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REVIEW ARTICLE

Status of Hepatozoonosis in Wildlife: A Global Overview

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Abstract

Hepatozoon spp. are parasites that are transmitted by ticks and can cause a range of diseases in both wild and domestic animals, varying in severity. These parasites have the ability to infect a diverse range of species such as birds, reptiles, amphibians, and mammals. They are frequently encountered in tropical and subtropical areas on all continents. Various carnivores, such as foxes (*Vulpes vulpes*), black-backed jackals (*Canis mesomelas*), golden jackals (*Canis aureus*), African wild dogs (*Lycaon pictus*), hyenas (*Crocuta crocuta*), cheetahs (*Acinonyx jubatus*), leopards (*Panthera iridus*), and lions (*Panthera leo*), have been identified as hosts for similar species of *Hepatozoon* based on their physical characteristics. This article aims to provide a comprehensive review of the findings regarding *Hepatozoon* spp. in both free-roaming and captive wild animals from America, Europe, and Asia. The discovery of *Hepatozoon* spp. in different types of mammals, including those that are domesticated and those that are wild, indicates that there is a possibility of diseases spreading between animals that live in the wild and those that are domesticated.

1. Introduction

The investigation of Hepatozoon, a genus containing over 300 species of apicomplexan parasites, has primarily focused on domestic animals and peri-urban areas, neglecting its occurrence in natural environments and wild hosts, which remains poorly explored (Ferrari *et al.*, 2022). The *Hepatozoon* genus includes a variety of species that can infect a diverse array of animals, spanning birds, reptiles, amphibians, and mammals. These infections are prevalent in tropical and subtropical regions across all continents (O'Dwyer, 1907). until a decade ago, histology methods were primarily used to study blood parasites, with a few exceptions. Nevertheless, since the mid-2010s, molecular methods have become increasingly popular for detecting and identifying Hepatozoon infections. Specifically, researchers have utilized the amplification of fragments of the small subunit ribosomal RNA (18S rRNA) gene. These advanced techniques have enabled the identification of numerous Hepatozoon species in wild mam-

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mals throughout Europe, America, and Asia, with a particular emphasis on carnivores. Although the 18S rDNA gene has been commonly used as a molecular marker for phylogenetic analyses, problems in separating closely related species might occur due to its high degree of conservation. All Hepatozoon species share a common life cycle, which includes gametogony and sporogony taking place in the definitive host, typically a bloodsucking invertebrate. In the intermediate host, usually a vertebrate, schizogony occurs, followed by the development of gametes (Hamšíková *et al.*, 2016). *Hepatozoon* spp. can be transmitted in multiple ways, but the most common method is through the ingestion of infected vectors or hosts. In mammals, for example, animals can become infected when they consume arthropod ectoparasites like ticks, fleas, and mosquitoes, which carry the parasite and are attached to another individual. This transmission often occurs during grooming behavior (Hamšíková *et al.*, 2016; Johnson *et al.*, 2010; Paperna *et al.*, 2004; Rigó *et al.*, 2016). Furthermore, transmission can happen within predator-prey food webs when predators consume prey that either carry infectious meronts or are infested by infected vectors. Additionally, vertical transplacental transmission has also been observed (Duscher *et al.*, 2015; Murata *et al.*, 1993). *Hepatozoon* spp. have been found in a wide range of habitats across the globe. These habitats include temperate broad-leaved forests, conifer forests, Mediterranean scrublands, subtropical forests, savannahs, and taiga. These findings suggest the presence of intricate transmission cycles that remain largely unexplored (Battisti *et al.*, 2020; Duscher *et al.*, 2014; Laakkonen *et al.*, 2001; Modrý *et al.*, 2021; Perles *et al.*, 2019; Tomé *et al.*, 2014). However; there is still a significant gap in our understanding of the occurrence and frequency of *Hepatozoon* spp. in essential ecosystems. Particularly, limited research has been conducted in exploring their presence across different altitude levels, where the swift habitat changes correspond to a diverse range of hosts that coexist to some extent. Mountainous habitats are highly vulnerable to both abiotic and biotic changes caused by global climate alterations. These changes can lead to the movement of mammal species to higher elevations, which in turn can cause significant alterations within local communities (Gilman *et al.*, 2010; Urban *et al.*, 2016). Consequently, there is a possibility for the expansion of parasites in both their geographic range and the variety of host species they infect (Garcia-Vozmediano *et al.*, 2020; Rizzoli *et al.*, 2019). Monitoring the presence of *Hepatozoon* spp., a parasite with a highly adaptable transmission cycle, is vital in habitats experiencing rapid changes. In rodents, *Hepatozoon* spp. infection can be pathogenic, but typically presents as a mild and asymptomatic condition (Brumpt *et al.*, 1946; Hoogstraal, 1961; Johnson *et al.*, 2008; Miller, 1908; Simpson *et al.*, 2006). In contrast, carnivorous animals like canids, felids, mustelids, and snakes may experience a weakened immune system due to *Hepatozoon* spp. This makes them more vulnerable to co-infection with other pathogens (Alencar *et al.*, 1997; Cunningham and Yabsley, 2011; Diakou *et al.*, 2020; Zechmeisterová *et al.*, 2021). This article aims to provide a comprehensive review of the findings regarding *Hepatozoon* spp. in both free-roaming and captive wild animals from America, Europe, and Asia. The sampling methods and materials used, as well as the laboratory detection methods employed, will be discussed in detail.

2. Reports of *Hepatozoon Species* in Domestic and Wild Canids

Dogs. Two species of Hepatozoon have been discovered in pet dogs: *Hepatozoon canis*, which has been observed in various regions including Europe, Asia, Africa, South America, and the United States; and *Hepatozoon americanum*, which has only been found in the United States so far. In Brazil, the only species found affecting dogs is *H. canis* (Forlano *et al.*, 2007; Vincent-Johnson *et al.*, 1997; Shkap *et al.*, 1994). *H. canis*, a protozoan belonging to the phylum Apicomplexa, was initially discovered in dogs' blood in India and identified as *Leukocytozoon canis* (Christophers, 1907; James, 1905). *H. canis* like *Theileria* spp. and *Rangelia vitalii* can infects both leukocytes and parenchymal tissues (Fig. 1).

