Original article

Detection of *Sarcocystis* Infection in Wild Ungulates in Tripoli Safari Park, Libya

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Abstract
Sarcocystosis is one of the most widespread muscle diseases of domesticated and non-domesticated wild mammals. To estimate the prevalence of *Sarcocystis* infection, muscles samples were obtained from79 freshly dead wild life herbivores species, 7 Addax (*Addax nasomaculatus*), 14 Barbary sheep (*Ammontragus lervia*), 17 Dorcas gazelle (*Gazella dorcas*), 12 Mouflon (*Ovis musimon*) and 29 Fallow deer (*Dama dama*) in Safari Park south of Tripoli, Libya. Individual sarcocysts were processed and optical examination was used for detection of macroscopic sarcocysts whereas light microscopy was used for microscopic sarcocysts.The examination of muscular tissue samples from different organs in 5 different wild animal species revealed an overall prevalence of 56.9% (45/79); 71.4% (5/7) in addax; 64.3% (9/14) in Barbary sheep; 58.6% (17/29) in fallow deer; 58.3% (7/12) in Mouflon and 41.0% in gazella for *Sarcocystis* spp.Macroscopic and microscopic sarcocysts were 3.8% (3/79) and 53.2% (42/79) respectively.Among the muscles infected with cysts, highest infection of *Sarcocystis* were recorded in heart 50.6%(40/79) and48.1%(38/79) in diaphragm whereas in oesophagus40.5% (32/79).Reported results highlight the high prevalence of *Sarcocystis* infection in wild animals in the Tripoli safari park and suggests the need of further molecular biology studies to characterize *Sarcocystis* species that infect captive wild animals existed in Libya, and investigate their possible role in zoonosis.

Key words: *Sarcocystis*; Necropsy; Wild animals; Tripoli; Libya

Introduction

Sarcocystis species are protozoan parasites with a heteroxenous life cycle, which produce cysts in the muscle of a wide range of herbivorous (Pipia *et al*. 2016). In these wild animal species, sarcocystosis is frequently asymptomatic, but in case of high intensity of parasite, the percentage of animals reported to lose weight (Dubey *et al*. 1989; Daugschies *et al*. 2000). Sarcocysistosis can represent a serious problem among wide range of wild-animal species worldwide, although a high prevalenceof this parasitic disease has been reported in domesticated and non-domesticated wild animals (Malakauskas and Grikienienë 2002).In addition, wild animals can play a crucial role in disseminating these parasites; either as a carries or reservoirs (Dovgalev and Posokhov, 1987). In addition to itsveterinary-economic significance effect on general health condition of wild animals, the *Sarcocystis* infection can be of zoonotic importance.

Sarcocystis is an obligate intracellular protozoan parasite with heterogeneous life cycles that requires both intermediate and definitive hosts. Definitive hosts (Predators) become infected, when they consume infected intermediate hosts. In return, Preys get infected when they consume the silages contaminated with the faeces containing oocysts of *Sarocystis* spp. (Pereira and Bermejo 1988; Fayer *et al*., 2015).Generally intestinal infection occurs in carnivorous definite host; while in intermediate host parasite invade tissues. However, birds, reptiles and wild mammals can be definite host for different species of *Sarcocystis* (OIE/CFSPH, 2005).*Sarcocystis* infection of muscle tissues (Muscular sarcocystosis) are usually found in muscular tissue of diaphragm, oesophagus, heart,tongue and skeletal muscles (Prakas and Butkauskas 2012).

Man can be the definitive host for *Sarcocystis hominis* and *Sarcocystis suihominis*. When cysts in muscle tissue (*Sarcocystis*) are ingested with raw or insufficient cooked beef (*S.hominis*) or pork (*S.suihominis*), the merozoites contained in the cysts are releasein the human intestine penetrating the intestinal epithelium wall, causing a serious health problem (Pedro and Acha 1989). The intestinal infection in humans has been reported in many countries throughout the world, with an incidence rate of 6-10% (WHO 1981).

