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Acute Fatal Toxoplasmosis in a Great Spotted Woodpecker (*Dendrocopos major*)

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ABSTRACT: A juvenile male Great Spotted Woodpecker (*Dendrocopos major*), found dead at a bird feeding station in central Norway in September 2011, was examined postmortem. Its lungs were consolidated and edematous, and its spleen was enlarged. The main histopathologic diagnoses included acute protozoal necrotizing interstitial pneumonia, splenitis, and hepatitis. *Toxoplasma gondii* parasites were identified with immunohistochemistry in all examined organs: lung, heart, liver, kidney, and spleen. Direct multilocus genotyping of the parasites revealed that the woodpecker was killed by a *T. gondii* strain belonging to genotype II. This is the first report of naturally acquired fatal generalized toxoplasmosis in a *Dendrocopos* species.

Key words: *Dendrocopos major*, genotyping, Piciformes, *Toxoplasma gondii*, wild bird, woodpecker.

The zoonotic protozoan *Toxoplasma gondii* infects warm-blooded animals, including birds. The infections are common in Falconiformes, Strigiformes, Columbiformes, Anseriformes, Passeriformes, and Galliformes, and clinical toxoplasmosis has been reported mainly in pigeons (Columbidae) and Island Canaries (*Serinus canaria*; Dubey 2002, 2010). Among woodpeckers (Picinae), there is one previous report of naturally acquired clinical toxoplasmosis in a Red-bellied Woodpecker (*Melanerpes carolinus*; Gerhold and Yabsley 2007). We describe naturally acquired acute fatal toxoplasmosis in a Great Spotted Woodpecker (*Dendrocopos major*).

The woodpecker, found dead at a bird feeding station in central Norway (63°21′N, 10°21′E) in September 2011, was submitted to the Norwegian Veterinary Institute for necropsy. Tissue samples of lung, heart, liver, kidney, and spleen were fixed in 10% buffered formalin and embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin and Gram stains for histologic examination. Standard bacteriologic examination on calf blood agar plates, incubated aerobically at 37 C for 24–48 hr, was performed on samples from lung and liver. Virologic examinations of frozen lung and liver samples included real-time reverse-transcription PCR for detecting avian paramyxovirus type 1, type A influenza virus, and gammacoronaviruses (Spackman et al. 2002; Fuller et al. 2010; Jones et al. 2011). The method for influenza virus was modified: The reaction volume was 25 μL and PCR conditions were 50 C for 30 min and 95 C for 15 min, followed by 40 cycles of 95 C for 10 sec and 60 C for 20 sec.

Immunohistochemic staining with specific anti–*T. gondii* antibodies was performed on all tissue samples to confirm toxoplasmosis and to evaluate the burden and spread of the parasites. The staining was performed according to Conley et al. (1981) with *Toxoplasma gondii* Ab-1 Rabbit Polyclonal Antibody (Thermo Fisher Scientific, Runcorn, United Kingdom) as the primary antibody, and repeated in parallel according to Jokelainen et al. (2011) with *Toxoplasma gondii* Epitope-Specific Rabbit Antibody (Thermo Fisher Scientific). DNA was extracted from the formalin-fixed, paraffin-embedded tissue samples for direct genetic multilocus characterization of the causative parasite strain (Jokelainen et al. 2011). DNA was extracted from frozen, cultivated *T. gondii* tachyzoites as positive control and from nuclease-free water as negative control. Genotyping was based on an analysis of length polymorphism at six microsatellite markers (B18, TUB2, TgM-A, W35, B17, and M33); a seventh marker (fingerprinting marker M48) was included for further

characterization (Blackston et al. 2001; Ajzenberg et al. 2005; Jokelainen et al. 2011). DNA from *T. gondii* strains belonging to genotypes I, II, and III served as references and positive controls, and DNA from *Neospora caninum* and nuclease-free water served as negative controls.

The woodpecker was a juvenile, male, weighing 80 g, and in slightly below normal body condition. The main gross findings were splenomegaly and dark, consolidated, edematous lungs. The lung tissue samples sank in formalin. Internal organs were congested, and the gastrointestinal tract was moderately filled with normal contents. The routine necropsy of a woodpecker without a history of neurologic signs did not include examination of the central nervous system. Toxoplasmosis was not suspected at necropsy.

Histopathologic examination revealed severe diffuse interstitial pneumonia with thickened alveolar septa, and macrophages and fibrinous exudate in the alveoli. Scattered foci of necrosis involved the alveolar septa, bronchiolar epithelial cells, and blood vessels. A marked perivascular edema infiltrated with macrophages was evident, as were tachyzoite-like structures in macrophages, bronchiolar epithelial cells, and the blood vessel walls. Irregular foci of coagulative necrosis were scattered throughout the spleen and liver tissues and associated with mild inflammatory reaction and tachyzoite-like structures, particularly along the periphery of the lesions. A few small necrotic foci with mild infiltration of mainly mononuclear inflammatory cells were detected in the myocardium. A mild to moderate multifocal perivascular infiltration dominated by mononuclear cells and scattered tachyzoite-like structures was present in the kidney. Scattered to multifocal mild accumulation of large, mainly Gram-positive, rod-shaped bacteria in the liver, kidney, and lung were unassociated with inflammatory reaction and were interpreted as a postmortem invasion. *Escherichia coli* were isolated from the liver, whereas no pathogenic bacteria were cultivated from the lung. No findings indicating a viral disease were detected and lung and liver samples were negative for the examined viruses.

