

# First epidemiological and molecular report of ticks and tick-borne pathogens in small ruminants across North and North-western Pakistan

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## Abstract

Mapping the distribution of ticks and tick-borne pathogens linked to their molecular information from previously unexplored geographic areas provide valuable information about the cryptic tick fauna and their associated pathogens of zoonotic and veterinary importance. The present research was designed to investigate the epidemiologic and phylogenetic aspects of ticks and tick-borne pathogens infesting goats and sheep in the north and north-western territories of Pakistan. A total of 1587 ixodid ticks were collected from goat and sheep herds (n = 600). The collected ticks were taxonomically classified using morphological keys and the molecular markers 16S rRNA and *COI*. The presence of tick-borne pathogens in collected ticks was assessed by PCR targeting the genetic markers 16S rRNA and *gltA* for bacterial pathogens, and 18S rRNA for Apicomplexan parasites. The morpho-molecular characterization of hard ticks revealed three ixodid tick genera ( *Hyalomma*, *Haemaphysalis*, and *Rhipicephalus* ) which included 11 tick species viz. *Hy. anatolicum* (5.9%) , *Hy. dromedarii* (9.1%), *Hy. excavatum* (2.8%), *Hy. isacii* (5.1%) , *Hy. scupense* (7.7%), *Hae. montgomeryi* (18.3%) , *Hae. sulcata* (6.5%), *Rh. decoloratus* (4.4%) , *Rh. haemaphysaloides* (13.2%) , *Rh. microplus* (10.8%) , and *Rh. turanicus* (16.2%). Amplified tick 16S rRNA and *COI* nucleotide sequences shared 99.74-100% similarity and clustered phylogenetically with similar tick species reported previously in China, India, Iran, Saudi Arabia, Sri Lanka, South Africa, Turkey, and Pakistan. The 16S rRNA , 18S rRNA and *gltA* nucleotide sequences of detected tick-borne pathogens, including *R. massiliae* (24.5%) followed by *T. ovis* (16.4%), *A. capra* (9.1%), *T. luwenshuni* (6.4%), *A. ovis* (5.9%), *R. hoogstraalii* (5.0%), *Anaplasma* spp. (4.5%), *Ehrlichia* spp. (4.5%), *Rickettsia* spp. (4.5%), *T. annulata* (4.1%) and *A. marginale* (3.6%), shared 99.48-100% similarity and grouped with sequences previously reported in South Asia, middle east Africa and USA. This study showed for the first time the presence of two tick ( *Hae. montgomeryi* and *R. decoloratus* ) and one tick-borne pathogen species ( *R. hoogstraalii* ) in Pakistan. Our results are the first available reports documenting important ticks and tick-borne pathogens of veterinary and public health concern from previously neglected parts of Pakistan. These results could be used as a baseline to facilitate future large-scale epidemiological and molecular studies about ticks and tick-borne pathogens of small ruminants at the local and global levels.

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