Gastrointestinal and respiratory parasites in Captive mammals at Rabat zoo in Morocco, with the first record of Capillaria *spp. in the Fennec fox* (Vulpes zerda)

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Prevalence.

Abstract
The aim of the present study was to broaden the understanding of parasitism in captive wild mammals in Morocco. For this purpose, an investigation on gastrointestinal and respiratory parasites in African mammals from Rabat Zoo was carried out. A total of 47 fecal samples were collected from 30 species from November 2021 to March 2022 and examined macroscopically and microscopically. Parasites were detected in 21 species at a prevalence of 70%, with a parasite positivity rate of 89% in artiodactyls, 50% in perissodactyls, 67% in both carnivores and primates. No parasitic infection was detected in proboscideans. The most frequent infection was by nematodes with a percentage of 50%, followed by both protozoa and mixed infection with a prevalence of 10%. In conclusion, the results demonstrated that parasite prevalence remains high, even in enclosed spaces like zoos. Therefore, in order to ensure animal welfare and staff safety, sanitary measures should be implemented in such facilities, including routine diagnostic tests followed by appropriate treatment.

Introduction

Zoological garden, wildlife sanctuaries and nature reserves are important for the conservation of wild animals, especially threatened ones since they provide an opportunity to study and monitor such species. Knowledge of their diseases is therefore essential, and parasitism is no exception (Barbosa *et al*. 2019).

In their natural habitats, wild animals are often infected with various types of parasites, and clinical signs related to parasitism are rarely observed due to the dispersal of animals over habitats, as well as the development of resistance against these agents, leading to an equilibrium between parasites and hosts (Mir *et al*. 2016). On the other hand, parasitic infection in captivity present a major concern for many reasons, two of which are the high density of animals in captivity, which promotes easy re-infection, together with the stress caused by captivity which weakens the immune system (Fischer *et al*. 2019), and makes animals more prone to parasitic diseases.

The study of parasitism inside enclosures is therefore relevant as it contributes to expand the knowledge of parasitic fauna present in wild mammals. In addition, the identification of parasites allows the evaluation of the risk they may pose to the captive animals and to the personnel.

The previously mentioned reasons were hence the principal objectives of this Investigation.

The aim of the present study was to determine for the first time the prevalence of gastrointestinal and respiratory parasites in thirty African mammal species from Rabat zoo, to identify them, and to correlate the findings with the taxonomic order of the studied mammals.

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Material and methods

Description of the Study Area and Animals

Located in the green belt of Temara (33.9553° N, 6.8943° W), Rabat Zoo is a state-owned facility of about 25 hectares housing more than 2000 animals of 150 different species by the time of the present study. Rabat Zoo is the largest zoo in Morocco and a destination for many visitors, receiving an average of 600,000 Moroccan and foreign tourists per year (Ançari 2019).

In an endeavour to ensure the well-being of the animals in captivity, the zoo presents a simulation of their natural habitats, divided into five main ecosystems: The Atlas Mountains, areas that include some of Morocco's famous animal species, such as the Atlas lion (*Panthera leo*), the Barbary sheep (*Ammotragus lervia*) and the Barbary macaque (*Macaca sylvanus*). The desert, a habitat that exhibits animal species native to the Saharan environment, with a particular focus on threatened antelopes, such as the Scimitar Oryx (*Oryx dammah*), Addax (*Addax nasomaculatus*) and Dorcas Gazelle (*Gazella dorcas*). The wetlands, a recreated ecosystem of lowland landscapes and home to aquatic animals such as crocodiles (*Crocodylus niloticus*), hippos (*Hippopotamus amphibius*), white pelicans (*Pelecanus onocrotalus*) and flamingos (*Phoenicopterus roseus*). The savannah, an area where some of Africa's most fascinating species are present, namely elephants (*Loxodonta africana*), giraffes (*Giraffa camelopardalis*), white rhinoceros (*Ceratotherium simum*), eland antelopes (*Taurotragus oryx*), ostriches (*Struthio camelus*) and baboons (*Papio Anubis*).