Ecological imbalances encountered many sub-Saharan countries including Libya, this was due to desertification, shortage of food and water during last three decades had led to immigration of wild animals from their natural geographical zone to nearby inhabited areas of man and domestic animals, accordingly, although a wide range of wild animals species such as; foxes, hedgehogs, common jackals, porcupines, hares and jerboa have been observed to resident and live beside domestic animals, and also sharing the same pastureland (FAO 1992). Under favourable zoo hygienic and sanitary conditions, their chances of contacting the *Sarcocystis* sporocysts through any kind of fodder, grass, soil, water reservoirs and other factors are suggestible maximized. In consequent, animals raises in the same area might have a great chance of getting infection with these parasites. This kind of direct contact providing a potential risk for transmission of infectious agents from wild animals to domestic

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animals, and could circulate of many parasitic diseases including sarcocystosis between wild and domesticated livestock animals in the region (Hosni, 2006).However, a literature search for previous published data revealed that transmission possibly to occur between different animal species. For instance,bovine cattle flocks raised in small individual farms in Lithuania were found infected and up to 90.6% of 940 cattle carcasses examined in the meat-packing plant proved to be infected with *Sarcocystis*. After a period of time, other animal species raised in individual farms within the same area appeared to be also infected with these parasites (Grikienienë 1994).

Domesticated herbivorous animal have reported to be infected with *Sarcocystis* in many regions. Previous studies throughout the world have indicated that 70-100% of sheep and cattle were infected with *Sarcocystis* (Salehi *et al.*2014; Zayed *et al*.2012; Mirella 2012; Dubey *et al*.1988; Aboudaya 1990; Munday 1975 and Saleque *et al.*1992).Studies on sarcocystosis in Libya are few, the only published study was exclusively conducted to detect the infection of *Sarcocystis* in herbivores livestock farm animals and not wild life animals. The reported results from that study revealed a high prevalence level of *Sarcocystis* infection in slaughtered sheep carcases examined postmortemly in main Tripoli abattoir belonging to the Tripoli municipality, the infection rate reported was 86.1% for microcysts (*S. ovicanis*) and 1.67% for macrocysts (*S. gigantea*) (El Hussein 2001).Similar findings was demonstrated by a number of studies conducted elsewhere have reported a high prevalence of sarcocystosis.

High prevalence of was reported in wild behaviours in Canada, up to 71% of 557 wild ungulates were infected (Jerome and Douglas, 1980), in America 79% of white-tailed deer infected with *Sarcocystis* (Karstad and Trainer 1969). *Sarcocystis* was also been reported in wide range of East African game animals (Kaliner *et al*. 1974). Wild life animals in Libya play an important role as harbours of diseases which can be transmitted to domestic animals and human. However, in the current study attempts was made to estimate the prevalence of sarcocystosis assessed by necropsy of recently died wild animals in wild ungulates in Tripoli safari park.

Materials and methods

Study area

This study was conducted in the Tripoli wild life Safari Park which is located approximately 25 Km south of Tripoli and comprises around 780 hectares. In range, the park climate is typically south Mediterranean. Winter is the main season for rainfall. The average annual rainfall is 150-200mm. The ambient temperature is temperate/cold in winter (4-44 °C) and hot in late spring and summer (15-44°C).

The park is habituated by hundreds of several species of wildlife herbivores Mountain zebra (*Hippotigris zebra*), impala (*Aepyceros melampus*), thomson's gazelle (*Eudoreas homsonii*), Scimitar horned Oryx (*Oryx dammah*), the domestic herbivores (Camels, sheep and goats), carnivores red fox (*Vulpes*) and fennec fox, *Fennecus zerda*) and native dogs. Such carnivores live in the park and/or wandering at surrounding areas.

Post-mortem examination

During a period (2001-2004) a total of 79 freshly dead wild life herbivores species, 7 Addax (*Addax nasomaculatus*), 14 Barbary sheep (*Ammontragus lervia*), 17 Dorcas gazelle (*Gazella dorcas*), 12 Mouflon (*Ovis musimon*) and 29 Fallow deer (*Dama dama*) were investigated for *Sarcocystis* infection. As a part of routine reporting procedure adopted for dead wild life animals in the park post-mortem examination was implemented.

