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Abstract: Bartonella species are emerging pathogens that have been isolated worldwide from humans and other mammals. Our objective was to estimate the prevalence of Bartonella infection in free-ranging African lions and cheetahs. Blood and/or serum samples were collected from a convenience sample of 113 lions and 74 cheetahs captured in Africa between 1982 and 2002. Whole blood samples available from 58 of the lions and 17 of the cheetahs were cultured for evidence of Bartonella spp., and whole blood from 73 of the 74 cheeths tested for the presence of Bartonella DNA by TaqMan PCR. Serum samples from the 113 lions and 74 cheetahs were tested for the presence of antibodies against B.henselae using an immunofluorescence assay. Three (5.2%) of the 58 lions and one (5.9%) of the 17 cheetahs were bacteremic. Two lions were infected with B.henselae, based on PCR/RFLP of the citrate synthase gene. The third lion and the cheetah were infected with previoulsy unidentified Bartonella strains. Twenty-three percent of the 73 cheetahs tested by TaqMan PCR were positive for Bartonella spp. Bartonella henselae antibody prevalence was 17% (19/113) for the lions and 31% (23/74) for the cheetahs. The prevalence of seropositivity, bacteremia, and positive TaqMan PCR was not significantly different between sexes and age categories (juvenile vs. adults) for both lion and cheetahs. Domestic cats are no longer the only known carriers of Bartonella spp. in Africa. Translocation of B.henselae seronegative wild felids might be effective in limiting the spread of Bartonella infection.

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Prevalence of Bartonella infection in free-ranging African lions (Panthera leo) and cheetahs (Acinonyx jubatus)

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1 **Abstract**

2 Bartonella species are emerging pathogens that have been isolated worldwide from
3 humans and other mammals. Our objective was to estimate the prevalence of Bartonella
4 infection in free-ranging African lions (Panthera leo) and cheetahs (Acinonyx jubatus). Blood
5 and/or serum samples were collected from a convenience sample of 113 lions and 74 cheetahs
6 captured in Africa between 1982 and 2002. Whole blood samples available from 58 of the
7 lions and 17 of the cheetahs were cultured for evidence of Bartonella spp., and whole blood
8 from 73 of the 74 cheetahs tested for the presence of Bartonella DNA by TaqMan PCR.
9 Serum samples from the 113 lions and 74 cheetahs were tested for the presence of antibodies
10 against B. henselae using an immunofluorescence assay. Three (5.2%) of the 58 lions and
11 one (5.9%) of the 17 cheetahs were bacteremic. Two lions were infected with B. henselae,
12 based on PCR/RFLP of the citrate synthase gene. The third lion and the cheetah were
13 infected with previously unidentified Bartonella strains. Twenty-three percent of the 73
14 cheetahs tested by TaqMan PCR were positive for Bartonella spp. Bartonella henselae
15 antibody prevalence was 17% (19/113) for the lions and 31% (23/74) for the cheetahs. The
16 prevalence of seropositivity, bacteremia, and positive TaqMan PCR was not significantly
17 different between sexes and age categories (juvenile vs. adult) for both lions and cheetahs.
18 Domestic cats are no longer the only known carriers of Bartonella spp. in Africa.
19 Translocation of B. henselae seronegative wild felids might be effective in limiting the spread
20 of Bartonella infection.

21 **Key words:** Bartonella, cheetahs, lions, serosurvey

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1 **1. Introduction**

2 Members of the genus Bartonella are aerobic gram-negative bacteria that have recently
3 been recognized as emerging pathogens (Anderson and Neuman, 1997). An increasing
4 number of species have been described in the last decade and the genus currently consists of
5 16 species, seven of which are associated with human diseases (Jacomo et al., 2002). Clinical
6 manifestations caused by Bartonella species include Carrion's disease, trench fever, cat
7 scratch disease, bacillary angiomatosis, hepatic peliosis, endocarditis, chronic bacteremia, and
8 neuroretinitis (Jacomo et al., 2002). The epidemiology of Bartonella infection is currently not
9 fully understood but most Bartonella species are believed to be hosted by animal reservoirs
10 and transmitted by arthropod vectors. Sand flies of the genus Lutzomyia are responsible for
11 the transmission of B. bacilliformis (Garcia-Caceres and Garcia, 1991). The human body
12 louse (Pediculus humanus) is the main vector for B. quintana (Maurin and Raoult, 1996). Cat
13 fleas (Ctenocephalides felis) have been shown to transmit B. henselae between cats (Chomel
14 et al., 1996), and various species of Bartonella have been identified in ticks (Schouls et al.,
15 1999; Chang et al., 2001; Sanogo et al., 2003).

16 Cat scratch disease (CSD) is the most common of the Bartonella-associated clinical
17 conditions in humans and is estimated to have an incidence of 22,000 cases per year in the
18 United States, representing an annual health care cost of more than $ 12 million (Jackson et
19 al., 1993). The main etiologic agent of CSD is Bartonella henselae (Regnery et al., 1992c;
20 Dolan et al., 1993), which was first identified as the agent of bacillary angiomatosis in
21 immuno-compromised patients (Regnery al., 1992a; Welch et al., 1992). Bartonella
22 clarridgeiae has also been involved in the etiology of CSD based on serology results (Kordick
23 et al., 1997; Margileth and Baehren, 1998).

