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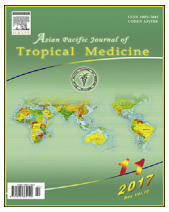

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Bartonella infection in asymptomatic horses and donkeys from Tuscany, Central Italy

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ABSTRACT

Objective: To investigate the occurrence of *Bartonella* sp. infection in asymptomatic horses and donkeys living in Tuscany, Central Italy.

Methods: Blood samples were collected from 77 horses and 15 donkeys and tested by indirect immunofluorescent test to detect antibodies against *Bartonella* sp. and by PCR to detect the pathogen.

Results: Fifty-four (58.69%; 95% *CI*: 47.95%–68.87%) animals, 9 donkeys and 45 horses, were seropositive with antibody titers ranging from 1:64 to 1:512. PCR assays detected 9 horses positive for *Bartonella* sp. and 3 donkeys for *Bartonella henselae* genotype I.

Conclusions: The detected sero-prevalence suggests a common and frequent exposure of equids living in Central Italy to bartonellae and PCR results show that *Bartonella* sp. infection is possible both in horses and donkeys. At the best of our knowledge, this is the first report of *Bartonella henselae* infection in donkeys.

1. Introduction

Bartonellosis is an emerging infectious disease of animals and human beings caused by several bacterial species of the genus *Bartonella*. These pathogens are facultative intracellular, haemotropic Gram-negative bacteria. They are usually transmitted by blood-sucking arthropods [1]. Currently, 22 named and numerous unnamed or *Candidatus* species are included in *Bartonella* genus [2]. Most of them are zoonotic causing human diseases with sub-clinical or severe forms. Several animal species act as reservoir hosts for bartonellae: cats for *Bartonella henselae* (*B. henselae*), *Bartonella clarridgeiae* and *Bartonella koehlerae*, dogs for *Bartonella vinsonii* subsp. *berkhoffii*, cattle for *Bartonella bovis*. However, bartonellae have been detected in numerous domestic and wild animal species [3].

Data about *Bartonella* infections in equids are very scant. The first case of equine bartonellosis was reported by Jones *et al.* [4] that detected *B. henselae* in the blood samples collected from one adult horse with chronic arthropathy and one with vasculitis in North Carolina (USA). Successively, a case of equine abortion was related to *B. henselae* [5]. Asymptomatic and sick adult horses and foals resulted infected by *B. henselae*, *Bartonella vinsonii* subsp. *berkhoffii* and a strain strongly related to *Candidatus Bartonella volans* during a survey carried out in North Carolina and Virginia (USA) [6]. Recently, *B. henselae* was found in the liver with suppurative cholangiohepatitis of a foal in Pennsylvania (USA) [7]. At the best of our knowledge, scientific literature does not report cases of equine bartonellosis in Italy.

For this reason, the aim of the present investigation was to evaluate with serological and molecular methods the occurrence of *Bartonella* infections among asymptomatic horses and donkeys living in Tuscany, Central Italy.

2. Material and methods

2.1. Samples

During the period March–May 2016, whole blood and EDTA-blood samples were collected from the jugular vein of 77 horses and 15 donkeys (*Equus asinus*) living in different horse centers. All the animals were clinically healthy at the sampling time and they were not recently treated with antibiotics.

Sera were obtained by centrifugation of whole blood samples at 1 500 × *g* for 15 min. EDTA-blood samples were centrifuged at 5 000 × *g* for 20 min to facilitate the erythrocytes breakage

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and the obtained cell concentrates were employed in DNA extraction executed with the DNeasy Tissue Kit (Qiagen, GmbH, Hilden, Germany) following the procedures reported in the kit. DNAs were kept at 4 °C until used in PCR assays and sequencing.

2.2. Serological analysis

The indirect immunofluorescence antibody test (IFAT) was performed following the procedures as previously described [8] and using *B. henselae* IFAT slides (MegaScreen *B. henselae*, MegaCor, Austria) and fluorescein isothiocyanate-conjugated sheep anti-Horse IgG (Sigma–Aldrich, St. Louis, Mo, USA) diluted a 1:30 in Evans Blue (Sigma–Aldrich) solution.

