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Case Report

CO-INFECTION OF *SARCOCYSTIS* SP. AND *HADJELIA TRUNCATA* IN FANTAIL PIGEONS (*COLUMBA LIVIA DOMESTICA*)

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Summary

Khordadmehr, M., V. R. Ranjbar, P. Shahbazi & M. Zeinoddin, 2018. Co-infection of *Sarcocystis sp.* and *Hadjelia truncata* in fantail pigeons (*Columba livia domestica*). *Bulg. J. Vet. Med.*, **21**, No 1, 115–121.

Hadjelia truncata belongs to the family Habronematidae which affects different groups of birds such as Columbiformes**.** A large number of *Sarcocystis* sp. may infect birds as intermediate hosts, but wild Columbiformes, include pigeons, are rarely affected. The present study describes mixed infection of two pigeon flocks with sarcocystosis and nematodiasis (*H. truncata*) which had neurologic and gastrointestinal clinical signs. The common clinical signs included progressive weight loss, pectoral muscle atrophy, white diarrhoea, depression, torticollis, paralysis, trembling, and 23.4% mortality. At necropsy, a large number of nematodes were detected in the gizzards and diagnosed as *H. truncata* in parasitological studies. For greater certainty, histopathological examination was conducted routinely. Different development stage of this nematode associated with severe inflammatory cells infiltration and necrosis were observed in tissue sections. Accidentally, the large number of *Sarcocystis* cysts was observed in tunica muscularis mucosa of gizzard associated with infiltration of inflammatory cells, hyaline degeneration and necrosis around degenerated cysts.

Key words: gizzard, *Hadjelia truncata,* inflammatory cells, pigeon, sarcocystosis

Pigeons are encountered in all regions of the world except for the poles. They live side by side with humans and other animal species in the nature and they are bred as a source of food as a hobby, symbol and for experimental aims (Harlin, 1994). Pigeons have a role in spreading some zoonoses to people and are a reservoir of many parasitic diseases for poultry (Piasecki, 2006).

Hadjelia truncata belonging to the family Habronematidae and order Spirurida (Anderson, 2000), affects different groups of birds such as Coraciiformes, Columbiformes and rarely Galliformes (Junker & Boomker, 2007). Clinical symptoms such as weight loss, diarrhoea, weakness and death were observed in infected pigeons (Geyhan, 2007; Appleby *et al.*, 1995). The adult parasite resides in the gizzard of the bird and the eggs are

present in the faeces. Various kinds of beetles, mainly *Alphitobius diaperinus*, have been identified as intermediate hosts (Junker & Boomker, 2007). There are several reports of pigeon infestation with this parasite from Egypt (Tadros & Iskander, 1997), Iraq (Al-Attar & Abdul-Aziz, 1985), Cyprus (Appleby *et al.*, 1995) and Iran (Razmi *et al.*, 2007; Radfar *et al.*, 2011; Nabavi *et al.*, 2013).

A large number of *Sarcocystis* spp. (Protozoan; Apicomplexa) may infect birds as intermediate hosts, but wild Columbiformes, which include pigeons, are rarely affected (Ecco *et al.*, 2008; Olias *et al.*, 2009). Among the few species affecting domestic poultry are *S. horvathi* and *S. wenzeli,* which infect chickens, and *S. rileyi*, for which ducks are intermediate hosts (Riley, 1931; Wenzel *et al.*, 1982). *S. falcatula* has been known to cause clinical disease in pigeons only after experimental infection; whether this species is pathogenic under natural conditions is not known (Box & Smith, 1982). In a previous study, *S. falcatula*-like infection have caused death in three free-roaming Victoria crowned pigeons (*Goura victoria*) in the USA (Suedmeyer *et al.*, 2001). An early literature report described an emerging neurologic disease with lethal outcome for domestic pigeons (*Columba livia* f. *domestica*) in Berlin, Germany, caused by a novel *Sarcocystis sp*. (Olias *et al.*, 2009). Moreover, newly characterised non-pathogenic species *S. columbae* have been found in wood pigeons (*Columba palumbus*) in Germany (Olias *et al.*, 2010b). Clinical signs in naturally infected pigeons, similar to those caused by *Paramyxovirus*-1 or *Salmonella* Typhimurium infection, were depression, polyuria, torticollis, opisthotonus, paralysis, trembling, and death. Pigeons had numerous parasitic cysts in their muscles (Olias *et al.*, 2010a).

The present study describes (for the first time) mixed infection of two pigeon flocks (with 94 pigeons) with sarcocystosis and nematodiasis (*Hadjelia truncata*) which had neurologic and gastrointestinal clinical signs.

