Journal of Bacteriology and Mycology

Open Access

Austin Publishing Group

Review Article

Molecular Detection of *Bartonella* in Lions, Zimbabwe

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Received: October 16, 2020; **Accepted:** November 25, 2020; **Published:** December 02, 2020

Abstract

Domestic cats around the world are commonly infected with *Bartonella* and there is growing evidence that wild felids can also be infected. To provide further data on *Bartonella* infections in African lions (Panthera leo). We used PCR and sequencing of the *16S rRNA* to detect *Bartonella* in whole blood DNA samples from a convenience sample of 84 lions. Four out of 84 (5%) lions were positive by PCR. The 305-bp sequence of the amplicons was identical in all four lions and had highest similarity to the recognized species *Bartonella grahamii* (304/305 matches) and *Bartonella henselae* (303/305 matches). It was identical to unidentified strains of *Bartonella* from mice in the US, and voles in Lapland and Alaska. Our Zimbabwe data adds to that available from South Africa to show infections with *Bartonella* are not uncommon in lions in Southern Africa and perhaps the continent. The range of *Bartonella* species that infect lions and their effects on the animals' health remains to be determined.

Keywords: Lion; *Bartonella*; Zimbabwe

Introduction

Bartonella are Gram-negative bacteria that are found principally in erythrocytes in their reservoir hosts. The organisms grow slowly on conventional agar media and with the advent of molecular testing there has been a dramatic increase in our knowledge of the species with the five known species in 1993 [1] expanding to over 33 today [2]. One of the first new species to be described was *Bartonella henselae*, the agent of cat scratch disease, bacilliary angiomatosis and endocarditis in people. The organism is commonly found in domestic cats that can remain bacteremic for long periods, even years, although only very infrequently, if ever, showing clinical signs [3].

Five *Bartonella* spp. have now been identified in domestic cats: *B. henselae, Bartonella clarridgeiae, Bartonella koehlerae, Bartonella bovis* (formerly *B. weissii*) and *Bartonella quintana* [2]. *Bartonella henselae* has also been found in wild felid species in Africa, in lions (*Panthera leo*) [4,5] and a cheetah [6]. Further, a species intermediate between *B. henselae* and *B. koehlerae* (Namibian cheetah strain 1178) was isolated from a wild cheetah in Namibia [5].

Studies of wild cats in the US have shown exposure to *Bartonella* spp. is high with seroprevalences of up to 53% [7]. *Bartonella henselae* has been shown to occur in wild felids [8] as well as a new subspecies, *B. koehlerae* subsp. *boulouisii* and *B. koehlerae* subsp. *bothieri*, for which mountain lions and bobcats are the natural reservoirs, respectively [2].

To provide more data on *Bartonella* infections in lions in southern Africa we used a PCR to analyzed DNA remaining from a study on tick-borne diseases in lions [9].

Materials and Methods

The DNA used in the study had been extracted from convenience samples of whole blood from 84 adult captive lions from Zimbabwe (Gweru, N=67; Masvingo, N=6; Dollar Block farm N=8; Hwange, N=3) for a previous study on tick-borne pathogens in lions [9]. That study had been reviewed and approved by the Institutional Animal Care and Use Committee of the Ross University School of Veterinary Medicine, St Kitts. For the current study, the DNA was analyzed by PCR as described previously [10] for a 305-bp section of the *16S rRNA* with forward (5'-AGCGCACTCTTTTAGAGTGAGCGG-3') and reverse primers (5'-CATGGCTGGATCAGGGTTGCC-3') that detect the recognized *Bartonella* in GenBank. Positive control DNA was from *B. henselae* and *B. clarridgeiae* while negative control DNA was from the related species *Brucella melitensis, Br. chilestrain, Wolbachia, Coxiella burnetii, Rickettsia felis, R. rickettsiae, Anaplasma phagocytophilum* and *A. marginale*. Amplicons of positive PCRs were sequenced (GenScript, Nanjing, Jiangsu, China) and compared with sequences available in GenBank.

