HHS Public Access

Author manuscript

J Wildl Dis. Author manuscript; available in PMC 2016 June 01.

Published in final edited form as:

J Wildl Dis. 2016 April 28; 52(2): 395–399. doi:10.7589/2015-10-292.

Histopathologic and Molecular Characterization of *Sarcocystis calchasi* Encephalitis in White-winged Doves (*Zenaida asiatica*) and Eurasian Collared Doves (*Streptopelia decaocto*), East-central Texas, USA, 2010–13

Carolyn L. Hodo[1,5], **Derick B. Whitley**[2], **Sarah A. Hamer**[3], **Wayne V. Corapi**[1], **Karen Snowden**[1], **J. Jill Heatley**[4], and **Aline Rodrigues Hoffmann**[1]

[1]Department of Veterinary Pathobiology, Texas A&M University, 4467 TAMU, College Station, Texas 77843-4467, USA

[2]Antech Diagnostic, 6166 Imperial Loop, College Station, Texas 77845, USA

[3]Department of Veterinary Integrative Biosciences, Texas A&M University, 4458 TAMU, College Station, Texas 77843-4458, USA

[4]Department of Small Animal Clinical Sciences, Texas A&M University, 4474 TAMU, College Station, Texas 77843-4474, USA

Abstract

Sarcocystis calchasi is a recently described apicomplexan parasite that causes encephalitis in avian hosts. We diagnosed one White-winged Dove (*Zenaida asiatica*) and two Eurasian Collared Doves (*Streptopelia decaocto*) in Texas, US, with a history of neurologic signs with protozoal encephalitis. On histologic examination, all three doves had moderate to severe meningoencephalitis characterized by large numbers of plasma cells, lymphocytes, and macrophages with gliosis and astrocytosis. Brain sections from two doves also contained numerous Mott cells. Protozoal schizonts with rosettes or clusters of individual merozoites consistent with *Sarcocystis* spp. were seen within areas of inflammation. Sarcocysts were also identified in the skeletal muscle of one dove. The PCR and sequencing of brain and skeletal muscle from two doves revealed 99% identity with *S. calchasi*. The presence of *S. calchasi* in fatal cases of encephalitis in doves in Texas suggests that the geographic and host ranges of *S. calchasi* are broader than previously reported.

Keywords

Avian; dove; encephalitis; protozoa; *Sarcocystis*; *Sarcocystis calchasi*

Organisms in the genus *Sarcocystis* are apicomplexan parasites that cause disease in a variety of species, often demonstrating a predilection for the central nervous system. The life cycle of *Sarcocystis* spp. includes both an intermediate and a definitive host. Predators

[5]Corresponding author (; Email: chodo@cvm.tamu.edu)

typically serve as definitive hosts and become infected upon ingestion of cysts in the tissue of their prey (Dubey 1976). Well known species of *Sarcocystis* of veterinary significance include *Sarcocystis neurona* and *Sarcocystis rileyi*, the causative agents of equine protozoal myeloencephalitis in horses and the nonpathogenic condition “rice breast” in waterfowl, respectively. *Sarcocystis falcatula*, closely related to *S. neurona*, can cause neurologic disease in wild birds (Wünschmann et al. 2009, 2010), but most commonly results in pulmonary lesions (Smith et al. 1990). Olias et al. (2009) described a novel disease syndrome of *Sarcocystis*-associated encephalitis in domestic pigeons *(Columba livia* f. *domestica*) in Germany and named the agent *Sarcocystis calchasi* (Olias et al. 2010a). Natural infection with *S. calchasi* has since been found in Northern Goshawks (*Accipiter gentilis gentilis*) in Germany (Olias et al. 2011), in domestic pigeons in Minnesota and Missouri, US (Wünschmann et al. 2011; Olias et al. 2014), and in three psittacine species in a zoologic park in California, US (Rimoldi et al. 2013). Here, we present a case series of *S. calchasi* encephalitis in two dove species in Texas.

