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Case report

A CASE OF NODULAR ENTERITIS DUE TO *CHAUNOCEPHALUS FEROX* IN A WHITE STORK (*CICONIA CICONIA*) IN BULGARIA

P. T. ILIEV[1], K. DIMITROV[2] & A. IVANOV[1]

[1]Department of Veterinary Microbiology, Infectious and Parasitic Diseases;
[2]Department of General and Clinical Pathology, Faculty of Veterinary Medicine,
Trakia University, Stara Zagora, Bulgaria

Summary

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The present report describes a case of chaunocephalosis due to *Chaunocephalus ferox* in a white stork (*Ciconia ciconia*) in Bulgaria. On July 2017, a malnourished and debilitated adult white stork was found in a field near Stara Zagora city. The bird was caught and transported to the Wildlife Rescue Center of Green Balkans organisation, with history of weakness, severe watery diarrhoea, loss of appetite, and cachexia. The stork died several hours after admission. The corpse was referred to the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University where post-mortem examination was performed. The necropsy revealed the presence of multifocal to coalescing mural intestinal nodular circumscribed lesions affecting all portions of the intestinal tract, most prominent and numerous in the jejuno-ileal segment. When sectioned, the nodules contained either single, or 2 to 3 small flattened tadpole-like trematodes which were identified as *Ch. ferox*. Histologically, a total traumatic destruction with ulceration and necroses of intestinal tissue layers were found at the site of fluke penetration. The trematodes also formed a marked granulomatous inflammatory reaction with inflammatory cellular infiltrate, consisting mainly of heterophilic and pseudoeosinophilic leukocytes, lymphocytes and histiocytes. In the area of infection, the intestinal villi and mucosa were totally eroded, and the surrounding mucosal crypts were distended by necrotic detritus.

Key words: Bulgaria, *Chaunocephalus ferox*, *Ciconia ciconia*, white stork

The white stork (*Ciconia ciconia*) is a large migratory bird, nesting and breeding in most of Europe as well as North Africa, the Middle East and Central Asia. The birds fly from their summer breeding areas in Europe in late August and September, heading for Africa, while the reverse migration occurs usually in late March and April (Johst *et al*., 2001). To avoid crossing over the Mediterranean Sea, the birds from Europe follow either the Eastern migration route by crossing the Bosphorus in Turkey, or follow the Western route over the Strait of Gibraltar (Leshem

& Yom-Tov, 1998). Most of the European stork population undertakes migration following the Eastern migration corridor which passes over the Bulgarian Black sea coast.

The white stork has cosmopolitan distribution in Europe with highest occurrence in Poland (51,700–53,900 pairs; 23%), Spain (33,217 pairs; 14%), Ukraine (26,200–32,400 pairs; 12%), Belarus (21,300–21,500 pairs; 9%) and Lithuania (19,500–20,500 pairs; 9%) (Anonymous, 2015). The territory of Bulgaria also represents an important site for breeding storks in the spring and summer. In the last few decades, there has been a decline in the population of white storks in nesting sites. A total of 11,000 breeding pairs were found on the territory of Bulgaria in the period from 1961 to 1963, while in 2004–2005 their number dropped to 4818 pairs (Petrov *et al*., 2007). Therefore, this bird is considered as a vulnerable species, under the protection of the Hunting Act over the whole territory of the country. Several reasons are considered responsible for decreasing stork population density, e.g. habitat modification due to extensive human activities (reduction of foraging areas and excessive use of pesticides); collision and electrocution from overhead power lines; burning of the nests; strangling by strings brought to the nest; ventricular obstruction due to ingestion of rubber as well as adult mortality in wintering areas (Höfle *et al*., 2003; Santoro *et al*., 2013). In this regard, distressed and sick birds in Bulgaria are temporarily kept and treated in Green Balkans – a leading organisation in the field of conservation of rare species and habitats in the country.

Being a carnivore, the white stork consumes a wide range of animal pray, including insects, fish, earthworms, small mammals, reptiles, amphibians, molluscs and aquatic vertebrates (Girisgin *et al*., 2017). All of these animal species may serve as intermediate hosts of numerous parasites affecting storks as definitive hosts. In this regard, the trematode *Chaunocephalus ferox* (Digenea: Echinostomatidae) is recognised as one of the most common helminths in ciconiiform birds, including the white stork (*C. ciconia*), black stork (*Ciconia nigra*), black-necked stork (*Xenorhynchus asiaticus*), and Asian open-billed stork (*Anastomus oscitans*) (Höfle *et al*., 2003). The life cycle of *Ch. ferox* requires two intermediate hosts, including water snails as first and amphibians and fish as second intermediate hosts (Höfle *et al*., 2003). The storks become infected following ingestion of frogs and fish carrying the infective larval stages – metacercariae. The larvae penetrate in the small intestinal mucosa where they mature and induce granulomatous nodules on the intestinal wall. Infected birds demonstrate non-specific clinical signs, e.g. weakness, diarrhea, loss of appetite and cachexia before dying (Choe *et al*., 2016). Chaunocephalosis due to *Ch. ferox* was first described as nodular disease (Patnaik *et al*., 1970), which usually lead to severe intestinal alterations, serious health disorders and high mortality, especially in heavily infected or young birds (Choe *et al*., 2016).

