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Introduction of Non-Autochthonous Cattle into a Farm in the Madonie Park (Sicily, Italy) and the Impact of Endemic Tick-Borne Diseases: A Serological, Molecular, and Entomological Study

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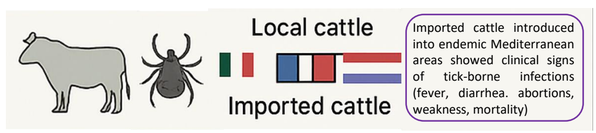
INTRODUCTION & AIM

Tick-borne diseases (TBDs) are a significant challenge for cattle farming in Mediterranean regions, where pathogens such as *Anaplasma marginale*, *Babesia* spp., and *Theileria annulata* are endemic. The introduction of non-native cattle into such environments can lead to severe clinical outcomes, particularly when animals lack prior exposure or immunity to local pathogens.

This study was conducted on a cattle farm located in the Madonie Natural Park (Sicily, Italy) (Figure 1), where animals imported from Austria, France, and the Netherlands showed clinical signs suggestive of tick-borne infections, including fever, diarrhea, weakness, and previous episodes of abortion and mortality.

Figure 1. Map of Sicily (Italy) showing the sampling area.



METHODS

A total of 26 out of 35 cattle on the farm as well as ticks from the animals when present were sampled. Blood samples were analyzed serologically for antibodies against *Anaplasma marginale* (VMRD), *Babesia bigemina (*Abbexa*)*, and Crimean-Congo Hemorrhagic Fever Virus (CCHFV) (IDVet) using ELISA. Antibodies against *Babesia bovis* (Fuller Laboratoires) and *Theileria annulata* (home-made slides) were detected using indirect immunofluorescence assay (IFA).

Genomic DNA was extracted individually from each sample (blood and ticks) using the DNeasy Blood and Tissue Kit (Qiagen, Germany).
PCR analysis was carried out on whole blood samples and on the spleen tissue from a deceased animal, targeting the *MSP4* gene for *A. marginale* [1], the restriction fragment *Spe I – Ava I* for *B. bigemina* [2], a fragment of the *rap-1* gene for *B. bovis* [2] and a fragment of the *30 kDa protein* coding gene for *T. annulata* [3].

Ticks collected from animals were morphologically identified and confirmed through mitochondrial cytochrome c oxidase subunit I (*COI*) gene (~710 bp) by polymerase chain reaction (PCR) using universal invertebrate primers [4]. Tick samples were also screened for *Rickettsia* spp. via PCR targeting the *ompA* [5]*, ompB* [6], and *gltA* genes [7], and amplicons were sequenced and compared to GenBank references.
All the used primers are reported in Table 1.

Pathogen	Primers	References
Anaplasma marginale	AmargMSP4Fw 5'-CTGAAGGGGGAGTAATGGG-3' AmargMSP4Rev 5'-GGTAATAGCTGCCAGAGATTCC-3'	[1]
Babesia bigemina	BilA 5'-CATCTAATTTCTCTCCATACCCCTCC-3' BilB 5'-CCT CGG CTT CAA CTC TGA TGC CAA AG-3'	[2]
Babesia bovis	BOF 5'-CACGAGGAAGGAACTACCGATGTTGA-3' BOR 5'-CCAAGGAGCTTCAACGTACGAGGTCA-3'	[2]
Theileria annulata	N516 5'-GTAACCTTTAAAAACGT-3' N517 5'-GTTACGAACATGGGTTT-3'	[3]
Mitochondrial cytochrome c oxidase subunit I (COI) of metazoan	LCO 1490 5'-GGTCAACAAATCATAAAGATATTGG-3' HCO 2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	[4]
Rickettsia spp. *ompA*	Rr190.70p 5'-ATGGCGAATATTTCTCCAAAA-3' Rr190.701n 5'-GTTCCGTTAATGGCAGCATCT-3' Rr190.602n 5'-AGTGCAGCATTCGCTCCCCCT-3'	[5]
Rickettsia spp. *ompB*	rompB OF 5'-GTAACCGGAAGTAATCGTTTCGTAA-3' rompB OR 5'-GCTTTATAACCAGCTAAACCACC-3' rompB SFG IF 5'-GTTTAATACGTGCTGCTAACCAA-3' rompB SFG/TG 5'-IR GGTTTGGCCCATATACCATAAG-3'	[6]
Rickettsia spp. *gltA*	877P 5'-GG GGC CTG CTC ACG GCG G-3' 1258N 5'-ATT GCA AAA AGT ACA GTG AAC A-3'	[7]

Table 1. The table summarizes the target genes, primer sequences, and corresponding literature references.

