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REVIEW ARTICLE

TITLE:

MORPHOLOGICAL IDENTIFICATION OF DIFFERENT TICKS IN DISTRICTS SWABI, MARDAN, AND CHARSADDA, KHYBER PAKHTUNKHWA, PAKISTAN

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Abstract

Ticks are ectoparasitic arthropods that play a crucial role in transmitting pathogens to humans and animals. This study focused on identifying the morphological diversity of ticks in Swabi, Mardan, and Charsadda districts, known for high tick prevalence in Pakistan. Field surveys were conducted over nine months, collecting tick specimen from forests, grasslands, and residential areas. Specimens were preserved and examined using a stereo-zoom microscope and taxonomic keys to classify them accurately. The study identified various tick species, including hard ticks (*Ixodidae*), in the study areas. The morphological characteristics of each species, such as body size, color patterns, capitulum shape, and scutum features, were documented. The results showed a diverse tick fauna in the study regions, with some species commonly found across all three districts and others having localized distribution patterns. This study provides valuable data on tick species in the studied districts, which can help in developing of targeted interventions and disease surveillance programs. Overall, the research offers important insights into the morphological identification of ticks in Swabi, Mardan, and Charsadda districts in Pakistan, contributing to tick taxonomy and epidemiology. These findings can serve as a basis for implementing preventive measures and protecting public health in the study regions and beyond.

Keywords: Morphological, Identification, Ticks, Distribution, Disease, Prevalence and Host.

1. Introduction

Pakistan's economy heavily relies on agriculture and livestock, with ticks posing a major threat to the latter. Ticks, hematophagous arthropod that feeds on the blood of their hosts, can damage livestock hides and reduce body weight (**Ali *et al*., 2014**). This parasitic infestation affects the economy by causing toxicosis, allergic reactions, and severe itching in livestock (***Shah et al*., 2015**). The prevalence of tick related disease is higher in tropical and subtropical regions (**Muraleedharan, 2005**). Given the economic impact of tick infestations, efforts are focused on controlling these parasites and the disease they transmit (**Jongejan and Uilenberg, 2004**).

Ticks first appeared in the Cretaceous period (146 to 66 million years ago) and continued to evolve and disperse until the Tertiary period (65 to 5 million years ago) (**De La Fuente *et al*., 2003**). In Aristotle's renowned book "Historia Animalium" dating back to 400 B.C., ticks were described as being produced from plants and as repulsive parasites. Throughout the mid-eighteenth century, parasitologists worldwide continued their studies on tick taxonomy, bionomy, prevalence, regional abundance, and seasonal patterns (**Shah *et al*., 2015**). The oldest known specimen of a tick, the Argasid bird tick, was found in Cretaceous New Jersey amber, while more recent specimens from Dominican and Baltic ambers can be classified into existing taxa (**Dunlop *et al*., 2016**). Some tick species discovered in preserved reptiles suggest an origin dating back around 99 million years, such as the Deinocroton Dracula tick found in a dinosaur feather (**Penalver *et al*., 2017**).

Ticks are common parasites found both in both humans and animals worldwide, especially in tropical and subtropical regions. They are significant disease vectors, spreading over 200 diseases through around 10% of the 899 tick species. Ticks not only suck blood but also have other direct negative effects on their hosts (**Jongejan and Uilenberg., 2004**).

Ticks play a significant role in the global spread of tick-borne animal diseases, serving as important vectors for various illnesses. In Pakistan, ticks, as ectoparasites, transmit different protozoans that affect the growth rate of domestic animals, contaminate animal products, and contribute the spread of diseases such as theileriosis and babesiosis (**Ghosh et *al*., 2007**; **Despommier, 2000; Jongejan and Uilenberg, 2004**). Additionally, tick can transmit zoonotic agents including bacteria (*Francisella tularensis, Borrelia burgdorferi*), rickettsia (*Rickettsia rickettsii, Coxiella burnetii, Ehrlichia canis*), and protozoa (*Babesia divergens, B. microti*), posing new challenges to the healthcare industry due to the increasing number of cases worldwide (**Kilpatrick and Randolph, 2012**).