The present paper aims to describe a case of massive infection by the digenean trematode *Ch. ferox* in a white stork in Bulgaria.

Case presentation

On July 2017, a malnourished and debilitated adult white stork was found in a field near Stara Zagora city. The bird was easily caught and transported to the Wildlife Rescue Center of Green Balkans organiza-

tion, where it died several hours after admission. The corpse was then referred to the Department of General and Clinical Pathology, Faculty of Veterinary Medicine, Trakia University, where postmortem examination was performed to determine the cause of the death. The volunteers of the Rescue Center recalled us that the bird was found injured and helpless, demonstrating weakness, severe watery diarrhoea, loss of appetite and cachexia.

During the necropsy, the whole gastrointestinal tract was examined for presence of parasites and gross pathological alterations. The helminths recovered were washed several times with tap water before further preparation, including fixation in 70% ethanol, staining with haematoxyline, dehydration in a series of ascending concentrations of ethanol, clearing in xylene, and mounting on slides in Canada balsam (Koinarski *at al*., 2014). Identification of the parasites was based on the morphological features according to Kostadinova (2005). Intestinal content was also examined for parasitic eggs, cysts and larvae by routine flotation with sodium chloride (sp. gr. 1.20), sedimentation with tap water, and larvaescopy by simplified Baermann technique (Koinarski *et al*., 2014). For histopathological examination, the infected parts of gastrointestinal tract were fixed in 10% neutral buffered formalin (48–72 h) and embedded in paraffin. Cross sections (5 μm thick) of the paraffin blocks were cut on a Leica RM 2235 microtome and conventionally stained with haematoxylin-eosin (H/E).

Necropsy revealed the presence of multifocal to coalescing mural intestinal nodular circumscribed lesions affecting all portions of the intestinal tract, most prominent and numerous in the jejunoileal segment (Fig. 1). Intestinal nodules appeared yellowish-brown to grayish-white, ranging in size from 0.2 cm to 0.5 cm in diameter. When incised, the intestines were almost empty and the remaining scant amount of intestinal content appeared watery to viscous. Intestinal mucosa showed many prominent roundish areas corresponding to the nodules with brownish-yellow to brownish-red discoloration with central ulceration and haemorrhages. When sectioned, these nodules contained either single, or 2 to 3 small flattened tadpole-like trematodes (Fig. 2). Recovered flukes were creamy white to pinkish in color, slightly protruding with their posterior narrow end in the intestinal lumen. Some of the nodules had thicker consistence and contained mainly necrotic detritus.

Fig. 1. Multifocal diffuse mural intestinal lesions due to *Ch. ferox* infection in a white stork (*C. ciconia*).

A total of 23 worms were subjected to morphological identification. The body of worms was divided into a bulbous anterior part and a narrow subcylindrical posterior part. The total body length varied between 4.0 and 7.2 mm (mean length 5.27±0.74 mm). The well-developed head collar was armed with 27 collar spines arranged in 4 pairs of large angle spines, 4 pairs of

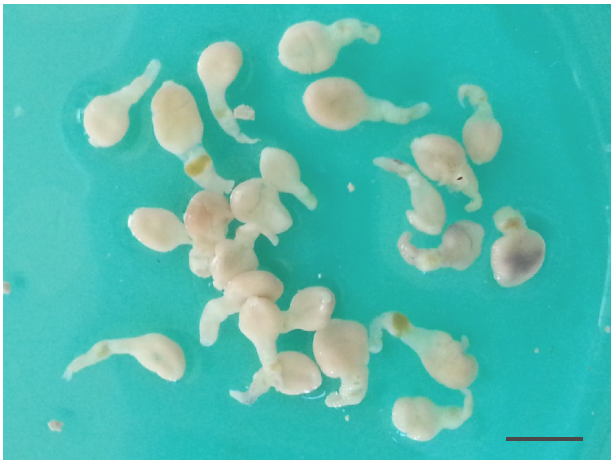

Fig. 2. Specimens of *Ch. ferox* (Bar=5 mm).