2.3. Molecular examinations

All DNA samples were tested by two different PCR protocols. The first assay allowed to identify the presence of *Bartonella* bacteria, amplifying a 296-bp fragment of the 16S rRNA gene of this genus. The protocol was carried out using the primers p24E and p12B, as previously described by Relman *et al* [9].

In order to confirm the positive reactions obtained with the first protocol, a second PCR assay was performed. This protocol employed the primers BhCs781 and BhCs1137 and detected a 380-bp fragment of the citrate synthase gene (*gltA*) specific for *Bartonella* sp., as reported by Maillard *et al* [10].

The positive DNA samples were successively submitted to the PCR protocol described by Bergmans *et al.* [11], that allows to identify *B. henselae* and distinguish genotype I and genotype II.

3. Results

IFAT detected 54 (58.69%; 95% *CI*: 47.95%–68.87%) animals positive to *B. henselae* antigen with antibody titers ranging from 1:64 (cut-off) to 1:512 (Table 1). Specifically, 19 (20.65%; 95% *CI*: 12.92%–30.36%) animals had 1:64 antibody titer, 22 (23.91%; 95% *CI*: 15.63%–33.94%) 1:128, 10 (10.86%; 95% *CI*: 5.34%–19.08%) 1:256 and 3 (3.26%; 95% *CI*: 0.68%–9.23%) 1:512.

The first PCR assay detected 12 (13.04%; 95% *CI*: 6.93%–21.68%) positive reactions specific for *Bartonella* sp. that were confirmed by the second PCR protocol specific for the same genus. All the PCR-positive animals, 9 horses and 3 donkeys, were serologically positive (Table 2). The 3 donkeys resulted infected by *B. henselae* genotype I.

Table 1
Horses and donkeys resulted positive to *B. henselae* antigen with indirect immunofluorescent antibody test.

Animals	No. tested animals	No. positive animals	Antibody titers			
			1:64	1:128	1:256	1:512
Horses	77	45	15	19	8	3
Donkeys	15	9	4	3	2	0
Total	92	54	19	22	10	3

Table 2
Horses and donkeys resulted *Bartonella* sp.-infected by PCR according to age and serological results.

No.	Animals	Gender	Age (years)	Antibody titers	PCR
1	Horse	Male	11	+1:128	*Bartonella* sp.
2	Horse	Female	16	+1:64	*Bartonella* sp.
3	Horse	Female	15	+1:256	*Bartonella* sp.
4	Horse	Female	12	+1:512	*Bartonella* sp.
5	Horse	Male	31	+1:64	*Bartonella* sp.
6	Horse	Female	12	+1:64	*Bartonella* sp.
7	Horse	Female	18	+1:256	*Bartonella* sp.
8	Horse	Female	13	+1:64	*Bartonella* sp.
9	Horse	Female	21	+1:128	*Bartonella* sp.
10	Donkey	Female	7	+1:128	*B. henselae*
11	Donkey	Female	22	+1:128	*B. henselae*
12	Donkey	Male	13	+1:256	*B. henselae*

4. Discussion

The results obtained during this investigation showed that *Bartonella* sp. infection occurs among equid population living in Tuscany.

The detected high sero-prevalence (58.69%; 95% *CI*: 47.95%–68.87%) suggests a common and frequent exposure of the tested animal population to bartonellae. IFAT was executed employing *B. henselae* antigen; the positive reactions could be due to the presence in the animal blood of antibodies against *B. henselae*, but also to other bartonellae because of cross-reactions [12].

Among the seropositive animals, only 12 (13.04%; 95% *CI*: 6.93%–21.68%) resulted PCR positive for *Bartonella* sp. In fact, serological tests are able to detect antibodies against *Bartonella* sp. in animals with current and past infections; otherwise, PCR not always can find infected animals, because bacteremia is not continuous and low level of bacteria are present in the blood [2].

