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Pathological findings in 61 free-ranging leopards (*Panthera pardus)* from the Greater Kruger National Park, South Africa. Part One: parasites

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ABSTRACT

The Kruger National Park and surrounding protected areas in South Africa are home to a core unmanaged population of approximately 1,200 free-ranging leopards (*Panthera pardus*). Leopard populations outside of protected areas are in decline due to trophy hunting, habitat fragmentation and human-wildlife conflict. Apart from single descriptions of parasites detected by faecal or necropsy examinations and serology, very little information is available on parasitic conditions in free-ranging leopards. This paper provides baseline information on parasites in free-ranging leopards that may be relevant to the management of threatened populations of leopards outside of the Greater Kruger National Park (GKNP). Opportunistic field necropsies with routine histological examination of formalin-fixed tissues and, where possible, identification of helminths preserved in ethanol or by histology were performed on 61 free-ranging leopards in the GKNP between 1998 and 2023 for long-term disease monitoring. Most (52/61, 85%) of these leopards were euthanized because they attacked or killed a person or had severe traumatic injuries or disease. Five leopards died during anaesthesia performed to evaluate traumatic injuries or tuberculosis status. Three animals died naturally due to traumatic injuries and one due to electrocution on an electric fence. Parasitic disease was seen in similar numbers of males (n = 29) and females (n = 23) and in animals ranging from 1 to 16 years old. Common conditions included myocardial hepatozoonosis (34/61, 56%), gastroduodenitis due to *Cylicospirura pardalis* (31/61, 51%), verminous pneumonia attributed to metastrongyles (14/61, 23%) and skeletal muscle sarcocystosis (17/61, 28%). Single cases of infestation with *Dirofilaria sudanensis*, *Armillifer armillatus* and *Linguatula serrata* were recorded. In the majority of these free-ranging leopards, even heavy parasitic infestations were associated with minimal inflammation and were likely subclinical. However, two leopards had gastric outflow obstruction due to *C. pardalis* lesions, two had severe mange and one had severe myocarditis due to hepatozoonosis, which may have significantly affected the health of the affected animals. The factors that trigger the transformation of common subclinical parasitic conditions to potentially life-threatening disease require further elucidation.

1. Introduction

Leopards (*Panthera pardus*) are large, elusive, powerful, mainly nocturnal and solitary felids with a black and tan to white spotted coat [1–3]. The Kruger National Park in South Africa (approx. 20,000 km^2) is home to a core unmanaged population of approximately 1,200 free-ranging leopards [4,5]. Leopard populations outside of protected areas are in decline, due to trophy hunting, habitat fragmentation and human–wildlife conflict [6].

Very little published information is available on parasitic conditions and their clinical significance in free-ranging leopards. The presence of helminths, protozoa and arthropods in African leopards has largely been documented by single taxonomic records with minimal information on the intensity of infection or associated lesions [7–9]. Nematodes described from leopards include *Dracunculus medinensis* [10], *Trogostrongylus subcrenatus* [11], *Ancylostoma braziliense, A. caninum* [12],

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Galonchus perniciosus [13], *Toxocara cati* [14], and *Trichinella spiralis* [15], as well as *Filaria martis* [16]. More recently, the identification of *Physaloptera praeputialis* in leopards from Zimbabwe [17] and a detailed description of the spirurid *Cylicospirura pardalis* from leopards from the Greater Kruker National Park (GKNP) have been described [18,19]. A variety of cestodes, including *Diphyllobothrium* [20] and *Taenia* spp [21], the trematode *Pharygostomum cordatum* [13] and the pentastome *Armillifer armillatus* [22] are reported to occur in free-ranging leopards.

Isopora and *Toxoplasma*-like oocysts as well as *Giardia* cysts have been recovered from the faeces of free-ranging leopards in Thailand [23] and of captive ones from zoological gardens in Egypt and Germany [24]. *Sarcocystis* cysts in the myocardium of an Indian leopard have been reported previously [24]. *Babesia leo* n. sp was detected in blood samples from healthy free-ranging leopards in the GKNP, South Africa [25]. *Hepatozoon* gamonts in neutrophils in blood smears were detected in a dehydrated, cachexic, anaemic free-ranging leopard from Iran [26]and have also been seen in apparently healthy captive and free-ranging leopards in South Africa [27]. *Trypanosoma congolense* and *T. evansi* have been detected in blood smears in African [28] and Indian [29] leopards. Positive IgM titres and *Toxoplasma gondii* tachyzoites and bradyzoites were found in the peritoneal fluid and brains of two free-ranging leopards that died due to trauma from road traffic in Iran [30]. *Ctenocephalides* and *Echidnophaga* spp fleas [31] have been recorded in leopards as well as *Amblyomma, Haemphysalis, Hyalomma, Ixodes, Rhipicentor and Rhipicephalus* ticks [32].

This paper describes the macroscopic and histological characteristics of parasitic conditions seen in 61 free-ranging leopards that died or were euthanized in the Kruger National Park and surrounding protected areas (GKNP) between 1998 and 2023.

2. Materials and methods

A wildlife disease database kept by the first author was searched for free-ranging leopards from the GKNP that died or were euthanized between 1998 and 2023. In total, 64 leopard cases were found. Two cases were discarded from this series due to severe autolysis obscuring pathological findings and one because it was an animal that had surgical repair for a bone fracture and was deemed not representative of a free-ranging animal.

Opportunistic field necropsies for long-term disease monitoring were conducted in the GKNP. The range of tissues that could be examined was influenced by ante- and post-mortem predation, facilities available at the site of discovery, time of day and the cause and circumstances of the death. Examination of internal organs was more common than evaluation of the nasal cavities, brain and spinal cord, endocrine glands, reproductive tract, skeletal muscle and bone marrow. Macroscopically visible helminths were collected during necropsy procedures using forceps and fixed and stored in 70% ethanol. Lactophenol was used to clear specimens for morphological examination [19].

Selection of tissues for histopathological evaluation during field necropsy examinations was variable. Formalin-fixed tissues were processed routinely through graded alcohols, embedded in paraffin wax, sectioned (10 μm) and stained with haematoxylin and eosin (HE) [33]. Helminths and protozoa were identified histologically using Gardiner and Poynton [34,35].

The sex, age, cause of death, clinical history and parasitic conditions of all 61 leopards were tabulated based on clinical and macroscopic information listed on the submission form and histopathological findings. Age was given variably as age class, years or a range of years: juvenile (less than 1 year old), subadult (1–2 years old), adult over 2 years old to 10 years old and elderly (over 10 years old) [5]. The middle year was used where an age range was given.

3. Results

Macroscopic and/or histological parasitic disease was identified in most (52, 85%) of the 61 animals. Table 1 shows the parasite, anatomical location, percentage of the animals affected and case number seen in this series. The case number, sex, age, cause of death, clinical history and parasitic lesions seen in all 61 animals are given in Supplementary Table 1.

