

**Studies on prevalence and status of acaricide
resistance in *Rhipicephalus (Boophilus) microplus*,
a common tick in U.P.**

THESIS

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IN

Applied Animal Sciences

SUBMITTED BY

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**UNDER THE SUPERVISION OF
PROF. KAMAL JAISWAL**

**BABASAHEB
BHIMRAO
AMBEDKAR
UNIVERSITY**



• LUCKNOW •

प्रज्ञा शील करुणा
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(SCHOOL FOR BIOSCIENCES AND BIOTECHNOLOGY)
BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY
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Year 2019

DECLARATION

I hereby declare that the thesis entitled “**Studies on prevalence and status of acaricide resistance in *Rhipicephalus (Boophilus) microplus*, a common tick in U.P.**” submitted to the Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow by me for the award of degree of the Doctor of Philosophy in Applied Animal Sciences is an outcome of my original work and the outcome of my own efforts under the supervision of Prof. Kamal Jaiswal, Department of Applied Animal Sciences, Babasaheb Bhimrao Ambedkar University, Lucknow. It has not been submitted in part or full to this or any other University for the award of any other diploma and degree. This is also declared that the thesis is essentially free from all kinds of plagiarism.

(Neeraj Kumar)

CERTIFICATE

This is to certify that the thesis titled “**Studies on prevalence and status of acaricide resistance in *Rhipicephalus (Boophilus) microplus*, a common tick in U.P.**” submitted by **Neeraj Kumar** is an original research work and has not been previously submitted in part or full for the award of any other degree or diploma to this or any other university.

The thesis submitted to Babasaheb Bhimrao Ambedkar University Lucknow, satisfies all the requirements as stipulated in the Doctor of Philosophy (Ph.D.) regulations -1999 as amended in 2008/2010/2013 and it is fit for submission and evaluation for the award of the degree of Doctor of Philosophy of the university.

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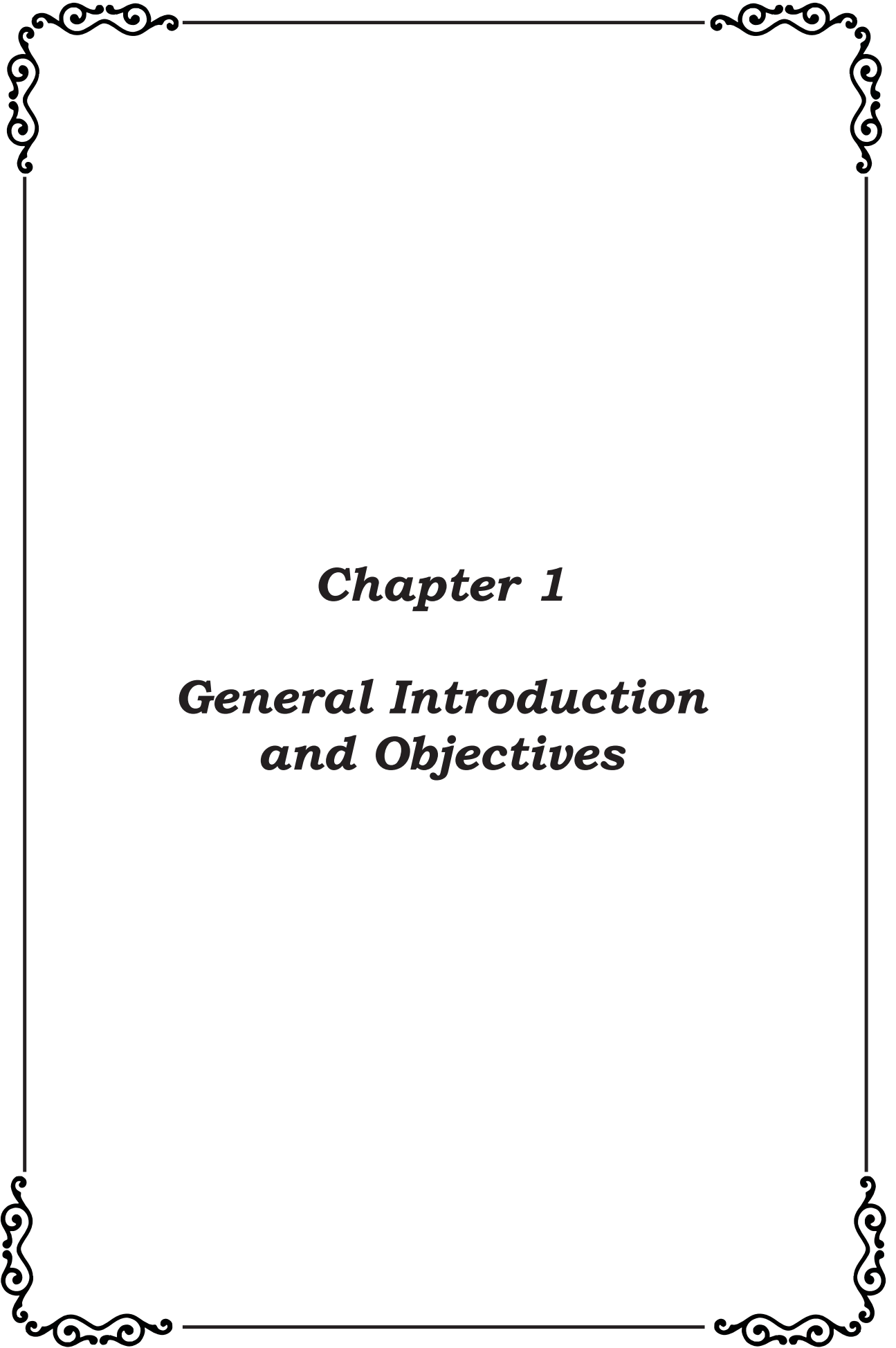
ABBREVIATIONS

