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Isolation of a New *Sarcocystis* Species from Sudanese Camels (*Camelus Dromedarius*)

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Abstract: Six weaned puppies 4-weeks-old were fed once a composite of raw cameline meat (oesophagus, diaphragm, heart and skeletal muscles) collected from camels slaughtered at El Gedarif city-Eastern Sudan. The puppies started to shed two types of *Sarcocystis* sporocysts 9-13 days after feeding raw meat. One type identified morphologically as *Sarcocystis cameli*, measured 13.2-13.6×6.5-9.5 μm with a patent period of 55-57 days. The second type had a larger size than the first one (vis *Sarcocystis camelocanis*), measured 16.0×9.9-11.5 μm and a shorter patent period (37-45 days). The two species which harvested from intestinal mucosae of the infected puppies were orally given to three naïve weand calf-camels (1×10^4 *Sarcocystis* sporocysts for each calf). The calves were medicated with amprolium at 100 gm kg^{-1} body weight to reduce the acute effects of *Sarcocystis*. Histological findings revealed the presence of two types of *Sarcocystis* tissue cysts ; one form had a thin cyst wall (0.5-1 μm in width), measured 72.5-264×9.9-29.5 μm, the ground substance extended inwards into the cyst in the form of narrow septae which divided the whole cyst into copartments. The other cyst measured 73-155×23-29.5 μm, had a thick cyst wall (2-3 μm in width) which divided the cyst into compartments.

Key words: Camel (*Camelus dromedaries*), body weight, calf

INTRODUCTION

There is a considerable confusion in the literature regarding the speciation of *Sarcocystis* of camels (*Camelus dromedaries*). Mason[1] in his original report on the occurrence of Sarcocystosis in the dromedary described two different types of tissue cysts but he interpreted the two cases to be different developmental stages of the same parasite. Abdel-Ghaphar *et.al.*[2] in their ultrastructural studies of *Sarcocystis* species of the camel in Egypt, described *Sarcocystis* cyst with peculiar structures. Hilali and Mohamed[3] described two types of *Sarcocystis* cysts from camel. Fatani *et al.*[4] described two types of *Sarcocystis* cysts from camels in Saudi Arabia but they isolated one type of *Sarcocystis* sporocysts from dogs experimentally fed cameline meat.

The aim of this stydu was to speciate *Sarcocystis* of camels depending upon morphology of sporocysts which isolated from dogs fed cameline meat and the cysts detected in muscles of experimentally infected camels.

MATERIALS AND METHODS

Experimental animals

Dogs: Seven weaned 4-week-old puppies were reared in a separate clean kennels at the department of pathology and diagnosis, CVR Laboratories. The puppies were kept on pre-boiled cow`s milk mixed with bread throughout the experimental period (75 days).

Six puppies were fed 400 gm minced raw cameline meat (composite of oesophagus, diaphragm, heart and skeletal muscle) collected from camels slaughtered at El-Gedarif city-Eastern Sudan. One puppy was not fed the camel`s meat and kept as a non infected control. Fecal samples were collected daily from each puppy and examined for presence of coccidian oocysts and/or sporocysts using the sugar floatation technique. Sporocysts dimensions were measured by a caliberated compound microscope. Prepatent periods of infection were recorded.

Camels: Four 2-weeks-old weaned calf-camels were purchased from a camel`s market and kept in a clean pens.

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During the experimental period the camels were fed on pre-boiled cow`s milk until six week of age and thereafter they fed on alfa hay and concentrates.

Preparation of inoculum: *Sarcocystis* sporocysts were obtained from an artificial digestion of intestinal mucosae of puppies fed on raw minced cameline meat (29 days post feeding) as described by Box and Smith[5]. The harvested sporocysts were stored at 4°C in 2.5% potassium dichromate until use for infection.

Experimental infection: The sporocysts were washed from potassium dichromate in which they were preserved. Counting of sporocysts was performed using Mcmaster chamber.Three calf-camel was inoculated orally with *Sarcocystis* sporocysts through a stomach tube, each calf received 1×10^4 *Sarcocystis* sporocysts (two types of sporocysts). One calf-camel was not dosed with *Sarcocystis* sporocysts and was left as an experimental control. The calves were medicated with amprolium at 100 kg body weight mixed with the food, the medication began on the day of inoculation and continued daily for 30 consecutive days according to the procedure described by Leek and Fayer[6]. Amprolium was dosed prophylactically to permite the development of immunity while protecting the host from severe disease and rapid death resulting from infection with *Sarcocystis* .

Histological methods: The three infected calf-camels were sacrified 95-97-and 100 days after infection (one calf at each day). Tissue samples from oesophagus, diaphragm, heart, tongue, brain and skeletal muscles were fixed in neutral buffered 10% formalin. Paraffin embeded tissues were cut at 5 µm, stained with haematoxylin and eosin (H and E) and examined by light microscope for detection of *Sarcocystis* cysts.

