Co-Infection with *Capillaria hepatica* and *Bartonella elizabethae* in a Brown Rat (*Rattus norvegicus*) from Nigeria

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ABSTRACT

During a survey for *Bartonella* species in rodents in Vom, Plateau State, Nigeria, a brown rat (*Rattus norvegicus*) with multiple subcutaneous nodules was captured. Gross pathology examination revealed multifocal pale areas in the spleen and liver. Histopathologic examination of the liver revealed numerous multifocal well demarcated and walled-off granulomas compressing and destroying the hepatic cord and parenchyma. Granulomas contained *Capillaria hepatica* nematodes in different developmental stages with numerous typical ova. There was degeneration and necrosis of hepatocytes, bridging fibrosis and biliary hyperplasia and reduplication, surrounded by inflammatory foci composed of lymphocytes, plasma cells and macrophages. Tiny smooth bacterial colonies with metallic sheen were isolated from blood of the rat cultured on blood chocolate agar. DNA extracted from the blood and spleen of the rat was found positive for *Bartonella* spp. citrate synthase gene (*gltA*) by PCR. Sequence analysis shows 99% similarity to *B. elizabethae*. The identification of two zoonotic agents in a rat highlights the importance of rats in the transmission cycle of zoonotic diseases in Nigeria.

Key words: *Rattus norvegicus, Capillaria hepatica, Bartonella elizabethae,* Nigeria.

INTRODUCTION

Rodents especially rats (*Rattus norvegicus*, *Rattus rattus*, *Cricetomyces gambianus*) abound in domestic and peri-domestic settings in Nigeria, all year round (1). Their role in the transmission of zoonotic diseases is well documented (2-4). Their role in disease transmission in Nigeria is even greater as rats are being hunted and consumed as a source of protein by some local ethnic groups. In addition, the act of trapping rats, bringing them to the homestead and preparing them for human consumption without proper protective clothing, litters the environment with rat feces and expose humans especially children, who play around contaminated playground, to zoonotic agents. *Capillaria hepatica* is a worldwide distributed zoonotic nematode of mammals, primarily infesting the liver of rats (5, 6). Consumption of *C. hepatica* unembryonated eggs may cause pseudoparasitism in man, with no apparent clinical signs or symptoms of disease. True infection, with liver localization of the parasite and the development of hepatic lesions, requires the ingestion of infective eggs. The contamination of playgrounds by infective eggs can explain the high susceptibility of children to capillariasis (7). *Rattus norvegicus* have also been found to carry various zoonotic *Bartonella* species (2, 8-10). In the United States and Portugal, 18% of rodents tested were *Bartonella* bacteremic, and some isolates from *R. norvegicus* were closely related to *B. elizabethae* (9). The latter species was associated with endocarditis in humans (9, 11).

Since rodents are the principal hosts of *C. hepatica* and also reservoir of *Bartonella* spp., contact between humans and rodents can be a potential health hazard. There is paucity of information of bartonellosis in man and animals in Nigeria.

We present in this report a co-infection of zoonotic agents; *C. hepatica* and *B. elizabethae* in a *Rattus norvegicus* in Nigeria.

CASE REPORT

During a survey for bartonellosis in rodents from Vom, Nigeria, one of the rats (*Rattus norvegicus*) trapped was noticed to be emaciated with poor body condition. Diffuse solitary nodules were present on its body and hard solid masses could be palpated in its abdomen.

The rat was humanely euthanized according to the guidelines of the Animal Welfare and Ethics Committee of the National Veterinary Research Institute (NVRI), Vom, Nigeria.

Approximately 2ml of blood was collected in sterile ethyelene diamine tetra acetic acid (EDTA) tube and stored at -20°C until further analyzed. The heart, liver, skin and spleen were harvested. Each tissue was divided into two parts. One part was preserved in buffered formalin while the other was placed in a sterile tube and kept at -20°C until further analyzed. Ticks were removed from its body and pooled into a universal bottle containing absolute ethanol. The ticks were identified as *Rhipicephalus sanguineus.*

Tissues in buffered formalin were processed at the Central Diagnostic Division, NVRI Vom. Five µm paraffin-wax sections of organs were dewaxed and stained with hematoxylin-eosin (H&E), mounted on charged microscope slides and observed under a Carl Zeiss light microscope for histopathological changes. The other samples were transported to The Koret school of Veterinary Medicine, Israel, for further analysis.

