Scholars Academic Journal of Biosciences
Abbreviated Key Title: Sch Acad J Biosci
ISSN 2347-9515 (Print) | ISSN 2321-6883 (Online)
Journal homepage: https://saspublishers.com

OPEN ACCESS

Zoology

Morphological and Molecular Characterization of Ticks Species Associated with Ruminants in Sargodha Division Pakistan

Mehwish Niazi[1*], Sidra khan[2], Kamran Ahmad Nasir[3], Samia Zahoor[4], Arooj Latif[5], Muhammad Saail Abbas[6], Dr. Shahzad Ali[7], Abdur Rehman Azam[8], Uzma Manzoor[9]

[1]Institute of Molecular Biology and Biotechnology, Department of Zoology, University of Lahore, Sargodha Campus, Pakistan
[2]Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan
[3]Department of Animal Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
[4]Department of Zoology, Government College University Lahore, Pakistan
[5]Department of Zoology, Punjab University Lahore, Pakitan
[6]Department of Zoology, Faculty of Engineering and Applied Sciences, Riphah International University, Faisalabad Campus, Faisalabad 38000, Pakistan
[7]Department of Wildlife and Ecology, University of Veterinary and Animal Science, Pakistan
[8]Department of Pathobiology, University of Illinois Urbana-Champaign
[9]Institute of Microbiology, Government College University Faisalabad, Pakistan

DOI: https://doi.org/10.36347/sajb.2025.v13i02.002 | **Received:** 28.12.2024 | **Accepted:** 05.02.2025 | **Published:** 08.02.2025

***Corresponding author:** Mehwish Niazi
Institute of Molecular Biology and Biotechnology, Department of Zoology, University of Lahore, Sargodha Campus, Pakistan

Abstract **Original Research Article**

Although Pakistan's economy depends heavily on the livestock industry, animal health is greatly impacted by tick infestation and illnesses spread by ticks. This study evaluated the morphological traits, risk factors, and prevalence of ticks in four districts within the Sargodha Division. Between February and June 2021, 1,067 ruminants (cows, buffaloes, goats, and sheep) from 40 farms were watched. The ITS-2 nuclear gene (750 bp) in 10 tick species was the object of PCR detection. The total infestation rate was 32.10%, with Khushab having the lowest percentage (24.71%) and Mianwali having the highest (54.44%). Goats (16.41%), sheep (27.38%), buffaloes (33.73%), and cows (41.5%) were the most affected species. Male buffaloes (59.37%) were more infected than females (33.58%), while female cows (45.10%) were more infested than males (31%). The distribution of ticks differed by species and sex; in cows and buffaloes, the most afflicted regions were the udder and tail, respectively. Ticks were mostly detected in the ears of sheep and goats and concentrated on the testicles of males. The major risk factors were the summer season, inadequate waste clearance, dogs afflicted with ticks, communal living, grazing systems, and poor tick control techniques. According to morphological studies, there is just one soft tick species, Ornithodoros erraticus, and hard ticks from the genera Rhipicephalus, Hyalomma, and Ixodes. Four tick species had positive bands (750 bp) validated by PCR testing. The study emphasizes the necessity for efficient tick management methods by highlighting the epidemiological implications of species, sex, and tick dispersion patterns in infection rates.

Keywords: Epidemiology, Tick-borne diseases, Ruminants, Cattle parasites, Tick identification, Geo-spatial distribution of ticks, One Health and tick ecology, Hemiparasites load in ruminants, Tick microbiome analysis, Vectorial capacity of Pakistani tick species, Rickettsial pathogens in ticks, Ixodid tick hotspots in Punjab, Climatic influence on tick proliferation.

INTRODUCTION

Ticks rank second only to mosquitoes as the most significant vectors of human diseases worldwide and are the primary vectors of disease-causing microbes in both domestic and wild animals (Wikel S. K., 2018). These ectoparasites possess a broad host range, and their preferred hosts vary depending on the tick species and habitat. As a subtropical country, Pakistan has a large rural population relies on livestock, including ruminants, for their livelihood (Ghafar A. *et al.*, 2020). The economic impact of ticks and tick-borne diseases is substantial, with estimated losses ranging from US $13.9 to $18.7 billion, along with an annual shortage of approximately 3 billion pieces of cattle hides and skins (de Castro J.J., 1997; Karim S. *et al.*, 2017). Ticks negatively affect livestock and human health by transmitting various microorganisms, including

protozoans, viruses, and bacteria. The most common tick genera infesting humans and animals in Pakistan include Rhipicephalus, Haemaphysalis, Hyalomma, and Ornithodoros, which are widely distributed throughout the country (Hoogstraal H. & Varma M.G.R., 1962; Robertson, R. G., *et al.*, 1970; Karim S. *et al.*, 2017). Taxonomically, ticks belong to the phylum Arthropoda and order Acarina, with approximately 899 recognized species classified as vertebrate parasites, including 185 species of Argasidae (soft ticks), 713 species of Ixodidae (hard ticks), and one species of Nutallilllididae (Kaur D. *et al.*, 2017). Pakistan has reported 75 tick species based primarily on morphological characteristics (Halliday *et al.*, 2018; Nasreen N. *et al.*, 2020). The seasonal occurrence of tick infestations in cattle, buffaloes, goats, and sheep shows periodic variation, with cows being the most commonly affected host, followed by buffaloes and other ruminants (Rehman *et al.*, 2017; Atif F.A. & Khan *et al.*, 2017; Moyo B. & Masika P.J., 2009; H. Guo *et al.*, 2019). Ticks cause significant economic damage through blood loss, which leads to reduced weight gain and lower milk production in livestock (Kaur D. *et al.*, 2017). The life cycle of ticks involves the infestation of host animals from early October through April, with peak populations observed between late October and mid-February (Kaiser M.N. & Hoogstraal H., 1964; Kiran A. *et al.*, 2019). The prevalence of ticks in domestic ruminants is influenced by climatic factors, with higher infestation rates recorded during summer and spring (Sajid M.S. *et al.*, 2007; Bianca N., 2016; Kiran A. *et al.*, 2019). A study conducted in two regions of Lower Punjab reported that bovine tick infestation exceeded 50% (Sajid M.S. *et al.*, 2008a & b; Kiran A. *et al.*, 2019).

Several species from the genera Dermacentor, Haemaphysalis, Hyalomma, and Rhipicephalus of the Ixodidae family infest domesticated animals, with some species acting as vectors for pathogens affecting livestock and humans. Haemaphysalis longicornis is a prominent vector transmitting pathogens responsible for anaplasmosis, babesiosis, and rickettsiosis (Ghafar A. *et al.*, 2020). The most frequently observed tick species infesting ruminants belong to the Ixodidae family (Bibi S. *et al.*, 2020). Accurate taxonomic classification of ticks is critical for effective tick-borne disease management. Morphological identification methods primarily focus on the size, shape, and distinctive features such as mouthparts, body outline, and the scutum, which covers the entire dorsal side in males but only a partial area in females (Abdel-Shafy, 2008; Hmoon M.M. *et al.*, 2018; Bibi S. *et al.*, 2020). Conventional light microscopy (LM) and scanning electron microscopy (SEM) are widely used for taxonomic identification of adult ticks; however, these methods present challenges when specimens are blood-engorged, physically damaged, or in immature stages such as eggs, larvae, or nymphs (Caporale D.A. *et al.*, 1995; Guglielmone A.A. *et al.*, 2006; Hmoon M.M. *et al.*, 2018). Furthermore, conventional microscopy techniques struggle to provide precise taxonomic identification at the subspecies or closely related species level (Liu G.H. *et al.*, 2013; Nava S. *et al.*, 2015). Ticks have been reported across all regions of Pakistan, including Sindh, Khyber Pakhtunkhwa (KPK), Balochistan, and the northern areas such as Gilgit-Baltistan (Soomro *et al.*, 2014; Kakar M.N. *et al.*, 2017; Sajid *et al.*, 2008; 2009; 2011; 2017; Sajid M.S. *et al.*, 2020). Numerous risk factors contribute to tick infestations in livestock, which in turn influence the epidemiology of tick-borne diseases (Karim S. *et al.*, 2017; Sajid M.S. *et al.*, 2018; Hassan M.A. *et al.*, 2018; Rashid I. *et al.*, 2019; Rizwan *et al.*, 2019). Small ruminants, particularly goats—commonly referred to as "the poor man's cow"—play a vital role in the livestock industry of Pakistan (Sajid M.S. *et al.*, 2020).

