

A taxonomic re-appraisal of *Sarcocystis nesbitti* (Protozoa: Sarcocystidae) from the monkey *Macaca fascicularis* in Yunnan, PR China

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Received 22 January 2004; accepted 17 December 2004

Abstract

The first detection of *Sarcocystis nesbitti* Mandour, 1969 in the Chinese mainland is reported and the morphology of the sarcocyst is described in detail. The parasite was detected in the monkey, *Macaca fascicularis*, maintained on a monkey farm in Yunnan Province; the infection may have occurred via faecal contamination from local rats, mice and/or birds. *S. nesbitti* was characterized as follows: a macroscopic sarcocyst, length of the cyst up to 2 mm; cyst wall smooth, thin and no perpendicular protrusion is seen under the light microscope; border of cyst wall wavy, primary cyst wall thin (38–65 nm) and invaginated; ground substance about 0.5–0.76 µm thick with electron-dense granules and concentric spherical bodies. The cyst wall is described as type 1 by electron microscopy. It is suspected that *S. nesbitti* may utilize *Macaca mulatta*, *M. fascicularis*, *Cercocebus atys*, and *Papio papionis*, as well as human as intermediate hosts. The taxonomy of *S. nesbitti* is re-appraised in the light of a consideration of possible experimental artefacts and examination of the past literature. Evidence is presented that *S. nesbitti* may be one of the species infecting humans in South Asia and that the monkey may be a potential reservoir host.

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Keywords: Protozoa; Sarcocystidae; *Sarcocystis nesbitti*; *Sarcocystis* cyst morphology; Monkey; *Macaca fascicularis*; No human–primate

1. Introduction

A number of cases of *Sarcocystis* infection in humans, monkeys, and other nonhuman primates, have been reported from South-East Asia, for example from Malaysia [1–7] and other parts of the world [8–10]. The prevalence of infection with this parasite in humans is believed to be higher in South Asia than in Europe [11]. However, in the Chinese

mainland, there are only three reported cases of *Sarcocystis* infection in humans [12–14] and no cases reported in monkeys or other primates. In view of the unexpected absence of *Sarcocystis* infection in monkeys in the Chinese mainland, and questions as to the host range of *Sarcocystis* and the problem of reservoir hosts for human disease, we examined tissues from 69 monkeys (*Macaca fascicularis*) that were killed for another experimental purpose (medical drug research by others in our College). *M. fascicularis* is not naturally occurring in mainland China and this study used tissue samples derived from animals bred at a monkey farm supplying the research community. Although the host

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range of *Sarcocystis nesbitti* is unknown, it is possible that infection of the monkeys (which are kept in very large outdoor cages) occurred via exposure to oocysts or sporocysts contaminating the faeces of birds, mice or rats, as were commonly seen around the cages. No imported food or other animals or material, to which the monkeys have access, is found on the farm and the monkeys have been bred in isolation in Yunnan for several decades and were noted to be free of *Sarcocystis* in the past. In view of this we are confident that the *Sarcocystis* found originated locally and further work is required to determine the exact final host.

Transmission electron microscopic (TEM) ultrastructural studies of the *Sarcocystis* cyst, especially of the cyst wall have helped to distinguish and identify several species of *Sarcocystis* infecting animals, as each *Sarcocystis* species has its own ultrastructural type [15–17]. The ultrastructural classes of *Sarcocystis* cyst wall are referred to as types 1 to 24 by Dubey [17].

There are two named *Sarcocystis* species infecting monkeys, *Sarcocystis kortei* Castellani and Chalmers, 1909 and *S. nesbitti* Mandour, 1969. Naturally, the first descriptions of these taxa were based on light microscopy (LM) and we have been unable to find any report using TEM. The taxonomic validity of *S. kortei* has been confirmed by many authors [18–24]. In contrast, the taxonomy of *S. nesbitti* is much less certain and the validity of *S. nesbitti* has been questioned by Levine [25]; the taxonomic uncertainty is mostly attributable to a lack of TEM studies. Mehlhorn et al. [16], and Kan [26] each described one type of *Sarcocystis* cyst from the monkey, *Macaca mulatta* and *M. fascicularis*, respectively, under LM and TEM, because in both cases the structure of the cysts looked different from named *S. kortei* and *S. nesbitti*, these authors thought there was a new third species infecting monkeys. The objective of the present paper was therefore to present the first report of *Sarcocystis* in monkeys in mainland China; to describe the species present, which resembled *S. nesbitti*, on the basis of LM and TEM studies; and to help clarify the taxonomy of *Sarcocystis* in humans and primates in China.

2. Materials and methods

Muscle samples were collected from the farmed monkeys. In the interests of animal welfare and the prevention of cruelty, all applicable governmental and institutional laws and regulations (in Yunnan, China) were followed, and the experimental procedures and animal care was designed so as to eliminate animal suffering.

69 monkeys (*M. fascicularis*) 38 males and 31 females, were raised on a monkey farm in a mountainous area of Yunnan, South China mainland. All the monkeys were laboratory-born, caged after birth and fed a meat-free diet. Muscle samples from the fresh cervix, scapular region,

abdomen, psoas, thigh, and diaphragm of each monkey were checked for *Sarcocystis* parasites under LM as previously reported [27–29]. The fresh cysts were examined both in situ in host muscle and as isolated preparations prepared from muscle tissue were observed and measured under LM at 40 \times , 100 \times and 400 \times magnification. For TEM work the parasites contained in muscle cells were fixed in glutaraldehyde and ultrathin sections were prepared and stained with uranyl acetate and lead citrate; these were subsequently examined using a JEM100-CX transmission electron microscopy at 80 kV.

3. Results

3.1. Light microscopy

Of the 69 monkeys examined, only one, a male, was found to be infected with *Sarcocystis* sp., thus giving an infection rate of 1.4%. *Sarcocystis* sp. cysts were found in the fresh cervix, scapular region, abdomen, psoas, and diaphragm muscle, but none were found in the thigh muscles.

Sarcocystis sp. cysts were seen in large numbers within the fibres of the striated muscles and reached a length of 2.3–14 mm and a in width of 0.05–0.1 mm. All cysts observed were limited by a fine membrane, which lacked perpendicular protrusions even when observed under 400 \times magnification. The cyst wall was 0.46–0.60 μ m thick. The interior of the cyst was packed full with cyst merozoites, the shape of which was typical of *Sarcocystis*. The cyst merozoites measured 7.5–10 \times 1.25 μ m. No clear septa-like structures or segmentation of the cyst's interior was observed, but the zoites did appear clustered under LM (Fig. 1). No histological changes attributable to a host immune response (e.g. a secondary cyst wall) were observed around the parasitized muscle fibre.

3.2. Electron microscopy

The cysts were always situated within a muscle fibre and were limited by a typical primary cyst wall. The cyst wall was slightly wavy in some areas (Figs. 2 and 3) and in other part of the same cyst it was thrown into broad, irregular folds or protuberances (Fig. 4). The primary cyst wall (PCW) consisted of a thin membrane supported by osmiophilic material, the latter appearing as a thin, electron-dense layer 38–65 nm thick (Fig. 2). The layer of osmiophilic material was frequently interrupted by small vesicular invaginations that averaged 19–380 nm in diameter and 65–120 nm in depth (Fig. 2). These invaginations were found over the entire surface of the PCW. Positioned immediately beneath the PCW, there was a zone of ground substance (GS) about 0.5–0.76 μ m thick (Fig. 2), where the whole cyst wall, including the PCW, was not folded. In the GS, there were electron-dense granules

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