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Original article

Three different *Hepatozoon* species in domestic cats from southern Italy

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ABSTRACT

Three species of *Hepatozoon*, namely, *Hepatozoon felis*, *Hepatozoon canis* and *Hepatozoon silvestris* may affect domestic and/or wild felids. Although hepatozoonosis has been documented in a wide range of mammal species, data on cats are limited. To investigate the occurrence of these pathogens in cats, blood samples were collected from animals living in three provinces of southern Italy (Bari, Lecce, and Matera), and molecularly analysed by PCR amplification and sequencing of segments of the 18S rRNA gene. Out of 196 blood samples collected, *Hepatozoon* spp. DNA was amplified in ten cats (5.1%, CI: 3%-9%), with the majority of infected animals from Matera (8/34, 23.5%) and one each from the other two provinces. BLAST analysis revealed the highest nucleotide identity with sequences of *H. canis*, *H. felis* and *H. silvestris* deposited in GenBank. Results of this study indicate that these three species of *Hepatozoon* infect domestic cats in Italy. This is the first report of *H. silvestris* infection in a domestic cat.

1. Introduction

Parasites of the genus *Hepatozoon* (Eucoccidiorida, Hepatozoidae) are protozoa whose life cycle is shared between a wide range of hematophageous invertebrate and vertebrates, acting as definitive and intermediate hosts, respectively (Smith, 1996). Although several differences exist in the ecology and biology of these protozoa, the primary route of *Hepatozoon* spp. transmission to vertebrate hosts is represented by the ingestion of the invertebrate host containing mature sporozoites (Smith, 1996; Baneth et al., 2007; Ferguson et al., 2012).

Although the distribution, pathogenic role and clinical features of hepatozoonosis caused by *Hepatozoon* spp. have been intensively studied in domestic and wild canids (Vincent-Johnson et al., 1997; Baneth, 2011; Allen et al., 2011), data on feline hepatozoonosis are more limited (Baneth, 2011; Lloret et al., 2015). Indeed, gamonts of *Hepatozoon* spp. or their DNA have been detected in several members of the Felidae family, including bobcats (*Lynx rufus*) (Mercer et al., 1988), cheetahs (*Acynonyx jubatus*) (McCully et al., 1975), flat-headed cats (*Prionailurus planiceps*) (Salakij et al., 2008), jaguars (*Panthera onca*) (Criado-Fornelio et al., 2009), leopards (*Panthera pardus*; *Leopardus tigrinus*) (Metzger et al., 2008; McCully et al., 1975; Averbeck et al., 1990), lions (*Panthera leo*) (Pawar et al., 2012), little-spotted cats (*Leopardus tigrinus*) (Metzger et al., 2008), ocelots (*Leopardus pardalis*) (Metzger et al., 2008), Pallas cats (*Felis manul*) (Barr et al., 1993), pumas (*Felis concolor*, *Puma yaguarondi*) (André et al., 2015), tigers (*Panthera tigris*) (Pawar et al., 2012), European wildcats (*Felis silvestris*) (Hodžić et al., 2016), and domestic cats (*Felis catus*) (Baneth et al., 2013). However, the exact identification of *Hepatozoon* spp. is often difficult as the morphology of the blood and tissue stages usually does not allow to accurately differentiate the pathogen at species level (Smith, 1996).

Three *Hepatozoon* species have been reported in felines, with *Hepatozoon felis* considered as the most prevalent (Baneth et al., 2013), and *Hepatozoon canis* reported sporadically (Jittapalapong et al., 2006; Baneth et al., 2013; Díaz-Regañón et al., 2017). In addition, *Hepatozoon silvestris* has been recently described as a novel species infecting wild cats (*Felis silvestris*) from Bosnia and Herzegovina (Hodžić et al., 2016). However, the host spectrum and possible pathogenic role of this species remains unknown (Bhusri et al., 2017). Information on the occurrence of *Hepatozoon* spp. in cats is minimal and would deserve additional investigation particularly in areas of the Mediterranean basin, such as Italy, where reports are limited to a single record (Otranto et al., 2017). In the present study, we investigated the occurrence of *Hepatozoon* spp. in blood samples collected from domestic cats living in southern Italy.