RESULTS

The puppies started to shed two morphologically different *Sarcocystis* sporocysts 9-13 DAI. A new type of sporocysts was detected and provisionally designated as *Sarcocystis camelocanis* ; the sporocysts measured 16.0 ×9.9-11.5 µm, had an ellipsoidal shape with one side being more convex than the other and had a smooth, colourless walls. Each sporocyst contained four banana-shaped sporozoites and residual body (Fig. 1). The patent period was 37-45 days. The second *Sarcocystis* sporocysts was designated as *Sarcocystis cameli* ; the sporocysts measured 13.2-13.6×6.5-9.5 µm, had an ellipsoidal shape with one side being more convex than the other and had a smooth colourless walls. Each

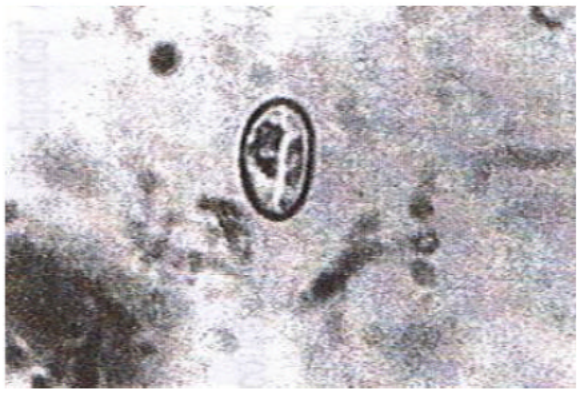

Fig. 1: *Sarcocystis camelocanis* sporocyst from puppies feces X 2000

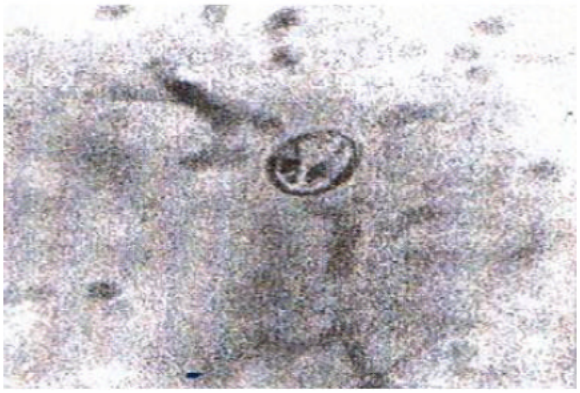

Fig. 2: *Sarcocystis cameli* sporocyst from puppies feces X 2000

Fig. 3: *Sarcocystis* cyst with thin wall of two layers x400 (H and E)

sporocyst contained four over lapping banana-shaped sporozoites with a clear sporocystic residuum (Fig. 2). The patent period was 55-57 days.

Two types of *Sarcocystis* cysts were appeared in the histosections, they were randomly distributed between the skeletal muscle fibres. One of the cysts had

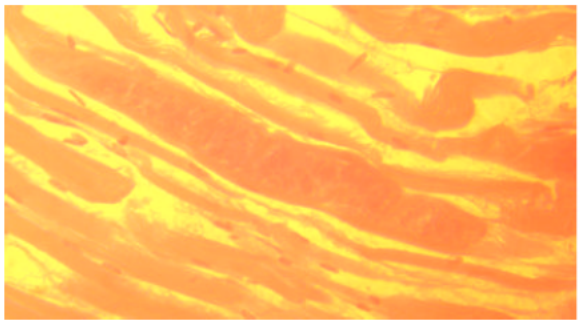

Fig. 4: *Sarcocystis* cyst with thick striated outer and smooth inner layers x400 (H and E)

a thin cyst wall of 0.5 µm, measured 72.5-264×9.9-29.5 µm. The cyst wall was formed of two layers ; an outer layer composed of radial spines and a smooth inner one. The ground substance extended inwards into the cyst in the form of narrow septae, which divided the whole cyst into copartments (Fig. 3). The other *Sarcocystis* cysts measured 73-155×23.0-29.5 µm, it had a thick striated outer and a smooth inner layers cyst wall measured 1-3 µm which divided the cyst into copartments (Fig. 4).

DISCUSSION

This study shows that at least two different species of *Sarcocystis* are transmissible between dromedary and domestic dog. The morphological and biological characteristics of one type of the isolated sporocysts were consistent with those reported by Hilali and Mohamed[5] and Hilali *et al.*[7] for *Sarcocystis cameli*. On the other hand, the second type of sporocysts refered to *Sarcocystis camelocanis*, had a larger dimensions (16.0×9.9-11.5 µm) and a shorter patent period (37-45 days). The differences in morphological and biological characteristics may confirm a presence of another species of *Sarcocystis* transmissible between dog and camels.

Description of *Sarcocystis* tissue cyst given by earlier workers in Egypt indicated the existence of two different types. Mason[1] in his original report of the occurrence of *Sarcocystis* infection in Egyptian camels, described two types of tissue cysts; one had a smooth non striated wall and the other one with a striated wall. Abdel-Gaphar *et al.*[2] described *Sarcocystis* tissue cysts in camels in Egypt that measured 130-180×60-110 µm and had a smooth wall with little or no striations. On the other hand, Hilali and Mohamed[3] described a *Sarcocystis* from camels, the cyst had a thick striated wall (1-2 µm) and measured 33-389×22-33 µm. Fatani *et al.*[4] described two types of *Sarcocystis* cysts from camels in Saudi Arabia but they isolated one type of *Sarcocystis* sporocysts from dogs experimentally fed cameline meat.

In this study two types of *Sarcocystis* sporocysts were isolated from puppies fed on cameline meat and thereby two different *Sarcocystis* cysts were detected in the skeletal muscles of camels which experimentally inoculated with those isolated *Sarcocystis* sporocysts. One of the cysts had a thin cyst wall of 0.5 µm, measured 72.5-264×9.9-29.5 µm the ground substance extended inwards into the cyst in the form of narrow septae, which divided the whole cyst into copartments. The other *Sarcocystis* cysts measured 73-155×23.0-29.5 µm, it had a thick striated outer and a smooth inner layers cyst wall measured 1-3 µm which divided the cyst into copartments.

The validity of the *Sarcocystis* isolated in this study as a new species needs further investigations which are now underway.

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