Results and Discussion

Four (5%) of the lions were PCR positive with amplicons that were all identical. Comparing the sequences of the amplicons with those of recognized species in GenBank revealed the *Bartonella* in the lions was closest to a number of strains of *Bartonella grahamii* with which it had 1 mismatch (304/305 matches, 99%, 4e-155). The flea-borne *B. grahamii* has been widely described in wild rodents, including in Nigeria, and has been associated with eye conditions in people [11]. The next most closely related species was *B. henselae* with which the lion strain had 2 mismatches (303/305; 99%; 2e-153). *Bartonella henselae* has previously been identified by PCR and sequencing in both wild (4%; 2/58) [5] and semi-captive (2%; 1/65) lions [12]. In the latter study, anti-*Bartonella* antibodies were detected by ELISA in 29% of the lions. In a survey in Zambia, however, none of 48 wild lions had evidence of *Bartonella* infection by PCR [13] and, in a survey in Zimbabwe, none of 4 lions were culture-positive [6]. *Bartonella henselae* has also been isolated from a captive cheetah in Zimbabwe [6] and a *Bartonella* intermediate between *B. henselae* and *B. koehlerae* (Namibian cheetah strain 1178) was isolated from a wild cheetah in Namibia [5].

Our lion *Bartonella* had identical GenBank sequences with a number of isolates that have not been definitively identified.

Citation: Huang K, Kelly PJ, Zhang J, Marabini L, Dutlow K, Yang Y, et al. Molecular Detection of *Bartonella* in Lions, Zimbabwe. J Bacteriol Mycol. 2020; 7(8): 1158.

These included ones found in voles from Lapland (KT961186) [14] and Alaska (EU97535) [15], a cat flea (FJ981670), and a mouse (*Peromyscus leucopus*) in the Midwestern USA (U71322) [16]. It is important to note that, although our PCR detected a variable region of the *16S rRNA*, it is recommended that a number of other genes, such as the *gltA* [10], are also sequenced to ensure the correct classification of newly identified strains. Shortcomings in using only one gene sequence for speciation are apparent from the data on the Midwestern mouse isolate referred to above. Although it had an identical *16S rRNA* sequence (U71322) to our *Bartonella* and to *B. grahamii*, when sequencing was performed on PCR products amplified from the *gltA* it was found the isolate was most closely related to *Bartonella vinsonii*. Sequencing of further genes would have been required to more clearly define the isolate. Unfortunately we had only very limited DNA available to us and were, then, unable to confirm the identity of the lion *Bartonella* by sequencing other genes.

In summary, our study shows lions in Zimbabwe can also be infected with *Bartonella* which together with data from South Africa indicate infections are widespread in the region and perhaps the continent. Further, our data is consistent with other studies showing there are as yet uncharacterized *Bartonella* in Southern African, not only in lions [5] but also bats [17], mice [18], and small mammals [12]. Further studies are needed to characterize the clinical significance of infections with *Bartonella* in lions, especially in relation to reportedly common coinfections with other vector borne agents [9] and FIV [19]. There are also possible public health implications, in particular as HIV infections are common in Southern Africa and studies have shown high prevalences of HIV-positive patients (up to 23%) are positive for *Bartonella* in PCRs on blood samples [20].

Acknowledgments

This project was supported by the Ross University School of Veterinary Medicine, the Animal and Wildlife Area Research and Rehabilitation (AWARE) Trust of Zimbabwe.

Conflict of Interest

The authors declared no competing interests.

Author Contributions

Ke Huang, Patrick Kelly, and Chengming Wang designed the study and wrote the manuscript. Lisa Marabini and Keith Dutlow collected the samples and Ke Huang, Jilei Zhang, and Yi Yang performed the laboratory analyses. All authors participated in the review of the manuscript.

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