Prevalence of *Sarcocystis* infection in camels (*Camelus dromedarius*) slaughtered at Addis Ababa abattoir, Ethiopia

Berhanu Mekbib

Faculty of Vetrinary Medicine,
Hawassa University, P.O.Box 05, Hawassa, Ethiopia

Abstract

Camels are very important animals for the livelihood of pastoralists in arid and semi-arid environments, but are highly neglected and poorly studied. A cross-sectional study was conducted from October 2018 to May 2019 to estimate the prevalence, associated risk factors and tissue distributions of *Sarcocystis* in one-humped camels slaughtered at Addis Ababa abattoir enterprise (Akaki branch). Esophagus, diaphragm and heart tissue samples were collected from 166 slaughtered camels and examined histopathologically for presence of *Sarcocystis*. Of the total camels examined, *Sarcocysts* were detected in 51 (30.72%) camels. Relatively higher proportions of males (33.33%), young ones (33.33%), those originated from Minjar (35.71%) and camels with good body condition (43.39%) had *Sarcocystis* infection compared to other categories. However, except for body condition score ($p = 0.039$), other considered risk factors did not show significant difference with the prevalence of *Sarcocystis* infection. The infection rate of esophagus, diaphragm and heart were 19.87%, 14.46% and 13.25%, respectively. Moreover, multiple tissue infections by this parasite were detected in 26 (51%) camels of the 51 Sarcocystis positive camels. In general, the observed high prevalence of camel *Sarcocystis* infection in the present study, coupled with lack of information on the public health and economic significance, warrants further investigation and community awareness creation about the control measures.

Key words: Addis Ababa abattoir, *Camelus dromedarius*, Prevalence, Risk factors, *Sarcocystis*.

Corresponding author's address: Berhanu Mekbib email: berhanumm2002@gmail.com**,**Tel**:**(+251) 92 630 8148

INTRODUCTION

One-humped Camels (*Camelus dromedarius*) are contributing to the livelihoods of large human population in marginal and arid areas of Africa, particularly in the arid lowlands of Eastern Africa namely, Somalia, Sudan, Ethiopia, Kenya and Djibouti. The roles they play towards resilience to the present climate change make them the preferred domestic animal species and are towards an increase (Behnke, 2010).

Ethiopia has been considered to have a large camel population, which ranks third in Africa next to Somalia and Sudan (FAO-OIE-WHO, 1993). However, the exact data on camel population in the country appears to be debatable as reports show variable figures where estimates were as high as 4.8 million (Behnke, 2010) while official government livestock data showed 1.2 million (CSA, 2017). Camels are widely distributed in the Southern, Eastern and Northeast lowland areas of the country (Mirkena et al., 2018) and play an important role as a primary source of subsistence through milk and meat production, draught power, transportation service and source of cash (Kissi and Assen, 2017). Recently, camels have become one of the national export animals to the Middle East countries (Tefera and Abebe, 2012). Although camel production was challenged by several factors including diseases, infections with *Sarcocystis* species are widely known to cause considerable economic losses due to condemnation of edible organs and decreased meat production and quality (Romazanvoc, 2001).

Sarcocystis species are intracellular protozoan parasites of the phylum Apicomplexa which affect the skeletal and cardiac muscles of man and many species of animals worldwide (Fayer et al., 2015). The parasite requires two hosts to maintain its lifecycle, an intermediate or prey host (herbivores) and final or predator host (carnivores). One humped camels are the intermediate hosts for *Sarcocystis cameli* and become infected after ingestion of the sporulated oocysts passed in the feces of carnivores (mainly dogs) as the final hosts (Stojecki et al., 2012; Fayer et al., 2015). Although the economic and public health impacts of *Sarcocystis* infection in camel are not fully understood, some researchers

speculate that heavy infection with *Sarcocystis* spp can result in loss of weight, anemia, abortion and even death in cases of very heavy infection (Valinezhad et al., 2008; Hamidinejat et al., 2013).

