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Zoonotic and vector-borne pathogens in tigers from a wildlife safari park, Italy

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ABSTRACT

Infectious diseases by pathogens, including those of zoonotic concern, may act as a primary or contributory cause of threat to wildlife conservation and may represent a risk for human health, mainly for people working at, or visiting the zoological parks. Given the paucity of data on pathogens infecting wild tigers, we investigated the occurrence of infectious agents in this animal species, with a special focus on those of zoonotic concern. Blood and serum samples from tigers (n = 20) living in a wildlife safari park of southern Italy were screened by serological and molecular tests. All animals scored positive for antibodies against *Toxoplasma gondii* (100%), whereas they displayed different prevalence of seropositivity for *Rickettsia conorii* (30%), *Bartonella henselae* (15%) and *Leptospira interrogans sv* Icterohaemorrhagiae and/or *Leptospira kirschneri sv* Grippotyphosa (15%). No antibodies against *Coxiella burnetii* were detected. In addition, 8 tigers (40%) tested molecularly positive to "*Candidatus* Mycoplasma haemominutum", and 3 (15%) to *Hepatozoon canis*. No DNA of *R. conorii*, *Bartonella* spp., *Ehrlichia/Anaplasma* spp. and piroplasmids was amplified. The occurrence of tiger infections by bacteria and parasites may represent a risk for morbidity and, in some circumstances, mortality in this endangered species and a source of infection for other animals, including humans. These findings indicate that the circulation of zoonotic pathogens such as *T. gondii, R. conorii, L. interrogans sv* Icterohaemorrhagiae, "*Candidatus* Mycoplasma haemominutum" and *B. henselae* in given environments may represent a relevant health issue considering the close association among animals and humans visiting, or working at, the wildlife safari park. Preventative measures are advocated in order to control ectoparasites and other sources of infection (e.g., small rodents), thus for minimizing the risk of infection for animals as well as for humans.

1. Introduction

The wild tiger (*Panthera tigris*) is the largest Felid species and a widely recognized symbol of wildlife conservation. Historically, tigers inhabited most of Asia representing an icon in the nature and culture of this geographical area (Mazak, 1981). However, wild tigers are on the brink of extinction, with only about 3200 to 3500 surviving today (Recovery Program Global Tiger, 2011). Although anthropogenic alterations of landscape, including urbanization, habitat destruction and poaching are the main factors affecting wildlife conservation, infectious diseases may, under specific captive conditions, act as a primary or contributory cause of threat for animal species and populations across local to global scales (Lewis et al., 2017; Pedersen et al., 2007). Indeed, infections by bacteria, viruses and parasites may pose significant risks to wildlife populations, in particular to endangered species (Cabello et al., 2013; Millán et al., 2009), leading in some cases to mortality (Barbón et al., 2019; East et al., 2008; Santos et al., 2018; Tiensin et al., 2005). In addition, wildlife infectious diseases caused by zoonotic pathogens may represent a risk for human health (Daszak et al., 2000). Wild animals are often kept in captivity in zoological parks or circuses in proximity to urban areas and in close contact with humans, in particular animal keepers, thus serving as indicators of human risk of exposure to zoonotic agents in certain environments (Scotch et al., 2009) and representing a source of infection for humans and others animals (Chomel et al., 2007). In this scenario, pathogens transmitted by vectors play an important role. For example, an active focus of *Leishmania*

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infantum infection has been recently studied in a group of tigers living in a wildlife safari park in southern Italy demonstrating the risk of zoonotic parasite transmission in this environment (Iatta et al., 2020). Therefore, considering the close association among zoo animals and humans, zoonotic diseases by bacteria and parasites, including those transmitted by arthropods, represent a relevant health issue that is scantly investigated in animals kept in captivity. In particular, knowledge about the importance of diseases in tiger mortality, as well as on the pathogens infecting this species needs to be filled. Although infections by zoonotic pathogens have been largely described in wild carnivores, both in nature and in captivity (André et al., 2012), reports on wild felids and in particular in tigers are scant. Only *Hepatozoon felis* (Pawar et al., 2012), *Mycoplasma haemofelis* (Haefner et al., 2003) and *Toxoplasma gondii* (Dorny and Fransen, 1989; Goodrich et al., 2012) were detected in tigers by molecular, parasitological and serological tests, respectively. Therefore, the aim of the present study was to carry out a serological and molecular survey of pathogens infecting tigers from southern Italy, with a special focus on those of zoonotic concern.

