ABSTRACT

Ticks are chelicerate arthropod ectoparasites of vertebrate hosts, parasitizing vertebrates including amphibians, reptiles, birds, and mammals. These creatures are haematophagous in nature and also act as vector sources to transfer a wide range of pathogens including protozoans, bacteria, and viruses. Ticks and tick-borne diseases (TBDs) are developing health-related issues among farmers of animals in the upper highlands of Balochistan, Pakistan. Given the limited research on this topic in the study region, this study aims to examine the current knowledge, attitudes, and practices (KAP) concerning ticks and tick-borne diseases by surveying 153 farmers with grazing animals using a socio-behavioral tool. This study also determines the spatiotemporal distribution of ticks on grazing and pet animals and identifies the associated risk factors including gender, age, and host influence on tick infestations. Odds ratios and 95% confidence intervals, based on Fisher's test, were used to assess risk factors for determining preventive measures. The findings revealed a low level of knowledge among the participants. For instance, there was a lack of awareness of the effects of climate change and the economic impact of ticks on animal health. The essential precautions, such as the non-indiscriminate use of acaricides, wearing dark-colored clothing, and limiting children's interaction with grazing animals, were often overlooked. However, the farmers had a positive attitude towards tick control, but they mostly relied on the knowledge of local communities. The neglect of such measures places these farmers and their children at risk of contracting TBDs. A total of 9548 hard ticks were randomly collected from 2668 out of 3628 hosts, including cows, buffaloes, goats, sheep, camels, and pet animals (dogs) in seven districts of Balochistan, Pakistan. Four genera of ticks, *Hyalomma* (5374/9548; 56.49%), *Haemaphysalis* (1021/9548; 11.15%), *Rhipicephalus* (3034/9548; 31.11%), and *Dermacentor* (119/9548; 1.25%) were morphologically identified. Of the 9548 collected ticks, the most abundant tick species was *Hyalomma anatolicum* (1528/9548; 16%), followed by *Hyalomma scupense* (1268/9548; 16.30%), *Rhipicephalus turanicus* (1065/9548; 13.78%), *Haemaphysalis sulcata* (1021/9548; 13.1%)., *Rhipicephalus haemaphysaloides* (1015/9548; 10.63%), *Rhipicephalus microplus* (954/9548; 9.99%), *Hyalomma dromedarii* (695/9548; 7.27%), *Hyalomma kumari* (689/9548; 7.21%), *Hyalomma issaci* (578/9548; 6.05%), *Hyalomma turanicum* (575/9548; 6.02), *Dermacentor* sp. (119/9548; 1.24%), and *Hyalomma asiaticum* (41/9548; 0.42%) and these ticks were overlooked in terms of pathogen research, including *Rickettsia*, *Anaplasma*, and