The owner of two pigeon (*Columbia livia*) flocks with 94 birds (in Yazd Province; central part of Iran) complained from gastrointestinal and neurological disease manifested by progressive weight loss specially with pectoral muscle atrophy, white diarrhoea, vomiting, weakness, depression, torticollis, paralysis, trembling, and 23.4% (22 cases) mortality which occurred 4–6 days after clinical signs. Blind antibiotic therapies were administered to treat the sick birds, but no advantage was observed.

Six new dead bird carcasses were chosen for postmortem inspection. According to the clinical signs, the neurotropic velogenic form of Newcastle disease was suspected at first. For this reason, the viscera and trachea were removed and opened for macroscopic examination. In all cases, the gizzard was larger and softer than normal and distortion was observed. A large number of nematodes was detected beneath the lining of the affected gizzards (Fig. 1). The other organs were macroscopically normal. According to the necropsy, the treatment was performed by levamisole hydrochloride and anhydrous niclosamide which did not appear to be an effective treatment for the nematode.

The worms were collected from the lining of the gizzards and washed by shaking in 0.9% saline and kept in 70% ethanol + 5% glycerin. A number of worms were counted and cleared in lactophenol for identification using a light microscope (Soulsby, 1982). Also, for pathological

examination, tissue blocks from various parts of the gizzards were processed by conventional methods for preparation of paraffin wax sections. The sections were stained with haematoxylin and eosin and studied with light microscope.

Fig. 1. Macroscopic appearance of the large number of nematodes beneath the lining of the affected gizzards.

A large number of nematodes were observed under the koilin layer of the gizzard during necropsy. The mean worm burden was 115 worms per case (30 male and 85 female). The morphology of nematodes was examined by light microscope. The length of male and female were 7–11 mm and 15–20 mm, respectively. Microscopical examination of cephalic region revealed two lateral lips with winged appearance and a cylindrical pharynx (Fig. 2A). Moreover, in the caudal region (Fig. 2B, C), two obvious unequal and dissimilar spicules were observed in the male nematode. The tail was coiled and two wide caudal alae and papillae were present. Pathological study of tissue samples was also done. Different developmental stages of this nematode (egg with length 43–45 µm and width of 20–30 µm, larva and adult) were observed in tissue sections (Fig. 3A, B). The parasite was more prevalent between tunica submucosa and tunica muscularis mucosa of gizzard (Fig. 3C). Severe different inflammatory cells infiltration (including heterophils, eosinophils and lymphocytes) (Fig. 3D) with necrosis was seen in mucosa and submucosa of gizzard.

Accidentally, at pathological study, we observed a large number of microscopic *Sarcocystis* cysts in the tunica muscularis mucosa of gizzard (Fig. 4A). In non-degenerated cysts (Fig. 4B), the cyst wall seemed smooth or slightly wavy by light microscope. Moreover, a heterophil-rich inlammatory cell population associated with hyaline degeneration and necrosis were observed around degenerated cysts (Fig. 4C, D).

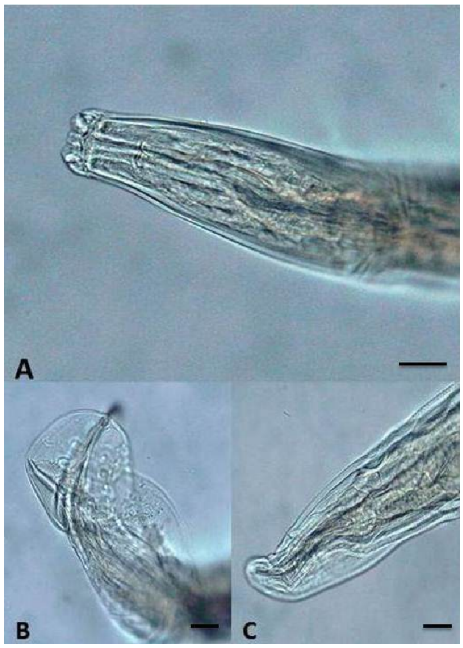

Fig. 2. Anterior and posterior end of *Hadjelia truncata*. In cephalic region, 2 lateral lips with winged appearance were seen (A). In tail region, two obvious unequal spicules with coiled tail are presented in male (B) but not in the tail of female (C) (bar=10 µm).