2. Material and methods

2.1. Sample collection

The samples tested were collected for a study investigating the prevalence of *L. infantum* infection in the same local tiger population (Iatta et al., 2020). Briefly, between March and June 2019, whole blood in EDTA tubes and serum samples were collected from 20 tigers (i.e., 8 males and 12 females) born in the safari Park (Apulia region, Brindisi Province, southern Italy, 40°50′ N, 17°20' E; 300 m above sea level), and living in an open enclosure without any other animal species. The samples were kept at −20 °C at the Unit of Parasitology at the Department of Veterinary Medicine, University of Bari (Italy) before being processed. Animal data (i.e., age, sex, weight, and microchip number) were recorded for each animal. The protocol of this study was approved by the ethical committee of the Department of Veterinary Medicine of the University of Bari (Prot. Uniba 9/19).

2.2. Serological testing

Serum samples were tested for antibodies against *Toxoplasma gondii*, *Bartonella henselae, Rickettsia conorii, Chlamydia abortus*, *Coxiella burnetii*, *Leptospira* spp. and *Babesia canis*. The tests were not specifically validated for tigers as detailed in the following. The detection of antibodies against *T. gondii* was performed by an IFAT commercial kit (VMRD Veterinary Medical Research & Development, Pullman, WA, USA) detecting IgG and IgM, following the technique described in the Manual of the OIE (World Organisation for Animal Health, 2017a). The anti-feline IgG and IgM conjugates were included in the kit and ready to use. An IFAT commercial kit (Biopronix, St Andrews, VI, Australia) was used for the detection of antibodies against *B. henselae* and the samples were classified as positive when a fluorescence was observed at serum dilution of 1:64 or higher. Both commercial IFAT tests are validated for domestic cats. The antibodies against *R. conorii* were detected by a modified IFAT commercial kit (*Rickettsia conorii* IFA Canine IgG Antibody Kit, Fuller laboratories, CA, USA) validated for dogs and employed for other animals by using specific conjugates, such as, in this case, anti-feline IgG (VMRD Veterinary Medical Research & Development, Pullman, WA, USA). A feline positive control for this pathogen was not available. The positive and negative controls of reaction were based on canine samples and relative specific conjugate provided by the kit. The detection of antibodies against *Chlamydia* spp. was performed by an in-house complement fixation test (CFT), following the procedures of the OIE (World Organisation for Animal Health, 2017a). The antigen was a purified culture of *Chlamydia abortus* strain S26/3 (Moredun Institute, UK).

The test for antibodies against *C. burnetii* was performed by an in-house complement fixation test (CFT), following the procedures of the Manual of the OIE (World Organisation for Animal Health, 2017b). Two different commercial antigens, Phase I and Phase II, were provided (Dolfinin, Slovak Republic) and the cut-off value was 1:10. The gold standard test microagglutination (MAT) was employed for the detection of antibodies against *Leptospira* spp.following the procedure described (World Organisation for Animal Health, 2017c), using a cut-off value of 1:100 and a panel of 11 *serovars* (*sv*), belonging to 8 serogroups (Australis *sv* Bratislava, Canicola *sv* Canicola, Grippothyphosa *sv* Grippotyphosa, Icterohaemorrhagiae *sv* Copenagheni and Icterohaemorrhagiae, Pomona *sv* Pomona, Sejroe *sv* Hardjo, Saxkoebing and Sejroe, Tarassovi *sv* Tarassovi, Ballum *sv* Ballum). Both CFT as well as MAT are not animal species dependent tests.

A modified IFAT commercial kit (Fluo *Babesia canis*, Biopronix, Product Line, Agrolabo Spa, Italy) was used for the detection of anti-*B. canis* antibodies with anti-feline IgG ready to use as conjugate (VMRD Veterinary Medical Research & Development, Pullman, WA, USA). The IFAT is validated for dog and adapted to other species by using specific conjugate. A feline positive control for this pathogen was not available, thus canine samples were used as positive and negative controls and relative specific conjugate provided by the kit.

2.3. Molecular procedures

DNA was extracted from whole blood collected in EDTA tubes using the GenUP DNA Kit (Biotechrabbit, Germany), following the producer's

Table 1
Targeted pathogens and list of primers used in this study.