The global rate of urbanization has increased in recent decades, leading to significant changes in the diversity of flora and fauna. This has impacted the transmission of human and animal diseases by vectors and altered the interactions between animals and pathogens. (**Bradley and Altizer, 2007**). Ticks, ectoparasites are found on animals, is the second-largest category of transmission vectors for zoonoses, capable of spreading various infections including bacteria, viruses, and parasites (**Boulanger *et al*., 2019**). Tick bites can harm the host's reproductive capacity, leading to anemia, emaciation, skin damage, inflammation, and rashes. In severe cases; tick bites can cause paralysis and even death (**Marchiondo *et al*., 2013**).

In the current study, morphology of various species from different fauna was studied in detail, certain tick species with known zoonotic potential were also identified, highlighting the public health significance of this research. Many investigators have carried out researches on different parameters of ticks in different regions in Pakistan but no morphological identification of ticks from district Swabi, Mardan and Charsadda have been described before so, there is a demand for the appropriate morphological identification of ticks from these regions. Understanding the morphological diversity and distribution of ticks in the region is essential for devising effective control and management strategies to mitigate tick-borne diseases' impact on human and animal health.

2. Methods and Materials

A cross-sectional study was conducted from June 2022 to March 2023, where tick samples were collected from various domestic animals like cows, dogs, buffalo, goats, sheep, and camels during routine visits to farms and grazing grounds in the research regions. A proforma was used to record details such as the animal's species, age, location, and sex. Different body parts of the animals were examined for ticks, including shoulders, dewlaps, bellies, heads, ears, necks, backs, legs, perineum, and tails. Ticks were carefully removed using forceps and preserved in Eppendorf tubes for transportation to the parasitology lab. The specimens were cleaned with distilled water and stored in a container with 70% ethanol. Adult ticks both (male and female) were morphologically identified using a stereomicroscope with 100–200-fold magnification and a digital microscope. Common taxonomic keys were used for classification; based on morphological traits according to Walker's classification.

2.1 Study Design

The aim of this study was to identify the hosts infested by ticks, determine the tick species present, and assess their abundance in relation to the host and geographic location in the districts of Charsadda, Mardan, and Swabi. Samples were collected from various host species in different areas of these districts.

2.2 Collection Sites for Sample

Ticks were collected from various areas in Charsadda, Mardan, and Swabi. In Charsadda, the collection sites included Umarzai, Turangzai, Utmanzai, Mera Turangzai, Dedarabad Turangzai, Esazai, Badra Khiel, Banda Turangzai, Rajjar, Ameer Abad, Mandani, Merozai Turangzai, Peran Turangzai and Malak Abad.

In Mardan, the collection sites were Rustam, Ale Landi, Daggar, Bhai Khan, Landai, Gujrat, Bakhshali, Maqam Chowk, Pakistan Chowk, Toru, Mayar, Shahbaz Garhi, Hussai, and Garyala.

In Swabi, the collection sites included Upper Maniray, Lower Maniray, Gulu Deray, Steppa Neer, Shwe Kali, Nawi Kali, Tolanday, Permolu, Hisha Marali, Sherdara, Dagai, Tarakay, Yar Husain, Dobian, Babu Derai and Khas Bazargi.