Despite the huge potential of one humped camels and their role in the livelihoods of pastoral communities and national economy, still very little attention has been given to camels' production and health care (Megersa, 2010). Except one study conducted about a decade ago (Woldemeskel and Gumi, 2001), information on the current prevalence of *Sarcocystis* infection in camels is lacking at national level. Therefore, the present study was designed to estimate the prevalence, identify the associated risk factors and assess tissue distribution patterns of *Sarcocystis* infection in camels slaughtered at one branch of Addis Ababa abattoir enterprise.

MATERIALS AND METHODS

Study Area

The study was conducted from October 2018 to May 2019 at Akaki abattoir, which is owned by the Addis Ababa abattoir enterprise and located in Addis Ababa city, the capital of Ethiopia. Although the camel meat is not popular in Addis Ababa, camels are slaughtered for the Somali and other Muslim communities who live in the city. The camels slaughtered in the abattoir originated from Borana and Kereyu pastoral areas and Minjar-Shenkora district.

Borana pastoral area is located at approximately 600 km South of Addis Ababa at an altitude ranges from 970 m.a.s.l in the south bordering Kenya to 1693 m.a.s.l. in the Northeast. The area is characterized by an arid and semi-arid climate, with pockets of sub-humid zones. The rainfall in the area is bimodal where the average annual rainfall varies between 350 mm and 900 mm. The rainfall of the area is erratic by nature and there are four distinct seasons interspersed by long rainy season (expected between March and May) and the short rainy season (between October and November) (Galma, 2015).

Kereyu Pastoral area, circumscribed in Fentale and Boset districts, is located at about 250 km East of Addis Ababa at an altitude of 930 m above sea level. The Kereyu pastoralists occupy the arid lands around the Awash River down in the rift valley for pasture for their cattle, goats and camel (Tefera and Abebe, 2012). It has an average annual rainfall of 504 mm. The mean annual maximum and minimum temperature are 32.40 and 18.5°C, respectively. Pastoralism and agro-pastoralism are the main livelihood systems in the area.

Minjar-Shenkora is one of the districts in the Amahara Regional state of Ethiopia, located at the southern end of the North Shewa Zone at about 129 km East of Addis Ababa. The district is bordered on the east, south and west by the Oromia Regional state and on the northwest by Hagere Mariam. Its altitude ranges from 1040 to 2,380 meters above sea level. The average temperature ranges from 14 ºC to 27 ºC while the annual rainfall ranges between 780 and 900 mm. The district is known with its scattered bushes, shrubs and acacia trees (Setotaw et al., 2014).

Study Population

The study population included the total number of camels slaughtered at Akaki abattoir. Camels purchased from different markets were transported to the abattoir by trucks and kept at lairage for 3 to 4 days.

The camels in the pastoral area (their original sites) brows on bushes and shrubs, but may rarely consume grass when shrubs or trees are not available. The browse species includes the family *Chenopodiaceae*, *Acacia brevispica*, *Opuntia ficus indica*, *Dichrostachys ciniarea* and *Euphorbia tirucalli*. Rivers, ponds and wells are the main sources of water for camels. The watering sites are usually visited by large numbers of camels and other animals at a time from the surrounding as well as from distant areas. The pond and river water sources are also shared by wild animals (Mirkena et al., 2018).

Study Animals and Sample Size

The study animals were selected from the study population using convenient sampling method. Since the abattoir usually slaughters on average six to eight camels per day, sampling was made once per week for 6 months. Accordingly, a total of 166 apparently health camels were selected for this study irrespective of their origin, sex, body condition and age. Data about the age, sex, origin and body condition score of the selected animals were recorded before slaughtering. The age of the camels was estimated using rostral dentition (Bello et al., 2013) and then categorized as young (less than 5 years) and old (≥ 5 years of age) for ease of data analysis. The body condition score of the camels

were assessed according to Faye et al. (2001) and then grouped as poor (score 1), medium (score 2 and 3) and good (score 4).