The animals with parasitic conditions were brought to the attention of the veterinarians by park staff or members of the public because they were displaying behaviour deemed a risk to humans (31/52, 60%): they attacked (2/52, 4%) or killed (3/52, 6%) a person; were suffering from traumatic injury (12/52, 23%) or as a result of electrocution on an electric fence while being surrounded by tourist vehicles (1/52, 2%). Three leopards (6%) had significant non-parasitic disease: severe pododermatitis of uncertain cause (M47), severe tuberculosis (M48) and severe lameness (M54). Most (46/52, 88%) of the leopards with parasites were euthanized, but small numbers died naturally (2/52, 4%). *Mycobacterium bovis* is endemic in the GKNP (see Part Two). Five (5/52, 10%) leopards suffering traumatic injury, severe disease or judged to be a risk to humans died during anaesthesia for ante-mortem comparative intra-dermal skin testing for tuberculosis conducted to screen for positive animals that would not be released after treatment.

Parasites were recorded in similar proportions of males (29/35, 83%) and females (23/26, 88%), and the age of affected animals ranged from one to 16 years. Some leopards had only one parasite (13/61, 21%) while more had two to three different parasites (29/61, 48%). Seven leopards (11%) had five or more different parasites (F12, M24, M26, F33, M37, M48, M52). No parasites were recorded from three female and six males ranging from a cub to an elderly leopard.

3.1. Respiratory system

An adult pentastome (*Linguatula serrata*) was recorded in the nasal cavity of F25 (Fig. 1). Mild to moderate multifocal verminous pneumonia was present in many leopards (15/61, 25%). Linear white pleural tracts were described macroscopically, which corresponded with mild diffuse acute hyperplastic, neutrophilic and eosinophilic bronchiolitis (Fig. 2a) with mild peri-bronchial mucous gland hyperplasia, mild multifocal to diffuse, mainly lymphohistiocytic interstitial pneumonia (F12, M26, M27, M29, F33, M52) and variable necrosis (F33) or atelectasis (M52). Adult nematodes (approx. 250–400 μm diameter) with platymyarian musculature and a multinucleate intestine without a brush border were present mainly in airways, with embryonated eggs and/or spiny larvae (approx. 30 μm diameter) in alveolar spaces (Fig. 2b). Tiny (15–20um) spiny nematode larvae, similar to those seen in the lung, were present in the colon lumen of two animals associated with minor lymphoplasmacytic colitis.

3.2. Cardiovascular system

Hepatozoon meronts (<100 μm diameter) were present in the myocardium in small to medium numbers in many leopards (34/61, 56%). The meronts had variably thick walls and contained bundles of nucleated bradyzoites, often with the nuclei arranged in a peripheral ring. The association between the presence of mild histological myocarditis (n = 29) and the presence of *Hepatozoon* meronts in the myocardium was variable. Macroscopically, M24 had short white streaks in the myocardium (Fig. 3a) and abundant *Hepatozoon* but minimal myocarditis histologically. M48 had similar streaks but only rare *Hepatozoon* and no inflammation. Myocardial inflammation associated with meronts was lymphoplasmacytic and occasionally histiocytic or neutrophilic (Fig. 3b). *Hepatozoon* meronts with no myocarditis were seen in F5, M34, F35, F45 and M55. M36 had severe myocarditis with a mixed inflammatory cell infiltration, including many eosinophils, and multifocal myocardial necrosis (Fig. 3c). Small numbers of *Hepatozoon* meronts and no other pathogens were present in the affected myocardium. F61 had myocarditis but no *Hepatozoon* in the sections examined.

Table 1
Parasite, anatomical location, percentage affected and case number recorded from 52 free-ranging leopards from the Greater Kruger National Park, South Africa[a]

Parasite	Identity	Anatomical location	Percentage affected	Case number
Nematode	*Cylicospirura pardalis*	Duodenum	36	M4, F5, M8, F10, M11, F12, F16, M17, M20, M21, M24, F28, F31, M36, M37, F44, M47, M48, M50, F57, M59, M60
		Pylorus	31	F2, M4, F12, F14, M15, M20, F23, F23, **M24**, M26, F28, F32, **M36**, M47, F49, M50, F51, M52, F57
		Oesophagus	3	M52, M59
		Jejunum	3	M24, M26
		Colon	3	M17, F28
	Metastrongyle	Lung	25	F12, M20, M24, M26, M27, M29, F32, **F33**, M36, M37, F38, F44, M48, M52, M60
		Kidney	2	F12
	Not identified	Small intestine	13	M21, F33, M37, F38, M48, F51, M52, F57
	Not identified	Colon	3	F38, **F51**
	Dirofilaria sudanensis	Skin	2	**M50**
Cestode	Not identified	Small intestine	11	M20, M21, **F31**, F33, M47, M52, F61
	Not identified	Colon	3	F38, F61
Trematode	Not identified	Small intestine	10	F5, M6, F12, M24, F25, F57
	Not identified	Duodenum	5	F12, F32, M48
	Not identified	Colon	5	F5, M37, F57, M60
Not Identified		Intestine	2	F23
Protozoa	*Hepatozoon*	Heart	56	F2, M4, F5, F7, **M11**, F12, F14, F15, F16, F17, F20, **M24**, M26, M27, M29, M30, F31, F33, M34, F35, **M36**, M37, F38, F40, M41, F44, F45, M47, M48, M50, **M53**, M54, M55, F58
		Lung	8	F2, M26, F31, M47, M53
		Lung	2	M22
		Diaphragm	2	F12
		Lymph node	2	F12
		Spleen	2	F14
		Peripheral lymphocyte	2	F7
	Sarcocystis	Skeletal muscle	18	F7, M24, M26, F28, M29, **F33**, F35, M43, M52, M55, F58
		Diaphragm	11	M17, M30, F35, M37, M43, M48, **F57**
		Periocular muscle	3	M37, M48
		Tongue	2	M30
		Longus colli muscle	2	M24
		Heart	2	F57
	Not identified	Skin	2	M37
Pentastome	*Linguatula serrata*	Nasal cavity	2	F25
	Armillifer armillatus	Pleura	2	M22
		Peritoneum	2	M22
Mites	*Sarcoptes scabiei*	Skin	3	**F12, M56**
Ticks	*Rhipicephalus*	Skin	2	F12
	Amblyomma	Skin	2	F12
	Not identified	Skin	3	F23, M29

F, female; M, male.
Case numbers in bold are those leopards in which there were many parasites present or the associated inflammation was severe.

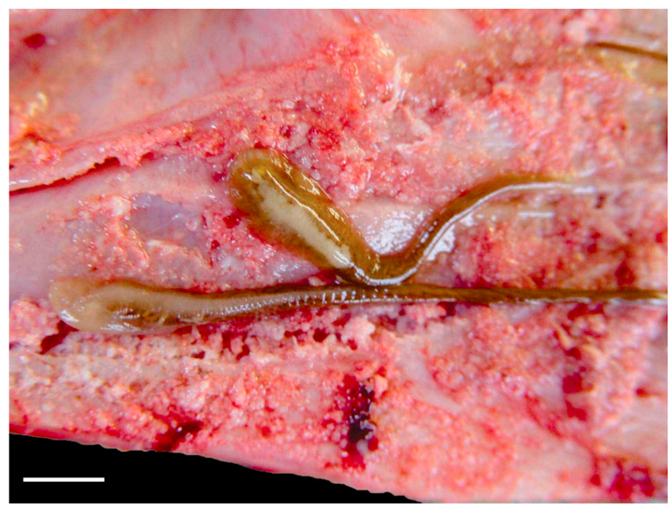

Fig. 1. Two pentastomes (*Linguatula serrata*), nasal cavity of a free-ranging leopard (F25). Bar, 5 mm.