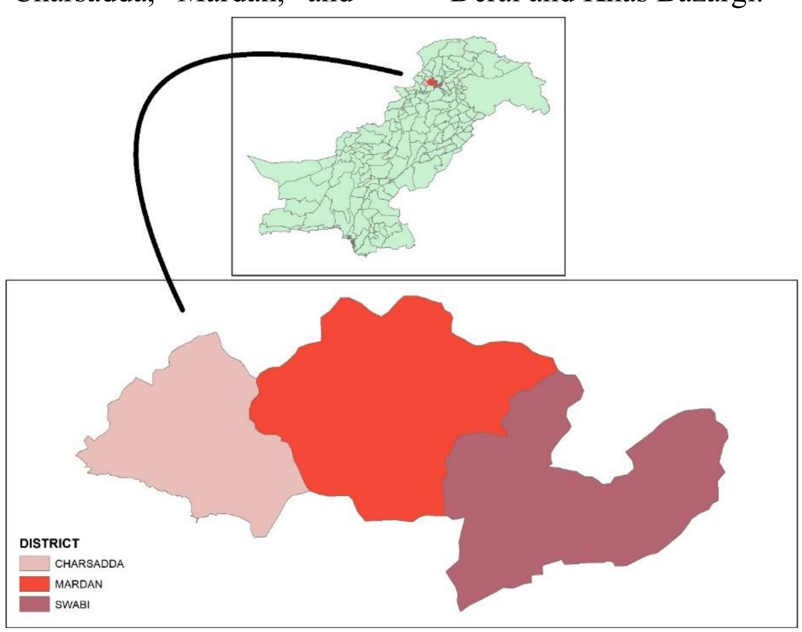


Fig 2.1. Shows the study map for district Charsadda, Mardan and Swabi.

2.3 Target Hosts

The target hosts in three districts, Charsadda, Mardan, and Swabi, included goats, camels, dogs, donkeys, cows, sheep, bulls, and calves. Our focus was on soft ticks, but unfortunately, the ticks were not found on the host.

Physical host control without chemical injection was carried out on domestic animals during tick isolation and search on their body surfaces.

2.4 Tabulation

The table was created using Excel and includes the tube number, target host's common name, scientific name, host gender, and location. It also includes the collection date and meteorological data such as temperature, humidity, and precipitation. The number of ticks removed from the host, their geographic coordinates, tick species, and life stage were recorded after identification.

2.5 Tick identification

To identify the tick genus, the dorsal portion of the tick was affixed to a microscope for examination. The primary characteristic examined was the mouth parts followed by the lateral groove, festoon, and scutum form on the dorsal side for species identification of males and females. The ventral side of the tick was then observed under a microscope to check the ventral plate of the males for species determination, along with investigations on the lateral side's spiracle plate. The morphology of the vaginal opening was investigated in both nymphs and adults to aid in species identification. Furthermore, the leg color and leg number were studied to identify species and determine developmental phases.

2.6 *Hyalomma* genus Morphological identification

In contrast to other genera, *Hyalomma* ticks are medium sized and unfed, measuring 5 to 6 millimeters in length, including the mouth. They have a medium angular lateral border on the basis capituli, long frontal teeth, three paired ventral plates in males, and equal paired spurs on the coxae. Their legs have rings of a light color, with Articles 1 and 3 of the Palp shorter than Article 2. The scutum and scutum color are brown, and they have convex eyes. Both males and females have a festoon, are not feed, and exhibit an anal groove and a sizable spiracle plate posterior to leg 4.

2.7 *Rhipicephalus* genus Morphological identification

The *Rhipicephalus* genus is characterized by medium-sized, unfed ticks measuring 3 to 5 millimeters, including the mouthpart. They have an anterior mouthpart with tiny palp articles, a hexagonal basis capituli with angular lateral borders, no rings of light color on the legs, and a dark black scutum in males and conscutum in females. The eyes can be flat or somewhat convex, males have festoons, and females have a large posterior spiracle plate on leg 4. Males have two paired ventral plates, uneven spurs on coxae 1, and a groove anterior to the anus.

2.8 *Haemaphysalis* genus Morphological identification

The *Haemaphysalis* genus is distinct from others due to its medium sized unfed ticks measuring 3 millimeters, including the mouthpart, and lacking eyes. The basis capituli has a rectangular shape with straight lateral borders and short anterior mouth-parts. In some species, palp 2 is wide and has a characteristic shape. These ticks have dark-colored legs, with males having a scutum and females having conscutum. Both males and unfed females exhibit festoons, and the posterior large spiracle plate is present on leg 4. Males do not have ventral plates, and Coxae 1 has a single spur.