And finally, the rainforest, simulating forest landscapes found near the equator, and housing species native to this type of environment such as chimpanzees (*Pan troglodytes*), mandrills (*Mandrillus sphinx*) and lemurs (*Lemur catta*).

This study covered various mammals housed in Rabat zoo. The main focus was on mammals from Africa. A total of 30 species were investigated and arranged according to their taxonomic orders (Table I).

After opening hours, all animals must be housed in solitary or shared cages that are specifically constructed for each species. Zoo keepers feed animals and clean enclosures daily in the morning.

Most of zoo mammals are treated for gastrointestinal parasites periodically throughout the year, usually with benzimidazoles and avermectins. In addition, occasional deworming is carried out at the opportunity, when animals are subjected to other veterinary procedures.

That being said, none of the studied animals had received anthelmintic treatment at least 40 days prior to the investigation.

Samples and sampling process

A total of 77 faecal samples were collected from 30 species of mammals at Rabat Zoo, all individuals belonging to these species are adult and clinically healthy.

The collection included 22 samples from nine species of *Artiodactyla*, 5 samples from two species of *Perissodactyla*, 3 samples from one species of *Proboscidea*, 33 samples from twelve species of *Carnivora* and 14 samples from six species of Primates.

Each week from November 2021 to March 2022, a visit to the zoo took place during which 10 to 20 grams of faeces were collected directly from the floor of the enclosures, giving priority to fresh faeces that had been emitted either in the morning or at night.

The faecal samples were placed in labelled plastic cups and carried to the Laboratory of Parasitology at Institut Agronomique et Vétérinaire Hassan II (Agronomic and Veterinary Institute Hassan II) where they were refrigerated for later examination.

In the zoo context, taking samples from each individual is nearly impossible.

Therefore, the study did not cover the entire population, but only subgroups of it, and the number of faecal samples was determined according to the numbers of individuals of each species. Furthermore, it should be kept in my mind that most animals are housed collectively, for this reason the faecal samples were pooled as they could not be assigned to specific individuals.

Laboratory techniques

Macroscopic examination

The faeces were routinely examined with the naked eye before microscopic examination to note their appearance (consistency, colour, presence of blood or mucus, etc.) and to detect the likely presence of roundworms or tapeworm proglottids.

Some nematodes may be observed with the naked eye (Family Ascarididae) while others, smaller in size, require microscopic techniques to be detected (Zajac *et al*. 2021).

Microscopic examination

The faecal material was analysed by three different methods in order to obtain a global screening for all potential present parasites.

The methods were centrifugal faecal flotation for nematode and cestode eggs together with oocysts, sedimentation for trematode eggs, and Baermann technique for nematode larvae (Bowman 2021). The techniques were performed as described by Zajac *et al*. (2021).

Parasite identification

After detection under the Leica DM500 microscope, which is equipped with a digital camera (Leica ICC50 W), eggs, larvae and oocysts were identified on the basis of measurements and description of morphology (colour, content, shell shape, etc.) as detailed by Thienpont *et al*. (1979), as well as illustrations, microscopic photographs and diagrams provided by Hasegawa *et al*. (2009) and Bowman (2021).