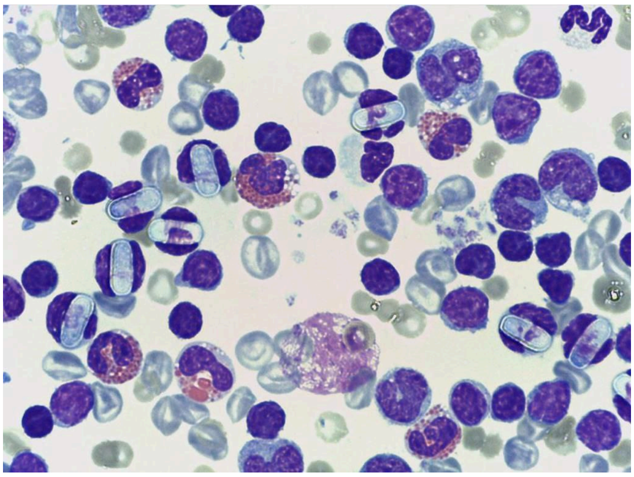

Fig. 1. Giemsa-stained blood smear showing gamonts of *Hepatozoon* spp in neutrophils (100x).

Dogs become infected with *H. canis* when they consume ticks that harbor mature oocysts (Baneth *et al.*, 2001). The brown dog tick, known as Rhipicephalus sanguineus, plays a vital role as the main host for *H. canis*. Although in Brazil, the main vector of *H. canis* is Amblyomma ovale. Dogs and wild canids, on the other hand, serve as intermediate hosts in this process.^{3} In general; there is a significant difference in how common

Hepatozoon spp. is between red foxes and dogs living in the same areas (Cassini *et al.*, 2009; Conceição-Silva *et al.*, 1988; Gabrielli *et al.*, 2010). Blood PCR analysis revealed the presence of *H. canis* in 21.1% of the 331 dogs surveyed in kennels and shelters located in southern Portugal (Menn *et al.*, 2010). Foxes are considered to be important carriers of *H. canis* for pet dogs. It is interesting to observe that foxes have been present in the world for a much longer time than the domestication of dogs (Lindblad-Toh *et al.*, 2005; Allen *et al.*, 2008). Conducted a study to examine the Hepatozoon species found in the blood of 274 domestic dogs in Oklahoma, United States. Nested PCR and Real-time PCR techniques were employed for the analysis. The findings of the study indicated that *H. americanum* was the most commonly found species in dogs residing in endemic areas, as well as in clinically ill and shelter dogs. Moreover, certain dogs (n=2) that presented with clinical signs of American canine hepatozoonosis or similar organisms referred to Oklahoma State University. These results indicate that *H. americanum, H. canis, and H. canis* could potentially be responsible for causing diseases in dogs residing in the southern part of the United States (Allen *et al.*, 2008). A study conducted in Brazil in 2016 by de Castro Demoner et al. uncovered a significant occurrence of *H. canis* in dogs. By employing both microscopic examination and molecular analysis, they determined that around 66.45% of the dogs were infected with *H. canis* (de Castro Demoner et al., 2016). *H. canis* infection is highly prevalent in dogs, and it is commonly found in areas where the tick vector *R. sanguineus* is present. This encompasses various regions, including Africa, Southern Europe, South America, Asia (including the Middle East), as well as islands in the Pacific and Indian Oceans (Table 1).

Hepatozoonosis is a disease that is commonly found in animals and its prevalence can vary. Previous research has shown different percentages of *H. canis* seroprevalence in dogs. For example, in Portugal, it was reported to be 36%, in Nigeria 17.6%, in India 2.5%, and in Thailand 2.1% (Gevrey, 1993). In order to accurately evaluate the degree and seriousness of *H. canis* infection in dogs, it is crucial to conduct comprehensive and systematic studies. This is because the infection often does not show any clinical signs. Furthermore, the occurrence of *H. canis* infection greatly differs in various regions. For example, 39% of dogs in rural areas of Rio de Janeiro state, Brazil, were found to have circulating *H. canis* gamonts in their blood. In Zaria, Nigeria, this percentage was 22% among dogs, whereas in Malaysia, only 1.2% of dogs were affected (Ezeokoli *et al.*, 1983; O'dwyer *et al.*, 2001; Rajamanickam et al., 1985). The PCR analysis did not thoroughly investigate the similarity among various *H. canis* isolates. A thorough examination of the 18S rRNA of Hepatozoon isolates from dogs in Japan and Israel showed a significant resemblance, with a similarity of up to 99% to *H. canis*. However, these isolates were distinct from *H. americanum* (Inokuma *et al.*, 2002). The initial clinical case of *H. canis* infection in Bulgaria was documented by Ivanov and Kanakov (Ivanov and Kanakov, 2003). Jittapalapong *et al.* were the first to molecularly confirm the presence of *H. canis* in Thailand (Jittapalapong *et al.*, 2006). Canine hepatozoonosis was initially discovered in 1978 around the Gulf Coast area of Texas, marking the first documented cases in the United States. Since then, the illness has been found in dogs throughout a large part of the southeastern United States. At first, it was believed to be caused by *H. canis*, but the disease showed a much more severe progression compared to cases of canine hepatozoonosis seen in other parts of the world. Significant variations were noted not just in the clinical symptoms and laboratory abnormalities, but also in the pathological characteristics, tissue specificity, parasite structure, and tick vectors. In 1997, this organism was officially acknowledged as a separate species called *H. americanum* (Craig *et al.*, 1978). Javad Khoshnegah *et al.* were the first to report the detection of *H. canis* infection in dogs in Iran. The diagnosis was confirmed under a microscope by identifying *H. canis* gametocytes within neutrophils in peripheral blood smears and bone marrow smears stained with Giemsa (Khoshnegah *et al.*, 2009). Zeinali *et al.* made a significant contribution by documenting the first-ever occurrence of *H. canis* infection in dogs living in northwestern Iran. The results of their research showed a significant occurrence of canine hepatozoonosis in the examined area, suggesting that the disease is widespread among dogs in that particular region (Zeinali *et al.*, 2022). Sanchez *et al* in 2021 determined the prevalence of Hepatozoon infections in 80 stray dogs in Cuba and confirmed the species identity and phylogenetic relationships of the causative agent. The DNA sequences of the *18S* rRNA gene of the *Hepatozoon* spp. from Cuban dogs showed a nucleotide identity >99% with those of *18S* rRNA sequences of *H.canis* isolates from Czech Republic, Brazil and Spain. Phylogenetic analysis revealed that obtained sequences clustered within the *H. canis* clade, different from the *Hepatozoon felis* or *H. americanum* clades (Díaz-Sánchez *et al.*, 2021).