Pathogen	Target gene	Primer	Sequence 5′-3′	Pc (μM)	Ta (°C)	Fragment length (bp)	Reference
Rickettsia spp.	*RompB*	RompB OFm RompB ORm	5' -GTA ACC GGA ART AAT CGT TTC GT- 3′ 5' -GCT TTA TAA CCA GCT AAA CCR CC- 3′	0.1	58	511	Choi et al., 2005
Piroplasmidae	18S rRNA	BJ1 BN2	5' -GTC TTG TAA TTG GAA TGA TGG- 3′ 5' -TAG TTT ATG GTT AGG ACT ACG -3′	0.1	60	500	Casati et al., 2006
Mycoplasma spp.	16S rRNA	MycE929f-MycE1182r	5′-ACGGGGACCTGAACAAGTGGTG-3′ 5′-AGGCATAAGGGGCATGATGACTTG-3′	0.1	60	259	Ravagnan et al., 2017
Bartonella henselae/ clarridgeiae	Citrate synthase	BART-LC-GEN-F BART-LC-HEN-R BART-LC-CLA-R	5′-ATGGGTTTTGGTCATCGAGT-3′ 5′-AAATCGACATTAGGGTAAAGTTTTT-3′ 5′-CAAGAAGTGGATCATCTTGG-3′	0.1	58	190	Staggemeier et al., 2014
Ehrlichia spp./ *Anaplasma* spp.	*GroEL*	GroEL643f GroEL1236r	5′-ACT GAT GGT ATG CAR TTT GAY CG-3′ 5′-TCT TTR CGT TCY TTM ACY TCA ACT TC-3′	0.2	60	593	Barber et al., 2010
Hepatozoon spp.	18S rRNA	Piroplasmid-F Piroplasmid-R	5′-CCAGCAGCCGCGGTAATT-3′ 5′-CTTTCGCAGTAGTTYGTCTTTAACAAATCT-3′	0.4	60	358	Tabar et al., 2008

Abbreviation: Pc = primer concentration; Ta = temperature of annealing; *RompB* = Rickettsial Outer Membrane Protein B gene; 18S rRNA = 18S ribosomal RNA gene; 16S rRNA = 16S ribosomal RNA gene; *GroEL* gene = heat shock protein gene.

recommendations. All DNA samples were tested for *Hepatozoon* spp., piroplasmids including *Babesia* spp. (except *Babesia bovis*), *Theileria* spp. and *Cytauxzoon* spp., and *Ehrlichia* spp./*Anaplasma* spp., *Rickettsia* spp., *Mycoplasma* spp. and *B. henselae/clarridgeiae* (Table 1). All PCR products were purified and sequenced in both directions using the same forward and reverse primers, employing the Big Dye Terminator v.3.1 chemistry in a 3130 Genetic analyser (Applied Biosystems, California, USA) in an automated sequencer (ABI-PRISM 377). Nucleotide sequences were edited, aligned and analysed using Geneious platform version 9.0 (Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012) and compared with available sequences in the GenBank database using Basic Local Alignment Search Tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Molecular detection of *Hepatozoon* spp. was performed by conventional PCR (cPCR) using primers targeting partial 18S rRNA gene (Table 1). Amplified PCR products were visualized by gel-electrophoresis in 2% agarose gel containing GelRed nucleic acid gel stain (VWR International PBI, Milan, Italy) and viewed on GelLogic 100 gel documentation system (Kodak, New York, USA).

Amplification of *Rickettsia* spp., Piroplasmidae, *Mycoplasma* spp., *B. henselae/B. clarridgeiae* and *Ehrlichia* spp./*Anaplasma* spp. DNA was performed by in-house SYBR Green real-time PCRs using primers and protocols described in Table 1. Each reaction was carried out in a total volume of 20 µl, containing 10 µl of Quanti-Fast SYBR Green PCR Master mix 2X (Qiagen GmbH, Germany), sense and reverse primers (concentration reported in Table 1) and 3 µl of DNA. Amplifications were performed in a StepOnePlus™ instrument (Applied Biosystems, Foster City, CA). The thermal profile consisted of 5 min of activation at 95 °C, followed by 40 cycles at 95 °C for 15 s (denaturation), specific annealing temperature (Table 1) for 30 s (annealing) and 60 °C for 30 s (extension). Following amplification, a melting curve analysis was performed by slowly raising the temperature of the thermal chamber from 60 °C to 95 °C to distinguish between specific and non-specific amplification products. For all PCR runs, negative (sterile water) and positive controls including synthetic DNA of *Rickettsia felis* and DNA of *B. canis* from dog blood, *M. haemofelis* and *B. henselae* from cat blood and *Anaplasma phagocytophilum* from bovine blood were employed.