Table I. *Studied mammals, and their population size up to January 2022.*

Order	Scientific name	Common name	Population size
Artiodactyla	*Syncerus caffer*	African buffalo	7
	Eudorcas thomsonii	Thomson's gazelle	3
	Gazella dorcas	Dorcas gazelle	22
	Gazella cuvieri	Cuvier's gazelle	4
	Ammotragus lervia	Barbary sheep	50
	Oryx dammah	Scimitar oryx	6
	Addax nasomaculatus	Addax	26
	Giraffa camelopardalis	Northern giraffe	4
	Hippopotamus amphibius	Hippopotamus	7
Perissodactyla	*Equus quagga*	Plains zebra	5
	Ceratotherium simum	White rhinoceros	3
Proboscidea	*Loxodonta africana*	African bush elephant	1
Carnivora	*Panthera leo*	African lion	38
	Leptailurus serval	Serval	7
	Caracal caracal	Caracal	2
	Vulpes vulpes	Red fox	3
	Vulpes zerda	Fennec fox	7
	Canis lupaster	African wolf	1
	Lycaon pictus	African wild dog	1
	Crocuta crocuta	Spotted hyena	3
	Hyaena hyaena	Striped hyena	1
	Suricata suricatta	Meerkat	5
	Herpestes ichneumon	Egyptian mongoose	6
	Genetta genetta	Common genet	3
Primates	*Lemur catta*	Ring-tailed lemur	10
	Macaca sylvanus	Barbary macaque	30
	Mandrillus sphinx	Mandrill	3
	Papio Anubis	Olive baboon	14
	Erythrocebus patas	Common patas monkey	3
	Pan troglodytes	Chimpanzee	5

Source: The inventory of mammals at Rabat Zoo (January 2022)

Results

Prevalence of global, mono and mixed infections

Overall, 21 out of 30 species were positive for parasites with a prevalence of 70% (21/30).

Gastrointestinal infection accounted for the majority of parasitic infections, with 15 (50%) of the animal species infected with nematodes, 3 (10%) with protozoa and 3 (10%) with mixed infections.

The prevalence of infection in each mammalian order was distributed as follows: 88.89% for artiodactyls, 66.67% for primates, 66.67% for carnivores, 50% for perissodactyls and no parasites for proboscideans (Table II).

Table II. *Prevalence of different parasites found in mammals at Rabat Zoo, Morocco*

		Artiodactyla n = 9	Perissodactyla n = 2	Proboscidea n = 1	Carnivora n = 12	Primates n = 6	Overall (30)
Nematodes	*Trichostrongylus* spp. (%)	4 (45)	-	-	-	-	4 (13)
	Strongyle-type (%)	5 (55)	1 (50)	-	-	1 (17)	7 (23)
	Nematodirus spp.(%)	5 (55)	-	-	-	-	5 (17)
	Oesophagostomum spp.(%)	1 (11)	-	-	-	-	1 (3)
	Toxascaris leonina (%)	-	-	-	1 (8)	-	1 (3)
	Toxocara canis (%)	-	-	-	1 (8)	-	1 (3)
	Ancylostoma spp. (%)	-	-	-	1 (8)	-	1 (3)
	Trichuris spp. (%)	-	-	-	1 (8)	2 (33)	3 (10)
	Strongyloides spp. (%)	-	-	-	3 (25)	1 (17)	4 (13)
	Enterobius spp. (%)	-	-	-	-	1 (17)	1 (3)
	Capillariid-type (%)	-	-	-	-	1 (17)	1 (3)
	Capillaria spp. (%)	-	-	-	2 (16.67)	-	2 (7)
Nematode positive (%)		**7 (78)**	**1 (50)**	**0**	**4 (33)**	**3 (50)**	**15 (50)**
Protozoa	*Eimeria* spp.(%)	1 (11)	-	-	-	-	1 (3)
	Cystisospora spp.(%)	-	-	-	4 (33)	-	4 (13)
	Entamoeba spp.(%)	-	-	-	-	1 (17)	1 (3)
Protozoa positive (%)		**0**	**0**	**0**	**3 (25)**	**0**	**3 (10)**
Mixed infection		**1 (11)**	**0**	**0**	**1 (8)**	**1 (17)**	**3 (10)**
Total		**8 (89)**	**1 (50)**	**0**	**8 (67)**	**4 (67)**	**21 (70)**

'-' negative, 'n' number of species, data are presented as number of positive species, with prevalence (%) in parentheses

Parasite identification

Artiodactyls

Species belonging to *Artiodactyla*, were infected with various intestinal parasites, namely nematodes belonging to the following families: *Trichostrongylidae*, *Molineidae* and *Strongylidae*, as well as protozoa belonging to *Eimeriidae* family (Table III, Figure 1).