1 Substantial evidence has accumulated identifying the domestic cat (Felis catus) as the
2 main reservoir for B. henselae. Natural and experimental infections of cats with B. henselae
3 were documented shortly after the bacterium was first identified (Regnery et al., 1992a;
4 Koehler et al., 1994; Abbott et al., 1997). No major clinical signs have been reported in cats
5 under natural conditions, although fever, transient anorexia, mild neurologic signs, myalgia,
6 lymphadenopathy, and reproductive failure have been described in experimentally infected
7 cats (Breitschwerdt and Kordick, 1995; Greene et al., 1996; Guptill et al., 1997; Kordick and
8 Breitschwerdt, 1997; Guptill et al., 1998; O'Reilly et al., 1999). Infected cats have been
9 shown to remain bacteremic for several months (Kordick et al., 1995; Abbott et al., 1997).
10 High B. henselae infection prevalence rates reported in cat populations (Koehler et al., 1994;
11 Chomel et al., 1995; Branley et al., 1996; Bergmans et al., 1997; Heller et al., 1997; Chomel
12 et al., 1999; Marston et al., 1999; Chomel et al., 2002), with a range of 16.5% to 61%
13 depending on the origin of the cats tested, have later confirmed the reservoir role of cats. Cats
14 have subsequently been reported to be reservoir for B. clarridgeiae (Kordick et al., 1997) and
15 B. koehlerae (Droz et al., 1999; Yamamoto et al., 2002), and have been shown to host B.
16 weissii (Regnery et al., 2000), which is now known as B. bovis (Bermond et al., 2002).
17 Free-ranging felid species may likely be reservoirs for Bartonella species, as many
18 free-ranging felids of North America have been shown to be infected with Bartonella spp.
19 based both on serological and bacteriological data (Chomel et al., unpublished data). In
20 California, 26 (35%) of 74 mountain lions (Puma concolor) and 33 (53%) of 62 bobcats
21 (Lynx rufus) had antibodies against B. henselae in their blood (Yamamoto et al., 1998). In
22 Florida, antibodies against B. henselae were detected in two (28%) of seven mountain lions
23 originating from Texas (Puma concolor stanleyana), and in five (18%) of 28 Florida panthers

1 (Puma concolor coryi) (Rotstein et al., 2000). Statistical testing of the association between
2 age and seropositivity showed that antibody prevalence was significantly higher in animals
3 less than two years old than in animals over two years old (Rotstein et al., 2000; Yamamoto et
4 al., 1998). Less clear is the association between sex and seropositivity. Yamamoto et al.
5 (1998) found that male mountain lions were more likely to be seropositive than females,
6 whereas Rotstein et al. (2000) found no significant difference in seropositivity status between
7 males and females. Culture isolation and PCR-based speciation of the Bartonella strains were
8 not performed in these two studies, and it is not known whether the Bartonella strains present
9 in wild felids are the same as those in domestic cats.

10 Some evidence suggests that African free-ranging felid populations could be infected
11 with Bartonella species. Infection of domestic cat populations with B. henselae has been
12 documented in South Africa (Kelly et al., 1996; Pretorius et al., 1999). Kelly et al. (1998)
13 isolated B. henselae from the blood of one of three cheetahs tested in Zimbabwe, and
14 antibodies to B. henselae have been found in seven species of African wild felids (genera
15 Acinonyx, Panthera and Felis) kept in different zoological parks of California (Yamamoto et
16 al., 1998). Estimating the prevalence of Bartonella infection in African free-ranging felids is
17 of interest to determine if wild felid populations could play a reservoir role for Bartonella
18 species and could potentially serve as a source of infection for domestic cats and humans.
19 Lions and cheetahs represent good candidate species for estimating the prevalence of
20 infection in sub-Saharan African felids because they are widespread. The objectives of this
21 study were 1) to estimate the prevalence of Bartonella infection in African lions and cheetahs
22 by culturing blood samples for evidence of bacteria and by testing sera for the presence of
23 antibodies against B. henselae using an immunofluorescence assay (IFA), 2) to identify the

1 Bartonella strains obtained, and 3) to test for association between Bartonella infection and
2 putative risk factors, including age, sex, and geographic location.

3 **2. Materials and methods**

4 2.1. Animals

5 Blood and serum samples were collected from 74 free-ranging cheetahs and 113 free-
6 ranging lions in Africa between September 1982 and February 2002. Fifty-eight lions had
7 serum and blood samples available and the other 55 lions only had serum samples available.
8 Whole blood was not available from one of the 74 cheetahs. The samples were obtained from
9 animals immobilized for disease surveillance or conservation purposes in Kruger National
10 Park in South Africa (112 lions), Masai Mara National Park (1 cheetah) and Nairobi National
11 Park (6 cheetahs) in Kenya, Ngorongoro crater (2 cheetahs) and Serengeti National Park in
12 Tanzania (1 lion, 13 cheetahs), and various regions of Namibia (52 cheetahs). Blood and
13 serum samples were stored at least at –20°C until tested.

14 2.2. Blood culture

15 Whenever available, whole blood samples collected in EDTA tubes were centrifuged
16 at 5000 x g for 30 min at room temperature. Blood pellets were resuspended in 125 μl of M
17 199 inoculation medium (Kohler et al., 1992) and plated onto heart infusion agar (Difco
18 laboratories, Detroit, MI) containing 5% fresh rabbit blood. The plates were then incubated at
19 35°C in 5% CO_2 for four weeks, and cultures were examined at least twice weekly for
20 bacterial growth. The number of colonies observed was recorded as the number of colony-
21 forming units per milliliter of blood. Colonies were sub-cultured, harvested, and frozen at -
22 70°C in M 199 inoculation medium containing 10% DMSO (Fisher Scientific, Fair Lawn,
23 NJ). Identification of the isolates as Bartonella spp. was based on the phenotypic

characteristics of the colonies, and species determination was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis of fragments of the citrate synthase gene.

