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Prevalence of *Hepatozoon* and *Sarcocystis* spp. in rodents and their ectoparasites in Nigeria

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ABSTRACT

Using polymerase chain reaction targeting the 18S rRNA gene and DNA sequencing the prevalence and diversity of Apicomplexa and Piroplasmida infections in rodents from Nigeria was studied. Overall, 13 of 194 (7.7%) rodent blood samples tested were positive for *Hepatozoon* spp. while 2 (1.0%) were positive for *Sarcocystis dispersa. Hepatozoon* spp. DNA was detected in all the rodentspecies tested except *Neotoma* spp., and was most prevalent (50%) in the African giant rat (*Cricetomys gambianus*), followed by *Mus musculus* (18.2%), *Rattus rattus* (6.3%) and *Rattus norvegicus* (4.1%). The *Hepatozoon* spp. DNA sequences from the rodents were 98–100% identical to each other and to *Hepatozoon* spp. DNA sequence from small mammals deposited in GenBank. Five of the sequences from *R. rattus* (n = 2) and *R. norvegicus* (n = 3) were 98–99% identical to *Hepatozoon felis* (KY649442.1). *Sarcocystis dispersa* DNA was detected in one *R. rattus* (2.1%) and one *R. norvegicus* (0.8%). These findings suggest that rodents are involved in endemic cycles of *Hepatozoon* spp. and *Sarcocystis* spp. agents of veterinary importance.

1. Introduction

Peri-domestic rodents do not only constitute a nuisance to humans because they destroy crops in storage and farms, but are host to a plethora of pathogens of veterinary and medical importance (Bengis et al., 2004). They serve as source of pathogens to humans either directly via exposure to their excreta or indirectly via arthropod vectors such as fleas, mites and ticks (Meerburg et al., 2009). Therefore, rodents play a role in the epidemiology of several diseases affecting man and animals including those caused by apicomplexan parasites. Haemogregarines of the genus *Hepatozoon* Miller, 1908 consist of apicomplexan blood parasites that have been described from a wide range of mammals, amphibians, reptiles and birds in different climes (Levine, 1982; Smith, 1996; Criado-Fornelio et al., 2006; Penzhorn, 2006). In the USA, 47.0% of rodents and 22.6% of wild canids screened in Oklahoma and Texas were positive for *Hepatozoon* spp. (Mercer et al., 1988; Johnson et al., 2007). A prevalence of 0–67% has been reported for various rodent species in Finland (Laakkonen et al., 2001) and 0–41.6% in Poland (Karbowiak et al., 2005). A molecular survey in Spain reported 17 and 28% *Hepatozoon* spp. infection in bank voles and red foxes, respectively, while wild house mice, wild boars, roe deer, hares, striped field mice and moles were negative for hematozoa infections (Gimenez et al., 2009). *Hepatozoon* spp. have been reported in dogs in Nigeria (Ezeokoli et al., 1983; Kamani et al., 2013a), but there is no reported survey on rodents.

Sarcocystis spp. are apicomplexan intracellular cyst forming parasites, which are characterized by an obligatory heteroxenous prey-predator two-host life cycle, with several species infecting rodents (Dubey et al., 2015). Sarcocystosis is caused by a variety of species and affects wild and domestic animals (Wong and Pathmanathan, 1992). Cats and dogs have been recognized as competent definitive hosts while cattle, pigs, horses, sheep, goats, birds, rodents, camelids, reptiles and humans are known as intermediate hosts (Fayer, 2004; Dubey et al., 2015). Human infection with *Sarcocystis* spp. is characterized by diarrhea, bloat, dyspnea, tachycardia, nausea and loss of appetite (Beaver et al., 1979; Fayer, 2004; Fayer et al., 2015). Sarcocystosis is considered as one of the opportunistic infections that are exacerbated in acquired immune deficiency syndrome (AIDS) patients (Velasquez et al., 2008).

In Nigeria, prevalence of *Sarcocystis* spp. has been reported in 1.8–12% of dogs, 37.5% of goats, 42.5% of cattle and 60% of pigs

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(Kudi, 1989; Adejinmi and Osayomi, 2010; Obijiaku, 2012; Okita et al., 2017). In Taiwan, *Sarcocystis* spp. were identified in the musculature of 34% of 95 rodents examined, while in Thailand it was found in 33% of 143, in Australia in 13% of 406 and in Lithuania in 12% of 778 rodents (Munday et al., 1978; Jakel et al., 1997; Grikienienė et al., 2001; Tung et al., 2009). There is paucity of published reports on the molecular detection of *Hepatozoon* spp. and *Sarcocystis* spp. infections in rodents in Nigeria. Therefore, the aim of this study was to determine the prevalence of *Hepatozoon* spp. and *Sarcocystis* spp. infections in rodents by molecular techniques and to discuss their implications on veterinary and human health in Nigeria.

2. Materials and methods

2.1. Ethics statement

The study protocol was approved by the Institutional Animal Care and Use Committee (IACUC), National Veterinary Research Institute Vom. Permission to place the traps in the study area was granted by the residents.

