

Cystoisospora orlovi (EIMERIORINA: SARCOCYSTIDAE) - A LITTLE KNOWN COCCIDIAN OF THE OLD WORLD CAMELIDS

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ABSTRACT

Based on the morphology of its oocysts *Cystoisospora orlovi* was described in camel calves in Kazakhstan in 1950. Over decades *C. orlovi* was considered a pseudo-parasite until alterations caused by this parasite in the colon of dromedary calves were described. During an observation period of 150 months (January 2005 – June 2017), 85 cases of coccidiosis due to *C. orlovi* were observed in 72 camel calves and 13 adult dromedaries in Dubai (United Arab Emirates). Most of the cases in camel calves were seen between the months of December to May in an age group of up to 35 days while the majority of cases in adult camels occurred in summer. Contrary to *Eimeria* infections in camelids, *C. orlovi* causes a diphtheroid colitis with multiple erosions of the mucosa of the large intestine. Most of the cases were diagnosed post mortem. Only 11 camels in which the parasite was diagnosed *intra vitam* survived. The sporadic occurrence of cystoisporosis in single suckling calves suggests a vertical transmission of the parasite from mother to calf.

Key words: *Cystoisospora orlovi*, coccidia, dromedary, Dubai, United Arab Emirates

During a survey on camel coccideans in the Almaty district of Kazakhstan, Cygankov¹ (1950) discovered a previously unknown species for the camel with oocysts containing two sporocysts. The new coccidian was named *Isospora orlovi*. It was found in 10 out of 19 camel calves in an age between 10 to 35 days. The validity of *I. orlovi* was doubted by protozoologists (Pellerdy, 1965; Kheysin, 1972; Levine, 1985) who regarded it as pseudo-parasite² of camels. A further finding of *Isospora* sp. in camels morphologically matching *I. orlovi* was made in India by Raisinghani *et al* (1987). *Isospora* oocysts described in camels in Syria by Dariush and Golemanskij (1993) had other dimensions and were, due to the presence of Stieda bodies, attributed to spurious parasites (Duszynski *et al*, 2001).

Kinne *et al* (2001) described for the first time alterations in the colon of camel calves in Dubai (UAE) caused by *Isospora* sp. suggesting that this

parasite was identical with *I. orlovi*. Based on these findings the material was used for a redescription of *I. orlovi* (Kinne *et al*, 2002). First cases of *I. orlovi* in camel calves from Rift Valley Province in Kenya were described by Younman *et al* (2002). Further cases were reported from the same locality by Bornstein *et al* (2008).

Morrison *et al* (2004) sequenced the 18s rRNA subunit and placed *I. orlovi* in one clade with the human pathogen *I. belli* in direct neighborhood with *I. suis* and *I. felis*. Based on morphological peculiarities and supported by molecular examinations the paraphyletic genus *Isospora* was split into 2 monophyletic genera: *Isospora* found in birds and *Cystoisospora* that infect mammalian hosts (Barta *et al*, 2005).

The aim of this paper is to summarise our experience with *Cystoisospora orlovi* in dromedaries in the UAE obtained over a 12½ year's period.

Materials and Methods

A total of 76,969 and 2,885 faecal samples of adult dromedaries and camel calves, respectively were examined at the Central Veterinary Research Laboratory (CVRL) in Dubai during a time period of 150 months (January 2005-June 2016). While the

1. There are several versions of spelling of the authors name: Zigankoff (Pellerdy, 1965), Tsygankov (Levine, 1985) but the right transcription from the Russian original reference would be Cigankov.

2. A pseudo-parasite or spurious parasite is a development stage of an apparent parasite that entered the alimentary tract by food or water and is passed with faeces.

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majority of these samples were sent for a routine parasitological checkup, 2,084 originated from necropsied camels (996 adults and 1,088 calves).

During the routine necropsy, organ samples including pieces of the large and small colons were taken and fixed in 10% neutral formalin, dehydrated through graded alcohols and embedded in paraffin wax. Sections (3 µm) were cut and stained with haematoxylin and eosin (HE) and Periodic acid schiff (PAS) and examined under the microscope (Olympus BX 53).