Advancements in molecular biology have facilitated the identification of genes involved in the physiological processes of blood-feeding arthropods and the pathogens they transmit to humans and animals (Burger *et al.*, 2014; J. Zeb *et al.*, 2020). Molecular techniques have recently been employed for taxonomic identification and phylogenetic analysis of ticks. The 18S rRNA gene is commonly used for genus-level identification, while 16S rRNA, COX-1, and ITS-2 are widely used for species-level differentiation. Additionally, the 12S rRNA marker is employed for analyzing relationships among newly diverged branches of tick phylogenies (Abdullah H.H. *et al.*, 2016). DNA barcoding is an innovative method for tick species identification, utilizing a short, conserved region of genetic material. Among molecular markers, the ITS-2 gene is approximately 750 base pairs long and is one of the most effective for tick identification and genetic characterization (Chitimia L. *et al.*, 2010). This marker has proven useful in identifying various species within the genera Rhipicephalus and Dermacentor (Abidgoudarzi M. *et al.*, 2011). As tick infestations and tick-borne diseases continue to pose significant economic and health burdens in Pakistan, integrating molecular techniques with traditional morphological methods can enhance species identification, improve surveillance, and aid in developing effective control strategies.

MATERIALS AND METHODS

Study area:

Location and climate of the study area:

This cross-sectional study was conducted in the Sargodha Division of the Province of Punjab, Pakistan. Sargodha Division comprises four Districts (Mianwali, Bhakkar, Khushab, and Sargodha). The total area of the Sargodha division is 26,360 km^2 and 6513697.9 acres with the GPS coordinates of 32° 4' 56.8776'' N and 72° 40' 8.8608'' E. The district Sargodha covers an area of 5.854 K km^2 with GPS coordinates of 32.0740° N, 72.6861° E. Sargodha's climate is extremely hot in summer and temperate cold in winter. The maximum range of temperature in summer is 50 $C^{o,}$ and in winter is 8 $C^{o,}$ while annual precipitation is 532.5 mm (per year).

The district Khushab covers an area of 6,511 km² with GPS coordinates of 32.2955° N, 72.3489° E. The climate of Khushab is extremely hot in summer, with a maximum temperature of 50 Co and a minimum temperature of -1 C° in winter. The average rainfall in winter is 100mm, and in summer is 424 mm. The district Bhakkar covers an area of 8,153 km² with GPS coordinates of 31.8621° N, 71.3824° E. The climate of Bhakkar is desert-like, with almost no rainfall. The district Mianwali covers an area of 5,840 km² with GPS coordinates of 32.6645° N, 71.4774° E. The climate of Mianwali is extremely hot in summer, with a maximum temperature of 50 C° and with average rainfall of 352mm.

Data collection:

Ethical considerations:

The ticks were collected from the domestic animals (ruminants) by the verbal consent of their owners. The verbal informed consent was obtained from the livestock farm owner for tick collection.

Sample collection and preservation:

The ticks were collected from different body parts of four ruminant species (cow, buffaloes, goat and sheep) from February to June 2021. The specimens were collected using fine-tipped tweezers and preserved in separate vials containing 70% ethanol. Besides these, all information regarding the collection date, District, and Farm number is also coded on vials.

Determining the epidemiological aspects:

Forty farms were included in this study, and a total of 1067 ruminants (Cows, buffaloes, goats and sheep) were included in the study to determine the tick infestation rate, Ruminant species prevalence, gender-wise prevalence of ticks, prevalence of ticks on various body parts, and associated risk factors in all districts of Sargodha Division.

The infestation rate of studied animals is calculated by using the given formula:

Infestation rate= $\frac{\text{No.of infested animals}}{\text{No.of observed animals}} \times 100$ and expressed in simple percentages. Ticks distribution on body parts of studied ruminants was also expressed in percentage using the formula. $\frac{\text{No.of body parts infested}}{\text{No.of infested animals}} \times 100$

Risk factor analysis

For Risk factor analysis, a questionnaire comprised of 18 questions was designed to collect the responses of farm keepers to assess the risk factors associated with tick infestation. Percentage analysis highlighted the major risk factors associated with high tick prevalence.

Morphological identification

A total of 761 ticks (Male and female) were collected from the infected animals. All tick specimens were morphologically characterized under a stereomicroscope using a MULTI-KEY, a windowing tool kit developed by the University of Edinburgh.

DNA Extraction

DNA was extracted from specimens by using a commercially available Thermo scientific Kit. DNA was extracted by following the manufacturer's instructions.

Procedure

i. Firstly, the specimen's cuticle was removed, and then grinded using a blade.
ii. Then, the ground specimen was placed in Eppendorf tubes with the help of a pipette.
iii. Then digestive solution and Proteinase K were added in eppendrofs containing samples.
iv. Samples were vortexed by using vortex.
v. Then, samples were placed in a Heat block for 24 hours.
vi. After 24 hours, samples were drawn from a heat block, and RNAase was added. Samples were placed at room temperature for 10 minutes.
vii. Then 50% chilled ethanol (400 µl) was added by using pipettes.

Note: Tips should be changed for another sample if it touches the Eppendorf.

i. Then, samples were added to DNA column tubes.
ii. Then centrifuge the samples for 2 minutes. Then, remove the liquid from the column. Moreover, again, centrifuge the samples. Then, the liquid was removed.
iii. Buffer solution I was added to samples and then centrifuged for 2 minutes. After that, again remove the liquid.
iv. Then Buffer solution II was added to samples and centrifuged for 2 minutes.
v. After that, columns were disposed of, and DNA was immediately stored in the refrigerator.

PCR Detection:

PCR based detection was performed by using a primer. The targeted nuclear ribosomal gene sequence of primer is given in Table 1-

Table 1: Targeted gene, primer sequence and its length

Targeted Gene	Primer Sequence	Base Pair	Reference
ITS-2	AGGACACACTGA GCACTGATTC ACTGCGAAGCACTTAGACCG	750 bp	Rehman. An *et al.*, 2017

Table 2: Total volume of the PCR reaction

Sr. No	Reagents	Volume
1.	Master Mix	12µl
2.	Forward primer	1 µl
3.	Reverse primer	1 µl
4.	Template DNA	2.5 µl
5.	Injection water	8.5 µl
	Final Volume	25 µl

Table 3: Conditions of Thermal Profile for PCR

Sr. No	Condition	Temperature C°	Time	Cycles
1	Initial Denaturation	94	4min	1x
2	Denaturation	94	1min	35x
3	Annealing	57	30s	35x
4	Extension	72	45s	35x
5	Final Extension	72	10min	1x

Agarose Gel Electrophoresis

The products of PCR were confirmed by subjecting 6ml of the amplification product mixture to electrophoresis. The fragments of the amplified DNA of specific sizes were visualized using UV fluorescence and a gel documentation system (Doc EZ imager).

