

On the presence of *Sarcocystis miescheri* sp. nov. in camels of Qena Governorate

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ABSTRACT

It is the 1st time to study sarcocysts of camels in Qena Governorate, 156 specimens of camels (*Camelus dromedarius*) were collected from different slaughtered- houses in Qena Governorate, the rate of infection with *Sarcocystis* was (42.3 %). Out of 126 males, 54 (42.85 %) were infected while the infected number of females were 12 (40 %) from 30 specimens. Morphological and ultrastructures of muscle stage (sarcocyst) have been reported and illustrated in this study by both light and electron microscope, TEM showed that there are two types of *Sarcocystis* in camels in Qena Governorate. Dogs that infected with camel's oesophagus muscles contain mature *Sarcocystis* excreted two different types of oocysts in their feces after 13 – 15 days of infection. Detailed description of oocysts has been reported.

Key words: *Sarcocystis*, camels, dogs, oocysts and prepatent period.

INTRODUCTION

Sarcocystis cysts were first described by (Miescher, 1843) and first named by Lankester (1882), since that time hundreds of species of *Sarcocystis* were discovered in a wide range of hosts such as rodents, cattle, sheep, pigs, goats, camels, reptiles and man, it is cosmopolitan. The morphology of *Sarcocystis* has received more attention, macroscopically, microscopically and ultramicroscopy. The fine structure of *Sarcocystis miescheriana* of buffaloes is described by Ludvik (1960). Mandour (1965a) described *Sarcocystis garnhami* n. sp. from opossum, *Didelphis marsupialis*, also Mandour (1969) described *Sarcocystis neshitti* n. sp. from the rhesus monkey. *Sarcocystis* in camels have been reported by Mason (1910). The life cycle of *Sarcocystis* of camels have been studied by (Hilali & Mohamed, 1980); Kuraev, 1981 and Dubey *et al.*, 1989) but the fine structure of *Sarcocystis* of camels has never been studied by previous authors. For this reason, the present work was suggested to study the electron microscopy of this

parasite. Also the dog was used experimentally to confirm its role in the life cycle of *Sarcocystis* of camels; with detailed study of the oocysts of this species.

Therefor the present work aims to study the morphological and structural characters of the muscle stage (sarcocysts) also, the oocysts passing with feces of experimentally infected dogs and also the ultra-structure of the cyst wall of this species using transmission electron microscopy (TEM).

MATERIALS AND METHODS

Random samples of oesophagus from camels in different ages were collected from freshly slaughtered camels in Qena slaughter house. Muscles of oesophagi were compressed between two slides and examined microscopically for detection of any Miescher's tubes. Pieces of the infected material of about 0.5 x 0.5 cm were taken, then they were fixed in 10 % formal saline for preparation of paraffin sections and were stained with hematoxylin and eosin (H & E). The infected dogs were dissected and

transverse sections about 5 μm thick of small intestine were prepared.

Preparations for electron microscopy:

Small pieces of highly infected muscles were immediately fixed in 3 % glutaraldehyde in 0.1 M cacodylate buffer for at least 4 h at 4° C. Fixed samples were post fixed in 2 % OsO_4 in the cacodylate buffer for 24 h, then washed four to five times in the buffer (10–15 min each). Specimens were dehydrated in graded ethanol, transferred to propylene oxide, and finally embedded in Araldite (sigma) embedding medium. Semi- and ultrathin sections were cut on a Reichert-Jung ultracut microtome. Ultrathin sections were stained with uranyl acetate and lead citrate before examination with a Zeiss A 902 transmission electron microscope.

Experimental infection:

In order to find the definitive host and to study sporogony, laboratory bred and coccidia free 3 young puppies (*Canis familiaris*) being 2-3 months old. Two were fed small segments of highly infected muscles of the camels, one non infected puppy was used as control; puppies used in the experimental infection were usually fed boiled milk and bread and had never been fed meat before infection. These animals were kept alive and examined daily for shedding of any coccidian oocysts for a period of 7 weeks post infection. Fecal samples from both infected and control animals were examined daily for a period of 7 weeks after feeding the dogs with the infected muscles by: Flotation without centrifugation.