Faecal samples or content of the colon taken per rectum were used for parasitological examination. Faecal samples were processed with the flotation method in centrifugation tubes using saturated NaCl/ZnCl₂ solution (density 1.24). Carbol fuchsin stained faecal smears (Potters and van Esbroeck, 2010) from suckling calves and diarrhoeic samples were examined in addition to exclude *Cryptosporidium* oocysts. In addition, scrapings from colon mucosa were examined by direct microscopy.

For morphometric studies, 10 positive faecal samples were suspended in a 2.4% aquatic potassium dichromate solution ad kept in petri dishes for one week at 25°C. Ten sporulated oocysts were microscopically measured from each sample. For this, one ml of the faecal suspension was then mixed with 12 ml of a concentrated ZnCl₂/ NaCl solution in a plastic tube covered by a cover slip. After 5 min, the coverslip was removed and placed on a glass slide

for microscopical examination. Measurements were taken with a microscope (Olympus BX 51) connected via a camera (Olympus DP 27) to a computer operated by the software "cellSense Dimension". The length and width of 100 oocysts and containing sporocysts were measured. In addition, 10 unsporulated oocysts freshly harvested from colon mucosa were also measured.

In order to establish the source of infection, the mother and all calves of the group that were in contact with 3 positive individuals were coproscopically checked in 2008, 2014 and 2016.

On two separate occasions, two adult female dromedaries were orally infected with 200,000 and 1,000,000 sporulated oocysts in 2010 and 2017, respectively. For a period of 3 weeks, faecal samples of these camels were examined daily for the presence of *Cystoisospora* oocysts.

Results and Discussion

During the 150 months' observation period *C. orlovi* was detected in faecal samples of 72 camel calves and 13 adult camels (Table 1). These samples, originated from 70 carcasses (64 calves, 6 adult camels) sent for necropsy and 15 from live camels (8 camel calves, 7 adult camels) suffering from diarrhoea. All calves were born in Dubai; of the 13 adult camels, 7 originated from the UAE, 6 others were imported from Saudi Arabia and Sudan. The majority of positive samples (n = 64) originated from The Emirates Industry for Camel

Table 1. Frequency of *Cystoisospora orlovi* positive samples in camel calves and adult camels in Dubai between January 2005 and May 2017.

Year	Camel calves				Adult camels			
	Examined	Positives			Examined	Positives		
		Total	EICMP	Other farms		Total	EICMP	Other farms
2005	25	0	0	0	5,540	0	0	0
2006	578	3	0	3	11,702	0	0	0
2007	399	3	1	2	11,301	0	0	0
2008	403	6	1	5	12,223	3	1	2
2009	86	4	1	3	11,474	0	0	0
2010	121	12	9	3	3,212	9	9	0
2011	191	13	12	1	3,174	0	0	0
2012	125	4	3	1	3,964	1	1	0
2013	114	0	0	0	2,718	0	0	0
2014	503	5	5	0	4,258	0	0	0
2015	145	4	3	1	3,182	0	0	0
2016	124	12	12	0	3,900	0	0	0
2017	71	6	6	0	321	0	0	0
2005-2016	2,885	72	53	19	76,969	13	11	2

Table 2. Distribution of *C. orlovi* in camel calves and adults throughout seasons of the year.

Age group	Month											
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Calves	17	17	16	7	5	1	2	0	0	1	1	5
Adults	1	0	0	1	0	3	6	0	2	0	0	0

Milk Products (EICMP), a large scale camel dairy farm that started operation in 2006 and has currently, a camel population of 4,500 animals. The remaining 21 positive samples came from camels of traditional breeding farms in Dubai. *Cystoisospora* cases were unequally distributed throughout the observation period. As seen from table 1, the parasite was not diagnosed in 2005 and 2013 while in 2010, 2011 and 2016, 21, 13 and 12 positive cases were seen. Most positive cases in calves occurred during winter and spring between December and May while in 11 out of 13 adult camels the parasite was diagnosed in June (n=3), July (n=6) and September (n=2) (Table 2).

