Molecular biodiversity and spatio-temporal distribution of hard ticks infesting cattle in district Swat Khyber Pakhtunkhwa Pakistan

By

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DEPARTMENT OF ZOOLOGY
FACULTY OF BIOLOGICAL SCIENCES
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"In the Name of ALLAH, the most beneficent, the most Merciful"

بسم الله الرحمن الرحيم

This thesis is dedicated to my parents' deepest gratitude whose love, & prayers have always been a source of strength for me.

DECLARATION

I hereby declare that the work presented in the following thesis is my own effort and the material contained in the thesis is original work. I have not previously presented any part of this work elsewhere for any other degree.

NIDA SIRAJ

CERTIFICATE

The dissertation "Molecular biodiversity and spatio-temporal distribution of hard ticks infesting cattle in district Swat, Khyber Pakhtunkhwa, Pakistan" submitted by **Ms. Nida Siraj** is accepted in its present form by the Department of Zoology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad as satisfying the thesis requirements for the degree of Master of Philosophy in Zoology (Parasitology).

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Nida Siraj

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ABSTRACT

In Pakistan, ixodid ticks have a variety of distribution patterns. Globally, ticks are the significant carrier of numerous diseases affecting people, animals, and livestock. Tick infestations generate serious issues in Pakistan's livestock industry, which is the country's principal source of rural income. They significantly harm the cattle business by causing hide loss and the spread of vector-borne diseases. About US$ 498.7 million in annual economic losses were attributed to tick-borne diseases. The distribution and way of life of ticks are significantly influenced by factors like the climate, host accessibility used by people and animals, and the environment. From March 2022 to October 2022, this study was carried out in seven tehsils of district Swat, Khyber Pakhtunkhwa (KPK). By using a practical sample technique, 2217 ticks overall were gathered from 561 infected cattle of diverse ages and sexes. With the aid of precise morphological characteristics, tick identification was done down to the genus and species level. *Rhipicephalus microplus* is exclusively to blame for annual loss through irritation, a decrease in milk/meat production frequently results in animal death, and the spread of contagious diseases such as babesiosis, theileriosis, and anaplasmosis. Numerous bacterial and viral illnesses, such as the tick-borne encephalitis virus and the Caribbean hemorrhagic fever virus, bacteria are spread by the *Rhipicephalus* genus. *Rhipicephalus microplus* (40.78%) had a larger prevalence than *Hyalomma anatolicum* (31.93%), while *Hyalomma marginatum* (27.29%) had the lowest prevalence, according to the findings. Several risk variables were substantially correlated with the prevalence of all ixodid tick species (age, sex, living conditions). Comparatively male, and young animals in plain, concrete buildings were less harmed than female and adult animals kept in mountainous, traditional rural dwellings. The distribution of tick infestation by body region showed that external genitalia (34.93%) was the most popular location, followed by the udder (26.02%), neck and head regions (20.68%), ear and tail (9.63%), shoulder (6.78%), and rear legs (1.96%). Additionally, mature, frail, and female animals are more susceptible to a tick infection. Tick infestation is worst on the body's concealed areas. Summer was the season with the highest tick prevalence, followed by spring and fall. The KPK province's cattle population was primarily infested by the cattle tick *Rhipicephalus microplus*, and the summer was the tick season with the highest tick load records.

INTRODUCTION

Ticks are compulsive blood-sucking ectoparasitic arthropods of the order *Acari* that infest both people and animals (Nasirian, 2022). They can infest mammals, birds, and occasionally reptiles (Jaffar *et al*., 2022). Three families, scattered over the globe, make up their composition. Many species are members of the Ixodidae family, popularly known as "hard ticks." Argasinae and Ornithodorinae collectively known as "soft ticks," are two subfamilies of the Argasidae family. There is only one species in the Nuttalliellidae family (Defaye *et al.,* 2022). *Dermacentor*, *Haemaphysalis*, *Rhipicephalus*, *Hyalomma*, and *Amblyomma* are all important veterinary genera. However, the *Ornithodoros*, *Argas*, and *Otobius* genera include the most significant populations of soft ticks. (Hurtado & Giraldo, 2018).Ticks are completely reliant on their host for all their essential needs, including food and shelter. They are particularly prevalent on the epidermis of the host and its outgrowths. Ticks are deadly dermal parasites that can either directly or indirectly impair blood loss, milk supply, and the development of numerous diseases. Diseases caused by protozoans, viruses, and bacteria that are spread by ticks within the animal in dairy and meat animals include theileriosis, babesiosis, and hemorrhagic fever. (Jaffar *et al*., 2022). The tick families Ixodidae, Argasidae, and Nuttalliellidae have been classed as economically significant. There are 949 recognized species in the Ixodidae, 200 in the Argasidae, and only one in the Nuttalliellidae (Hussain *et al*., 2021). All around the world, terrestrial animals are obligately infected by ticks (Acari: Ixodida), which feed on blood. They can directly harm cattle by inducing itchiness, sensitivities, and immobilization, or they can implicitly harm livestock by spreading infections like protozoa, viruses, and bacteria (Ghafar *et al*., 2020). Through the direct consequences of their feeding on humans and as carriers of numerous disease agents in both humans and cattle, ticks are vital to human health. There are currently known descriptions of over 19 tick-borne diseases in cattle and companion animals, in addition to over 16 human diseases caused by ticks or transmitted by ticks (Zhang *et al*., 2019). Ticks and tick-borne disease (TTBD) are thought to be a threat to 80% of the world's livestock population, which is primarily found in the subtropics and tropics. It is estimated that in these nations, the TBDs in cattle cost between US$ 13.9 and US$18.7 billion every year, demonstrating that ticks are a crucial concern for both farmers and the country. The reemergence and dissemination of TTBDs in animals and humans are also thought to be influenced by continuing seasonal and climatic changes (Theron & Magano, 2022).

Over 17% of illnesses and over 700,000 fatalities worldwide are a result of vector-borne diseases (VBDs) each year. Ticks and mosquitoes are the two most common vectors, in order of significance. In terms of human pathogens, ticks (Ixodida) rank second to mosquitoes in terms of veterinary vector-borne pathogens (Defaye *et al.,* 2021). The detrimental consequences of ticks extending beyond humans and cattle are crucial from a conservation standpoint since they pose a significant threat to wildlife. Wild animals also act as conduits for the spread of pathogens between wildlife and people (Ali *et al*., 2022).

Ticks are regarded as the most significant vectors of diseases affecting humans, cattle, and companion animals because they transmit a considerably broader spectrum of harmful bacteria, protozoa, rickettsiae, spirochaetes, and viruses globally. These ticks are known to transmit several diseases, including anaplasmosis, babesiosis, theileriosis, and rickettsia (Farooqi *et al*., 2017). Ticks are important carriers of several diseases that seriously endanger public health, cause large financial loss, and reduce animal health and production. Numerous microbes, such as viruses, bacteria, and protozoa, are transmitted by ticks (Sultankulova *et al*., 2022). A sizable portion of the world's economy is made up of domestic ruminants like buffalo, cattle, goats, sheep, and camels. Though hard tick infections in the domestic ruminant group are frequently observed around the world. The transmission of various diseases, blood loss, hypersensitivity, inflammation, and irritation, reduction in livestock weight and milk production, impact on respiratory rates, appetite, body condition, blood composition, damage to the hide and udder, and open wounds that result in secondary infections make ticks one of the most significant parasites that negatively impact livestock health, performance, and productivity. They may be a significant contributor to the emergence and reemergence of tick-borne illnesses such as babesiosis, rickettsiosis, anaplasmosis, ehrlichiosis, Lyme disease, relapsing and Q-fever diseases, and lethal arboviruses (Nasirian, 2022). According to estimates, 80% of the world's cattle are subjected to tick infestations (Theron & Magano, 2022). Household ruminants, including camels (Artiodactyla: Camelidae), cattle, goats, sheep, and buffaloes (Artiodactyla: Bovidae), are found all over the world and play an important role in the global economy, primarily in developing nations. As a result, domestic ruminants supply crops with manure, food, raw materials, and energy. Consequently, it is not surprising that the domestic ruminant industry, particularly the dairy industry, has grown to be a significant source of income for most rural residents and a target for agribusiness in the dairy, meat, and various other products sectors of the processed food industry. They give the proprietors of small-scale dairy farms with limited resources an essential source of revenue (Ghafar *et al*., 2020). Particularly in areas of the world's tropics and subtropics, tick infestations are the main issue for animal health that significantly reduces the income of farming communities with few resources. An estimated

US$14,000–18,000,000 in annual economic losses worldwide resulting from tick infestations (Nasirian, 2022). In 1974, cattle tick (*Rhipicephalus microplus*) losses were estimated to be USD 62 million in Australia alone. Brazil loses roughly USD 2 billion every year (Rajput *et al*., 2006). The annual global economic burden of complex ticks and tick-borne diseases is estimated to be US$ 22-30 billion (Shakya *et al*., 2020).

1: Economic significance of ticks in Pakistan

Rural areas of Pakistan are home to most of the population. In many communities, raising and trading cattle is a significant source of revenue and employment. Numerous obstacles beset Pakistan's livestock and dairy industries, such as owners' ignorance of feed, tick management, artificial insemination, financial limitations, and the dearth of appropriate medical services. In this area, ticks are one of the most significant ectoparasites. This is encouraged by Pakistan's climate, which offers ideal circumstances for tick development and reproduction (Zafar *et al*., 2022). With an 11.7% contribution to GDP and a 60.6% contribution to the agriculture sector, livestock is one of the primary drivers of the Pakistani economy (Rehman *et al*., 2017). Pakistan ranks third in milk output, after India and the United States of America. In 2017-2018, the agriculture industry grew by 3.8%, beating its aim of 3.5 % (growth was 2.9 % the last year) (Hussain *et al*., 2021). Infestations of ticks have negative impacts on people, animals, and the environment. Blood is drawn from numerous human and animal body parts by these arthropods. The output of milk, meat, and wool is reduced because of these microorganisms, which spread during feeding and cause diseases. Through immune system suppression, blood loss, irritability and stress, damage to the skin, and other negative effects, they have a detrimental effect on animals. Tick and TBD control in India's and Pakistan's cattle industries costs $498.7 million annually (Rahman *et al*., 2022). *Rhipicephalus* and *Hyalomma* ticks infest cattle and buffalo in Pakistan, transmitting infections such as *Anaplasma marginale*, *Babesia bigemina*, *Babesia bovis*, and *Theileria annulate*. Pakistan is home to 49.6 million cattle, 41.2 million buffalo, 78.2 million goats, and 30.9 million sheep (Khan *et al*., 2022). Animal husbandry provides 53.2% to the agricultural sector of Pakistan's economy and 11.4% of Pakistan's gross domestic product, demonstrating the country's importance as a livestock-raising nation in Asia (Aziz *et al*., 2022). The four most common illnesses spread by ticks are anaplasmosis, theileriosis, babesiosis, and cowdriosis (TBDs). Bovines and small ruminants are affected. The economy of Pakistan is most affected by the first three of these diseases. In Pakistan, the tick genera Rhipicephalus, Hyalomma, Haemaphysalis, Ixodes, Ornithodoros, and Argas are reported to transmit a variety of TBPs. The Khyber Pakhtunkhwa (KPK) province of Pakistan is a hotspot for emerging and recurrent TBDs with importance to veterinary and public health (Khan *et al*., 2022). Several studies from Pakistan have found that more than 80% of bovines were infested with ticks of the *Hyalomma* and *Rhipicephalus* species (Hussain *et al*., 2021).

In Africa, ticks, and the diseases they carry have been identified as the most prevalent and significant issues harming animals. They are responsible for 8.5% of the nation's economic issues. The amount of milk produced per cow decreased significantly during a 15-week period due to a rising tick infection with an estimated loss of 92.5 liters of milk per cow (Jonsson *et al.,* 1998). According to estimates made in Australia, each fully gorged female cattle tick prevents cattle from gaining between 0.6 and 0.9 grams of weight, causing a single animal's weight to drop by roughly 600g to 900 g during low infestations and by 2 kg during medium- to high-infestation periods during the three-week feeding session (Theron & Magano, 2022). Tick-borne diseases (TBDs) such as Lyme disease are the end outcome. About 500,000 diagnoses are in the United States each year (Keesing *et al*., 2022). In Nepal, the economic loss caused by ticks and TBDs in livestock accounted for 18.71% of the overall cost (Theron & Magano, 2022). Cases of tick-borne infections (Lyme disease, spotted fever group rickettsiosis or SFGR, babesiosis, Powassan virus, anaplasmosis/ehrlichiosis, and tularemia) in the United States totaled 48,610 in 2016 and 59,349 in 2017. *B. miyamotoi* and *B. mayonii* were identified as novel disease agents in Canada in 2013 and 2014, respectively. Rocky Mountain spotted fever (RMSF), the most common TBD in Mexico, has been documented in 30 states across the country (Alkishe *et al*., 2021). Tick population control is widely regarded as a promising method of limiting human exposure to TBDs. In general, field investigations demonstrate that using chemical or biological acaricides can lower the number of ticks by 50%-90% (Keesing *et al*., 2022). TBDs are the most common and widespread of all vector-borne diseases in various areas of Europe, experiencing enormous economic losses in patient care (estimated at EUR 19.3 million in the Netherlands and EUR 80 million in Germany) (Vasic *et al*., 2022). Hard tick infestations in small and big ruminants have been predicted to cause USD 7.0 billion in annual losses. *Rhipicephalus microplus* has caused significant economic losses (USD 22-30 billion) in the livestock industry. The zoonotic species *Hyalomma anatolicum, Rhipicephalus microplus, Hyalomma hussaini,* and *Rhipicephalus annulatus* are thought to be involved in the spread of Crimean Congo hemorrhagic virus infection in Pakistan (Iqbal *et al*., 2022). A detailed epidemiological examination in different agro-climatic zones has not been conducted in the KPK province

1.1: Ticks' ecology

Fire suppression has permitted broad biological changes in the composition, structure, and function of fire-dependent landscapes in the eastern United States over the last century, which are now in decline. These changes have most certainly led to the improvement of habitat conditions that favor disease-carrying tick species, critical tick wildlife hosts, and interactions that have facilitated pathogen transmission among them and to people (Gallagher *et al*., 2022). Tick distribution and pathogen transmission differ depending on species and geographic region. Vectors of tick-borne zoonoses such as *Anaplasma phagocytophilum*, *Babesia microti*, *Borrelia burgdorferi*, and *Powassan* virus are transferred by Ixodes species in North America. The worldwide spread of tick species is influenced by a variety of factors, including environmental issues, frequency in habitats that serve as sources, and host transferability. According to one research of migrating bird ticks, up to 22 tick species have been imported into Canada, with some originating as far south as Brazil. These ticks, once on Canadian soil, have the potential to spread across the ecosystem and, in some cases, bring novel zoonotic infections into local tick populations (Kanji *et al*., 2022).

1.2: Tick as vector of the pathogen

Ticks can be infection carriers, transmitting germs from host to host during blood-sucking and causing a wide range of diseases. The existence, dynamics, and amount of parasite stock in ticks have a significant impact on the kinetics of TBD transmission. Ticks typically acquire infected with disease-causing organisms when feeding on afflicted animals. The organism may then be passed from stage to stage within the tick. or from the female tick to the egg to the larvae an increase in vector potential of thousands of times. If the next stage or generation feeds on another animal that is susceptible to the disease, the organism is transmitted to that animal. Ticks cause significant economic losses both directly through blood sucking and indirectly as vectors of infections and poisons (Rajput *et al*., 2006).

1.3: Tick-Associated Diseases of Humans

I: Lyme Disease

Lyme disease is caused by *Borrelia burgdorferi*, which is spread by *Ixodes pacificus* and *Ixodes scapularis*. In the late twentieth century, Lyme disease was identified as a significant infectious disease. Since then, the CDC has documented an upsurge in Lyme disease cases across the US. For example, 18,000 cases of Lyme disease were registered in 2000, but the number of cases increased to 42,743 in 2017 and 33,666 in 2018 (Alkishe *et al*., 2021). Annual Lyme disease cases in the United States have increased from a few hundred in the early 1980s to more than 30,000 in recent years, according to data from the CDC. According to a recent study, real clinician diagnoses of Lyme disease in the last decade have surpassed 450,000 every year. Personal behaviors such as using repellents, checking for ticks, and avoiding tick habitats can help prevent exposure to B. burgdorferi and other tickborne infections (Keesing *et al*., 2022). As with bovines, the most common tick-borne diseases are babesiosis, anaplasmosis, theileriosis, and heartwater (Hurtado & Giraldo, 2018).

II: Babesiosis

Babesiosis, commonly known as piroplasmosis.(24)Babesiosis is caused by the protozoan genus Babesia (species *B. microti, B. duncani, B. divergens, and B. venatorum*), which can induce influenza-like symptoms (chills, fever, headache, lethargy, and body aches) and thrombocytopenia. It is spread by the ixodid tick Ixodes scapularis bites. Babesiosis is a major developing infectious disease. Globally significant sickness that affects both people and farmed animals. Babesia is most transmitted to mammals through tick bites. but additional methods of transmission (e.g., vertical transmission, blood transfusion, or organ donation) have been recorded in both people and animals, including wildlife reservoir hosts (Bajer *et al*., 2022). This disease is widespread in the Northeast and Midwest of the United States, with a total of 7612 cases identified between 2011 and 2015 (Alkishe *et al*., 2021). The number of human cases has recently increased, particularly in the United States, where approximately 2000 new cases are reported each year, as well as in Canada and China (Bajer *et al*., 2022).

III: Anaplasmosis and Ehrlichiosis

Anaplasma marginale produces bovine anaplasmosis. It harms red cells which leads to an initial illness with inflammation, elevated bacteremia levels, leukopenia, lethargy, reduced growth and milk production, miscarriage, and responsible for death in rare cases. *A. marginale* has been discovered to be spread globally by at least 200 distinct tick species. Under the perfect circumstances adequate flora and sufficient humidity that prevent ticks from drying out, male ticks can endure in the surroundings for a few months to a year or more, which serves as a reservoir in the wild for *A. marginale*.(Hurtado & Giraldo, 2018). Microbiological pathogens *Ehrlichia* species and *Anaplasma phagocytophilum* strains are hazardous to human health. Following Lyme disease, the second most typical tick-borne diseases in the US are ehrlichiosis and anaplasmosis. (Alkishe *et al*., 2021).

IV: Spotted Fever Rickettsiosis (SFR)

Some species of the bacterium Rickettsia, which includes R. rickettsii, the cause of RMSF, are responsible for this human disease. Ticks including *D. andersoni, Dermacentor variabilis,* and *Rhipicephalus sanguineus* are responsible for its transmission. *Amblyomma maculatum* transmits Rickettsia parkeri, which produces a milder kind of RMSF. In 2010, the CDC created a new category called spotted fever rickettsiosis that encompassed all rickettisial infections (RMSF, Rickettsia parkeri rickettsiosis, Pacific Coast tick fever, and rickettsialpox) (Alkishe *et al*., 2021). Annually, more than 6000 cases of tickborne spotted fever group (SFG) rickettsioses are documented based on passive surveillance (Walker *et al*., 2022).

V: Tularemia

Tularemia is a bacterial infection that can affect the skin, eyes, lungs, and lymph nodes. F. tularensis has five subspecies, although only *Ft. tularensis and Ft. holarctica* elicit illnesses in North America. Ticks *(Dermacentor variabilis, D. andersoni, and Amblyomma americanum*), deer flies, and mosquitos can all spread this disease. Direct interaction with infected animals or consuming contaminated water while biting flies are uncommon in the central United States, most of the threat is associated with tick bites and animal interaction (Alkishe *et al*., 2021).

VI: Powassan Virus

Powassan virus (genus Flavivirus) is a tick-borne encephalitis virus that produces severe brain damage and a high case fatality rate. The first human case was described in 1958 in Powassan, Ontario, Canada, with the virus isolated from the brain tissue of a 5-year-old boy who died of acute encephalitis (Alkishe *et al*., 2021).

VII: Theileriosis

Economically important Theileria species that infect cattle and small ruminants are transmitted by ixodid ticks of the genera *Rhipicephalus*, *Amblyomma*, *Hyalomma*, and Haemaphysalis. Worldwide, the most commercially important tickborne infections that cause bovine theileriosis are *Theileria annulata* (cause of tropical theileriosis) and *Theileria parva* (cause of east coast fever). Around 250 million cattle are threatened by Tropical theileriosis which is a severe constraint on livestock production and productivity in many developing countries (Hurtado & Giraldo, 2018). Several Theileria species have been described from cattle, water buffalo, sheep, and goats worldwide, transmitted by ixodid ticks of the genera *Rhipicephalus*, *Hyalomma*, *Amblyomma*, and *Haemaphysalis* (Aktas *et al*., 2006).

