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Molecular and Serological Investigation of Tick-Borne Pathogens in Equine Populations in Sicily, Italy

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INTRODUCTION & AIM

Tick-borne diseases (TBDs) are an emerging concern in equine medicine worldwide. They can cause clinical signs ranging from mild fever to severe chronic conditions, affecting animal welfare, athletic performance, and productivity. TBDs are transmitted by various tick species and involve multiple pathogens, including bacteria (*Anaplasma* spp., *Rickettsia* spp.), protozoa (*Babesia caballi, Theileria equi*), and viruses (e.g., Tick-Borne Encephalitis Virus, TBEV). Sicily (Italy), characterized by a Mediterranean climate, diverse habitats, and abundant tick fauna, provides ideal conditions for the persistence and transmission of these pathogens.

Nevertheless, epidemiological data on TBDs in local equine populations remain limited.

This study aimed to assess the circulation of major tick-borne pathogens in horses and donkeys from Sicily using a combined molecular and serological approach, to enhance understanding of the regional epidemiological scenario and support evidence-based surveillance and control strategies.

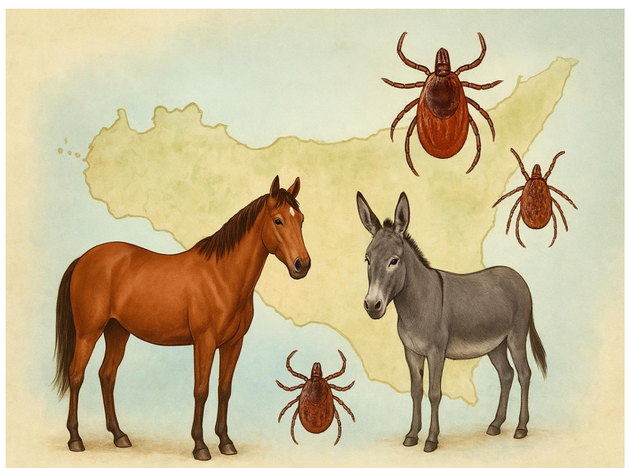

Figure 1. Map of Sicily (Italy) showing the sampling area.

METHODS

Between 2024 and 2025, blood, serum, and tick samples were collected from horses and donkeys located in different areas of Sicily (Figure 1). Ticks found attached to animals were carefully removed using sterile forceps and stored individually in 70% ethanol until further processing. Morphological identification of tick species was performed under a stereomicroscope following standard taxonomic keys, based on adult and nymphal morphological features [1].

Genomic DNA was extracted from whole blood and individual tick samples using the DNeasy Blood and Tissue Kit (Qiagen, Germany) according to the manufacturer's instructions. Before extraction, ticks were mechanically homogenized in tissue lysis buffer and digested overnight with proteinase K at 56 °C to ensure complete lysis. DNA was then purified, eluted and stored at −20 °C until further use.

Molecular screening was performed by polymerase chain reaction (PCR) assays targeting specific genes of the main tick-borne pathogens, including *Rickettsia* spp. (*ompA, ompB, gltA*) [2, 3, 4], *Anaplasma* spp. (*16S rRNA*) [5], *Borrelia burgdorferi* (*OspA*) [6], *Babesia caballi* (*48 kDa rhoptry protein*) [7], and *Theileria equi* (*EMA-1*) [7]. Reactions were carried out in a final volume of 50 µL containing GoTaq G2 DNA Polymerase (Promega Italia s.r.l., Milan, Italy) and 5 µL of DNA template, using a SimpliAmp Thermal Cycler (Thermo Fisher Scientific, USA). Positive and negative controls were included in each run to ensure analytical reliability. Amplified products were separated by electrophoresis on 2% agarose gels stained with SYBR Safe DNA gel stain and visualized under ultraviolet light. Amplicons of the expected size were purified and sequenced bidirectionally by Macrogen Europe (Amsterdam, The Netherlands). Sequence chromatograms were checked and edited manually using Chromas software (Technelysium Pty Ltd., Australia), and consensus sequences were generated in FASTA format. The obtained sequences were compared with reference data available in GenBank using the BLAST algorithm (version 2.13.0; NCBI, USA) to confirm pathogen identity.