Study Methodology

Post-mortem Examination.
Following slaughter and evisceration, the cardiac muscle, esophagus and diaphragm were examined for macroscopic sarcocysts using visualization, palpation and multiple incisions, when required. The pathological lesions were differentiated according to the guidelines on meat inspection for developing countries (Herenda et al., 1994).

Histopathological examinations
Representative tissue samples from oesophagus, diaphragm and heart of 166 slaughtered camels were collected and fixed with 10% neutral buffered formalin solution. The samples were labeled immediately and transported later to the parasitology and pathology laboratory of the Faculty of Veterinary Medicine, Hawassa University. In the lab, the specimens were trimmed, washed with water, dehydrated in ascending series of ethanol (70%, 80%, 85%, 90% and 99%), cleared in xylene, and embedded in paraffin. Sections of 5 µm thickness were prepared, stained with Hematoxylin-Eosin (HandE) stain and then examined under light microscope (Makhija, 2012).

Statistical Analysis
Data were entered into Microsoft Excel spreadsheet coded and then analyzed using STATA statistical software (STATA, 2013; window version 13.1). Association between various risk factors (sex, age, origin and body condition score) and the prevalence of *Sarcocystis* infection was estimated using chi-square independent test and one way ANOVA. In all the analysis, significance was set at $p< 0.05$.

RESULTS

The overall prevalence of *Sarcocystis* infection in camels in the current study was 30.72% (51 out of 166 camels). All of the cysts observed in the examined tissue were microscopic (Fig. 1 and 2) and had morphological difference on their wall, some were thick walled (Fig. 3a) and the majorities were thin walled (Fig. 3b).

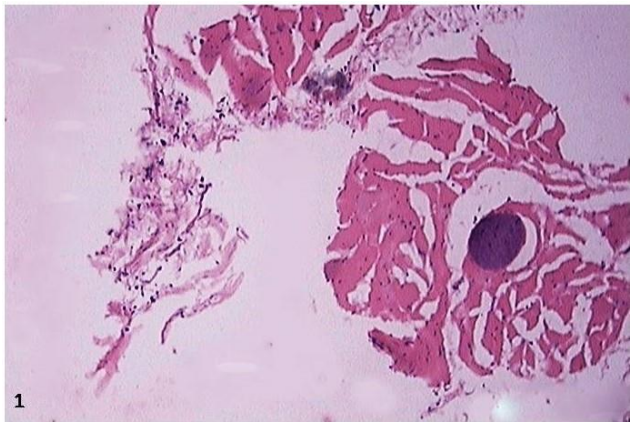

Figure 1. Cardiac muscle containing oblong shaped *Sarcocystis*, Haematoxylin and Eosin stain, 10X Objective magnification.

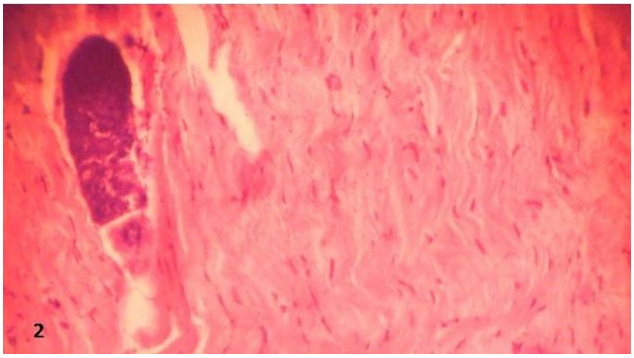

Figure 2. Muscle tissue from esophagus containing oblong shaped *Sarcocystis*, Haematoxylin and Eosin stain, 40X Objective magnification