Macroscopic sarcocysts

During the post-mortem inspection of dead animal carcases, a systemic examination of the organs according to Georgi (1980) was performed; heart, diaphragm, and esophagus (laryngeal/pterygoid) muscles were examined in these 79 dead animals, respectively. Macroscopic cysts were identified visually by naked eye (Soulsby 1986) and classified according to Dubey *et al*. (1989).

Collection of samples

For microscopic sarcocysts, muscles specimens collected were cut and preserved in plastic containers and transported to veterinary laboratory of Tripoli Zoo for laboratory diagnosis using the peptic digestion method described by (Dubey *et al*., 1989).

Statistical analysis

Data were analysed using the statistical program for social sciences (SPSS-PC version 10.0), to assess if there was association between the *Sarcocystis* infection and wild animal species type. The Chi-squared test ($\chi 2$) was used, to determine whether there were significant differences on prevalence value between the different wild animals species examined. Differences were considered with statistical significance when the P value was < 0.05.

Results

Seventy nine wild life herbivores examined at Tripoli Safari Park were infected with *Sarcocystis*. The overall prevalence of Macroscopic and microscopic sarcocysts were 3.8% (3/79) and 53.2% (42/79) respectively (Table 1).An over infection rates obtained for macroscopic sarcocysts among the five wild animal species was 3.8% (3/45); in addax 14.9% (1/7) and 6.9% (2/29) in fallow deer. A 53.2% microocysts was detected in 42 out of total 79 examined animals; 4/7 (57.1%) in *A. Nasomaculatus*, 64.9% (9/14)in *A. Lervia*, 41.2% (7/17) in *G. Dorcas* and 58.7% (7/12) in (*O. musimon*) and 51.7% (15/29) in *D. Dama* (Table 1).The overall prevalence of microcysts infection among muscular tissue were(50.6%)in heart, 48.1% in diaphragm and 40.5% in esophagus. The highest rates of infection in organs were detected in heart (64.9%), diaphragm (64.3%) and esophagus (57.1%). The rates of infection of the organs of the examined animal were showed in table (2).

Table 1: Prevalence of macroscopic and microscopic *Sarcocystis* spp. in infected mammals.

Common name of animal	Latin name	No. of animal		Prevalence of *Sarcocystis* infection %	No. of positive (%)	
		examined	infected		macrocysts	microcysts
Addax	*Addax nasomaculatus*	7	5	71.4	1(14.3)	4 (57.1)
Babary sheep	*Ammontragus lervia*	14	9	64.3	0	9 (64.3)
Dorcas gazella	*Gazella dorcas*	17	7	41.2	0	7 (41.2)
Mouflon	*Ovis musimon*	12	7	58.3	0	7 (58.3)
Fallow deer	*Dama dama*	29	17	58.6	2 (6.9)	15 (51.7)
Total		79	45	56.9	3 (3.8)	42 (53.2)

Table 2: Prevalence of *Sarcocystis* infection found in examined tissues of wild different animal species.

Common name of animal	Latin name	No. of animal examined	Heart (%)	Diaphragm (%)	Esophagus (%)
Addax	*Addax nasomaculatus*	7	4 (53.1)	3 (42.9)	3 (42.9)
Babary sheep	*Ammontragus lervia*	14	9 (64.9)	9 (64.9)	8 (57.1)
Dorcas gazella	*Gazella dorcas*	17	7 (41.2)	7 (41.2)	6 (35.3)
Mouflon	*Ovis musimon*	12	6 (50)	7 (58.3)	6 (50)
Fallow deer	*Dama dama*	29	14 (48.3)	12 (41.4)	9 (31.0)
Total		79	40 (50.6)	38 (48.1)	32 (40.5)