Serum samples were analyzed to assess previous exposure to selected tick-borne pathogens. Antibodies against *Anaplasma phagocytophilum* and *Babesia caballi* were detected using indirect immunofluorescence assay (IFA) commercial kits (Fuller Laboratories, USA), following the manufacturer's instructions. In addition, antibodies against TBEV were investigated using an enzyme-linked immunosorbent assay (ELISA; Gold Standard Diagnostics, USA). Since this assay is not fully specific for TBEV and may cross-react with other flaviviruses, positive results were interpreted as indicative of exposure to flavivirus antigens rather than confirmation of TBEV infection.

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RESULTS & DISCUSSION

A total of 163 blood samples, 196 serum samples, and 29 ticks were collected and analyzed from equine hosts across different areas of Sicily. The identified tick species included *Ixodes ricinus* (n = 20), *Haemaphysalis punctata* (n = 6), and three unidentified nymphs.

Molecular analyses detected *Theileria equi* DNA in 16% of the blood samples, confirming its active circulation among Sicilian equine populations. No amplification was obtained for *Rickettsia* spp., *Anaplasma* spp., *Babesia caballi*, or *Borrelia burgdorferi*. Among the collected ticks, one *Haemaphysalis punctata* specimen tested positive for *Anaplasma* spp. DNA.

Serological analyses revealed antibodies against *Anaplasma phagocytophilum* in 12.2% of samples, against *Babesia caballi* in 3.6%, and against flaviviruses in 6,1% (Table 1). The detection of antibodies to multiple agents suggests that equines in Sicily are frequently exposed to diverse tick-borne pathogens, although active infections appear relatively uncommon. The discrepancy between molecular and serological results may reflect transient parasitemia, low pathogen loads below the detection threshold, or past exposure followed by immune clearance.

Overall, these findings confirm that *Theileria equi* remains the predominant tick-borne pathogen affecting equines in Sicily, in agreement with reports from other Mediterranean regions. The detection of *Anaplasma* spp. DNA in *Haemaphysalis punctata* highlights the presence of this bacterium within local tick populations and suggests a possible epidemiological link with equine hosts. However, further studies are needed to clarify its clinical relevance and transmission dynamics in this region.

Pathogen	Test	Sample	Number of samples	Positive	Prevalence (%)
Theileria equi	PCR	EDTA blood	163	26	16
Rickettsia spp.	Nested PCR	EDTA blood	163	0	0
Anaplasma spp.	Nested PCR	EDTA blood	163	0	0
Babesia caballi	PCR	EDTA blood	163	0	0
Borrelia burgdorferi	Real time PCR	EDTA blood	163	0	0
Anaplasma spp	Nested PCR	Tick	29	1	3.4
Rickettsia spp.	Nested PCR	Tick	29	0	0
Anaplasma phagocytophilum	IFA	Serum	196	24	12.2
Babesia caballi	IFA	Serum	196	7	3.6
Flavivirus (TBEV or related)	ELISA	Serum	196	12	6,1

Table 1. Serological and molecular detection of tick-borne pathogens in equine population from Sicily.

CONCLUSION

This study provides both molecular and serological evidence of the circulation of tick-borne pathogens among equine populations in Sicily. The detection of *Theileria equi* DNA, together with serological evidence of exposure to *Anaplasma phagocytophilum*, *Babesia caballi*, and flaviviruses, highlights the complex and multifactorial epidemiology of tick-borne diseases in this Mediterranean region.

These findings emphasize the need for continuous surveillance and integrated vector control strategies to reduce infection risks and protect equine health and productivity. Future research should aim to clarify the clinical impact of these pathogens in horses and donkeys and to further investigate the ecological dynamics of their tick vectors under Mediterranean environmental conditions.

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