3. Reports of *Hepatozoon Species* in Foxes

In 2023, Uiterwijk *et al.* conducted a European study which discovered the existence of Hepatozoon in red foxes throughout Europe (Uiterwijk *et al.*, 2023). A study conducted by Cardoso *et al.* in 2014 examined the occurrence of *H. canis* in red foxes from different regions of Portugal.

Table 1
The worldwide occurrence of Hepatozoon species in various animal species within the realm of wildlife.

Animal	Number of sampling and animal	Country/ location of sampling	species	The type of material sampled	prevalence	laboratory method	Reference
Dog	274 dogs	USA	Hepatozoon	Blood species	Out of 77 canine blood samples that tested positive for 68 (24.8%) were found to have H. americanum, Hepatozoon spp., 2 (0.7%) had H. canis, Hepatozoon spp., both H. americanum and H. canis.	Nested PCR and real-time PCR	(Allen *et al.* 2008).
Dog	158 dogs	Brazil	Hepatozoon spp.	Blood samples	The occurrence of H. canis was found to be quite high, at 66.45%.	microscopy and molecular analysis	(de Castro Demoner *et al.* 2016).
Cat	37 free-roaming domestic cats	Brazil	Anaplasmataceae, Hepatozoon sp. And.	Blood	Hepatozoon spp. 0%	PCR	(André *et al.* 2014).
Red fox	91 Red foxes (Vulpes vulpes)	Portugal	Hepatozoon canis	Blood and tissues including bone marrow heart, ..	PCR: 68 out of 90 foxes (75.6%)	PCR and histopathological detection	(Cardoso *et al.* 2014).
Grey wolf	107 grey wolf (Canis lupus L.1758)	Serbia	Hepatozoon canis	Spleen samples	Out of the 30 locations that were tested, 26 of them had confirmed cases of H. canis, with a total of 62 individuals (57.94%) testing positive for the disease.	The 18S ssrRNA gene was analyzed genetically by sequencing a fragment of it using PCR.	(Kuručki, Tomanović, Sukara & Ćirović 2022).
Jackal	An Indian jackal	India	Hepatozoon Canis	Blood sample	After undergoing eight weeks of rehabilitation, the animal experienced a significant decline in its haemoglobin concentration, platelet count, and weight, and was subsequently diagnosed with Hepatozoonosis.	CBC	(Kolangath *et al.* 2022).
Giant panda	14 giant pandas (Ailuropoda melanoleuca)	USA, china and UK	a novel Hepatozoon species	Blood smear	100% prevalence (14/14) across		(Yu *et al.* 2019).
Short-beaked echidna	18 short -beaked echidna	-	Hepatozoon tachyglossi	Blood samples	Nine (50%) of these animals showed parasitaemia with H tachyglossi.	CBC and multiple biochemical analysis electron microscopy	(Ploeg *et al.* 2008).
Captive cranes	3 Young captive cranes	Japan	Hepatozoon-like organisms	Liver, spleen, blood films and bone marrow	-	microscope	(YASUDA, KONC & KOYAMA 1987).
Grey squirrel	Grey Squirrel (Sciurus carolinensis Gmelin, 1788)	USA	Hepatozoon griseisciuri n. sp.	-	-	-	(Clark 1958).
chuckwalla Sauromalus spp.	-	USA	Hepatozoon sauromali sp.	Blood	-	Blood film test	(Lewis & Wagner 1964).
Snake	Eighty-three Neotropical Rattlesnake, Crotalus durissus collilineatus	Brazil	Hepatozoon	Blood	Wild snakes that were caught had an infection rate of 38.3%, but no Hepatozoon sp. was found.	spectrophotometery	(Motta *et al.* 2011).
Snake	1 Southern Water Snake (Nerodia fasciata pictiventris).	USA	Hepatozoon sp.	Peripheral blood	-	Gross and histopathologic examination of the liver	(Wozniak *et al.* 1998).
wild mammals and ticks	2801 wild mammals and 754 ticks	Europe	Hepatozoon species	Ticks and a piece of spleen From each wild mammal.	Out of the total number of wild mammals observed, 370 (13.2%) were found to be carrying ticks. In the study, a total of 754 ticks were collected and tested for Hepatozoon. The results showed that none of the 35 Hyalomma sp. ticks tested positive for Hepatozoon, while 4 out of 25 Dermacentor spp. ticks (16.0%) and none of the 23 Haemaphysalis spp. ticks tested positive. Additionally, 24 out of 50 Ixodes ticks (5.3%) and 3 out of 221 Rhipicephalus spp. ticks (1.4%) tested positive for Hepatozoon, with an overall positive rate of 4.2% (32/754). The most commonly found species of Hepatozoon was H. canis, with a total of 22 positive cases.	PCR	(Uiterwijk **et al.** 2023).
Small mammal species	30 Ear samples mammal species	Italy	Hepatozoon spp.	Ear samples	an overall prevalence of 35.9%	PCR	(Ferrari *et al.* 2022).
Sika deer (Cervus nippon centralis) and Japanese wild boar (Sus scrofa leucomystax)	63 Sika deer (Cervus nippon centralis) and 30 Japanese wild boar (Sus scrofa leucomystax) 63 heads were found.	Japan	Sarcocystis cyst (sarcocyst) and Hepatozoon sp.		During the survey, it was discovered that Hepatozoon sp. was present in the muscles of boars.	An examination of the 18S ribosomal DNA through phylogenetic analysis	(Matsuo, Uetsu, Takashima & Abe 2016).