From 2010 to 2013, the necropsy service at the Texas A&M University (TAMU) Veterinary Medical Teaching Hospital, College Station, Texas, US, received eight pigeons and doves with neurologic signs, with final diagnoses ranging from trauma to viral encephalitis. Three of these birds, a White-winged Dove (*Zenaida asiatica*, dove 1, presented on 15 September 2010) and two Eurasian Collared Doves (*Streptopelia decaocto*, doves 2 and 3, presented on 12 May 2011 and 3 May 2013, respectively), had lesions consistent with *Sarcocystis* encephalitis. Doves 1 and 2 were found by members of the public in the Houston, Texas, area and were brought to the Wildlife Center of Texas in northwest Houston before transfer to TAMU. Dove 3 was found in a yard in College Station, Texas (30°36′N, 96°18′W). Reported neurologic signs included ataxia, head tilt, circling, head pressing, and inability to stand or fly. The birds were in fair to poor body condition. At necropsy, dove 1 had a pulmonary granuloma and enlarged liver; dove 3 had a moderate amount of hemorrhage surrounding the left orbit.

Following necropsy, formalin-fixed sections of brain, heart, lung, liver, spleen, skeletal muscle, digestive system, and kidneys were processed routinely for histology and stained with H&E. Histologically, all three doves exhibited severe multifocal histiocytic and lymphoplasmacytic meningoencephalitis, affecting both the white and gray matter (Fig. 1A). Perivascular cuffing and gliosis was evident in the surrounding areas, along with foamy macrophages (gitter cells). The inflammation extended into the meninges, especially around vessels. Plasma cells congested with immunoglobulin (Mott cells) were often seen in the brain of doves 1 and 2 (Fig. 1B). Within the areas of inflammation in all three doves were rare protozoal schizonts, approximately 5–10 μm in diameter, with an indistinct wall and containing numerous basophilic, 2×5–μm merozoites (Fig. 1C). In the skeletal muscle of dove 2 were multifocal, 20–30-μm intramyocytic thin-walled protozoal cysts filled with 2×5–μm bradyzoites (Fig. 1D). Additional findings in dove 1 were pulmonary and hepatic fungal granulomas and a fibrinous coelomitis. *Aspergillus fumigatus* was cultured from the pulmonary granulomas and is presumed to be unrelated or secondary to debilitation from *Sarcocystis* infection.

Using a commercial extraction kit (E.Z.N.A. Tissue DNA Kit; Omega Bio-Tek, Norcross, Georgia, USA) and following the manufacturer's protocols for fresh and formalin-fixed, paraffin-embedded (FFPE) tissues as appropriate, we extracted DNA from FFPE brain of all three birds, plus FFPE muscle of dove 2 and fresh frozen brain of dove 3. The internal transcribed spacer 1 (ITS1) and the D2 region of the 28S ribosomal RNA gene were targeted for amplification with a series of PCRs using previously published primers (Wünschmann et al. 2009, 2011; Olias et al. 2011). Amplification products were separated on agarose gels, purified (ExoSAP-IT; Affymetrix, Santa Clara, California, USA), and sequenced in both directions at either Eton Bioscience, Inc. (San Diego, California, USA) or the TAMU genetics lab. Resulting sequences were analyzed, aligned using MEGA6.06 software (Tamura et al. 2013), and compared to a national sequence database (GenBank) using the BLAST program (Altschul et al. 1990). A phylogenetic tree was constructed in MEGA using the neighbor-joining method, with bootstrap consensus trees inferred from 1,000 replicates (Fig. 2).

We obtained DNA amplification products from the brain and muscle of dove 2 and brain of dove 3 with all three PCR reactions, but amplification was not successful with any primers from the formalin-fixed brain of dove 1. Sequence results from the D2 region of the 28S rRNA gene from doves 2 and 3 were 100% homologous with *S. calchasi* (GenBank accession FJ232949). From the ITS1 region, sequences from doves 2 and 3 were 99% identical to *S. calchasi* (GenBank accessions FJ232949, KC733715-8). The D2 region sequences from the two doves were aligned and trimmed to 323 base pairs. The neighbor-joining phylogenetic tree grouped these sequences together with *S. calchasi*, demonstrating significant distance from other published *Sarcocystis* spp. sequences and from a sequence generated from a Mourning Dove (*Zenaida macroura*) necropsied at TAMU in 2011 and suspected of being infected with *S. falcatula* (Fig. 2). Sequences generated from this study were deposited in GenBank as accessions KT945019–KT945022.