RESULTS

Results of the Epidemiological Aspects

District-wise infestation rate:-

In each District, Ten farms were selected for sampling. (Table-1, Fig-1) In District Mianwali, The tick infestation percentage recorded was (54.44%), which is the highest infestation percentage, followed by Sargodha, where the infestation rate was (28.92%), and Bhakkar (25.69%) and the lowest tick infestation was observed in ruminants of District Khushab (24.71%) as compared to other districts of Sargodha Division. In Sargodha Division, including four Districts, the overall tick infestation rate was (32.61%).

Table 1: District-wise Tick Infestation Percentage in Sargodha Division

District	Number of animals observed	Number of animals infested	infested Percentage
Mianwali	270	147	54.44%
Bhakkar	288	74	25.69%
Khushab	267	66	24.71%
Sargodha	242	70	28.92%
Total	1067	348	32.61%

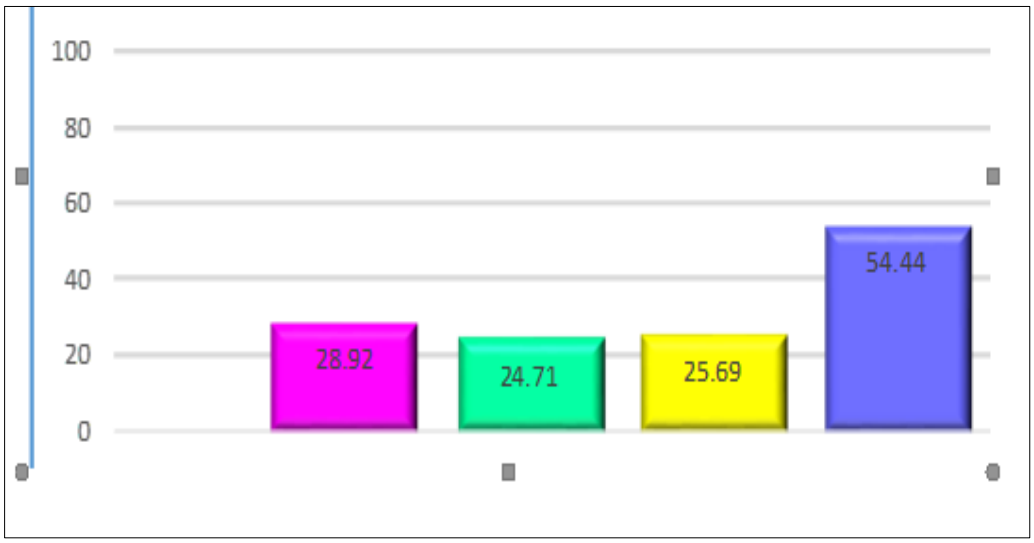


Figure 1: District-wise Percentage analysis of Tick infestation in Sargodha Division Pakistan

Ruminants specie wise ticks infestation rate in studied districts:-

(Table-2., Fig-2) In Mianwali, the most infested ruminant was cows (65.97%), followed by buffaloes (64.55%), goats (35.41%) and sheep (32.60%). In Bhakkar, the most infested species was also cow (36.78%), followed by sheep (22.85%), (22.5%) and Goats (16.27%). In Khushab, the most infested species was buffalo. (32.69%) followed by cows (29.31%), Sheep (16.98%) and Goats (13.04%). In Sargodha, the Highest tick infestation was observed in sheep (56.52%), followed by cows (36.04%), buffaloes (33.73%), and less infested ruminant goats (10.9%).

(Table-3., Fig-6) revealed the results of the whole division, including four districts. Cows were found more infested with ticks (39.40%), and the second most highly infested species was buffalo (33.73%). Small ruminants were found less infested sheep (27.38%). In addition, the least infested ruminant species was goat (17.61%).

Table 2: Ruminant-wise Tick infestation percentage in four Districts in the Sargodha Division

District	Species			
	Cow	Buffalo	Goat	Sheep
Mianwali	64/97=65.97%	51/79=64.55%	17/48=35.41%	15/46=32.60%
Bhakkar	32/87=36.78%	27/120=22.5%	7/46=16.27%	8/35=22.85%
Khushab	34/116=29.31%	17/52=32.69%	6/46=13.04%	9/53=16.98%
Sargodha	31/86= 36.04%	20/78=25.64%	6/55=10.9%	13/23= 56.52 %

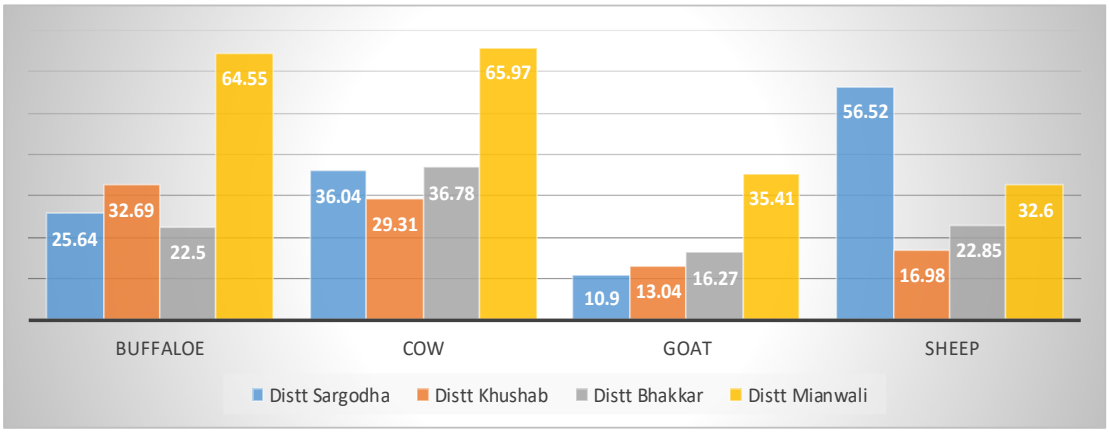


Figure 2: Percentage of Tick infestation in studied ruminant species of Sargodha Division

Table 3: Overall Tick infestation percentage of ruminants in the Sargodha Division

S. No	Ruminant Species	Number of observed animals	Number of animals infested	Infestation rate
1.	Cow	386	160	41.45%
2.	Buffalo	329	111	33.73%
3.	Sheep	157	43	27.38%
4.	Goat	195	34	16.41%

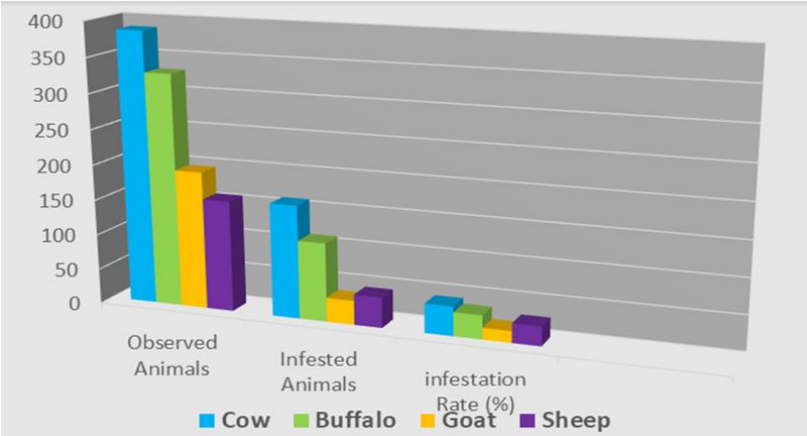


Fig. 3: Overall Tick infestation percentage of ruminants in Sargodha Division

Sex-wise tick infestation rate in ruminants:

Table-4 In district Mianwali, female cows (69.33%) were found more infested as compared to male cows (50%), while male buffaloes (73.33%) were found more infested than female buffaloes. Male sheep (35.48%) were more infested than female sheep (20%). Male goats (44.44%) were more infested than female goats (26.66%).