The wild felids cand anids found in India include the Asiatic lion, Indian tiger, Indian leopard, Indian, wild dog as well as domestic and cats that are dogs native to India.	-	India	Hepatozoon spp.	Blood samples	PCR testing revealed the presence of Hepatozoon spp. in the blood samples of several animals. Specifically, out of the 9 Asiatic lions tested, 5 were positive for Hepatozoon spp. Additionally, 2 out of 5 Indian tigers, 2 out of 4 Indian leopards, and 2 out of 2 Indian wild dogs were also found to have the presence of Hepatozoon spp. Furthermore, 2 out of 4 domestic cats and 2 out of 3 domestic dog samples were positive for Hepatozoon spp.	PCR.	(Pawar *et al.* 2012).
Wild carnivores from Brazil and other exotic locations, including captive wild cat and dogs	In Brazil, there are 165 captive wild felids and 100 captive wild canids, which a re exotic carnivorous animals.	Brazil	Hepatozoon spp.	Blood samples	Six types of cats, including four little spotted cats, one jaguarondi,. and one puma, as well as five types of canids, including two bush dogs, one fox, one crab-eating fox, and one maned wolf, were found to be infected with Hepatozoon spp.	PCR.	(André *et al.* 2010).
36 distinct species of wild animals, including reptiles, birds, and mammals.	A total of 181 wild animals were examined, consisting of 36 distinct species. These species included 2 reptiles, 5 birds, and 29 mammals.	Brazil	Piroplasmida (genera Babesia, Theileria, Cytauxzoon) or Hepatozoon.	Blood samples	The study found various types of Piroplasmida and Hepatozoon agents in different animals. Specifically, Cytauxzoon felis was detected in one ocelot, Theileria cervi in two red brocket deer, Theileria spp. in three nine-banded-armadillos, one agouti, and four lowland pacas, and Babesia spp. in one common opossum and one white-lipped peccary. Additionally, Hepatozoon sp. (possibly Hepatozoon caimani) was found in three spectacled caimans, Hepatozoon felis in an ocelot, and Hepatozoon spp. in one scorpion mud turtle and one lowland paca.	PCR.	(Soares *et al.* 2017).

The researchers utilized both histopathology and molecular analysis to investigate this prevalence. This research involved the examination of blood and tissue samples obtained from 91 red foxes (Vulpes vulpes). PCR and sequencing methods were used to detect *H. canis* in 75.6% of the foxes that were sampled. Histopathological analysis revealed the presence of *H. canis* meronts in the bone marrow and spleen of certain foxes. Ultimately, it was concluded that PCR is more sensitive than histopathological examination in diagnosing *H. canis* (Cardoso *et al.*, 2014). PCR investigations carried out in different countries in southern Europe have shown a considerable prevalence of *H. canis* infection in foxes. In the case of red foxes from northern Spain, the use of blood PCR revealed a significantly lower occurrence of *H. canis* at 28.6% (2 out of 7) compared to the results obtained from this study in Portugal, where it reached 70.5%. When the spleen PCR method was used, the occurrence of *H. canis* in red foxes showed significant increase. In central Spain, the prevalence rate reached as high as 90% (18 out of 20), while in Croatia it was 23.0% (44 out of 191), and in Italy it was 16.4% (16 out of 119) (Gabrielli *et al.*, 2010; Criado-Fornelio *et al.*, 2006; Dežek *et al.*, 2010; Gimenez *et al.*, 2009). During a previous study carried out in southern Portugal, it was noted that among the 301 red foxes analyzed using blood, bone marrow, and spleen and lymph node smears under a microscope, a considerable proportion of 143 (48%) were found to be infected with meronts or gamonts (Conceição *et al.*, 1988). Meanwhile, in Israel, an enzyme-linked immunosorbent assay was employed to test 84 fox sera, and it was found that 20 (23.8%) of them exhibited positive results for antibodies to *H. canis* (Fishman *et al.*, 2004). The differences in research findings from various studies might suggest that the rates of occurrence vary depending on the geographical location. Nevertheless, these disparities could also arise due to the usage of different methods, like serology for identifying exposure and PCR for detecting infection, as well as variations in the tissues sampled (Criado-Fornelio *et al.*, 2007). There have been debates surrounding the detection of *H. canis* in striated muscle. This organism has been identified through PCR analysis in the heart and striated muscle tissues of red foxes from Slovakia (Majláthová *et al.*, 2007). Generally, there is a noticeable difference in the occurrence rates of *Hepatozoon* spp. between red foxes and dogs living in the same areas. *Hepatozoon* spp. is more commonly found in red foxes, while dogs have considerably lower rates of infection (Cassini *et al.*, 2009; Conceição-Silva *et al.*, 1988; Gabrielli *et al.*, 2010).