Discussion

The results demonstrated that *Sarcocystis* are extremely widespread muscle parasites of dissected wild ungulates in Tripoli safari park. The prevalence of *Sarcocystis* infection in postmortem examined wild animal species proved to be high at 56.9%, with a highest prevalence was reported in addax at 71.4% and Barbary sheep at 64.3%, whereas the lowest was reported in Dorcas gazella 41%. However, the prevalence of sarcocystosis in other two ungulate species was alike, 58.6% in fallow deer, and 58.3% in Mouflon (Table1). Statistical estimation revealed no significant differences in the prevalence of this parasite among the examined animal species (p= 0.614).Most often sarcocysts were detected equally in both muscles; the heart and diaphragm of Barbary sheep at 64.9%, whereas in other examined animals (prevalence of infection in heart muscles were ranged between 41.2% and 53.1%, respectively).The location of *Sarcocystis* infection was different accordingly, and the infection of *Sarcocystis* in heart and diaphragm muscles of investigated animal species was higher than in those of esophagus (Table2).

Our data on the prevalence of wild animals sarcocystosis are in accordance with those data furnished by Arnastauskienë (1989), revealed 49.4% of wild ungulates were *Sarcocystis* infected in 1981-1986.However, sarcocystosis infection was reported at higher level of87.2% among ungulates in central, northern and eastern Lithuania (Malakauskas and Grikienienë 2002). In Poland, high-level infection rate of 88.7%-94.3% was reported among roe deer and red deer respectively (Tropilo *et al*. 2001), also these authors point out a relatively lower infection rate among wild boar at 24.7%. Although, high-rate (up to 100%) of wild boar were infected with these parasites in earlier study established in the Netherlands and Germany (Tadros and Laarman 1976; Erber 1978).

The results herein reported indicate the high distribution of macroscopic *Sarcocystis* species infection in wildlife animals of Tripoli, Libya. *Sarcocystis* spp. Infection rate was obtained for macroscopic and microscopic in different wild animal species. Lower frequency of macroscopic sarcocysts infection reported (3.8%) compared with microscopic sarcocysts (53.2%).The highest frequency of microscopic *Sarcocystis* infection was reported in Barbary sheep (*A. lervia*), whilst the highest frequency of macroscopic sarcocysts at 14.3%was detected in Addax *A. Nasomaculatus*.

The overall prevalence for *Sarcocystis* infection in present study was varied between 41.0% (Dorcas gazella) and 71.4% (addax).Prevalence of *Sarcocystis* infection in wild animal populations reported here are similar to the results obtained by some authors from investigations performed in many European countries. But, the result on macroscopic and microscopic sarcocystosis infection are different to those provided by other workers in Sarda breed sheep slaughtered in different abattoirs of Sardinia, Italy, which revealed much higher an overall prevalence of infection for macroscopic forms of sarcocysts23.3% (Pipia *et al.*

2016) in compare to only 14.3% macroscopic infection rate obtained in the current study.

The postmortem examination of recently died animals allowed us to identify morphology of cysts comparable with those described by Dubey *et al*. (1989), respectfully. Cysts were mainly localized in the heart (50.6%), and diaphragm (48.1%) in compare to (40.5%) in the esophagus muscles. This finding is in contrast with those results obtained by other authors (Tenter 1995; Oryan *et al*. 1996; Pipia *et al*. 2016) whom reported higher number of cysts mainly was localized in the esophagus (57%) while lower rate of cysts infection were detected in the abdominal muscle at (12.3%).

In the present morphological study for detection of *Sarcocystis* spp. infection in wild animals has been applied for the first time in an official veterinary postmortem inspection in Libya, but not in domestic animal such as sheep, that had previously investigated by EL hussein (2001)who reported results highlight the high prevalence of *Sarcocystis* infection detected in sheep carcasses slaughtered in Tripoli abattoir with infection rate of86.1% for microcysts (*S. ovicanis*) and 1.67% for macrocysts (*S. gigantea*) (EL hussein, 2001).*Sarcocysits* have been also reported in a numerous studies on wild animal species worldwide: in Lithuania, *Sarcocystis* cyst was firstly detected in moos by Arnastauskien and Kazlauskas (1984). Although, infection with *Sarcocystis* species has been commonly reported at a high prevalence figure in herbivores, but it was rarely reported in bears. In a study conducted in Pennsylvania, black bears (*Ursus americanus*) were reported to be infected with *Sarcocystis* (Dubey *et al*. 2008). An outbreak of sarcocystosis had been reported in parrots and pigeon in Belo Horizonte Zoo in Brazil (Ecco *et al.*2008), the same authors attributed to the fact that the same parasite species transmitted through feed contaminated by feces of opossum which is common inhabitant of the forest surrounding the zoo.

