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Seasonal Variation and identification of Ixodid ticks in cattle, buffalo, sheep and goats in Lower Sindh

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Abstract: Tick-bornediseases are a significant human public health as well a livestock health problem in Pakistan. A survey study was carried out in lower Sindh (Tharparkar, Badin, Hyderabad, Karachi, Tando Muhammad Khan, Thatta, and Mirpurkhas). Thirteen hundred and sixty (1360) "tick samples" were collected randomly from infested Cattle, buffalo, sheep, and goats and then transported to the Parasitology laboratory, Sindh Agriculture University, Tandojam. the study was designed to investigate seasonal variation in prevalence of ticks and genus wise tick identification using the key for the tick samples of cattle, Buffalo, sheep and goats.

Methodology: Investigations were performed throughout the year to record tick prevalence in various seasons, Ticks were identified on the basis of mouth parts, particularly position of capitulum, genital aperture, festoons.

Results:1360(Mean=544) were observed animals, 528(Mean=132) were the infested animals. The Study reveals the highest rate of tick infestation in the month of July(74.0, 47.5, 50,65.21%) in Cattle, Buffalo, Sheep and Goats respectively, while the lowest rate of tick infestation was observed in the month of December(11.7,18.1, 16, 21.42%) in Cattle, Buffalo, Sheep and Goats respectively-.Ticks collected from all districts of lower Sindh were subjected to genus wise identification and the most commonly infested ticks were belonging to genus *Hyalomma* (27.5%) followed by *Rhipicephalus* (24.29%), *Boophilus* (22.80%), and *Amblyomma* (14.20%).

Conclusion: Mild temperature and high relative humidity were identified supporting abiotic factors for tick infestation. Intensity of infestation increase with age and female host were more prone to infestation, Weak animals and cottage structures or houses were more considered as risk factors tick infestation.

Keywords: Seasonal variations of Ticks (Vectors) infestation, taxonomic identification, Lower Sindh.

1. INTRODUCTION

Ticks are not only a potential vector, but also act as reservoirs of many infectious agents. Tick-borne diseases are a significant human public health and livestock health concern in Pakistan. Being a subtropical country, Pakistan carries rich fauna of disease vectors, including mosquitoes, soft and hard ticks, the latter of which transmit a piece of major vector-borne diseases. However, research work on vector-borne disease is rare. Among the major tick-borne diseases in Pakistan are Crimean-Congo hemorrhagic fever (HCC) as well as human and animal anaplasmosis caused by the agents *Anaplasma phagocytophilum* and *Anaplasmamarginal*, Respectively. The Crimean-Congo hemorrhagic fever virus (CCHFV) causes a very deadly disease transmitted by ticks, and outbreaks occur mainly in the cold and arid regions of Pakistan. *Pasteurella multocida*, *brucella abortus* and *Salmonella typhimurium* are transmitted by Ticks and are life threatening vectors of many infectious agents of humans and animals. (Irfan, 1984) Ticks have a tremendous ability to adapt to changing geo-climatic conditions and can therefore expand their range. (Lindblom 2013) They are known to be the primary vectors for the transmission of many pathogens of almost all origins such as viral, bacterial, rickettsial, and parasitic. (Sonenshine *et al.*, 2017) To date, 899 species of ticks are known to belong to three families: Ixodidae, Argasidae, and Nuttalliellidae (represented by a typical mono species limited to South Africa. (Rahlenbeck, *et al.*, 2016) After sucking blood, the outer surface of a tick reaches 200-600 times more than its unfed body weight. (Little 2018) The prevalence of tick-borne pathogens and their emergence in cattle have been found throughout Pakistan. ("(PDF) Prevalence) *Hyalomma anatolicum* has transmitted some of the tick pathogens that are of zoonotic importance (for example, Crimean Congo hemorrhagic fever). Parasitic diseases are the most common in Pakistan and cause enormous damage to animals' health. Ecto- and endo parasites cause 40% production losses for our herds. (Chen, *et al.*, 2010)