Small numbers of *Hepatozoon* meronts were also seen in the lung, with (F2, F31) or without (M26, M47, M53) inflammation, as well as in the diaphragm with associated mild lymphocytic myositis, in a lymph node with no inflammation and in the spleen. An *Hepatozoon* gamont was seen in the cytoplasm of a lymphocyte in a peripheral blood smear in one animal (Fig. 4).

3.3. Gastrointestinal system

Verminous gastroduodenitis, characterized by single to multiple coalescing raised smooth nodules (>4 cm diameter) that greatly distended and distorted the wall of the pylorus and/or cranial duodenum, was recorded in many (31/61, 51%) leopards (Fig. 5a). On cut section, tangled red nematodes filled irregularly shaped cavities in these nodules. Histologically, these transmural lesions consisted of thick layers of loose immature to dense mature granulation tissue infiltrated by small to medium numbers of macrophages, lymphocytes, plasma cells and eosinophils. Intralesional large spirurid nematodes (<2 mm diameter) were often surrounded by necrotic tissue debris, large foamy macrophages and mixtures of eosinophils and neutrophils (Fig. 5b).

Verminous gastroduodenitis was associated with necrosis of the duodenum submucosa or tunica muscularis (F5, F12, F28, M37, M47), variably hyperplastic mucosal-associated lymphoid tissue at the pyloric-

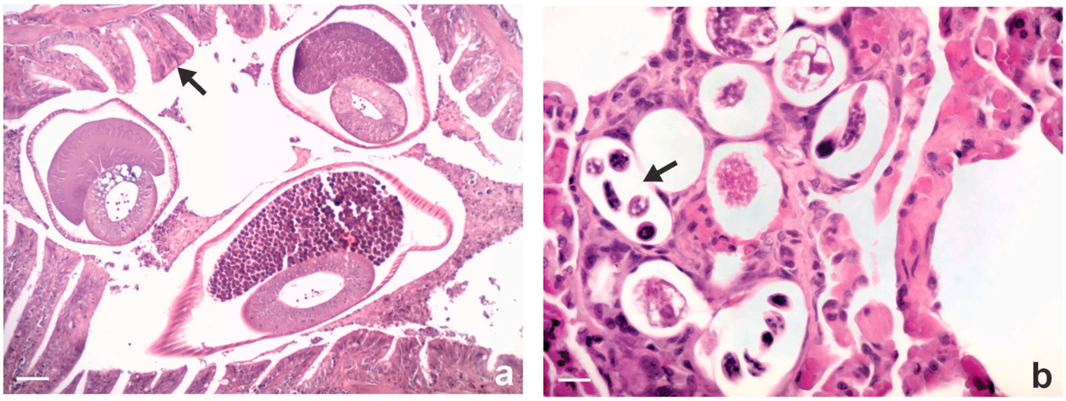

Fig. 2. Verminous pneumonia, free-ranging leopards. **(a)** Adult metastrongylid nematodes in bronchiole lined by hyperplastic epithelium (arrow, M26). HE. Bar, 10 µm. **(b)** Multiple sections of spiny nematode larvae in alveolar spaces (arrow, M52). HE. Bar, 45 µm.

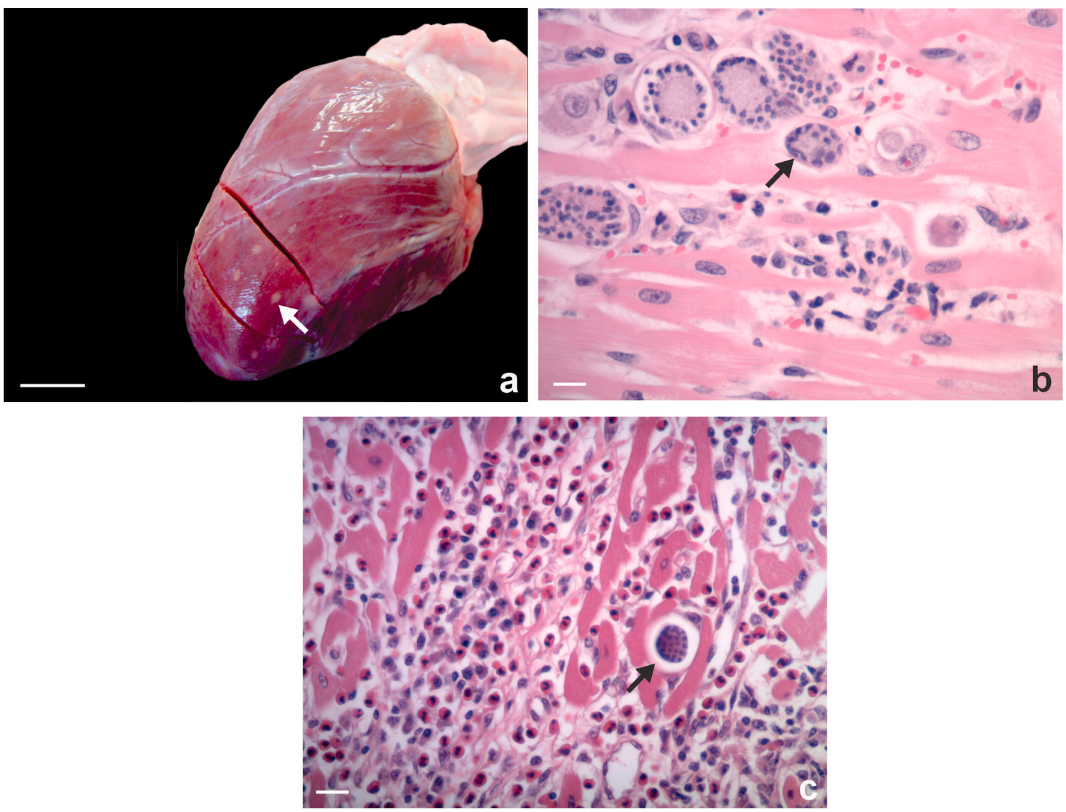

Fig. 3. *Hepatozoon* meronts, myocardium, free-ranging leopards. **(a)** Raised pale tan foci beneath the epicardium (arrow, M24). Bar, 28 mm. **(b)** Multiple meronts containing bundles of nucleated bradyzoites, often with the nuclei arranged in a peripheral ring (arrow), and associated mild lymphoplasmacytic and histiocytic myocarditis (F16). HE. Bar, 200 µm. **(c)** Severe eosinophilic and lymphoplasmacytic myocarditis associated with an *Hepatozoon* meront (arrow, M36). HE. Bar, 100 µm.