2.4. Phylogenetic analysis

The phylogenetic analysis based on 316 bp 18S rRNA gene sequences of *Hepatozoon* spp. detected in tigers and in other animal hosts available from the GenBank database was carried out and inferred using the Neighbor-Joining (NJ) method (Saitou and Nei, 1987). Evolutionary analyses were conducted on 8000 bootstrap replications using the MEGA6 software (Tamura et al., 2013). Homologous sequence from *B. canis was* rused as outgroup (accession number AY649326).

3. Results

Tigers enrolled in the study aged from 6 months to 11 years old, weighed from 70 to 220 kg and were apparently healthy and free of ectoparasite infestations. The prevalence of tigers which scored positive to the tested pathogens by serology and PCR is reported in Table 2 along with the antibody titres for each pathogen considered. In particular, out of three samples positive to antibodies against *Leptospira* spp., one showed a titre of 1:400 and 1:800 against *Leptospira interrogans sv* Icterohaemorrhagiae and *Leptospira kirschneri sv* Grippotyphosa respectively, one against *L. interrogans sv* Icterohaemorrhagia (1:100) and one to *L. kirschneri sv* Grippotyphosa (1:200).

No antibodies against *C. burnetii* were detected. Concurrent antibody positivity against different pathogens were detected in 11 tigers with the most prevalent seropositivity combination represented by IgG against *T. gondii* and *R. conorii* (4/11, 36.4%), followed by *T. gondii* and *Leptospira* spp. (2/11, 18.2%).

Eleven tigers (55%) were positive by molecular testing, in particular 8 (40%) to "*Candidatus* Mycoplasma haemominutum" DNA and 3 (15%) to *Hepatozoon* spp. DNA. Consensus sequences of the 18S rRNA genes analysed displayed 99–100% nucleotide identity to *Hepatozoon canis* sequences (accession nos. MK645965, MK091088 and MK673842) available in the GenBank database.

The NJ analysis based on the 18S rRNA partial sequences (Fig. 1) indicated that 3 tigers were infected with *H. canis* which clustered with high bootstrap value (i.e., 99%) together with those of domestic and wild carnivores including 2 cats from Italy (KY649446) and Israel (KC138532). Sequences obtained of 18S rRNA gene of *H. canis* were deposited in GenBank under accession numbers from MT232062 to MT232064.

No coinfections with the two pathogens were molecularly diagnosed in these 11 tigers. No DNA of *R. conorii*, *Bartonella* spp., *Ehrlichia/Anaplasma* spp. and piroplasmids was amplified.

4. Discussion

The occurrence of bacterial and parasitic infections in tigers indicates that these animals living in the wildlife safari park are exposed to multiple pathogens of zoonotic concern such as *T. gondii* (100%) followed by *R. conorii* (30%), *B. henselae* (15%) and *L. interrogans sv* Icterohaemorrhagiae and/or *L. kirschneri sv* Grippotyphosa (15%).

The detection of anti-*T. gondii* antibodies in all the tigers analysed is supported by the high prevalence reported in captive felids from zoological parks in Brazil (54.6%, Silva et al., 2001; 66.7%, Ullmann et al., 2010), in Australia (69.6% Hill et al., 2008), in Portugal (75%, Tidy et al., 2017) and in Mexico (81.4%, Alvarado-Esquivel et al., 2013), including tigers (Alvarado-Esquivel et al., 2013; Yang et al., 2017). The seropositivity to *T. gondii* in zoo animals is most likely related to the dietary habits or environment factors since these animals were generally fed with raw meat and may hunt birds and rodents that may act as intermediate hosts (Yang et al., 2017). Although the time length of *T. gondii* oocyst shedding by infected tigers is unknown and may take place during a relatively short period after an initial acute infection, as in the domestic cat (Zulpo et al., 2018), millions of oocysts could be shed and survive in soil for years (Dorny and Fransen, 1989). Furthermore, oocysts may be transported via freshwater or rainwater into other animal enclosure representing a source of infection to them as well as to caretakers.