2.3. DNA extraction and PCR-RFLP analysis

Bacterial DNA was extracted from blood isolates by hot water extraction (15 min at 100°C). The extracted DNA was then used as a template for amplification of fragments of the citrate synthase gene (gltA). Approximately 380 basepairs (bp) of the gltA gene were amplified using previously described primers and methods (Norman et al., 1995). The amplified products were verified by gel electrophoresis and then enzymatically digested overnight, at 65°C using TaqI restriction endonuclease, and at 37°C using HhaI, MseI, and AciI restriction endonucleases. Control samples included DNA from a strain isolated from a naturally infected cat, which was previously confirmed positive for B. henselae, and a sample with no DNA template. The digested fragments were separated by electrophoresis in a 3% Nusieve GTG agarose gel (Biowhittaker Molecular Applications, Rockland, ME). Fragment sizes were estimated by comparison with a 100-bp ladder (Invitrogen, Carlsbad, CA). Banding patterns were compared with those of other strains in the American Type Culture Collection and our laboratory.

2.4. TaqMan PCR

Blood samples collected in 1985, 1992, and 1993 from 56 cheetahs were too contaminated with fast-growing bacteria to allow for the culture of Bartonella spp., and were therefore tested by a Bartonella real-time TaqMan PCR system. In order to be consistent in our methodology, the 17 blood samples tested by blood culture were also submitted to TaqMan PCR testing. The real-time TaqMan PCR method is based on a combination of the

TaqMan technology and the ABI PRISM 7700 (Applied Biosystems, Foster City, CA) real-time sequence detection system. The use of two specific primers and an internal labeled TaqMan probe combined with the 5'-3' nuclease activity of Taq DNA polymerase allows direct quantification of the PCR product accumulation during the exponential amplification phase of the PCR. Quantification of the number of organism present in each sample is obtained by using the standard curve method. Standard curves are optimized for the proposed pathogens. The PCR mastermix contains a hot start enzyme (AmpliTaq Gold), an internal dye (ROX) to normalize variations caused by manual pipetting and an AmpErase UNG nuclease system to prevent PCR product carryover. Data collection and processing is performed in an attached computer using ABI Sequence Detection System software.

2.4.1. Sample preparation for TaqMan PCR

One hundred µl of whole anticoagulated blood was used to extract genomic DNA (gDNA) using DNeasy Tissue Kit (Qiagen, Valencia, CA). Briefly, whole blood was mixed with proteinase K, PBS and lysis buffer AL to a final volume of 420 µl and digested for 10 min at 70°C. DNA was extracted according to the manufacturer's instruction.

2.4.2. TaqMan PCR systems

Two TaqMan PCR systems were used: first, a TaqMan PCR system specific for feline glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Leutenegger et al., 1999) was used as a quality control to test for the integrity of the gDNA extracted from the aged blood samples. In addition, a Bartonella specific TaqMan PCR system for detection of Bartonella spp. at the genus level was used targeting the citrate synthase gene: forward primer Bart.738f: 5'-GTGCTAATCCATTTGCATGTATT-3', reverse primer Bart.831r: 5'-

1 GTAACATTTTTAGGCATGCTTCATTA-3', TaqMan probe: Bart.772p: 5'-6-FAM-

2 AGCTGGTCCCCAAAGGCATGCAA-TAMRA-3'.

3 2.5. Serology

4 Sera from lions and cheetahs were tested by immunofluorescence assay (IFA) for the

5 presence of antibodies against B. henselae. For antigen preparation, a B. henselae strain "U-

6 4" (University of California, Davis) originally isolated from a naturally infected cat was

7 cultivated on Vero cells. Infected Vero cells were then suspended in L15/MEM tissue culture

8 medium (Invitrogen, Carlsbad, CA). Forty μl of the suspension were dotted onto each well of

9 12-well polytetrafluoroethylene-coated slides (Cel-line Associated, Newfield, NJ), and

10 incubated for 24 hours. The slides were then washed twice in phosphate-buffered saline

11 (PBS, pH 7.4, SIGMA, St Louis, MO), fixed 20 min in acetone, air dried, and then stored at -

12 20°C. Sera from lions and cheetahs were diluted 1:64 in PBS containing 5% skim milk, and

13 then 20 μl of the 1:64 dilutions were added to the test wells of the slides. Positive and

14 negative control sera from domestic cats were included on all slides. Slides coated with test

15 sera were incubated 30 min at 37°C and then washed three times in PBS. Fluorescein-

16 conjugated goat anti-cat immunoglobulin (whole-molecule immunoglobulin G; Cappel,

17 Organon Teknika Corp., Durham, NC) was diluted 1:800 in PBS containing 5% skim milk

18 and 0.001% Evan's blue, and 20 μl of the mix were applied to each well. The slides were

19 incubated 30 min at 37°C and then washed three times in PBS. Three μl of glycerol were

20 applied to each well and the slides then covered with a cover slip prior to interpretation with a

21 fluorescence microscope (magnification x 400). The intensity of bacillus-specific

22 fluorescence was scored subjectively from one to four, and a fluorescence score greater than

23 two at a dilution of 1:64 was considered to be a positive result (Childs et al., 1994). A

double-blinded reading of each slide was performed by the same two readers. All serum samples positive at 1:64 dilution were two-fold serially diluted and tested with the same IFA technique to obtain an endpoint titer.