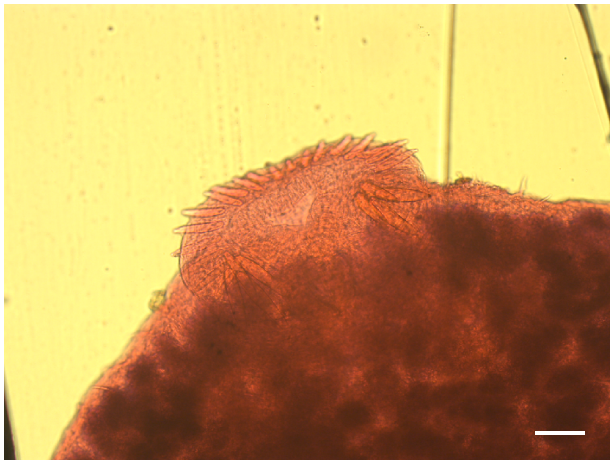

Fig. 3. Head collar of *Ch. ferox* (Bar=200 μm).

smaller lateral spines, and 11 dorsal spines (Fig. 3). The oral sucker was spherical, well-developed and smaller than the ventral sucker. Vitellaria were well-developed, occupying the whole anterior part of the body.

The eggs were oval, yellowish-brown, operculated, undeveloped and measured 86.5–99.6 by 51.6–57.7 μm (Fig. 4). The morphology of the parasites and eggs generally agreed with the descriptions of the digenean trematode *Ch. ferox* by Kostadinova (2005) and Greben *et al*. (2016).

Histology revealed that there was a total traumatic destruction with ulceration and necroses of intestinal tissue layers at the site of fluke penetration. The mucosa, submucosa and muscle layers were severely distended by the parasites. Also, trematodes formed a marked granulomatous inflammatory reaction with inflammatory cellular infiltrate, consisting main-

ly of heterophilic and pseudoeosinophilic leukocytes, lymphocytes and histiocytes. In the area of infection, the intestinal villi and mucosa were totally eroded, and the surrounding mucosal crypts were distended by necrotic detritus (Fig. 5).

Fig. 4. Egg of *Ch. ferox* (Bar=30 μm).

Necrotic processes were most prominent in the areas surrounding the flukes. Older nodules consisted mainly of necrotic cellular detritus, segments of the fluke cuticle, degenerated heterophils, abundance of macrophages (especially closer to necroses) presented by a rim of giant multinucleated foreign-body type macrophages, and followed again by mixed infiltration of heterophils, eosinophils, lymphocytes and prominent perilesional fibrosis (Fig. 6).

Ch. ferox is one of the most common parasites in storks. The wide distribution of the fluke may be due to several reasons, including numerous different intermediate hosts, formation of large flocks of young and adult storks before migration, nesting in different countries and continents twice a year, etc. In this regard, the external development of *Ch. ferox* requires water snails acting as first intermediate hosts and amphibians and fish as second intermediate hosts (Höfle *et al*., 2003). As a carnivore, the diet of the white storks mainly includes snails, amphibians and fish, in which the parthenogenesis (cercariae formation) and encystation (infec-

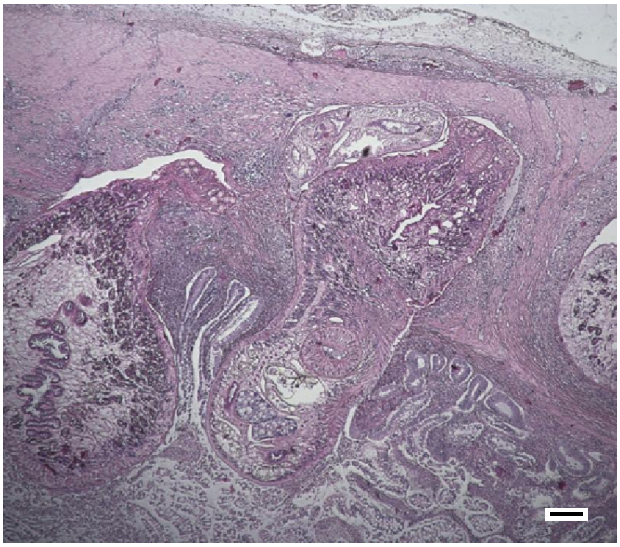

Fig. 5. Cross section of multiple nodular lesions containing adult *Ch. ferox* parasites in the intestine of white stork, H&E (Bar=100 μm).

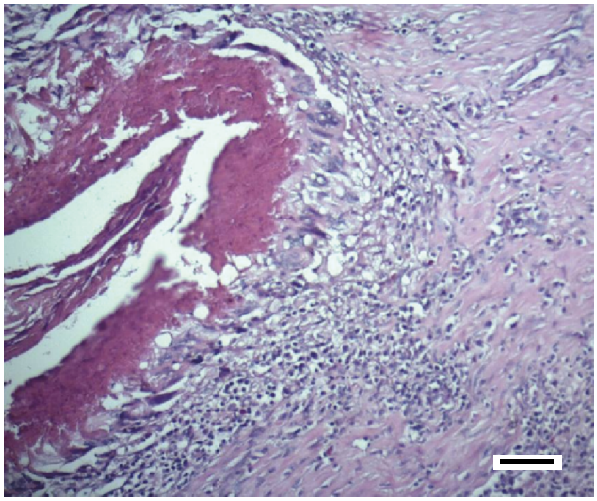

Fig. 6. Presence of characteristic trematode cuticle among necrotic detritus surrounded by prominent granulomatous inflammation. Older intestinal parasitic nodule, white stork, H&E (Bar=50 μm).