Perissodactyls

Only Grant's zebra (*Equus quagga*) of the two studied perissodactyls revealed positive for intestinal nematodes which belongs to *Strongylidae* and *Trichostrongylidae* families (Strongle-type) (Figure 2). On the other hand, the results for White Rhinoceros (*Ceratotherium simum*) were negative.

Table III. *Parasites detected in different species of the order Artiodactyla*

Mammal species	Parasite species detected
Syncerus caffer	*Oesophagostomum* spp.
Eudorcas thomsonii	*Trichostrongylus* spp., *Strongyle-type*
Gazella dorcas	*Trichostrongylus* spp., Strongyle-type, *Nematodirus* spp.
Gazella cuvieri	*Trichostrongylus* spp., *Nematodirus* spp.
Ammotragus lervia	*Trichostrongylus* spp., Strongyle-type, *Eimeria* spp.
Oryx dammah	Strongyle-type, *Nematodirus* spp.
Addax nasomaculatus	*Nematodirus* spp.
Giraffa camelopardalis	Strongyle-type, *Nematodirus* spp.
Hippopotamus amphibius	Negative

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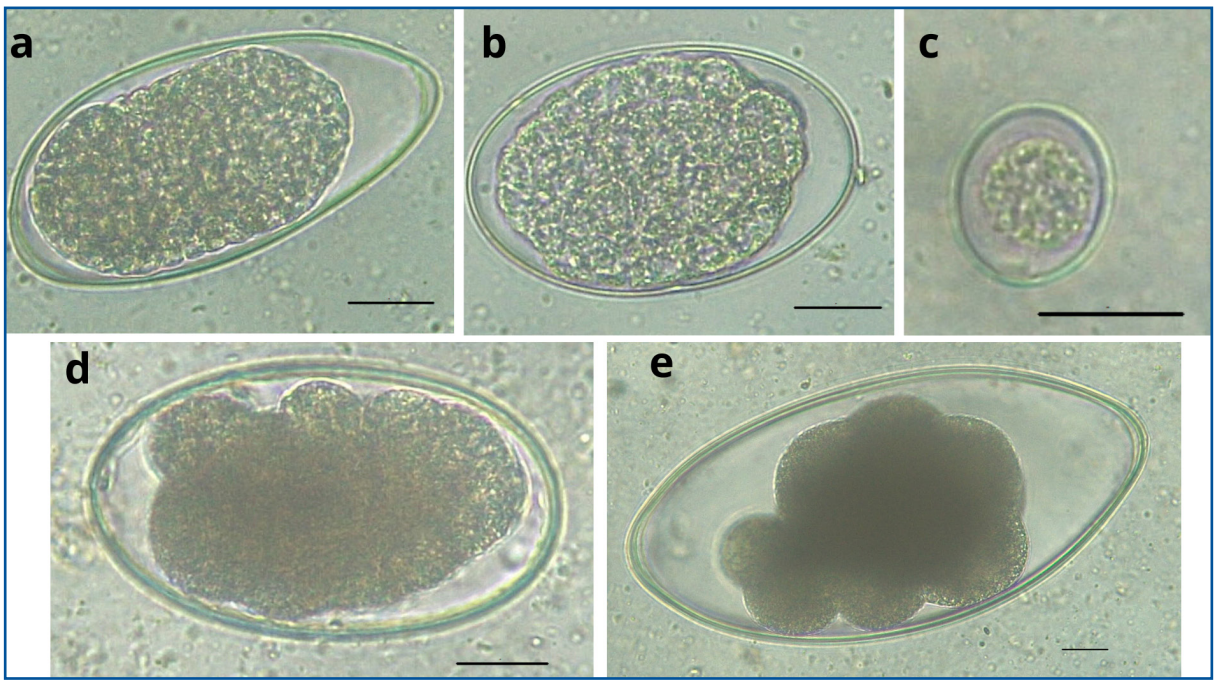

Figure 1. *Different intestinal parasites recovered from artiodactyls.(a)* Trichostrongylus *spp., (b)* Strongyle-type, *(c)* Eimeria *spp., (d)* Oesophagostomum *spp., (e)* Nematodirus *spp. (Scale bar = 20 µm)*