Ehrlichia spp. The highest number of ticks were collected from district Nushki (2213/9548; 23.1%), followed by Loralai (1740/9548; 18.2%), Quetta (1633/9548; 17.1%), Pishin (1610/9548; 16.8%), Zhob (1149/9548; 12.0%), Sherani (621/9548; 6.5%) and Musakhail (582/9548; 6.0%). Seasonal analysis revealed the highest number of tick collections was in summer (3105/9548; 32.51%) followed by spring (2611/9548; 27.34%), autumn (2096/9548; 21.95%), and winter (1736/9548; 18.18%). Infestation rates in hosts were highest in sheep (887 of 2668; 33.24%) followed by goats (665/2668; 24.92%), cows (586/2668; 21.96%), camels (218/2668; 8.17%), buffaloes (181/2668; 6.78%) and dogs (131/2668; 4.91%). More female ticks (6248/9548; 65.43%) were collected than male ticks (3300/9548; 34.56%), while male hosts (1533/2668; 57.45%) were more in number than females (1135/2668; 42.54%). Age-based analysis indicated that older animals had the highest infestation rate (1461/2668; 54.76%), followed by adults (781/2668; 29.27%), and younger animals (426/2668; 15.96%). The highest tick counts were observed on the forelimbs (2744/9548; 28.73%), followed by the hind limbs (1339/9548; 14.02%). Univariate logistic regression identified key risk factors such as grazing patterns, water resources, and seasonal influences. Subsequently, a subset of microscopically identified ticks were subjected to DNA extraction and PCR to amplify 16S rDNA and cytochrome c oxidase subunit 1 (*cox1*) fragments. Furthermore, *gltA, ompA*, and *ompB* fragments were targeted for *Rickettsia* spp. and 16S rDNA fragments for both *Anaplasma* and *Ehrlichia* spp. The BLAST analysis of the 16S rDNA and *cox1* sequences from *Dermacentor* sp. showed maximum identities of 98.42% and 97.45% with *D. pavlovskyi* from China. The 16S rDNA and cox1 sequences of *H. turanicum* demonstrated 100% identity with those of the same species previously reported from Pakistan. The 16S rDNA and cox1 sequences of *H. asiaticum* had shown 99.52 and 100% identities respectively with their corresponding species reported from Asian countries including China, Kazakhstan, and Turkey. The 16S rDNA sequences of *H. anatolicum* showed 100% identity with those of the same species previously reported from Pakistan, China, India, Turkey, and Iraq. Similarly, the 16S rDNA and *cox1* sequences of *H. anatolicum* and *H. scupense* showed 100% identity with those of the same species previously reported from China. The *gltA, ompA*, and *ompB* fragments are associated with *H. turanicum* and showed 100% identities with *Rickettsia aeschlimannii* reported from Egypt, Italy, Kazakhstan, Kenya, Pakistan, and Senegal. The 16S rDNA sequences of *Anaplasma* sp. and *Ehrlichia* sp. are associated with both *H. asiaticum* and *H. turanicum* exhibited 99.67 and 100%

identities with unknown *Anaplasma* sp. and *Ehrlichia* sp. reported from Morocco and Pakistan, respectively. The phylogenetic analysis based on 16S rDNA and *cox1* *Dermacentor* sp. indicated a close evolutionary relationship with *D. pavlovskyi. H. turanicum, H. asiaticum, H. anatolicum* and *H. scupense* from the current study clustered with their respective species. Similarly, in *gltA, ompA*, and *ompB* phylogenetic trees of *Rickettsia*, *R. aeschlimannii* of the present study clustered with the same species, whereas *Anaplasma* sp. and *Ehrlichia* sp. of this study clustered with undetermined *Anaplasma* spp. and *Ehrlichia* spp. in the 16S rDNA phylogenetic tree of Anaplasmataceae. Among the DNA samples from the screened ticks, a coinfection rate of *R. aeschlimannii, Anaplasma* sp., and *Ehrlichia* sp. (2 out of 80, 2.5%) was observed in *H. turanicum*, whereas individual infection rates were noted as follows: *R. aeschlimannii* (8 out of 80, 10%), *Anaplasma* sp. (5 out of 80, 6.3%), and *Ehrlichia* sp. (5 out of 80, 6.3%). This study for the first time reported on the morphological and molecular bases of three tick species, namely *Dermacentor* sp., *H. turanicum,* and *H. asiaticum* with two known previously reported species *H. anatolicum* and *H. scupense,* and molecular assessment of associated Rickettsiales bacteria (*Rickettsia, Anaplasma*, and *Ehrlichia* spp.) in *H. turanicum* and *H. asiaticum* in Balochistan, Pakistan. This study indicates minimal involvement from the government in educating farmers and controlling ticks. The role of stakeholders, including the government, non-governmental organizations, veterinary doctors, and local farmer communities, is crucial to address these issues and to implement effective training programs that address misconceptions about ticks and TBDs. The present study also highlights how tick fauna has been updated with the identification of a variety of tick species from the different districts and territories of Balochistan, Pakistan, and may help to understand the identity, molecular epidemiology, and geographic distribution of ticks and associated pathogens as well as provides valuable insights into the distribution and risk factors of ticks and emphasizes the need for their effective control strategies.