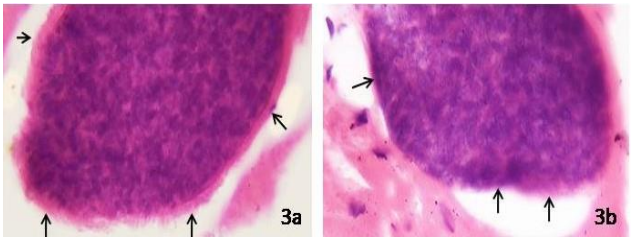

Figure 3. Higher magnification of *Sarcocystis* with thick wall (arrow) (a) and *Sarcocystis* with thin wall (arrow) (b). Haematoxylin and Eosin stain, 100X Objective magnification

Relatively higher prevalence of infection was observed in male camels (33.33%), young camels (33.33%), camels originated from Minjar (35.71%), and camels with good body condition (43.39%) than in other categories. However, with the exception of the body condition score ($p = 0.039$), the difference in the prevalence of *Sarcocystis* infection between or among the categories of the other considered risk factors (origin, age and sex) were not statistically significant ($p> 0.05$) (Table 1).

Table 1. Prevalence of *Sarcocystis* infection in camels by the putative risk factors

Variable		№ examined	№ (%)positive	**F**/χ2	p value
Origin	Borana	62	21 (33.87)	1.01	0.368
	Kereyu	62	15 (24.19)		
	Minjar	42	15 (35.71)		
BCS*	Poor	61	17 (27.87)	3.31	0.039
	Medium	52	11 (21.15)		
	Good	53	23 (43.39)		
Sex	Female	64	17 (26.56))	0.847	0.357
	Male	102	34 (33.33)		
Age group	Young	75	25 (33.33)	0.438	0.508
	Old	91	26 (28.57)		
Overall		166	51 (30.72)		

*BCS = body condition score

Out of the 166 tissues each of esophagus, diaphragm and heart tissue examined histopathologically, the cysts were observed in 33 (19.87%), 24 (14.46%) and 22 (13.25%) tissues, respectively. The cysts were observed concurrently in two or three tissues of 26 (51%) of the 51 *Sarcocystis* positive camels. There was no statistically significant difference (p = 0.0758) in detecting *Sarcocystis* between the tissues examined; the higher being in esophagus (Table 2, Fig. 2).

Table 2. Frequency and proportion of *Sarcocystis* in different tissues (n=498) of camels examined

Tissue /organ	No positive	Proportion	95% CI
Oesophagus	33	19.87	13.75–26.01
Diaphragm	24	14.46	9.05–19.86
Heart	22	13.25	8.04–18.46
Over all (Average)	79	15.86	12.76–19.37

DISCUSSION

The prevalence of *Sarcocystis* reported in this study (30.7%) is lower than the previous reports made in Ethiopia (45.5%; Woldemeskel and Gumi, 2001) and other countries like Saudi Arabia (88.35%; Fatani et al., 1996), Iran (83.6%; Valinezhad et al., 2008; 51.5%; Hamidinejat et al., 2013) and Iraq (91.6%; Latif et al., 1999). The difference in the prevalence of *Sarcocystis* in camels between the present study and other previous reports could be due to the variations in animal husbandry, the ecology and the sensitivity of the diagnostic methods used in each study. In Ethiopia, since there is no grazing grass land or pasture intentionally kept for camels and the camels are left to browse on the bush and shrubs, the chance of infection with sporulated oocyst/usually sporocysts/ is very minimal except at the watering points. However, the large number of sentinel dogs kept by the pastoralist and the wild carnivores such as hyenas, wolves, and jackals are known to contaminate the pastures and especially the drinking water with *Sarcocystis* sporocysts (Valinezhad et al., 2008).