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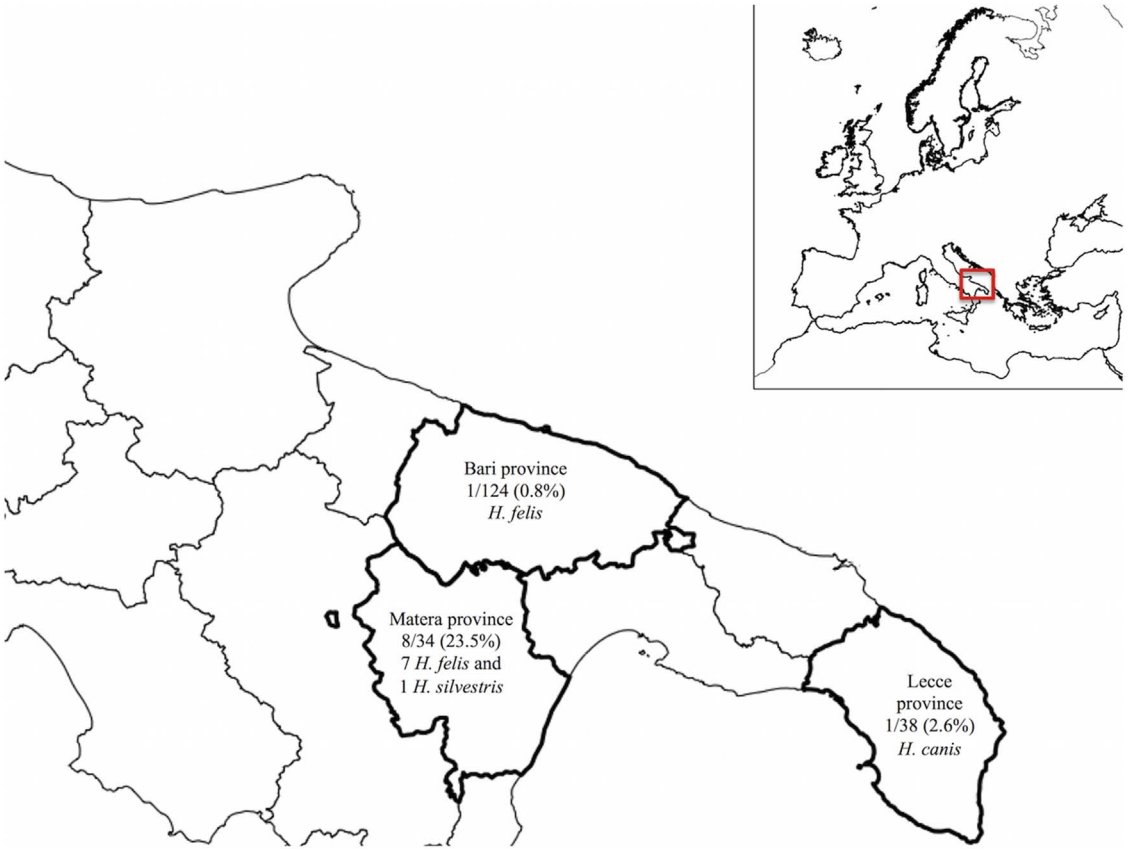

Fig. 1. Map of the three study areas, with local prevalence of *Hepatozoon* infection in cats and the distinct species reported from each sampling area.

2. Materials and methods

Blood samples were collected from cats living in three different provinces of southern Italy (i.e., Bari, Lecce, and Matera) (Fig. 1) and sent to the Department of Veterinary Medicine (University of Bari, Italy) for diagnostic purposes, following a general physical examination. Samples were collected into EDTA tubes and frozen at −20 °C until further analyses. For each sample, data on cat age, gender, occurrence of concomitant ectoparasite infestation, and positivity for feline leukaemia virus (FeLV) using a commercial serological kit (SNAP Feline Triple Test, IDEXX Laboratories, USA) were recorded. In addition, one cat succumbed to feline infectious peritonitis. DNA was extracted using a commercial extraction kit (Qiagen, DNeasy Blood & Tissue Kit, Germany) following the manufacturer's instructions, eluted in 100 µl and used as template for PCR amplification. The reactions were performed using the Piroplasmid-F/Piroplasmid R primers (Tabar et al., 2008) targeting a partial sequence of the 18S rRNA gene of *Hepatozoon* spp. Conventional PCR was performed in total volume of 25 µl using the PCR-ready High Specificity mix (Syntezza Bioscience, Jerusalem, Israel) with 500 nM of each primer and sterile DNase/RNase-free water (Sigma, St. Louis, MO, USA). Amplification was performed using a programmable conventional thermocycler (Biometra, Göttingen, Germany). Initial denaturation at 95 °C for 5 min, was followed by 35 cycles of denaturation at 95 °C for 30 s, annealing and extension at 64 °C for 30 s and final extension at 72 °C for 30 s. After the last cycle, the extension step was continued for a further 5 min. For all PCR tests, positive sample (DNA extracted from cat blood positive by PCR and confirmed by sequencing) and non-template controls (using ultra pure water) were included. PCR products were electrophoresed on 1.5% agarose gels stained with ethidium bromide and evaluated under UV light for the size of amplified fragments by comparison to a 100 bp DNA molecular weight marker. All positive PCR products were sequenced using the BigDye Terminator v 3.1 Cycle Sequencing Kit and an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA), at the Center for Genomic Technologies, Hebrew University of Jerusalem, Israel. The products were sequenced in both directions with the same primer as for PCR, sequences were assembled using BioEdit software and aligned using ClustalW program (Larkin et al., 2007), and compared for similarity with sequences available in GenBank, using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

The percentage of nucleotide variation (Pwc, %) amongst sequences identified was calculated using the Kimura 2 Parameter substitution model with Gamma Distributed (G) rates among sites (Kimura, 1980), included in the MEGA6 software (Tamura et al., 2013).

Chi square test was used to correlate the effect of cat gender, age (< 1 year, 1–2 years, > 2 years), the occurrence of ectoparasite infestation and FeLV with the positivity for *Hepatozoon* DNA. The significance level was set at $p < 0.05$.

3. Results

Hepatozoon spp. DNA was amplified from the blood of ten out of 196 (5.1%, CI: 3%-9%) cats, with the majority of animals infected sampled in Matera (8/34, 23.5%), one from Bari (1/124, 0.8%) and another one from Lecce (1/38, 2.6%) (Fig. 1). No significant statistical correlation was observed among gender, age and presence of ectoparasite infestation ($p > 0.05$). The positive cats (5 males and 5 females) were aged from 1 to 8 years (median 2.5 years) and were in apparent good physical condition, with eight of them infested by *Ctenocephalides felis* (Table 1).

BLAST analysis performed on 388 bp long sequences revealed the highest nucleotide identity with those of *H. canis* (n = 1), *H. silvestris* (n = 1), and *H. felis* (n = 8) available from GenBank (Table 1). For the latter species, three sequences (mean nucleotide difference among them = 0.52%) were identified and named as sequences I, II, and III.