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Infections with *Sarcocystis wenzeli* are prevalent in the chickens of Yunnan Province, China, but not in the flocks of domesticated pigeons or ducks

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ABSTRACT

The distribution and prevalence of infections with species of *Sarcocystis* in domestic fowl in Asia are poorly known. Here, ducks, pigeons, and chickens from Yunnan Province, China were examined for evidence of parasitic infection with *Sarcocystis* spp. One hundred and ninety one chickens, 514 ducks, and nine pigeons were investigated. Whereas the ducks and pigeons lacked tissue cysts in their muscle, brain or peripheral nervous system, cysts of *Sarcocystis wenzeli* were identified in 17 of 191 chickens (8.9%). Morphologically, the cysts were thread-like, ranging in size from 334–3169 × 41–117 μm (mean 1093 × 65 μm). Cysts were septate with dense, short finger-like protrusions which appeared radially striated. The cyst wall was 1.4–3.5 μm (mean 2.4 μm) thick. The bradyzoites were lancet shaped and measured 12.2–17.7 × 1.8–2.9 μm (mean 14.6 × 2.5 μm). Ultrastucturally, the primary sarcocyst wall had stubby villar protrusions, corresponding to the 'type 9' class previously designated. The protrusions measured 0.87–1.89 × 0.47–0.91 μm (mean 1.27 × 0.59 μm; $n = 57$). These findings confirm previous work from the vicinity of Kunming concerning the occurrence of *S. wenzeli* in chickens, and its use of both cats and dogs as definitive hosts, but indicate that corresponding infections may not occur in the regional domestic flocks of other types of fowl.

1. Introduction

The family Sarcocystidae comprise cyst-forming coccidia, which parasitize a wide variety of vertebrates, including humans (Zuo, 1992). *Sarcocystis* species are among the most common and widespread protozoan parasites of livestock and poultry, rendering them of veterinary and economic importance. The prevalence of infections with *Sarcocystis* species in domestic animals, such as cattle, water buffalo and swine, can approach 100% in southern China (Zuo, 1992). Some species of *Sarcocystis* can cause reduced weight gain, poor feeding efficiency, anorexia, fever, anemia, muscle weakness, reduced milk yield, abortion, and death in intermediate hosts such as cattle, sheep, goats, and swine. For poultry, an important economical food source, *Sarcocystis* is an important pathogen, which can cause frequent mortalities in chickens (Mao et al., 1994).

To date, there are only limited reports concerning *Sarcocystis* of chickens in China, and none of other domesticated birds (Zuo, 1992; Mao et al., 1994). Instead, reports from other countries form the basis of our understanding of infection in these animals (Zaman, 1976; Munday, 1977; Munday et al., 1977; Brehm and Frank, 1980; Bergler et al., 1980; Wenzel et al., 1982; Box and Smith, 1982; Dubey et al., 1987; Clubb and Frenkel, 1992; Mutalib et al., 1995; Saito et al., 1995; Graczyk et al., 1996; Barta et al., 1997; Jäkel et al., 1999; Olias et al., 2010a,b). In this study, we investigated chickens, ducks, and pigeons for *Sarcocystis* infection in Yunnan, China in 1998–2004.

2. Materials and methods

2.1. Sampling of animals

We examined the infection status of 191 free-ranging domestic chickens (*Gallus gallus*), 514 domestic ducks (*Anas platyrhynchos*) and nine domestic pigeons (*Columba livia* f. *domestica*) that had

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been slaughtered for commercial purposes in Kunming, Wenshan, Chuxiong, Yuxi, Dehong, Lincang districts in Yunnan province, China.

2.2. Cyst isolation

To investigate *Sarcocystis* infection, muscle samples were taken from the neck, leg, breast, wing, heart, esophagus and stomach muscles, brain, and the peripheral nervous system of all birds during April 1998–January 2004. The cysts were isolated as previously described (Yang et al., 2001a,b).

2.3. Microscopic examinations

Samples were prepared and checked for *Sarcocystis* cysts by light microscopy (LM) as previously reported (Yang et al., 2001a,b). Fresh cysts were examined both in situ in host muscles and as isolated preparations. For transmission electron microscopy (TEM), the encysted parasites were fixed in glutaraldehyde. Thereafter, ultrathin sections were prepared and stained with uranyl acetate and lead citrate. These sections were subsequently examined using a JEM100-CX transmission electron microscope at 80 kV. For scanning electron microscopy (SEM), parasite cysts were separated from muscle cells and fixed in glutaraldehyde and examined using a JEM100-CX scanning electron microscope at 15 kV.

3. Results

3.1. Infection rate

No *Sarcocystis* were found in any of 514 ducks or nine pigeons. However, 17 of 191 chickens were found to be infected with *Sarcocystis* sp. (prevalence rate of 8.9%). Cysts were located in neck (8.4%), leg (8.9%), breast (5.7%), and wing (2.1%) muscles. No cysts were identified in the heart, esophagus, stomach muscles, brain, or peripheral nervous system.