RESULTS & DISCUSSION

Serological analysis revealed a high level of exposure to tick-borne pathogens among the examined cattle. Specifically, 21 out of 26 animals tested positive for *Anaplasma marginale*, while 20 animals showed antibodies against *Babesia bovis*. Antibodies for *Babesia bigemina* were detected in 3 animals and 14 animals were seropositive for *Theileria annulata*. None of the animals tested positive for antibodies against CCHFV (Figure 2).

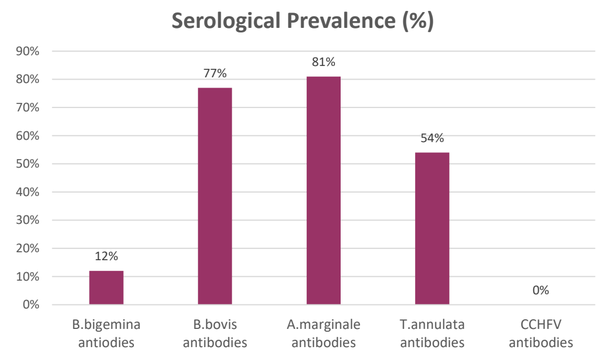


Figure 2. Serological prevalence of tick-borne pathogens in cattle.

Molecular analyses

Sample Analyzed / Pathogen	No. of Samples	Positive (n)	Positive (%)
Whole blood / Anaplasma marginale	26	9	35%
Whole blood / Babesia bigemina	26	1	3.8%
Whole blood / Theileria annulata	26	1	3.8%
Whole blood / Babesia bovis	26	0	0%
Spleen / A. marginale + T. annulata (deceased animal)	1	1	100%
Ticks identified / Hyalomma lusitanicum	–	Present	100% (of collected ticks)
Ticks PCR / Rickettsia aeschlimannii	–	Present	Detected in some tick samples

Table 2. Results of molecular analyses on blood, spleen, and tick samples from cattle.

Molecular testing using PCR confirmed active infections in several animals. *Anaplasma marginale* DNA was detected in 9 out of 26 blood samples, confirming ongoing infection in a subset of animals that were also seropositive. *Theileria annulata* and *Babesia bigemina* were each detected in 1 animal, while *Babesia bovis* was not detected in any sample despite the high seroprevalence, suggesting past exposure rather than active infection. Additionally, spleen tissue from one deceased animal tested positive for both *A. marginale* and *T. annulata*, supporting the clinical suspicion of a tick-borne etiology contributing to mortality.
Entomological investigations identified the ticks collected from the animals as belonging to the species *Hyalomma lusitanicum*, a well-known vector in Mediterranean regions. Molecular analysis of tick DNA revealed the presence of *Rickettsia aeschlimannii*, a zoonotic pathogen of increasing public health interest (Table 2). These findings further underscore the potential for co-infections and the broader epidemiological significance of tick populations in this area.

CONCLUSION

The results confirm the endemic circulation of tick-borne pathogens in the Madonie Park area. High seroprevalence of *A. marginale* and *T. annulata* suggests widespread exposure among the herd, while the presence of active infections detected by PCR highlights ongoing transmission. None of the animals tested positive for antibodies against CCHFV, indicating no evidence of viral circulation in the herd at the time of sampling.
Clinical signs and mortality were observed only in the non-native cattle, whereas local animals, likely immunocompetent due to prior exposure, showed no significant clinical disease. This underlines the importance of assessing local infectious risks before introducing animals into endemic areas.
The detection of *Hy. lusitanicum* infected with *R. aeschlimannii*, a zoonotic agent, emphasizes potential public health implications. The presence of a competent vector, combined with climate change and increased animal movement, could favor future emergence of novel or re-emerging diseases.
This study highlights the value of an integrated approach, combining serology, molecular diagnostics, and entomological surveillance, for evaluating and managing the risks associated with tick-borne diseases in both animal and human populations.

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