It is pendant health issue called Tick-borne illness mostly found in temperate regions of the northern hemisphere. It is hard tick called *Ixodes ricinus* of the main European vector for active ingredients such as *Borrelia burgdorferi*, *Anaplasma phagocytophilum*, tick-borne encephalitis (TBE) virus and highest species of the Spotted Fever Group of Rickettsiae (SFG), of at least nine, known pathogens in humans in this part of

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the world. (Al-Adhami, *et al.,* 2011) Lyme disease, Rocky Mountain typhus, human granulocytic anaplasmosis, babesiosis, tick-borne encephalitis, Crimean Congo hemorrhagic fever spread by ticks because they are hematophagous arthropods causes diseases in humans and animals. (Sonenshine *et al.,* 2017) Ticks are very much linked to mites and are included in the class Arachnida, to which spiders belong. There are two morphologically and biologically different groups of ticks: the "soft ticks" (Argasidae) and the "hard ticks" (Ixodidae). The castor bean (or sheep tick) is a hard tick commonly found in Europe, *Ixodes ricinus*, which is not only the most common tick species but can also transmit a variety of pathogens that cause human diseases, including tick-borne encephalitis (TBE) and Lyme disease. (Rahlenbeck, *et al.,* 2016) Ticks are common and important ectoparasites, and many species easily feed on a variety of vertebrate hosts. (Little 2018) It is known that *Ixodes ricinus* is the most common tick species in Europe and Poland. In these areas, these ectoparasites are also the main vector of *Borrelia burgdorferi*, *Anaplasma phagocytophilum* and *Babesia microti*. ("(PDF) Prevalence) Ticks are mandatory hematological arthropods, currently considered only as vectors of pathogenic pathogens for humans, and animals. They transmit a variety of pathogens, including bacteria, viruses, blood prototypes, spirochetes, Rickettsiales, etc. (Chen, *et al.,* 2010) Tick-borne pathogens are a growing problem worldwide, including the blood-borne bacterial species Anaplasmataceae, which causes disease in animals and humans. Ehrlichia and Anaplasma species are structurally, genetically, and immunologically similar. (Al-Adhami, *et al.,* 2011) In the endemic areas of Turkey, Crimean-Congo hemorrhagic fever (FCH) has been related to numerous deaths since 2002 and peaked in 2008 and 2009, consequential in a total of 126 deaths. (Inci, *et al.,* 2016) Although ticks are known to transmit a variety of pathogens, vector competencies for the transmission of Bartonella spp. by ticks were speculative until recently, when the in vivo transmission of *B. birtlesii* by Ixodes ricinus ticks was detected in mice. (Janecek, *et al.,* 2012) Many species easily feed on a variety of vertebrate hosts, as ticks are common and significant ectoparasites

2. MATERIALS AND METHODS

Collection of Ticks samples

Investigations were performed throughout the year to record tick prevalence in various seasons. Ticks were collected (a) from open pastures and (b) farm animals. Each tick group was tested for PCR to detect the pathogen, ticks were collected from infested animals and kept in vials were properly labeled with the required information. Ticks were gently collected with consideration so that the mouthparts may not get detached (a problem that may invite secondary bacterial infection). For the collection of ticks, fine point forceps were used.

Labeling and storing of Ticks

Collected samples were subjected to identification in the Molecular Parasitological Laboratory at the Department of Veterinary Parasitology, Sindh Agriculture University. If the identification was desired, later on, the ticks were transferred to autoclaved test tubes containing 70% Ethyl alcohol as a preservative as suggested by (Soulsby, 1986).

Processing of Ticks

For identification and reference record, the ticks were removed from 70% ethyl alcohol and were boiled for 10 to 20 minutes in tubes containing 10% Potassium hydroxide (KOH to remove the exoskeleton (Scutum). Boiled ticks were further passed through grades of ethanol series for 2 hours in 20%, 70%, 90%, and 100% for dehydration. Dehydrated ticks were washed with tape water and transferred to clove oil for 24-48 hours to keep the muscles soft and bring the shine to the sample.

Preparation of Potassium Hydro Oxide(KOH)

10 parts of Potassium hydro oxide were taken in a graded measuring cylinder (Borosil, Germany) and dissolved in 90 parts of distilled water to get a 10% solution of KOH.