3. Results

In district Charsadda, a total of 530 ticks were collected from 132 hosts. The distribution of tick species was as follows: *R. microplus* accounted for 41.50% (220 ticks), *R. haemaphysaloides* for 4.5% (24 ticks), *R. sanguineus* for 10.3% (55 ticks), *R. turanicus* for 18.3% (97 ticks), *Ha. bispenosa* for 0% (0 ticks), *Hy .anatolicam* for 18.7% (100 ticks), *Hy. dromedarii* for 11.88% (63 ticks), and *Hy. scupense* for 4.1% (22 ticks). The P-Value of 0.05 was calculated using Chi square test, indicating a correlation between the observed frequencies of the tick species and their distribution. This suggests that the distribution of tick species is not random, indicating that certain species may be more prevalent than others (Fig 3.1).

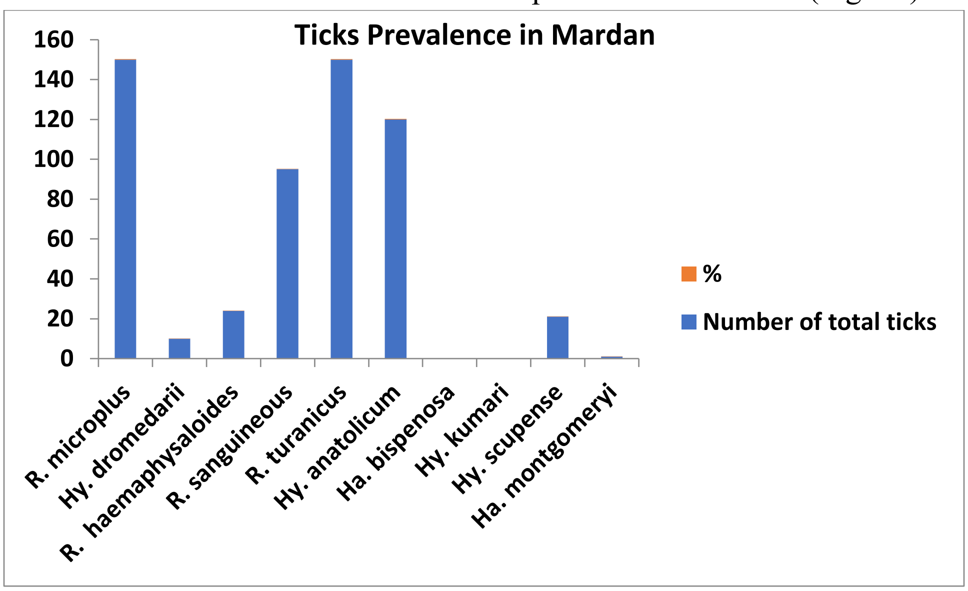


Fig. 3.2 Shows number of spp. with percentages in district Mardan.

A total of 721 ticks were collected from 197 hosts in the Swabi distict. Among them, *R. micro plus* accounted for 26.26% (330 ticks), *R. haemaphysaloides* for 3.74% (27 ticks), *R. sanguineous* for 13.86% (100 ticks), *R. turanicus* for 23.57% (170 ticks), *Ha. bispenosa* for 0.27% (2 ticks), *Hy. anatolicam* for 4.85% (35 ticks), *Hy. dromedarii* for 3.46% (25 ticks), *Hy. sucpense* for 1.80% (26 ticks).

In the entire sample collected from Jun 2022 to March 2023, a total of 89 animals were observed, including cows (870 ticks), dog (67 ticks), sheep and goats (534 ticks), camels (47 ticks), and buffaloes (304 ticks), totaling 1822 ticks (Fig. 3.3).