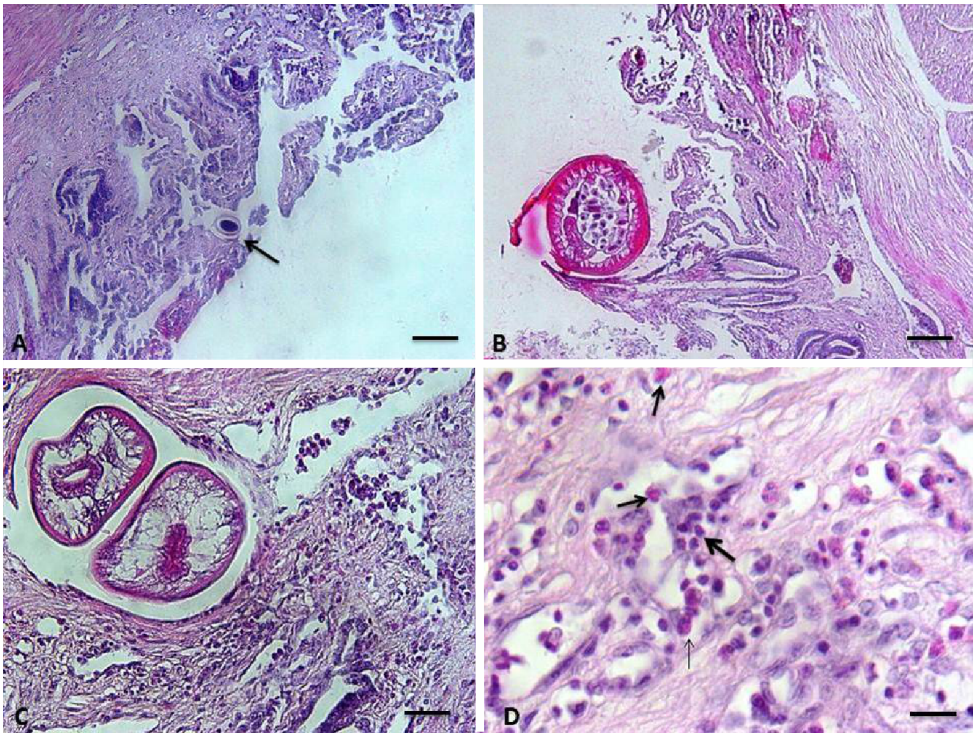

Fig. 3. Gizzard, pigeon, nematodiasis. Different developmental stage of this nematode. A) Egg with length 43–45 µm and width of 20–30 µm; arrow (H&E, bar=300 µm). B) Adult was observed in tissue sections (H&E, bar=300 µm). C) The parasite was predominant between tunica submucosa and tunica muscularis mucosa (H&E, bar=200 µm). D) Inflammatory cells infiltration: heterophil (thin arrow); eosinophil (semithin arrows); lymphocyte (thick arrow) in gizzard mucosa and submucosa (H&E, bar=60 µm).

Both sarcocystosis and nematodiasis (due to *Hadjelia truncata*) are lethal in pigeons (Olias *et al.*, 2010a; Nabavi *et al.*, 2013). *Sarcocystis sp.* and *Hadjelia truncata* in pigeons cause neurological and gastrointestinal symptoms which were seen in the pigeons simultaneously in this study.

In the present study, the nematodes in the gizzard, were identified as *Hadjelia truncata.* Recently, this parasite has been reported from countries such as Cyprus (Appleby *et al.*, 1995) and Iran (Razmi *et al.*, 2007; Radfar *et al.*, 2011; Nabavi *et al.*, 2013). The most common clinical signs reported by these researchers were nonspecific gastrointestinal symptoms and included body weight loss, poor feed consumption, diarrhoea, weakness and increased mortality. Also, diagnosis is based on the necropsy and observation of the nematodes under the koilin layer of the gizzard. Nabavi *et al.* (2013) reported severe clinical signs and 9.58% mortality rate in three pigeon flocks containing 637 birds. In another study from Iran however, slight infection (1.096%) by this parasite has been reported in a survey of parasites