Preparation of Ethanol Series

1. 20% ethanol

20 cc of absolute ethanol (Merck, Germany) was added to 80 ccs of distilled water.

2. 70% ethanol

70 cc of absolute ethanol (Merck, Germany) was added in 30 ccs of distilled water.

3. 90% ethanol

90 cc of absolute ethanol (Merck, Germany) was added in 10 ccsof distilled water.

4. 100% ethanol

Company-made absolute ethanol or 100% ethanol (Merck, Germany) was used directly.

Permanent Mounting of Ticks

Processed ticks were placed on a glass slide and seen under the dissecting microscope. With the help of a pointer, their body parts were properly expanded. A drop of Canada balsam (SYN, England) was placed in the middle of the slide, and the sample was covered with a coverslip to make a permanent mount.

Tick Identification

Ticks were identified with the help of published keys Location of mouthparts, particularly position of the capitulum, genital aperture, festoons, and appearance were considered as the basis of identification as suggested by(Hove *et al.* 2008).

Data Analysis

Data on tick infestation was pooled and analysed by following formula. % age of Tick infestation=Number of Infested animals × 100Number of observed animal Data was also analysed by using excel 2016.

3. RESULTS AND DISCUSSION

To generate baseline data on tick infestation; research was carried out in the cities of Lower Sindh, in Sindh province of Pakistan on cattle, buffalo, sheep, and goat, Animals included all ages, and both sexes i.e. male and female.

As the first parameter of study, data on tick infestation was pooled and analyzed. Table 1 reveals the rate of tick infestation in cattle, buffalo, sheep, and goats. According to this table, the highest rate of tick infestation (49.26%) was found in cattle, (34.97%)followed by buffalo, (41.48%) in sheep (33.02%) in goats in different districts of lower Sindh. (Sajid, *et al.*, 2007).

Table -1: Rate of Tick Infestation in Cattle, Buffalo, Sheep and Goat

S.No	Type of Animal	No. Observed	No. Infested	Percentage (%)
1	Cattle	340	132	38.82
2	Buffalo	350	136	38.85
3	Sheep	330	130	39.39
4	Goat	340	130	38.23
Total		1360	528	38.82

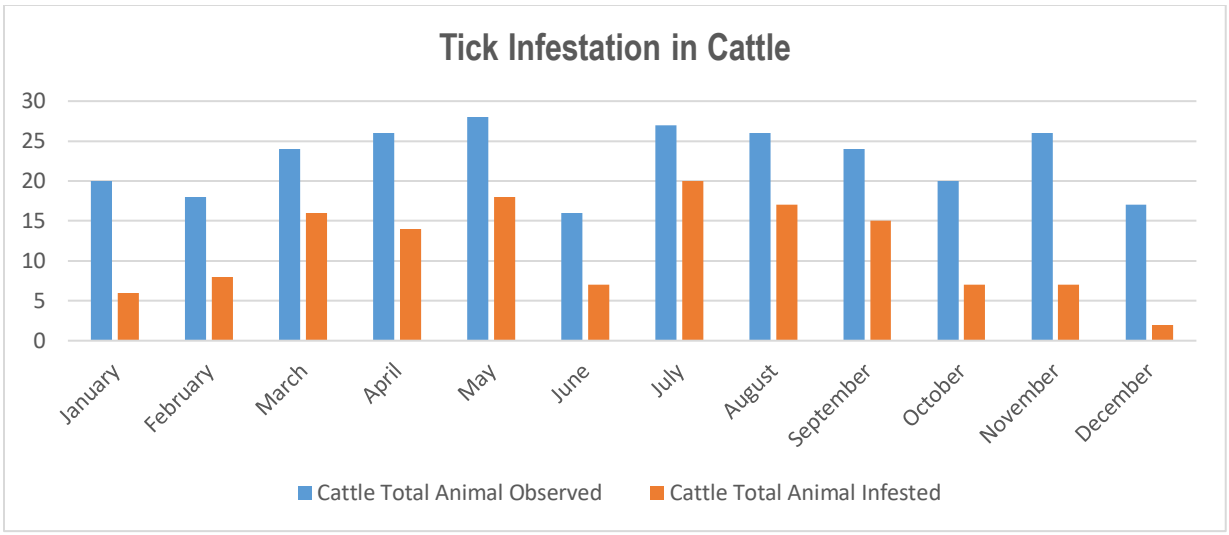
Overall prevalence of ticks infesting domestic animals was 69.4% (536/772). Among animal hosts, cattle were found high infestation (87.2%, 157/180) followed by buffalos (79%, 91/114), domestic fowls (74.7%, 112/150), goats (68.3%, 82/120), dogs (66.7%, 32/48), horses (61.3%, 49/80), and sheep (16.3%, 13/80) (Ali 2019)