For bacterial culture, 200µl of thawed whole blood sample was plated onto blood chocolate agar. The plate was incubated at 35°C in 5% CO_2 and checked every other day for growth of *Bartonella* species. Suspected colonies were randomly selected and separately sub-cultured onto different fresh agar plates. The resulting pure cultures were stained using Gram stain.

DNA was extracted from the spleen, liver, heart and ticks using Illustra tissue and cell genomicPrep miniSpin kit (GE Healthcare, UK, Limited) and from blood using the Bi*O*stic™ Bacteremia DNA Isolation Kit (MO Bio Laboratories, Inc., Carlsbad, CA, USA) according to manufacturer's instructions.

Polymerase chain reaction (PCR) was performed using the primers, BhCS871p: (5'-GGGGACCAGCTCATGGTGG-3') and BhCS1137n: (5'-AATGCAAAAAGAACAGTAAACA-3') for amplification of a 379 bp fragment of the citrate synthase gene (*gltA*) of *Bartonella* spp. Positive and negative controls were included in each PCR run. PCR was performed in 25 µl total volume as follows: 3µl of DNA template, 1µl of 10mM each primer, 1µl $Mgcl_2$, 20µl of ultra pure PCR water. PCR was performed using Syntezza PCR-Ready High Specificity (Syntezza Bioscience, Israel). Amplification was performed using a programmable conventional thermocycler (Biometra, Goettingen, Germany) using the following program parameters: an initial denaturing at 95°C for five minutes, and 35 cycles at 95°C for one minute, 56°C for one minute, and 72°C for one minute.

The PCR products were analyzed for the presence of amplicons of the correct size by electrophoresis of 6µl of the products in 1.5% agarose gels containing ethidium bromide. Amplicons of the proper size were identified by comparison to the positive control lane on the agarose gel and comparison to a 50bp DNA molecular ladder.

DNA amplicons from positive samples were purified (EXOSAP-IT, USB, Cleveland, Ohio, USA) and sequenced using forward primers at the Center for Genomics Technologies, Hebrew University of Jerusalem.

DNA sequences obtained were evaluated with MEGA 5 software and compared for similarity to sequences in GenBank, using the BLAST program hosted by NCBI, National Institutes of Health, Bethesda, MD USA http:/www.ncbi.nlm.nih.gov/BLAST.

Sequences of *Bartonella* spp. derived from *Rattus norvegicus* in this study were deposited in GenBank under the following accession numbers JX065633 and JX065634.

Approximately 80% of the liver was studded with multifocal to coalescing pale granulomas. Abscesses were seen under the skin with marked infiltration of polymorphonuclear cells. There were no gross or histopathologic lesions in the heart, lungs and kidneys. However, in the liver there was compression and destruction of the hepatic cord and parenchyma by numerous multifocal well demarcated and walled-off granulomas containing nematode parasites in different developmental

stages and ovoid typical *C. hepatica* ova of up to 20-30µm in diameter. Multifocally cross sections of whole larva and slender adult stages of the nematode parasites were noticed. The larvae and adult stages had thick curticle, narrow rim of clear space, body wall, hypodermis and a coelomyrian striated muscle (Figures 1 & 2). Well developed digestive and reproductive organs (esophagus and a gravid uterus) containing numerous ova with polar eminence at each end, consistent with *Capillaria hepatica*, were seen. Degeneration and necrosis of hepatocytes, bridging fibrosis and biliary hyperplasia and reduplication, surrounded by inflammatory foci composed of lymphocytes, plasma cells and macrophages were present (Figures 1 & 2).

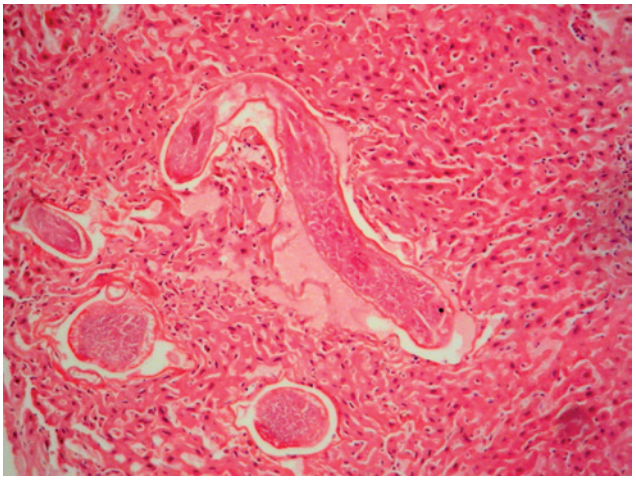

Figure 1: Liver histopathology (×10); Multifocal cross sections, whole larva and slender adult stage of the nematode parasites, *Capillaria hepatica*. (H&E)