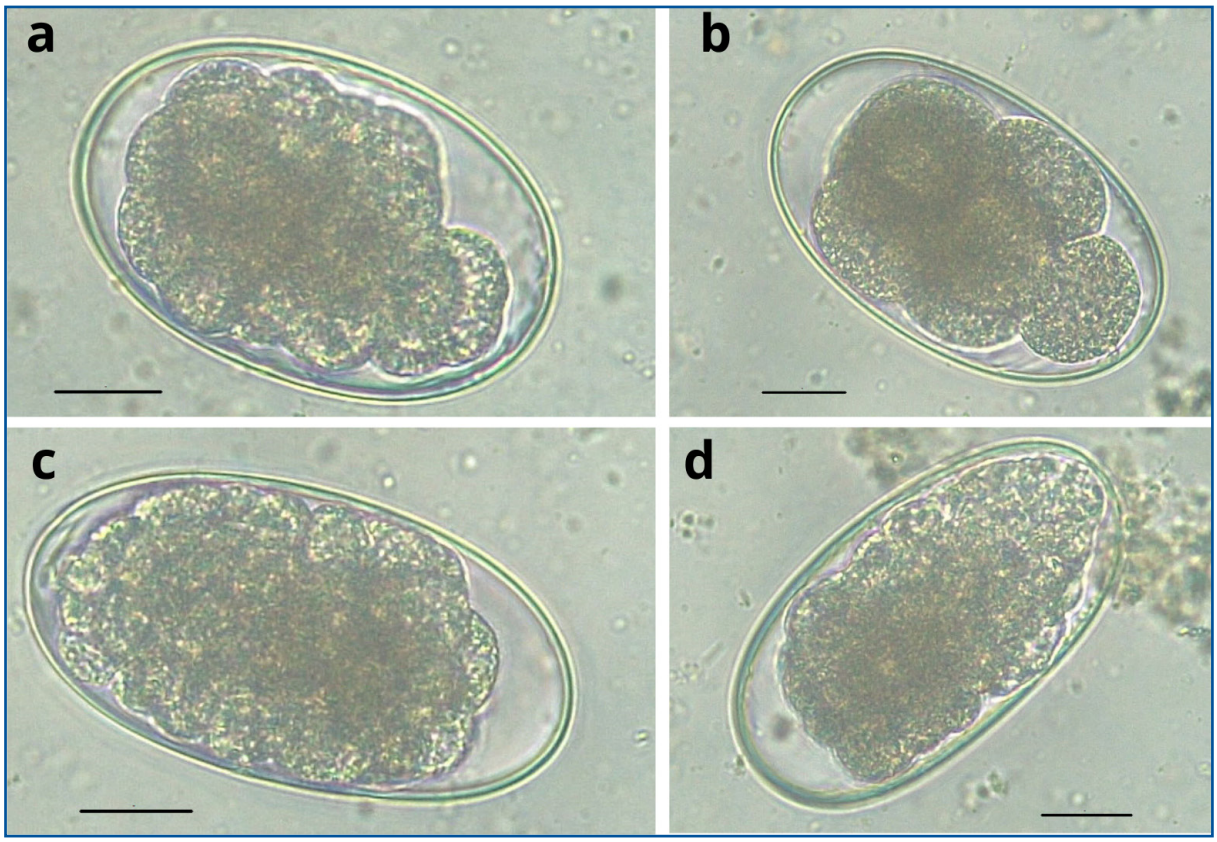

Figure 2. *Different intestinal parasites recovered from perissodactyls. (a, b, c, d) Strongyle-type (Scale bar = 20 µm)*

Carnivores

Ascarididae, Strongyloididae, Ancylostomatidae, Trichuridae, Capillariidae and *Eimeriidae* families account for the parasitic infection within order Carnivora (Table IV, Figure 3,4).

