

Clinical Bovine Piroplasmosis Caused by *Babesia occultans* in Italy

Nicola Decaro,^a Vittorio Larocca,^a Antonio Parisi,^b Michele Losurdo,^{a,b} Riccardo Paolo Lia,^a Maria Fiorella Greco,^a Antonio Miccolis,^c Gianpiero Ventrella,^a Domenico Otranto,^a Canio Buonavoglia^a

Department of Veterinary Medicine, University of Bari, Valenzano, Italy^a; Istituto Zooprofilattico Sperimentale di Puglia e Basilicata, Foggia, Italy^b; Veterinary Practice, Bari, Italy^c

A clinical outbreak of bovine piroplasmosis was reported in Italy. The etiological agent was characterized as *Babesia occultans*, a parasite regarded as apathogenic and never detected before in continental Europe. This report paves the way for further studies to assess the occurrence of this tick-transmitted protozoan in other European regions.

Bovine babesiosis is a tick-borne disease of cattle caused by apicomplexan protozoa of the genus *Babesia* which may induce clinical conditions characterized by hemolytic anemia and fever, with occasional hemoglobinuria and even death of animals. *Babesia* parasites are responsible for severe economic losses in cattle industry, with large social and epidemiological impacts (1). Among the main *Babesia* species infecting cattle, *Babesia bovis* and *Babesia bigemina* cause a severe and often fatal disease in untreated cattle of tropical and subtropical regions whereas *Babesia divergens* is associated with bovine and human infection in Europe (2). In addition, *Babesia major* (3) and *Babesia occultans* are less pathogenic. The latter species was detected more than 30 years ago in southern Africa, being transmitted by a *Hyalomma marginatum* tick (4). *Babesia occultans* has never been associated with clinical signs in animals, even after experimental infection of splenectomized individuals (4). For a long time, the distribution of *B. occultans* was believed to be confined to sub-Saharan Africa (4, 5), and this protozoan was detected only recently in *H. marginatum* in Tunisia (3) and in cattle in the Balearic Islands (6). However, even in this case, clinical disease was not associated with *B. occultans* infection. This study reports on a clinical outbreak of bovine piroplasmosis by *B. occultans* in continental Europe.

In May and June 2012, an outbreak of clinical piroplasmosis occurred in a cattle herd from the Apulia region of southern Italy following a severe tick infestation. At that time, the herd consisted of 48 brown Swiss cows kept in loose housing and fed with forages and purchased feeding stuffs. In early May of each year, all cows were subjected to deltamethrin treatments against ticks and facilities were treated with azamethiphos against flies. However, despite the antiparasitic treatments, tick infestation was repeatedly reported in the farm. Clinical signs were observed in 26 lactating cows (mainly primiparous animals) and consisted of marked pallor of the oral and genital mucosae, fever (up to 40.8°C), and a drop in milk production. Neither gastroenteric nor respiratory signs were reported. Two days after the onset of fever, the animals received oxytetracycline hydrochloride orally (p.o.) at a dose of 11 mg/kg of body weight every 12 h (q12h) for 7 days and two administrations, 36 h apart, of imidocarb dipropionate intramuscularly (i.m.) at a dose of 1.7 mg/kg. Deltamethrin treatments were also repeated in all animals at the farms. The therapy resulted in complete recovery of animals within about 10 days, although further clinical cases were sporadically reported in July and a single case occurred in August.

