DOI: 10.7589/JWD-D-20-00184

Journal of Wildlife Diseases, 57(3), 2021, pp. 712–714

Encephalitis Associated with *Sarcocystis halieti* Infection in a Free-ranging Little Owl (*Athene noctua*)

Kristina Maier-Sam,[1,5] Taina Kaiponen,[2] Anna Schmitz,[3] Christoph Schulze,[4] Sabine Bock,[4] Andreas Hlinak,[4] and Philipp Olias[2] [1]Clinic for Birds, Reptiles, Amphibians and Fish, Justus Liebig University Giessen, Frankfurter Strasse 114, 35392 Giessen, Germany; [2]Institute of Animal Pathology, University of Bern, Länggassstrasse 122, 3012 Bern, Switzerland; [3]Institute for Poultry Diseases, Faculty of Veterinary Medicine, Freie Universität Berlin, Königsweg 63, 14163 Berlin, Germany; [4]Berlin-Brandenburg State Laboratory, Gerhard-Neumann-Str. 2, 15236 Frankfurt (Oder), Germany; [5]Corresponding author (e-mail: kristina.maier@vetmed.uni-giessen.de)

ABSTRACT: A juvenile Little Owl (*Athene noctua*) was diagnosed with granulomatous encephalitis and muscular sarcocysts. *Sarcocystis halieti* was identified in the brain and muscle tissue by PCR and subsequent sequencing. This is the first report of *S. halieti* as a potential encephalitis-causing pathogen in birds.

Apicomplexan parasites of the genus *Sarcocystis* are characterized by an obligate two-host life cycle. In the definitive host, sexual multiplication takes place in the intestines, whereas asexual replication and formation of sarcocysts occurs in the intermediate host (Mehlhorn and Heydorn 1978). More than 25 *Sarcocystis* spp. using birds as intermediate hosts have been described (Dubey et al. 2016) with some of them (e.g., *Sarcocystis falcatula*, *Sarcocystis calchasi*) being highly pathogenic for their intermediate hosts (Godoy et al. 2009; Olias et al. 2009). Sarcocysts of *Sarcocystis halieti* were first reported in 2012, in skeletal muscles of hunted Great Cormorants (*Phalacrocorax carbo*) in Lithuania (Prakas et al. 2018). As definitive hosts of *S. halieti*, the Eurasian Sparrowhawk (*Accipiter nisus*) and the White-tailed Sea Eagle (*Haliaeetus albicilla*) were detected (Mayr et al. 2016; Gjerde et al. 2018). Recently, Herring Gulls (*Larus argentatus*) were identified as another intermediate host (Prakas et al. 2020). Because neither of the known intermediate host species belongs to the natural prey of sparrowhawks, a large host spectrum of *S. halieti* is suspected (Mayr et al. 2016). So far, *S. halieti* has not been associated with clinical disease in its hosts (Prakas et al. 2018, 2020).

A free-ranging Little Owl (*Athene noctua*) nestling was found dead next to its nest site near Michendorf (52°17′03.7″N, 13°01′43.5″E), Germany, in June 2018. Three days earlier the nestling had been banded by professional bird ringers and appeared healthy. At necropsy, the bird was in a good body condition. Cause of death was a blunt thoracic trauma. Except for traumatic lesions, the inner organs were without further macroscopic lesions. A few adult *Syngamus trachea* were present within the tracheal lumen. Histopathologic examination of H&E-stained sections of the pectoral muscle revealed multiple slender, thin-walled sarcocysts containing lancet-shaped bradyzoites (Fig. 1A). In the brain, a severe multifocal, granulomatous encephalitis was found (Fig. 1B). Organ samples, including the brain, tested negative for *Chlamydia* spp., herpesviruses, avian avulavirus serotype 1 (avian paramyxovirus serotype 1), influenza A viruses, West Nile virus, and Usutu virus by PCR and virus culture using standard protocols approved by the German veterinarian National Reference Laboratories (Friedrich-Loeffler-Institut 2019). With DNA extracted from the pectoral muscle and brain, a *Sarcocystis*-specific PCR, amplifying the complete D2 region of the 28S rRNA was performed as previously described (Wünschmann et al. 2011). Subsequent agarose gel electrophoresis of the PCR products revealed amplicons of the same size as the positive controls (*S. calchasi* and *S. halieti*) in samples from both brain and muscle. Sequence analysis of both amplicons showed 100% sequence identity with the previously published sequence of the *S. halieti* Dkorm16 isolate (GenBank accession no. MH130210). The closest related species were *Sarcocystis columbae* and *Sarcocystis corvusi*, with 99.05% and 98.1% sequence identity, respec-