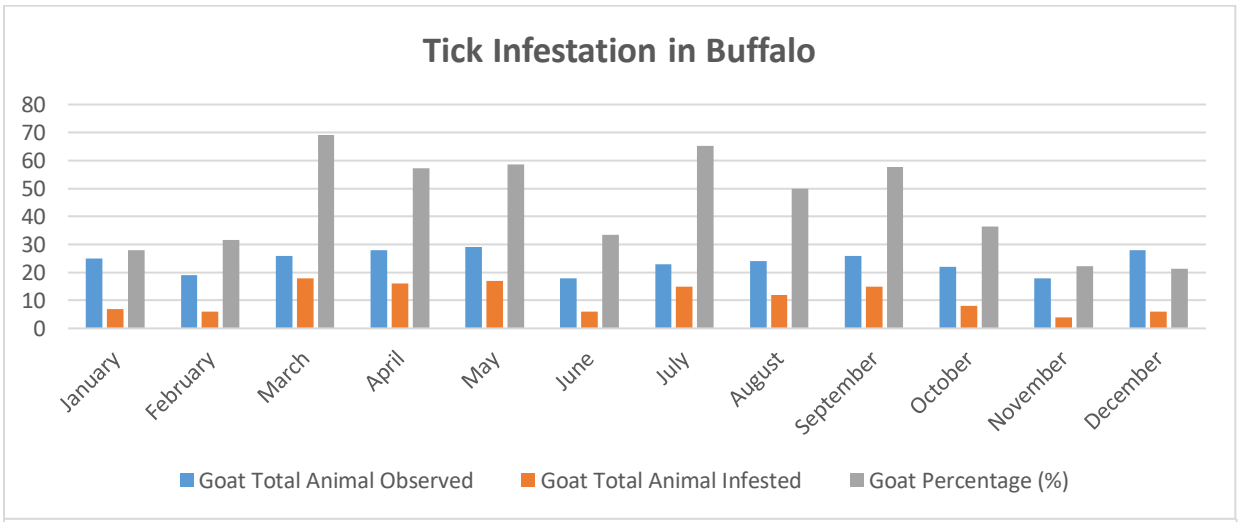
Table-2: Month-Wise Tick Infestation in Cattle, Buffalo, Sheep, and Goats