Histologic findings in all three doves included protozoal schizonts within areas of encephalitis. This contrasts to some previous studies of *S. calchasi* in birds in which organisms were not observed within areas of inflammation (Olias et al. 2009, 2010a). Protozoa were rare in most sections and sometimes difficult to find, but the histiocytic to granulomatous nature of the inflammation combined with the presence of Mott cells was suggestive of a protozoal infection and warranted a thorough search. While Mott cells are a nonspecific finding and are often seen with chronic inflammation, their presence has been described in cases of protozoal encephalitis associated with *Trypanosoma evansi* infection in horses (Rodrigues et al. 2009). Other differentials for encephalitis in doves include pigeon paramyxovirus type 1, avian influenza virus, St. Louis encephalitis virus, West Nile virus, *Salmonella*, and neural migration of larval nematodes (Marlier and Vindevogel 2006; Harlin and Wade 2009). Here, the histiocytic to granulomatous inflammation surrounding protozoal schizonts together with the absence of evidence of other etiologic agents allowed us to make a diagnosis of *Sarcocystis*-associated encephalitis. Additionally, although intramuscular protozoa are often incidental findings in birds, the organisms in the muscle of dove 2 were genetically identical to those in the brain.

The DNA sequencing results of the ITS1 and 28S rRNA gene segments from tissues of doves 2 and 3 confirm significant homology to *S. calchasi*. Because of a long region of repeated bases in the targeted ITS1 region, which effectively truncated sequencing from both directions, double-stranded (overlapping) sequences were not obtained from this region. However, the marked heterozygosity in the ITS1 region of *Sarcocystis* spp. and the fact that our sequences showed such high homology with each other and with *S. calchasi*, while differing from closely related *Sarcocystis* spp., lends additional credibility to the diagnosis. Although we were not able to confirm the genetic identity of the *Sarcocystis* species from dove 1, most likely due to degradation of target DNA, the similarity of the microscopic findings to those of doves 2 and 3 suggests the same etiology. Although there have been no previously published reports of *S. calchasi* in wild birds in the southern US, our findings show that it has been present in Texas since at least 2011 (dove 2), possibly 2010 (dove 1).

The definitive host of *S. calchasi* in the southern US is unknown. The Northern Goshawk was shown to be a definitive host in previously published reports (Olias et al. 2010b, 2011), but the Goshawk's range does not extend as far south as Texas. Wünschmann et al. (2011) suggested the Cooper's Hawk (*Accipiter cooperi*) as the definitive host in more southern regions, but this has not been fully investigated. A DNA sequence from the 18S ribosomal RNA gene of a *Sarcocystis* spp. in a Cooper's Hawk in Georgia, US, published before *S. calchasi* was recognized (Yabsley et al. 2009) shows high homology to *S. calchasi*, but sequencing of additional genes would be necessary to confirm its identity. Accipiters such as the Northern Goshawk, Cooper's Hawk, and Sharp-shinned Hawk (*Accipiter striatus*) prey almost exclusively on small to midsized birds, including pigeons and doves, making them good candidates for definitive hosts of *S. calchasi*.

Our results implicate *S. calchasi* in two cases of fatal encephalitis in doves in east-central Texas in 2010–13. There is no evidence to suggest infections are having a significant impact on dove populations, but anecdotal reports from a wildlife rehabilitation center suggest the parasite may be associated with local outbreaks that could be of concern if they affect any of the threatened dove species in Texas. Additional investigation into the host range of *S. calchasi* is warranted, both for the definitive and intermediate hosts. *Sarcocystis calchasi* has been found in wide-ranging areas of the US over the last 3 yr, and it is likely that the parasite has been present in the US for some time and is only recently being recognized. Testing of archived samples may contribute additional information on the distribution and history of this parasite and examination of local *Accipiter* species may help determine the definitive host in the southern US.

Funding was provided by the Texas A&M University department of Veterinary Pathobiology (VTPB). We acknowledge Lisa Auckland for assistance with laboratory methods and the VTPB histology laboratory for slide preparation. We also acknowledge the Wildlife Center of Texas for their enthusiastic support of this project and contribution of avian patients.

LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. J Mol Biol. 1990; 215:403–410. [PubMed: 2231712]

Dubey J. A review of *Sarcocystis* of domestic animals and of other coccidia of cats and dogs. J Am Vet Med Assoc. 1976; 169:1061–1078. [PubMed: 824260]

Harlin R, Wade L. Bacterial and parasitic diseases of Columbiformes. Vet Clin North Am Exot Anim Pract. 2009; 12:453–473. [PubMed: 19732704]

Marlier D, Vindevogel H. Viral infections in pigeons. Vet J. 2006; 172:40–51. [PubMed: 16772130]

Olias P, Gruber AD, Hafez HM, Heydorn AO, Mehlhorn H, Lierz M. *Sarcocystis calchasi* sp. nov. of the domestic pigeon (*Columba livia* f. *domestica*) and the Northern Goshawk (*Accipiter gentilis*): Light and electron microscopical characteristics. Parasitol Res. 2010a; 106:577–585. [PubMed: 20033211]

Olias P, Gruber AD, Heydorn AO, Kohls A, Mehlhorn H, Hafez HM, Lierz M. A novel *Sarcocystis*-associated encephalitis and myositis in racing pigeons. Avian Pathol. 2009; 38:121–128. [PubMed: 19322710]

Olias P, Gruber AD, Kohls A, Hafez HM, Heydorn AO, Mehlhorn H, Lierz M. *Sarcocystis* species lethal for domestic pigeons. Emerg Infect Dis. 2010b; 16:497–499. [PubMed: 20202429]

Olias P, Maier K, Wünschmann A, Reed L, Armien AG, Shaw DP, Gruber AD, Lierz M. *Sarcocystis calchasi* has an expanded host range and induces neurological disease in cockatiels (*Nymphicus hollandicus*) and North American rock pigeons (*Columbia livia* f. *dom.*). Vet Parasitol. 2014; 200:59–65. [PubMed: 24360290]

Olias P, Olias L, Krücken J, Lierz M, Gruber AD. High prevalence of *Sarcocystis calchasi* sporocysts in European *Accipiter* hawks. Vet Parasitol. 2011; 175:230–236. [PubMed: 21074324]

Rimoldi G, Speer B, Wellehan JFX, Bradway DS, Wright L, Reavill D, Barr BC, Childress A, Shivaprasad HL, Chin RP. An outbreak of *Sarcocystis calchasi* encephalitis in multiple psittacine species within an enclosed zoological aviary. J Vet Diagn Invest. 2013; 26:775–781. [PubMed: 24081928]

Rodrigues A, Fighera RA, Souza TM, Schild AL, Barros CS. Neuropathology of naturally occurring *Trypanosoma evansi* infection of horses. Vet Pathol. 2009; 46:251–258. [PubMed: 19261636]

Smith JH, Neill PJ, Dillard EA, Box ED. Pathology of experimental *Sarcocystis falcatula* infections of canaries (*Serinus canarius*) and pigeons (*Columba livia*). J Parasitol. 1990; 76:59–68. [PubMed: 2105389]

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 2013; 30:2725–2729. [PubMed: 24132122]

Wünschmann A, Armien AG, Reed L, Gruber AD, Olias P. *Sarcocystis calchasi*–associated neurologic disease in a domestic pigeon in North America. Transbound Emerg Dis. 2011; 58:526–530. [PubMed: 21848932]

Wünschmann A, Rejmanek D, Conrad PA, Hall N, Cruz-Martinez L, Vaughn SB, Barr BC. Natural fatal *Sarcocystis falcatula* infections in free-ranging eagles in North America. J Vet Diagn Invest. 2010; 22:282–289. [PubMed: 20224094]

Wünschmann A, Rejmanek D, Cruz-Martinez L, Barr BC. *Sarcocystis falcatula*–associated encephalitis in a free-ranging Great Horned Owl (*Bubo virginianus*). J Vet Diagn Invest. 2009; 21:283–287. [PubMed: 19286517]

Yabsley MJ, Ellis AE, Stallknecht DE, Howerth EW. Characterization of *Sarcocystis* from four species of hawks from Georgia, USA. J Parasitol. 2009; 95:256–259. [PubMed: 18576854]