However, the detected positive molecular reactions show that *Bartonella* sp. infection is possible both in horses and donkeys.

All the animals lived in areas where the presence of hematophagous arthropods is common. At the sampling time, no vectors were observed in the animals; however, owners and/or veterinarians reported frequent contact of the animals with ticks, biting flies and fleas.

Bartonella sp. DNA has found in several tick species worldwide. In particular, several studies have demonstrated that hard ticks of the genera *Ixodes*, *Rhipicephalus*, *Dermacentor*, and *Haemaphysalis* can harbor bartonellae, even though natural vector capacity has been not showed for *Bartonella* transmission by ticks [1].

Transmission by fleas is typical for bartonellosis. Cat fleas *Ctenocephalides felis* are the main vectors of *B. henselae* [1]; moreover, *Bartonella* sp. DNA has been found in *Xenopsilla* sp., *Ctenophthalmus* sp. fleas [13], *Lipoptena* sp., *Hippobosca* sp., and *Melophagus* sp. biting flies [14]. Bartonellae can also be transmitted mechanically through scratches, bites and wounds contaminated by arthropod feces, as demonstrated for cat scratch disease [2].

Data present in literature on equine bartonellosis report *B. henselae* as etiologic agent of a case of cholangiohepatitis in a foal [7] and of an equine abortion [5]; moreover, asymptomatic *B. henselae* infections in some adult and young horses were observed [6].

B. henselae has been related to severe hemolytic anemia in a 2-year-old mare in Germany [15], and chronic arthropathy and vasculitis in two adult horses in USA [4].

The sensitivity of equids to *B. henselae* has been studied by Palmero *et al.* [16] with an experimental infection: three horses of the four intradermally inoculated with *B. henselae* developed

regional lymphadenopathy and limb edemas, seroconverted and became bacteremic.

Our results confirm that this pathogen is able to infect horses and show that this infection is possible in donkeys too. *B. henselae* genotype I has been found in this survey; in a previous study, this genotype resulted more widespread than genotype II among cat population of Central Italy [8]. More epidemiological data about the spreading of bartonellae in this geographic area are not available; however, the present results seem to confirm the higher occurrence of genotype I that is considered more pathogenic for humans than type II [17].

B. henselae is the etiologic agent of cat scratch disease, hepatic peliosis and bacillary angiomatosis in humans, mainly immunocompromised patients, other than atypical manifestations including Parinaud's oculoglandular syndrome, encephalitis, osteomyelitis, pneumonia, glomerulonephritis, hemolytic anemia, and endocarditis [18]. Infected cats, considered reservoirs of this pathogen, usually are asymptomatic, but sometimes can develop fever, lymphadenopathy, gingivitis and endocarditis. Hepatic disorders and pyogranulomatous lymphadenitis have been observed in infected dogs [19,20].

Considering that all the animals tested in this investigation were asymptomatic at the sampling time, it could be supposed that bartonellae may infect horses and donkeys without inducing specific pathologies. Some authors suggest that bartonellae can contribute to aggravate pathological situation induced by other causes mainly in immunocompromised animals [6].

The significant prevalence of *Bartonella* sp. infection detected in this survey, suggests that equine bartonellosis could be underestimated. Even though validated methods for testing equine blood are not available, horses and donkeys with vasculitis, arthropathy, hepatitis, lymphadenopathy and reproductive disorders should be examined for bartonellosis with both serological and molecular tests.

At the best of our knowledge, this is the first report of *Bartonella* infections in equids in Italy and the first one in donkeys worldwide. Horses and donkeys are frequently exposed to hematophagous arthropods and consequently to several vector-borne pathogens, including *Bartonella* sp. bacteria. For this reason, diagnosis of bartonellosis should be considered for equids with clinical signs consistent with bartonellosis in other animal species. Humans which share the same environments with *Bartonella*-infected equids are exposed to the same risk to contract bartonellae through ticks, biting flies and fleas.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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