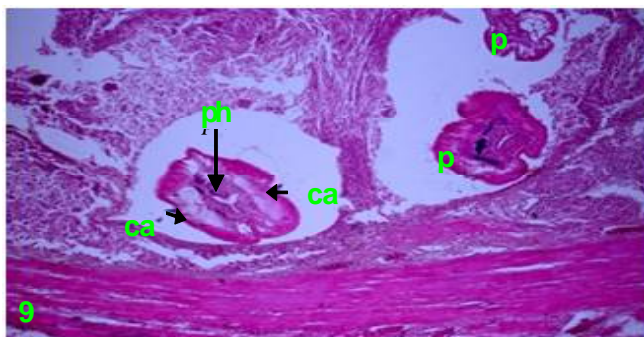


Fig.9: Granulomas containing cross sections of *H. isolonche* at the level of pharynx. *p*: parasite; *ph*: pharynx; *ca* : cervical ala. H&E, 10 X

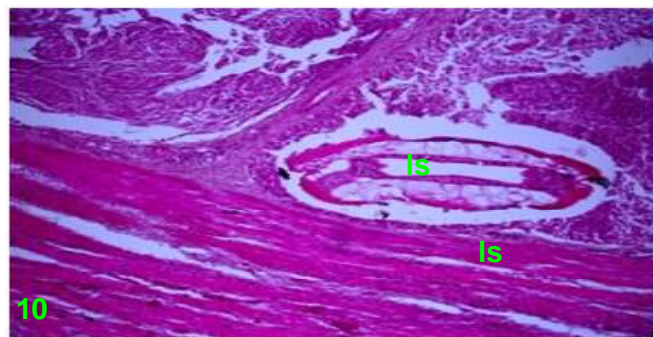


Fig.10: Longitudinal section (*ls*) of a developing *H. isolonche* with massive fibroblastic proliferation, surrounded by a zone of cellular reaction. *p*: parasite. H&E, 10 X.



Fig.11: Longitudinal section of a *H. isolonche* inside a nodule. H&E. 40X

of benign neoplasm of smooth muscle origin or leiomyoma (Balaguer *et al.*, 1992) corroborates the present findings. As reviewed by Griner *et al.* (1977) various authors have attributed the caecal lesions due to heterakiasis as verrucose typhlitis, nodular typhlitis, parasitic neoplasia, parasitic granuloma and leiomyoma. Menezes *et al.* (2003) reported haemosiderosis and granulomas with necrotic centre in the submucosa associated with *H. gallinarum* infection in pheasants. The hemosiderosis in the present study indicates breach of sub-mucosal vessels due to invasion by migration of the developing stages of parasites. In the present context infiltration of inflammatory cells, and granulomatous reaction was evidenced indicating a severe nodular typhlitis which differed from lesions described in experimental heterakiasis (*H. gallinarum*) in domestic chicken (Choudhury and Das, 1993).

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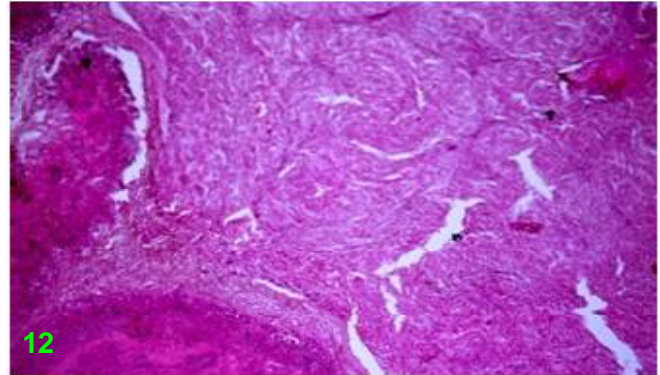


Fig.12: Massive fibroblastic proliferation in the submucosal layer. H&E. 40X

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