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Sarcocystis calchasi has an expanded host range and induces neurological disease in cockatiels (*Nymphicus hollandicus*) and North American rock pigeons (*Columbia livia* f. *dom.*)

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ABSTRACT

Pigeon protozoal encephalitis (PPE) is an emerging central nervous system disease of pigeons (*Columba livia* f. *domestica*) caused by the apicomplexan parasite *Sarcocystis calchasi*. The intermediate host specificity of *S. calchasi* had been considered high, as domestic chickens were resistant to experimental infection. Here, we have re-evaluated this concept and expanded the known host range of *S. calchasi* by experimental infection of cockatiels (*Nymphicus hollandicus*), a species distantly related to pigeons. In this work, a group of eight cockatiels were experimentally infected with *S. calchasi*, which resulted in a biphasic central nervous system disease that paralleled PPE in many aspects, albeit with a more diverse pathology. All cockatiels became lethargic and polyuric between days 7 and 13 pi and during that time schizonts of *S. calchasi* were found primarily in the liver and spleen accompanied by necrosis and inflammation. As with pigeons, neurological signs occurred during a chronic phase of the disease in three cockatiels between 57 and 63 dpi. However, all five cockatiels necropsied in that period, or at the end of the trial at 76 dpi, had a severe lymphohistiocytic and necrotizing encephalitis. No tissue cysts were found in the heart, and cockatiels infected with 10^5 sporocysts only had a negligible parasite load in skeletal muscles despite the presence of severe central nervous system lesions. Notably, intralesional schizonts were identified in the brain of one cockatiel. In contrast to previous results, intralesional schizonts were also identified in the brains of three of six naturally infected pigeons from Minnesota and Missouri examined as part of an epidemiological investigation. In both the cockatiel and the pigeons, tissue cysts were found concurrently with schizonts suggesting an uncommon phenomenon in the *Sarcocystis* life cycle. Based on the results of this study, transmission of *S. calchasi* to avian species other than the domestic

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pigeon is possible. These findings suggest a, so far, unmonitored prevalence of *S. calchasi* in avian populations and highlight a possible ongoing dissemination of this parasite in the Northern Hemisphere.

1. Introduction

Pigeon protozoal encephalitis (PPE) is an emerging central nervous system disease of the domestic pigeon (*Columba livia* f. *domestica*) caused by the apicomplexan parasite *Sarcocystis calchasi* (Olias et al., 2010a,b). First reported in Germany in 2009 (Olias et al., 2009), *S. calchasi* has since been found in the muscle tissue of a pigeon in Minnesota in 2011 (Wünschmann et al., 2011), suggesting the potential of an ongoing worldwide spread of the parasite. In Germany, the definitive host for the parasite is the Northern goshawk (*Accipiter g. gentilis*) and presumably also the European sparrowhawk (*Accipiter n. nisus*) (Olias et al., 2010a, 2011). Presently the definitive host of *S. calchasi* in North America is unknown but *Accipiter* species seem likely (Wünschmann et al., 2011).

In general, *Sarcocystis* spp. are relatively host specific in their obligate two-host life cycle between its definitive host (predator species) and intermediate host (prey species) (Dubey et al., 1989). However, some species of avian origin, including *Sarcocystis neurona* and its sister taxon *S. falcatula* (Mansfield et al., 2008), are less specifically adapted and may infect a wide spectrum of intermediate hosts including mammals such as horses and sea otters in the case of *S. neurona* (Dubey et al., 2001; Miller et al., 2010). *S. neurona* causes equine protozoal myeloencephalitis (EPM), one of the most common neurologic diseases of horses in North America.

The pathophysiology of the central nervous system lesions caused by *S. neurona* is, so far, unexplained but metabolites or cytokines have been presumed to be involved (Dubey et al., 2001). The extensive brain lesions in pigeons caused by infections with *S. calchasi* have been shown to be associated mainly with T-cell infiltration and an overexpression of MHC-II, IFN-γ and TNF-α-related cytokines. Since no parasites have been found associated within these brain lesions previously, a T-cell mediated delayed-type hypersensitivity reaction had been proposed as a potential cause (Olias et al., 2013).

To further assess the cross-species infectious potential of *S. calchasi*, we experimentally infected cockatiels (*Nymphicus hollandicus*) due to it being an avian species that is only distantly related to pigeons (Hackett et al., 2008) but more importantly because members of this bird order include many highly endangered species. Of note is that African, Asian, Pacific and Australian parrots are highly susceptible to *S. falcatula* infections, because these members of the psittacine group have not evolved with the parasite (Clubb and Frenkel, 1992; Hillyer et al., 1991; Page et al., 1992). Furthermore, it is known that the *S. falcatula* group of parasites (Britton et al., 2010) is capable of causing severe acute and lethal disease in multiple avian species including pigeons and a central nervous system disease in birds of prey (Wünschmann et al., 2009, 2010). The findings of the present study suggest a broader host spectrum and increased virulence potential of *S. calchasi* in birds than previously thought. For the first time, schizonts of *S. calchasi* were identified associated with extensive brain lesions in one cockatiel and three of six naturally infected pigeons from North America that were investigated as part of the epidemiological approach of this study.

2. Material and methods

2.1. Experimental infection of cockatiels with S. calchasi

All experiments in cockatiels were governmentally approved (permit number V54-19c 20-15 (1) GI 18/9 Nr. 01/2010).

All cockatiels originated from a controlled flock maintained at the Clinic for Birds, Reptiles, Amphibians and Fish, Justus-Liebig-University Giessen, Germany. They were clinically healthy. Crop and cloacal swabs had been tested twice a year for the absence of Avian Bornavirus and *Chlamydophila* sp. by PCR. Serological tests had been performed to exclude the presence of specific antibodies against Avian Bornavirus and Paramyxovirus 1. Fecal material of the housings had been examined monthly for endoparasite stages and for *Salmonella* sp. by cultivation on selective media. Prior to the trial, pectoral muscle biopsies tested negative for parasitic stages by histological examination. Eight cockatiels were separated in four groups of two birds each and were orally inoculated with different doses of *S. calchasi* sporocysts suspended in water: 3×10^6 (group 1), 1×10^5 (group 2), 1×10^4 (group 3) and 1×10^2 (group 4). The cockatiels were assigned to each group on a random blinded basis. Sporocysts belonged to the same *S. calchasi* strain used in previous trials in pigeons (Olias et al., 2010a,c, 2013) (Table 1). Three cockatiels, either inoculated with water or with filtered storage medium of sporocysts served as controls. For humane reasons, birds with compromised general condition and neurological signs were euthanized. All surviving animals were euthanized on day 76 pi.

2.1.1. Histopathological and molecular analysis of cockatiels

All cockatiels underwent a complete necropsy. Tissue samples from skeletal muscle (pectoral and thigh muscle), lung, heart, liver, spleen, kidneys, oesophagus, crop, intestine, bone marrow, brain, spinal cord and ischiadic nerve were fixed in 4% phosphate-buffered formalin and sections of 4 μm thickness were stained with hematoxylin and eosin (HE). Five serial sections were immunohistochemically analyzed with an anti-*S. calchasi* antibody (anti-*Sc*B) as described previously (Olias et al., 2013). DNA was extracted from liver, spleen, lung, skeletal muscle and brain of all cockatiels and a polymerase chain reaction (PCR) assay was performed with *S. calchasi*-specific primers Sca1 and Sca2