4. Reports of *Hepatozoon Species* in Wolves, Coyotes, Jackals and Hyenas

Research conducted by Mercer *et al.* in the USA in 1988 discovered that twelve coyotes (*Canis latrans*) from the Texas Gulf Coast had been infected with *Hepatozoon sp.* It is crucial to acknowledge that this study had certain limitations. These limitations encompassed a small sample size and a dearth of long-term follow-up data (Mercer *et al.*, 1988). In a more recent study conducted in 2022 by Kuručki *et al.* a thorough examination was performed on spleen samples taken from 107 gray wolves in Serbia. Using PCR, it was determined that 57.94% of the animals examined tested positive for *H. canis*. The presence of *H. canis* was confirmed in 62 out of 107 individuals (57.94%)

across 30 distinct locations. Genetic analysis has revealed the presence of five unique sequence types of *H. canis* in grey wolves, indicating a notable degree of diversity. The dietary habits of grey wolves in Serbia might play a role in fostering this diversity of *H. canis.* To enhance our comprehension further, further research is needed to explore the transmission mechanisms, potential sources of infection, and the impact of this pathogen on carnivores in the wild. This study is particularly noteworthy as it represents the first confirmation of *H. canis* presence in grey wolf populations in Serbia, highlighting the important role of grey wolves in the spread of this pathogen within natural ecosystems (Kuručki *et al.*, 2022). Hepatozoonosis in wild canids in India has been infrequently documented. In a study conducted by Kolangath *et al.* in 2022, a paraplegic Indian jackal with a decubital ulcer was diagnosed with *H. canis* following rehabilitation. The jackal was effectively treated using doxycycline and received supportive care, which led to a full recovery (Kolangath *et al.*, 2022). In a European study conducted by Uiterwijk *et al.* in 2023, it was found that *H. canis* had the highest prevalence among carnivores, specifically gray wolves and golden jackals, as well as in ticks. Out of the ticks tested, 32 ticks (4.2%) were identified as positive for *Hepatozoon*, with *H. canis* being the most frequently detected species. These findings indicate the potential for the pathogen to spill over into domestic animals, including domestic dogs (Uiterwijk *et al.*, 2023). In certain cases, the occurrence of clinical disease and mortality in hyenas has been associated with *Hepatozoon* spp. infection in young coyotes (East *et al.*, 2008; Garrett *et al.*, 2005; Kocan *et al.*, 2000). A thorough examination of the 18S rRNA of Hepatozoon isolates from coyotes in the south-central United States revealed 19 distinct sequences comprising 3 major clusters of *Hepatozoon* spp., i.e., 1 most closely related to *H. americanum*, another most closely related to *H. canis*, and the third an intermediate between the 2 groups and it was found the diversity of *Hepatozoon* spp. in coyotes appears greater than previously recognized (Starkey *et al.,* 2013).

5. Reports of *Hepatozoon Species* in Domestic and Wild Felids

Cats. In 1988, Mercer *et al.* conducted a study in the USA which revealed that three bobcats (Lynx rufus) from the Texas Gulf Coast were infected with *Hepatozoon sp* (Mercer *et al.*, 1988). Nevertheless, the study encountered some limitations, including a limited number of participants and the lack of data for long-term monitoring. In a separate study conducted in northern and central Portugal, the molecular evaluation of 320 cats revealed that none of them tested positive for *H. canis* using PCR analysis. However, it was discovered that 15.6% of the animals were PCR-positive for *H. felis* (Vilhena *et al.*, 2013). In a study conducted by André *et al.* in Brazil in 2014, they examined 37 free-roaming domestic cats using the PCR method, but they were unable to identify any *Hepatozoon* spp. Infections (André *et al.*, 2014). Before this study in Brazil, there were a few instances where phylogenetically related species of *Hepatozoon*, similar to *H. canis* and *H. felis*, had been found in cats residing in the state of São Paulo. Although these occurrences were infrequent. (de Bortoli *et al.*, 2011; Rubini *et al.*, 2006). Further research is crucial to enhance our understanding of how cats contribute to the spread of vector-borne diseases. André *et al.* (2010) conducted a study using molecular techniques to identify *Hepatozoon* spp. in different felids (including four little spotted cats, one jaguarondi, and one puma) and several canids (including two bush dogs, one fox, one crab-eating fox, and one maned wolf) in Brazil. This is the first time that *Hepatozoon* spp. has been identified in pumas, jaguarondis, bush dogs, and maned wolves in Brazil. Using PCR analysis, it was found that the prevalence of *Hepatozoon* spp. in wild felids in Brazil is lower compared to that in Neotropical felids in Maranhao and Ceara, domestic cats in Barcelona, and Iriomote wild cats and Tsushima leopard cats in Japan. The study also found that various species or genotypes of *Hepatozoon* spp. could infect wild carnivores in Brazil. The existence of *Hepatozoon* spp. in endangered Brazilian wild cats and dogs is a cause for concern due to the possible risks it poses to these animals. This is especially true for individuals with weakened immune systems or concurrent infections (André *et al.*, 2010).

6. Reports of *Hepatozoon Species* in Lions, Tigers and Leopards

In a study carried out by Mercer *et al.* in 1988, it was discovered that *Hepatozoon sp.* had infected six ocelots (*Felis pardalis*) from the Texas Gulf Coast (Mercer *et al.*, 1988). This particular wild species has the capability to act as a reservoir of infection for domestic dogs. Nevertheless, it is crucial to acknowledge that the study had certain limitations, such as a small sample size and a lack of long-term follow-up data. Pawar *et al.* conducted a study in 2012 where they identified the presence of *Hepatozoon* spp. in a diverse range of animals, encompassing Asiatic lions, Indian tigers, Indian leopards, Indian wild dogs, domestic cats, and domestic dogs. The specific species of *Hepatozoon* identified varied depending on the animal. *H. felis* was identified as the detected species in Asiatic lions, Bengal tigers, Indian leopards, and domestic cats. On the other side, *H. canis* was found to be the identified species in Indian wild dogs and domestic dogs. The detection of *H. felis* and *H. canis* in both domestic and wild animals indicates that these species are not confined to specific hosts and possess the capability to infect a range of felids and canids (Pawar *et al.*, 2012). Soares *et al.* conducted a study in 2017 in the Brazilian Amazon, where they identified the presence of *H. felis* in an ocelot (Soares *et al.*, 2017). It is evident based on morphological and molecular data that *Hepatozoon luiperdjien* and *Hepatozoon ingwen* which infect felines such as leopards are distinct species. These species are also distantly related to *H. felis* (GenBank: AY628681) based on 18SrDNA sequence comparisons, isolated by Criado Fornelio *et al.* from domestic cats from Spain. The *H. felis* isolates from Spain are widely regarded as the representative *H. felis* isolates to be used for comparison (Criado-Fornelio *et al.*, 2006).[55] Thus, based on the phylogenetic relationships and comparisons of *H. felis* and *H. felis*-like species of Hepatozoon, the identity of the Hepatozooninfecting large carnivores, currently identifed as H. felisby Pawar *et al.* is questioned.[73]