2.6. Statistical analysis

Statistical analyses were performed using Epi Info 2000 version 1.1.2 (CDC, Atlanta, GA) and SPSS version 11.0 (SPSS Inc., Chicago, IL). Nonparametric tests (Chi-square, Fisher's exact test) were used to test for an association between bacteremia or seropositivity, and putative risk factors such as sex, age, and geographic origin of the animals. The association between bacteremia and seropositivity was evaluated by paired analysis using the McNemar Chi-square test.

3. Results

3.1. Description of the lion and cheetah populations

One hundred and thirteen lions were included in the study. A precise age estimate was available for 34 (30.1%) of them, ranging from 14 months to 15 years old (mean 69.9 months, standard deviation 33.8 months). The age status was only known as juvenile (≤ 24 months old) or adult (> 24 months old) for 78 (69.0%) lions, and was undetermined for one (0.9%) lion. Sixty-three (55.8%) lions were female, 49 (43.4%) were male, and the sex of one (0.9%) lion was not recorded. Seventy-four cheetahs were included in the study and a precise age estimate was available for 64 (86.5%) of them, ranging from six months to 16 years old (mean 42.9 months, standard deviation 35.1 months). The age status was only known as juvenile (≤ 24 months old) or adult (> 24 months old) for six cheetahs, and was undetermined for four cheetahs. Thirty-six (48.6%) cheetahs were female, 34 (45.9%) were male, and the sex of four (5.4%) cheetahs was not recorded.

1 3.2. Blood culture

2 Bartonella were isolated from the blood of three (5.2%) of the 58 lions for which
3 whole blood samples were available. Bacteria of the genus Bartonella were isolated from the
4 blood of one (5.9%) of the 17 cheetahs for which non-contaminated blood samples were
5 available. The prevalence of bacteremic lions was not significantly different between males
6 (5.0% or 1/20) and females (5.3% or 2/38) or between juveniles (8.3% or 1/12) and adults
7 (4.3% or 2/46).

8 3.3. DNA extraction and PCR-RFLP analysis

9 Bartonella henselae was isolated from the blood of two of the bacteremic lions, and a
10 previously unidentified Bartonella strain, temporarily named "Lion 98-215", was isolated
11 from the blood of the third bacteremic lion (Table 1). The three bacteremic lions were from
12 different locations in the Kruger National Park in South Africa (Table 1). The Bartonella
13 strain isolated from the blood of the bacteremic cheetah from Gobagos province in Namibia
14 was also a previously unidentified strain temporarily designated as "Cheetah 1178".

15 3.4. TaqMan PCR

16 Seventeen (23.3%) of the 73 cheetahs tested by TaqMan PCR had a positive result.
17 The prevalence of positive TaqMan PCR result was not significantly different between males
18 (20.6% or 7/34) and females (22.9% or 8/35), between juveniles (32.1% or 9/28) and adults
19 (14.6% or 6/41), or between cheetahs from eastern Africa (28.6% or 6/21, of which 2/7 for
20 Kenya and 4/14 for Tanzania) and Namibia (21.2% or 11/52).

21 3.5. Serology

22 Antibodies against B. henselae were found in 19 (16.8%) of the 113 lions and in 23
23 (31.1%) of the 74 cheetahs. The prevalence of B. henselae seropositive lions was not

1 significantly different between males (12.2% or 6/49) and females (20.6% or 13/63) or
2 between juveniles (3.8% or 1/25) and adults (20.9% or 18/86). The prevalence of B. henselae
3 seropositive cheetahs was not significantly different between males (29.4% or 10/34) and
4 females (33.3% or 12/36), between juveniles (25% or 7/28) and adults (35.7% or 15/42), or
5 between cheetahs from eastern Africa (45.5% or 10/22, of which Kenya 1/7 and Tanzania
6 9/15) and Namibia (25% or 13/52). Titers of serum antibodies against B. henselae ranged
7 between 1:64 and 1:1024 in both lions and cheetahs. No antibodies against B. henselae were
8 detected in one of the two lions found to be bacteremic with B. henselae. No antibodies
9 against B. henselae were detected in the lion found to be bacteremic with the Bartonella strain
10 "Lion 98-215", or in the cheetah found to be bacteremic with the Bartonella strain "Cheetah
11 1178" (Table 1). A summary of the culture, TaqMan PCR, and serology results is provided in
12 Table 2.

13 3.6. Paired analysis

14 No significant association was detected between positive IFA and positive blood
15 culture for the lions' samples. No association was detected between positive IFA and positive
16 blood culture, or between positive IFA and positive TaqMan PCR for the cheetahs' samples.
17 For the animals tested by both IFA and culture (58 lions and 17 cheetahs), 96.2% of the
18 seronegative lions and 92.9% of the seronegative cheetahs were abacteremic. For the 73
19 cheetahs tested by both IFA and TaqMan PCR, 76% of the seronegative cheetahs had a
20 negative TaqMan PCR result.