1.4: Economic and health impact of the ticks in other animals

Many countries rely heavily on small ruminants for their meat and milk supplies, as well as for the income generated by the purchase of their skins and fibers. Over 35 million small ruminants in China are thought to be infected by Theileria, Anaplasma, and Babesia. In sheep, Anaplasma phagocytophilum produces tick fever, which results in fever, neutropenia, coughing, appetite loss, exhaustion, calorie restriction, and decreased milk supply. In goats, A. phagocytophilum can cause infection and a drastic reduction in milk production. Domestic ticks are also affected by ticks. (Hurtado & Giraldo, 2018). A virus known as African swine fever is transmitted by several species of soft ticks of the genus Ornithodoros and results in major financial damage to the swine industry. (Gaudreault *et al*., 2020). The two most encountered species regarded to be poultry pests are Argas persicus and Argas radiatus. (Yang *et al*., 2014). The bird tick, sometimes referred to as the "blue bug," is the tick that feeds on poultry the most frequently. Several other hard tick species also sporadically feast on poultry (Hinkle & Corrigan, 2020). Argas persicus outbreaks in economic birds cause gastrointestinal discomfort, sleepiness, ruffled feathers, weight loss, diminished egg production, and leukemia which in chronic infestations can be fatal. The fowl tick has been shown to treat wide range of illnesses in many different parts of the world. As a result of their isolation from *Argas persicus*, *Staphylococcus aureus*, *Borrelia anserina*, *Escherichia coli*, and *Salmonella Pullorum* are assumed to be particularly important in the pathogenesis of these illnesses. *Dermacentor*, *Ixodes*, and *Amblyomma* are the hard ticks that are most frequently found on horses. Two of the most prevalent diseases resulting from tick infestation in horses are equine granulocytic anaplasmosis and equine piroplasmosis. At the bite site, there are abscesses, intrusions, enlarged pores, scar tissue, lesions, and baldness. (Hurtado & Giraldo, 2018).

1.5: Ticks control

Acaricides remain the primary technique for tick management in bovine cattle (chemical control). Additionally, it has long been suggested that the prospect of resistance development makes the sole method of chemical management inadequate (Hurtado & Giraldo, 2018). An inclusive strategy to controlling ticks should rotate acaricides with active ingredients that have diverse modes of action and no potential for cross-resistance, and it should be applied correctly at appropriate doses and periods to decrease the likelihood of resistance (Rodriguez *et al*., 2018). Commercial farmers in South Africa frequently employ a strict tick management approach that entails up to 37 acaricide sprays year. In South Africa, the estimated cost of cattle dipping in 1999 was between US$1.60 and US$9.60 for five to thirty applications. Additionally, the cost of a vaccination dosage may range from US$3.30 to US$12.60 and treating heartwater illness of cattle may cost US$1.50 to US$3.00 per individual per year. Chemical acaricides are now used in tick control tactics to reduce tick populations over the long term as well as temporarily. Avermectines and milbemycin are among the synthetic acaricides that are used, with amitraz, picaridin, and N,N-diethyl-m-toluamide (DEET) being the most popular ones. Other synthetic acaricides include carbamates, cyclodines, cyclodines, macrocyclic lactones, amidines, and pyrethroids. Chemical acaricides are most frequently applied to the host animals via dipping, spraying, hand spraying, injecting, hand dressing, and pouring on. (Theron & Magano, 2022). The ability of the livestock owner to effectively manage tick populations with acaricides depends on his or her knowledge of tick biology, acaricide chemical classes, and application instructions. To acquire the required amount of active ingredients for efficient tick management, it is important to know the best way to combine acaricides and water. Through dipping and spraying, acaricides are implemented to host animals. Spraying is still the most prevalent approach (Mutavi *et al*., 2021). Other tick-control measures include physical eradication (practical only on farms with a small number of infested animals), the adoption of tick-resistant breeds, and the use of pathogen-resistant breeds, Physical eradication (possible only on farms with a small number of infested animals), the adoption of tick-resistant breeds, and the use of pathogen-resistant breeds are other tick-control techniques. Grasslands being burned, exposing ticks' many life phases to extreme heat, and clearing away the flora that serves as protection; good animal feed to increase tick resistance; use of essential oils and plant extracts with acaricidal properties; vaccination; among other things, biological control using worms, entomopathogenic fungi, ants, and birds (Rodriguez *et al*., 2018).

(a): Organochlorines

Since 1946, organochlorines have been used as acaricides. These chemicals are considered to work by binding to the picrotoxinin site in the aminobutyric acid (GABA) chloride ionophore complex (Abbas *et al*., 2014).

(b): Organophosphates and Carbamates

Organophosphates were one of the first chemical classes utilized to control arachnids. Both OPs and carbamate acaricides kill ticks by blocking AChE, a crucial enzyme required for nervous system function (Abbas *et al*., 2014).

(c): Amidines (Amitraz)

Amitraz, a triazapentadiene molecule, belongs to the amidine group. Amitraz has proven to be an effective treatment for cattle ticks. For more than 30 years, Amitraz has been in use. The toxic effects of amitraz on a receptor for the neuromodulator octopamine are thought to be its method of action (Abbas *et al*., 2014).

(d): Pyrethrins/Pyrethroids

Pyrethrins are naturally occurring chemicals obtained from chrysanthemums. Strong neurotoxins include pyrethroids and pyrethrins. They act on sodium ion channels, causing neuronal excitement due to changes in the permeability of nerve membranes to sodium and potassium ions (Abbas *et al*., 2014).

(e): Macrocyclic lactones

Avermectins and milbemycins are examples of macrocyclic lactone acaricides. Actinomycetes of the genus Streptomyces produce avermectins and milbemycins, which are substances that are found in nature. Macrocyclic lactones prevent the transmission of electrical activity in neurons and muscle cells by enhancing the production and adsorption of gamma-aminobutyric acid (GABA) at nerve endings. (Abbas *et al*., 2014).

1.6: Application of chemicals

Chemicals have been applied to animals using a variety of methods, including dipping, spraying, ear tagging, and pouring. Acaricide applications to tick-infested cattle through dipping or sprayer can be equally successful under ideal conditions with competent equipment management without harming animals and subsequent product dilution (Rajput *et al*., 2006).

(a): Dipping

Animals are dipped in a dipping tub with a chemical solution in this procedure. To control ticks and tick-borne diseases that affected cattle in Australia, Africa, and the United States by 1893, "dipping-vats" were used to submerge tick-infested livestock in a variety of chemical substances. Cottonseed oil, fish oil, crude oil, kerosene, creosote, a tobacco extract, soap, and a mixture of Sulphur and kerosene were among the hundreds of potential acaricides that were considered for dipping (Rajput *et al*., 2006).

(b): Spray

Spraying equipment is lightweight, and just a tiny amount of acaricide is required for a single application (Rajput *et al*., 2006).

(c): Spot treatment or hand dressing

The areas between the legs and the teats, the tail brush, the underside of the tail, and the inner regions of the ear are the most susceptible to escape treatment in calves with enormous udders. Hand dressing or spot treatment refers to the use of acaricides to these areas (Rajput *et al*., 2006).

(d): Some other applications

Additional ways to administer acaricides include using pour-one, ear tags, tail bands, and pyrethroids with prolonged residual activity. In addition, a mechanical applicator was developed**.** An intraruminal ivermectin slow-release device offered 90 days of tick protection in Kenya (Rajput *et al*., 2006).

1.7: Ticks and climate

Another complicated element affecting ticks' survival is climate change, which may allow ticks to establish themselves in formerly inhospitable locations. Climate change-induced changes in plant cover and other habitat characteristics may provide new possibilities for tick larvae survival and possible northward translocation of these and other key tick species (Osbrink *et al*., 2022). Their survival is aided by hot, humid weather, but development is hampered by cold climes. (Hurtado & Giraldo, 2018). Since they spend a large portion of their life cycle exposed to local elements, such as abiotic (such as moisture, warmth, soil humidity) and biotic (such as humid leaves, vegetative cover, dense shade, and host interaction), ticks must adapt to these circumstances. Climate change-induced temperature increases will almost certainly result in an increase and/or alterations in the possible ranges of several tick species, typically at higher elevations and higher latitudes (Alkishe *et al*., 2021). According to the Köppen classification, Pakistan is in a temperate climate and has hot weather across a large portion of its landmass. Additionally, Pakistan is one of the top 10 nations in the world that are being negatively impacted by anthropogenic climate change (Eckstein *et al*., 2018). Although Pakistan has recently done a variety of ecological and genomic studies on ticks and diseases transmitted by ticks, but all eco-epidemiological and the taxonomic aspects have not been covered. For example, an assessment of tick distribution across different biological zones indicated that *Hyalomma anatolicum* and *Rhipicephalus microplus* are the two most common tick species infesting ruminants in Pakistan (Zeb *et al*., 2019).

1.8: Ticks and Host

Ticks are both carriers and true repositories of the bacteria, and they can horizontally spread it to other healthy hosts or to their offspring by transovarial and transestadial transmission. (Hurtado & Giraldo, 2018). Ticks regularly consume blood, yet there are long intervals between meals. When they bite their hosts, they damage the tissues of the animals at their feeding site by inducing itchiness, swelling, or sensitization (Taylor *et al*., 2015). Traditionally, ticks prefer the groin area and external genitals because they have thin, delicate epidermis and abundant blood flow. (Hurtado & Giraldo, 2018). Blood loss from tick infestations can cause anemia. The host's immune response is lowered, and stress is produced when a tick bites it to feed, which affects productivity. As a result, there is a decline in the output of meat and milk, an upsurge in sickness, and in many cases fatalities, in addition to the consequent financial damage brought on by the manufacturers' costs for management and controls. The commercial value of skin with the condition drops. Sores caused by tick bites may develop into isolated inflammation, bacterial infections later on, or an infestation of flies enticed to areas with blood. (Eskezia & Desta, 2016). *Ixodes rubicundus*, *Dermacentor andersoni*, and *Ixodes holocyclus*, among others, have paralyzing poisons in their saliva that is able to behead vulnerable animals (Hurtado & Giraldo, 2018).

1.9: Ticks History

Ticks are regarded as parasites in Aristotle's classic book "Historia Animalium," and it is mentioned that ticks are created from plants and differentiated parasites (Durrani *et al*., 2008). Ticks were first discovered in the Cretaceous period (146-66 million years ago) according to the fossil record. Tick species had rapid spread and evolution throughout the tertiary period (65-5 million years ago) (Fuente, 2003). Ticks' origins, however, are most likely pre-middle Cretaceous, given the two major lineages of ticks, Argasidae and Ixodidade, were substantially differentiated in the middle Cretaceous. Being premature, the original hosts were most likely amphibians or reptiles (Nava *et al*., 2009).

1.10: Ticks’ morphology

Many tick larvae are now unable to be recognized as species, therefore their medical veterinary significance, especially their role in pathogen transmission, remains unknown. Ticks of all stages (larvae, nymphs, adult males, and females) have specific physical characteristics that allow them to be reliably identified. Ticks of all stages and species can be identified by morphological characteristics of the capitulum (including the hypostome), leg coxae, and scutum (Coley, 2015). Scutum, which covered the entire dorsal surface of the male but only a portion of the female, is the main physical characteristic of the hard tick. While it is absent from the Argasidae family and has a leathery body (Ismael & Omer, 2021).

1.11: Life cycle

Ixodid ticks go through four developmental stages: egg, larva, nymph, and adult. Depending on how many hosts they feed on during their life cycle, ixodid ticks are classed as one-, two-, or three-host species (Yang *et al*., 2022). The availability of hosts is the most important element influencing tick populations. Fewer than a dozen of the world's 700 hard tick species (family Ixodidae) are one-host ticks, the majority of which are Boophilus or the similar genus Margaropus. The remaining 99% are three-host ticks, which means that each of the three feeding stages, larva, nymph, and adult, feeds on a separate individual host (Alkishe *et al*., 2021). Only adult ticks can reproduce, either on the host or on the ground before they go in search of a host. After mating, the male perishes, while the female perishes after laying eggs. In most situations, a tick will change hosts at each of these stages of its life cycle. Depending on how many hosts a tick feeds on, their life cycles can be three hosts, two hosts, or one host (3 meals and 2 molts) (Ndongo *et al*., 2022).

1.12: Genus Hyalomma

This genus contains species that range in size from medium to big and infect wild mammals, domestic animals, and birds. Geographically, the Middle East, Asia, and North Africa are the most affected. Most of these tick species have been documented from non-endemic areas such as the United Kingdom, Austria, Poland, Germany, and France, whereas immature stages of these species were discovered in migrating birds, adults were discovered in dogs, horses, people, and sheep (Lebon *et al*., 2021). The genital opening is typically narrowly U-shaped, the adanal plates are straight, the legs have yellow color strips, and the cervical grooves are deep (Apanaskevich *et al*., 2010). Because of their vector potential for livestock and impact on public health, *Hyalomma* ticks are one of the most important disease-transmitting genera of Ixodidae (Zaheer *et al*., 2022). Identification features include narrow U-shaped genital apertures, straight adanal plates, yellow color streaks on the legs, and deep cervical grooves (Apanaskevich *et al*., 2010).

1.13: *Hyalomma marginatum*

Hyalomma marginatum is a tick with two hosts. Larvae consume small to medium-sized animals. Adult ticks parasitize a second host, primarily wild and domestic ungulates, after molting nymphs, which normally remain and feed on the same host. It may occasionally infest dogs, cattle, and people (Valcarcel *et al*., 2010). These ticks are quite active and primarily exophilic, utilizing the 'hunting' method to find their host. Ticks of *H. marginatum* can endure a wide range of relative humidity, from dry to humid circumstances (Shyma *et al*., 2021).

1.14: *Hyalomma anatolicum*

A multi-host tick called *Hyalomma anatolicum* is a common vector of various illnesses that are dangerous to both biomedical science and human health. There are about 10 different species of Hyalomma multi-host ticks known to exist in India, the most prevalent of which is *Hyalomma anatolicum*. Additionally, the crucial blood parasite Theileria annulata in cattle is transmitted mostly by *Hyalomma anatolicum*. The virus that causes Crimean-Congo Hemorrhagic Fever (CCHF) in humans has been linked to this species, according to reports from several nations. (Shyma *et al*., 2021).

1.15: Life Cycle of genus Hyalomma

Adult Hyalomma spp. oviposition occurs after mating on the host body. The larvae look for their first meal in their hosts (small animals and birds) (hematophagy). Depending on the species, larvae remain connected to the same host for engorgement and molt in the same location on the host body (two host ticks) or molt to drop off (three-host ticks). In the case of two-host ticks, the nymphs began to feed on the same host, while in the case of three-host ticks, they began to attach to a new host. After being fed and engorged, all species leave their host and mature into adults. Adults will then look for large hosts for their hematophagy. After feasting on their host blood, the adult females' ticks drop off and look for a good location for their eggs hatching. Pathogenic agents are transmitted between tick species and hosts during the life stages. Humans beings can become infected by the bite of a pathogenic tick or through infected animal bodily fluid as shown in figure 1 (Bente *et al*., 2013).

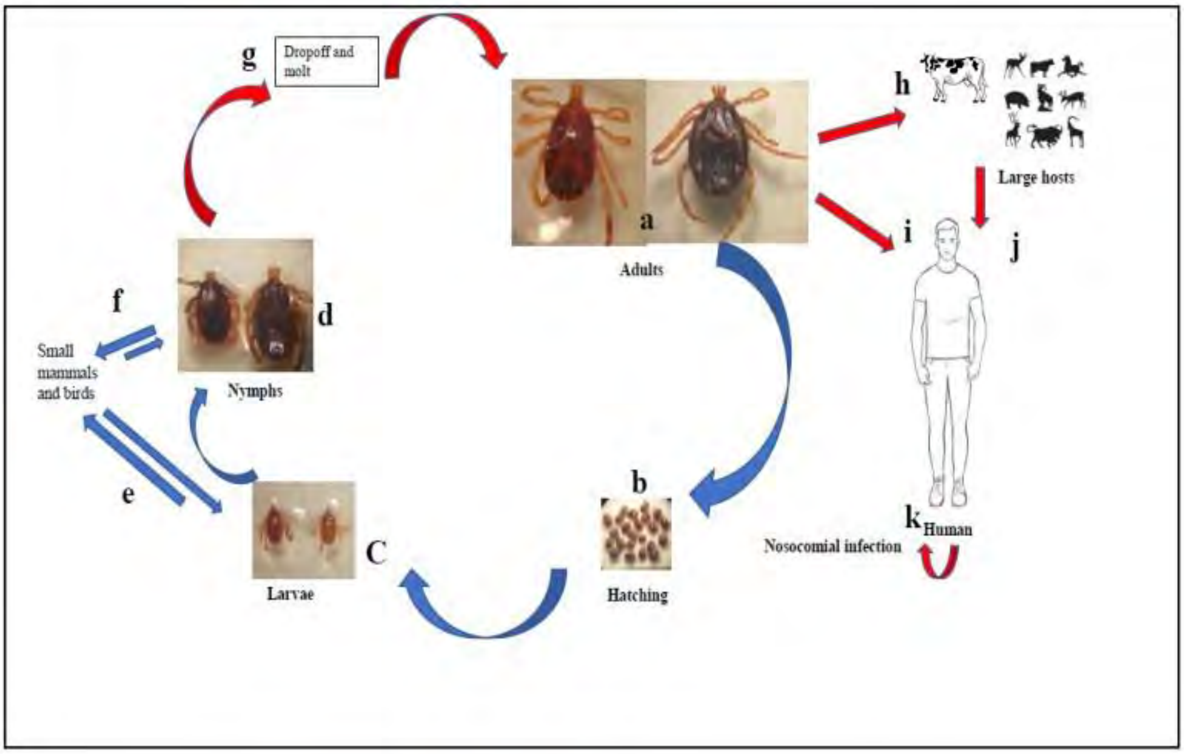

Fig (1.1): Life cycle *Hyalomma Spp*, with the horizontal and vertical transmission of pathogenic agent

1.16: Genus *Rhipicephalus*

This genus has approximately eighty-four species, many of which are medically and veterinary significant, and act as a vector for numerous pathogens such as Ehrlichia Canis, Rickettsia conorii, and others. Because of the physical resemblance, correctly identifying members of such a genus is a challenging undertaking, especially for inexperienced taxonomists. Numerous illnesses, including Crimean-Congo hemorrhagic fever, Lyme disease, anaplasmosis, east coast fever, and Rocky Mountain spotted fever, are caused by this genus. (Latrofa *et al*., 2013).

1.17: *Rhipicephalus microplus*

The cattle tick, *Rhipicephalus microplus*, is the most important tick of livestock, causing enormous economic losses. *R. microplus* is a hematophagous arthropod that is found worldwide in tropical and subtropical climates. It is monogenic, feeds mostly on cattle, and is the major tick for livestock, causing enormous economic losses (Pereira *et al*., 2022). *Rhipicephalus microplus* infests cattle raised for meat and milk. Aside from the direct impact of tick feeding on victims, this species serves as a vector of infections that cause babesiosis and anaplasmosis (Shakya *et al*., 2022).

The livestock industry has suffered enormous financial losses as a result of *Rhipicephalus microplus* (USD 22-30 billion). Because it is believed that *Rhipicephalus microplus*, *Hyalomma anatolicum*, Hyalomma hussaini, and *Rhipicephalus annulatus* are responsible for the transmission of Crimean Congo hemorrhagic virus disease in Pakistan, these species have zoonotic relevance. (Iqbal *et al*., 2020). *Rhipicephalus microplus* is the tick with the greatest economic impact due to its extensive dispersion, vector capacity, blood-sucking activities, and the number of cattle it affects. Because of their remarkable versatility and capacity for reproduction, ticks of the genus *Rhipicephalus* have been able to disperse throughout the planet. In areas where these parasites pose a problem, there are about a billion cattle. The transmission of infections including *B. bovis*, *Anaplasma marginale*, and *Babesia bigemina*, as well as the clinical condition known as "tick fever" is attributed to the most prominent cattle tick in the world, *Rhipicephalus microplus*. (Hurtado & Giraldo., 2018).

1.18: Life Cycle

The teletropic life cycle of the cow tick *Rhipicephalus microplus* (a single-host cycle). R. microplus lives primarily on the host. Female ticks (completely engorged) fall off the host and lay eggs before dying. The immature larvae can survive for up to 15 days before finding a suitable host. After acquiring the host, the parasitic stage of their life cycle begins and progresses through three stages: larvae, nymphs, and adults. Except for adult males, all three stages are hematophagous. *R. microplus* can reproduce three to four times each year (Jain *et al*., 2020) as shown in figure 1.1.