7. Reports of *Hepatozoon Species* in Pandas,

Sloth Bears and Wild Boar

In a separate study by Yu *et al.* in 2019, which focused on the blood of 14 giant pandas, a previously unknown species of *Hepatozoon* was identified in all of the pandas. Notably, all of these animals showed no symptoms of infection at the time of sampling (Yu *et al.*, 2015). In this study, Ribosomal DNA sequences indicated a previously undescribed Hepatozoon species. Phylogenetic and distance analyses of the sequences placed it within its own branch, clustered with Old World species with carnivore (primarily ursid and mustelid) hosts A significant number of Indian sloth bears have been found to be infected with *Hepatozoon ursi*, which poses a potential threat to their health and survival (Pawar *et al.*, 2011). Additionally, a new species of *Hepatozoon* called *Hepatozoon apri* has been discovered in Japanese wild boar (*Sus scrofa leucomystax*), and it is closely related to the *Hepatozoon species* found in carnivores (Yamamoto *et al.*, 2017). Furthermore, in a study carried out by Matsuo *et al.* in Japan in 2016, *Hepatozoon sp.* was discovered during an examination of pig muscles molecularly, and authors suggested Hepatozoon isolates from wild boars might be host-specific (Matsuo *et al.*, 2016).

8. Reports of *Hepatozoon Species* in Short-Beaked Echidna and Martens

Infection with *Hepatozoon tachyglossi* in echidnas is primarily accidental, with only one weakened individual displaying clinical symptoms. In a study conducted by Ploe *et al.* in 2008, which examined the blood of 18 short-beaked echidnas, *H. tachyglossi* was detected in 50% of the cases using various biochemical analyses and electron microscopy. However, in this review, authors do not observe consistent serum biochemical changes associated with parasitemia or monocytosis (Ploeg *et al.*, 2008). In short-beaked echidnas, the presence of *H. tachyglossi* as an intracellular parasite may be an accidental discovery during hematologic examination and is unlikely to serve as a reliable prognostic indicatorIn in order to find out the presence of the mentioned parasite (Hill *et al.*, 2022). The *Hepatozoon species* found in the hearts of Japanese martens is probably the same species found in pine martens in Scotland (Kubo *et al.*, 2009). Pine martens in Scotland were found to be infected with a newly discovered species of *Hepatozoon*, which is closely related to *H. canis*. This infection led to myocarditis and myositis, but did not result in any noticeable illness (Simpson *et al.*, 2005). In a European study carried out by Uiterwijk *et al.* in 2023, it was observed that *H. martis* was predominantly found in stone martens and pine martens (Uiterwijk *et al.*, 2023). The newly identified species, *Hepatozoon martis*, primarily infects martens and has a strong affinity for muscle tissue. It is also capable of transmission through non-vectorial routes such as transplacental, transmission by predation and carnivorism (Hodžić *et al.*, 2013).

9. Reports of *Hepatozoon Species* in Small Mammals

Hepatozoon perniciosum was discovered in laboratory (white rats) in Washington, D.C. The schizogonic cycle was identified in the liver, while the sporogonic cycle occurred in the mite (*Echinolaelaps echidninus)*, which served as the vector (Clark, 1985). Moreover, a sequence resembling *Hepatozoon ayorgbor* was identified in three spleen samples taken from great gerbils in northwestern China (Ji *et al.*, 2021). Several species from the *Hepatozoon* genus, including *H. lavieri*, *H. erhardovae*, *H. sylvatici*, *H. griseisciuri*, and on occasion *H. muris* (formerly known as *H. perniciosum*), have been identified in (European rodents) (Laakkonen *et al.*, 2001; Al-Quraishy *et al.*, 2021; De Monte, 1971; Karbowiak *et al.*, 2005; Pawelczyk *et al.*, 2004; Sebek, 1978). In a 2023 European study conducted by Uiterwijk *et al.*, rodents were found to harbor *Hepatozoon sp.* and *H. ayorgbor* (Uiterwijk *et al.*, 2023). In a study conducted in the North-Eastern Italian Alps, *Hepatozoon* spp. was found in 35.9% of the 830 ear samples collected from 11 various small mammal species. Among these species, *My. glareolus* and *Apodemus* spp. demonstrated the highest rates of infection. Significantly, this study represented the initial identification of *Hepatozoon* spp. in *C. nivalis*, an alpine species (Ferrari *et al., 2022*). *H. canis*, a parasite transmitted by ticks, is prevalent in rural areas of Brazil. Although the specific vectors responsible for transmitting *H. canis* remain unidentified, this has sparked speculation about alternative methods of transmission. In forest fragments located near rural regions in Botucatu County, Brazil, it was found that small rodents were harboring distinct *Hepatozoon* spp. infections that were different from *H. canis.* While sylvatic rodents may not function as reservoirs for *H. canis*, they have the potential to act as paratenic hosts for *Hepatozoon* spp. This means they can carry infective stages of the parasite, which can subsequently be transmitted to intermediate host predators (Demoner *et al.*, 2016). A high prevalence of *Hepatozoon* infection was observed among wild rodents in southeastern Brazil, reaching 55%. Merino *et al.* conducted a study in 2009 where unidentified species of *Hepatozoon* were detected in 47% of 17 rodents, namely (*Abrothrix olivaceus)* and (*Abrothrix sanborni)* (Merino *et al.*, 2009). Likewise, in 2014, Maia *et al.* reported a prevalence rate of 41% for *Hepatozoon* parasites among wild rodents from North Africa (Maia *et al.*, 2014). *Hepatozoon* spp. are commonly found in wild rodents in North America. For example, a study conducted by Johnson *et al.* in 2007 discovered *Hepatozoon sp.* in 58% of cotton rats and 33.3% of white-footed mice in the United States (Johnson *et al.*, 2007). A groundbreaking study conducted by Wolf *et al.* in 2016 revealed the first-ever identification of *Hepatozoon sp.* in sylvatic rodents in Brazil. The study found that the prevalence of (*Calomys callosus)* rodents was relatively low, specifically at 7% (Wolf *et al.*, 2016). The *Hepatozoon* genus, was initially documented in grey squirrels in Maryland, USA, as well as in California ground squirrels in the nearctic region. Multiple species of *Hepatozoon* have been identified within the Sciuridae family; however, specific differentiation remains unclear due to limited knowledge regarding their life cycles. The detection of *Hepatozoon* in grey squirrels in Maryland presented a challenge due to the coexistence of Miller's species in the same area. Further investigation is necessary to understand the sexual and asexual cycles of *Hepatozoon* within arthropod vectors and infected squirrels. This research will aid in determining the exact identity of *Hepatozoon* found in grey squirrels (Clark, 1958). Te role of rodents in the epidemiology of Hepatozoonsp. Should be further investigated to evaluate if genotypes circulating in rodents are specifc of this group of mammals or might also circulate in wild carnivores and reptiles.