21 **4. Discussion**

22 This study reports the first isolation of Bartonella species from free-ranging African
23 lions and the first prevalence estimate of Bartonella infection in free-ranging African lions

and cheetahs. Bartonella henselae seroprevalence detected here for lions (16.8%) and cheetahs (31.1%) are in the range of those reported in previous studies for North American free-ranging felids. Rotstein et al. (2000) found a seroprevalence of 18% in Florida panthers and 28% in mountain lions from Texas. In California, Yamamoto et al. (1998) reported a seroprevalence of 35% in mountain lions and 53% in bobcats. Unlike these two studies where juvenile animals had higher seropositivity prevalence than adults, but as observed in some reports for domestic cats (Haimerl et al. 1999; Gurfield et al., 2001), seropositivity prevalence was not significantly different between juvenile and adult lions or cheetahs. Similar to the study in Florida (Rotstein et al. 2000), but unlike the study in California (Yamamoto et al., 1998) where male mountain lions were more likely to be seropositive than females, antibody prevalence was evenly distributed between sexes for both African lions and cheetahs.

The prevalence of Bartonella positive blood culture in lions (5.2%) and cheetahs (5.9%) were low compared to those reported in previous studies of domestic and stray cats (Koehler et al., 1994; Chomel et al., 1995; Branley et al., 1996; Bergmans et al., 1997; Heller et al., 1997; Chomel et al., 1999; Marston et al., 1999; Chomel et al., 2002). Kelly et al. (1998) recently reported the isolation of B. henselae from one of three cheetahs tested in Zimbabwe. The low prevalence of positive blood culture in free-ranging cheetahs contrasts with the higher prevalence of positive TaqMan PCR results (23.3%). Bartonella are fastidious slow-growing organisms, and neither serology nor PCR methods have been shown to be more sensitive than culture to detect Bartonella infection in humans (La Scola and Raoult, 1999). It is therefore possible that the true bacteremia prevalence in our sample of wild cheetahs may be more accurately estimated by TaqMan PCR than by blood culture.

1 No serum antibodies against Bartonella were detected for the bacteremic cheetah and
2 for two of the three bacteremic lions. Isolation of Bartonella from seronegative cats has
3 previously been reported (Chomel et al., 1995; Kordick et al., 1995; Pretorius et al., 1999;
4 Gurfield et al., 2001). Possible reasons for the absence of serological response include
5 immunosuppression (especially in case of feline immunodeficiency virus (FIV) co-infection),
6 collection of sera at an early stage of infection, or lack of antibody cross-reaction among
7 strains.

8 The prevalence estimates reported here should be handled with precautions because of
9 the limitations of the sampling scheme. The sample size was low and it could not be
10 determined how representative our sample was of the free-ranging African lion and cheetah
11 populations. These limitations, which are frequently encountered in studies of wildlife
12 diseases, should be taken into account when readers make use of the results reported here.

13 Characterization of the two previously unidentified Bartonella strains, "Lion 98-215"
14 and "Cheetah 1178", is currently in progress.

15 The existence of Bartonella infection in free-ranging felids of Africa has implications
16 for public health. Transmission of CSD from cats to humans mainly occurs through scratches
17 and bites. Flea-borne transmission has been suspected in humans and recent evidence of the
18 presence of B. henselae in ticks suggests the possible existence of an arthropod vector
19 (Sanogo et al., 2003). The arthropod-based transmission of Bartonella species from free-
20 ranging felids to humans and domestic cats could occur when the large cats roam around
21 human settlements to prey on livestock and small ruminants, or when people have access to
22 national parks and other wildlife sanctuaries.

1 Seventy percent of the worldwide HIV cases are reported in Africa (De Cock et al., 2 2002) and Bartonella infection is likely to be a common secondary infection. Bacillary 3 angiomatosis, a severe manifestation of Bartonella infection, has previously been reported in 4 different countries of southern Africa (Pretorius et al. 1999). Frean et al. (2002) recently 5 reported that 10% of 188 HIV-positive outpatients in Johannesburg, South Africa, were 6 Bartonella bacteremic, as determined by nested PCR.

7 The existence of Bartonella infection in free-ranging African felids also has 8 implications in wildlife conservation because health data are indispensable for efficient 9 population management. Further study should be done to investigate the clinical significance 10 of Bartonella infection in African lions and cheetahs, and the possible effects of co-infection 11 with other pathogens such as FIV. Bartonella henselae has been shown to contribute to 12 chronic disease manifestations in domestic cats (Breitschwerdt and Kordick, 1995; Greene et 13 al., 1996; Guptill et al., 1997; Kordick and Breitschwerdt, 1997; Guptill et al., 1998; O'Reilly 14 et al., 1999), and could similarly affect wild felids.

15 Translocation of free-ranging felids can be a useful tool to reduce conflicts with 16 expanding human populations, to repopulate protected areas in which animals have been 17 decimated by poaching, and to maintain genetic diversity in metapopulations (Nowell and 18 Jackson, 1996). Special care must be given to ensure that potential pathogenic agents are 19 clearly identified and are not introduced into populations where they could have harmful 20 effects. The existence of Bartonella infection should be taken into account prior to relocating 21 lions and cheetahs. We reported that a negative serology result was highly predictive of a 22 negative blood culture (96.2% in lions and 92.9% in cheetahs), and predictive of a negative 23 TaqMan PCR (76% in cheetahs). Translocation of B. henselae seronegative wild felids might

1 therefore be effective in limiting the translocation of Bartonella species together with their

2 hosts.

