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Genomic search of *Rickettsia* species in the blood of dogs and cats in Tabriz region

Abstract:

Members of the genus *Rickettsia* are Gram-negative, obligate intracellular parasites transmitted by arthropods to a wide range of vertebrates, including humans. As emerging and re-emerging pathogens, these bacteria pose a serious threat to public health. Companion animals, particularly dogs and cats, which are in close contact with humans and arthropod vectors, can act as potential reservoirs in the ecological cycle of these infectious agents, increasing the risk of zoonotic disease transmission. The present study aimed to investigate the molecular prevalence of bacteria from the Rickettsiaceae family and to specifically identify the *Rickettsia* genus in blood samples from domestic dogs and cats in the Tabriz region of Iran.

In this descriptive cross-sectional study, a total of 180 blood samples (105 from dogs and 75 from cats) were collected from veterinary clinics in the city of Tabriz. Following genomic DNA extraction and confirmation of its quality and quantity, a two-step diagnostic approach based on quantitative Real-time Polymerase Chain Reaction (Real-time PCR) was employed. In the first step, all samples were screened for members of the Rickettsiaceae family using primers targeting the 16S rRNA gene. Subsequently, positive samples from the screening stage were re-tested for confirmation and specific identification of the *Rickettsia* genus using primers targeting the citrate synthase gene (*gltA*).

The initial screening results using the 16S rRNA gene showed that 3 out of the 180 samples (two dogs and one cat) were positive, indicating an overall prevalence of 1.67% for the Rickettsiaceae family in the studied population. In the confirmatory stage with the *gltA* gene, the presence of *Rickettsia* genus DNA was confirmed in two of the three initial positive samples (one dog and one cat). Therefore, the specific and confirmed prevalence for the *Rickettsia* genus in the entire population was estimated to be 1.11% (0.95% in dogs and 1.33% in cats). Notably, one canine sample that was positive in the initial screening tested negative in the confirmatory assay, which may indicate the presence of another bacterium from the Rickettsiaceae family (but not of the *Rickettsia* genus).

This study provides the first definitive molecular evidence of the presence of bacterial agents of the *Rickettsia* genus in the dog and cat population of Tabriz city. Although the detected prevalence is low, this finding confirms that these animals can act as silent reservoirs for Rickettsial agents and may play a role in their transmission cycle within an urban area. The use of a dual-target diagnostic method is of great importance for the accurate differentiation of the *Rickettsia* genus from other members of its family and is recommended for future epidemiological studies.

Keywords: *Rickettsia*, Blood, Dog, Cat, Tabriz