Table-5 In District Bhakkar, The female cows (41.42%) were more infested than male cows (29.41%). Buffaloes male (30%) were found more infested than buffaloes female (20%). Sheep males (46.15%) were more infested than sheep females (9.09%). Goat males (20%) were more infested than females (11.53%).

Table-6 In District Khushab, female cows (34.11 %) were more infested than male cows (16.12%). Buffaloes males (33.33%) were more infested than females (32.60%). Sheep males (21.87%) were more infested than sheep females (9.52%). Goat males (23.52%) were more infested than females (6.89%).

Table-7 In District Sargodha, Cow females (33.92%) were more infested than males (33.33%). Buffaloes male (30.76%) were found more infested than buffaloes females (24.61%). Sheep females (66.66%) were more infested than males (50%). Goat males (30.76%) were more infested than females (4.76%).

Table 8 shows the results of gender-wise infestation of ruminants in the whole division. Female cows (45.10%) were more infested than males (31%). Male buffaloes (59.37%) were more infested than female buffaloes (33.58%). Male sheep (34.44%) were found more infested than female sheep (19.40%) and also male goats (29.41%) were found more infested than female goats (11.8%).

Table-4: Ruminants (Sex) wise Tick infestation percentage in Districts Mianwali

S. No	Ruminant Species (Female)	Number of observed animals	Number of animals infested	Infestation rate
1.	Cow	75	52	69.33%
2.	Buffalo	64	40	62.5%
3.	Sheep	15	3	20%
4.	Goat	30	8	26.66%
S. No	**Ruminant Species (Male)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1.	Cow	22	11	50%
2.	Buffalo	15	11	73.33%
3.	Sheep	31	11	35.48%
4.	Goat	18	8	44.44%

Table-5 Ruminants (Sex) wise Tick infestation percentage in Districts Bhakkar

S. No	Ruminant Species (Female)	Number of observed animals	Number of animals infested	Infestation rate
1	Cow	70	29	41.42%
2	Buffalo	90	18	20%
3	Sheep	22	2	9.09%
4	Goat	26	3	11.53%
S. No	**Ruminant Species (Male)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1	Cow	17	5	29.41%
2	Buffalo	30	9	30%
3	Sheep	13	6	46.15%
4	Goat	20	4	20%

Table-6 Ruminants (Sex) wise Tick infestation percentage in Districts Khushab

S. No	Ruminant Species (Female)	Number of observed animals	Number of animals infested	Infestation rate
1	Cow	85	29	34.11%
2	Buffalo	46	15	32.60%
3	Sheep	21	2	9.52%
4	Goat	29	2	6.89%
S. No	**Ruminant Species (Male)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1	Cow	31	5	16.12%
2	Buffalo	6	2	33.33%

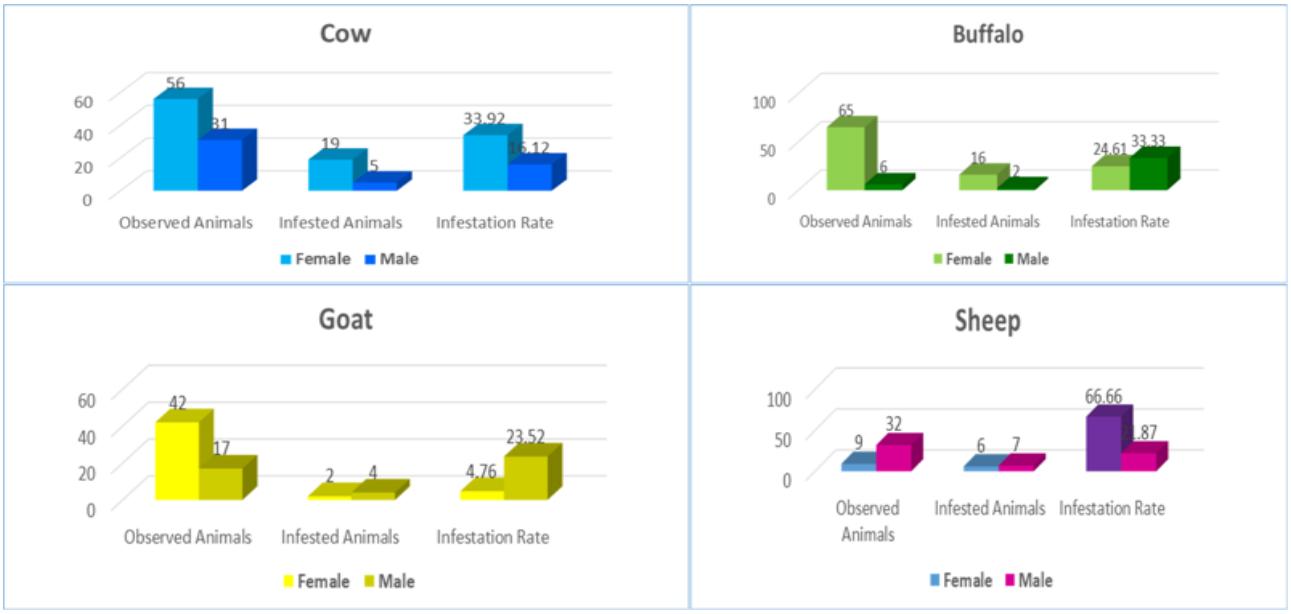
3	Sheep	32	7	21.87%
4	Goat	17	4	23.52%

Table-7 Ruminants (Sex) wise Tick infestation percentage in Districts Sargodha

S. No	**Ruminant Species (Female)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1	Cow	56	19	33.92%
2	Buffalo	65	16	24.61%
3	Sheep	9	6	66.66
4	Goat	42	2	4.76
S. No	**Ruminant Species (Male)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1	Cow	30	10	33.33%
2	Buffalo	13	4	30.76%
3	Sheep	14	7	50%
4	Goat	13	4	30.76%

Table-8- Ruminants (Sex) wise Tick infestation percentage in Sargodha Division

S. No	**Ruminant Species (Female)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1.	Cow	286	129	45.10%
2.	Buffalo	265	89	33.58%
3.	Sheep	67	13	19.40%
4.	Goat	127	15	11.8%
S. No	**Ruminant Species (Male)**	**Number of observed animals**	**Number of animals infested**	**Infestation rate**
1.	Cow	100	31	31%
2.	Buffalo	64	38	59.37%
3.	Sheep	90	31	34.44%
4.	Goat	68	20	29.41%



District-wise results of the distribution of ticks on different body parts

Table 4.9 shows the overall infested parts of all ruminants in the Sargodha Division. Results reveal that the most infested body part of cows (female) is the udder (36.75%), inner thighs (35.04%), all over the body (33.33%), and the least infested part following the tail (50.42%) was external genitalia (14.52%). No ticks were found distributed in the ears. While in buffaloes (females), ticks were highly distributed on the udder (79.77%) followed by the tail (70.78%), inner thighs (28.08%), all over the body (11.23%) and the least infested part was external genitalia (4.49%). No ticks were found distributed in the ears. All infested goats and