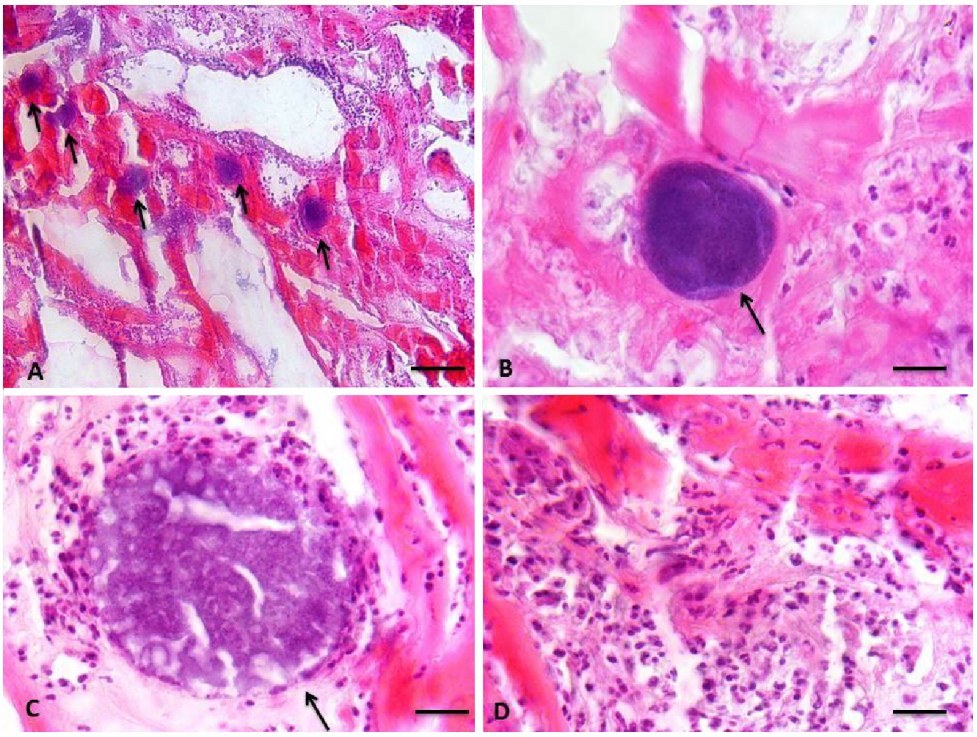

Fig. 4. Gizzard, pigeon, sarcocystosis. A) Large number of microscopic *Sarcocystis* cysts in tunica muscularis mucosa of gizzard (H&E, bar= 200 µm). B) In non-degenerated cysts (H&E, bar= 60 µm), the cyst wall seemed smooth or slightly wavy. C) Heterophil-rich inflammatory cell population around degenerated cysts (H&E, bar=60 µm). D) Hyaline degeneration and necrosis associated with inflammatory cells around degenerated cysts (H&E, bar= 60 µm).

of domestic pigeons (Radfar *et al.*, 2011). It seems that the severity of symptoms seen in affected pigeons of the present study, are more similar to the findings of Nabavi *et al.* (2013). The cephalic and caudal morphology of *Hadjelia truncata* nematode was described by Appleby *et al.* (1995). Moreover, parasitological investigation of the present study, similar to that of Nabavi *et al.* (2013), showed that the collected nematodes were longer than those described by Razmi *et al.* (2007). Probably, this resemblance could be because of the similar weather conditions of these two areas in Iran (Sistan and Yazd Provinces).

At histopathological examination, the nematodes were seen in all parts of the gizzard; mucosal surface, serosal surface, junction of tunica submucosa and tunica muscularis mucosa and even between of muscle fibres of muscularis mucosa. There was an inflammatory response in all places where the nematodes were present. Gross and histopathological findings were similar to those reported by other reserchers (Razmi *et al.*, 2007).

Sarcocystis calchasi is the causative agent of pigeon protozoal encephalitis

(PPE), an emerging neurological disease of the domestic pigeon (*Columba livia f. domestica)* (Olias *et al.*, 2009; Wünschmann *et al.*, 2011). To date, little research has been done in this area. Recently, some researchers reported an emerging neurologic disease with lethal outcome for domestic pigeons (*Columba livia* f. *domestica*) in Berlin, Germany, caused by a novel *Sarcocystis sp.* (Olias *et al.*, 2009). Also, in an experimental study, pigeons infected with high doses of *Sarcocystis sp.* died 7–12 days after infection and showed multifocal severe necrosis with numerous parasitic stages while those infected with lower doses had central nervous signs, which did not develop until 8 weeks after infection and at pathological examination, exhibited marked encephalitis, myositis, and *Sarcocystis* cysts in skeletal muscles (pectoral, gastrocnemius and neck) but not in the brain. The late occurrence of brain lesions and the absence of parasitic stages in the brain suggested an indirect and currently unknown mechanism of encephalitis (Olias *et al.*, 2010a). The results of another recent study suggested an immune evasion strategy of *S. calchasi* during the early phase and a delayed-type hypersensitivity reaction as cause of the extensive cerebral lesions during the late neurological phase of disease. In this study, according to unsuccessful antibiotic therapy, probably due to the use of levamisole and its effect on the nematodes, gastrointestinal symptoms improved, but neurological symptoms still continued and the pigeons with neurological signs died after 2–3 weeks. In the present study, *Sarcocytis* cysts were found in the gizzard accidentally, therefore only the gizzard showed a large number of cysts with severe inflamatory cell response in tunica muscularis mucosa. The results of this part suggest that the muscles of the gizzard could be a convenient place for future research of *Sarcocystis* sp. in the pigeons which has not been reported before.

The present study described a mixed infection of pigeon with sarcocystosis and nematodiasis (*Hadjelia truncata*) both of which lethal for pigeons, with neurologic and gastrointestinal clinical signs. Because of the severe disorders caused by these two parasites and little knowledge about them in pigeons, especially on *Sarcocystis* sp., more research in these fields is needed.

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