RESULTS

About 156 specimens of oesophagi of camels (*Camelus dromedarius*) were collected from different slaughter-houses from different regions in Qena Governorate, Egypt in (2009-2010), 66 (42.3 %) were found infected with *Sarcocystis*.

Only microscopic sarcocysts were observed in the examined camels as shown in fig. (1), cysts measured (229 μm – 2 mm) in length and (77.76–137.85 μm) in width.

Longitudinal and transverse sections were obtained and stained with PAS and H & E stains as in figs (2 &3). And there were no macroscopic cysts. The cysts located within muscle fibers was surrounded by a spiny primary cyst wall which was about (1.7- 2.13 μm) in thickness.

Merozoites in the present study that was obtained from the digested material mixed with human blood and stained with Giemsa's stain were banana shaped with one end more pointed than the other, the nucleus located centrally or shifted towards the less pointed end and measured (21.56 – 32.88 μm) x (7.76 – 17.76 μm) as in fig. (4).

Transmission electron microscope examination of ultrathin sections of different Cysts showed that the primary cyst wall appeared as a thick electron dense layer and there are more than one type of cyst walls

1- Cysts showed small papillae, projecting outwards from the cyst wall as in figure. (5) villi-like protrusions within which fine fibrils as in figures (6).

2- Cysts showed spine-like protrusions with a broad base and with more or less blunt apex, the core of these protrusions contained longitudinally extended microfibrils throughout the whole length of the protrusion as shown in figures (7). While the interior of the cyst contained mostly cyst merozoites (cystozoites) and only a few

Metrocytes lying closely together, the ground substance underneath the primary cyst wall appeared rather homogenous and extended into the interior of the cyst forming thin septa separating the whole cyst into a number of compartments enclosing the metrocytes.

Experimental infection:

The experimental feeding of two puppies (*Canis familiaris*) on infected camel meat started shedding of sporulated oocysts and sporocysts after a prepatent period of 13-15 days, and the patent period extends to the day 22 of infection (7-9 days). The control dog remained negative for any coccidian oocysts during the period of experiment. And the transverse sections in different regions of the small intestine of the infected dog showed the oocyst that contains two sporocysts as in fig. (8).

Oocysts pass into the intestinal lumen and then pass from the body in the feces. Two types of immature oocysts without residum passed in the feces of the infected dogs, oocyst contains two sporocysts, the first type of oocysts is globular, with two oval, elongated sporocysts, lie transverse in their position inside the oocyst, the oocyst wall is thick, oocyst measured (21.62–23.69 μm) with a mean of (22.65) in length and (18.18 – 23.11 μm) with a mean of (20.64) in width as in fig. (9). The second type is ellipsoidal in shape, contains two rounded sporocysts, lie longitudinal in their position inside the oocyst, oocyst measured (20.84–26.79 μm) with a mean of (23.81) in length and (18.5–20.72 μm) with a mean of (19.61) in width as in fig. (10). Sporocysts contain residum and differ in the two types of oocysts. In the first type measured (10.13–13.95 μm x 8.59–9.94 μm) with a mean of (12.04 x 9.26 μm).

And in the second type, they measured (8.7–14.35 μm x 8.48–11.6 μm) with a mean of (11.52 μm x 10.04 μm).

DISCUSSION

This is the first study on *Sarcocystis* of camels by both light and electron microscope and it is the first study on the successful production of the intestinal stages of *Sarcocystis* in dogs in Qena, Upper Egypt. The study included the morphology of the oocysts.

Interpretation on the merozoites

The merozoites in the present study resembled that of Fatani *et al.*, (1996) in shape where both of them were banana shaped with one end more pointed than the other, the nucleus located centrally or shifted towards the less pointed end, but their dimensions are different where in the present study it was longer and wider than in case of Fatani *et al.*, (1996).

Interpretation on the cyst wall

The present cysts cannot be considered as *Hammondia*, *Toxoplasma* or *Besnoitia*. Since the cyst of *Sarcocystis* is divided internally into compartments by septa while *Hammondia*, *Toxoplasma* and *Besnoitia* are not. Mandour, (1964) stated that the structure of the cyst wall is essential to differentiate the different species of the *Sarcocystis*.