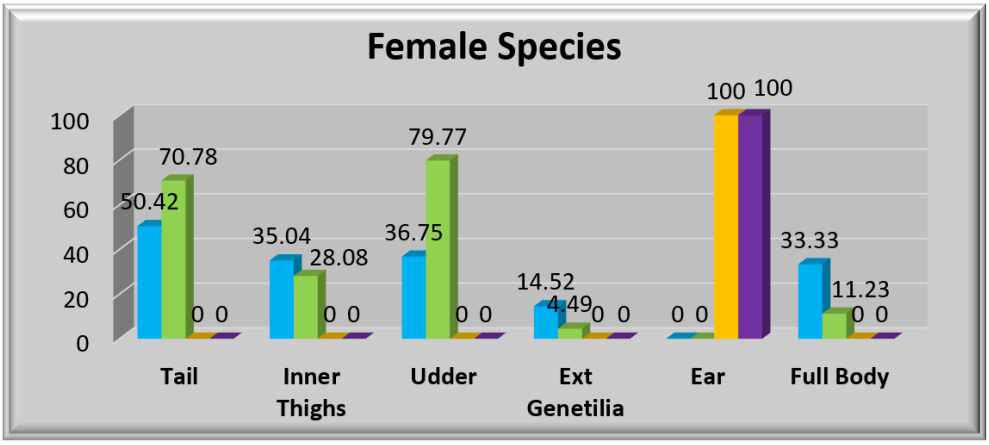
sheep (Females) ticks were found in ears. In males, ticks were highly distributed on testicles and dewlap (55.88%), followed by the tail and all over the body (14.70%), and the least infested part was external genitalia (2.94%). In buffalo (males), the most infested part was the testicles (73.07%), followed by dewlap (61.53%), the external genitalia (15.38%), and the least infested part, the tail (7.69%). No ticks were found distributed in the ears. In all infested goats and sheep (males), ticks were found in the ears.

A- Udder **B- Tail** **C- Ear**

Fig 4: Body parts infested with ticks

Table-9: Infestation percentage of distribution of ticks on different body parts of all observed ruminants of Sargodha Division

	Species (Females)			
	Cow	**Buffalo**	**Goat**	**Sheep**
Observed animals	129	89	13	15
Body parts				
Tail	50.42%	70.78%	-	-
Inner thighs	35.04%	28.08%	-	-
Udder	36.75%	79.77%	-	-
Ext. genetilia	14.52%	4.49%	-	-
Ear	-	-	100%	100%
Full body	33.33%	11.23%	-	-
	Species (Males)			
	Cow	**Buffalo**	**Goat**	**Sheep**
Observed animals	31	38	31	20
Body parts				
Tail	14.70%	7.69%	-	-
Inner thighs	-	-	-	-
Dewlap	55.88%	61.53%	-	-
Ext. genetilia	2.94%	15.38%	-	-
Ear	-	-	100%	100%
Testicles	55.88%	73.07%	-	-
Full body	14.70%	-	-	-



Morphological Identification Results:

Ten tick species were identified, out of which 9 species were hard ticks, and one was soft ticks. The identified hard tick species belong to the two most prominent genera, Rhippicephalus and Ixodese, While the identified soft tick species belongs to the genus Argasidae. The overall prevalence of the identified species in a Sargodha Division and the prevalence percentage of the tick species on ruminants are given in Table 13.

Table 10: Name of the identified species and their prevalence percentage on the studied ruminants

Name of Ticks	Ruminant Species				
	Buffalo	Cow	Goat	Sheep	Total
Boophilus annulatus (Hard Tick, Rhipicephalus)	59 (39.9%)	36(24.3%)	35 (23.6%)	48 (32.5%)	148 (19.4%)
Rhipicephalus sanguineus (Hard Tick, Rhipicephalus)	37(30.3%)	61 (50%)	9 (7.8%)	15 (12.3%)	122 (16.3%)
Ixodese gibosus (Hard Tick, Ixodese)	18 (32.7%)	28 (51%)	4 (7.8%)	5 (9%)	55 (7.22%)
Rhipicephalus cameras (Hard Tick, Rhipicephalus)	40 (20.3%)	81 (41%)	47 (23.9%)	29 (14.7%)	197 (25.9%)
Hya. Anatolicum (hard tick, Hyalomma)	18 (36.8%)	23 (47%)	6 (12.2%)	2 (4.8%)	49(6.43%)
Hyalomma dromedarri (Hard Tick, Hyalomma)	10 (30.3%)	17 (51.5%)	4 (12.10%)	2 (6.6%)	33 (4.33%)
Ornithodorous erratics (soft Tick, Argasidae)	11 (18.3)	30 (50%)	12 (20%)	7 (11.7%)	60 (7.9%)
Hya. Detritum scupense (Hard tick Hyalomma,)	9 (30%)	9 (30%)	3 (10%)	9 (30%)	30 (3.9%)
Ixodese hexagons (Hard Tick, Ixodese)	2 (18.2%)	8 (72%)	0 (0%)	1 (9.9%)	11 (1.5%)
Rhipicephalus turanicus (Hard Tick Rhipicephalus,)	13 (23.2%)	26 (46.4%)	2 (3.6%)	15 (26.8%)	56 (7.4%)
Total Tick count					**761**

RISK FACTOR ANALYSIS:

Keeping ruminant species under the same shed, Grazing system, ignorant about tick-borne diseases, infested dogs at the farm, closed housing system and soft flooring, absence of rural poultry, crevices in houses, not removing animal waste, summer season and ignoring the ticks at the ground are significant factors associated with high tick infestation. These factors are the leading causes of tick infestation on animals and badly affect the productivity of dairy animals.

PCR ASSAYS:

After PCR amplification of the targeted sequence ITS-2, four positive bands of the 750 bp were excised from the gel. 1 KB ladder was used in this gel, M representing marker or ladder.

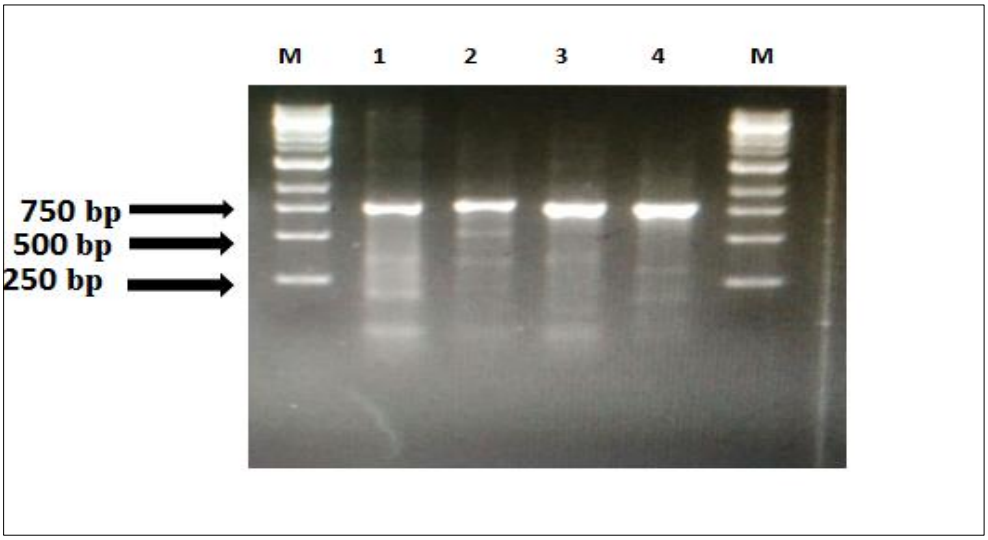


Figure 5: Gel Picture of amplified sequences of the targeted Gene ITS-2

DISCUSSION

The results of the study showed the highest tick infestation rate in Mianwali (54.44%), followed by Sargodha (27.38%) and Bhakkar (24.40%) and the lowest infestation rate was observed in Khushab (22.47%). In Mianwali, this is the first study to determine ruminants' tick infestation rate. The results of this study correlate with the results of Batool M *et al.*, 2019. They reported that the lowest tick infestation (33.47%) was seen in the Northern zone of Punjab (Khushab and

Bhakkar). In the whole Sargodha Division, the tick infestation rate in ruminants was (32.10%). Hussain. S *et al.,* 2021 in their epidemiological survey regarding ticks and tick-borne diseases, reported that the least tick infestation was observed in District Khushab (12.5%) compared to other studied districts of their survey. The reason for the lowest tick infestation in District Khushab is farmers' knowledge about tick infestation and taking care as well as cure of tick infestation. There are professional farm keepers to look after having experienced acaricide management in Khushab district.