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1 **References**

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3 Abbott, R.C., Chomel, B.B., Kasten, R.W., Floyd-Hawkins, K.A., Kikuchi, Y., Koehler, J.E.,
4 Pedersen, N.C., 1997. Experimental and natural infection with Bartonella henselae in
5 domestic cats. Comp. Immunol. Microbiol. Infect. Dis. 20, 41-51.

6 Anderson, B.E., Neuman, M.A., 1997. Bartonella spp. as emerging human pathogens. Clin.
7 Microbiol. Rev. 10, 203-219.

8 Bergmans, A.M.C., de Jong, C.M.A., van Amerongen, G., Schot, C.S., Schouls, L.M., 1997.
9 Prevalence of Bartonella species in domestic cats in The Netherlands. J. Clin.
10 Microbiol. 35, 2256-2261.

11 Bermond, D., Boulouis, H.J., Heller, R., Van Laere, G., Monteil, H., Chomel, B.B., Sander,
12 A., Dehio, C., Piemont, Y., 2002. Bartonella bovis Bermond et al. sp. nov. and
13 Bartonella capreoli sp. nov., isolated from European ruminants. Int. J. Syst. Evol.
14 Microbiol. 52, 383-390.

15 Branley, J., Wolfson, C., Waters, P., Gottlieb, T., Bradbury, R., 1996. Prevalence of
16 Bartonella henselae bacteremia, the causative agent of cat scratch disease, in an
17 Australian cat population. Pathology 28, 262-265.

18 Breitschwerdt, E.B., Kordick, D.L., 1995. Bartonellosis. J. Am. Vet. Med. Assoc. 206, 1928-
19 1931.

20 Chang, C.C., Chomel, B.B., Kasten, R.W., Romano, V., Tietze, N., 2001. Molecular evidence
21 of Bartonella spp. in questing adult Ixodes pacificus ticks in California. J. Clin.
22 Microbiol. 39, 1221-1226.

1 Childs, J.E., Rooney, J.A., Cooper, J.L., Olson, J.G., Regnery, R.L., 1994. Epidemiologic
2 observations on infection with Rochalimaea species among cats living in Baltimore,
3 Md. J. Am. Vet. Med. Assoc. 204, 1775-1778.
4 Chomel, B.B., Abbott, R.C., Kasten, R.W., Floyd-Hawkins, K.A., Kass, P.H., Glaser, C.A.,
5 Pedersen, N.C., Koehler, J.E., 1995. Bartonella henselae prevalence in domestic cats
6 in California: risk factors and association between bacteremia and antibody titers. J.
7 Clin. Microbiol. 33, 2445-2450.
8 Chomel, B.B., Kasten, R.W., Floyd-Hawkins, K., Chi, B., Yamamoto, K., Roberts-Wilson, J.,
9 Gurfield, A.N., Abbott, R.C., Pedersen, N.C., Koehler, J.E., 1996. Experimental
10 transmission of Bartonella henselae by the cat flea. J. Clin. Microbiol. 34, 1952-1956.
11 Chomel, B.B., Carlos, E.T., Kasten, R.W., Yamamoto, K., Chang, C.C., Carlos, R.S., Abenes,
12 M.V., Pajares, C.M., 1999. Bartonella henselae and Bartonella clarridgeiae infection
13 in domestic cats from The Philippines. Am. J. Trop. Med. Hyg. 60, 593-597.
14 Chomel, B.B., Boulouis, H.J., Petersen, H., Kasten, R.W., Yamamoto, K., Chang, C.C.,
15 Gandoin, C., Bouillin, C., Hew, C.M., 2002. Prevalence of Bartonella infection in
16 domestic cats in Denmark. Vet. Res. 33, 205-213.
17 De Cock, K.M., Mbori-Ngacha, D., Marum, E., 2002. Shadow on the continent: public health
18 and HIV/AIDS in Africa in the 21st century. Lancet 360, 67-72.
19 Dolan, M.J., Wong, M.T., Regnery, R.L., Jorgensen, J.H., Garcia, M., Peters, J., Drehner, D.,
20 1993. Syndrome of Rochalimaea henselae adenitis suggesting cat scratch disease.
21 Ann. Intern. Med. 118, 331-336.
22 Droz, S., Chi, B., Horn, E., Steigerwalt, A.G., Whitney, A.M., Brenner, D.J., 1999. Bartonella
23 koehlerae sp. nov., isolated from cats. J. Clin. Microbiol. 37, 1117-1122.

1 Frean, J., Arndt, S., Spencer, D., 2002. High rate of Bartonella henselae infection in HIV-
2 positive outpatients in Johannesburg, South Africa. Trans. R. Soc. Trop. Med. Hyg.
3 96, 549-550.

4 Garcia-Caceres, U., Garcia, F.U., 1991. Bartonellosis. An immunodepressive disease and the
5 life of Daniel Alcides Carrion. Am. J. Clin. Pathol. 95, S58-66.

6 Greene, C.E., McDermott, M., Jameson, P.H., Atkins, C.L., Marks, A.M., 1996. Bartonella
7 henselae infection in cats: evaluation during primary infection, treatment, and
8 rechallenge infection. J. Clin. Microbiol. 34, 1682-1685.

9 Guptill, L., Slater, L., Wu, C.C., Lin, T.L., Glickman, L.T., Welch, D.F., HogenEsch, H.,
10 1997. Experimental infection of young specific pathogen-free cats with Bartonella
11 henselae. J. Infect. Dis. 176, 206-216.