Primates

Primates were infected with parasites belonging to *Oxyuridae, Strongyloididae, Capillaridae, Trichuridae* and *Entameobidae* families (Table V, Figure 5).

Table IV. *Parasites detected in different species of the order Carnivora*

Mammal species	Parasite species detected
Panthera leo	*Toxascaris leonina, Cystisospora* spp.
Leptailurus serval	Negative
Caracal caracal	*Cystisospora* spp.
Vulpes vulpes	*Strongyloides* spp., *Capillaria aerophila*
Vulpes zerda	*Toxocara canis*, *Strongyloides* spp., *Capillaria aerophila*
Canis lupaster	Cystisospora spp.
Lycaon pictus	Negative
Crocuta crocuta	Negative
Hyaena hyaena	Negative
Suricata suricatta	*Cystisospora* spp.
Herpestes ichneumon	*Trichuris* spp.
Genetta genetta	*Ancylostoma* spp., *Strongyloides* spp.

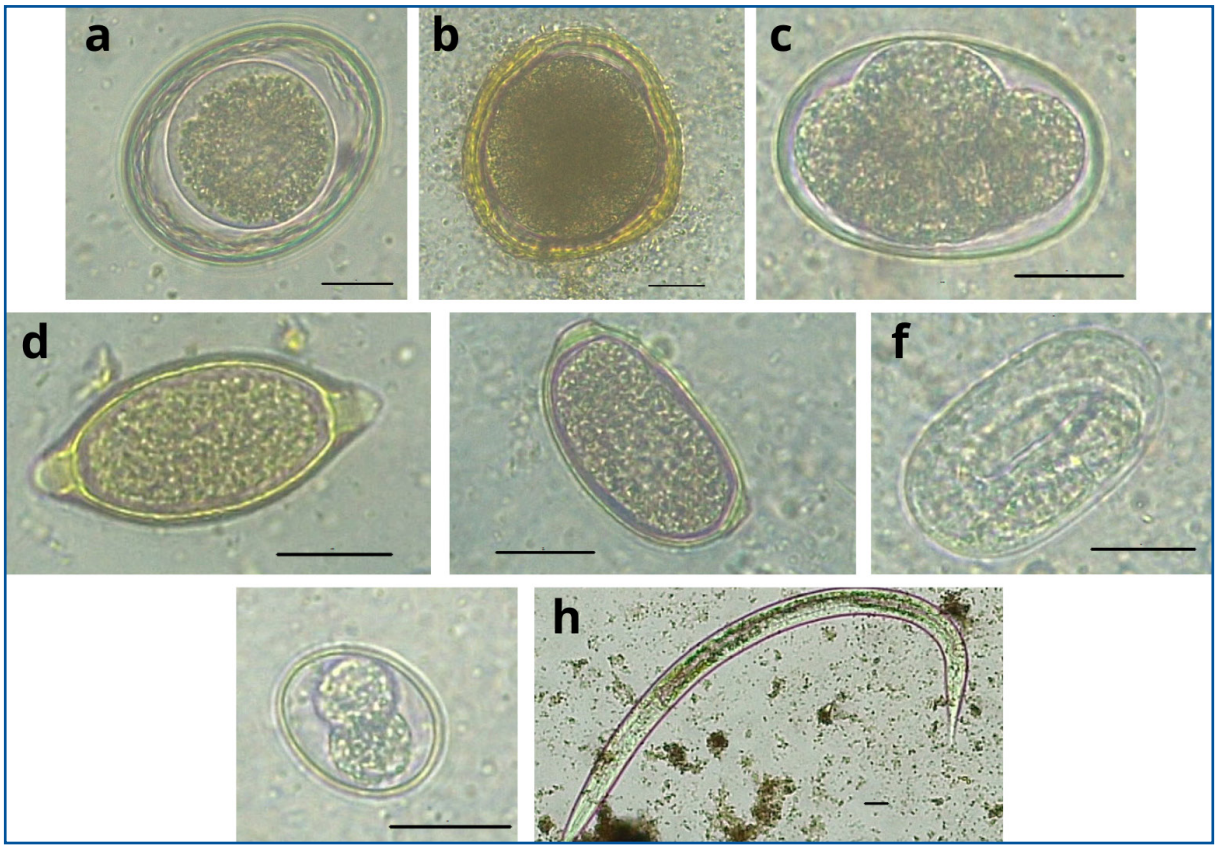