duodenum junction, small pleocellular perivascular cuffs in the submucosa and muscle layers adjacent to these granulomas, a large diverticulum on the greater curvature of the stomach proximal to the pylorus (M24) and a constricted pylorus plugged with a large bolus (approx. 2L) of undigested hair and small bone fragments (M36). Colonies of coccobacilli occurred in the necrotic material of M48. Similar eosinophilic necrogranulomas were present in the oesophagus, jejunum or colon walls, as well as eosinophilic and lymphoplasmacytic peritonitis and mesenteric steatitis in the adjacent tissues (F28). Two unencapsulated foci of severe necrotizing and eosinophilic granulomatous colitis were seen in M17, consisting of two large unencapsulated separate foci of necrosis, fibrin deposition and large numbers of eosinophils and smaller numbers of macrophages in the colon wall. The surrounding musculature was oedematous and infiltrated by large numbers of eosinophils with small scattered, often perivascular, aggregates of plasma cells and lymphocytes and prominent immature collagen, lipofuscin-laden macrophages and activated fibrocytes extending to the margins of the section and the serosa. Moderate multifocal dystrophic gastric mineralization in both the gastric lamina propria and smooth muscle was seen in M20 and a focus of necrosis and mineralization associated with lymphoplasmacytic inflammation and fibrosis was seen in the tunica muscularis of the duodenum of M26.

Some (9/61, 15%) animals had eosinophilic intestinal lesions without evidence of parasites. Mild to moderate, focal to multifocal

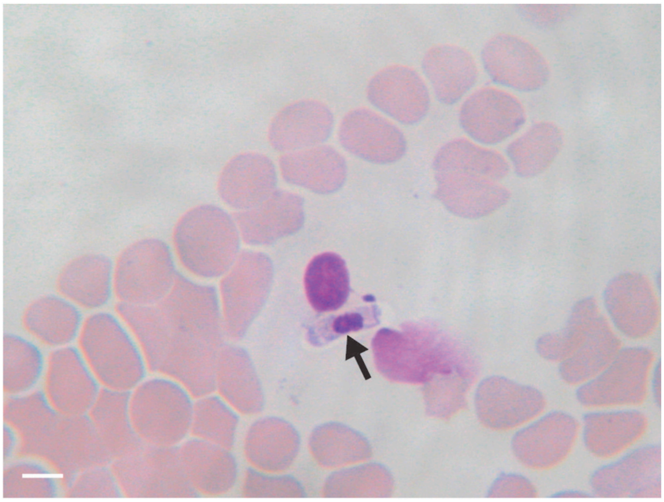

Fig. 4. *Hepatozoon* gamont, cytoplasm of a lymphocyte, peripheral blood smear (arrow, F7), KyroQuick. Bar, 7.5 µm.

transmural eosinophilic and variably lymphoplasmacytic enteritis was present in eight animals (F28, M29, F31, F40, M42, M52, F58, M59) and F51 had similar colitis. Mild multifocal lymphoplasmacytic pancreatitis was seen histologically in M53.

Adult nematodes and nematode eggs, resembling *Toxocara cati,* were seen (8/61, 13%) histologically in the small intestinal lumen or the colon in two animals. Adult trematodes in the intestinal lumen were recorded rarely and were associated with variable minimal to mild lymphoplasmacytic enteritis, mild mucous cell hyperplasia, many globule leukocytes in the lamina propria (10/61, 16%) and, in one case (F32), with mild multifocal necrotizing neutrophilic duodenitis. Trematode eggs with yellow–brown pigmented thick capsules were seen occasionally in the large intestinal lumen (M21, M48, F57, M60). Adult cestodes were seen histologically in the small or large intestinal lumina of eight (13%) animals, with prominent mucosal-associated lymphoid follicular hyperplasia (M20, F33), cestode eggs in the colon (F61), eosinophilic enteritis (F31, M52) and encysted larvae (250–300µm) in the large intestinal mucosa (F38). Many unidentified endoparasites were recorded in F23.

M22 had a severe pleural and peritoneal infestation with pentastomes (Fig. 6a). Histologically, pentastome nymphs occurred in cysts in the small intestine tunica muscularis. The pentastomes were pseudosegmented with cuticular spines, sclerotized openings in the body wall, striated musculature and bright eosinophilic intestinal glands (Fig. 6b). Cyst walls were lined by mature fibrous connective tissue infiltrated with small numbers of lymphocytes, plasma cells and eosinophils. One degenerating parasite was surrounded by an intense infiltration of eosinophils, macrophages and multinucleate giant cells and mineralized necrotic debris.

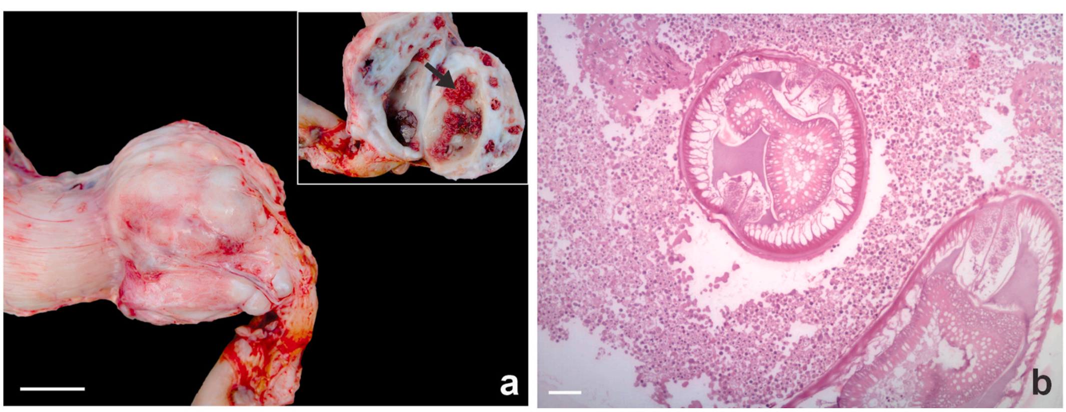

Fig. 5. Verminous gastroduodenitis, free-ranging leopards. (**a**) Multiple coalescing white firm nodules in the distal pylorus and cranial duodenum. Bar, 4 cm. Inset: myriad filamentous red nematodes fill cavities in the dense connective tissue of these nodules (F12). (**b**). Large spirurid nematodes with a ridged cuticle, coelomyarian musculature, a large intestine of columnar cells with a brush border, eosinophilic fluid in the pseudocoelom, and large embryonated eggs in the uterus are surrounded by necrotic inflammatory cells (M36). HE. Bar, 450 µm.