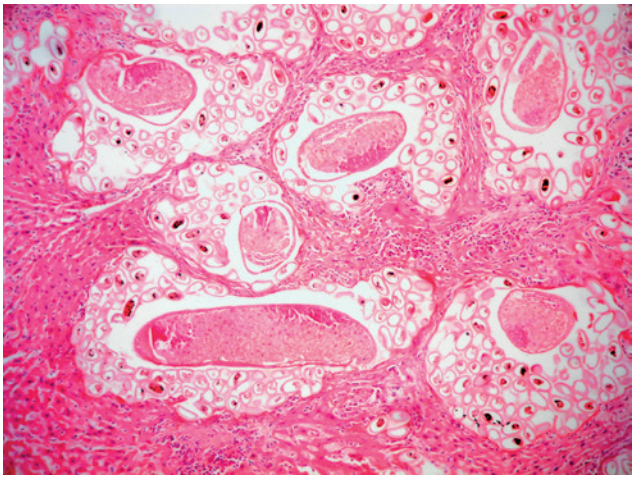

Figure 2: Liver histopathology (×10); Within individual fibrous connective tissue walled-off peri portal areas are numerous *Capillaria hepatica* ovoid ova of up to 20-30µm, with polar eminence at each end. Note the degeneration and necrosis of hepatocytes with bridging fibrosis. (H&E)

Tiny smooth bacterial colonies with metallic sheen were isolated from blood of the rat cultured on blood chocolate agar. The colonies were Gram negative cocci to rod shaped bacteria. *Bartonella gltA* DNA with 99% sequence homology with GenBank deposited *B. elizabethae* was identified from the blood and spleen of the *Rattus norvegicus* rat. Phylogenic analysis performed using the maximum likelihood method showed that the sequences from this study formed a distinct clade with *B. elizabethae* accession number GU056192 from the Genbank (Figure 3).

DISCUSSION

Pathologies consistent with *C. hepatica* were evident on gross and histopathology of the liver. However, the rat appeared to be emaciated and in poor body condition contrary to the report by Ceruti *et al.* (7) in which *C. hepatica* infected rats, appeared to be in a good body condition. The pathologies observed in this rat could be as a result of synergistic effects between *C. hepatica* and *B. elizabethae* since pathologies due to bartonellae infections alone are not commonly observed in rodents (12). *Bartonella* DNA has been isolated from rodent spleens in Israel (13) which is consistent with our finding and may be due to pooling of infected erythrocytes in the spleen.

Rattus norvegicus have been shown to harbor several *Bartonella* species, including *B. tribocorum* and *B. elizabethae* which were implicated as a cause of human diseases (9, 11, 14). It has been shown that rodent-associated *Bartonella* species could act as human pathogens and suggested that the predominantly urban rat *R. norvegicus* could be a reservoir for human infection among inner-city residents living in poor social conditions (9).

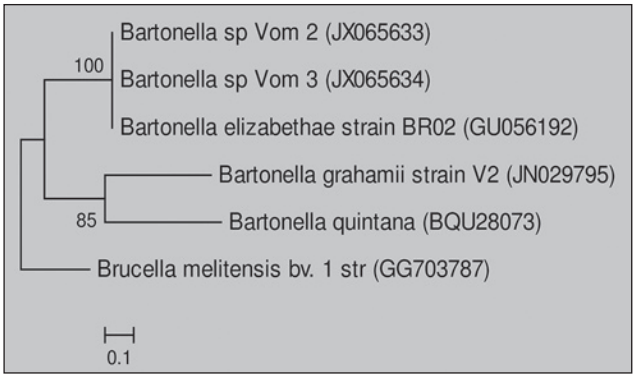

Figure 3: The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. Stability of inferred phylogeny was assessed by using bootstrap analysis of 1000 randomly generated trees. Sequences from this study are in dotted lines.

In conclusion, the finding of these zoonotic agents in a rat captured in the vicinity of human habitation coupled with the fact that people in this area consume rat meat highlights the serious danger posed by rodents to human health. Physicians and medical workers should consider bartonellosis in the diagnosis of cardiac conditions in Nigeria. Further investigation is needed to determine the role of *Bartonella* spp. in general and *Bartonella elizabethae* in particular in human endocarditis cases in Nigeria.

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CONFLICT OF INTEREST

There was no conflict of interest in this study.

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