Figure 3. *Different intestinal parasites recovered from carnivores. (a)* Toxascaris leonina*, (b)* Toxocara canis*, (c)* Ancylostoma *spp., (d)* Trichuris *spp., (e)* Capillaria *spp., (f)* Strongyloides *spp. (Egg form), (g)* Cystoisospora *spp., (h)* Strongyloides *spp. (Larva form) (Scale bar = 20 µm)*

Figure 4. *Anterior end of Adult Toxascaris leonina, recovered directly from Lion Feaces (Scale bar = 200 µm)*

Table V. *Parasites detected in different species of the order Primates*

Mammal species	Parasite species detected
Lemur catta	Negative
Macaca sylvanus	Strongyle-type, Capillariid-type, Entamoeba *spp.*
Mandrillus sphinx	Negative
Papio Anubis	*Trichuris* spp., *Strongyloides* spp.
Erythrocebus patas	*Trichuris* spp.
Pan troglodytes	*Enterobius* spp.

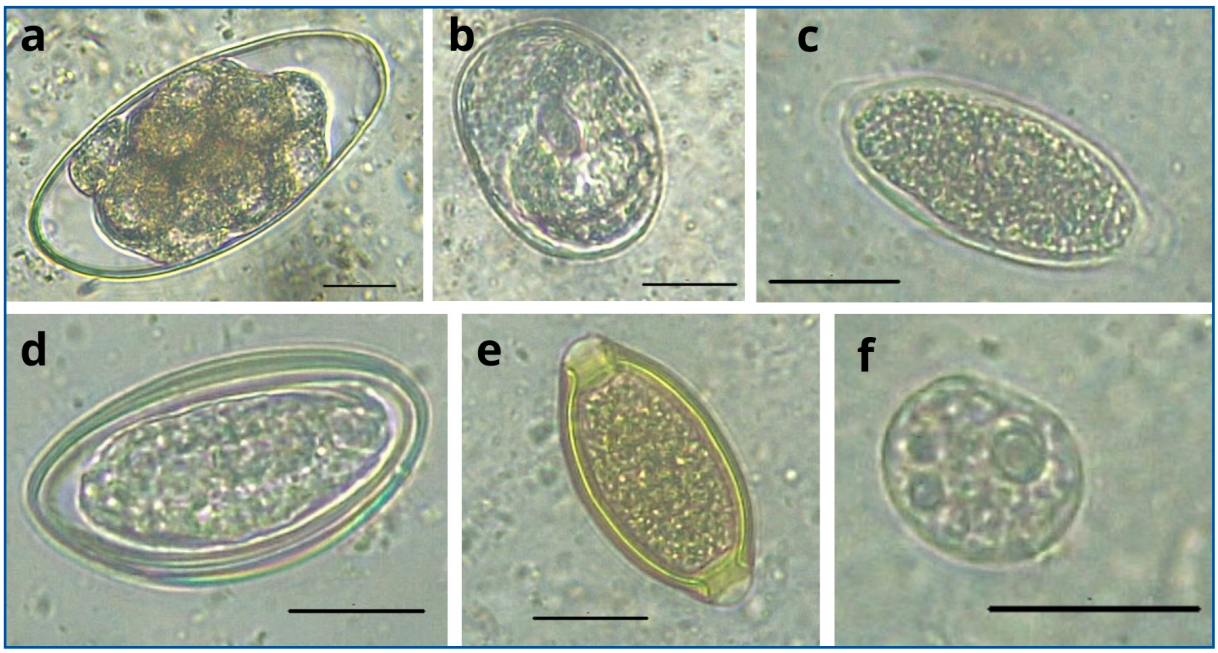

Figure 4. *Anterior end of Adult Toxascaris leonina, recovered directly from Lion Feaces (Scale bar = 200 µm) Figure 4: Anterior end of Adult* Toxascaris leonina*, recovered directly from Lion Feaces (Scale bar = 200 µm)*