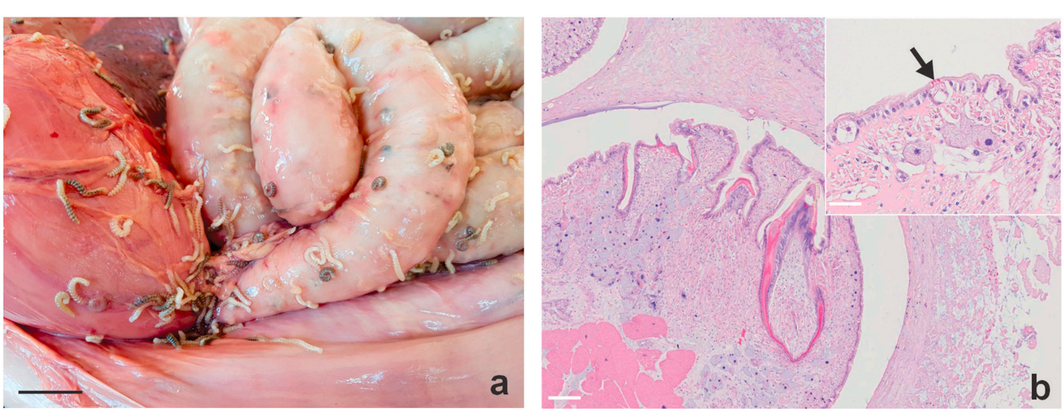

Fig. 6. Pentastomiasis, free-ranging leopard (M22). (**a**) Myriad pentastome nymphs with coiled cuticles on peritoneal surfaces of small intestine. Bar, 19 mm. (**b**) Anterior portion of a pentastome nymph with large acidophilic glands. HE. Bar, 110 µm. Inset. Sclerotized opening in the cuticle (arrow). HE. Bar, 20 µm.

3.4. Musculoskeletal system

Small to large numbers of large (approx. 250 µm diameter) sarcocysts were often seen (17/61, 28%) histologically in adult leopards in the skeletal, diaphragm, periocular, longus colli and tongue muscles as well as in the heart. The cysts were associated with mild lymphoplasmacytic (M17, M50, F57, F58) and, rarely (F57), neutrophilic inflammation, as well as small foci of myofibre degeneration (F29). In the remaining cases the cysts were not associated with inflammation (Fig. 7).

3.5. Other systems

A section of a nematode with a large, multinucleated intestine and accessory hypodermal cords (likely a metastrongyle) was present in the renal cortical interstitium of F12, with minimal associated inflammation. In F28 the renal cortex contained a medium number of foci of eosinophilic inflammation and cortical inflammation.

Two animals had severe mange and sparse hair coats (F12, M56). Macroscopically the skin was extensively crusted with deep thick folds on the head, neck and upper shoulders (Fig. 8). Histologically, alopecia, epidermal hyperplasia with marked parakeratotic hyperkeratosis and epidermal lichenification was associated with abundant mites in the stratum corneum, small numbers of random intra-epidermal pustules, oedema of teh stratum corneum and abundant large bacterial cocci (likely *Staphylococcus*) and yeasts (likely *Malassezia*), mild superficial diffuse lymphocytic dermatitis and moderate sebaceous gland hyperplasia. The mites had chitinized appendage bases, cuticular triangular pegs and fine spines, characteristic of *Sarcoptes scabiei*.

Severe ectoparasitism due to *Rhipicephalus* and/or *Amblyomma* spp was recorded in F12, F23 and M29. M37 had unidentified protozoan cysts in the subcutis adjacent to the popliteal lymph node with mild lymphoplasmacytic inflammation. M50 had wet crusted skin with alopecia on the ears and ventral neck and many *Dirofilaria sudanensis* in the subcutis (Fig. 9).

4. Discussion

To the authors' knowledge this is the first detailed record of parasitic conditions affecting free-ranging leopards in southern Africa. As expected, these free-ranging leopards had multiple parasitic infections. The conditions recorded in this case series are likely not completely representative of the prevalence of such conditions in free-ranging leopards for several reasons. This group of leopards is biased towards animals at the human–wildlife interface. Also, these elusive animals may die away from human observation, and tissue sampling was variable. Large amounts of data are missing, either because organs or tissues appeared normal macroscopically or because tissues were not sampled for histological examination.

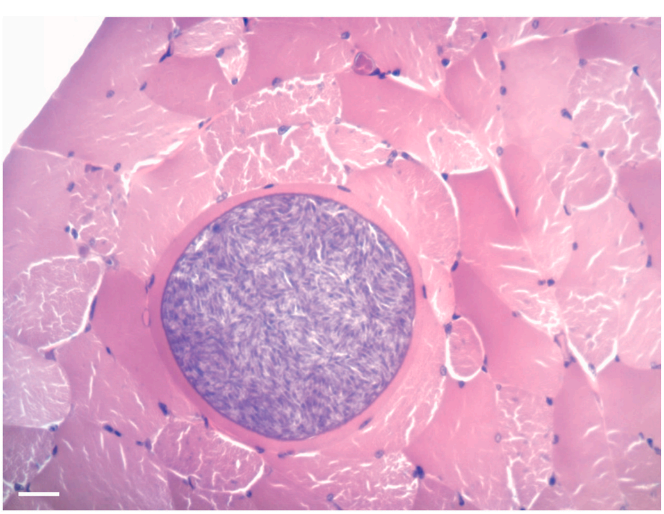

Fig. 7. *Sarcocystis* in skeletal muscle, with no associated inflammation, free-ranging leopard (M26). HE. Bar, 58 µm.

Fig. 8. Mange, free-ranging leopard (F9). Hyperplastic crusted skin on the face Bar

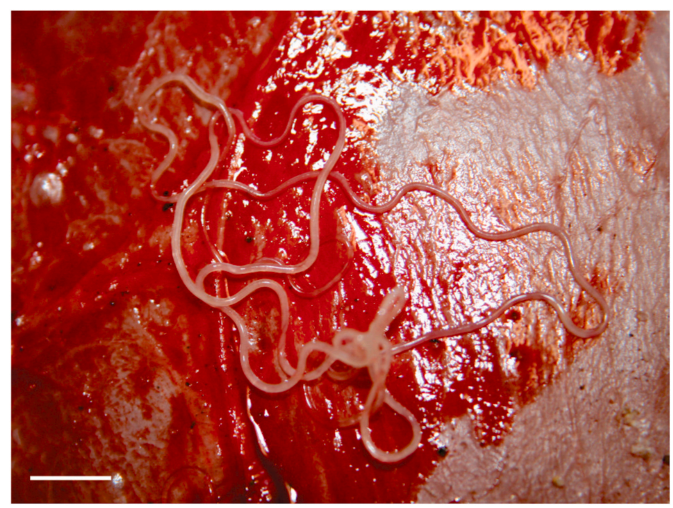

Fig. 9. Subcutaneous filariasis, free-ranging leopard. Long coiled white filarid helminths in the subcutis (M41). Bar, 24 mm.

While the presence of various helminth, coccidian, arthropod and protozoal parasites of leopards [7] and the morphology of the duodenal nematode *C. pardalis* [18,19] and the pentastome *A. armillatus* [36] have been reported previously, this paper contains the first detailed descriptions of the macroscopic and histological pathology of verminous pneumonia and gastroduodenitis as well as trematode, pentastome, filarid, *Hepatozoon* and *Sarcocystis* infestations in free-ranging leopards.