EDTA-blood samples collected from 8 febrile animals were used for hematological investigations, revealing mean hematolog-

ical parameters generally below the reference ranges (red blood cells [RBC], $3.75 \times 10^{12} \pm 1.24 \times 10^{12}$ liter⁻¹; lower reference limit, 5.0×10^{12} liter⁻¹; packed cell volume (PCV), 19.95% \pm 4.78%; lower reference limit, 24%; hemoglobin (Hb), 66.1 ± 1.61 g liter⁻¹; lower reference limit, 80 g liter⁻¹). Blood samples were also submitted to a PCR assay for the detection of bovine piroplasms using the generic primers RLB-F2 (5'-GACACAGGGAG GTAGTGACAAG-3') (7) and 18STBR (5'-GATCCTTCYGCAG GTTCACC-3') as described elsewhere (8). Reactions were performed using the LA PCR kit version 2.1 (TaKaRa Bio Inc., Shiga, Japan) in 25- μ l volumes containing 1 μ mol liter⁻¹ of primers, 1 \times LA PCR buffer (Mg²⁺ plus), 4 μ l of a deoxynucleoside triphosphate (dNTP) mixture (corresponding to 400 μ mol liter⁻¹ of each dNTP), 1.25 units of TaKaRa LA *Taq*, and 5 μ l of template DNA. The cycling protocol consisted of preheating at 94°C for 3 min followed by 40 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 10 min. To rule out other pathogens responsible for anemia and/or fever in cattle, molecular analyses were carried out to detect *Anaplasma marginale* (9), *Anaplasma centrale* (10), bovine pestiviruses (11), bovine coronavirus (12), Schmallenberg virus (13), and bluetongue virus (14). Other eubacteria were searched for through amplification of 16S ribosomal DNA (15).

All samples tested positive for *Babesia* spp./*Theileria* spp. 18S rRNA genes without any evidence of coinfection by other pathogens of cattle. PCR products were purified using Montage PCR filter units (Millipore, Milan, Italy) and sequenced by BigDye 3.1 Ready reaction mix (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequences were imported and assembled with the BioNumerics 5.0 software (Applied Maths, Saint-Martens-Latem, Belgium) and analyzed using the BioEdit software package (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>) and NCBI's (<http://www.ncbi.nlm.nih.gov>) and EMBL's (<http://www.ebi.ac.uk>) analysis tools. The 18S rRNA gene sequences obtained from the diseased cows were 100% identical to each other and matched with the best nucleotide identity (99.7 to

Received 15 March 2013 Returned for modification 22 April 2013

Accepted 1 May 2013

Published ahead of print 8 May 2013

Address correspondence to Nicola Decaro, nicola.decaro@uniba.it.