Discussion

The investigation of gastrointestinal and respiratory parasites of wild mammals in zoological gardens has been reported from different parts of the world by many Authors (Atanaskova *et al*. 2011; Aviruppola *et al*. 2016; Kvapil *et al*. 2017; Li *et al*. 2015; Thawait *et al*. 2014). Although all of these studies, including the current one, was done on apparently healthy individuals, the screening revealed a high infection prevalence with various types of parasites, this is explained by the fact that wild animals naturally harbours a fair burden of parasite without developing clinical signs, unless in stress conditions (Mir *et al.* 2016).

Concerning gastrointestinal parasites, the results agree with reports of other studies which concluded that nematodes and protozoa with direct life cycles were the sole parasites to be present (Dashe and Berhanu 2020). Additionally, the species detected had also been reported by several authors. In Artiodactyla, *Oesophagostomum* spp. and other strongyles were reported in the Kafue lechwe (*Kobus leche kafuensis*) by Phiri *et al.* (2010). *Nematodirus* spp. was reported in a variety of wild ruminants of Tunisia by Said *et al.* (2017), and *Eimeria* spp. was recorded in African buffalo by Gorsich *et al.* (2014). In Perissodactyla, *Trichostrongylus* spp. and other strongyles have been reported by Mukanga *et al.* (2019). In Carnivora, *Ancylostoma* spp., *Toxascaris* spp. and *Cystoisospora* spp. were found in lions by Mukarati *et al.* (2013). *Strongyloides* spp., *Toxocara* spp. and *Trichuris* spp. were reported in various African carnivores by Berentsen *et al.* (2012), Flacke *et al.* (2010) and Bjork *et al.* (2000) respectively. In primates, Strongyles, Capillariids, *Strongyloides* spp., *Trichuris* spp. and *Entamoeba* spp. were all described in seven non-human primates by Kouassi *et al.* (2015). Similarly the current survey, *Enterobius* spp. was found in chimpanzees by Yaguchi *et al.* (2014).

As for pulmonary parasites, Red fox has already been described to be a final host of *Capillaria* spp. (Karamon *et al.* 2018), but to the best of the authors' knowledge, this research is the first to report this lungworm in the Fennec fox.

Only African mammals were chosen for this study in order to compare the findings to those of other researchers who have studied free-living mammals. For gastrointestinal parasites, the nematodes and protozoa detected on the African wilderness were similar to the present findings (Horak *et al.* 2021). However, the dissimilarity was in the presence of Plathylminthes in free-ranging mammals (Berentsen *et al.* 2012; Van Wyk and Boomker 2011), unlike captive ones in Rabat zoo, in which no mammal has been reported to harbour heterexenous parasites. These conclusions agree with many other studies done on captive mammals (Goossens *et al.* 2005; Kamel and Abdel-Latef 2021), and can be explained by the unlikely presence of intermediate hosts in enclosures, which are required for the transmission of trematodes and cestodes (Mir *et al.* 2016).

Conclusion

This paper revealed that African mammals in Rabat zoo were infected with different gastrointestinal and lung parasites at an overall mean rate of 70%. Nematodes were the most prevalent parasites, followed by protozoa, while there was no trematode or cestode species recovered from the faecal samples.

Since investigated animals were apparently healthy and showed no symptoms, the high prevalence indicates subclinical infection that can emerge under stress conditions and cause pathogenicity. Additionally, some carnivores and primates were infected with nematode genera such as *Enterobius*, *Trichuris* and *Strongyloides*, all of which can be transmitted to humans. Therefore, in order to reduce the risk of outbreaks and zoonoses inside zoos, sanitary measures need to be reinforced, deworming programmes should be reconsidered and finally, further epidemiological investigations according to season, age, climate, etc. should be performed.

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