Despite serological evidence of exposure to spotted fever group (SFG) *Rickettsia* species has been largely reported in domestic cats from the Mediterranean area with seropositivity up to 48.7% (Alves et al., 2009; Persichetti et al., 2018; Solano-Gallego et al., 2006), but rarely in wild felids (de Sousa et al., 2018), the seroprevalence to *R. conorii* has been herein firstly detected in tigers (30%). Although the IFAT was conceived for the detection of antibodies against *R. conorii*, cross-reactions with other SFG *Rickettsia* species cannot be ruled out (Persichetti et al., 2018). Similarly, antibodies against *B. henselae* were firstly detected in tigers (15%) rather than in other wild felids previously reported with similar seroprevalence in puma (*Felis concolor*, 11.8%) and African lions (*Panthera leo*, 17%) but higher in cheetahs (*Acinonyx jubatus*, 31%) and bobcats (*Lynx rufus*, 37%) (Chomel et al., 2004; Molia et al., 2004). Although the bacterial detection has not been confirmed by molecular tests for both zoonotic pathogens, further investigations about the transmission route and the pathogenic role of SFG *Rickettsia* species and *B. henselae* in wild felids as well as their potential role as reservoir should be investigated. For instance, the vector role of ticks and fleas could be crucial in the transmission of these pathogens to other animals and humans.

The exposure of tigers (15%) to *Leptospira serovars* of zoonotic concern has been already observed in other wild felids (Esson et al., 2019; Ullmann et al., 2012) and domestic cats (Rodriguez et al., 2014). The high antibodies titers detected in one tiger against more than one serogroup may be suggestive of a recent field contact with those pathogens. Specifically, considering that *L. interrogans sv*

Table 2
Prevalence of infections by bacterial and parasitic agents determined by serology and PCR among the 20 tigers.

Pathogen	N. seropositive tigers (%)	N (Ab titre)	N. PCR-positive tigers (%)
Single infections			
Toxoplasma gondii	20 (100)	1 (1:32); 6 (1:128); 3 (1:256); 4 (1:512); 4 (1:1024); 2 (1:2048)	nt
Rickettsia spp.	6 (30)[a]	3 (1:64); 1 (1:128); 2 (1:256)	0
Bartonella henselae/B. clarridgeiae	3 (15)[b]	2 (1:64); 1 (1:128)	0
Leptospira spp.	3 (15)	1 (1:400 *sv* Ict + 1:800 *sv* Grip); 1 (1:200 *sv* Grip); 1 (1:100 *sv* Ict)	nt
Mycoplasma spp.	nt		8 (40)
Hepatozoon spp.	nt		3 (15)
Chlamydia abortus	2 (10)	1 (1:128); 1 (1:32)	nt
Babesia canis	1 (5)	1 (1:32)	nt
Coxiella burnetii	0		nt
Ehrlichia spp./*Anaplasma* spp.	nt		0
Piroplasmids	nt		0

nt, not tested.
sv Ict, *Leptospira serovar* Icterohaemorrhagiae; sv Grip, *Leptospira serovar* Grippotyphosa.
[a] IFAT only for the detection of antibodies against *Rickettsia conorii*.
[b] IFAT only for the detection of antibodies against *Bartonella henselae*.