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doi:10.1128/JCM.00713-13

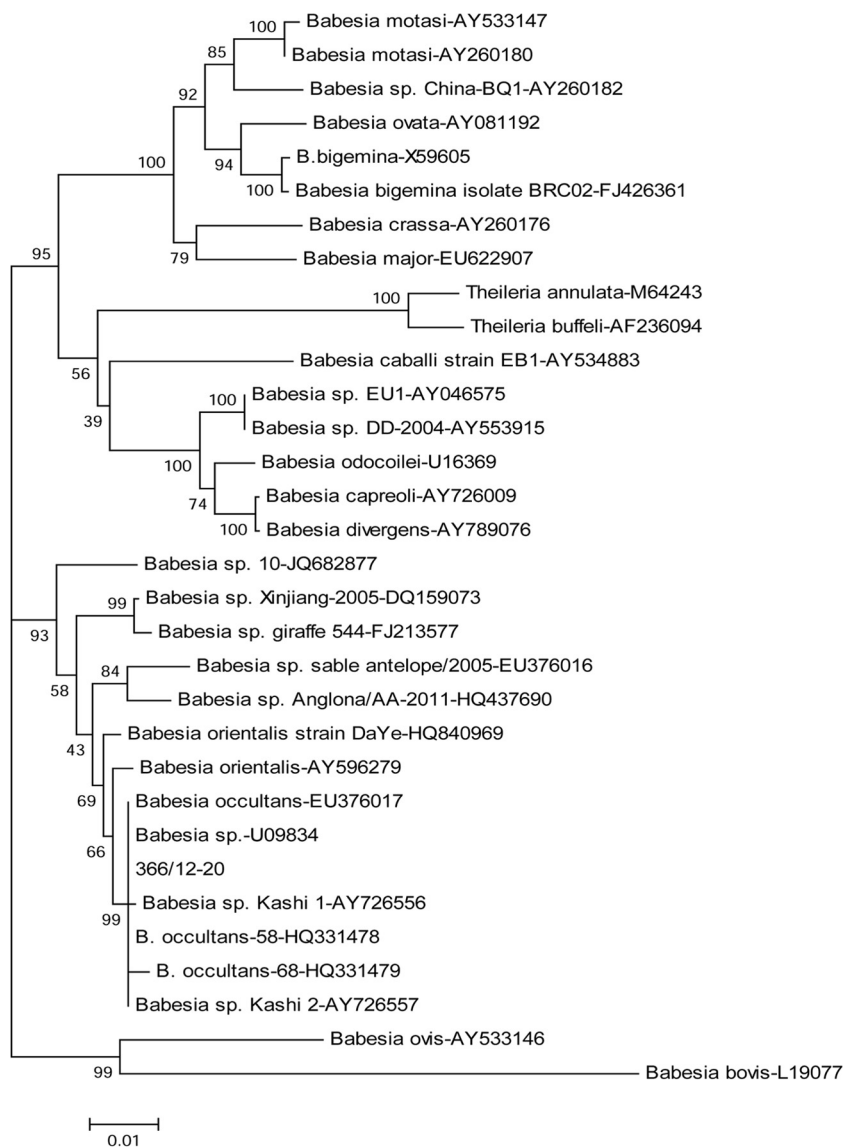


FIG 1 Neighbor-joining tree based on partial 18S rRNA gene sequences of ruminant piroplasms. Statistical support was provided by bootstrapping more than 1,000 replicates. The scale bar indicates the estimated numbers of nucleotide substitutions per position.

100%) to analogous sequences of *B. occultans* retrieved from the GenBank database but not those of other protozoa within the genus *Babesia*. Phylogenetic analysis was carried out on a 1,050-nucleotide sequence generated from sample 366/12-20 (GenBank accession number [KC157568](http://www.ncbi.nlm.nih.gov/nuccore/KC157568)), which was considered the prototype strain (Italy-366/12-20). The neighbor-joining tree obtained with the Mega4.1 software (<http://www.megasoftware.net/mega4/mega41.html>) over 1,000 replicates showed that strain Italy-366/12-20 clustered with the *B. occultans* clade, which also comprises *Babesia orientalis*, *Babesia* sp. Kashi 1, and *Babesia* sp. Kashi 2, but not with other piroplasms (Fig. 1). *Babesia* sp. Sable antelope and *Babesia* sp. Anglona, which was recently isolated from pigs in Italy (16), were also strictly related to the *B. occultans* cluster. This pattern of segregation was confirmed by the maximum-parsimony method (data not shown).

In this article, we report a clinical outbreak of bovine piroplasmosis caused by *B. occultans*. Although there was no evidence of

coinfections with other pathogens, less-common infectious agents of bovine anemia were not able to be ruled out definitively. *Babesia occultans* is considered a completely apathogenic or low-pathogenic parasite of cattle with a geographical distribution restricted to sub-Saharan Africa (4, 5). Although this piroplasm has been recently detected in the Balearic Islands (3, 6), no reports from continental Europe were available. Nonetheless, *H. marginatum* is diffused in southern Italy, where the infection by *B. occultans* might be more spread out than currently acknowledged (17). *Babesia occultans* was molecularly detected in cows that displayed fever, anemia, and severe alteration in the hematological parameters. Circumstantial evidence for the condition caused by this protozoan is also represented by the efficacy of imidocarb dipropionate in the animals' recovery. Phylogeny showed that other *Babesia* strains that have not been classified so far may likely belong to the species *B. occultans*. Importantly, the detection of this protozoan in continental Europe suggests that thorough surveil-

lance programs should be undertaken for this tick-borne disease in order to implement effective control measures in cattle populations in which the proper tick species and the vectored pathogen occur.

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