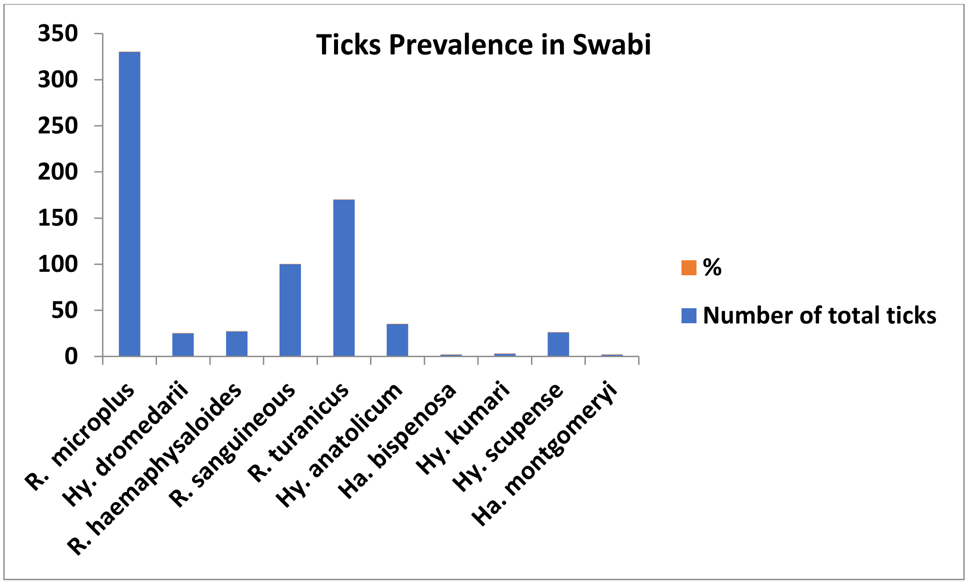


Fig. 3.3 Shows number of spp. with percentages in district Swabi.

Rhipicephalus microplus, commonly known as the southern cattle tick, is a one-host tick native to Asia but has been introduced to various regions worldwide, such as Africa, Australia, North and South America, and Europe. It is a significant pest of cattle and other livestock and can transmit diseases babesiosis, anaplasmosis, and theileriosis. Controlling *R. Microplus* is challenging due to its resistance to numerous acaricides. *Rhipicephalus microplus* differs from other common tick species in its host range, life cycle, geographical distribution, and the diseases it transmits. It is important to be aware of the tick species present in a specific area to take necessary precautions to prevent tick bites and avoid tick-borne diseases (Fig. 3.4).

. A, *Rhipicephalus microplus* female dorsal view. B, *Rhipicephalus microplus* female ventral view.

Fig. 3.4 Shows the *Rhipicephalus microplus* female collected from a cow. A, *Rhipicephalus microplus* female dorsal view. B, *Rhipicephalus microplus* female ventral view.

Male *Rhipicephalus turanicus* ticks play a vital role in the ecosystem by regulating host populations and serving as a host for various parasites and diseases. However, they also pose a threat as potential vectors of infections to human and animals, emphasizing the importance of research and management efforts for public health and veterinary purposes. Studying male *Rhipicephalus turanicus* ticks collected from sheep can offer valuable understandings into their behavior, biology, and role in disease transmission. The *Rhipicephalus turanicus* tick is a common species that infests domestic animals such as goats, sheep, and cattle. The female *Rhipicephalus turanicus* tick is typically larger than the male. It has a hard, sclerotized exoskeleton, similar to other hard ticks. The body of the tick is flattened and elongated dorsoventrally before feeding, but becomes engorged and more rounded after meals (Fig. 3.5).

A. Rhipicephalus turanicus male *B. Rhipicephalus turanicus* female

Fig. 3.5 Shows the *Rhipicephalus turanicus* male collected from sheep. A, *Rhipicephalus turanicus* male dorsal view. B, *Rhipicephalus turanicus* male ventral view.

Adult *Rhipicephalus sanguineous* ticks relatively small compared to other tick species, with a color range from dark brown to reddish brown. The female tick body swells more after feeding; wherase the male tick body is typically longer and flatter from top to bottom. Due to its excellent adaptability to dogs, *Rhipicephalus sanguineous* commonly infest them, especially in places like kennels and dogs shelters where dogs are kept in close quarters. Ticks are often removed directly from dog's skin during veterinary visits. Dogs and human can contract various infections from *Rhipicephalus sanguineous*, including ehrlichiosis and babesiosis which seriously impact a dog's health (Fig. 3.6).