The lungworms were only identified histologically as metastrongylids. *Troglostrongylus subcrenatus* is recorded in leopards [13] while *Aelurostrongylus abstrusus* has been identified from caracals (*Caracal caracal)*, lions (*Panthera leo*) and serval (*Leptailurus serval*) in South Africa [37,38]. Both are snail-borne pathogens that may be transmitted to their hosts by paratenic hosts such as rodents and reptiles [39]. Vertical transmission may explain why *Troglostrongylus* spp are more common in domestic kittens than in adults [39]; in contrast, *Aelurostrongylus*spp are more common in adult cats [38]. Lungworm infestation was recorded only in leopards over 3.5 years old and not in the 11 younger animals; however, young animals were underrepresented in this case series. The lesions seen in this series were similar to those described in domestic cats [40].

Gastroduodenal nodules caused by nematodes of the genus *Cylicospirura* are common in domestic and free-ranging carnivores [19]. *Cylicospirura pardalis* parasitizes leopards [18]. The lesions seen in these leopards were similar to those described in the stomach of jaguarundis (*Herpailurus yagouaroundi*) [41], cougars (*Puma concolor*) [42], bobcat (*Felis rufus*) [43], lynx (*Felis canadensis*) and both domestic (*Felis domesticus*) [44] and wild (*Felis sylvestris*) cats [45]. Similar lesions in other parts of the gastrointestinal tract in these leopards may represent aberrant migration, as is recorded for other spirurids, such as *Spirocerca lupi* in domestic dogs [46]. The identity of the subcutaneous filarids was not determined in the one case recorded; *Filaria martis* and *F. russeli* have both been recorded in leopards [16]. *Acanthocheilonema reconditum* and *Dirofilaria repens* microfilaria are recorded in blood smears from domestic dogs from Mpumalanga, the province in which the GKNP is located [47].

The identity of the trematodes in the intestinal tract was not established. *Pharyngostomum cordatum* is recorded as occurring in leopards [13]. The pulmonary cysts associated with trematode infestations in Asian leopards and tigers were not seen [48,49]. Pentastome adults occur in the lungs of various snakes, commonly pythons [50], and are zoonotic pathogens through ingestion of infective ova in soil or water contaminated by snake faeces or consumption of intermediate hosts such as rodents [51,52]. The pathology caused by the *Armillifer* nymphs in M22 was similar to that described in humans [53]. No histological samples were submitted for the *L. serrata* adults in the nasal passages of one leopard. These parasites are recorded from the upper respiratory tract in lions, and larval cysts are common in the lymph nodes, liver and cardiac chambers of African buffalo in the GKNP [54].

Hepatozoonosis is caused by an intracellular coccidian parasite transmitted transplacentally and by ingestion of infected ticks [55]. *Hepatozoon* meronts were common in the heart and other tissues of these leopards and myocarditis could theoretically affect myocardial contractility and electrical signal transduction, but none of the leopards had evidence of chronic congestive left-sided heart failure to support this. Two species of *Hepatozoon* have been described in peripheral neutrophils (*H. luiperdjie*) and lymphocytes (*H. ingwe*) of South African leopards [27]; it is not known which species was present in the tissues of these leopards. The predilection of *Hepatozoon* for the myocardium is also recorded for the bobcat [56] as well as tissue cysts with mild to moderate inflammation in multiple organs in spotted hyaena (*Crocuta crocuta*) [57].

Sarcocystis parasites are also intracellular coccidian parasites with a predator-prey life cycle. Infective oocysts or sporocysts shed in predator faeces are ingested by the prey and form infective bradyzoites within sarcocysts in their muscle or nervous tissue. However, wild and domestic carnivores, including leopards, and humans can also be intermediate hosts [58–62]. Although clinical muscular disease is described in domestic dogs [59] and people [60], muscle infestations are thought to be largely incidental in felids since, as in these leopards, sarcocysts are associated with only mild lymphoplasmacytic inflammation and/or myofibre necrosis [63].

The identity of the mite causing the mange in two animals was likely *S. scabiei*, given the cuticular features. However, mites were not submitted for identification. Both *Notoedres cati* and *S. scabiei* are recorded in free-ranging carnivores [63–65], including lions [66] but not leopards, in the GKNP. Definitive identification of the lung nematodes, intestinal trematodes, *Hepatozoon* and *Sarcocystis* spp as well as the mites seen in leopards from the GKNP is needed, as is the life cycle of the *Sarcocystis* spp. Blood-borne parasites, apart from *Hepatozoon*, were not recorded in these animals.

Eosinophilic leucostasis and the presence of relatively large numbers of eosinophils in many tissues, not always in association with parasites, may be more common in free-ranging than confined animals exposed to fewer parasites. Similarly, eosinophilic inflammation in the intestine, lung, renal interstitium, coronary artery and pancreas may have been related to parasite infestations.

The clinical significance of parasite infestations in these free-ranging leopards is uncertain since many of the animals were euthanized due to behavioural rather than clinical conditions. In the majority of the animals, the parasites were associated with only minimal inflammation and therefore they likely did not cause clinical disease. However, two leopards had gastric outflow obstruction due to *C. pardalis* lesions, two had severe mange and one had severe myocarditis due to hepatozoonosis, which may have significantly affected the health of the affected animals. In addition, four of these five animals had four or more additional parasite infestations. The factors that trigger the transformation of common subclinical parasitic conditions to potentially life-threatening disease, which possibly increases the risk of affected leopards to humans, requires further elucidation. This paper provides baseline information on parasites in free-ranging leopards that may be relevant to the management of threatened populations of leopards outside of protected areas.

Statement of author contributions

Conceptualization (**EPM, RB**); data curation, investigation, methodology, resources, review and editing (**all authors**); formal analysis, funding acquisition, project administration, software and original draft (**EPM**).

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Declaration of competing interest

The authors declared no financial or personal conflicts of interest in relation to the research, authorship or publication of this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcpa.2026.02.006.

References

[1] Bothma JP. Leopard ecology in Southern Africa. In: van Heerden J, editor. Proceedings of a symposium on lions and leopards as game ranch animals. Onderstepoort: Wildlife Group of the South African Veterinary Association; 1997. p. 27–36.

[2] Stuart C, Wilson V. The cats of Southern Africa. Bulawayo: Chipangali Wildlife Trust; 1988:8–9.