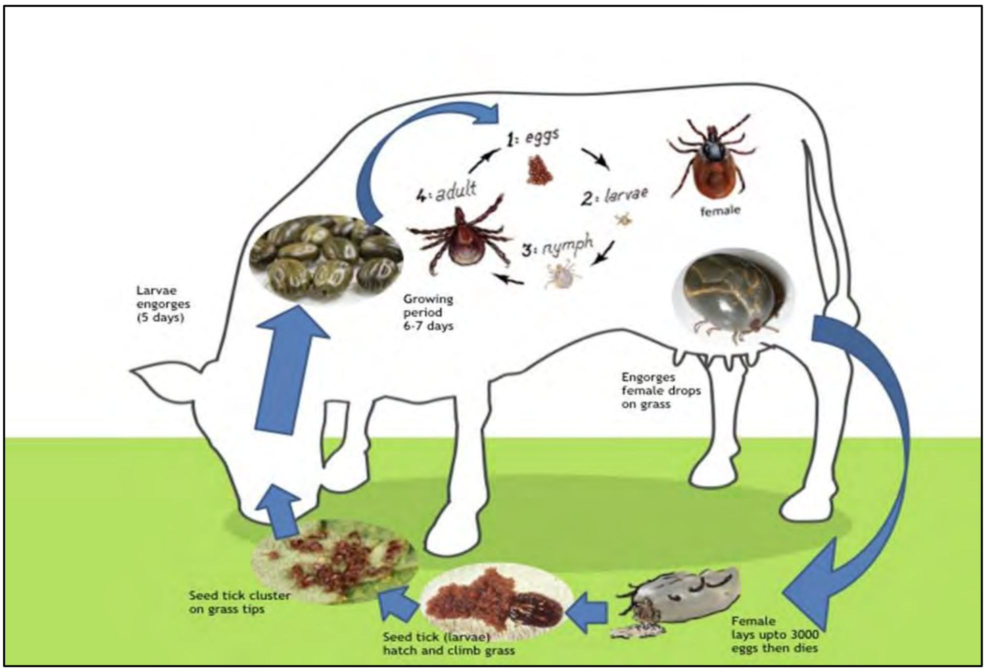

Fig 1.2: Life cycle of *Rhipicephalus microplus.*

Aim and Objectives

Aim

The aim of this study was to identify Ixodidae family ticks in the cattle population morphologically and molecularly, to evaluate their spatiotemporal distribution and associated animals' risk factors.

Objectives

The Objectives of the present study were:

- To find out the biogeographical distribution of cattle ticks in Swat, Khyber Pakhtunkhwa, Pakistan, and their association with risk factors.
- To identify hard ticks morphologically and molecularly by using specific genes.

MATERIALS AND METHODS

2.1: Study area

Northern Khyber Pakhtunkhwa's Swat valley is encircled by soaring mountains. Indus River separates it from Hazara in the east, while Chitral and Gilgit are to the north, Dir is to the west, and Mardan is to the south. Based on physical characteristics, Swat is separated into two geographical zones: mountain ranges and plains. The seven tehsils of Swat are Babuzai, Barikot, Kabal, Matta, Charbagh, Khwazakhela, and Bahrain. The number of union councils that make up each tehsil is further divided (Rasool *et al*., 2018).

2.1.a: Geography

The 5,337 km^2 Swat Valley is situated between 34°-40′ and 35° N latitude and 72′ to 74°-6′ E longitude. The Hindukush Mountain range's hills surround it in a nest. Swat is bordered by Chitral, Upper Dir, and Lower Dir in the east, Buner and Kohistan in the south, and Gilgit-Baltistan in the north. In 1991, the southern tehsil of Buner was declared an independent district. Mountain ranges surround the Swat Valley, which is in Northern Khyber Pakhtunkhwa (Ullah & Zahid, 2022).

Swat is physically separated into two parts.

I) Mountainous Series

II) Plains

2.1.b: Habitats and environmental condition

Climate

The weather in Swat is affected by all climatic factors, including ", latitude, and rain-bearing winds," because it is in the temperate region of the Northern Mountain ranges of the Indo-Pak Subcontinent. During the summer, monsoon rains arrive in Swat. Due to cyclonic currents from the Mediterranean Sea, it is magnificent throughout the winter (Ullah & Zahid, 2022).

Temperature

The altitude of Swat, which runs from 2500 feet to 7500 feet, results in a cooler territory in terms of temperature. Because there isn't a meteorological component, we can't determine the actual documented data. January is the chilliest month of the year in District Swat. Normally, the temperature hovers between 2°C and -2°C. The very last week of December to the end of February is the water-freezing season. In hilly locations, it takes longer than three months. The hottest months are July and August, with lowland temperatures exceeding 39°C during those times. But in steep areas, it is lower. Consequently, the region is home to high ranges of mountains, hills, plains, riversides, woods, marshes, fields, and grassy terrain, all of which are abundant in biodiversity (Ullah & Zahid, 2022).

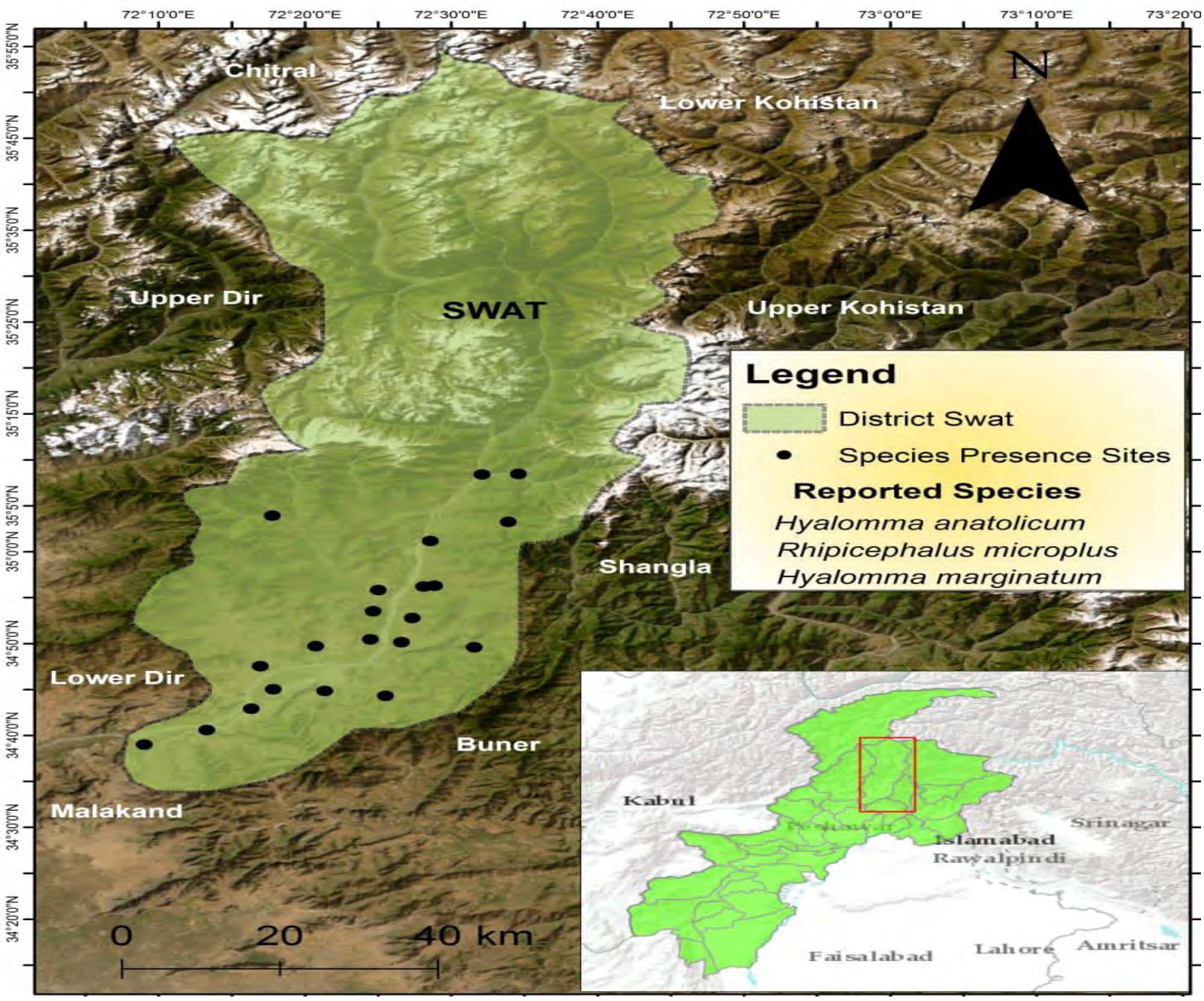

Fig 2.1 Map of Khyber Pakhtunkhwa shows the selected regions of our collection.

2.2: Ticks Collection and Preservation

A total of 770 cattle were selected randomly from different villages in seven tehsils of District Swat for the presence of tick infestation for a period of eight months with effect from March to October 2022. The animals' entire body, including their skin, as well as their head, belly, back, udder/scrotum, genital regions, leg, and tail, were meticulously studied. With the aid of forceps and hand-picking adult ticks and ticks in various phases of development were removed from infected animals and placed in separate glass bottles containing 70% alcohol for preservation. Each tube was labeled with the date, location, bodily parts, sex, season, living and health condition and age of the animal. By hand picking, ticks were removed from various body sections of individual cattle. Ticks were cleaned with distilled water, followed by 70% ethanol, to remove pollutants and tissues from their bodies. Finally, the specimens were kept in 100% ethanol for future research.

2.3: Morphological Identification of Ticks

Tick specimens were identified using established taxonomic keys based on morphological traits using a stereo zoom microscope (SZ61, Olympus, Tokyo, Japan) (Khan *et al*., 2022). Tick species were recognized morphologically using hard tick morpho-taxonomic features. Using a stereomicroscope and a standard identification key, the morphological traits were photographed (Walker, 2003). For further molecular identification, the specimens were preserved in 100% absolute ethanol.

2.4: Statistical analysis

Microsoft Excel 2019 was used to compile and manage the raw data. The difference between two distributions (i.e., non-infested and tick-infested animals) was calculated using the chi-square test, and $p < 0.05$ was deemed a significant level across groups. Tick prevalence data was compiled based on the season, breed, age, sex, and different body sections of the cattle. According to accepted techniques (Thrusfield, 2007), the prevalence (P) was calculated using the following formula:

$$P = \frac{Number\ of\ infested\ cattle\ during\ specific\ time\ period}{total\ number\ of\ cattles\ surveid} \times 100$$

2.5: Molecular identification by the phenol-chloroform method

A polymerase chain reaction (PCR) was used to confirm the species-level identification of the tick species detected morphologically.

(a): DNA Extraction

The genomic DNA of ticks was extracted using the phenol-chloroform method. Ticks were ground and cut in 1.5ml Eppendorf tubes using an autoclaved mortar and piston. A volume of 400µl buffer solution A was added (tris HCl, $MgCl_2$, sucrose, triton 100X). The vertex samples are then centrifuged for 1 minute at 13000rpm. The upper layer was discarded after centrifugation, and the preceding steps were repeated. After discarding the upper layer, add 400µl buffer solution B (NaCl, tris HCl, and EDTA) and 17µl 20% SDS was added and spined for 30 seconds at 4000rpm. The samples were then treated with 4.5µl protein kinase and stored at 37 °C overnight. On the next day of incubation, add 500mL of buffer solution C+D (beta-mercaptoethanol, phenol, and distal water iso-amyl alcohol and chloroform) was added to each sample and thoroughly mix. The tubes were then centrifuged at 13000rpm for 10 minutes to generate layers; collect the upper layer in already prepared eppendorf tubes without disturbing the lower layer to avoid contaminants. A volume of 500µl of solution D was then added to each upper layer and centrifuge for 10 minutes at 13000rpm. Layers were produced again, and the upper layer will be collected in new, labeled eppendorf tubes. Now, in each tube, add 55µl of sodium acetate and 450µl of isopropanol and gently shake, after the appearance of DNA threads, centrifuge for 10 minutes at 13000rpm, discard the materials, and the pellet remained at the bottom of the tubes. Pellet was washed with 200µl ethanol (70%) and centrifuge at 13000rpm for 5 minutes. After centrifugation, ethanol was carefully removed and then at 45 °C dry the tubes in an incubator for 40 minutes. Finally 50µl autoclave water was added and incubate for 30 minutes at 37°C before storing in a fridge. The sample was run in 1% agarose gel for confirmation.

2.6: Polymerase chain reaction and detection of targeted DNA

(b): Agarose gel preparation

To make 2% agarose gel, 1.2 gram of agarose was obtained and poured into a beaker with 1X Tris-borate-EDTA buffer (60ml). A hot plate stirrer was used to thoroughly mix the beaker containing agarose + distal water. The agarose gel was then cooled before adding 4µl ethidium bromide and gently mixing with a micropipette tip before being allowed to cool further. The agarose gel was then put into a combed tray that had already been prepared and allowed to settle. After cooling for 30 minutes, gently remove the comb and insert the gel in a gel electrophoresis tank containing TBE buffer at 120V for 40 minutes. A gel documentation machine was used to examine the PCR bands.

(c): PCR amplification

Throughout the experiment, the following 20µl of reaction mixture were used.

Forward primer = 1µl,

Reverse prier = 1µl

Master mix = 12µl

PCR water = 4µl

Template DNA = 2µl

To avoid contamination, the PCR reaction mixture was done in an ice container in a biosafety cabinet. The primer sequences were generated using the primer-blast NCBI program, and the parameters of the primers (GC content and melting temperature) were evaluated using the PCR-Primer stats tool. The PCR was performed using a manual thermocycler (kyratec SC300). The primers of the 460-bp fragment(16S+1=CCGGTCTGAAACTCAGTCAAGT),(16S-1= GCTCAATGATTTTTAAATTGCTGT) of tick's species were used for amplification of the 16s rRNA gene under the following conditions: Initial denaturation was carried out at 94.0°C for 2:30 minutes, annealing at 54.0°C for 0.30s, extension at 72.0°C for 0.45s, post cycling extension at 72.0°C for 7 minutes, and final hold at 4°C. The thermocycler's top heater was set to 105 (deg C) for this reaction, and the reaction was completed in 34 cycles. For the amplification of the ITS2 region primers of the 800-bp segment were employed. (ITS2+1=CCATCGATGTGAATGCAGGACA),(ITS21=GTGAATTCTATGCTTAAATCA GGGGGT). The total PCR reaction took 35 cycles, with the following conditions for such primers: initial denaturation at 95.0°C for 2:45 minutes, annealing temperature and time at 55.0°C for 1 minute, extension at 72.0°C for 1.5 minutes, and post cycling extension at 72.0°C for 7.00 minutes. For amplification of the COX1 area, primers of 800bp segment COX1+1= CCGGTCTGAACTCAGATCAAG), COX1-1= (TCAATGATTTTTAAATTGCTGT) were used. The complete PCR process took 34 cycles, with initial denaturation at 95.0°C for 5 minutes, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and final elongation at 72°C for 5 minutes. The PCR process took 2 hours and 15 minutes to complete. After the PCR reaction was completed, the sample tubes were removed and placed on an ice container to protect the product from harm during the following phase (gel electrophoresis). To check the PCR product, 2.5µl was taken from each sample and combine with 2.5µl loading dye before loading directly on agarose gel wells. 5µl of DNA gene ruler was loaded into one well for measuring amplicon size in base pairs. For 40 minutes, a 400-ampere current was applied to the gel electrophoresis equipment at 120V. The dye front was used to track the movement of samples. Following completion, the gel was carefully taken from the gel tank and placed on gel documentation system for band analysis.

RESULTS

A total of 770 cattle were examined, of which male and female cattle accounted for (46.1%) and (53.8%). The present study is indicating the maximum ratio of females (53.8%). The overall prevalence of tick infestation was 72.8% in cattle of district Swat. The only predominant tick species found were *Rhipicephalus microplus* (40.78%), *Hyalomma anatolicum* (31.93%) and *Hyalomma marginatum* (27.37%). The present study revealed tick infestation was higher in female cattle 11-15 years age group (19.09%) than 6-10 years (11.95%) than 1-5 years (10%) and <1 year (7.27%), and in male cattle of age group11-15 years (11.03%), than 6-10 years (5.71), than 1-5 years (4.81%) and <1 year (2.99%).

3.1: Distribution of ticks in tehsil Babuzai

In the current study, three villages were studied from tehsil Babuzai: Saidu Sharif, Odigram and Kokarai. The altitude, longitude, and latitude of the village Saidu Sharif are 3106, 72°21'23.52"E, and 34.44'55.28"N respectively. Ticks collected from Saidu Sharif were *H. anatolicum* (n=17), *Rh.* microplus (n=16) and *H. marginatum* (n=14).The altitude, longitude, and latitude of the village Odigram are 2906, 72.17547"E, and 34.45'9.51"N. Ticks collected from Odigram were *H. anatolicum* (n=31), *Rh. microplus* (n=18) and *H. marginatum* (n=22).The altitude, longitude, and latitude of the village Kokarai are 3596, 72.25302"E, 34.44'19.36"N. Ticks collected from Kokarai are *H. anatolicum* (n=20), *Rh. microplus* (28) and *H. marginatum* (n=18). The most prevalent specie of the tehsil was *H. anatolicum* (3.07%) followed by *Rh. microplus* (2.88%) and *H. marginatum* (2.44%) as shown in table 3.1 and figure 3.1.

Table 3.1: Ticks' distribution in tehsil Babuzai

Tehsil		**Babuzai**			**Percentage %**
Villages		**Saidu Sharif**	**Odigram**	**Kokarai**	
Species					
H. anatolicum	M	10	10	7	3.07
	F	4	16	9	
	N	9	5	4	
Rh. microplus	M	5	3	6	2.89
	F	4	6	10	
	N	17	9	12	
H. marginatum	M	6	15	3	2.44
	F	4	2	12	
	N	19	5	3	
Total		47	71	66	

Note: M=male, F=female, N=nymphs.

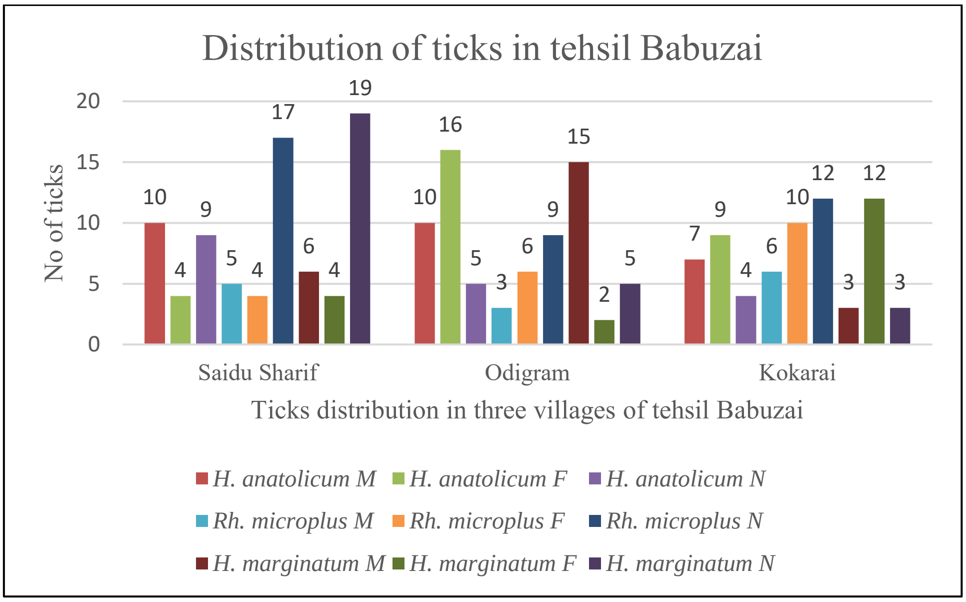

Figure 3.1: Hard ticks' distribution in tehsil Babuzai.

3.2: Distribution of ticks in Tehsil Barikot

In the current study, three villages were studied from tehsil Babuzai: Kota, Manyar and Barikot.. Ticks collected from Kota were *H. anatolicum* (n=42), *Rh. microplus* (n=33) and *H. marginatum* (n=27).The altitude, longitude, and latitude of the village Manyar are 2768, 72°16'20.46"E and 34.42'55.27"N. Ticks collected from Manyar were *H. anatolicum* (n=48), *Rh. microplus* (n=29) and *H. marginatum* (n=33).The altitude, longitude, and latitude of the village Barikot are 3626, 72°12'45.69"E, and 34.39'24.49"N respectively. Ticks collected from Barikot were *H. anatolicum* (n=24), *Rh. microplus* (36) and *H. marginatum* (n=26).The most prevalent specie of the tehsil was *H. anatolicum* (5.14%) followed by *Rh. microplus* (4.43%) and *H. marginatum* (3.89%) as shown in table 3.2 and figure 3.2.