A, *Rhipicephalus sanguineus* male dorsal view. B, *Rhipicephalus sanguineus* male ventral view

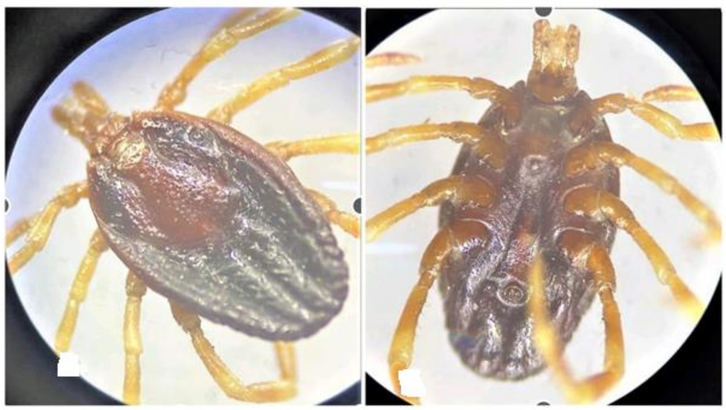
Fig. 3.6 Shows the *Rhipicephalus sanguineus* male collected from a dog. A, *Rhipicephalus sanguineus* male dorsal view. B, *Rhipicephalus sanguineus* male ventral view.

Similar to other ticks, the male *Rhipicephalus haemaphysaloides* can be identified by its distinctive features. It has a scutum or shield on the dorsal side that covers part of its body. The mouth parts, including the chelicerae and hypostome, are visible on the ventral side and are adapted for piercing the host skin to feed on blood (Fig. 3.7).

A. Haemaphysaloides male B. *Haemaphysaloides* Female

Fig. 3.7 *R. haemaphysaloides* male adult collected from cattle. A, *R. haemaphysaloides* male adult dorsal view. B, *R. haemaphysaloides* male adult ventral view.

Hyalomma anatolicum ticks are commonly found infecting cattle, among other hosts such as sheep, goats, and wild animals. Compared to other tick species, the female *Hylomma anatolicum* tick is relatively large, measuring several millimeters in length. It has a hard sclerotized exoskeleton typically of hard ticks, covering an elongated, flattened dorsovental body. The body color range from brown to grey before feeding, but darkens after meal (Fig. 3.8).



A, *Hyalomma anatollicam* male dorsal view. B, *Hyalomma anatollicam* female adult ventral view.

Fig. 3.8 Show *Hyalomma anatollicam* female collected from cattle. A, *Hyalomma anatollicam* male female dorsal view. B, *Hyalomma anatollicam* female adult ventral view.

Female *Hyalomma scupense* ticks can grow up to 15 millimeters in length when fully engorged, larger than males. Unfed females typically measure between 4 to 6 millimeters. They exhibit a range of colors typically ranging from dark brown to reddish brown (Fig. 3.9).

A, *Hyalloma scupensi* female adult dorsal view. B, *Hyalomma scupensi* female adult ventral view

Fig. 3.9 *Hyalomma scupensi* female adult collected from sheep. A, *Hyalloma scupensi* female adult dorsal view. B, *Hyalomma scupensi* female adult ventral view.

4. Discussion

In Pakistan, studies have revealed that ticks are rare in terms of their morpho-taxonomy, distribution, prevalence, molecular phylogeny, and management methods (appropriate immunization), as well as the research region. Unverified tick species at the molecular level in a recent large-scale investigation have been reported in several publications (**Karim *et al*., 2017**). The lack of understanding in the area of tick taxonomy employing fundamental keys may be the major cause of the discrepancy.

Since most farmers in Pakistan are concerned with livestock production and these ticks are notorious for carrying ectoparasites and important pathogens, such as *Babesia bovis, Babesia bigemina, Borrelia theileri,* and *Anaplasma marginale*, they are a major source of frustration for them (**Karim et al., 2017**; **Roy *et al*., 2018**). (**Jamil *et al*., 2022**) identified eight genus (*Amblyomma, Boophilus, Dermacentor*, *Hyalomma, Haemophysalis, Ixodes, Margaropus* and *Rhipicephalous*) in domestic animals of Pakistan. The morphology-based taxonomy of the *Boophilus* subgenus is complicated, mainly due to the occurrence of large intraspecific variation in morphological features (**Jongejan and Uilenberg, 2004; Walker, 2003**). Bovine tropical theileriosis is vectored by *Hyalomma* species (e.g. *Hy*. *anatolicum*, *Hy*. *dromedarii*, *Hy*. *ku mari*, and *Hy*. *scupense*), and is considered an economically important disease in cattle, resulting in high morbidity and economic losses (**Jabbar *et al*., 2015**)