In an early stage of the infection examination of faecal samples gives a negative result but unsporulated oocysts and zygotes can be seen in direct microscopy of mucosal scrapings (Fig 1). In the majority of cases however, oocysts were already in an advanced stage of sporulation. Due to the thin (1 μm), smooth, two-layered oocysts wall, the shape of the oocysts (ellipsoid, ovoid or 8-shaped) was determined by the configuration of the 2 sporocysts within the oocyst (Fig 2, 3). Sporulated oocysts had an average length of 31.3 (range: 27.0-34.8) μm and a width of 20.8 (range: 17.8-24.4) μm . Micropyle, polar granule and oocyst residuum were absent. The ellipsoidal shaped sporocysts were 18.4 (range: 15.0-21.9) μm long and 14.9 (range: 12.6-19.6) μm wide. Stieda, substieda and parastieda bodies were absent.

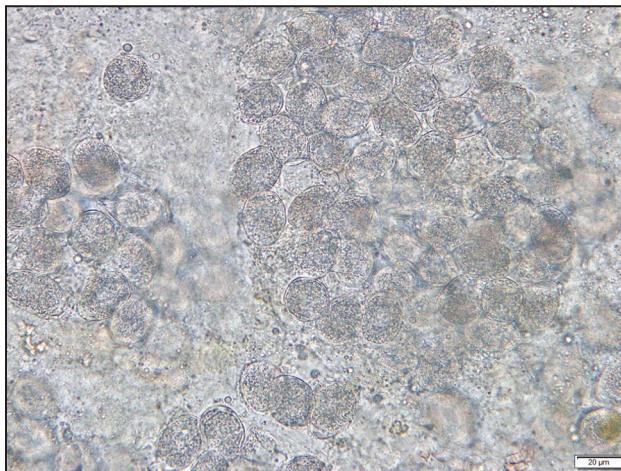


Fig 1. Unsporulated oocysts in a mucosal scraping from small colon.

The subsequent length/width indices for oocysts and sporocysts were 1:1.5 and 1:1.1, respectively. Sporocysts contained 4 elongated, banana shaped sporozoites (12-15 \times 4.5-5 μm) with rounded ends and a sporocyst residuum formed by cluster of small granules. Most of the unsporulated oocysts were oval in shape. Their average length and width was 28.6 (range: 25.4-30.2) and 20.5 (range: 19.4-21.5) μm , respectively.

Most cases of cystoisosporosis were diagnosed post mortem. Only 6 calves and 5 adults from EICMP farm in which the parasite was diagnosed *intra vitam* survived. The fate of 2 adult camels and 1 calf from other Dubai farms could not be followed further.

Alterations caused by *C. orlovi* were concentrated in the colon that contained crumbly yellowish to grey content (Fig 4). A diphtheroid to haemorrhagic colitis with multiple erosions in the mucosa (Fig 5) is pathognomonic for *C. orlovi* infections. At the end of the prepatent period, micro- and macrogamonts can be spotted in histological sections of the colon (Fig 6). During the patent period histocuts contain a large amount of sporulated and to a lesser extent unsporulated oocysts (Fig 7).

For 48 camel calves born on the EICMP farm, the exact age was submitted with the requisition form (Table 3). Thus, in 85.5% *C. orlovi* was diagnosed in calves at an age of up to 35 d. The youngest calf

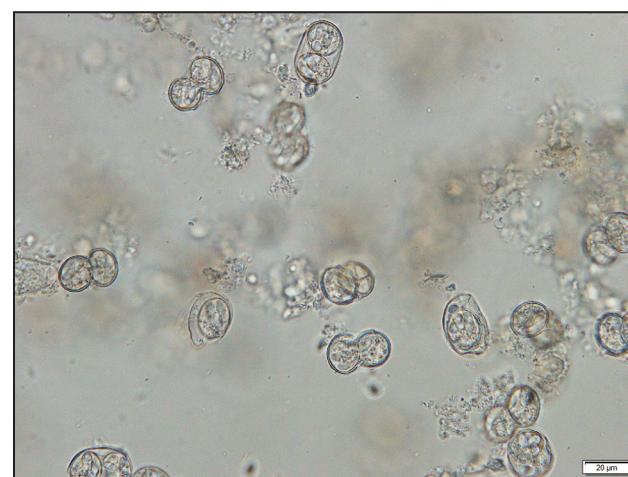


Fig 2. Sporulated *C. orlovi* oocysts. Oocyst contain 2 ellipsoid sporocysts the configuration of which determines the shape of the oocyst.

