

# Tick-Borne Zoonoses in Northern Portugal: Molecular Screening of Ixodid Ticks from Wild Mammals

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**Objectives:** Ixodid ticks are globally distributed hematophagous arthropods, known vectors of several pathogens that threaten both animal and human health. Wild mammals may act as reservoirs for tick-borne pathogens such as *Ehrlichia* spp., *Anaplasma* spp., and *Borrelia* spp., contributing to their persistence in natural ecosystems. This study aimed to characterize ixodid tick infestation in wild mammals admitted to a Wildlife Rehabilitation Centre in northern mainland Portugal and to screen collected ticks for *Borrelia*, *Anaplasma*, and *Ehrlichia* species.

**Materials and Methods:** Ticks were collected from wild mammals admitted to the Centre and identified morphologically to the species level using stereomicroscopy and taxonomic keys. A representative subset of ticks was screened by PCR for *Anaplasma/Ehrlichia* spp. and *Borrelia* spp., targeting the 16S rRNA and *flaB* genes, respectively. Positive amplicons were sequenced and analyzed using BLAST and phylogenetic methods for species identification.

**Results:** A total of 374 ticks were collected from 51 hosts of six different species of wild mammals. The tick species identified included 51 *Ixodes ricinus* (62% adults), 23 *Ixodes hexagonus* (52% adults), 280 *Rhipicephalus sanguineus* (85% adults), 1 adult *Rhipicephalus pusillus*, 11 *Rhipicephalus* spp. (73% larvae) and 7 nymphs and 1 larva of unidentified species. A total of 24 ticks from the species *I. ricinus* (n=4), *R. sanguineus* (n=19) and *Rhipicephalus* spp. (n=1) yielded PCR amplicons consistent with *Borrelia* spp., while 7 ticks from *R. sanguineus* (n=6) and *I. ricinus* (n=1) generated amplicons compatible with *Anaplasma/Ehrlichia* spp. For one *Borrelia* spp. sample, the phylogenetic tree revealed that the sequence clustered in a monophyletic group supported by 100% bootstrap, along with reference sequences of *Borrelia garinii*. The *B. garinii* DNA was detected in a *R. sanguineus* tick collected from a *Genetta genetta*.

**Conclusions:** These findings confirm the presence of *Borrelia*, *Anaplasma*, and *Ehrlichia* spp. in ticks from wild mammals in northern Portugal. The detection of the zoonotic *B. garinii* highlights the potential role of wild mammals as reservoirs, emphasizing the importance of integrated tick-borne pathogen surveillance within a One Health framework.

**Keywords:** *Anaplasma*, *Borrelia garinii*, *Ehrlichia*, ixodid ticks, wild mammals, Polymerase Chain Reaction, molecular epidemiology

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