The main hard tick species recorded on bovine animals during the present study in Swabi, Mardan and Charsadda belong to three tick genera. *Hyalomma* (*Hy.*

anatolicum, *Hy. kumari*, *Hy. dromedarii* and *Hy. scupense*), (*R. microplus*, *R. sanguineous* and *R. turanicus*). Most past reviews on *R. sanguineus* have either focused on the tick in relation to particular disease agents or have been broad ranging, focusing on the practicalities of identification, prevention, and control, as well as on transmitted diseases (**Parola *et al*., 2001**; **Dantas-Torres, 2010**). (**Nisa and Pour, 2005**).

In most breeds of cow, the infestation of *H. anatolicum* was found to be maximum. The relative prevalence of such ticks was*; Hy. Scupensi* (1.80%), *Hy. anatolicum* (4.85%), *Hy. dromedari* (3.46%), *R. Microplus* (26.26%), and *R. sanguinueos* (13.86%), *R. turanicus* (23.57%), *R .haemaphysalides*(3.74%), *Hy. Kumara* (0.41%), *Ha. Bispinosa* (0.27%), *Ha. Montgomeryi* (0.27%) and *Ha. Punctata* (0.13%). The percentage of tick infestation in young cattle was lower than the adult cattle (**Mattioli *et al*., 1998**). The study results described the face and ears of the host as the most advantageous predilection site for ticks with a prevalence of 62%, whereas they have also been found on the udder, scrotum, tail, leg, and belly in cattle (**Rafique *et al*., 2015**). Another research done in Kerman showed that the major kind of tick is Hyalomma (**Islam *et al*., 2006**).

The hard tick genera *Hyalomma* and *Rhipicephalus*, as well as a few others, were found on cattle and other livestock in the Peshawar, Pakistani Frontier region, and were previously described by (**Manan Khan and Ahmad, 2007**).

The largest prevalence of ticks was seen in July, August, and September according to seasonal tendencies of tick dispersal due to the predominant high temperatures and humidity throughout these months. Our findings are almost similar to those of (**Ali *et al*., 2019**). The maximum number of cows was found to be infested by tick infection in the summer season (N: 133). 238 ticks were collected in the summer. The infestation was highest in July (N: 56), which was followed by June (N: 50). The minimum infestation was noted in the winter season in which only two animals were found to be infested with seven ticks. Who noted the highest peak of infestation in July (57.3%) In the meantime, the infestation rate was observed at a minimum in November at 36.4%.

The study of the different researchers also confirmed our results (**Islam *et al*., 2006**). The gender, age, and type of the host as well as environmental variables including temperature, humidity, rainfall, and wind speed all had an impact on the tick population. The quantity of domestic and wild animals, geographical features like mountains and plains, and the kind of flora in that location all had an impact on the tick population (**Tadesse *et al*., 2012**).

5. Conclusion

Ticks of the genera *Haemaphysalis*, *Hyalomma*, *Rhipheciphalus* and are common in Charsadda Swabi, and Mardan, districts, infesting various animal hosts. *R. sanguineus* and *R. microplus* are the most frequent tick species found on animals. *Hyalomma* species like *Hy. Suspense*, *Hy. anatolicum*, and *Hy. dromedarii* are also widespread. The identification of these species was done using a stereomicroscope and an identification key. Face-to-face interviews with farmers were conducted to collect data on tick infestation frequency. *R. sanguineus* ticks are prevalent in dogs throughout the year due to factors like lack of acaricide treatment, traditional rural housing, hot and humid climate, grazing and host susceptibility. Efforts to control tick populations and the diseases they spread must persist.

6. Acknowledgment

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7. Conflict of Interest

The authors declare no conflict of interest.

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