Later David *et al.*, (1995) stated that the structure of the *Sarcocystis* wall is the most important feature used in species identification, Light microscopic examination can be used to distinguish some species within an intermediate host, although examination with transmission electron microscopy (TEM) is often necessary. New species should be described on the basis of the ultra-structure of the *Sarcocystis* using transmission ultra microscopy. The structure of the cyst wall of *Sarcocystis* of camels is not identical with that of sheep, cattle or pigs. The second type of cyst wall is only identical with *Sarcocystis garnhami* (Mandour, 1964 in the opossum *Didelphis marsupialis* (kangaroo) from South America. The first type of cyst wall is similar with that of Al-Goraishy *et al.*, 2004.

Interpretation on the final host and oocysts

The definitive host in the case of *Sarcocystis cameli* has been shown to be one of the family: Canidae according to (Hilali and Mohammed, 1980; Kuraev, 1981 and Dubey *et al.*, 1989). After an incubation period multiplication

involving asexual and sexual reproduction, oocysts pass in the feces of the final host according to (Dubey *et al.*, 1989). Mohammed and Hussein, (1983) showed the *Sarcocystis* sporocysts after 15 days of feeding dogs with infected camel meat and this agree with the present study. Fatani *et al.*, (1996) studied the experimental feeding of dogs with camel meat infected with sarcocysts showed that dogs excreted *Sarco-cystis* sp. sporocysts measured (10.7 – 14.3 μm x 8.3 – 10.7 μm) after a prepatent period of 9 - 10 days but in the present study there are two types of oocysts.

When compared with the results of the present study with *Isospora canis* (Hilali *et al.*, 1995) it was found that the two species of oocysts in the present study are smaller than in *Isospora canis*, in addition to the prepatent period and the patent period which in the present study longer than in case of *Isospora canis*.

When compare the results of the present study with *Hammondia heydorni*, Hilali *et al.*, (1995) there are a difference in a prepatent period, patent period and in dimensions of oocysts, where the oocysts in the present study is longer than that in *Hammondia heydorni*.

By comparison the present study with *Sarcocystis cameli* by Hilali *et al.*, (1995), it was found that the patent period may be similar and the measurements of the first type of oocysts in our study is similar with oocysts in case of (Hilali *et al.*, 1995, Mason., 1910 and Fatani *et al.*, 1996). It is worthwhile mentioning that Hilali *et al.*, (1995) study did not refer to any description of the cyst wall that was a very important factor in the identification of *Sarcocystis* species. The second type of oocysts has never been described before. For this reason it might be suggested that it probably result of feeding the new species of *Sarcocystis* (with spiny cyst wall).

For these reasons; the *Sarcocystis* in camels of Qena suggested to be a new species with the following characters:

1. Intermediate host: Camels.

2. Definitive host: Dogs.

3. Cyst size: (229.0 μm – 2 mm) in length and (77.76 μm – 137.85 μm) in width.

4. Habitat: Skeletal muscles.

5. Cyst wall: Showed spine-like protrusions with a broad base and with more or less blunt apex, the core of these protrusions contained longitudinally extended microfibrils throughout the whole length of the protrusion.

6. Trophozoite: It is banana shaped with one end more pointed than the other, the nucleus located centrally or shifted towards the less pointed end, and measured (21.56 – 32.88 μm) in length and (7.76 – 17.76 μm) in width.

7. The prepatent period: 13 – 15 days.

8. The patent period: 7 – 9 days.

9. Oocysts and sporocysts: Immature oocysts pass in the feces of the infected dog, each oocyst contains two sporocysts, oocysts were ellipsoidal with thin visible wall, lack micropyl, polar granule and residu-um. Oocysts measured (20.84 – 26.79 μm) with a mean of (23.81) in length and (18.5 – 20.72 μm) with a mean of (19.61) in width, Sporocyst: measured 8.7 – 14.35 x 8.48 – 11.6 with a mean of (11.52 x 10.04).