12 Guptill, L., Slater, L.N., Wu, C.C., Lin, T.L., Glickman, L.T., Welch, D.F., Tobolski, J.,
13 HogenEsch, H., 1998. Evidence of reproductive failure and lack of perinatal
14 transmission of Bartonella henselae in experimentally infected cats. Vet. Immunol.
15 Immunopathol. 65, 177-189.

16 Gurfield, A.N., Boulouis, H.J., Chomel, B.B., Kasten, R.W., Heller, R., Bouillin, C., Gandoin,
17 C., Thibault, D., Chang, C.C., Barrat, F., Piemont, Y., 2001. Epidemiology of
18 Bartonella infection in domestic cats in France. Vet. Microbiol. 80, 185-198.

19 Haimerl, M., Tenter, A. M., Simon, K., Rommel, M., Hilger, J., Autenrieth, I.B., 1999.
20 Seroprevalence of Bartonella henselae in cats in Germany. J. Med. Microbiol. 48, 849-
21 856.

1 Heller, R., Artois, M., Xemar, V., De Briel, D., Gehin, H., Jaulhac, B., Monteil, H., Piemont,
2 Y., 1997. Prevalence of Bartonella henselae and Bartonella clarridgeiae in stray cats. J.
3 Clin. Microbiol. 35, 1327-1331.

4 Jackson, L.A., Perkins, B.A., Wenger, J.D., 1993. Cat scratch disease in the United States: an
5 analysis of three national databases. Am. J. Public. Health. 83, 1707-1711.

6 Jacomo, V., Kelly, P.J., Raoult, D., 2002. Natural history of Bartonella infections (an
7 exception to Koch's postulate). Clin. Diagn. Lab. Immunol. 9, 8-18.

8 Kelly, P.J., Matthewman, L.A., Hayter, D., Downey, S., Wray, K., Bryson, N.R., Raoult, D.,
9 1996. Bartonella (Rochalimaea) henselae in southern Africa--evidence for infections
10 in domestic cats and implications for veterinarians. J. S. Afr. Vet. Assoc. 67, 182-187.

11 Kelly, P.J., Rooney, J.J.A., Marston, E.L., Jones, D.C., Regnery, R.L., 1998. Bartonella
12 henselae isolated from cats in Zimbabwe. Lancet 351, 1706.

13 Koehler, J.E., Quinn, F.D., Berger, T.G., LeBoit, P.E., Tappero, J.W., 1992. Isolation of
14 Rochalimaea species from cutaneous and osseous lesions of bacillary angiomatosis. N.
15 Engl. J. Med. 327, 1625-1631.

16 Koehler, J.E., Glaser, C.A., Tappero, J.W., 1994. Rochalimaea henselae infection. A new
17 zoonosis with the domestic cat as reservoir. J. Am. Med. Assoc. 271, 531-535.

18 Kordick, D.L., Breitschwerdt, E.B., 1997. Relapsing bacteremia after blood transmission of
19 Bartonella henselae to cats. Am. J. Vet. Res. 58, 492-497.

20 Kordick, D.L., Wilson, K.H., Sexton, D.J., Hadfield, T.L., Berkhoff, H.A., Breitschwerdt,
21 E.B., 1995. Prolonged Bartonella bacteremia in cats associated with cat-scratch
22 disease patients. J. Clin. Microbiol. 33, 3245-3251.

1 Kordick, D.L., Hilyard, E.J., Hadfield, T.L., Wilson, K.H., Steigerwalt, A.G., Brenner, D.J.,
2 Breitschwerdt, E.B., 1997. Bartonella clarridgeiae, a newly recognized zoonotic
3 pathogen causing inoculation papules, fever, and lymphadenopathy (cat scratch
4 disease). J. Clin. Microbiol. 35, 1813-1818.
5 La Scola, B., Raoult, D., 1999. Culture of Bartonella quintana and Bartonella henselae from
6 human samples: a 5-year experience (1993 to 1998). J. Clin. Microbiol. 37, 1899-
7 1905.
8 Leutenegger, C.M., Mislin, C.N., Sigrist, B., Ehrengruber, M.U., Hofmann-Lehmann R., and
9 Lutz, H., 1999. Quantitative real-time PCR for the measurement of feline cytokine
10 mRNA. Vet. Immunol. Immunopathol., 71, 291-305.
11 Margileth, A.M., Baehren, D.F., 1998. Chest-wall abscess due to cat-scratch disease (CSD) in
12 an adult with antibodies to Bartonella clarridgeiae: case report and review of the
13 thoracopulmonary manifestations of CSD. Clin. Infect. Dis. 27, 353-357.
14 Marston, E.L., Finkel, B., Regnery, R.L., Winoto, I.L., Graham, R.R., Wignal, S.,
15 Simanjuntak, G., Olson, J.G., 1999. Prevalence of Bartonella henselae and Bartonella
16 clarridgeiae in an urban Indonesian cat population. Clin. Diagn. Lab. Immunol. 6, 41-
17 44.
18 Maurin, M., Raoult, D., 1996. Bartonella (Rochalimaea) quintana infections. Clin. Microb.
19 Rev. 9, 273-292.
20 Norman, A.F., Regnery, R., Jameson, P., Greene, C., Krause, D.C., 1995. Differentiation of
21 Bartonella-like isolates at the species level by PCR-restriction fragment length
22 polymorphism in the citrate synthase gene. J. Clin. Microbiol. 33, 1797-1803.