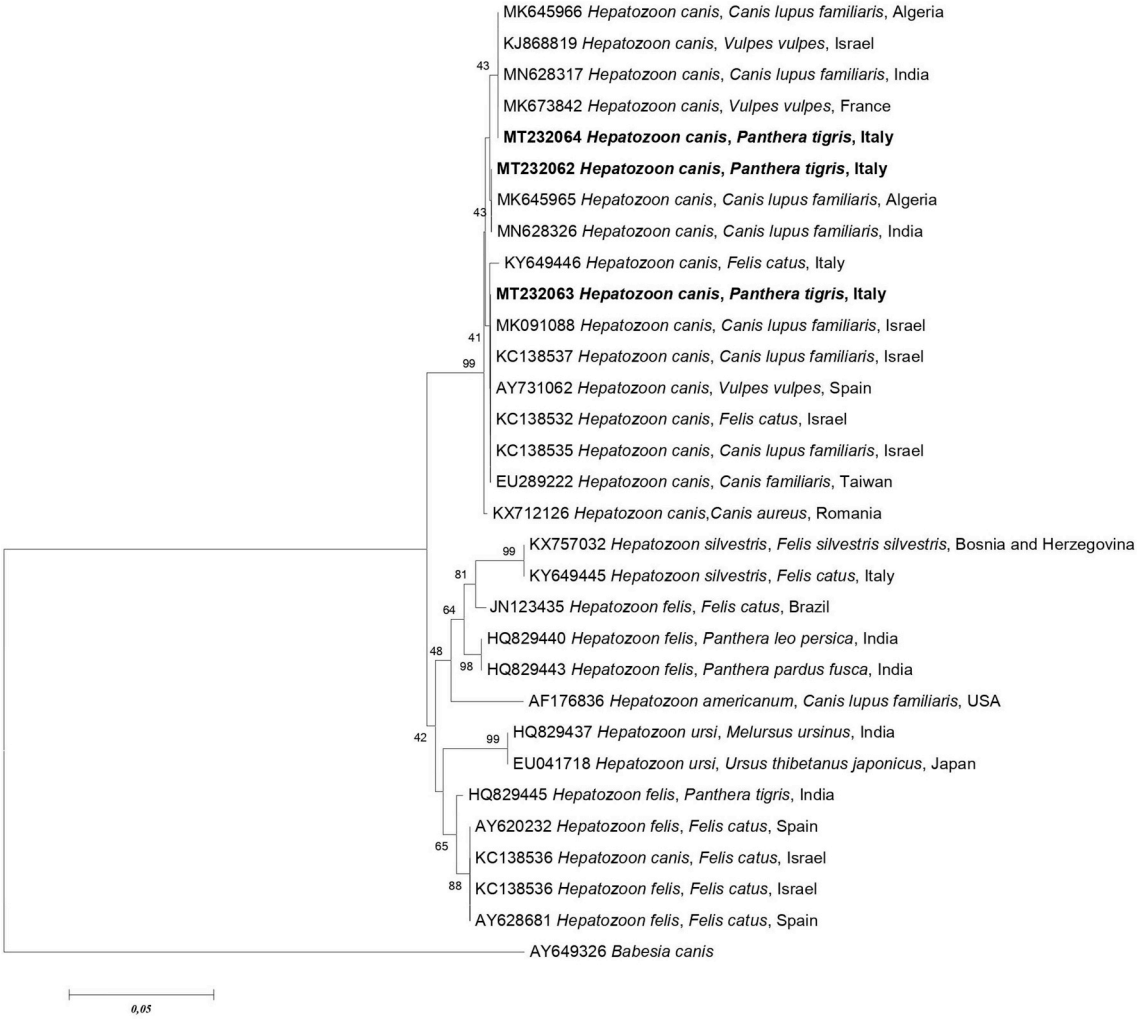

Fig. 1. A Neighbor Joining tree phylogram comparing 316 bp 18S rRNA DNA *Hepatozoon canis* sequences from tigers, herein in bold, to other *Hepatozoon* spp. GenBank deposited sequences with *Babesia canis* as outgroup. Sequences are presented by GenBank accession number, host species and country of origin.

Icterhaemorrhagiae is the most pathogenic serovar to humans (Bharti et al., 2003; Dupouey et al., 2014), the results indicate the potential risk of infection for other animal species, including humans. Although the transmission of leptospires occurs by direct or indirect contact, the circulation of rodents and wild mammals (i.e., the main reservoirs for these spirochetes) in the safari park may represent a source of infection for other susceptible hosts. The hygiene status of the tiger enclosure may represent a risk factor for the spreading of spirochetes since spraying water favors aerosolizing and diluting urines, leading to a longer survival of leptospires in the environment (Bierque et al., 2020).

No antibodies against *C. burnetii*, the aetiological agent of Q fever, have been recorded. Very few species of wild felids such as lions

(*Panthera leo*), European wildcats (*Felis silvestris silvestris*) and snow leopards (*Panthera uncia*) were positive to this pathogen (Candela et al., 2017; Esson et al., 2019; Torina et al., 2007).