According to the findings of some other authors, Clubb and Frenkel (1992); birds pick up the sporocysts or oocysts from undigested plant materials in mammals feces. For instance; the migratory Canadian geese (*Branta canadesis*) followed cattle and fed on undigested plant materials in the cattle's feces. In south coastal New Jersey (USA), Gary (1990) found macrocysts of *Sarcocystis* spp in 28 of 173 American black ducks (*Anas rubripes*) examined during winters of 1984–1985, 1985–1986 and 1986–1987. The latter author pointed out that even no macrocysts were detected in 80 juvenile black ducks. High-level macrocysts prevalence in adults was reported to increase from 3% (1 of 37) in 1984–1985, to 36% (12 of 33) in 1985–1986, and 65% (15 of 23) in 1986–1987 (Gary, 1990).Sarcocystosis has been investigated by a number of authors, Latif *et al*. (2010) examined 20 necropsied captive wild mammals and 20 birds in 2 petting zoos in Malaysia. In that study only microscopically visible cysts were detected in 8 animals, however, those authors stated that species identification was not possible to conduct. According to the data available in literature, the largest numbers of *Sarcocystis* species were ascertained for even-toed ungulates and rodents (Odening, 1998). Predatory mammals are the most important distribution agents of *Sarcocystis* species and usually serve as definitive hosts. Also, they can act as an intermediate hosts for several *Sarcocystis* species (Dubey *et al*. 1992; Odening *et al*. 1994).

From zoonotic stand point view, humans are susceptible to sarcocysts (Heydorn 1977; Schulze 1988).Previous studies on *Sarcocystis* parasites in game animals highlighted the importance of sarcocysts as potential zoonotic threat for human. Sarcocystoisis was diagnosed in two human cases in Germany, due to consumption of roe deer meat intensely infected with sarcocysts (Schulze 1988).Symptoms of diarrhea, nausea and vomiting has also been reported by the clinicians (Schultze 1988). Postmortem inspection proved to be practical methods for detection infections for many diseases including sarcocystosis. Particularly when subclinical conditions might not detected in a live animals, can be evaluated in postmortemed animals, and therefore it is possible to tell whether or not an animal was previously infected or exposed.

The present morphological observation study provided information on the status of sarcocysts in wild animals in Tripoli safari park. The study had two limitations; First, postmortemed inspected animals were not a true random sample of population at risk, because a large number of live wild ungulates animals were not possible to include in the study. However, direct detection method of sarcocystosis based on autopsy is a gold-standard, but is known to have disadvantages according to the data available in literature; as animals represented are a selected population, and only a limited number of animals can be examined, so necropsy technique is inappropriate for a large-scale population study. Second limitation, is that molecular methods to characterize sarcocysts to confirming the morphological description was enabled to conduct, due to lack of finance resources. Therefore macroscopic and microscopic cysts of genus *Sarcocystis*, was not identified at species level. Nevertheless, with the present study it was possible to report the frequency of sarcocysts in five different ungulate species and to describe the organs muscles most frequently infected with sarcocysts.

It is very difficult to draw a comparison between the frequency of *Sarcocystis* infection obtained from our study and sparse data available in literature as different estimation methods were used by different authors. Despite the fact that *Sarcocystis* species have been broadly studied, and increased interest in its biology, life cycles, taxonomy, veterinary as well as medical significance of these parasites; the prevalence of infection, ways of its distribution in many wild animal species being not clear. Further molecular studies are required to identify and differentiate the species of *Sarcocystis* that infect captive wild animals and investigate their possible role in zoonosis.

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