Hepatozoon sequences detected in rodents in Slovakia and the Czech Republic were phylogenetically related to sequences detected in lizards and snakes, positioning apart from H. canis detected in dogs (Hamšíková *et al.*, 2016). Sousa obtained similar results when analyzing Hepatozoon sequences from rodents sampled in Brazilian Pantanal. These results suggest that rodents might play an important role as intermediate or paratenic hosts for Hepatozoon infections in reptiles (de Sousa *et al.*, 2017). The sequences detected in rodents by Parles *et al* in Brazil in 2019 were positioned in a large clade comprising Hepatozoon sequences previously detected in rodents and reptiles. In addition, Hepatozoon sequences from felids and canids were grouped in another large clade. They reported a variety of Hepatozoon haplotypes associated with small mammals in three Brazilian biomes: Cerrado, Atlantic Forest, and Pantanal. Through phylogenetic analyses, the Hepatozoon agents grouped in the rodent-marsupial-reptile large clade of Hepatozoon spp. from the world (Perles *et al.*, 2019).

In the latter case, rodents would play a role as intermediate or paratenic hosts in the epidemiological cycles.

10. Reports of *Hepatozoon Species* in Wild Bbirds

The identification of *Hepatozoan* species in birds has been discussed in multiple studies (Bennett *et al.*, 1992; Merino *et al.*, 2014). In a study conducted by Shimizu *et al.* in 1987, microscopic examination of the liver, spleen, blood film, and bone marrow of captive cranes in Japan revealed the presence of *Hepatozoan-like* organisms. These organisms were associated with significant clinical symptoms, such as hepatomegaly (enlarged liver) and splenomegaly (enlarged spleen), in these animals (Shimizu *et al.*, 1978). Birds of prey can also become infected with *Hepatozoon* by consuming rodent baits that contain the parasites (Peirce *et al.*, 2005). In Tanzania, a previously unknown species of avian blood parasite called *Hepatozoon ellisgreineri* was recently identified in (secretary birds). What sets this species apart is its unique development within granulocytes, particularly heterophils, which distinguishes it from other *Hepatozoon* parasites found in birds. This discovery significantly enhances our understanding of avian *Hepatozoon* species and other blood parasites in birds, thereby advancing our knowledge of the diverse range of haematozoa (Valkiūnas *et al.*, 2016). In seabirds, blood parasites of the genus Hepatozoon had first been found in 4 species of albatrosses (*Hepatozoon albatrossi*). Subsequently, an undetermined Hepatozoon sp. was reported from 2 seabird species breeding at offshore islands of northern Mexico: 1 of 15 magnificent frigate birds Fregata magnificent and 1 of 5 black storm petrels Oceanodroma Melania (Clark *et al.*, 1969). However, neither *H. albatrossi* nor the undetermined Hepatozoon from Mexico have been mentioned in the later literature, and no molecular data are available. The sequences detected in rodents by Merino *et al.* in USA in 2014 specified that *Hepatozoon peircei* appears phylogenetically clustered with Hepatozoon species infecting rodents, marsupials, reptiles, and amphibians and is distant from a clade including those infecting carnivores and a group of species infecting lizards. However, the position of this avian Hepatozoonis not resolved.