Since *C. abortus* infection has never been reported in wild animals, the seropositivity detected in two tigers by an in-house complement fixation test may be due to a cross reaction with antibodies against *Chlamydia felis* which is the most common cause of feline chlamydiosis and different diseases in humans (Halánová et al., 2019). Similarly, the presence of a low antibody titer against *B. canis* in only one tiger resulted negative to piroplasmid's DNA does not allow any conclusion.

Noteworthy is the first report of *H. canis* infection in tigers (15%), considering that *Hepatozoon felis* is a very common species infecting wild and domestic felids worldwide (Baneth, 2011; Baneth et al., 2013; Giannelli et al., 2017; Otranto et al., 2019). In particular, *H. felis* was molecularly detected in captive wild felids from Brazil and Zimbabwe (André et al., 2010; Furtado et al., 2017; Kelly et al., 2014) including tigers from India (Pawar et al., 2012). Only a few reports of domestic cats positive for *H. canis* from Thailand, Israel, Italy and Spain are available (Baneth et al., 2013; Díaz-Regañón et al., 2017; Giannelli et al., 2017; Jittapalapong et al., 2006). Therefore, the occurrence of *H. canis* in felids may due to the low host specificity of this protozoan (Smith, 1996). Remarkably, the role of some tick species feeding on both canids and felids, such as *Rhipicephalus sanguineus* group (Dantas-Torres and Otranto, 2015), as well as the predation on prey infected by *Hepatozoon* cysts (Smith, 1996) may explain the circulation of this pathogen in these animals. Although *Hepatozoon* infections are usually subclinical in wildlife (André et al., 2010; East et al., 2008; Furtado et al., 2017; Metzger et al., 2008), they have occasionally been associated with clinical disease in young wild canids (Davis et al., 1978; Garrett et al., 2005), mortality in hyenas (East et al., 2008) and recently in domestic cat in Austria and Switzerland (Basso et al., 2019; Kegler et al., 2018). In addition, this parasite may be a potential threat in immunocompromised animals or during concomitant infections (Baneth et al., 1998; Kubo et al., 2006).

The phylogenetic analysis of 18S rRNA *Hepatozoon* sequences amplified from tigers in this study revealed that *H. canis* in tigers from Italy are closely related to those reported in wild canids and domestic cats from Israel, India, Spain, Brazil, Algeria and France.

The high prevalence of *Hemoplasma* infection in tigers (40%) was expected since it is very frequent in several wild felid species from different geographical areas (André et al., 2011; Haefner et al., 2003; Willi et al., 2007) and up to 40% in domestic cats worldwide (Díaz-Regañón et al., 2017; Walker Vergara et al., 2016). Among the three hemoplasma species known to infect felids, "*Candidatus* Mycoplasma haemominutum" (CMhm) is the most prevalent species in domestic cats, as the only species herein identified in tigers, followed by *Mycoplasma haemofelis* (Mhf) and "*Candidatus* Mycoplasma turicensis" (CMt) (Ravagnan et al., 2017; Walker Vergara et al., 2016). The transmission patterns of hemoplasmas are poorly understood although vector roles have been suggested for ticks and fleas (Barrs et al., 2010; Woods et al., 2006). Nonetheless, predation by wild felids on domestic cats has been hypothesized as a route for pathogen transmission (Kellner et al., 2018). Even though no *Ehrlichia* spp. and *Anaplasma* spp. DNA was amplified in this study, the risk of infection by these bacteria can not be ruled out, since their occurrence has been reported in other wild felids (Kelly et al., 2014; Torina et al., 2007).

Limited data on the pathological significance of the studied pathogens for tigers is available. A single case of toxoplasmosis has been reported in a Siberian tiger (*Panthera tigris altaica*) from a zoo in Belgium with a profuse diarrhea and a high oocyst shedding (Dorny and Fransen, 1989). Furthermore, a mild anemia associated with an intermittent parasitemia has been described in two sibling female tigers infected by *Mycoplasma felis* (Haefner et al., 2003).

In conclusion, although these tigers were apparently in good health condition, infections caused by bacteria and parasites in some circumstances such as in animals under stress, impaired immune system responses and concurrent infections, might predispose animals to clinical disease thus increasing the risk of morbidity and mortality due to these pathogens. Therefore, preventative measures for controlling ectoparasites and small mammalians, thus for minimizing the risk of infection for animals as well as for humans working in the zoo are advised.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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