Table 3.2: Ticks' distribution in tehsil Barikot

Tehsil		**Barikot**			**Percentage %**
Villages		**Kota**	**Manyar**	**Barikot**	
Species					
H. anatolicum	M	6	7	5	5.14
	F	23	34	8	
	N	13	7	11	
Rh. microplus	M	11	5	6	4.42
	F	12	7	21	
	N	10	17	9	
H. marginatum	M	3	6	4	3.89
	F	11	18	16	
	N	13	9	6	
Total		102	110	86	

Note: M=male, F=female, N=nymphs.

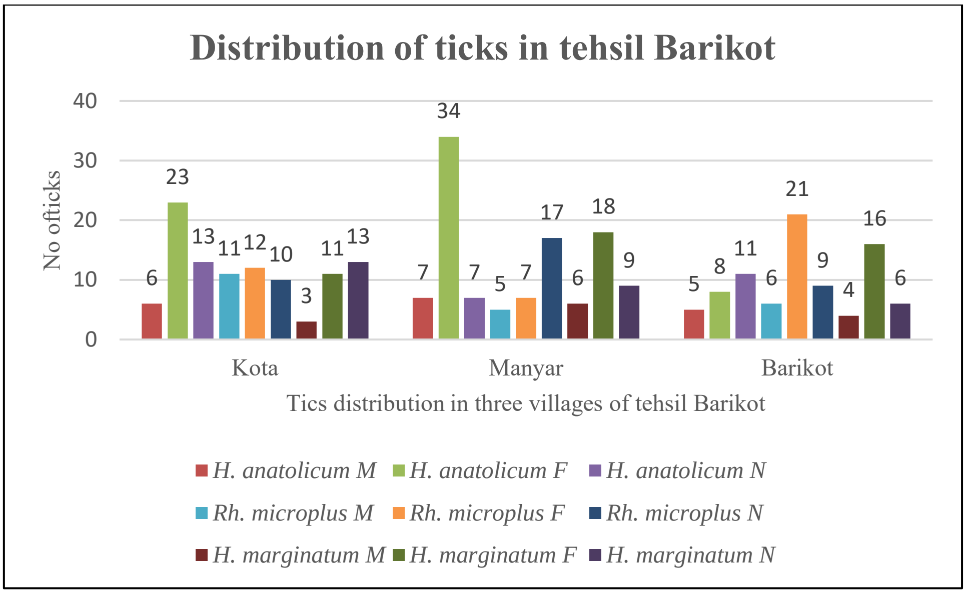

Figure 3.2: Hard ticks' distribution in tehsil Barikot.

3.3: Distribution of ticks in tehsil Kabal

In the current study ticks collected from Kanju were *H. anatolicum* (n=20), *Rh.* microplus (n=20) and *H. marginatum* (n=29).The altitude, longitude and latitude of the village Kabal are 2991, 72°16'57.07"E, 34.47'32.22"N.Ticks collected from Kabal were *H. anatolicum* (n=19), *Rh. microplus* (n=27) and *H. marginatum* (n=25).The altitude, longitude, and latitude of the village Ningolai are 3490, 72°24'29.02"E, and 34.50'28.27"N respectively. Ticks collected from Ningolai were *H. anatolicum* (n=23), *Rh. microplus* (26) and *H. marginatum* (n=29). The most prevalent specie of the tehsil was *Rh. microplus* (3.38%) followed by *H. marginatum* (3.74%) and *H. anatolicum* (2.89%) as shown in table 3.3 and figure 3.3.

Table 3.3: Ticks' distribution in tehsil Kabal

Tehsil		Kabal			Percentage %
Villages		Kanju	Kabal	Ningolai	
Species					
H. anatolicum	M	5	3	10	2.89
	F	10	9	4	
	N	5	7	9	
Rh. microplus	M	5	8	5	3.38
	F	7	13	4	
	N	8	6	17	
H. marginatum	M	14	8	6	3.74
	F	2	14	4	
	N	13	3	19	
Total		69	71	78	

Note: M=male, F=female, N=nymphs.

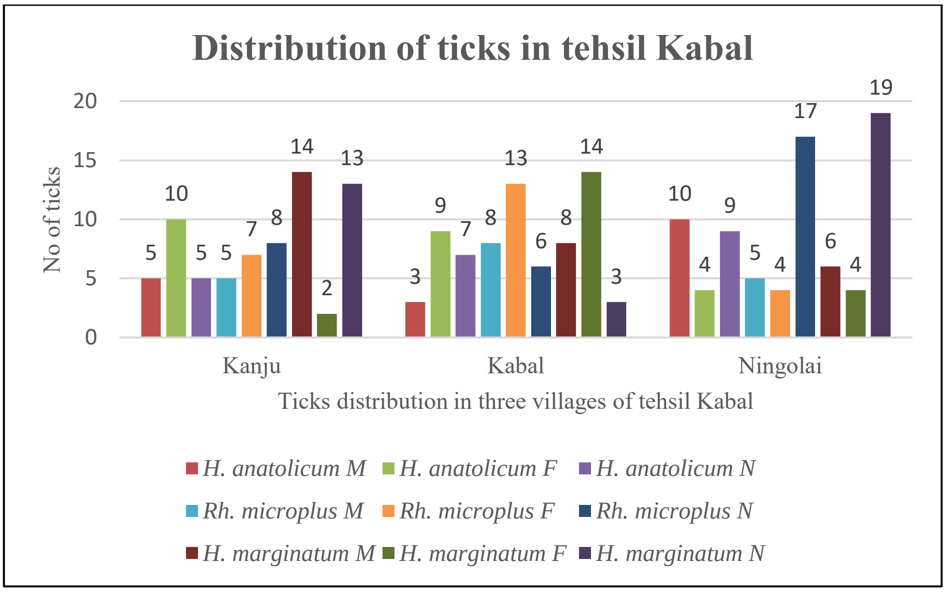

Figure 3.3: Hard ticks' distribution in tehsil Kabal

3.4: Distribution of ticks in Tehsil Matta

In the current study, three villages were studied from tehsil Matta: Sherpalam, Matta and Biha. Ticks collected from Sherpalam were *H. anatolicum* (n=30), *Rh.* microplus (n=36) and *H. marginatum* (n=36). Ticks collected from Matta were *H. anatolicum* (n=49), *Rh. microplus* (n=58) and *H. marginatum* (n=26). Ticks collected from Biha were *H. anatolicum* (n=10), *Rh. microplus* (136) and *H. marginatum* (n=28). The most prevalent specie of the tehsil was *Rh. microplus* (10.37%) followed by *H. marginatum* (4.05%) and *H. anatolicum* (4.01%) as shown in table 3.4 and figure 3.4.

Table 3.4: Ticks' distribution in tehsil Matta

Tehsil		**Matta**			**Percentage %**
Villages		**Sherpalam**	**Matta**	**Biha**	
Species					
H. anatolicum	M	7	14	4	4.01
	F	11	19	6	
	N	12	16	0	
Rh. microplus	M	16	14	87	10.3
	F	10	22	13	
	N	10	22	36	
H. marginatum	M	10	8	7	4.05
	F	13	9	10	
	N	13	9	11	
Total		102	133	174	

Note: M=male, F=female, N=nymphs.

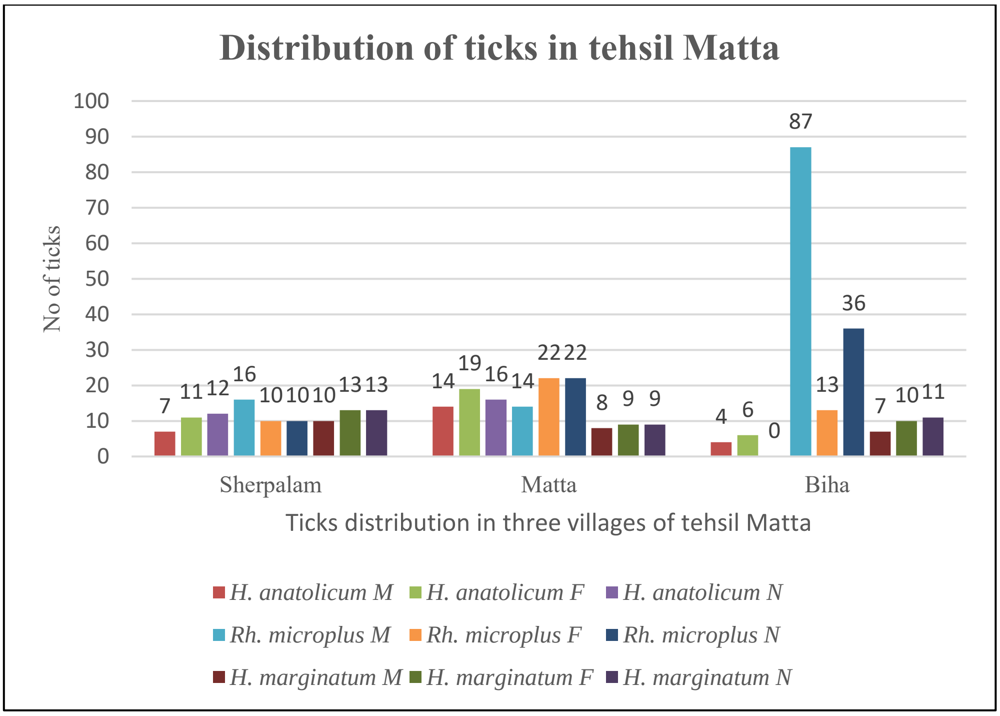

Figure 3.4: Hard ticks' distribution in tehsil Matta.

3.5: Distribution of ticks in Tehsil Charbagh

In the current study, three villages were studied from tehsil Matta: Charbagh, Gulibagh and Taligram. The altitude, longitude, and latitude of the village Charbagh are 3381, 72°26'36.84"E and 34.52'49.08"N respectively. Ticks collected from Charbagh were *H. anatolicum* (n=29), *Rh. microplus* (n=27) and *H. marginatum* (n=23).The altitude, longitude, and latitude of the village Gulibagh are 3946, 72°27'19.45"E, and 34.52'49.08"N respectively. Ticks collected from Gulibagh were *H. anatolicum* (n=21), *Rh. microplus* (n=65) and *H. marginatum* (n=56).The altitude, longitude, and latitude of the village Taligram are 4284, 72°31'35.03"E ,and 34.49'35.82"N respectively. Ticks collected from Biha were *H. Anatolicum* (n=34), *Rh. microplus* (36) and *H. marginatum* (n=20). The most prevalent specie of the tehsil was Rh. microplus (5.77%) followed by Hy. marginatum (4.46%) and Hy. anatolicum (3.78%) as shown in table 3.5 and figure 3.5.

Table 3.5: Ticks' distribution in tehsil Charbagh

Tehsil		Charbagh			Percentage %
Villages		Charbagh	Gulibagh	Taligram	
Species					
H. anatolicum	M	14	4	14	3.78
	F	8	4	6	
	N	7	13	14	
Rh. microplus	M	16	47	12	5.78
	F	2	7	14	
	N	9	11	10	
H. marginatum	M	9	43	6	4.46
	F	3	6	9	
	N	11	7	5	
Total		79	142	90	

Note: M=male, F=female, N=nymphs.

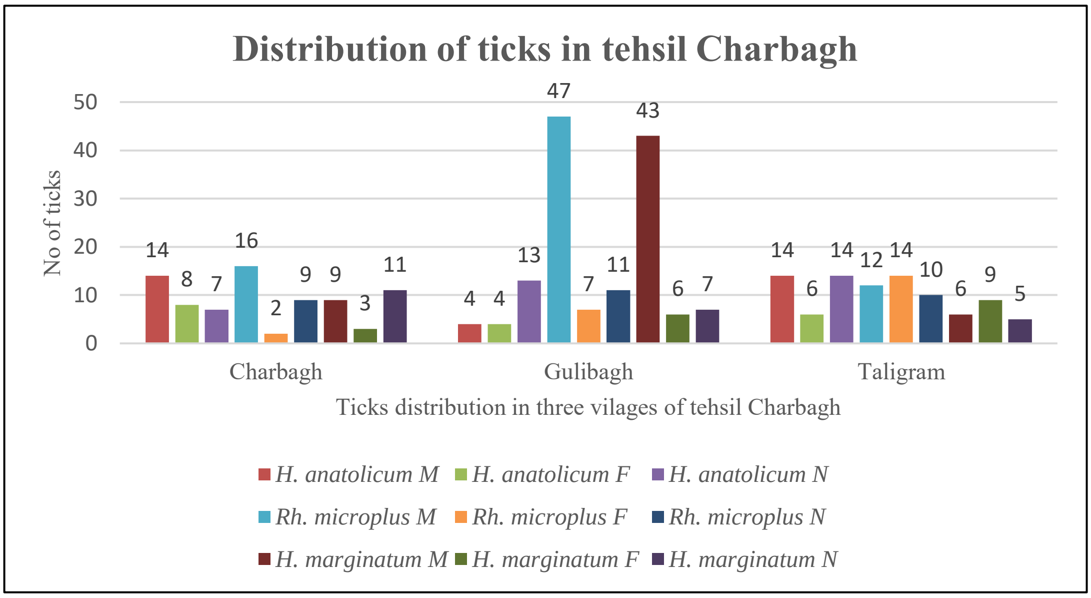

Figure 3.5: Hard ticks' distribution in tehsil Charbagh.

3.6: Distribution of ticks in Tehsil Khwazakhela

In the current study, three villages were studied from tehsil Khwazakhela: Khwazakhela, Shin and Shalpin. The altitude, longitude, and latitude of the village Khwazakhela 3740, 72°28'7.37"E, and 34.56'13.42"N respectively. Ticks collected from Khwazakhela were *H. anatolicum* (n=51), *Rh.* microplus (n=52) and *H. marginatum* (n=52).The altitude, longitude and latitude of the village Shin are 4561, 72°28'34.00"E, and 72.28'7.37"E respectively. Ticks collected from Shin were *H. anatolicum* (n=23), *Rh. microplus* (n=41) and *H. marginatum* (n=13**)**.The altitude, longitude, and latitude of the village Shalpin are 4481, 72°31'48.59"E and 34.56'54.33"N respectively. Ticks collected from Biha were *H. Anatolicum* (n=46), *Rh.*

microplus (68) and *H. marginatum* (n=30). The most prevalent specie of the tehsil was *Rh. microplus* (7.26%%) followed by *H. anatolicum* (5.41%) and *H. marginatum* (4.28%) as shown in table 3.6 and figure3.6.

Table 3.6: Ticks' distribution in tehsil Khwazakhela

Tehsil		Khwazakhela			Percentage %
Villages		Khwazakhela	Shin	Shalpin	
Species					
H. Anatolicum	M	16	3	13	5.41
	F	22	4	10	
	N	13	16	23	
Rh. microplus	M	11	24	9	7.26
	F	23	11	18	
	N	18	6	41	
H. marginatum	M	19	5	8	4.28
	F	26	7	13	
	N	7	1	9	
Total		155	77	144	

Note: M=male, F=female, N=nymphs.

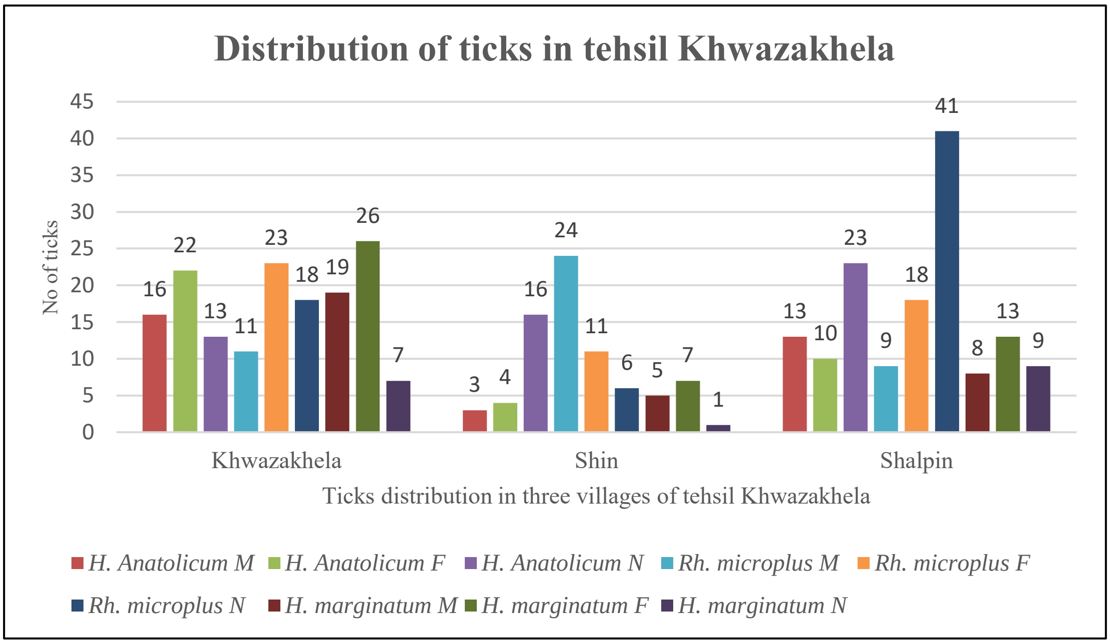

Figure 3.6: Hard ticks' distribution in tehsil Khwazakhela.

3.7: Distribution of ticks in Tehsil Bahrain

In the current study, three villages were studied from tehsil Bahrain: Madyan, Miandam and Chail. The altitude, longitude and latitude of the village Madyan are 4473, 72°32'6.98"E and 35. 8'25.48"N respectively. Ticks collected from Madyan were *H. anatolicum* (n=43), *Rh.* microplus (n=65) and *H. marginatum* (n=25). The altitude, longitude, and latitude of the village

Miandam are 6200, 72°33'53.45"E and 35. 3'14.73"N respectively. Ticks collected from Miandam were *H. anatolicum* (n=67), *Rh. microplus* (n=55) and *H. marginatum* (n=46).The altitude, longitude, and latitude of the village Chail are 5092, 72°34'36.03"E and 35. 8'28.33"N respectively. Ticks collected from Chail were *H. anatolicum* (n=61), *Rh. microplus* (32) and *H. marginatum* (n=27). The most prevalent specie of the tehsil was *H. anatolicum* (7.71%)followed by *Rh. microplus* (6.85%) and *H. marginatum* (4.42%) as shown in table 3.7 and figure 3.7.

Table 3.7: Ticks' distribution in tehsil Bahrain

Tehsil		**Bahrain**			**Percentage %**
Villages		**Madyan**	**Miandam**	**Bahrain**	
Species					
H. Anatolicum	M	11	10	13	7.71
	F	23	54	46	
	N	9	3	2	
Rh. microplus	M	35	45	19	6.85
	F	28	5	12	
	N	2	5	1	
H. marginatum	M	4	17	3	4.42
	F	7	19	11	
	N	14	10	13	
Total		133	168	120	

Note: M=male, F=female, N=nymphs.

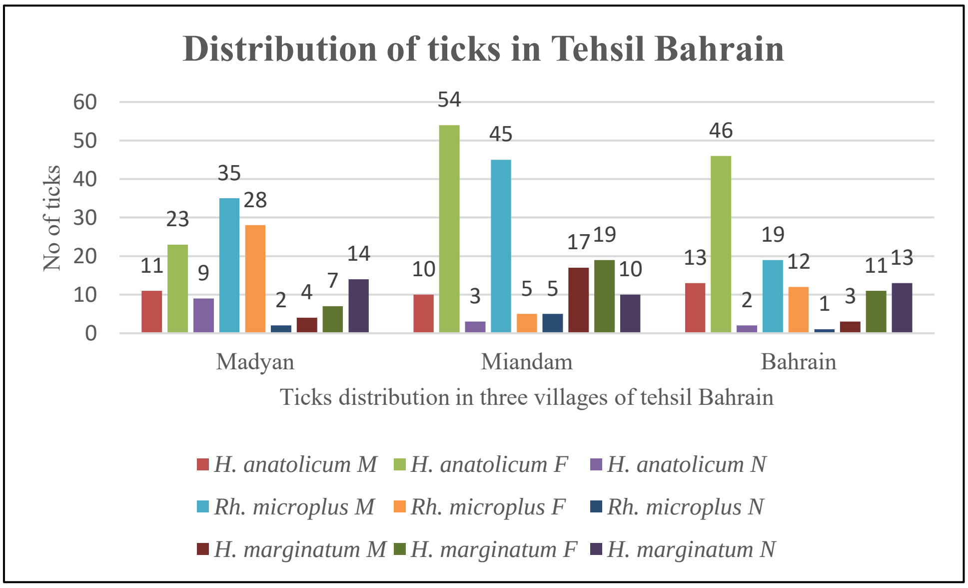

Figure 3.7: Hard ticks' distribution in tehsil Bahrain.