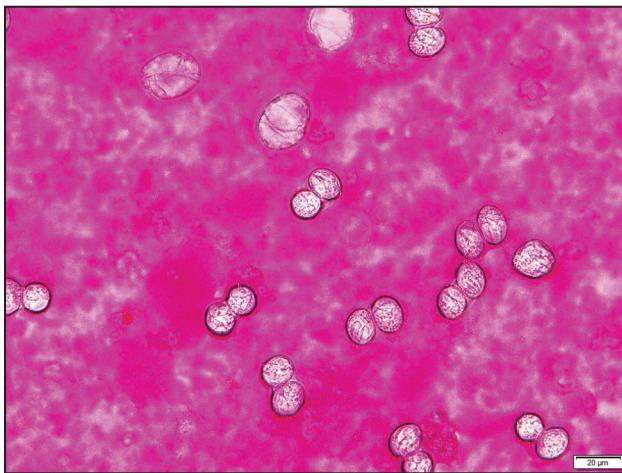


Fig 3. Sporulated and unsporulated *C. orlovi* oocysts in a fresh carbol fuchsin stained faecal smear.



Fig 4. The small colon of a *C. orlovi* infected camel calf with crumbly grey content.

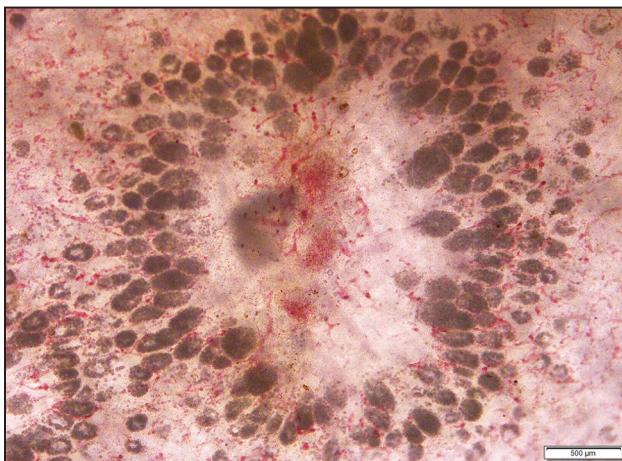


Fig 5. Mucosa of the colon with erosions and bleedings is pathognomonic for *C. orlovi* infections.

diagnosed with a *Cystoisospora* infection was 9 d old while the oldest calves were 115, and 150 d old. The age of the adult camels was between 4 and 8 years. It is noteworthy that all 5 fatal cases in adult camels



Fig 6. Histological sections of the small colon with microgamonts (a) and macrogamonts (b).

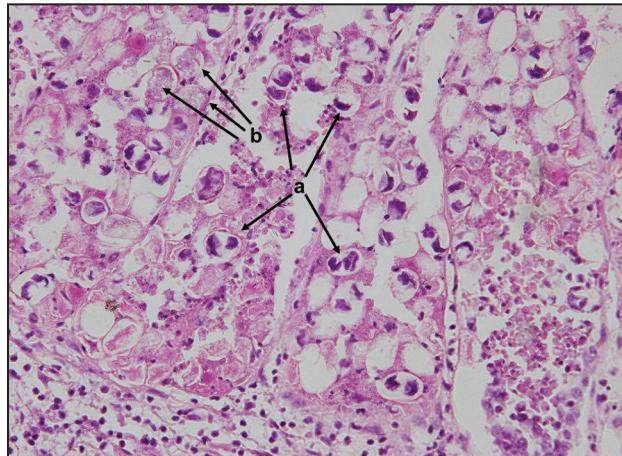


Fig 7. Histological sections of the small colon of a camel calf that died with a patent infection. Multiple sporulated (a) and unsporulated (b) oocysts can be seen between the villi.

in 2010 had severe concurrent bacterial infections (*Rhodococcus*, *Corynebacterium*, *Mycoplasma*) or suffered from clostridial enteroxemia.