1 Nowell, K., Jackson, P. (Eds.), 1996. Wild cats, status survey and conservation action plan.
2 International Union for Conservation of Nature and Natural Resources, Gland,
3 Switzerland, pp. 263-270.
4 O'Reilly, K.L., Bauer, R.W., Freeland, R.L., Foil, L.D., Hughes, K.J., Rohde, K.R., Roy, A.F.,
5 Stout, R.W., Triche, P.C., 1999. Acute clinical disease in cats following infection with
6 a pathogenic strain of Bartonella henselae (LSU16). Infect. Immun. 67, 3066-3072.
7 Pretorius, A.M., Kelly, P.J., Birtles, R.J., Raoult, D., 1999. Isolation of Bartonella henselae
8 from a serologically negative cat in Bloemfontein, South Africa. J. S. Afr. Vet. Assoc.
9 70, 154-155.
10 Regnery, R.L., Anderson, B.E., Clarridge III, J.E., Rodriguez-Barradas, M.C., Jones, D.C.,
11 Carr, J.H., 1992a. Characterization of a novel Rochalimaea species, R. henselae sp.
12 nov., isolated from blood of a febrile, human immunodeficiency virus-positive patient.
13 J. Clin. Microbiol. 30, 265-274.
14 Regnery, R.L., Martin, M., Olson, J., 1992b. Naturally occurring "Rochalimaea henselae"
15 infection in domestic cat. Lancet 340, 557-558.
16 Regnery, R.L., Olson, J.G., Perkins, B.A., Bibb, W., 1992c. Serological response to
17 "Rochalimaea henselae" antigen in suspected cat-scratch disease. Lancet 339, 1443-
18 1445.
19 Regnery, R.L., Marano, N., Jameson, P., Marston, E., Jones, D., Handley, S., Goldsmith, C.,
20 Greene, C., 2000. A fourth Bartonella species, Bartonella weissii, species nova,
21 isolated from domestic cats. In: Proceedings of the 15th Meeting of the American
22 Society for Rickettsiology, April 30-May 3 2000, pp. 15.

1 Rotstein, D.S., Taylor, S.K., Bradley, J., Breitschwerdt, E.B., 2000. Prevalence of Bartonella
2 henselae antibody in Florida panthers. J. Wildl. Dis. 36, 157-160.
3 Sanogo, Y.O., Zeaiter, Z., Caruso, G., Merola, F., Shpynov, S., Brouqui, P., Raoult, D., 2003.
4 Bartonella henselae in Ixodes ricinus ticks (Acari: Ixodida) removed from humans,
5 Belluno province, Italy. Emerg. Infect. Dis. 9, 329-332.
6 Schouls, L.M., Van De Pol, I., Rijpkema, S.G., Schot, C.S., 1999. Detection and identification
7 of Ehrlichia, Borrelia burgdorferi sensu lato, and Bartonella species in Dutch Ixodes
8 ricinus ticks. J. Clin. Microbiol. 37, 2215-2222.
9 Welch, D.F., Pickett, D.A., Slater, L.N., Steigerwalt, A.G., Brenner, D.J., 1992. Rochalimaea
10 henselae sp. nov., a cause of septicemia, bacillary angiomatosis, and parenchymal
11 bacillary peliosis. J. Clin. Microbiol. 30, 275-280.
12 Yamamoto, K., Chomel, B.B., Lowenstine, L.J., Kikuchi, Y., Phillips, L.G., Barr, B.C., Swift,
13 P.K., Jones, K.R., Riley, S.P.D., Kasten, R.W., Foley, J.E., Pedersen, N.C., 1998.
14 Bartonella henselae antibody prevalence in free-ranging and captive wild felids from
15 California. J. Wildl. Dis. 34, 56-63.
16 Yamamoto, K., Chomel, B.B., Kasten, R.W., Hew, C.M., Weber, D.K., Lee, W.I., Droz, S.,
17 Koehler, J.E., 2002. Experimental infection of domestic cats with Bartonella koehlerae
18 and comparison of protein and DNA profiles with those of other Bartonella species
19 infecting felines. J. Clin. Microbiol. 40, 466-474.
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Table 1

Characteristics of Bartonella bacteremic animals out of 58 African free-ranging lions and 17 African free-ranging cheetahs tested for evidence of Bartonella by culturing whole blood samples

Species	ID	Sex	Age	Place of capture	Blood culture (CFU/ml[c])	Strain isolated	Bartonella henselae antibody titer
Lion	98-215	M	Juv[a]	Undetermined (KNP[b])	2000	B. "Lion 98-215"	0
Lion	150.41	F	6 y	Gomondwane (KNP)	93	B. henselae	0
Lion	7069	F	3.5 y	Nkulumbeni (KNP)	35	B. henselae	1:128
Cheetah	1178	M	7 y	Gobagos province (Namibia)	49	B. "Cheetah 1178"	0

[a] Juvenile (≤ 24 months old)

[b] Kruger National Park, South Africa

[c] Colony forming unit / ml of blood

Table 2

Prevalence of Bartonella infection in a convenience sample of 113 African free-ranging lions and 74 African free-ranging cheetahs tested by blood culture, TaqMan PCR and antibody immunofluorescence assay

Species	Number of Bartonella bacteremic animals/ number tested (%)	Number of animals with Bartonella DNA in their blood/ number tested (%)	Number of Bartonella henselae seropositive animals/ number tested (%)
Lion	3/58 (5.2%)	ND[a]	19/113 (16.8%)
Cheetah	1/17 (5.9%)	17/73 (23.3%)	23/74 (31.1%)

[a] Not done