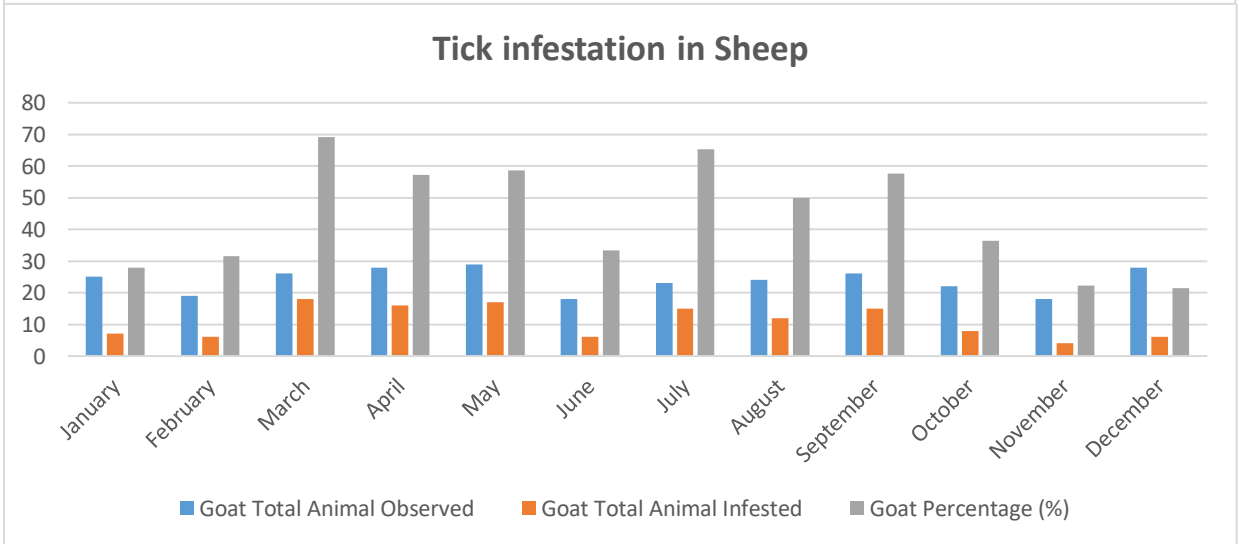
Month	Cattle			Buffalo			Sheep			Goat		
	Total Animal Observed	Total Animal Infested	Percentage (%)	Total Animal Observed	Total Animal Infested	Percentage (%)	Total Animal Observed	Total Animal Infested	Percentage (%)	Total Animal Observed	Total Animal Infested	Percentage (%)
January	20	6	30	36	7	19.4	26	5	19.23	25	7	28
February	18	8	44.4	30	10	33.3	22	6	27.27	19	6	31.57
March	24	16	66.6	42	17	40	26	7	26.9	26	18	69.23
April	26	14	53.8	46	16	34.7	28	10	37.5	28	16	57.14
May	28	18	64.2	45	20	44.4	30	8	26.66	29	17	58.62
June	16	7	43.7	34	9	26.4	22	7	31.81	18	6	33.33
July	27	20	**74.0**	40	19	**47.5**	30	15	**50**	23	15	**65.21**
August	26	17	65.3	43	17	39.5	29	14	48.27	24	12	50
September	24	15	62.5	42	18	42.8	32	10	31.25	26	15	57.69
October	20	7	35	37	10	27.0	34	10	29.41	22	8	36.36
November	26	7	26.9	34	8	23.5	36	8	22.22	18	4	22.22
December	17	2	**11.7**	33	6	**18.1**	25	4	**16**	28	6	**21.42**
Total	272	137	50.36	462	157	33.98	340	104	31.17	286	130	45.45
Mean	22.66	21.07		71.07	24.15		52.30	16.15		44	20	