2.2. Trapping of rodents

Locally fabricated wire cage traps baited with smoked fish and other food scraps were used for the trapping of rodents in domestic and peridomestic areas in Vom, (9°44′N/8 ° 47′ E), Plateau State, Nigeria in 2011 and 2015. The traps were set out in the evenings when rodents leave their burrows to search for food in farmlands or nearby human habitations. Traps were checked for rodents the following morning. In the laboratory, the rodents were anaesthetized using halothane gas and identified using standard taxonomic keys Smithers, (1986). Thereafter, 0.5–3 ml of blood was drawn from each rodent by cardiac puncture, aliquoted into EDTA tubes, and labeled as previously described (Kamani et al., 2013b). Rodents captured in 2015 were placed in plastic bags, anaesthetized using halothane gas and euthanized by cervical dislocation. Spleen samples were aseptically harvested from each rodent and placed in labeled sterile tubes containing absolute ethanol. All the samples were transported to the Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Israel, for further molecular analyses.

2.3. Collection of ectoparasites

Ectoparasites were collected from the anaesthetized rodents using either fine brush, blunt forceps or combs. The collected ectoparasites were introduced into labeled vials containing 70% ethyl alcohol and their identification was done using standard taxonomic keys (Rehn and Rehn, 1937; Costa, 1961, 1966; Lewis, 1967, 1974; Pegram et al., 1987; Apanaskevich et al., 2007).

2.4. DNA extraction from rodent tissues and ectoparasites

DNA extraction from samples collected in 2011 was performed on individual rodent blood samples and pools of 2–3 ectoparasites of the same species and hosts as described elsewhere (Kamani et al., 2013b). The Illustra tissue and cell genomic Prep miniSpin kit (GE Healthcare Ltd. UK) was used for DNA extraction from the additional spleen samples collected in 2015 according to the manufacturer's instructions.

2.5. Screening for Apicomplexa by conventional PCR

An initial molecular screening of all the samples was done using the general piroplasmid primers Piroplasmid F (5'-CCA GCA GCC GCG GTA ATT C-3') and Piroplasmid R (5'-CTT TCG CAG TAG TTY GTC TTT AAC AAA TCT-3') targeting 360 bp of the 18S ribosomal RNA (18S rRNA) gene (Tabar et al., 2008).

2.6. Conventional PCR assays for Babesia, Hepatozoon and Sarcocystis spp

All samples that were positive in the initial screening reactions were further subjected to specific PCR reactions using primers Piro A and B for *Babesia* spp. and Hep 18S F and R for *Hepatozoon* and *Sarcocystis* spp, targeting 405 bp and 625 bp of the 18S rRNA gene, respectively, as previously described (Olmeda et al., 1997; Inokuma et al., 2002; Rubini et al., 2005). Positive controls of DNA extracted from the blood of naturally infected dogs with *Babesia vogeli* and *Hepatozoon canis*, as well as negative DNA controls from colony-bred dogs negative by PCR for vector-borne pathogens were run with each corresponding PCR reaction.

Non-template control (NTC) reactions were run using the same procedures and reagents described above but without DNA added to the PCR to rule out contamination. PCR was performed using the Syntezza PCR-Ready High Specificity kit (Syntezza Bioscience, Israel). Amplification was performed using a programmable conventional thermocycler (Biometra, Goettingen, Germany).

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.

2.7. Sequencing

Positive PCR products were purified using a PCR purification kit (Exo-SAP, NEB; New England Biolabs, Inc., Ipswich, MA). Sequencing was performed at the Center for Genomic Technologies, Hebrew University of Jerusalem, Israel. Purified amplicons were sequenced in the forward direction except where the identity of the products could not be ascertained and then the reverse directions were also sequenced. DNA sequences obtained were evaluated by the MEGA 6 software (Tamura et al., 2013) and compared for similarity to sequences deposited in GenBank, using the BLAST program hosted by the National Center for Biotechnology Information (NCBI), National Institutes of Health, Bethesda, MD (www.ncbi.nlm.nih.gov/BLAST).

Bayesian Inference trees were built using MrBayes 3.2.6 (Huelsenbeck and Fredrik Ronquist, 2001) in Geneious software version 7.1.9 (Kearse et al., 2012). The substitution model Hasegawa-Kishino-Yano (HKY) with gamma distribution (G = 5) was chosen according to the Bayesian inference criterion (BIC)".

3. Results

3.1. Rodents

Overall, 194 rodents including the black rat (*Rattus rattus;* n = 48), brown rat (*Rattus norvegicus;* n = 121), house mouse (*Mus musculus;* n = 6) and the African giant rat (*Cricetomys gambianus;* n = 2) were trapped in 2011. Additional 17 rodents of 2 species were trapped in 2015: house mice (*M. musculus;* n = 16) and bushy tail rat (*Neotoma* spp.; n = 1).

3.2. Ectoparasites

A total of 170 ectoparasites were collected from the rodents trapped in 2011. Two species of ticks (Ixodoidea), i.e., the brown dog tick (*Rhipicephalus sanguineus* sensu lato; n = 79) and the yellow dog tick (*Haemaphysalis leachi*; n = 6) were identified. Two species of fleas (Siphonaptera), i.e., the rat flea (*Xenopsylla cheopis*; n = 7) and the rodent flea (*Ctenophthalmus* spp.; n = 6) as well as one species of Mesostigmata, gamasid mites (*Haemolaelaps* spp.; n = 62) and a member of the Dermaptera, earwigs (*Hemimerus talpoides*; n = 10) were also collected (Table 1). However, no ectoparasites could be found on the rodents trapped in 2015.