The absence of macroscopic *Sarcocysts* in the present study could be partly explained by the inherent nature of the *Sarcocystis* species affecting camels. Moreover, since there is a very rare to no trend of keeping cats by most pastoralists in the country, there is no probability of detecting macroscopic sarcocysts as they are feline origin (Nourollahi-Fard et al., 2015). The microscopic cysts encountered during the current study were both thin and thick walled. In line with this, both thin-walled and thick-walled sarcocysts were reported by Fatani et al. (1996) and Dubey et al. (1989) from camels in Saudi-Arabia and Egypt, respectively. In contrary, Woldemeskel and Gumi (2001) identified only thin walled sarcocyst from camels originated from southern Ethiopia. Whether these morphologically distinct Sarcocysts represent two different species, as argued by Dubey et al. (1989) and Saeed et al. (2018), or they are strains of the same species named *Sarcocystis cameli* needs further investigation preferably on its ultrastructural and molecular features. Dubey et al. (2015) redescribed Sarcocystis and reported only two Sarcocystis species in camel, namely *Sarcocystis cameli* and *Sarcocystis ippeni*, which both appear thin-walled on light microscope but the former had thick wall and the later thin wall on transmission electron microscope (ultrastructural basis). On the other hand, reports about the

molecular criteria and immunodiagnoses of different species of *Sarcocystis* in camels are lacking (Valinezhad et al., 2008).

The prevalence was higher in males (33.33%) than in females (26.56%), young camels (33.33%) than old camels (28.57%) and in camels originated from Minjar (35.71%) than from Borena (33.87%) and Kereyu (24.19%) although these differences were not statistically significant. This finding is in line with several studies including the once conducted by Woldemeskel and Gumi (2001), Shekarforoush et al. (2006), Valinezhad et al. (2008) and Hamidinejat et al. (2013). These findings suggest the presence of other potential risk factors particularly associated with the husbandry system. In this regard, the prevalence of *Sarcocystis* infection in the current study among the three body condition scores was statistically significant ($p = 0.039$) and could be emanated from the difference in the husbandry and management systems. Camels with good body condition were more infected (43.39%) than camels with poor (27.87%) and medium (21.15%) body condition scores probably because they got additional feed and care by maintaining homesteads where dogs are freely roaming.

Of the three tissues/organs examined for the presence of sarcocyst, esophagus was the most frequently infected organ/tissue (19.87%), followed by diaphragm and heart. Similar report was also made by Shekarforoush et al. (2006), Woldemeskel and Gumi (2001) and Hamidinejat et al. (2013). In contrary, Fatani et al. (1996) reported that diaphragm is the most commonly affected tissue. The detection of the cyst concurrently in two or three tissues of the camels we examined (51%) probably confer more or less equal affinity *Sarcocystis* species for any organ with striated muscle. According to Valinezhad et al. (2008), these differences may be due to differences among *Sarcocystis* strains of various definitive host origin and the methods applied in the sarcocyst detection. In the diagnosis of *Sarcocystis* infection, the sensitivity is known to increase in the order of squash technique, histopathology and polymerase chain reaction. According to Calero-Bernal et al. (2015) the detection ratio of *Sarcocystis* infection can be improved two-fold by examining histological sections rather than squashed muscle.

CONCLUSIONS AND RECOMMENDATIONS

Results of this study showed a high prevalence of *Sarcocystis* infection in camel irrespective of their sex, origin and age group. Based on light microscopy, both thin and thick walled Sarcocysts were detected for the first time in Ethiopia. With the increasing curiosity of consumers for food safety and the increasing export competitors, the presence of this infectious agent could be a deterring factor for export of meat. Therefore, awareness need to be created among the pastoral community and actors involved in the value chain of camel production and marketing to minimize risks of economic loss associated with camels' infection/disease. Moreover, further molecular-based investigations and experimental trials with best animal models are needed to better clarify the encountered species/s and its/their zoonotic implication, if there is any. Further studies are also required to identify husbandry related risk factors by involving large number of camels slaughtered both in backyard and other abattoirs found in different parts of the country.

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