The results of the study of ruminant-wise tick infestation rate showed the highest infestation on cows (39.40%) followed by buffalo (33.73%), sheep (27.38%) and the least infested species was goat (17.61%). These results show a resemblance with the one study conducted by Bibi, A. *et al.,* 2020 in Baluchistan that the most infested ruminant was a cow (65%), followed by buffaloes (55), then sheep (30%) and the least infested was a goat (27.5%). The factor that could be responsible for higher tick infestation in cows is their thin skin compared to buffaloes having thick skin (Sajid, M.S *et al.,* 2009).

The results of ruminant specie (sex wise) infestation rate showed the highest infestation rate in female cows (45.10%) as compared to male cows (31%). Kakar M.E. *et al.,* 2017 also reported high tick infestation in female cows (36%) compared to males (32%). The tick infestation rate in female cows is higher may be due to some factors like hormonal imbalance and poor body conditions in pregnancy or the lactation period, which makes them more prone to tick infestation (Shekhar, S and Singh, J.P *et al.,* 2020). The highest infestation rate was observed in male buffaloes (59.37%) compared to female buffaloes (33.58%). These findings are related to Tasawar Z *et al.,* 2014. They also reported a higher tick infestation in male buffaloes (85%); females (43.33%) were less infested than males.

The highest tick infestation rate in male buffaloes can be related to the to a factor that they have poor immune systems as compared to females due to the chemical composition of their male hormone steroid, which may be responsible for weakening the defense mechanism of the male host and making it more susceptible to parasitic attacks (Khalil, M.I *et al.,* 2018). The male host of small ruminants (goats and sheep) was more infested than females. These findings align with the study of Noor, J *et al.,* 2016 that male goats (58.33%) were found to be more infested than female goats (41.67%).

The overall results of the distribution of ticks on various body parts of ruminants showed that in cows and buffaloes (females), the predilection sites of tick infestation were the tail, followed by the udder, inner thighs and least infested parts of all external genitalia and distribution on all over the body was observed very low and no ticks were observed on ears. These results align with the findings of research by H.I. Musa *et al.,* 2016 that the most infested part of the female cows was the udder (84.3%) and the tail (69.81%), and least infested parts were ears and all over the body. However, Mustafa. I *et al.,* 2014 reported the same results that in buffalo (females), ticks were found highly distributed on the udder. The smooth tissues of the predicted sites for tick infestation are very beneficial for ticks. They can easily feed on blood at the smooth tissues and reach blood capillaries. Female ticks get an advantage by feeding on such parts for reproduction, as the sites like the udder and undertail are not exposed, so the poultry or birds cannot prey upon ticks (Mustafa. I *et al.,* 2014). In male cows and buffaloes, the most infested sites were testicles and dewlap, followed by the tail and all over the body, and the least infested part was external genitalia. In the current study, in all small ruminants (goats and sheep) ticks were found distributed only in ears. These studies are in line with the studies of Mustafa. I *et al.,* 2014 in their studies, ticks were mostly found in goats' ears. Noor. J *et al.,* 2016 reported the highest prevalence of ticks in the ears of small ruminants compared to other body parts. Another author, Ramzan.M *et al.,* 2019 reported in their study that the predilection site of tick infestation in goats and sheep was ears.

The morphological results of our study align with the study of (Mustafa, I *et al.,* 2014) reported that the most prevalent tick species are B. annulatus, R.sanguineus, and Hya. marginatum and Hya. anatolicum. Our morphology results showed the highest prevalence of the genus Rhippicephalus of hard ticks. These studies are compatible with Shoaib M *et al.,* 2020 who also reported a higher prevalence of Rhippicephalus.

The results of the risk factor analysis are also in line with the study of (Ali A. *et al.,* 2019) that more prevalence of ticks is recorded in the summer season, and the summer season is associated with high tick prevalence. Our results showed that the highest tick infestation in those grazing animals is in a closed housing system. These findings also align with the study of Iqbal A *et al.,* who also reported that these factors are highly associated with tick infestation.

The PCR detection results of excising positive bands of 750 base pairs of the targeted sequence of ITS-2 are also in line with the study of Roman. M. *et al.,* 2021 molecularly characterize the hard ticks using the primer ITS-2 in their study. A fragment of 750 bp was amplified and successfully characterized the hard ticks.

CONCLUSION

It is concluded that the tick infestation is going beyond in the Sargodha Division and affecting the yield of dairy animals by causing tick-borne disease, reduction of milk in lactating animals, reduced growth in young animals and serious economic losses. Farm keepers

ignore the risk factors associated with tick infestation, thus contributing to more animal infestation. A stereomicroscope proved to be a reliable instrument for the morphological identification of tick species. However, for confirmation, PCR assays are proven authentic using a ribosomal gene ITS-2 to characterize hard ticks.