[3] Skinner JD, Chimimba CT. The mammals of the southern African subregion. 3rd revised edition. Cape Town: Cambridge University Press; 2005. p. 385–90.
[4] Swanepoel LH, Somers MJ, Van Hoven W, Schiess-Meier M, Owen C, Snyman A, et al. Survival rates and causes of mortality of leopards *Panthera pardus* in Southern Africa. Oryx 2015;49(4):595–603.
[5] Friedmann Y, Traylor-Holzer K. Leopard (*Panthera pardus*) case study: WG 5 - mammals. Case study 4. Mexico. 2008.
[6] Williams ST, Williams KS, Lewis BP, Hill RA. Population dynamics and threats to an apex predator outside protected areas: implications for carnivore management. R Soc Open Sci 2017;4:1–10.
[7] Boomker J, Penzhorn BL, Horak IG. Parasites of lions (*Panthera leo*) and leopards (*Panthera pardus*): a documentation. In: van Heerden J, editor. Proceedings of a symposium on lions and leopards as game ranch animals. Onderstepoort; South African Veterinary Association Wildlife Group; 1997. p. 131–42.
[8] Meltzer DGA. Disease overview of large felids. In: van Heerden J, editor. Proceedings of a symposium on lions and leopards as game ranch animals. Onderstepoort: South African Veterinary Association Wildlife Group; 1997. p. 143–51.
[9] Burroughs R. Veterinary management and care of large carnivores in captivity. In: van Heerden J, editor. Proceedings of a symposium on lions and leopards as game ranch animals. Onderstepoort: South African Veterinary Association Wildlife Group; 1997. p. 127–30.
[10] Leiper R. Guinea worm (*Dracunculus medinensis*) in a leopard at Broken Hill, Northwest Rhodesia. Vet News 1910;7:113.
[11] Raillet A, Henry AC. Un Haemostrongylus des bronches du leopard. Bull la Soc Pathol Exot 1913;6:451–4.
[12] Adler S. Ancylostomes in a leopard. Ann Trop Med Parasitol 1924;16:293–4.
[13] Baer JG. Helminthes parasites. Exploration des Parcs Nationaux du Congo Belge: JG Baer - W Gerber Mission (1958). Brussels: Belgian Congo National Parks Institute; 1959.
[14] Vulsteke C. Note sur quelques nematodes parasites avec description de neuf especes nouvelles. Rev Zool Bot Afr 1956;53:441–77.
[15] Nelson GS, Guggisberg CWA, Mukundi J. Animal hosts of *Trichinella spiralis* in East Africa. Ann Trop Med Hygeine 1963;57:332–46.
[16] Tadros G. On *Filaria martis* Gmelin, 1790, newly recorded from the leopard with the description of a new species of the Genus *Filaria* Mueller, 1787. J Helminthol 1964;38:125–8.
[17] Jooste R. A checklist of helminth parasites of the larger domestic and wild mammals of Zimbabwe. Trans Zimbabwe Sci Assoc 1988;64:15–32.
[18] Junker K, Lane EP, Mcree AE, Foggin C, Schalk Van Dyk D, Mutafchiev Y. Two new species of *Cylicospirura vevers*, 1922 (Nematoda: Spirocercidae) from carnivores in Southern Africa, with validation of the related genera *Gastronodus* Singh, 1934 and *Skrjabinocercina* Matschulsky, 1952. Folia Parasitol 2013;60(4):339–52.
[19] Junker K, De Klerk-Lorist LM, Foggin C, Martin C, Mutafchiev Y. Further studies on the diversity of *Cylicospirura Vevers*, 1922 (Nematoda: Spirocercidae) in African carnivores, with description of a new species. Parasitology 2024:151(7);692–707.
[20] Hudson JR. A list of cestodes known to occur in East African mammals, birds and reptiles. J East African Ugandan Nat Hist Soc 1934;49–50:2015–217.
[21] Verster A. A taxonomic revision of the genus *Taenia* Linnaeus, 1758 s. str. Onderstepoort J Vet Res 1969;36:3–58.
[22] Zumpt F. Myiasis in man and animals in the old world. A textbook for physicians, veterinarians and zoologists. London: Butterworths; 1965. p. 267.
[23] Patton S, Rabinowitz A. Parasites of wild Felidae in Thailand: a coprological survey. J Wildl Dis 1994;30:472–5.
[24] Hasslinger MA, El-Assaly T, Selim M. Comparative studies on coprological results of carnivorous animals in zoological gardens of Giza, Egypt, and Munich, Germany. Assiut Vet Med J 1992;26:102–9.
[25] Penzhorn BL, Kjemtrup A, Lopez-Rebollar LM, Conrad PA. *Babesia leo* n. sp. from lions in the Kruger National Park, South Africa, and its relation to other small piroplasms. J Parasitol 2001;87:681–5.
[26] Khoshnegah J, Mohri M, Mirshahi A, Mousavi SJ. Detection of *Hepatozoon* sp. in a Persian leopard (*Panthera pardus ciscaucasica*). J Wildl Dis 2012;48(3):776–80.
[27] Van As M, Netherlands EC, Smit NJ. Molecular characterisation and morphological description of two new species of *Hepatozoon* Miller, 1908 (Apicomplexa: Adeleorina: Hepatozoidae) infecting leukocytes of African leopards *Panthera pardus pardus* (L.). Parasites Vectors 2020;13(1):1–16. https://doi.org/10.1186/s13071-020-3933-6.
[28] Baker J. A trypanosome of the *T. congolense* group in African lion and leopard. Trans R Soc Trop Med Hyg 1960;54:2.
[29] Dasgupta B, Pal N. Blood film of leopard from Darjeeling, showing trypanosomes. Trans R Soc Trop Med Hyg 1977;71:386.
[30] Namroodi S, Gholami A, Shariat-Bahadori E. Toxoplasmosis may lead to road kills of Persian leopards (*Panthera pardus saxicolor*) in Golestan National Park, Iran. J Wildl Dis 2016;52(2):436–8.
[31] Haeselbarth E, Segerman J, Zumpt F. Insecta, excluding Phthiraptera. In: In: Zumpt F., editor. The arthropod parasites of vertebrates in Africa South of the Sahara. Johannesburg: The South African Institute for Medical Research; 1966. p. 283.
[32] Theiler G. The *Ixodidea* parasites of vertebrates in Africa South of the Sahara (Ethiopian Region). 1962. p. 1–260. Project report S9958, Onderstepoort, South Africa.
[33] Suvarna S, Layton C, Bancroft J, editors. Bancroft's theory and practice of histological techniques. 8th ed. St. Louis: Elsevier; 2019.
[34] Gardiner C, Poynton S. An Atlas of metazoan parasites in animal tissues. Washington D.C.: Armed Forces Institute of Pathology; 1999. p. 1–64.
[35] Gardiner C, Fayer R, Dubey J. An atlas of protozoan parasites in animal tissues. 2nd ed. Washington D.C.: Armed Forces Institute of Pathology; 1998. p. 41–6.