Locality: Qena (Upper Egypt).

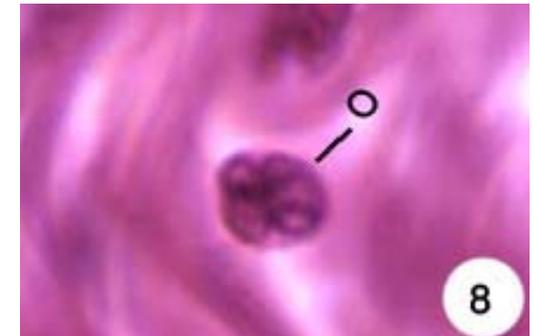
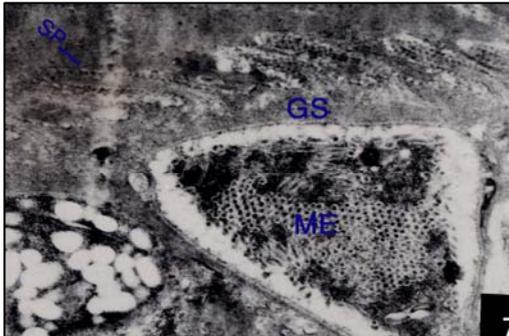
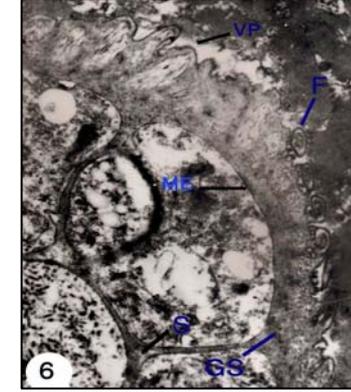
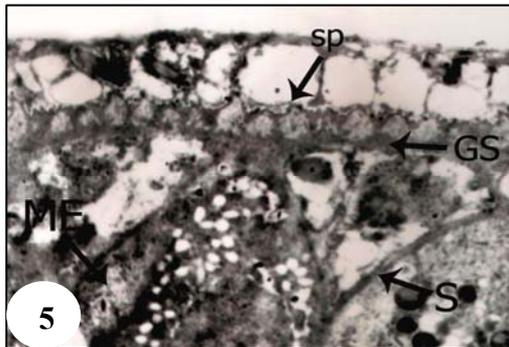
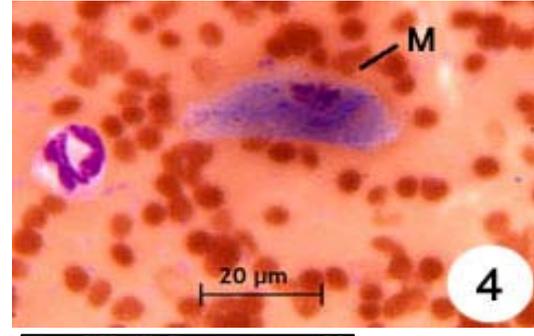
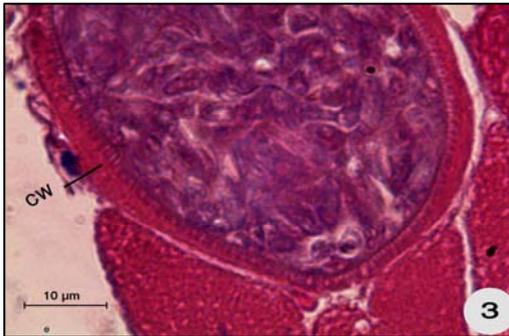
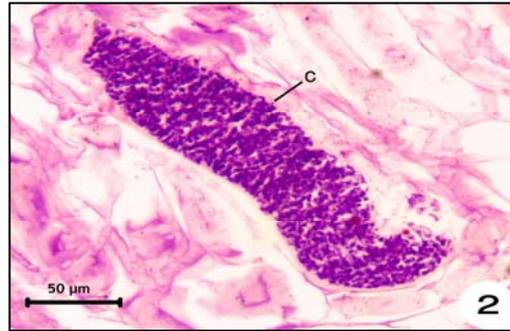
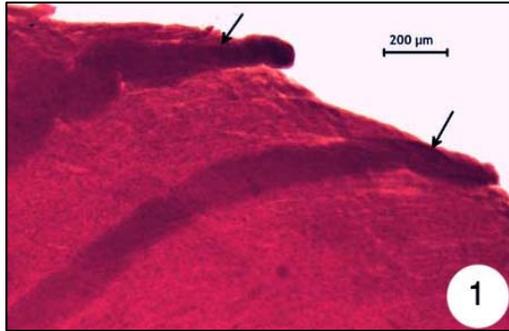
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FIGURE LEGENDS