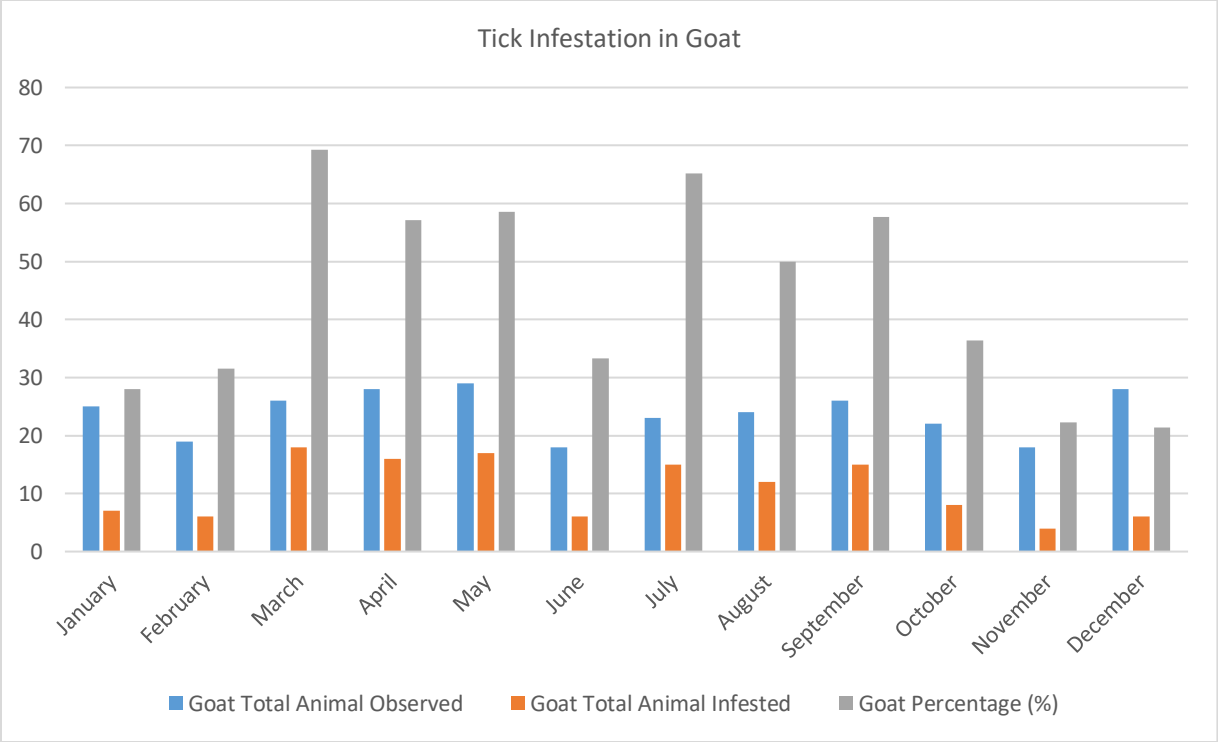
Table-3: Tick Infestation in Male and Female animals (Cattle, Buffalo, Sheep and Goats)

Sex	Cattle			Buffalo			Sheep			Goats		
	Observed	Infested	Percentage (%)	Observed	Infested	Percentage (%)	Observed	Infested	Percentage (%)	Observed	Infested	Percentage (%)
Female	47	25	53.19	64	27	42.19	34	12	35.29	49	22	44.89
Male	225	112	49.77	398	130	32.66	37	10	27.02	47	17	36.17
Total	272	137	50.37	462	157	33.98	71	22	30.98	96	39	40.62
Mean	136	68.5		231	78.5		35.5	11		48	19.5	


Tick Infestation in Cattle
30
25
20
15
10
5
0
January
February
March
April
May
June
July
August
September
October
November
December
Cattle Total Animal Observed
Cattle Total Animal Infested


Tick Infestation in Buffalo
80
70
60
50
40
30
20
10
0
January
February
March
April
May
June
July
August
September
October
November
December
Goat Total Animal Observed
Goat Total Animal Infested
Goat Percentage (%)


Tick infestation in Sheep
80
70
60
50
40
30
20
10
0
January
February
March
April
May
June
July
August
September
October
November
December
Goat Total Animal Observed
Goat Total Animal Infested
Goat Percentage (%)



The results are the ticks were classified into 2 genera and 9 species including: *Hyalomma dromedarii* (17.3%), *Hy. schulzei* (1.8%), *Hy. marginatum* (0.5%), *Hy. anatolicum excavatum* (12.60%), *Hy. anatolicum anatolicum* (11.2%), *Hy. Asiaticumasiaticum* (11.0%), *Rhipicephalus sanguineus* (21.2%), Rh. bursa (10.2%) and (13.911%). The frequency of the genus Hyalomma (54.6%) was higher than *Rhipicephalus. Rh. Sanguineus* was the predominant tick species and accounted for 21.26% of ticks .(Ganjali, *et al.*,2014)

Table-4: Temperature wise rate of tick infestation in Cattle, Buffalo, Sheep and Goat

Animal species	Amblyomma	Boophilus	Hyalomma	Rhipicephalus
Buffalo	39(24.84)	37(23.56)	41(26.11)	40(25.44)
Cattle	34(25.37)	33(24.62)	35(26.11)	32(23.88)
Sheep	5(21.7)	4(17.39)	8(34.7)	6(26.00)
Goat	7(22.00)	4(12.90)	11(35.4)	9(29.00)
Total	85(14.20 %)	78(22.80 %)	95(27.5%)	87(24.29%)

According to this table, ticks prefer low and mild temperatures to infest the host animal. The highest number of animals (47.2%) were identified at a temperature ranging between 31-40 ºC and lowest (33.33%) at 10-20ºC.