REFERENCES

- Abbasi, F., Abbasi, I. H. R., Nissa, T. F., Bhutto, Z. A., Arain, M. A., Soomro, R. N., ... & Fazlani, S. A. (2017). Epidemiological study of tick infestation in buffalo of various regions of district Khairpur, Pakistan. *Veterinary world*, *10*(6), 688.
- Abdel-Shafy, S. (2007). Scanning electron microscopy and comparative morphology of Hyalomma anatolicum excavatum, H. dromedarii and H. marginatum marginatum (Acari: Ixodidae) based on nymphs.
- Abdigoudarzi, M., Noureddine, R., Seitzer, U., & Ahmed, J. (2011). rDNA-ITS2 Identification of Hyalomma, Rhipicephalus, Dermacentor and Boophilus spp.(Acari: Ixodidae) collected from different geographical regions of Iran. *Advanced studies in biology*, *3*(5), 221-238.
- Abdullah, H. H., El-Molla, A., Salib, F. A., Allam, N. A., Ghazy, A. A., & Abdel-Shafy, S. (2016). Morphological and molecular identification of the brown dog tick Rhipicephalus sanguineus and the camel tick Hyalomma dromedarii (Acari: Ixodidae) vectors of Rickettsioses in Egypt. *Veterinary World*, *9*(10), 1087.
- Akhtar, K., Anees, R., Karim, T., Gul, S. U., Rehman, H. U., Ali, A., ... & Achakzai, S. K. (2019). Prevalence of tick infestation in cows of various Regions of district Karak, Pakistan. *J Entomol Zool Stud*, *7*(2), 791-795.
- Ali, A., Khan, M. A., Zahid, H., Yaseen, P. M., Qayash Khan, M., Nawab, J., ... & Ibrahim, M. (2019). Seasonal dynamics, record of ticks infesting humans, wild and domestic animals and molecular phylogeny of Rhipicephalus microplus in Khyber Pakhtunkhwa Pakistan. *Frontiers in physiology*, *10*, 793.
- Atif, F. A. (2012). Prevalence of cattle tick infestation in three districts of the Punjab, Pakistan. *Pakistan Journal of Science*, *64*(1).
- Ayaz, S., Shams, S., Abdel-Reheem, M. A., Khan, S., & Ullah, R. (2013). Epidemiology and molecular detection of babesiosis in household dairies in districts Kohat and Karak, Khyber Pakhtunkhwa Pakistan. *Life Science Journal*, *10*(SPL. ISSUE10), 188-193.
- Niazi, M., Ahmed, Z., Ahmed, N., Hafeez, S., Fatima, E., Farooq, U., ... & Rehman, A. (2025). An Epidemiological Study on Infestation Rate of Ticks in Ruminants of Sargodha Division Pakistan. *Indus Journal of Bioscience Research*, *3*(1), 539-548.
- Burger, T. D., Shao, R., & Barker, S. C. (2014). Phylogenetic analysis of mitochondrial genome sequences indicates that the cattle tick, Rhipicephalus(Boophilus) microplus, contains a cryptic species. *Molecular Phylogenetic Evolution*. 76, 241–253.
- Caporale, D. A., Rich, S. M., Spielman, A., Telford, S. R., 3rd, & Kocher, T. D. (1995). Discriminating between Ixodes ticks through mitochondrial DNA sequences. *Mol. Phylogenet. Evol*. 4: 361-365.
- Chen, X., Yu, Z., Guo, L., Li, L., Meng, H., Wang, D., ... & Liu, J. (2012). Life cycle of Haemaphysalis doenitzi (Acari: Ixodidae) under laboratory conditions and its phylogeny based on mitochondrial 16S rDNA. *Experimental and applied acarology*, *56*, 143-150.
- Chitimia, L., Lin, R. Q., Cosoroaba, I., Wu, X. Y., Song, H. Q., Yuan, Z. G., & Zhu, X. Q. (2010). Genetic characterization of ticks from southwestern Romania by sequences of mitochondrial cox 1 and nad 5 genes. *Experimental and Applied Acarology*, *52*, 305-311.
- De Castro, J. J., James, A. D., Minjauw, B., Di Giulio, G. U., Permin, A., Pegram, R. G., ... & Sinyangwe, P. (1997). Long-term studies on the economic impact of ticks on Sanga cattle in Zambia. *Experimental & applied acarology*, *21*, 3-19.
- de Castro, J. J. (1997). Sustainable tick and tickborne disease control in livestock improvement in developing countries. *Veterinary parasitology*, *71*(2-3), 77-97.
- de la Fuente, J., Estrada-Pena, A., Venzal, J.M., Kocan, K.M., Sonenshine, D. E., Ernieenor, Faraliana, Che. Lah/, SalmahYaakop. (2016). Precise identification of different stages of a tick, IxodesgranulatusSupino, 1897 (Acari: Ixodidae), *Asian Pacific Journal of Tropical Biomedicine*, *6*(7), 597-604, ISSN 2221-1691.
- de la Fuente, J., Estrada-Pena, A., Venzal, J. M., Kocan, K. M., & Sonenshine, D. E. (2008). Overview: Ticks as vectors of pathogens that cause disease in humans and animals. *Frontiers in bioscience: a journal and virtual library*, 13, 6938–6946.
- Erster, O., Roth, A., Hadani, Y., & Shkap, V. (2013). First detection of Ixodes ricinus on beef cattle in Israel. *Veterinary parasitology*, *191*(3-4), 394–399.
- Estrada-Peña, A., Venzal, J. M., Nava, S., Mangold, A., Guglielmone, A. A., Labruna, M. B., & De La Fuente, J. (2012). Reinstatement of Rhipicephalus (Boophilus) australis (Acari: Ixodidae) with redescription of the adult and larval stages. *Journal of Medical Entomology*, *49*(4), 794-802.
- Farooqi, S. H., Ijaz, M., Saleem, M. H., Rashid, M. I., Oneeb, M., Khan, A., ... & Mahmood, S. (2017). Distribution of Ixodid Tick Species and Associated Risk Factors in Temporal Zones of Khyber Pakhtunkhwa Province, Pakistan. *Pakistan Journal of Zoology*, *49*(6).

- Ghafar, A., Khan, A., Cabezas-Cruz, A., Gauci, C. G., Niaz, S., Ayaz, S., ... & Jabbar, A. (2020). An assessment of the molecular diversity of ticks and tick-borne microorganisms of small ruminants in Pakistan. *Microorganisms*, *8*(9), 1428.
- Guglielmone, A. A., Venzal, J. M., Gonzalez-Acuna, D., Nava, S., Hinojosa, A., & Mangold, A. J. (2006). The phylogenetic position of Ixodes stilesi Neumann, 1911 (Acari: Ixodidae): morphological and preliminary molecular evidences from 16S rDNA sequences. *Systematic Parasitology*, *65*, 1-11.
- Guo, H., Moumouni, P. F. A., Thekisoe, O., Gao, Y., Liu, M., Li, J., ... & Xuan, X. (2019). Genetic characterization of tick-borne pathogens in ticks infesting cattle and sheep from three South African provinces. *Ticks and tick-borne diseases*, *10*(4), 875-882.
- Halliday, B., Kamran, M., & Bashir, M. H. (2018). Checklist of the mites of Pakistan. *Zootaxa*, *4464*(1), 1-178.
- Hassan, M. A., Liu, J., Rashid, M., Iqbal, N., Guan, G., Yin, H., & Luo, J. (2018). Molecular survey of piroplasm species from selected areas of China and Pakistan. *Parasites & vectors*, *11*(1), 457.
- Hmoon, M. M., Htun, L. L., Wai, S. S., Thu, M. J., Aung, S. T., Chel, H. M., ... & Bawm, S. (2018). Morphological and molecular identification of ticks infested in stray dogs within Nay Pyi Taw Area, Myanmar. *South Asian J. Life Sci*, *6*, 41-45.
- Hoogstraal, H., & Varma, M. G. R. (1962). Haemaphysalis cornupunctata sp. n. and H. kashmirensis sp. n. from Kashmir, with Notes on H. sundrai Sharif and H. sewelli Sharif of India and Pakistan (Ixodoidea, Ixodidae). *The Journal of Parasitology*, *48*(2), 185-194.
- Iqbal, A., Sajid, M. S., Khan, M. N., & Khan, M. K. (2013). Frequency distribution of hard ticks (Acari: Ixodidae) infesting the bubaline population of district Toba Tek Singh, Punjab, Pakistan. *Parasitology Research*, *112*(2), 535–541.
- Kaiser, M. N., & Hoogstraal, H. (1964). The Hyalomma ticks (Ixodoidea, Ixodidae) of Pakistan, India and Ceylon, with keys to subgenera and species.
- Kakar, M.N., & Kakar sulemankhel, J. (2008). Prevalence of endo (trematodes) and ectoparasites in cows and buffaloes of Quetta, Pakistan. *Pakistan Veterinary Journal*, *28*, 34-36.
- Karim, S., Budachetri, K., Mukherjee, N., Williams, J., Kausar, A., Hassan, M. J., Adamson, S., Dowd, S. E., Apanskevich, D., Arijo, A., Sindhu, Z. U., Kakar, M. A., Khan, R., Ullah, S., Sajid, M. S., Ali, A., & Iqbal, Z. (2017). A study of ticks and tick-borne livestock pathogens in Pakistan. *PLoS Neglected Tropical Diseases*, *11*(6), e0005681.
- El Kammah, K. M., & El-Fiky, Z. A. (2005). Molecular markers of some tick genera in Egypt based on the internal transcribed spacer (ITS-2): 1-Ixodidae (Boophilus and Hyalomma). *Arab Journal of Biotechnology*, *6*(1), 39-48.
- Khalil, M. I., Lashari, M. H., Akhtar, M. S., & Tasawar, Z. (2018). PREVALENCE OF TICKS INFESTING BUFFALOES IN AND AROUND JAMPUR DISTRICT RANJANPUR, PAKISTAN. *FUUAST Journal of Biology*, *8*(2).
- Labruna, M. B., Naranjo, V., Mangold, A. J., Thompson, C., Estrada-Peña, A., Guglielmone, A. A., ... & De La Fuente, J. (2009). Allopatric speciation in ticks: genetic and reproductive divergence between geographic strains of Rhipicephalus (Boophilus) microplus. *BMC Evolutionary Biology*, *9*, 1-12.
- Liu, G. H., Chen, F., Chen, Y. Z., Song, H. Q., Lin, R. Q., Zhou, D. H., & Zhu, X. Q. (2013). Complete mitochondrial genome sequence data provides genetic evidence that the brown dog tick Rhipicephalus sanguineus (Acari: Ixodidae) represents a species complex. *International journal of biological sciences*, *9*(4), 361–369.
- Moyo, B., & Masika, P. J. (2009). Tick control methods used by resource-limited farmers and the effect of ticks on cattle in rural areas of the Eastern Cape Province, South Africa. *Tropical animal health and production*, *41*(4), 517–523.
- Nava, S., Estrada-Peña, A., Petney, T., Beati, L., Labruna, M. B., Szabó, M. P., Venzal, J. M., Mastropaolo, M., Mangold, A. J., & Guglielmone, A. A. (2015). The taxonomic status of Rhipicephalus sanguineus (Latreille, 1806). *Veterinary parasitology*, *208*(1-2), 2–8.
- Nava, S., Guglielmone, A. A., & Mangold, A. J. (2009). An overview of systematics and evolution of ticks. *Frontiers in bioscience (Landmark edition)*, *14*, 2857–2877.
- Ogo, N. I., de Mera, I. G., Galindo, R. C., Okubanjo, O. O., Inuwa, H. M., Agbede, R. I., Torina, A., Alongi, A., Vicente, J., Gortázar, C., & de la Fuente, J. (2012). Molecular identification of tick-borne pathogens in Nigerian ticks. *Veterinary parasitology*, *187*(3-4), 572–577.
- Rashid, I., Saqib, M., Ahmad, T. & Sajid, M. S. (2019). Sero-prevalence and associated risk factors of fever in cattle and buffaloes managed at institutional dairy farms. Pak Vet J xx(xx):xx-xx.
- Rehman, A., Nijhof, A. M., Sauter-Louis, C., Schauer, B., Staubach, C., & Conraths, F. J. (2017). Distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in Pakistan's semi-arid and arid agro-ecological zones. *Parasites & vectors*, *10*(1), 190.
- Rizwan, H. M., Sajid, M. S., Abbas, H., Qamar, M. F., Akram, Q., & Maqbool, M. (2019). Epidemiology and control of Congo fever in sacrificial animals of Pakistan. *Veterinary Science Research*, *1*(2), 18-24.
- ROBERTSON, R. G., WISSEMAN JR, C. L., & TRAUB, R. (1970). Tick-borne rickettsiae of the