3.8:The overall abundance of tick species in seven tehsils

In the current study, a total of n=2217 ticks were collected from seven tehsils: Babuzai, Barikot, Kabal, Matta, Charbagh, Khwazakhela, and Bahrain of the district Swat. A high number of ticks (n=421) were collected from tehsil Bahrain. Among all tehsils, two genera of Hyalomma and Rhipicephalus were identified. Among two genera, three species; *H. Anatolicum, Hy. marginatum and Rh. microplu*s were identified. Although all three species were collected from all the tehsils, but the most occurring specie was *Rh. microplus* (40.78%) followed by *H. Anatolicum* (31.93%) and *H. marginatum* (27.29%) respectively as shown in table 3.8 and figure 3.8.

Table 3.8: Ticks' species overall abundance in 21 villages

Tehsils	Villages	*Hy. anatolicum*	*Rh. microplus*	*Hy. marginatum*	Total
Babuzai	Saidu	17	16	14	47
	Odigram	31	18	22	71
	Kokarai	20	28	18	70
Barikot	Kota	42	33	27	99
	Manyar	48	29	33	110
	Barikot	24	36	26	86
Kabal	Kanju	20	20	29	69
	Kabal	19	27	25	71
	Ningolai	23	26	29	78
Matta	Sherpalam	30	36	36	102
	Matta	49	58	26	133
	Biha	10	136	28	174
Charbagh	Charbagh	29	27	23	79
	Gulibagh	21	65	56	142
	Taligram	34	36	20	90
Khwazakhela	Khwazakhela	51	52	52	155
	Shin	23	41	13	77
	Shalpin	46	68	30	144
Bahrain	Madyan	43	65	25	133
	Miandam	67	55	46	168
	Chail	61	32	27	120
Total		708	904	605	2217
Percentage		31.93	40.78	27.29	100%

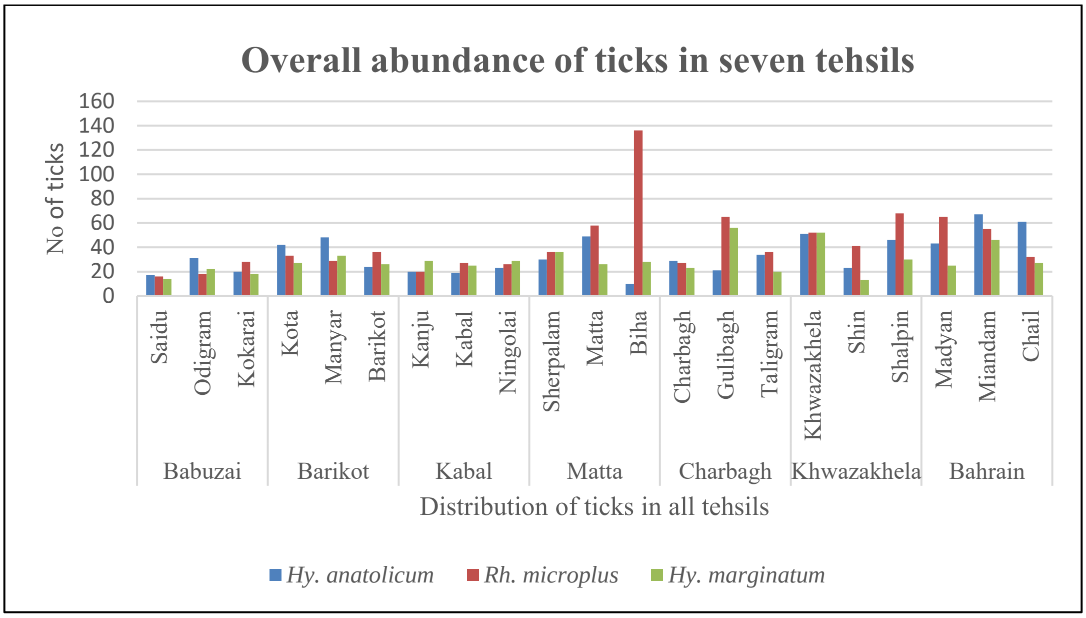

Figure 3.8: Overall abundance of ticks in seven tehsils.

3.9: Sex Factor

A total of 770 cattle were observed for tick infestation of which 415 (53.89%) were female and 355 were male (46.10%). In district Swat, a higher prevalence of tick infestation (89.64%) was recorded in females than in males (53.24%). Statistical analysis showed that tick infestation was significantly higher in female than in male animals (P <0.05) as shown in table 3.9 and figure 3.9.

Table 3.9: Sex-wise prevalence of tick infestation

Sex	Cattle examined (N)	Cattle positive (N)	Non infested (N)	Prevalence (%)	Ticks number	Chi-Square ($\chi 2$)	p-value
Male	355	189	166	53.24	909	128.186	0.0001
Female	415	372	43	89.64	1308		

*Statistical analysis. The difference of the prevalence of tick infestation in either sex groups was not statistically significant (P<0.05).

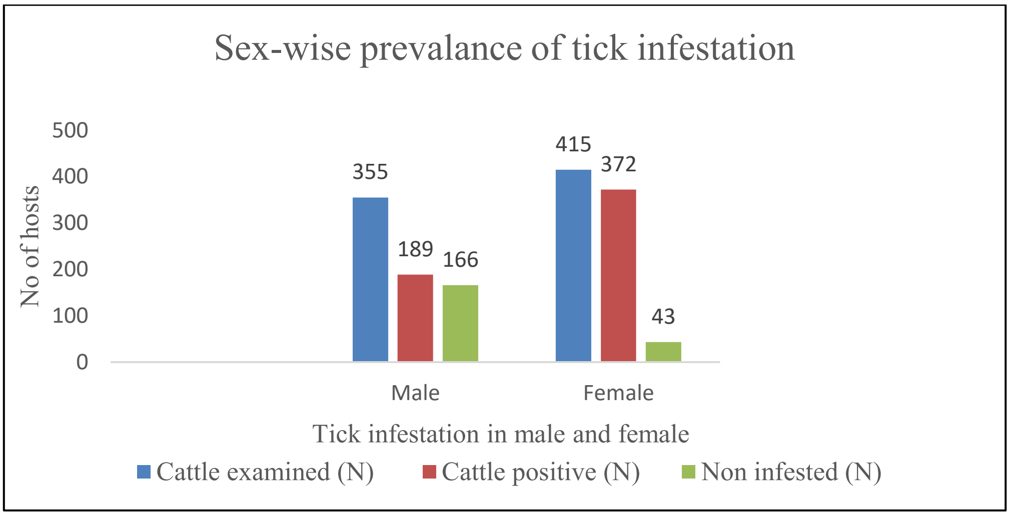

Figure 3.9: Sex-wise prevalence of tick infestation in cattle.

3.10: Gender-wise distribution of tick species

In this study, a total of 2217 ticks were collected belonging to two genera Hyalomma and Rhipicephalus of which three species were identified. Among 2217 ticks, *H. Anatolicum* were 708 (31.93), *Rh. microplus* were 904 (40.78%) and *H. marginatum* were 605 (27.28%). In *H .anatolicum*, male were (25.70%), female were (47.18%) and nymphs were (27.12%). In Rh. Microplus, male were (27.87%), female (43.03%) and nymphs (29.09%). In *H. marginatum*, male were (34.21%), female (35.54%) and nymphs (30.25%) as shown in table 3.10 and figure 3.10.

Table 3.10: Distribution of tick's gender-wise

Species	**Adults (N)**		**Nymphs (N)**	**Total (N)**	**Percentage (%)**
	Male	**Female**			
Hy. anatolicum	182	334	192	708	31.93%
Rh. microplus	252	389	263	904	40.77%
Hy. marginatum	207	215	183	605	27.28%

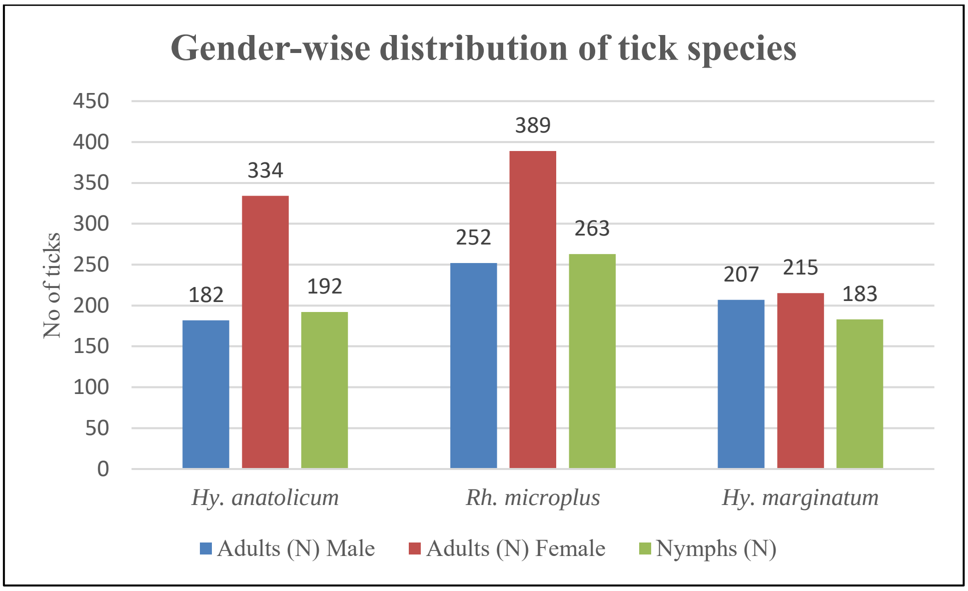

Figure 3.10: Distribution of tick's gender-wise.

3.11: Age-related differences in tick prevalence

A tick infestation was discovered in 561 out of 770 cattle. During the study, the overall prevalence (72.85%) was noted. Younger female cattle of age <1 year had the lowest tick infestation than 1-5 year (45.45%), 6-10 years (68.47%) and 11-15 years (93.89%). Similarly younger male cattle of age <1 year had the lowest (21.74%) tick infestation than 1-5 year (29.73%), 6-10 years (56.82%) and 11-15 years (74.12%) as shown in table 3.11, figure 3.11 and table 3.12 and figure 3.12. The statistical analysis revealed a difference in tick infestation prevalence (P<0.05) between age groups of cattle.

Table 3.11: Age-wise prevalence of tick infestation in female cattle.

Age group (year)		Cattle examined (N)	Cattle positive (N)	Healthy Cattle (N)	Prevalence	Ticks number (N)	X_2	p-value
Young cattle	<1	56	17	39	30.35	86	99.719	0.001
Adult cattle	1-5	77	35	42	45.45	112		
	6-10	92	63	29	68.47	498		
	11-15	147	138	9	93.88	612		

*Statistical analysis. Significant difference (P<0.05) in prevalence of tick infestation in different age groups of female cattle.

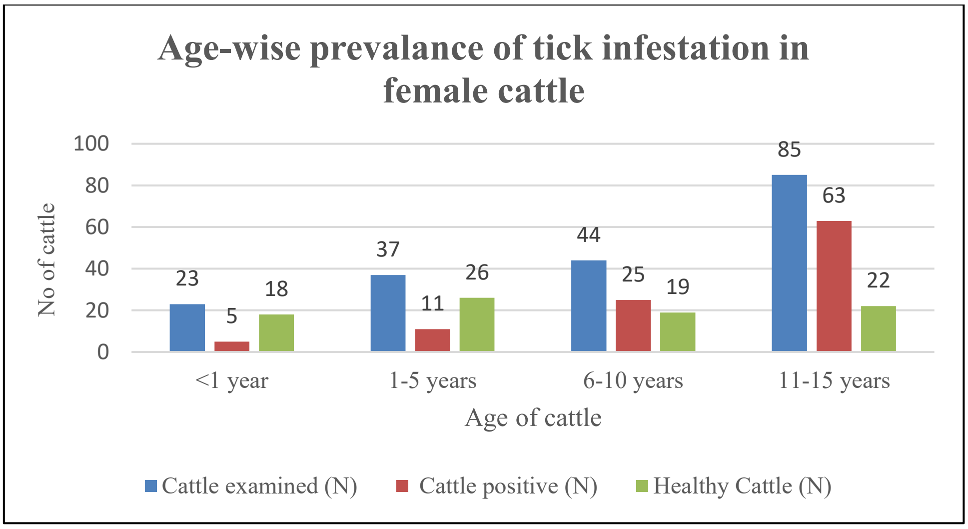

Figure 3.11: Ticks infestation in female animals age-wise

Table 3.12: Age wise prevalence of tick infestation in male cattle

Age group		Cattle examined (N)	Cattle positive (N)	Healthy cattle (N)	Prevalence (%)	Ticks number (N)	X_2	*P*-value
Young cattle	<1	23	5	18	21.74	72	32.441	0.001
Adult cattle	1-5	37	11	26	29.73	92		
	6-10	44	25	19	56.82	339		
	11-15	85	63	22	74.12	407		

*Statistical analysis. Significant difference ($P<0.05$) in prevalence of tick infestation in different age groups of female cattle.

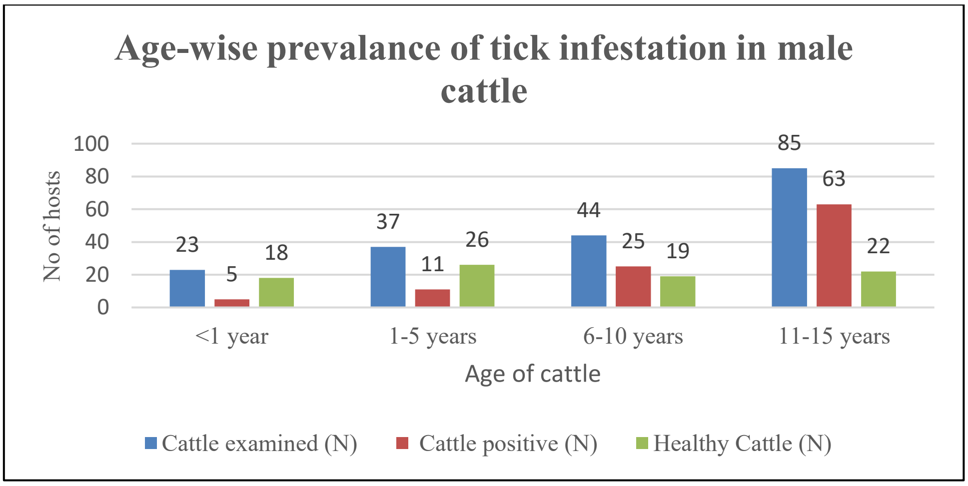

Figure 3.12: Ticks infestation in male animals age-wise.

3.12: Identification of the most prevalent tick species and their preferred places in District Swat

The present study showed that the most commonly occurring tick *Rh. microplus* (40.78%) with predilection sites shoulder, external genitalia, abdominal area, neck, and udder, followed by *H. anatolicum* (31.94%) with predilection sites external genitalia, udder, ear, and tail, while *H. marginatum* (27.29%) was noted with predilection sites, external genitalia, udder, hind legs, head, and especially neck regions as shown in table 3.13. Among these external genitalia and udder were observed in most tick-infested regions of the body.

Table 3.13: Most prevalent tick species along their preferred sites

Ticks identified	Preference sites	No of ticks	Percentage (%)
Rh. microplus	Shoulder, external genitalia, udder, neck, and abdomen	904	40.78 %
H. anatolicum	External genitalia, udder, ear and tail	708	31.94 %
H. marginatum	Head regions, hind legs and external genitalia	605	27.29%

3.12: Distribution percentage (%) of hard ticks (Ixodid) on cattle various body parts

The present study revealed that among the 561 infested cattle, the most infested body regions was external genitalia (34.93%) followed by udder (26.02%), neck and head region (20.68%), ear and tail (9.63%), shoulder (6.78%) and hind legs (1.96%) as shown in table 3.14 and figure 3.13.

Table 3.14: Hard tick distribution rate (%) on various cattle body parts

Body regions	Infested cattle	Cattle with infested sites	Percentage %
External genitalia	561	196	34.93%
Udder	561	146	26.02%
Neck &head regions	561	116	20.68%
Ear & tail	561	54	9.63%
Shoulder	561	38	6.78%
Hind legs	561	11	1.96%

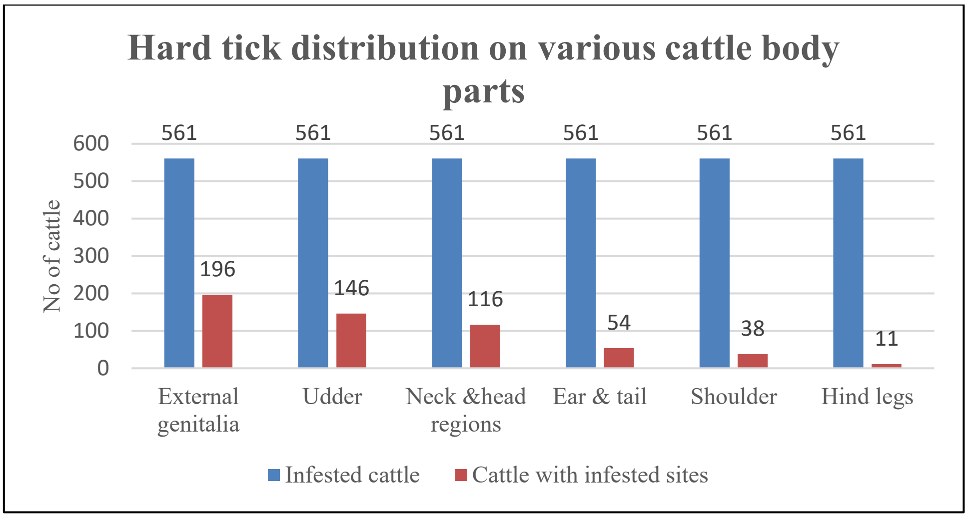

Figure 3.13: Percentage of hard ticks on various body parts of cattle.

3.14: Tick prevalence based on an animal's body condition

The highest tick infestation was recorded in those cattle whose body condition is poor, in the present study out of 770 observed cattle, cattle who (35.45%) were found in poor health condition had the highest tick infestation (96.46%), then those who were in good health (28.05%) and their tick infestation was (70.13%) recorded, followed by excellent body condition cattle (9.35%), their tick infestation rate was (40.22%) noted. Statistically, tick infestation was highest among cattle with poor body condition ($p < 0.05$) as shown in table 3.15 and figure 3.14.

Table 3.15: Tick infestation based on an animal's physical state

Risk factors		Cattle observed (N)	Infested cattle (N)	Uninfested cattle (N)	Ticks (N)	Percentage (%)	X_2	*P*- value
Body condition	Excellent	179	72	107	375	40.22%	177.32	0.001
	Good	308	216	92	786	70.13%		
	Poor	283	273	10	1056	96.46%		

* Significant ($p < 0.05$).

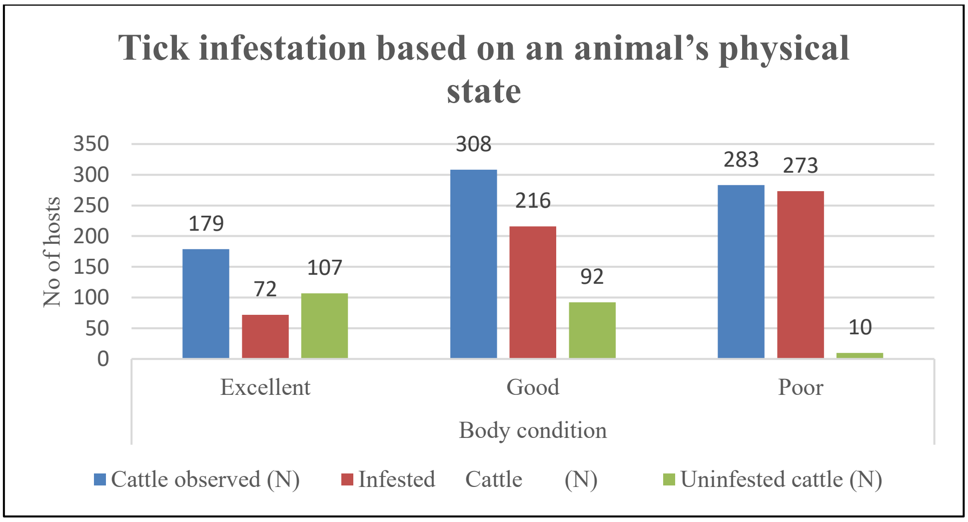

Figure 3.14: Association of tick infestation with cattle body conditions

3.15: Animal's house-wise and feeding pattern-wise prevalence of tick

Out of 770 animals, 456 were raised in standard (earthen/wooden type) and 314 were in cement concrete houses, respectively. Compared to the animals kept in the cement concrete house (51.91%), the animals raised in standard-type houses had significantly ($p<0.05$) greater tick infestations (87.28%). The animal maintained on an earthen floor had the highest ($p<0.05$) tick incidence (89.10%), followed by cement concrete (54.06%).The cattle feeding in group were significantly more tick infested (62.65%) than feeding individually (8.920%) as shown in table 3.16 and figure 3.15.