Table-5: Humidity wise rate of tick infestation in cattle, buffalo, sheep, and goat.

S. No	Temperature	Cattle,Buffalo, Sheep and Goats		
		Total Animal Observed	Total Animal Infested	Percentage (%)
1	10 - 20 ^{0}C	300	100	33.33
2	21 - 30 ^{0}C	350	136	38.85
3	31 - 40 ^{0}C	250	118	47.2
Total		900	**354**	39.33

Table-5: depicts the effect of humidity on the rate of tick infestation. Data shows that, humidity rates between 31-40 % are more suitable for tick infestation (47.62%) and when humidity increases (41-50), the infestation rate decreases (39.14%).Study showed, the highest prevalence (12%) of Hyalomma ticks observed followed by Boophilus (8.1%), Haemaphysalis (5%), and Rhipicephalus (3.5%).who also reported infestation of similar genera of ticks on Friesian cattle in district Kasur, Punjab. The species of *Hyalomma* were also reported by (Khan,1996). who studied the prevalence of tick infestation 28.2% (1269/4500) in cattle and 14.7% (662/4500) in buffaloes in the Faisalabad district of Pakistan. Seven species of ticks i.e. *Rhipicephalus*

sanguineus, *Boophilus microplus*, *B. annulatus*, *Hyalomma a. anatolicum*, *H. marginatum marginatum*, *Hyalomma aegyptium* and *Dermacentor marginatus*were identified by them. All species of ticks were found infesting buffaloes. (Walker, 2007). studied the incidence of ectoparasites in cattle, sheep, goats, and poultry in Northern areas of Pakistan.

Table-6: Infestation Rate (%) Of Different Identified Genera in Buffalo, Cattle, Sheep and Goats

S. No.	Humidity	Cattle, Buffalo, Sheep and Goat		
		Total Animal Observed	Total Animal Infested	Percentage (%)
1	10-20 %	258	116	44.96
2	21 – 30 %	308	105	39.14
3	31 – 40 %	147	70	47.62
4	**41 – 50 %**	**21**	**3**	**14.29**
Total		734	294	40.05

Ticks collected from all districts of Lower Sindh were subjected to genus identification and a total of four genera were found infesting in cattle, buffalo, sheep, and goat in the study area viz. *Hyalomma, Boophilus, Rhipicephalus*, and *Amblyomma*. The most commonly infested ticks were belonging to the genus *Hyalomma* (27.5%) followed by *Boophilus* (22.80%), *Rhipicephalus* (24.29%), and *Amblyomma* (14.20%) Different authors have reported different tick genera in various geographical locations. (Sharif,1928).

4. CONCLUSION

Cattle were more susceptible to tick infestation as compared to buffalo, Sheep, and goats. The intensity of infestation increase with age, Female host were more prone to infestation, Weak animals were more infested, Cottage structures or animal houses were considered favorable for tick infestation, Mild temperature and high relative humidity are supporting abiotic factors for tick infestation.According to month wise investigations, the highest rate of tick infestation was identified in the month of July and lowest rate was in the month of December. According to genus wise identification and the most commonly infested ticks were belonging to genus *Hyalomma* followed by *Rhipicephalus*, *Boophilus* and *Amblyomma* were found in the areas of Lower Sindh.

5. ACKNOWOLEGMENT

Iram Shaikh: Conceived the idea, recorded data in the field, prepared draft, and finalized the manuscript
Naheed Baloch: Helped in formulating the study and supervised the work
Abdullah G. Arijo: Co-supervised the work and helped in field experiment planning
Riffat Sultana: Co-supervised and helped in execution of field experiment
Saleem Raza Solangi: Helped in Sampling.

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