[36] Junker K, de Klerk-Lorist LM. Severe infection caused by nymphs of *Armillifer armillatus* (Pentastomida, Porocephalidae) in a leopard, *Panthera pardus*, in the Kruger National Park, South Africa. Parasitol Int 2020;76:102029. https://doi.org/10.1016/j.parint.2019.102029.
[37] Traversa D, Morelli S, Di Cesare A, Diakou A. Felid cardiopulmonary nematodes: dilemmas solved and new questions posed. Pathogens 2021;10(1):1–17.
[38] Di Cesare A, Laiacona F, Iorio R, Marangi M, Menegotto A. *Aelurostrongylus abstrusus* in wild felids of South Africa. Parasitol Res 2016;115(10):3731–5. https://doi.org/10.1007/s00436-016-5134-y.
[39] Bezerra-Santos MA, Mendonza-Roldan JA, Abramo F, Lia RP, Tarallo VD, Salant H, et al. Transmammary transmission of *Troglostrongylus brevior* feline lungworm: a lesson from our gardens. Vet Parasitol 2020;285:1–7. https://doi.org/10.1016/j.vetpar.2020109215.
[40] Gianelli A, Passantino G, Nascimento Ramos RA, Lo Presti G, Lia R, Brianti E, et al. Pathological and histological findings associated with the feline lungworm *Trogostrongylus brevior*. Vet Parasitol 2014;202(3–4):104–12.
[41] Matos MR De, Faccin M, Dahm V, Rafael C, Walter IM, Zabott MV, et al. Veterinary Parasitology : regional studies and reports: macroscopic and microscopic aspects. Vet Parasitol 2023;40:1–3.
[42] Ferguson JA, Woodberry K, Gillin CM, Jackson DH, Sanders JL, Madigan W, et al. *Cylicospirura* species (Nematoda: Spirocercidae) and stomach nodules in cougars (*Puma concolor*) and bobcats (*Lynx rufus*) in Oregon. J Wildl Dis 2011 Jan;47(1): 140–53.
[43] Pence DB, Samoil HP, Stone JE. Spirocercid stomach worms (Nematoda: Spirocercidae) from wild felids in North America. Can J Zool 1978;56(5):8–10. https://doi.org/10.1139/z78-146.
[44] Crossland NA, Hanks CR, Ferguson JA, Kent ML, Sanders JL, Del Piero F. First report of *Cylicospirura felineus* in a feral domestic shorthair cat in North America. J Feline Med Surg Open Reports 2015;1(2). 2055116915593964.
[45] Diakou A, Migli D, Dimzas D, Morelli S, Di Cesare A, Youlatos D, et al. Endoparasites of European wildcats (*Felis silvestris*) in Greece. Pathogens 2021;10 (5):1–23.
[46] Harrus S, Harmelin A, Markovics A, Bark H. *Spirocerca lupi* infection in the dog: aberrant migration. J Am Anim Hosp Assoc 1996;32:125–30.
[47] Schwan EV. Filariosis of domestic carnivores in Gauteng, KwaZulu-Natal and Mpumalanga provinces, South Africa, and Maputo province, Mozambique. Doctorate for department of veterinary tropical diseases in the faculty of veterinary science. University of Pretoria; 2009.
[48] Dharanesha NK, Saminathan M, Mamta P, Ramesh KR, Ananda KJ, Giridhar P, et al. Parasitic pneumonia caused by *Paragonimus* spp. in a wild royal Bengal tiger, Mysuru, South India. J Parasit Dis 2019;43(3):528–33. https://doi.org/10.1007/s12639-019-01112-8.
[49] Daengsvang S, Papasarathorn T, Tongkoom B. *Paragoniumus westermani* (Kerbert, 1878) in Thai leopards. Ann Trop Med Parasitol 1964;58(3):304–6.
[50] Young E. Pentastomiasis (*Armillifer and Linguatula* Sp.) infestations of wild animals in the Kruger National Park. J S Afr Vet Assoc 1975;46(4):335–6.
[51] Latif B, Omar E, Heo CC, Othman N, Tappe D. Case report: human pentastomiasis caused by *Armillifer moniliformis* in Malaysian Borneo. Am J Trop Med Hyg 2011;85 (5):878–81.
[52] Odeniran PO, Ademola IO. Zoonotic parasites of wildlife in Africa: a review. African J Wildl Res 2016;46(1):1–13.
[53] Tappe D, Büttner DW. Diagnosis of human visceral pentastomiasis. PLoS Negl Trop Dis 2009;3(2):e320.
[54] Shamsi S, Halajian A, Barton DP, Zhu X, Smit WJ, Roux F, et al. Occurrence and characterisation of tongue worms, *Linguatula* spp., in South Africa. Int J Parasitol Parasites Wildl 2020;11:268–81. https://doi.org/10.1016/j.ijppaw.2020.03.002.
[55] Baneth G. Perspectives on canine and feline hepatozoonosis. Vet Parasitol 2011; 181(1):3–11. https://doi.org/10.1016/j.vetpar.2011.04.015.
[56] Dubey JP, Gupta A, de Araujo LS, Kwok OCH, Rosenthal BM. *Hepatozoon rufi* n. sp. (Apicomplexa: Hepatozoidae) of bobcats (*Lynx Rufus*) from Mississippi. J Parasitol 2024;110(6):607–18.
[57] East ML, Wibbelt G, Lieckfeldt D, Ludwig A, Goller K, Wilhelm K, et al. A *Hepatozoon* species genetically distinct from *H. canis* infecting spotted hyenas in the Serengeti ecosystem, Tanzania. J Wildl Dis 2008;44(1):45–52.
[58] Somvanshi R, Koul GL, Biswas JC. *Sarcocystis* in a leopard (*Panthera pardus*). Indian Vet Med J 1987;11(3):174–5.
[59] Dubey JP, de Araujo LS, Gupta A, Kwok OCH, Rosenthal BM. *Trichinella* and at least three species of *Sarcocystis* parasitize the muscles of bobcats (*Lynx rufus*) from Mississippi. J Parasitol 2024;110(4):402–11.
[60] Arness MK, Brown JD, Dubey JP, Neafie RC, Granstrom DE. An outbreak of acute eosinophilic myositis attributed to human *Sarcocystis* parasitism. Am J Trop Med Hyg 1999;61(4):548–53.
[61] Dubey JP, Gupta A, Calero-Bernal R, de Araujo LS, García-Gil ML, Battle J, et al. Gray fox (*Urocyon cinereoargenteus*) identified as a new intermediate host for *Sarcocystis neurona*. Vet Parasitol Reg Stud Reports 2024;56:1–9.
[62] Greiner EC, Roelke ME, Atkinson CT, Dubey JP, Wright SD. *Sarcocystis* sp. in muscles of free-ranging Florida panthers and cougars (*Felis concolor*). J Wildl Dis 1989;25(4):623–8.
[63] Terio KA, Mcaloose D, Mitchell E. Felidae. In: Terio KA, McAloose D, St Leger J, editors. Pathology of wildlife and zoo animals. St. Louis: Elsevier; 2020. p. 263–85.

[64] Ryser-Degiorgis M-P, Ryser A, Bacciarini LN, Angst C, Gottstein B, Janovsky M, et al. Notoedric and sarcoptic mange in free-ranging lynx from Switzerland. J Wildl Dis 2002;38(1):228–32.
[65] Najera F, Crespo E, Garc A, Grande-Gomez R, Herrera-Sanchez F, Gentil M, et al. First description of sarcoptic mange in a free-ranging European wildcat (*Felis silvestris silvestris*) from Spain. Animals 2021:1–8.
[66] Young E. Some important parasitic and other diseases of lion. J S Afr Vet Assoc 1976;46(2):181–3.