Table 3.16: Tick infestation based on cattle housing and feeding pattern

Housing/Feeding		**Cattle observed (N)**	**Infested cattle (N)**	**Non- infested cattle (N)**	**Percentage (N)**	**Ticks number**	X_2	**P value**
House type	Muddy	456	398	58	87.28%	1539	117.637	0.001
	Cement	314	163	151	51.91%	678		
Floor type	Earthen	413	368	45	89.10%	1398	118.902	0.001
	Cement	357	193	164	54.06%	819		
Feeding	Group	557	349	208	62.65%	1667	178.319	0.001
	Indisivual	213	19	194	8.920%	550		

* Significant ($p < 0.05$).

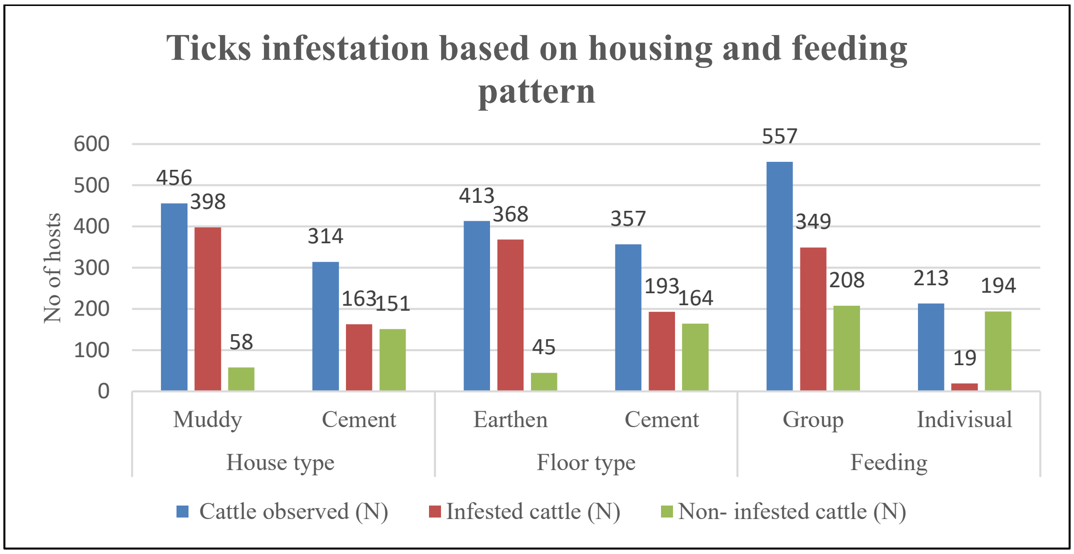

Figure 3.15: Percentage of tick infestation based on cattle housing.

3.16: Environment-related elements and tick infestation

3.16.1: Effect of grazing on tick infestation

In case of the effect of environmental factors high tick infestation was observed in free grazing cattle (97.20%) than semi-grazing cattle (61.42%) followed by non-grazing cattle as shown in table 3.17 and figure 3.16. Tick infestation was statistically substantially greater (P < 0.05) in free-grazing cattle compared to semi-grazing and non-grazing cattle in terms of the management approach.

Table 3.17: Effects of grazing on tick infestation

Grazing mode	Observed host (N)	Infected host (N)	No-infested cattle (N)	Percentage (%)	Ticks (N)	X_2	*P*-value
Free	286	278	8	97.20	994	138.997	0.001
Semi	267	164	103	61.42	756		
Zero	217	119	98	54.84	467		

* Significant ($p < 0.05$).

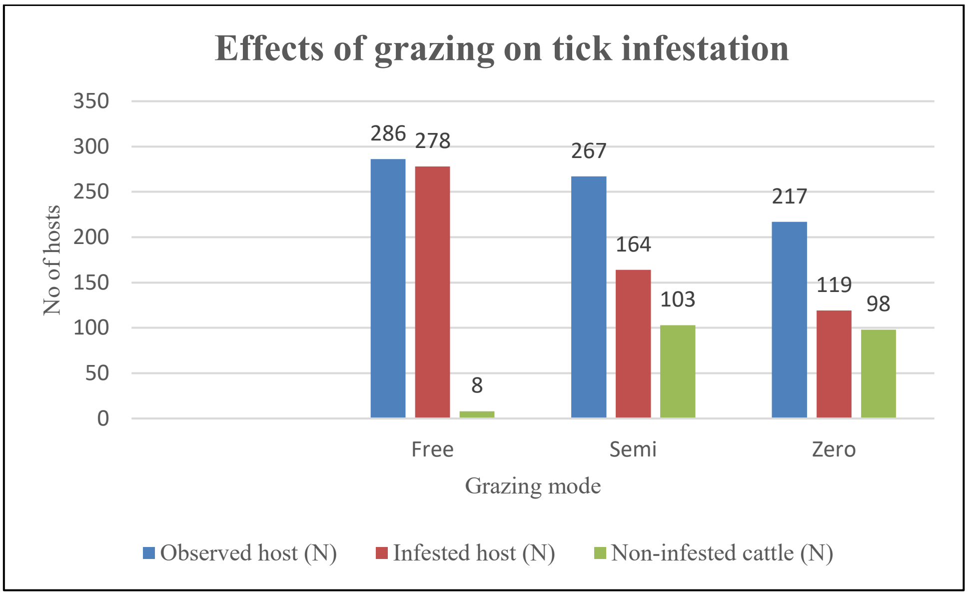

Figure 3.16.: Percentage of tick infestation based on grazing.

3.16.2: Effect of acaricide on tick infestation

In terms of acaricide use ticks infestation was lower in those cattle who were regularly treated (43.71%) than irregularly treated cattle (76.81%) and highest tick infestation was recorded in those cattle who were not treated with acaricide (88.81%) respectively as shown in table 3.18 and figure 3.17. Tick infestation was significantly (P <0.05) lower in cattle who received regular acaricide treatment compared to those that did not receive therapy or only occasionally received acaricides.

Table 3.18: Association of acaricides use with tick infestation

Acaricides	Observed host (N)	Infected host(N)	Non- infected cattle (N)	Infestation rate	Ticks (N)	X_2	*P*-value
No use	295	262	33	88.81	314	125.603	0.001
Irregular	276	212	64	76.81	892		
Regular	199	87	112	43.71	1011		

* Significant ($p < 0.05$).

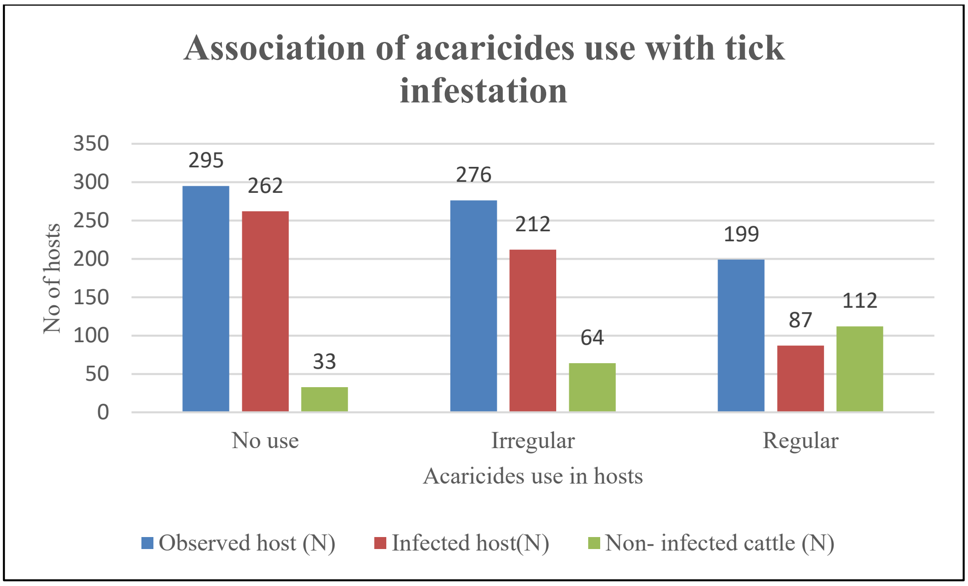

Figure 3.17: Percentage of tick infestation based on acaricide use.

3.17: Tick infestation relation with elevation in cattle

According to the results of present study, the cattle living in hilly areas were more tick infested (90.43%) than those living in plain area (51.99%) as shown in table 3.19 and figure 3.18. In this study, it was found that cattle raised in mountainous regions had significantly higher tick prevalence ($p < 0.05$) than cattle raised in plain areas.

Table 3.19: Relationship between elevation and tick infestation

Living Area	Ticks (N)	Cattle observed (N)	Cattle infested (N)	Non-infested cattle (N)	Percentage (%)	X_2	p-value
Hilly Area	1421	418	378	40	90.43	142.797	0.001
Plain Area	796	352	183	169	51.99		

* Significant ($p < 0.05$).

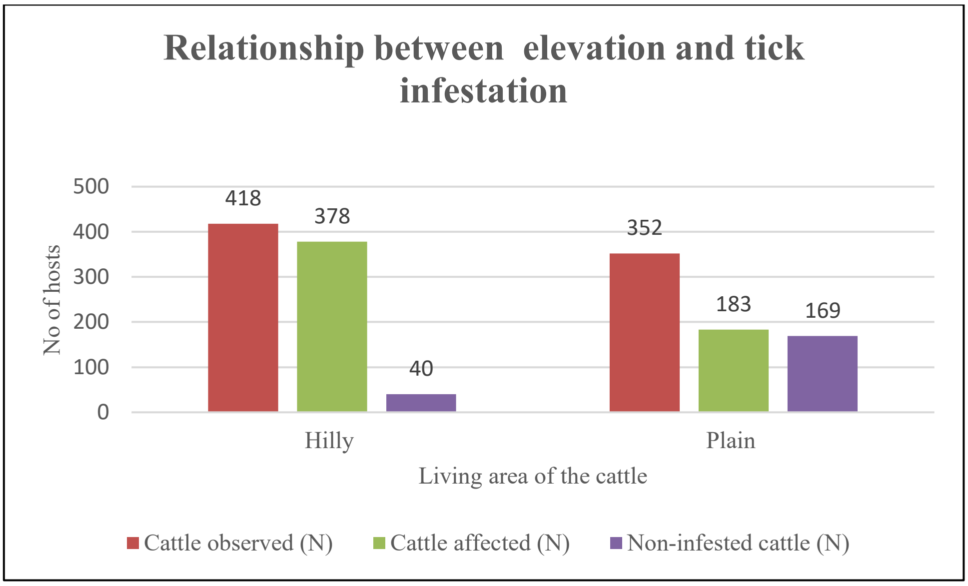

Figure 3.18: Prevalence of tick infestation based on topography.

3.18: Seasonal prevalence of tick infestation

The result of present study showed highest tick infestation in summer (June (89.10%), July (93.37%) and August (84.68%), followed by Spring (March (49.43%), April (71.14%), May (73.19%) and lowest in Autumn (September (47.83%), October (45.45%) as shown in table 3.20 and figure 3.19. Ticks started appearance in March and their number increased till the end of August and then gradually decreased in number. Seasonal variations in the proportions of non-infested and tick-infested animals were found to be significant ($p < 0.05$).

Table 3.20: Seasonal fluctuation in tick prevalence

Seasons	**Months**	**Cattle examined (N)**	**Cattle infected (N)**	**Cattle not infected (N)**	**Infestation rate**	***X*2**	***p*-value**
Spring	March	87	43	44	49.43	130.610	0.001
	April	70	54	16	71.14		
	May	97	71	26	73.19		
Summer	June	101	90	11	89.10		
	July	134	125	9	93.37		
	August	124	105	19	84.68		
Autumn	September	69	33	36	47.83		
	October	88	40	48	45.45		

*Statistical analysis. Highly significant difference ($P<0.05$) of prevalence of tick infestation during different seasons of the year.

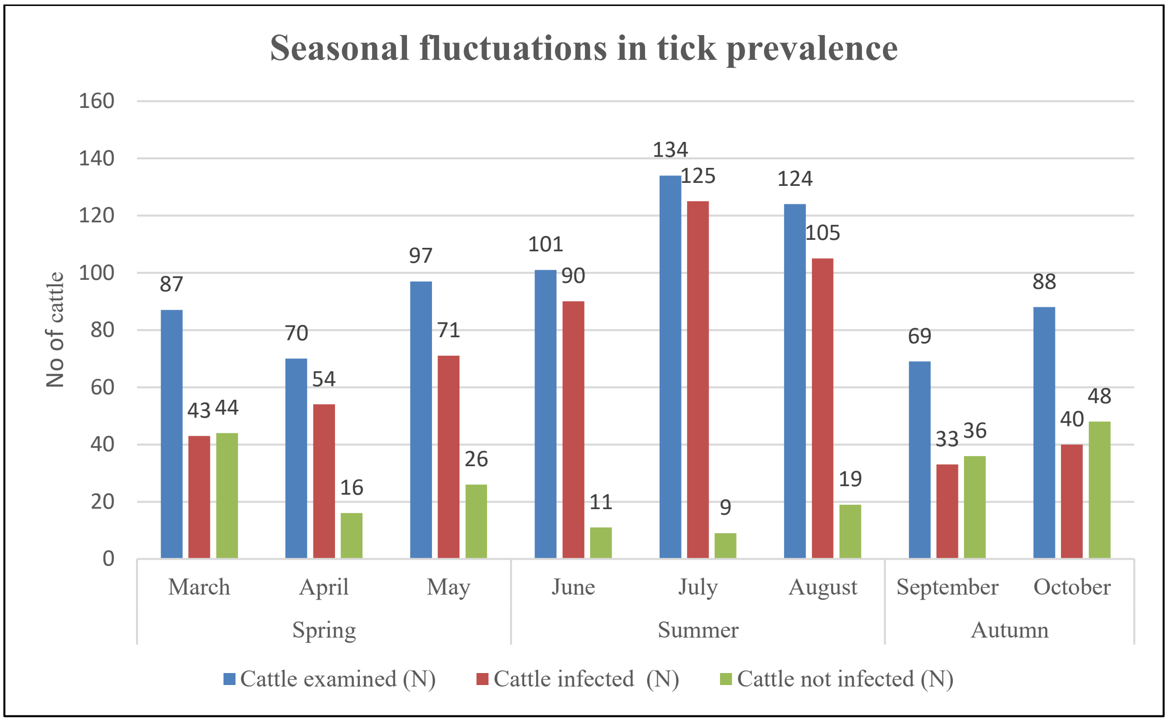

Figure 3.19: Prevalence of tick infestation based on seasonal fluctuations.

3.19. Morphological observations of tick species:

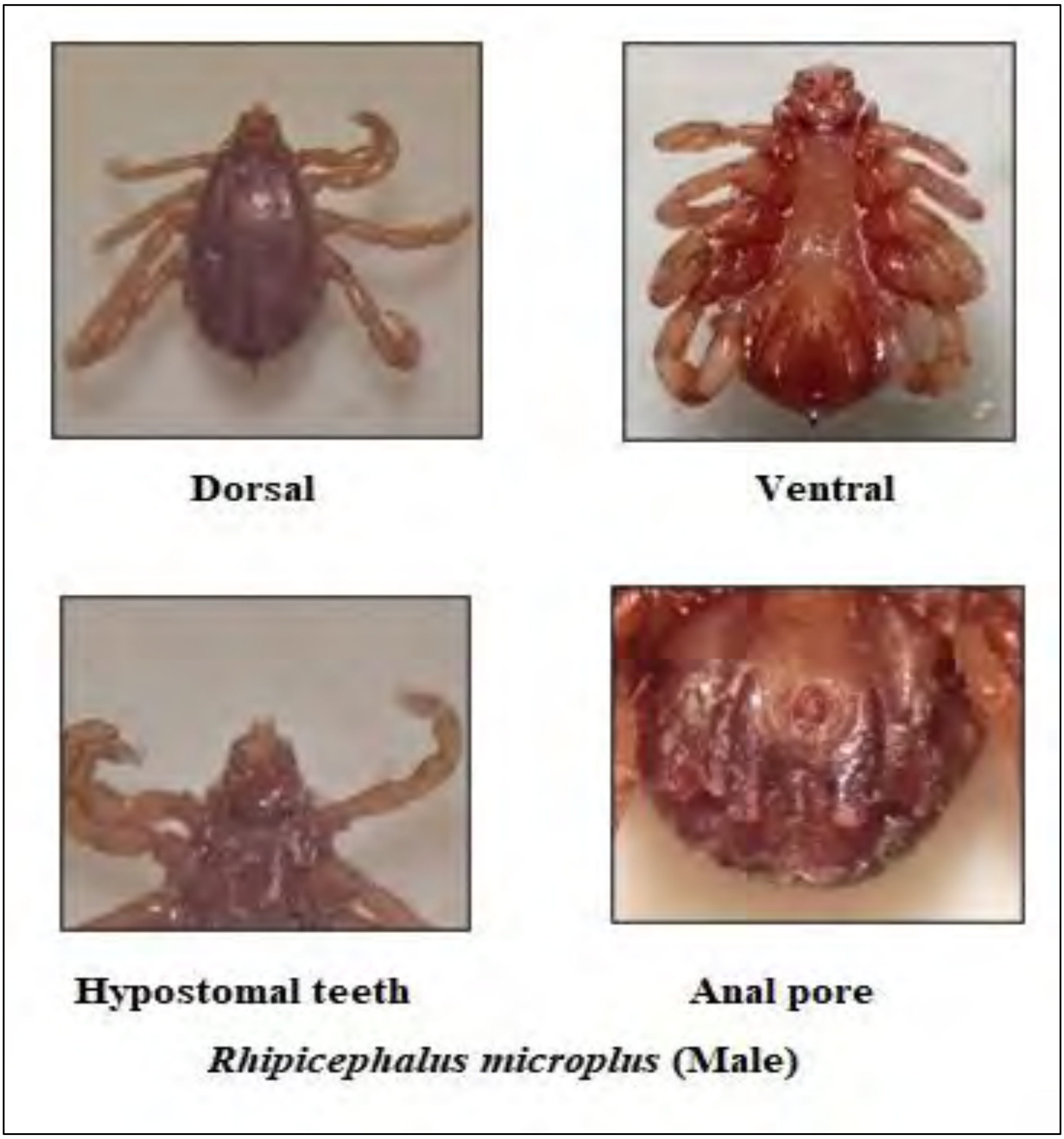

Figure 3.20: Showing morphological identification characters of *Rh. Microplus* male.