Immunohistochemistry for *T. gondii* demonstrated large to massive numbers of positively stained tachyzoites in lung, spleen, and liver tissue. The parasites were present in the necrotic areas and intracellularly in various cells, including bronchiolar epithelial cells, hepatocytes, and phagocytic cells, as well as in vessel walls. Immunopositive tachyzoites were less numerous in the kidney and myocardium. The number of parasites in the tissues would have been underestimated without immunohistochemistry.

Genotyping of the causative parasite strain was partly or fully successful from samples of the liver, spleen, kidney, and heart. The results at the genotyping markers were all consistent with *T. gondii* genotype II. The height of the peak obtained at marker M33 was consistently low in the samples, but not in the positive controls. The length of the PCR product at M48 was 223 base pairs, and there were no signs of a mixed infection with more than one strain.

We conclude that the woodpecker died from acute, generalized toxoplasmosis caused by a *T. gondii* strain of genotype II. This is the first report of naturally acquired fatal toxoplasmosis in a *Dendrocopos* species.

Clinical toxoplasmosis has been diagnosed previously in a woodpecker from the genus *Melanerpes* (Gerhold and Yabsley 2007). It was lethargic and suffered intermittent seizures. Severe meningoencephalitis was diagnosed, but no gross lesions were observed and no information was available on whether histopathologic lesions were detected in other organs. Because we did not examine the brain of the woodpecker we describe, whether it was also affected is unknown.

Toxoplasma gondii has been isolated by bioassay from trapped, presumably healthy appearing Himalayan Woodpeckers (*Dendrocopos himalayensis*) and a

Brown-fronted Woodpecker (*Dendrocopos auriceps*; Pande et al. 1961). The isolated strain was inoculated into another two Himalayan Woodpeckers and one Brown-fronted Woodpecker, all of which died within 3 days. *Toxoplasma gondii* parasites were detected in impression smears of their brain, spleen, liver, and lungs; the disease following the experimental infection was generalized, acute, and fatal. Differences between the original naturally acquired, mild infections and these rapidly fatal experimental infections might include different route of infection and infection dose, changed stage and virulence of the parasites, and concurrent diseases and captivity-related stress.

The parasites that proved fatal to the *Dendrocopos* woodpeckers upon experimental infection were highly virulent in mice (Pande et al. 1961). Such virulence in mice is considered typical of *T. gondii* strains of genotype I (Maubon et al. 2008). In contrast, the causative *T. gondii* strain of the fatal case described herein belonged to genotype II, and genotype II strains are typically nonvirulent in mice (Maubon et al. 2008). However, genotype II strains caused fatal infections in Europe in several other host species as well (Prestrud et al. 2008; Jokelainen et al. 2011; Spycher et al. 2011; Jokelainen et al. 2012; Jokelainen and Nylund 2012; Herrmann et al. 2013), including domestic cats (*Felis catus*), European brown hares (*Lepus europaeus*), mountain hares (*L. timidus*), arctic foxes (*Vulpes lagopus*), Eurasian red squirrels (*Sciurus vulgaris*), and a European beaver (*Castor fiber*). The *T. gondii* strain from the Red-bellied Woodpecker was classified as genotype III based on an analysis of PCR products of 5′ and 3′ ends of SAG2 (Gerhold and Yabsley 2007), but a result from an analysis with few markers should be interpreted cautiously.

The immune status of the host affects the outcome of *T. gondii* infection (Maubon et al. 2008). No signs of immunosuppression were evident and no other concurrent, possibly predisposing diseases were diagnosed in this woodpecker, yet the course of the generalized toxoplasmosis appeared acute.

The diet of woodpeckers consists mainly of insects plucked from trees, but many species are omnivorous and opportunistic. The Great Spotted Woodpecker eats insects, seeds from pine and spruce, and occasionally bird eggs, chicks, and small rodents. The species is a common guest on Norwegian bird feeding stations and appears to prefer nuts and suet. The woodpecker described here was found dead in a suburban area; the source of its infection may have been *T. gondii* oocysts shed by domestic cats frequenting the area. Ground feeding of spilled seeds and suet-balls contaminated with oocysts from the feet of previous visitors provide opportunities for ingestion of oocysts. Additionally, insects may mechanically transport *T. gondii* and be another possible route of infection (Gerhold and Yabsley 2007). Another possibility is that the woodpecker ingested tissues of another *T. gondii* host harboring the parasite.

The role of woodpeckers in the epidemiology of *T. gondii* is limited. On a bird feeding station, woodpeckers may become infected and contribute to the spread of oocyst contamination. An infected woodpecker may itself be a source of infection if eaten by another host. Preventing feline fecal contamination of feeding stations is relevant to protect their visitors from encountering *T. gondii*.

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