spotted fever group in West Pakistan: I. Isolation of strains from ticks in different habitats. *American journal of epidemiology*, *92*(6), 382-394.

- Rooman, M., Assad, Y., Tabassum, S., Sultan, S., Ayaz, S., Khan, M. F., ... & Ali, R. (2021). A cross-sectional survey of hard ticks and molecular characterization of Rhipicephalus microplus parasitizing domestic animals of Khyber Pakhtunkhwa, Pakistan. *PLoS One*, *16*(8), e0255138.
- Sajid, M. S., Asif Iqbal, A. I., Khan, M. N., Zafar Iqbal, Z. I., & Faisal Siddique, F. S. (2013). Descriptive epidemiology of insects infesting domestic sheep (Ovis aries) of district Toba Tek Singh, Punjab, Pakistan.
- Sajid, M. S., Iqbal, Z., Khan, M. N., Muhamamd, G., & Iqbal, M. U. (2007). Effect of Hyalomma ticks (Acari: Ixodidae) on milk production of dairy buffaloes (Bos Bubalus Bubalis) of Punjab (Pakistan). *Italian Journal of Animal Science*, *6*(sup2), 939-941.
- Sajid, M. S., Iqbal, Z., Khan, M. N., Muhammad, G., Needham, G., & Khan, M. K. (2011). Prevalence, associated determinants, and in vivo chemotherapeutic control of hard ticks (Acari: Ixodidae) infesting domestic goats (Capra hircus) of lower Punjab, Pakistan. *Parasitology research*, *108*, 601-609.
- Sajid, M. S., Kausar, A., Iqbal, A., Abbas, H., Iqbal, Z., & Jones, M. K. (2018). An insight into the ecobiology, vector significance and control of Hyalomma ticks (Acari: Ixodidae): A review. *Acta tropica*, *187*, 229-239.
- Sajid, M., Iqbal, Z., Shamim, A., Siddique, R., ul Hassan, M., & Rizwan, H. (2018). Distribution and abundance of ticks infesting livestock population along Karakorum highway from Mansehra to Gilgit, Pakistan. *Journal of the Hellenic Veterinary Medical Society*, *68*(1), 51-58.
- Sajid, M. S., Iqbal, Z., Khan, M. N., Muhamamd, G., & Iqbal, M. U. (2007). Effect of Hyalomma ticks (Acari: Ixodidae) on milk production of dairy buffaloes (Bos Bubalus Bubalis) of Punjab (Pakistan). *Italian Journal of Animal Science*, *6*(sup2), 939-941.
- Sajid, M. S., Rizwan, H. M., Khan, M. K., Qudoos, A., Atif, F. A., Malik, M. A., & Maqbool, M. (2020). Association of herd management with the infestation of ticks in domestic goats. *Journal of the Hellenic Veterinary Medical Society*, *71*(3), 2283-2290.
- Shah, A., Shah, S. R., Rafi, M. A., Noorrahim, M. S., & Mitra, A. (2015). Identification of the prevalent ticks (Ixodid) in goats and sheep in Peshawar, Pakistan. *J Entomol Zool Stud*, *3*, 11-14.
- Shoaib, M., Rashid, I., Akbar, H., Sheikh, A. A., Farooqi, S. H., Asif, M., ... & Khan, F. A. (2021). Prevalence of Rhipicephalus and Hyalomma ticks in cattle and associated risk factors in three districts of Khyber Pakhtunkhwa, Pakistan. *Pakistan Journal of Zoology*, *53*(2), 777.
- Soomro, M. H., Soomro, S. P., Bhutto, M. B., Akbar, Z., Yaqoob, M., & Arijo, A. G. (2014). Prevalence of ticks in buffaloes in the upper Sindh Pakistan. *Buffalo Bull*, *33*(3), 323-7.
- Wikel, S. K. (2018). Ticks and tick-borne infections: complex ecology, agents, and host interactions. *Veterinary Sciences*, *5*(2), 60.
- Young, M. R., & Hebert, P. D. (2015). Patterns of protein evolution in cytochrome c oxidase 1 (COI) from the class Arachnida. *PLoS One*, *10*(8), e0135053.
- Zeb, J., Shams, S., Ayaz, S., Din, I. U., Khan, A., Adil, N., ... & Raza, A. (2020). Epidemiology of ticks and molecular characterization of Rhipicephalus microplus in cattle population in North-Western Pakistan. *International Journal of Acarology*, *46*(5), 335-343.