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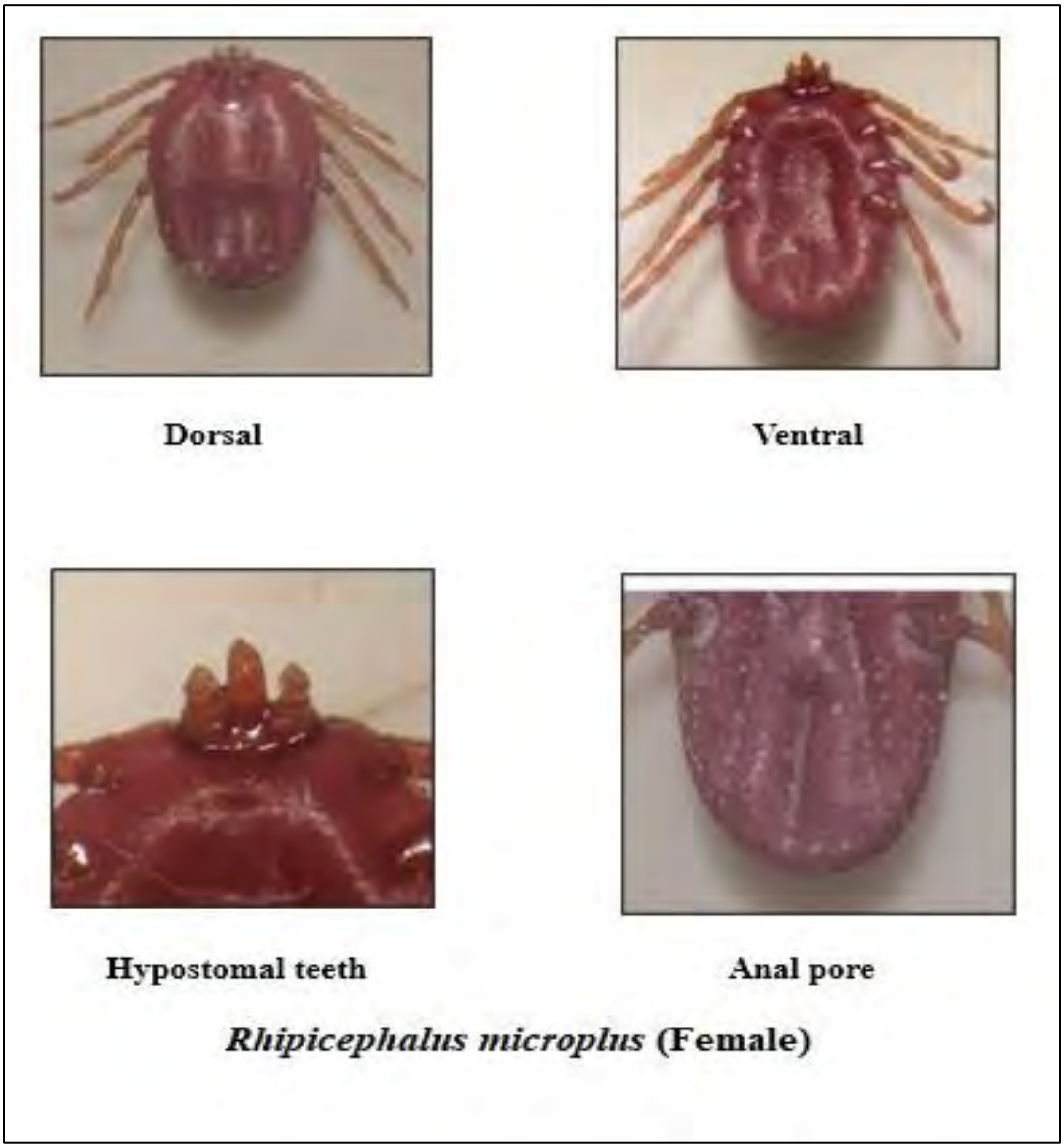


Fig 3.21: Showing morphological identification characters of *Rh. microplus* female

3.19.1: Characters for identification

Genital pore is U-shaped, porose area is oval shape and hypostomal teeth are 4+4.

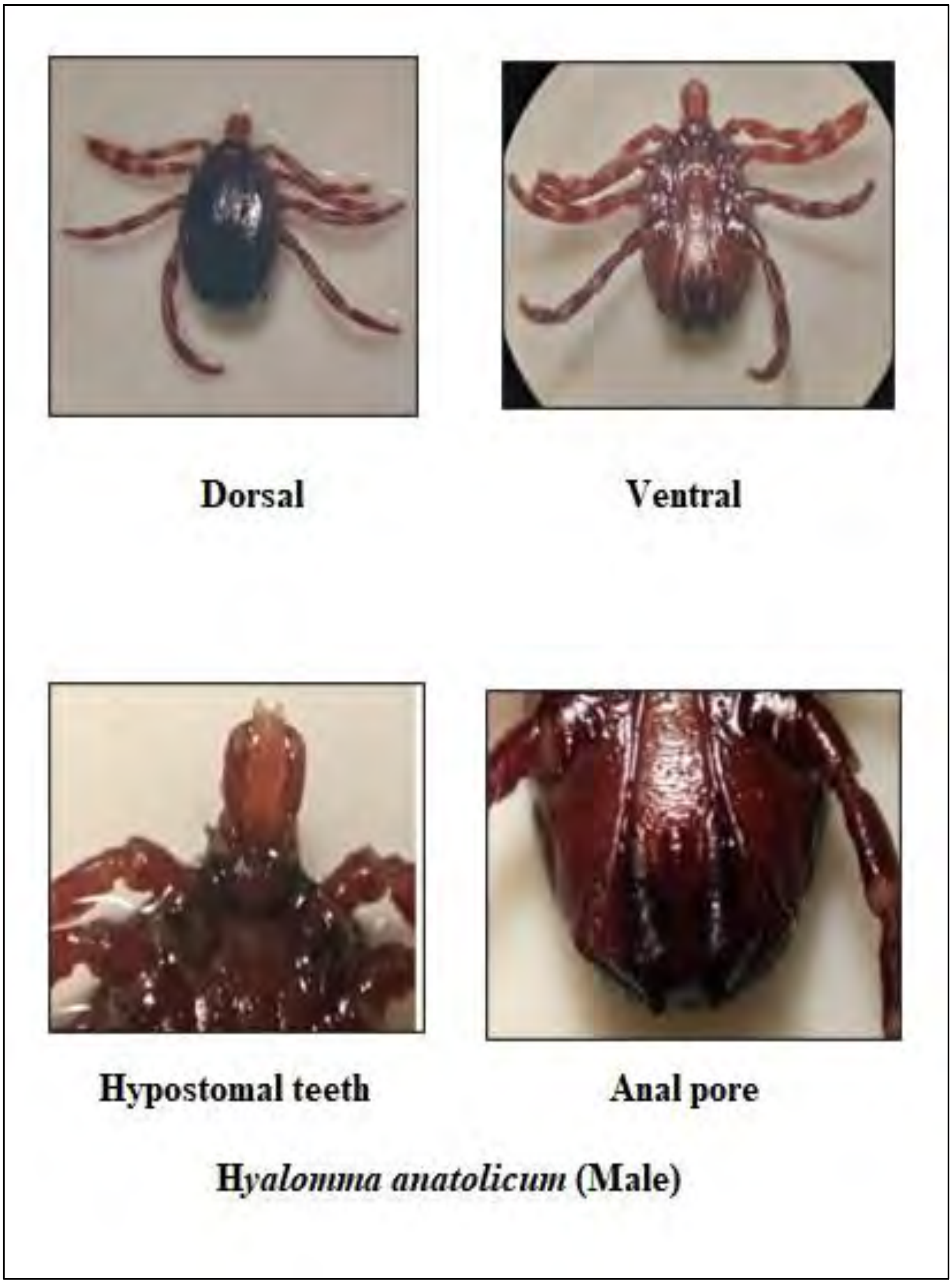


Fig 3.22: Showing morphological identification characters of *H. anatolicum* male

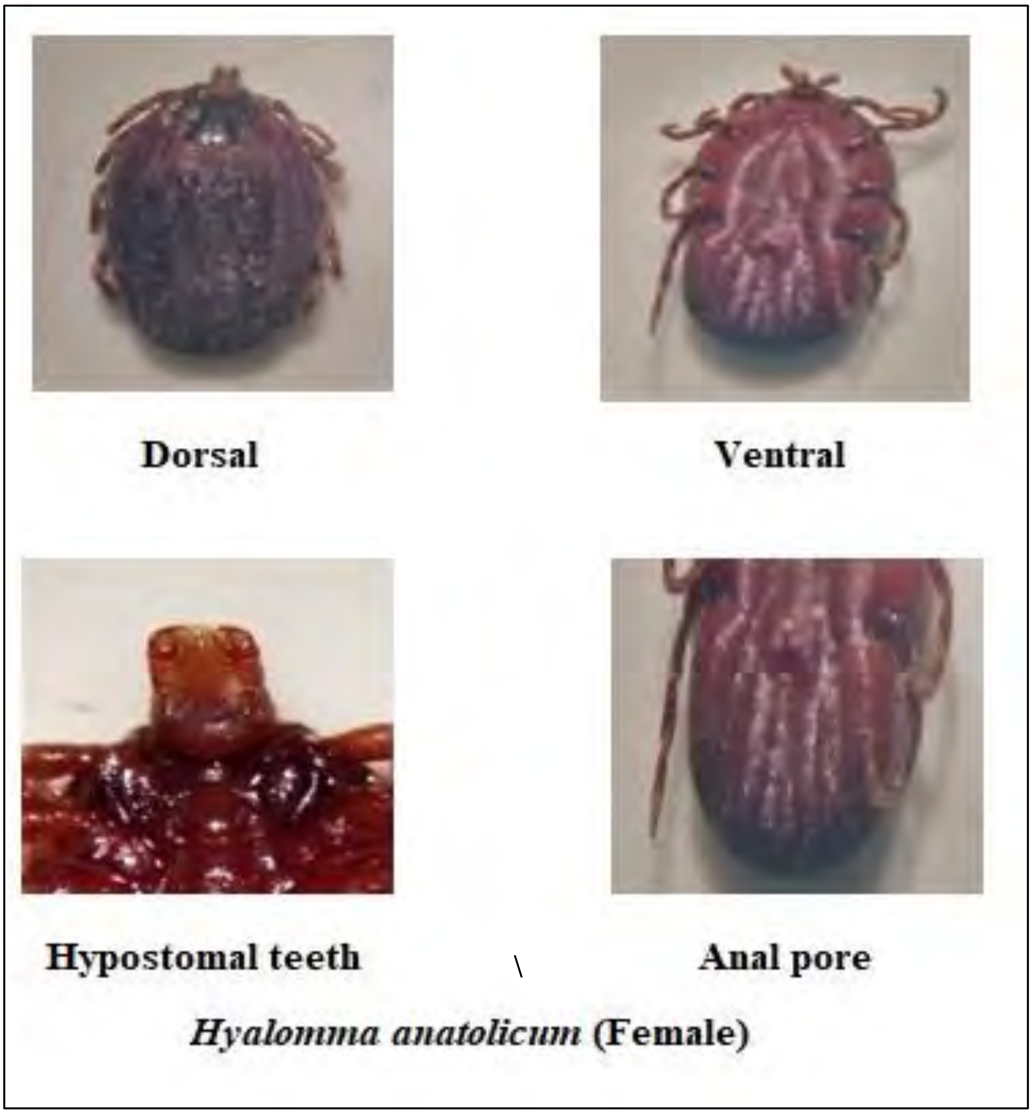


Fig 3.23: Showing morphological identification characters of *H. anatolicum* female

3.19.2: Characters for identification

legs are pale yellow, hypostome is long and genital pore is U-shaped.

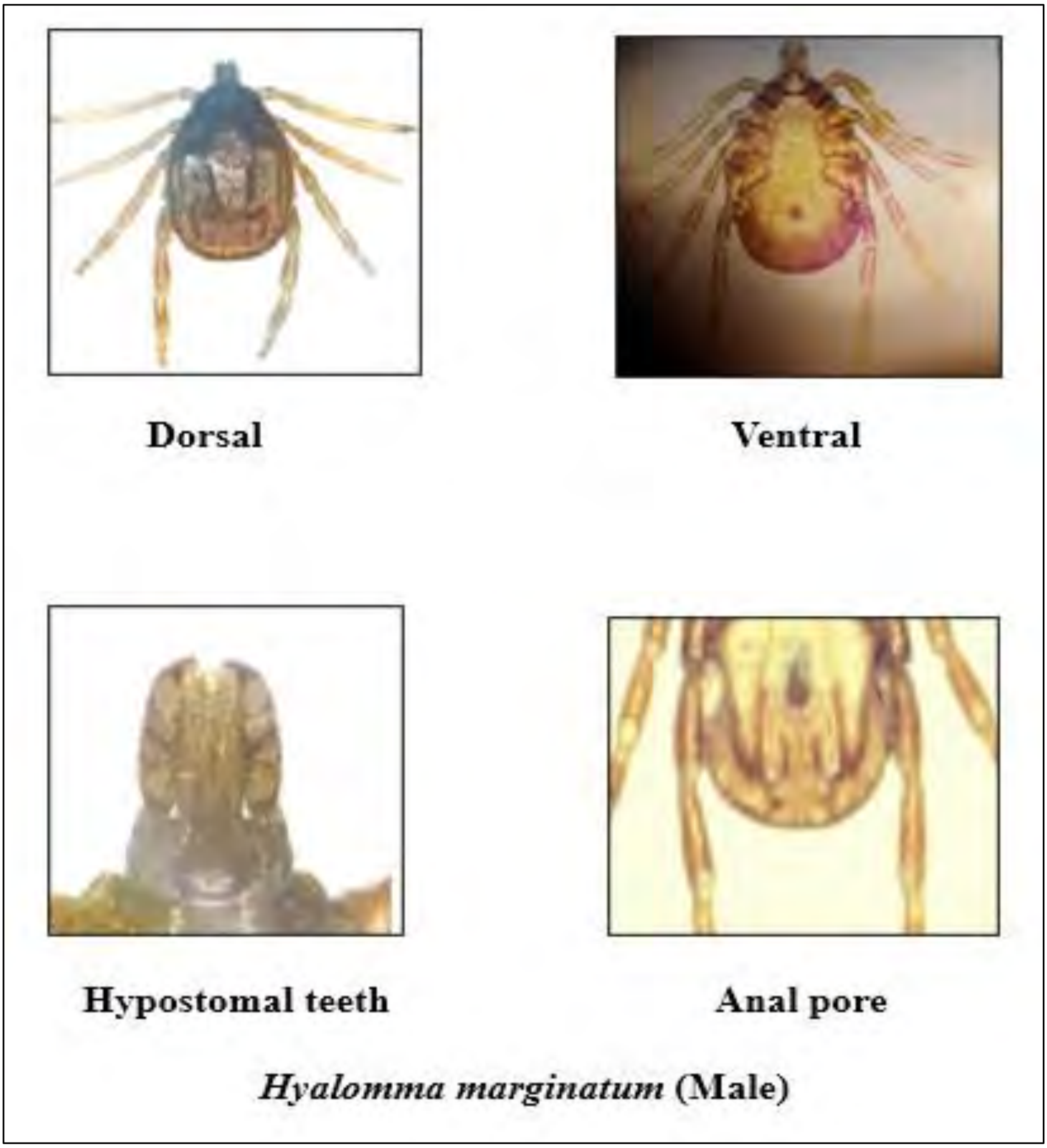


Fig 3.24: Showing morphological identification characters of *H. marginatum* male.

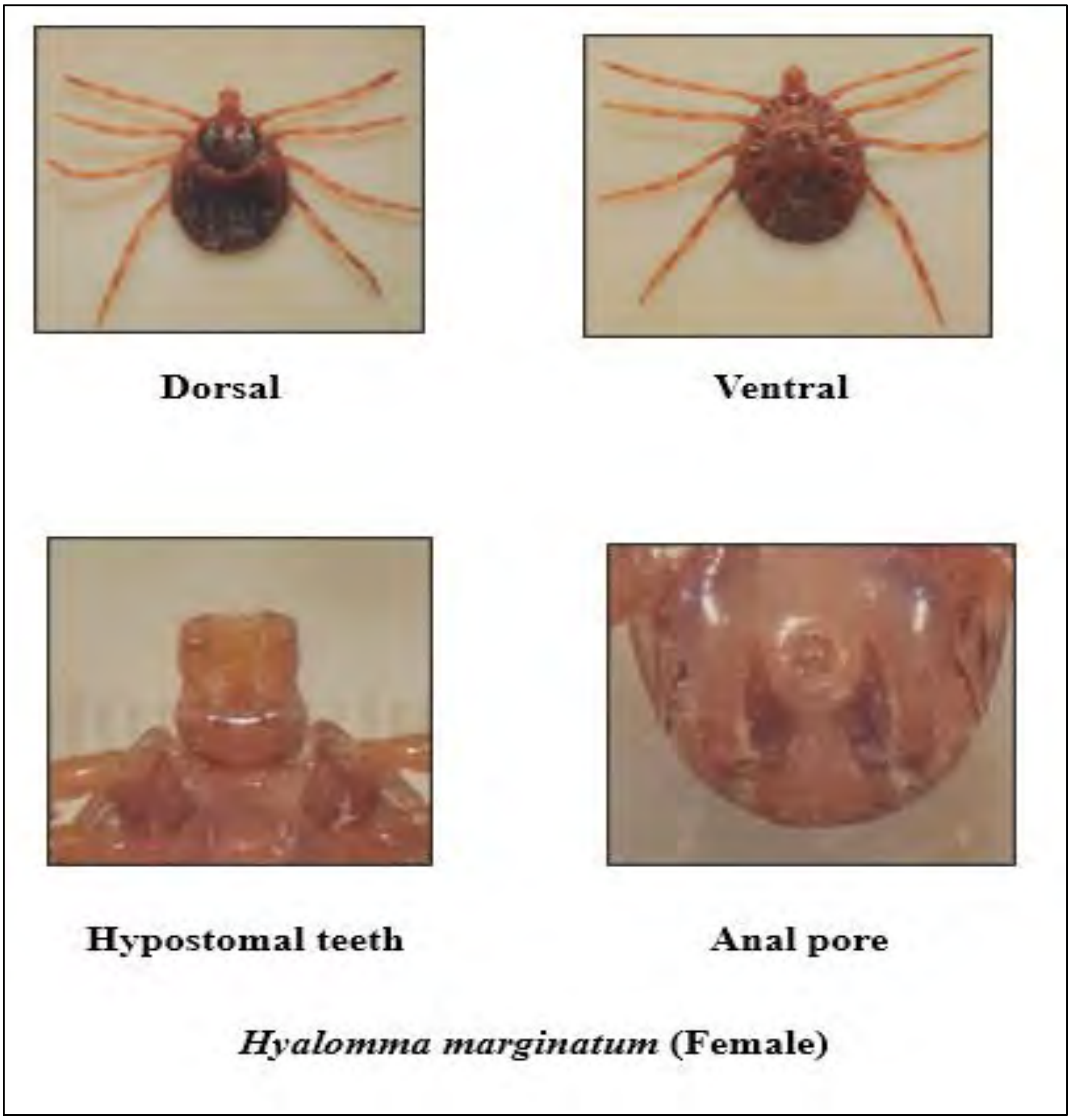


Fig 3.25: Showing morphological identification characters of *H. marginatum* female.

3.19.3: Characters for identification

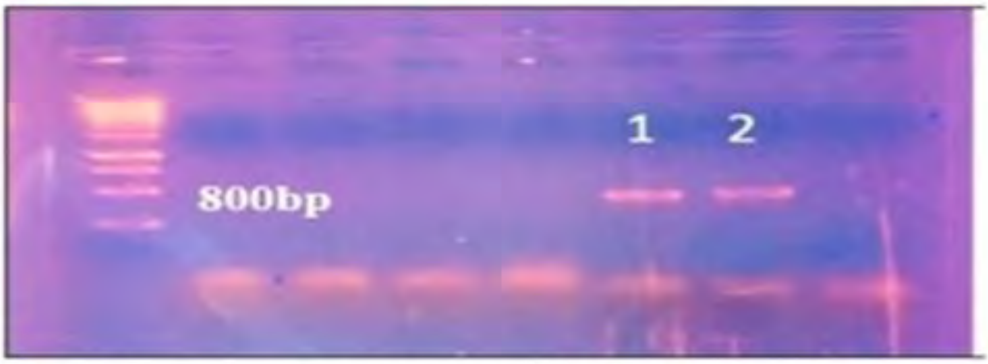
Hypostome is long, genital pore is U-shaped and scutum is dark colored.

3.20: PCR confirmation of ticks that have been taxonomically identified

Mitochondrial genes of morphologically recognized tick species (16S rRNA), (ITS2), and (COX1) were successfully amplified from *Rh. microplus*(38), *H. anatolicum*(23), and *H. marginatum* (18).

PCR results of ITS₂ gene of tick's species (800bps)

PCR results of 16s gene of tick's species (460bps)



PCR results of COX1 gene of tick's species (800bps)

Figure 3.26: PCR amplification of ITS_2, 16S and COX1 genes.

DISCUSSION

Ticks which are most prevalent in tropical and subtropical regions of the world, parasitize virtually all grassland and semi-aquatic vertebrates (Khan *et al.,* 20221). Livestock is important to the economy of Pakistan, a predominantly agricultural nation. A variety of tick species can grow and survive in Pakistan due to the country's favorable climate (Ali *et al.,* 2019). A hotspot for new and recurring TBDs that are significant to veterinary and public health is the Pakistani province of Khyber Pakhtunkhwa (KPK) (Khan *et al.,* 2021). The livestock industry experiences considerable loss due to diseases spread by ticks on a global scale. They have a significant economic impact since they result in decreased productivity, decreased worker effectiveness, and fatalities (Shoaib *et al*., 2022). Numerous studies have indicated that more than 80% of cattle are subject to heavy infestations of the *Hyalomma* and *Rhipicephalus* tick species in Pakistan (Ghafar *et al.,* 2020). Ticks, which are a crucial factor in the biological transmission of TBDs, are more likely to reproduce and survive in Pakistan's favorable geoclimate. (Atif *et al*., 2012). The two main tick genera that transmit hemoparasitic illnesses to livestock in Pakistan are Rhipicephalus and Hyalomma.

The primary goal of the current study is to examine the geographical distribution of ticks in cattle, their interactions with hosts and ecological factors, as well as the investigation of species-level identification using genetic markers in KPK, Pakistan. Tick species were imaged using a stereomicroscope (Optika:45k1637) for this study. Various body parts were examined with the aid of standard identifying keys.