- Fig. 1: Microphotograph of whole mount stained with potassium alum carmine stain showed the sarcocysts.
- Fig. 2: Microphotograph of longitudinal sections with thickness 5 μm of sarcocysts stained with PAS stain showed that sarcocysts are elongate (C).
- Fig. 3: Microphotograph of transverse section with thickness 3 μm stained with H & E stain showed sarcocyst within muscle fibers and surrounded by a spiny primary cyst wall (CW).
- Fig. 4: A smear of digested material mixed with human blood stained with Giemsa's stain showed the merozoite (M) with one end more pointed than the other; the nucleus was located centrally or shifted towards the less pointed end.
- Fig. 5: Transmission electron micrograph of the cyst showed small papillae (SP), projecting outwards from the small cyst wall, and showed that the ground substance (GS) underneath the primary cyst wall appeared rather homogenous and extend into the interior of the cyst forming thin septa (S) separating the whole cyst into a number of compartments enclosing the metrocytes (ME).
- Fig. 6: Transmission electron micrograph of another cyst showed villi-like projections (VP) within which fine fibrils (F) and, also showed the ground substance (GS), septa (S) separating the whole cyst into a number of compartments enclosing the metrocytes (ME).
- Fig. 7: Transmission electron micrograph of different cyst showed that the primary cyst wall appeared as a thick electron dense layer, this layer was folded giving rise to successive spine-like protrusions (SP) and their surface was not branched, The ground substance (GS) underneath the primary cyst wall appeared rather homogenous and extend into the interior of the cyst forming thin septa separating the whole cyst into a number of compartments.
- Fig. 8: Microphotograph of transverse section of small intestine of infected dog stained with H & E stain showed the presence of oocyst contains two sporocysts inside the intestine.
- Fig. 9: Showed that the two sporocysts lie transverse in their location inside the oocyst.
- Fig. 10: Showed that the two sporocysts lie longitudinally in their location inside the oocyst, and the sporocysts are rounded in shape.



ARABIC SUMMARY

وجود نوع جديد من الساركوسيست (ساركوسيست ميشاراي) فى الجمال بمحافظة قنا

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٣- كلية العلوم التطبيقية - جامعة أم القرى – المملكة العربية السعودية

لقد تم دراسة ساركوسيست الجمال لأول مرة فى محافظة قنا حيث تم تجميع ١٥٦ عينة من مناطق مختلفة من محافظة قنا، ١٢٦ عينة من الذكور و ٣٠ عينة من الإناث وقد كان عدد العينات المصابة من العدد الكلى ٦٦ وهذا العدد يمثل نسبة ٤٢,٣% بينما كان العدد المصاب من الذكور ٥٤ عينة بنسبة ٤٢,٨٥% أما عدد الإناث المصابة فكان ١٢ عينة بنسبة ٤٠,٠%. وتم دراسة وتوضيح الشكل المورفولوجى والتركيب الدقيق لمرحلة العضلات (الساركوسيست) بالميكوسكوب الضوئى والإلكترونى. حيث وضح الميكروسكوب الإلكتروني النافذ وجود نوعين مختلفين من الساركوسيست. وكذلك تم فى هذه الدراسة إصابة كلبين بأجزاء من مرىء الجمال التى تحتوى على ساركوسيست ناضجة وتم فحص الأوسيست الناتج فى براز الكلاب المصابة فوجد نوعان من الأوسيست بعد ١٣- ١٥ يوم من الإصابة وتم دراسة ووصف كل منهما.