Table 3. Age of *Cystoisospora orlovi* infected camel calves.

Age in days	Number of positives	
	n	in (%)
< 14	1	2.1
14 - 21	13	27.1
22 - 28	18	37.5
29 - 35	9	18.8
36 - 42	2	4.2
43 - 49	1	2.1

Coproskopical examination of the mothers and contact calves of three *Cystoisospora* positive calves gave negative results. None of these 25 samples revealed the presence of *Cystoisospora* oocysts. Also, the oral inoculation of 2 adult female camels with

sporulated *Cystoisospora* oocysts did not result in shedding of oocysts during the observation period of 21 days.

In the first description of *C. orlovi* by Cygankov (1950) the host species Bactrian or dromedary camel was not mentioned. Both species of Old World camels are kept in Kazakhstan. All later findings in India, UAE and Kenya however, were made in dromedaries.

In our material, most cases occurred during the main calving period in winter and spring (December to May) mainly in calves at an age between 2 and 5 weeks with a history of diarrhoea. Kinne *et al* (2001) reported 8 fatal cases of camel cystoisosporosis occurring between January and March 2001 in traditional camel farms in Dubai. The age of the calves was estimated to be 4 to 8 weeks. Similar findings were made in Kenya (Bornstein *et al*, 2008), where the parasite was diagnosed in 19 to 30 day old camel calves in ranched herds. The age of infected calves in pastoral herds in Kenya was 20 to 56 days. The excretion of *C. orlovi* oocysts is not compulsory restricted to young calves since we saw the parasite also in single calves at an age of up to 5 months. Also, the only camel calf from India that was diagnosed with *C. orlovi* was 6 months old (Raisinghani *et al* (1987) and our material included findings of *Cystoisospora* oocysts also in 13 adult camels.

Contrary to *Eimeria* spp. *C. orlovi* occurs sporadically mainly in young suckling calves and was, surprisingly detected so far only in 4 countries. Despite several attempts, the source of infection was not determined. A horizontal transmission as known for *Eimeria* infections can be excluded since contact calves and the calf mothers were coproscopically negative for *C. orlovi* oocysts.

In *Cystoisospora* species of carnivores extra-intestinal monozoic cysts occur in lymph nodes, liver, spleen or muscles of paratenic (rodents and farm animals) as well as of definitive hosts and play a role in the life cycle of these coccidians.

The porcine *C. suis*, the best investigated representative of the genus, has a direct life cycle (Harleman and Meyer, 1983). The two-peaked excretion of oocysts after a single experimental infection however, suggested the existence of extra-intestinal development stages in piglets. So far, these stages have not been visualised. Still, gnotobiotic piglets shed oocysts after intraperitoneal inoculation of tissue homogenates from experimentally infected piglets (Shrestha *et al*, 2015). Extra-intestinal stages were also described in the human pathogen, *C. belli*

(Lindsay *et al*, 2014). The close genetical relation between *C. belly* and *C. orlovi* (Morrison *et al*, 2004) suggested the existence of extra-intestinal stages also in *C. orlovi*.

In our opinion, it must be considered that the transmission of *C. orlovi* is vertically from mother to calf, probably via milk and, that an ingestion of *C. orlovi* oocysts by adult camels does not lead to a patent infection but most probably leads to a dissemination of sporozoites in extra-intestinal tissues. Stress situations like calving could activate these stages. Rare cases of cystoisosporosis in adult camels during the hot summer months might be attributed to heat stress or to the depression of the immunity due to other conditions as it was the case in 5 adult camels in 2010.

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