Tick and TBDs distribution have greatly impacted by environmental fluctuations (temperature, animals' interaction, habitat changes), as the evidence shows that the mean temperature of the world will likely increase by 1.5°C (2.7°F) (IPCC, 2021), such rising in the temperature has a long-term impact on ticks' distribution (Leger *et al.,* 2013). The highest tick species infection during this study was recorded in June to August (summer) due to rainfall and vegetation availability, which creates a suitable environment for ticks' life stages. Mean temperature and relative humidity were detected, which demonstrate a strong correlation-ship. The lowest infestation was observed from September to October, and like earlier findings, the same reports were made during that time (Kamran *et al.,* 2021 and Ali *et al*., 2021).

The takedown observations were organized and put together on a 2016 Microsoft Excel sheet. For comparisons and variables, the chi-square test (X_2) was employed. Seven tehsils of district Swat were studied in which Bahrain district has highest tick burden (18.98%) followed by Matta (18.55%), Khwazakhela (16.96%), Charbagh (14.04%), Barikot (13.44%), Kabal

(9.83%) and Babuzai (8.29%) of which three medically important ticks' species having two genera (*Rh. microplus, H. anatolicum* and *H. marginatum*), collected from 770 cattle hosts.

It was possible to identify ticks from the genera Rhipicephalus and Hyalomma. The results show that *Rhipicephalus*, followed by *Hyalomma*, is the most prevalent tick genera. This study revealed that *Rhipicephalus* is the most prevalent genus followed by *Hyalomma*, hence our results correlate with (Shoaib *et al.,* 2022; Shoaib *et al.,* 2021; Farooqi *et al*., 2017; Haque *et al*., 2011and Mustafa *et al.,* 2014). Hard ticks of the Ixodidae family, including *Rhipicephalus* and *Hyalomma*, have previously been described by researchers like Muhammad *et al*. (2008) in Pakistan and Ghosh *et al.* (2007) in Bangladesh, India, and Pakistan. Due to seasonal fluctuations over the study periods and climatic and topographic variances in Pakistan, India, and Bangladesh, these researchers' studies generally concur with our project's findings with a few minor exceptions. Pakistan's mixed species farming practices and lack of infrastructure can be blamed for the higher occurrence of hard tick genera there. Animal infections spread among animals under these circumstances due to overcrowding and mixing of sick and healthy animals (Shoaib *et al*., 2021). Our findings correlate with (Zeb *et al*., 2020). Our results are consistent with earlier research conducted at the national level that found a similar distribution of tick species. (Siddiqi and Jan, 1986; Farooqi *et al*., 2018; Ali *et al*., 2019; Khan *et al*., 2019; Nasreen *et al*., 2020). For instance, *Rh. microplus* was shown to be the most prevalent tick species in a study carried out in Northern Pakistan (Gilgit and Kohistan; region with a chilly and humid climate) (Shamim, 2017). Additionally, Taiwan and India have both been reported to have greater *Rh. microplus* prevalence rates (Tsai *et al*., 2011; Singh and Rath, 2013). *Rh. microplus*, however, was the second-most prevalent species after *H. anatolicum*, according to studies from other Pakistani provinces (Punjab and Baluchistan) (Sajid *et al*., 2009; Ali *et al*., 2013; Sultana *et al*., 2015; Rafiq *et al*., 2017 and Rehman *et al*., 2017). The varying ecological conditions in the several provinces may be the cause of this variance in species distribution. For instance, higher tick prevalence is favored by the temperature in arid and semi-arid regions (Estrada-Pena *et al*., 2006; Kapur *et al*., 2008; Jabbar *et al*., 2015; Rehman *et al*., 2017 and Ali *et al*., 2019). In the studied area, hard tick prevalence in cow populations is demonstrated by the current study. Due to the existence of a rich host species, dense vegetation, and favorable geoclimatic conditions, cattle had a higher tick infestation (72.85%) (Teel *et al*., 1996; Gray,2002; Bianchi *et al*., 2003; Jouda *et al*., Greenfield, 2011; Nicholson *et al*., 2011 and Karim *et al*., 2017).

The prevalence of tick infestation is also influenced by the sex of the cow. Compared to male animals (53.24%), female animals in this study had a higher prevalence of tick infection (89.64%). Similar results were confirmed by (Kabir *et al*., 2011), who discovered that female cattle had a significantly higher prevalence of tick infection (59.37%) than male cattle (35.83%). Female animals were discovered to be 2.61 times more vulnerable than males. Although the precise reason for the higher incidence of tick infestation in females is unclear, hormonal effects, immunosuppression during pregnancy and lactation, and stress all contribute to this higher prevalence (Kakar *et al*., 2017). The verdicts, however, do not support (Atif *et al*., 2012) conclusion, who found that males (56.46%) had a higher frequency of tick infestation than females (54.17%).The latest findings disagree with (Musa *et al*., 2014; Opara and Ezeh, 2011; and Hitcheock, 1993), who argued that male cattle in the tropics become more tick-infested than female cattle because they are used for the majority of farming duties and moved around in search of food. Tick infestations are less common in the tropics because the females are primarily restricted for reproductive purposes. To attach to passing hosts, primarily males, while feeding, tick larvae are said to climb grass and shrub blades. Our findings correlate with Kabir et al., 2011; Asmaa *et al*., 2014); Rony *et al*., 2010 and Sarkar, 2007). According to (Kabir *et al*., 2011), female cattle were more likely to have tick infestations than male cattle. They found that 95 female cattle (59.37%) had significantly ($p<0.01$) more ticks than 43 male cattle (35.83%). Tick infestation risk was 2.61 times higher in female cattle than in males. Even still, the precise reason for the greater frequency of tick infestation in female cattle remains a mystery, although it is conceivable that this phenomenon may be linked to some hormonal factors. The body's prolactin and progesterone levels increase an individual's susceptibility to infections. Additionally, production-related pressures like pregnancy and breastfeeding render female animals more vulnerable to infections. However, our results mismatch with (Musa *et al*., 2014 and Shoaib *et al*., 2021). Who observed that males are more likely than females to have tick infestations. The potential causes of increased male infestation. Mostly males are employed in drought-related activities, when they go to work in fields and other locations, their exposure to vectors remains high. The fact that farmers pay more attention to female cattle used for milk production than to male cattle (Bullocks) used primarily for meat and draught purposes and thus receiving less attention, is another explanation for these findings (Musa *et al*., 2014 and Shoaib *et al*., 2021).

Tick development and growth are facilitated by these favorable environmental variables, which results in greater prevalence rates. The lack of knowledge among farmers on tick control and host susceptibility may also contribute to the high prevalence of ticks (Pinheiro *et al*., 2010).The study revealed that male ticks had a lower ratio than female ticks. Our results mismatch with (Telmadarraiy *et al*., 2010; Tadesse and Sultan, 2014) and Shoaib *et al*., 2022). Male ticks stay attached to the host for a longer period than female ticks do because they feed on the host for a longer period of time and spend more time mating with other female ticks before dropping off to the ground after finishing their blood meal, this characteristic may be the reason why male ticks are more dominant than females (Gebre *et al*., 2001).

Age and grazing were identified as two potential risk factors for greater tick infestation after analysis of host and environmental factors related to tick infestation. Similar outcomes have also been recorded in the past from several regions of Pakistan (Durrani and Kamal, 2008; Khan *et al*., 2013; Karim *et al*., 2017 and Rafiq *et al*., 2017). The host animal's age significantly affects the pattern of tick infection (Manan *et al*., 2007). Current study showed that younger animals of age <1 year had the lowest infestation that was 30.35% for young female and 21.74% for young males than adult animals of age group 1-5 years female (45.45%), male (29.73%), 6-10 years age group female (68.47%), male (56.82%) and age group of 11-15 years female (93.88%), Male (74.12%). Similar outcomes were also reported by (Patel *et al*., 2013); Kaur *et al*., 2015; Gosh *et al*., 2019; Zeb *et al*., 2020 and Rehman *et al*., 2017).Tick infestation is higher in old animals than other age groups. This is presumably related to older animals' decreased immunity and resistance (Kemal *et al.,* 2020). The precise cause of the low infection rate in newborn calves is very difficult to pinpoint. Improvements in newborn calves' management techniques and the placement of the calves in separate pens may have something to do with the minimal tick infestation on them (Gosh *et al*., 2019). Compared to young animals, mature cattle had a higher prevalence of tick infestation. Calves are only allowed to graze in regions close to their homes, which are less infested, whereas adult cattle are allowed to travel around far in search of food and water. This may be the cause of the difference between the two. The findings of the current study are consistent with those of (Atif *et al*., 2012; Khan *et al*., 2022 and Kakar *et al*., 2017). who found that adult animals older than 5 years old had the highest prevalence of tick infestation (71.61%). whereas the youngest animals (20.80%) had the lowest percentage. Barnett and Bailey's (1955) observation lends confirmation to our findings that young calves exhibit age-related resistance to most tick-borne protozoan infections. In a similar vein, (Randuz, 2008) acknowledged the significance of colostrum feeding in calf production of antibody against illnesses. Strong innate immunity in calves is thought to be advantageous for reducing tick exposure. Colostrum is the first source of nutrition after delivery and has a crucial role in the health of newborns. Early in a calf's life, colostral antibodies protect it from sickness until its own defense system is ready (Kakar *et al*., 2017). The decreased surface area of animals and the grooming of calves may contribute to the lower tick burdens (Mooring *et al*., 2000). The adult hosts' free grazing habits and wide surfaces make them more vulnerable to tick attachment, as opposed to the younger hosts, who have less tick load because of their reduced grazing habits, low body surface area, and robust immune systems (Swai *et al.,* 2005). In contrast to the findings of the present study, Manan et al. (2007) presented data showing higher tick infestation in young animals under one year (24.5%), followed by animals between one and two years (20.5%), and adult farm animals over two years (19.20%), respectively. while contraindicating the observation made by (Vatsya *et al*., 2007; Singh and Rath, 2013; Patel *et al*., 2013; Dehuri *et al*., 2017; Debbarma *et al*., 2017; Patel et al. 2019) who found that animals older than three years had the lowest tick infestation rate.

The results of the current investigation demonstrated that ticks can be found in various body parts in varying numbers. Tick infestations were observed in abundance throughout the udder, including the teats, perineum, and external genitalia. The most prevalent tick species found in the current study was *Rhipicephalus microplus* (40.78%) with preference sites including the neck, udder, shoulder area, and external genitalia, fondness sites for *Hyalomma anatolicum* (31.94%) include the external genitalia, udder, ear, and tail. Similarly, the external genitalia, udder, rear legs, and head regions are preferred locations for *Hyalomma marginatum* (27.29%). These findings are in line with those made by Moges *et al*., (2012), who discovered tick infection in 169 local cattle in the Chilga area of Ethiopia. Our results are consistent with (Kakar *et al*., 2017), whose study found that Boophilus (35.5%) was the most common tick species, with preference areas including the shoulder area, dewlap, external genitalia, udder region, legs, especially the rear legs, abdominal area, and in some cases head regions, particularly the neck. Likely predilection areas for *Hyalomma* (26.5%) were the perineum region, udder, and external genitalia. They noted nearly the same tick preference locations as those found in the current study. The fact that ticks favors warm, moist, concealed areas with a good vascular supply and thin skin could potentially be a contributing factor to the increased tick infestations on the external genitalia and udder (Muchenje *et al.,* 2008). For infestation, ticks typically favors skin with shorter hair and thinner layers. This facilitates feeding by allowing mouth parts to easily enter a highly vascular location (Sajid, 2007). Like our findings, Atif *et al*., (2007) found that the udder and external genitalia (98%) were the most often infested areas, followed in decreasing order by dewlap (92%), inner thighs (90%), neck and back (54%), tail (26%), ears (13%), around eyes (10%), flanks (4%) and legs (2%) . Asmaa and colleagues in 2014 also noticed udder and external genitalia as the most tick infested regions.

In the current study, there was a strong correlation between bodily condition and tick prevalence among the variables considered. Animals with poor body conditions had higher tick infection rates than the other groups, according to body condition. This might be because unfit animals had little resistance to tick infection and insufficient bodily capacity to develop resistance. although animals in good physical condition fought the parasite reasonably well, according to (Manan *et al.,* 2007). The present study showed that cattle had the highest tick infestation (96.46%) with poor body condition followed by good (70.13%) and excellent body condition (40.22%). A major management risk factor for tick prevalence is the host's poor physical condition (Patel *et al* 2019). Researchers from across the world report that tick infestations have caused cattle mortality due to anaemia (Jonsson, 2006).

Cattle who were reared in ordinary type houses recorded with high tick infestation (87.28%) in comparison to those who were kept in concrete houses (51.91%).The animal maintained on an earthen floor had the highest tick incidence (89.10%), preceded by cement concrete (54.06%). In keeping with the current finding, Farooqi *et al*., (2017), Patel *et al*., (2019), and Rehman *et al*., (2017) also found that animals raised in wooden/traditional rural houses had considerably higher tick prevalence than animals kept in concrete-style houses. The earthen/wooden homes' flaws and crevices give ticks a place to hide and promote their growth. Patel *et al.,* (2019). The current study's findings revealed that the prevalence of ticks was lowest (8.92%) in the animals who were fed alone and highest (62.65%) in the animals that were allowed to feed in groups. Our findings were consistent with those of Patel *et al*., (2019), who found that tick prevalence was lowest (8.92%) in animals fed singly and highest (62.65%) in those allowed to eat in groups. Tick frequency was much lower in free-range grazing farms (32.4%) compared to beef (79.2%) and dairy farms (82.3%), according to research by Abdella and colleagues in 2017.

Tick infestation was found to be higher in cattle kept in free-grazing (93.28%) and semi-grazing (83.17%) regions in the grazing system than it was in cattle kept in zero-grazing areas (44.94%). Likewise, compared to farms where cattle were managed with stall feeding, tick infection was noticeably greater in roaming animals. This might be because grazing cattle are exposed to more ticks than non-grazing cattle are (Ghosh *et al*., 2007; Iqbal *et al*., 2013; Rehman *et al*., 2017 and Zeb *et al*., 2020). Our results coincides with Zeb *et al*., 2020, compared to livestock housed in zero grazing regions (59.5%), they found more ticks in cattle from semi-grazing (86.0%) and free-grazing (85.7%) areas. *B. microplus* was discovered in cattle that were pastured in irrigated or river delta environments by Hussain and Kumar in 1986. Although the precise reason for the increased frequency of tick infestation in cattle is unknown. However, it is possible to speculate that routine barn cleanings and acaricide treatments will lessen the likelihood of a tick infection in stall-feeding animals. While grazing cattle can graze everywhere, this increases their susceptibility to tick infestation (Kabir *et al*., 2011).

Likewise, when it came to the usage of acaricides, cattle treated routinely had lower rates of tick infestation (29.38%) than those treated infrequently or not at all (97.70%) or never at all (77.83%). The prior research is supported by the observation that cattle not frequently treated with acaricides were substantially more likely to experience a tick infestation than were cattle that were (Rehman *et al.*, 2017). Our results match with (Zeb *et al*., 2020). They discovered that the prevalence of tick infestation was significantly ($P < 0.001$) lower (25.2%) in cattle that received frequent acaricide treatment compared to those that did not receive treatment (80.1%) and received treatment very sometimes (75.3%). Additionally, farms that used acaricides inconsistently had greater rates of tick infestation, which may be a sign of acaricide resistance. The incidence of acaricide resistance in cattle ticks in Pakistan, however, is not well known. In other regions of the world, reports of widespread acaricide resistance in cattle ticks have been made (Abbas *et al*., 2014). According to these results, a nationwide survey should be carried out to look into acaricide resistance in cattle ticks in Pakistan.

According to the current study, cattle raised in mountainous areas had considerably ($p<0.01$) higher tick prevalence (90.43%) than animals raised in plain areas 66 (51.9%). Kabir *et al*., (2011) reported similar findings, that tick prevalence was considerably (p0.01) greater in cattle raised in hilly area 72 (44.44%) than in cattle raised in plain area 66 (30.27%). In comparison to plain areas, cattle in hilly areas were 1.84 times more sensitive to tick infestations. Because of the existence of various types of imperata grass, shrubs, and herbs, which provided a favorable environment for all ticks to lay their eggs and hatch throughout the year, the intensity of infestations in mountainous and flat zones varied (MacLeod, 1970). In a hilly location, 65.5% of cattle were tick-infested, according to Kamal *et al*., (1996). Due to their activity in hilly areas and the need for a high volume of oxygen to survive, hypothetically, cattle reared in hilly areas have a high concentration of RBC cells in their blood volume. As a result, due to ticks' active appetite for blood, tick infestation risk is higher. Another presumption was that there would be a water shortage in steep areas but not in flat areas. Animals are regularly bathed and rubbed in plain places, but this is uncommon in steep areas (Kabir *et al*., (2011).

Tick population dynamics are greatly influenced by season, and there is a discernible shift in prevalence rates between seasons. Seasonal temperature fluctuations have an impact on annual patterns of tick activity, which affects tick and TBD dynamics. Variations in tick occurrence in the same area may be caused by changes in the seasons (Hancock *et al.,* (2011). Since all stages of ticks hibernate in cold climates, the winter season hinders tick infestations. These findings corroborate earlier observations from the area Ali *et al.,* 2019; Khan *et el.,* 2022 and Ali *et al.,* 2021. The present study revealed highest tick infestation in the summer followed by Spring and autumn. July and August are the wettest months because of the rising temperatures and rising humidity. Similar results were published by Sanjay *et al*., 2007; Patel *et al*., 2013 and Kaur *et al*., (2015). Our findings match with Rony *et al*., 2010; Sajid *et al.,* 2009 and Mohanta *et al*., 2011) revealed a rise in infection rates in the summer. The monsoon season's hot and muggy weather is ideal for the development of ticks in all their developmental phases. However, due to the harsh winter weather that makes it difficult for them to survive, ticks spend the season lurking in cracks and crevices as engorged females, nymphs, larvae, and unfed adults Singh and Rath, (2013). The increasing prevalence of ticks during the monsoon season shows that humidity may be a macroclimatic element that affects the rate of tick infestation (Vatsya *et al*., 2008). Ticks hibernate over the winter by hiding in cracks and crevices, which results in a low infestation level, because the cold, dry winters are unfavorable for their existence (Singh and Rath, 2013). Our results mismatch with Lahkar *et al*., (1994), the peak tick season was from November to February, and the lowest was from May to June, according to their findings. The macroclimatic influence of precipitation (humidity) on seasonal variations in tick infestation appeared to be significant (Vatsya *et al*., 2007), as it was observed in our study that highest tick infestation was noted in July which is the most humid month. The current results correlate with Atif *et al*., (2012), came to the conclusion that June and July saw the highest levels of tick infestation across all study districts, and they found that mean maximum temperature was substantially correlated with month-by-month tick prevalence throughout all study locations. In contrast, Stuti *et al*., (2008) reported that tick infection on animals occurred throughout the year, with the rainy season experiencing the highest infestation followed by the summer, and the winter experiencing the lowest. According to Sanjay *et al*., (2007), cattle had greater rates of tick infestation in rainy seasons than in summer or winter. According to research by Rony *et al*., (2010), the summer season (78.46%) had a significantly ($p<0.001$) higher seasonal prevalence than the winter (62.85%) and rainy season (52.11%).Typically, during droughts, tick populations remain low (Urquhart, 1996). According to (Khan *et al*., 1993), higher temperatures and humidity during the summer months contributed to a rise in tick infestation.

To distinguish closely related species of ticks, many types of genetic markers, including COX1, ITS, 12S rRNA, and 16S rRNA, have been utilized to identify ticks with high accuracy (Abdullah *et al*., 2016). Three genetic markers 16s rRNA, ITS_2, and COX1 were used in this investigation and successfully amplified the necessary genes. All three primers amplified *Rh. microplus, H. anatolicum, and H. marginatum* under circumstances. The PCR results of the specified amplified species were sent for sequencing for additional evolutionary relationship and phylogenetic analysis.

CONCLUSION

This study concludes that *Rhipicephalus* (*Boophilus*) was the predominant tick genus of cattle population in district Swat followed by *Hyalomma*. There was a significant association ($p<0.05$) between tick infestation and certain factors like age, sex, living and health factors. Tick prevalence was highest in tehsil Bahrain, Female and adult cattle, mountainous areas, diseased and those cattle who were kept in concrete houses. There was higher female tick ratio than male. Prevalence of tick species were at their peak during summer and rainy seasons and remained low during winter season. In the current investigation, genetic markers (16S rRNA, ITS2, and COX1) were employed to successfully amplify the targeted genes of the three tick species.

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Hard ticks

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