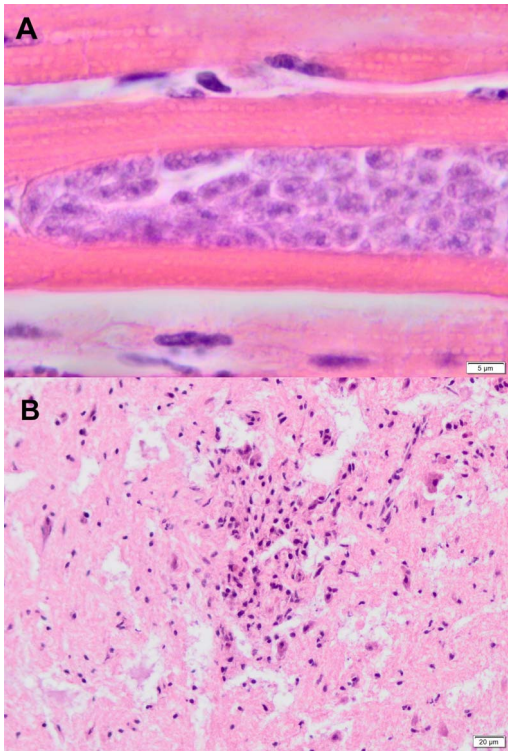

FIGURE 1. Histopathologic photomicrographs of tissues from a juvenile Little Owl (*Athene noctua*), found dead near Michendorf, Germany, in June 2018, which tested positive for *Sarcocystis halieti* by PCR. (A) Pectoral muscle with a section of thin-walled sarcocyst containing multiple lancet-shaped bradyzoites. (B) Cerebrum showing severe, multifocal granulomatous encephalitis. Tissue artifacts are due to previous freezing of the carcass. H&E stain.

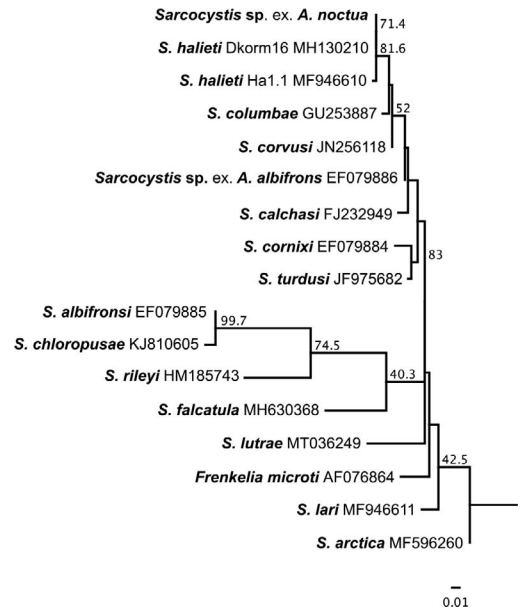

FIGURE 2. Phylogenetic tree rooted on *Besnoitia besnoiti* (AY833646; not shown) and based on the maximum-likelihood method (PhyML 3.3 under the HKY85 substitution model [Guindon et al. 2010]). Posterior clade probabilities are shown. The amplified 316-base pair of the D2 region of the 28S rRNA gene of a *Sarcocystis* sp. identified in the brain and pectoral muscle of a Little Owl (*Athene noctua*), found dead near Michendorf, Germany, in June 2018, is identical to the sequence of the Dkorm16 isolate of *Sarcocystis halieti* and clusters with other avian *Sarcocystis* spp. transmitted by raptors.

tively. In a phylogenetic analysis, the sequence from the Little Owl clustered with two 28S rRNA sequences of *S. halieti* within a basal clade comprising *Sarcocystis* spp. using birds as intermediate hosts and mostly raptors of the family Accipitridae as definitive hosts (Fig. 2).

Muscular sarcocysts of *S. halieti* have been reported from Great Cormorants and Herring Gulls (Prakas et al. 2018, 2020), but not previously, to our knowledge, from an owl. Because oocysts of *S. halieti* have been detected in Eurasian Sparrowhawks (a raptor preying on small-sized birds), a larger intermediate host spectrum of the parasite had previously been suspected (Mayr et al. 2016). Although not frequently preyed on, Little Owls belong to the prey spectrum of Eurasian Sparrowhawks (Brüll 1984). Therefore, small owl species may function as intermediate host in the natural life cycle of *S. halieti*. The Great Horned Owl (*Bubo virginianus*) is the only other bird of the order Strigiformes from which a *Sarcocystis* spp. infection with associated encephalitis has been reported. The generated sequence of the internal transcribed spacer 1 region from the brain of the diseased Great Horned Owl showed 99% sequence similarity with *S. falcatula*. Because only encephalitis-associated schizonts and no muscular stages of the parasite were demonstrated, it remains to be shown whether owls serve as true intermediate or only aberrant hosts for *S. falcatula*. The Little Owl showed multiple muscular sarcocysts but no encephalitis-associated pathogens by light microsco-

py, although both muscle and brain tested positive for *S. halieti* by PCR. This is not unexpected, because the closely related *S. calchasi* has frequently been reported to cause severe encephalitis without or with only rare occurrence of schizonts in the affected brain regions in both experimental and natural infections (Olias et al. 2009, 2013; Bamac et al. 2020). A delayed-type hypersensitivity reaction has been suggested as a cause for *S. calchasi*–induced encephalitis, although the exact pathomechanism is not completely understood (Olias et al. 2013). A similar mechanism might be the cause of severe encephalitis in true intermediate hosts of *S. halieti*. Because *S. calchasi*–induced encephalitis is associated with severe neurologic signs (e.g., tremor, torticollis, and paralysis; Olias et al. 2009), similar clinical signs may be assumed for the Little Owl in the present case. It remains speculative, however, whether the encephalitis caused the nestling to fall out of the nest and, by that, contributed to the diagnosed lethal thoracic trauma. Infections with *Sarcocystis* spp. are sporadically reported as causes for epizootic events in wildlife species, with high mortality (Miller et al. 2010; Bamac et al. 2020). Given that *Sarcocystis* spp. parasites may be absent or rare in the brain, *S. halieti* should be considered as a cause of encephalitis in free-ranging owls even in the absence of microscopically detected parasites in the brain. The effect of *S. halieti* on the population of Little Owls is the subject of further investigations.

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Submitted for publication 28 October 2020.
Accepted 14 January 2021.