tive metacercariae formation) take place (Santoro *et al*., 2013). The wide distribution of these intermediate hosts, as well as their close ecological relationship with storks favour completion of the life cycle of *Ch. ferox* and increase the probability of transmission of the fluke to the offspring (Höfle et al., 2003). In Europe, the fluke was reported in Germany (Schuster *et al*., 2002), Turkey (Girisgin *et al*., 2017), Poland (Michalczyk *et al*., 2020), Italy (Santoro *et al*., 2013), Spain (Höfle *et al*., 2003), Hungary (Murai *et al*., 1986) and Ukraine (Greben *et al*., 2016). In the Czech Republic, *Ch. ferox* has been considered as a dominant species with relative prevalence of 34% in the white stork and 71% in the black stork (Sitko & Heneberg 2015). Apart from aforementioned distribution, *Ch. ferox* was also reported in Austria, Belarus, Britain, Bulgaria, Moldova, Slovakia, Sweden and Switzerland (de Jong *et al*., 2014).

Ch. ferox may severely affect the health of the infected bird, causing nodular enteritis, accompanied with diarrhoea, weakness, loss of appetite, and cachexia. According to Poonswad *et al*. (1992), the pathogenicity of *Ch. ferox* depends on the number of flukes and nodules present in the intestines of birds. Heavily infected or younger storks appeared debilitated and cachectic as a result from malnutrition due to an inability of the intestinal mucosa to absorb nutrients from food. Additionally, damaged mucosa may favour the infection with other pathogens such as *Salmonella* spp., as seen in 2 storks in a study of Höfle *et al*. (2003). Generally, infections with highly pathogenic helminths may also predispose to secondary mechanical injuries during hunting as the flight ability and therefore predatory effectiveness of birds are impaired (Santoro *et al*., 2013). Other researchers reported 4 cases of easily caught Asian open-billed storks, heavily infected with *Ch. ferox* (Poonswad *et al*., 1992).

An interesting theory concerning the appearance of intestinal nodules due to *Ch. ferox* and the gizzard impaction in a sick Oriental white stork was published

(Choe *et al*., 2016). According to the authors, these intestinal lesions might act as a risk factor for intestinal obstruction, leading to gastric impaction. Also, they concluded that *Ch. ferox* may kill its host by inducing gastric impaction, even if the intensity of infection is low, represented only by 7 nodules containing 9 flukes. In addition, larger nodules and adult flukes may cause more severe disease than smaller granulomas and juvenile flukes respectively (Poonswad *et al*., 1992). However, the infection with *Ch. ferox* in nestlings of white storks may also be responsible in some cases of parental infanticide ("throwing out of the nest") behaviour of the adult storks (Höfle *et al*., 2003). In this connection, the number of confirmed victims in a study by Höfle *et al*. (2003) has been low (3 cases) to confirm this hypothesis.

With regard to histological findings of stork chaunocephalosis, several researchers reported similar changes at the site of the fluke penetration, including traumatic destruction of tissues with haemorrhages; increased thickness of mucosa, submucosa and muscle layer; heavy mixed inflammatory cell infiltration composed of heterophils, lymphocytes and histiocytes in *lamina propria* and *tunica muscularis*; necrosis of the muscle layer around the parasite; accumulation of necrotic material, degenerate heterophils and cellular debris in older nodules; loss of intestinal villi and necrosis; enlargement of intestinal crypts; fibrosis of the nodular capsules and smooth muscles of *tunica muscularis* (Patnaik *et al*., 1970; Poonswad *et al*., 1992; Höfle *et al*., 2003; Santoro *et al*., 2013). These findings are in general agreement with the results of current examination. Also, the presence of giant multinucleated foreign body type macrophages, heterophils, eosinophils, lymphocytes and perilesional fibrosis in our observations indicated a long-term immunological interaction between *Ch. ferox* and its host.

In conclusion, the present case report provides additional data on the distribution of *Ch. ferox* in white storks in Bulgaria. Also, such studies are rare in the country and may be carried out only on sick or dead birds admitted to rescue centres or veterinary clinics.

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Correspondence:

Petar T. Iliev
Department of Veterinary Microbiology, Infectious and Parasitic Diseases,
Faculty of Veterinary Medicine,
Trakia University,
6000 Stara Zagora, Bulgaria
tel: +359 42 699 587
e-mail: petyo_todorow@abv.bg

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