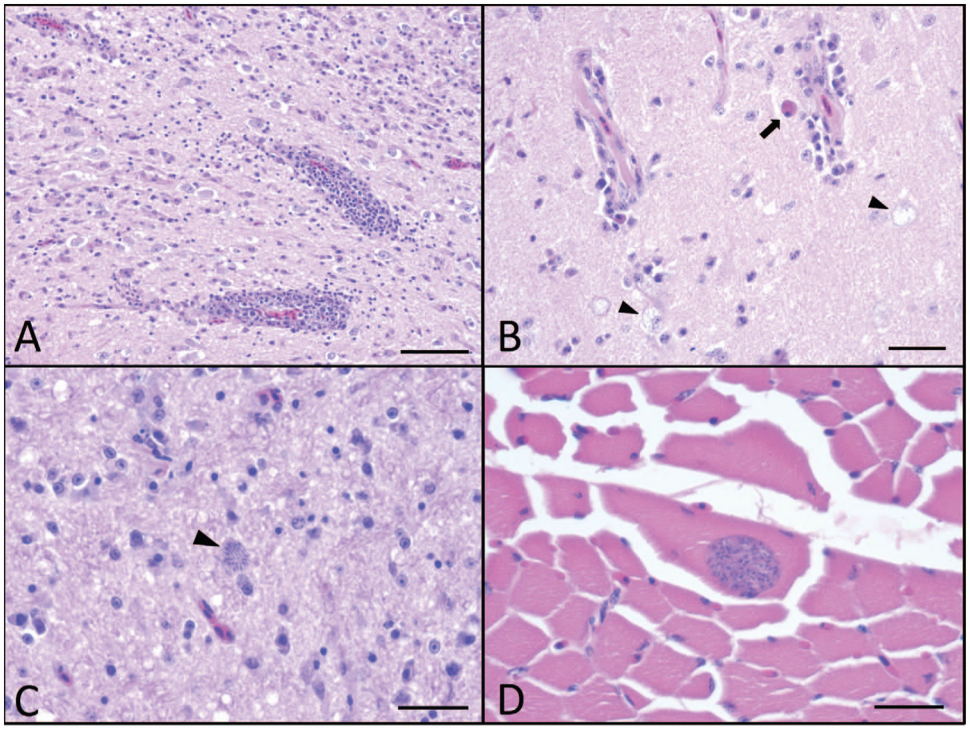

Figure 1.

Histologic changes in *Sarcocystis calchasi*–affected doves from east-central Texas, USA, 2010–13. H&E stain. (A) Cerebrum of a Eurasian Collared Dove (*Streptopelia decaocto*; dove 3). Inflammatory infiltrate composed of macrophages, lymphocytes, and plasma cells, with perivascular cuffing and gliosis. Bar=25 μm. (B) Brainstem of a White-winged Dove (*Zenaida asiatica*; dove 1). Gitter cells (arrowheads) and Mott cells (arrow) are common in the areas of inflammation. Bar=25 μm. (C) Cerebrum of a Eurasian Collared Dove (dove 3). A protozoal schizont consistent with *Sarcocystis* sp. (arrowhead). Bar=20 μm. (D) Skeletal muscle of a Eurasian Collared Dove (dove 2). An intramyocytic thin-walled sarcocyst filled with numerous bradyzoites. Bar=15 μm.

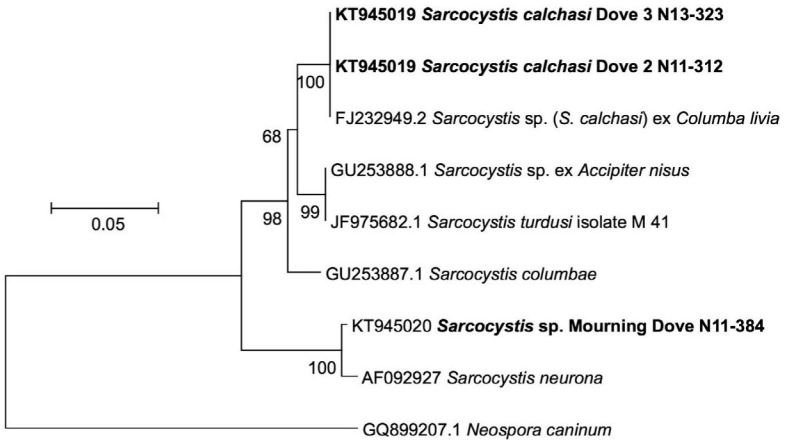

Figure 2.

Phylogenetic tree based on a 323–base-pair region of the D2 region of the 28s rRNA gene of *Sarcocystis* spp. isolates, rooted on *Neospora caninum*. Sequences generated in this case series are in bold. The tree was generated using the neighbor-joining method, with bootstrap consensus trees inferred from 1,000 replicates.