3.2. LM

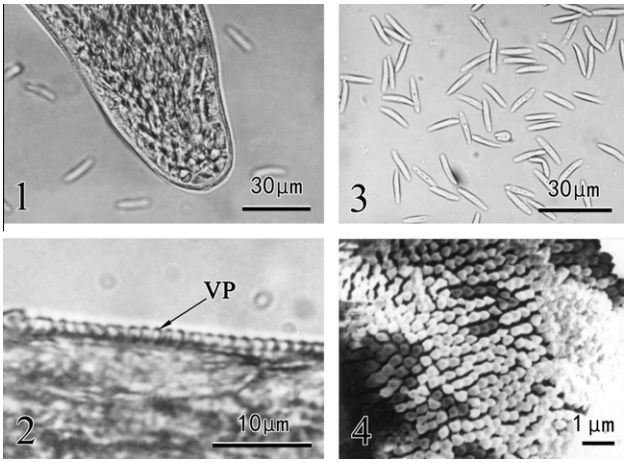
The cysts were thread-like with a size of 334–3169 × 41–117 μm (mean 1093 × 65 μm, $n = 86$) (Fig. 1). Sarcocysts were septate and showed dense, short finger-like protrusions, and the cyst wall appeared radially striated (Fig. 2). The cyst wall measured 1.4–3.5 μm in thickness (mean 2.4 μm, $n = 79$). The cyst interior was packed with myriad lancet-shaped bradyzoites, which measured 12.2–17.7 × 1.8–2.9 μm (mean 14.6 × 2.5 μm, $n = 62$) (Fig. 3). In the cyst, no clear septa-like structure or segmentation of the cyst's interior was observed, but the zoites appeared clearly clustered.

3.3. TEM and SEM

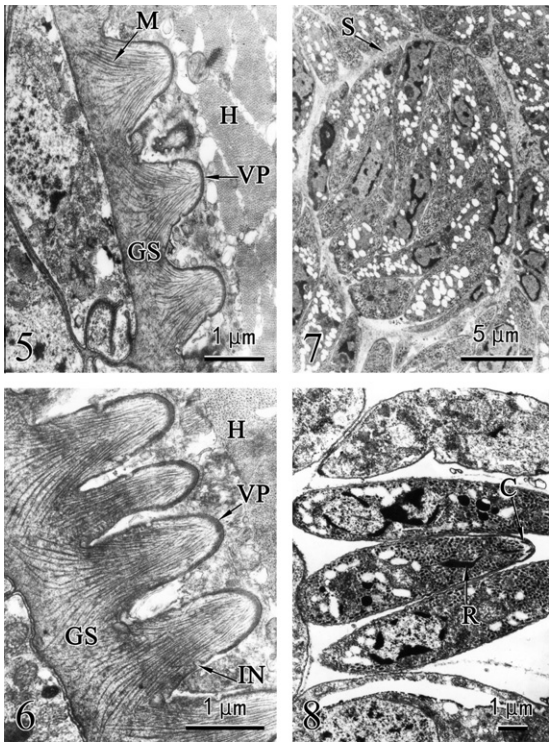
By TEM, cysts were abundant within the fibers of skeletal muscles. They were limited by a fine membrane, the primary sarcocyst wall (Pw), which had stubby conical villar protrusions (VP) with criss-crossing microtubules. These attributes correspond to TEM type 9 described by Dubey (1989) (Figs. 5 and 6). The protrusions measured 0.87–1.89 × 0.47–0.91 μm (mean 1.27 × 0.59 μm, $n = 57$). The primary cyst wall (PCW) consisted of a thin membrane supported by osmiophilic material, the latter appearing as a thin, electron-dense layer, 0.02–0.07 μm (mean 0.05 μm, $n = 20$) thick on the surface of the VP. The layer of osmiophilic material was occasionally interrupted by small vesicular invaginations that averaged 0.05–0.08 μm (mean 0.06 μm, $n = 12$) in depth. There was a zone of ground substance (GS) about 0.32–1.09 μm (mean 0.73 μm, $n = 45$) thick. Clear septa-like structures or segmentation of the cyst's interior was observed (Fig. 7), which was filled with myriad lancet-shaped bradyzoites (Fig. 8). By SEM, the surface of the cyst was seen to be covered by densely packed, short finger-like protrusions (Fig. 4).

4. Discussion

According to previous reports, at least two named species of *Sarcocystis* occur in chickens: *Sarcocystis horvathi* and *Sarcocystis wenzeli* (Zaman, 1976; Munday, 1977; Brehm and Frank, 1980; Bergler et al., 1980; Wenzel et al., 1982; Box and Smith, 1982; Dubey et al., 1987; Clubb and Frenkel, 1992; Mutalib et al., 1995;



Figs. 1–4. (1) LM image of a sarcocyst of *Sarcocystis wenzeli* from chicken muscles. (2) Stubby villar protrusions (VP) under LM. (3) Lancet-shaped bradyzoites. (4) Cyst surface covered by densely packed, short finger-like protrusions under SEM.



Figs. 5–8. (5 and 6) TEM images of sarcocyst wall of *Sarcocystis wenzeli*. (7 and 8) TEM images of interior of sarcocyst. GS: ground substance; H: host cell cytoplasm; IN: invagination; M: microtubules; S: septa-like structures or segmentation; C: conoid; R: rhoptry.