11. Reports of *Hepatozoon Species* in Lizards, Snakes and Crocodiles

Lewis and Wagner carried out a study in the USA in 1964, where they discovered a previously unknown species of *Hepatozoon* called *Hepatozoon sauromali sp.* This parasite was found in the blood of (chuckwalla lizards) belonging to the *Sauromalus species.* The study revealed that gametocytes, the sexual stages of the parasite, develop within the red blood cells, while schizogony (asexual reproduction) occurs in the liver. The mature schizont contains an average of 24 merozoites, which are the resulting daughter cells. The parasite is transmitted by a newly identified mite species called *Hirstiella*, and the transmission takes place through the oral route. Sporogony occurs within the body cavity of the mites, resulting in the formation of large oocysts containing around 12 sporozoites. When mites feed on chuckwallas infected with the parasite, they can transmit the infection to "clean" (Sceloporus lizards). Approximately 30 days later, these previously uninfected lizards exhibit blood cell infections (Lewis and Wagner, 1964). In 1938, Lavier and Callot described *H. burneti* as a novel species discovered in the gecko species known as *Tarentola mauritanica.* These researchers hypothesized that a genus of sand fly called *Phlebotomus sp.* served as the invertebrate host responsible for transmitting the parasite (Lavier and Callot, 1938). In 1962, Mackerras identified a novel species called *H. breinli* in a spotted goanna known as (*Varanus tristis orientalis*). The reproductive phases of this parasite were observed to develop within Culex fatigans mosquitoes, indicating that this particular mosquito species serves as the vector for *H. breinli* (Mackerras, 1962). *Hepatozoon* spp. has been detected in 8% of ticks found on mammals and 96% of ticks found on snakes in Thailand (Sumrandee *et al.*, 2015). In Shanghai, China, (King rat snakes) have been found to be infected with a new species of *Hepatozoon* called *Hepatozoon chinensis.* This species is unique from other known *Hepatozoon species* and alters the shape of the erythrocytes that it infects (Han *et al.*, 2015). In a study conducted by Wozniak *et al.* in 1998 in the USA, *Hepatozoon sp.* was identified in the bloodstream of a southern water snake (*Nerodia fasciata pictiventris*). One of the clinical symptoms observed in this case was the presence of granulomatous hepatitis, which was associated with *Hepatozoon sp* (Wozniak *et al*, 1998). In 2011, Motta *et al.* conducted a study in Brazil, focusing on eighty-three neotropical rattlesnakes known as Crotalus durissus collilineatus. The researchers found that the infection rate of *Hepatozoon* spp. in wild-caught snakes was 38.3%, whereas no infection was detected in captive-bred snakes. It was observed that infected snakes tended to have lower weight, length, and weight-length ratios. Furthermore, it was observed that these infected snakes had an increase in immature red blood cells (Motta *et al.*, 2011). In 2017, Soares *et al.* conducted a study in the Brazilian Amazon which discovered the existence of *Hepatozoon sp.* in (spectacled caimans).[74] In 2006, a study conducted by Vilcins *et al* related to blood smears of 5 reptiles, including the water python, and their ticks examined for the presence of the Hepatozoon sp. DNA was detected in all tick and reptile species, with 57.7% of tick samples (n = 187) and 35.6% of blood smears (n=35) showing evidence of infection (Vilcins *et al.*, 2009). Tome *et al.* 2016 analyzed nearly 600 samples from several species of the Wall Gecko genus Tarentola of the Mediterranean Basin and the Canary Islands for the presence of these parasites using haemogregarine-specific primers to assess the diversity and distribution of Hepatozoon spp. in this group of hosts. Their results show that geckos of *Tarentola* spp. harbor a great diversity of haemogregarines (Tomé *et al.*, 2016). Junsiri *et al* in Thailand identify and evaluate the phylogenetic relationships of *Hepatozoon* sp. in crocodile monitors. The phylogenetic results revealed that *Hepatozoon* sp. in crocodile monitors had 99% similarity with *H. caimani*, and the *Hepatozoon* 18S rRNA gene was grouped in the same clade as crocodiles, reptiles, rodents, and amphibians (Junsiri *et al.*, 2024). In a study conducted by Bouer *et al.* in 2017 in the Brazil, *Hepatozoon* spp.-gametocytes were found in 70.8% (17/24) and 88.8% (8/9) of blood smears from free-ranging and captive caimans (*Caiman crocodilus yacare)*, respectively. *Hepatozoon* spp. 18S rRNA DNA was found in 79.2% (19/24) and 88.8% (8/9) of free-ranging and captive caimans, respectively.

12. Conclusion and future perspective

Apicomplexa, a group of protozoa, continues to be one of the least explored groups, making accurate identification of *Hepatozoon* spp. challenging. To tackle this issue, we carried out an extensive investigation to establish how common *Hepatozoon* spp. is among different types of animals living in the wild or in captivity in various parts of the globe. This study examined case reports and articles that used PCR-based methods with sequencing to identify *Hepatozoon* spp. The epidemiology of *Hepatozoon* spp. In wild and domestic animals is still not fully elucidated around the world. According to the research, *Hepatozoon* spp. infections are prevalent in alpine environments and are commonly found in various species of small mammals that reside in these regions. The prevalence of *Hepatozoon* spp. varies across different mammal species, with the bank vole (*My. glareolus*) exhibiting the highest prevalence, followed by the yellow-necked mouse (*A. flavicollis*). Additionally, research has shown that *Hepatozoon* spp. can spread through the consumption of infected vectors, indicating that it serves as a vector-borne pathogen for small mammals. The discovery of *Hepatozoon* spp. in different types of mammals, including those that are domesticated and those that are wild, indicates that there is a possibility of diseases spreading between animals that live in the wild and those that are domesticated. Studies have shown that *H. canis*, a parasite that affects dogs, is present in North America. This discovery contradicts previous beliefs that the parasite was not found in the region. The occurrence of *Hepatozoon* spp. varies among mammal species in different regions, with a higher prevalence observed in Croatia and the Netherlands/Belgium compared to Austria and Bosnia. These studies underscore the significance of comprehending the prevalence and transmission patterns of *Hepatozoon* spp. to evaluate the risk of infection and potential consequences for wildlife and domestic animals. Our research has found that wildlife can be a potential source of infection in environmental settings. Moreover, they can spread parasites to both humans and domestic animals. With the rise in deforestation and urbanization, and the increasing proximity between humans, domestic animals, and wildlife, it is imperative to conduct further research in this field to prevent any outbreaks.

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None

Conflicts of interest

The authors affirm that they do not have any known conflicts of interest or personal relationships that could have influenced the work presented in this paper.

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