S. No	Abbreviations	Full Name
1	AIT	Adult Immersion Test
2	LPT	Larval Packet Test
3	FAO	Food and Agriculture Organization
4	DPX	Dextrin Plasticized Xylene
5	SPSS	Statistical Package for the Social Sciences
6	sp.	Species
7	spp.	Multiple species
8	M	Molar
9	Kg	Kilogram
10	G	Gram
11	Mg	Milligram
12	Mg	Microgram
13	M	Micron
14	Cm	Centimetre
15	ml	Millilitre
16	m	Meter
17	mm	Millimetre
18	Hrs	Hours
19	Min	Minute
18	pH	Potential Hydrogen
20	Ppm	Parts Per Million
21	ANOVA	Analysis of Variance

22	LC50	Lethal Concentration resulting in 50% mortality
23	LC95	Lethal Concentration resulting in 95% mortality
24	SEM	Standard Error of the Mean
25	Fig.	Fig.
26	etc.	Et cetera
27	viz.	Namely
28	i.e.	That is
29	e.g.	For example
30	b.wt.	Body weight
31	<i>et al.</i>	And others

SYMBOLS

Symbols	Meaning
%	Percentage
°C	Degree Celsius
±	More or Less analogous (Range of Standard error)
χ^2	Chi- square
≤	Less than or equal to
≥	Greater than or equal to
>	Greater than
<	Less than
@	At the rate



Chapter 1

General Introduction and Objectives

CHAPTER – 1

INTRODUCTION

General Introduction

Parasitism is the term used for the condition where parasites live on or inside the body of their host for nutrition and reproduction. Parasites which harbour on the body surface of the host are known as ectoparasites. Among all ectoparasites, ticks are most renowned parasites affecting wide range of vertebrate hosts. Tick infestation in livestock lead to compromised quality of animal products and often responsible for livestock death (Elango and Rahuman, 2010). Economical loss due to tick infestation also is widely reported, where major damage has been reported in leather industries due to frequent piercing of tick on hide of animals (Biswas, 2003; Jongejan and Uilenberg, 2004). As per leather industry report, the leather sector of India is suffering from a huge short fall of 3000 million pieces of hide and skin per year.

Dermatitis is the secondary infection which is highly prevalent in diseased livestock due to tick bite. Erythematous nodules (Kim *et al.*, 2003), erosion, and ulcers are some of the examples of secondary systemic infections.

India contribute remarkable share of world's livestock resources with nearly 57% of world's buffaloes, 16.5% of cattle, 16.3% of goats, and 5.7% of sheep (FAO, 2004). It has been reported that almost every genera of livestock are prone to infestation of ticks. The annual control cost of TTBDs has been evaluated as 498.7 million US\$ (Minijaw and McLeod, 2013). The most widely recognized joint impact of TTBDs in Indian dairy framework is decrease in milk yield i.e., loss of 14% of the lactation and reduced quality of hides for leather industry.

Ticks are potent vectors of several disease causing agents such as bacteria, viruses, and protozoa. Ticks has occupied second position after mosquitoes for spreading pathogens in humans, domestic and wild animals (Boldbaatar *et al.*, 2006). In accordance to a recent report on tick borne diseases, India is found to be the source country of emerging tick borne diseases. In India, around 106 species of ticks have been recorded till

date. Among them *Rhipicephalus (Boophilus) microplus*, previously known as *Boophilus microplus* (CFSPH, 2007), is the most common cattle tick is widely popular in tropical & subtropical region. They show one host life cycle (Soulsby,1982) and are the most prevalent cattle tick including horse, sheep, goat and deer etc (Ghose *et al.*, 2007) found in a variety of agro climatic zones of India.

Highest prevalence of *B. microplus* is reported from the Vidarbha region of Maharashtra (MH) (Maske *et al.*, 2012), Tamil Nadu (Kaushal *et al.*, 2002), Chattishgarh (Das and Shrivastava, 2002). It has also been reported from Pantnagar (Das, 1994), the Dharwad region of Karnataka (Kamble and Hiregoudar, 1988), Jammu and Kashmir (Kaul *et al.*, 1990), Bhubaneswar (Pratap *et al.*,1991), Tirunelveli (Sundaram *et al.*,1986), Bangalore (Jagannath *et al.*, 1979), north eastern India (Miranpuri, *et al.*, 1978), Punjab (Gill and Gill, 1977), Delhi (Raizada and Nagar, 1979) and Andhra Pradesh (Sivasankar and Rao, 1984), etc.

Economic impact of *Rhipicephalus (Boophilus) microplus* on cattle includes reduced weight of cattle, low quality of milk production and transmitting agent of various diseases such as babesiosis (*Protozoalpiroplasms Babesiabovis* and *Babesia bigemina*) and anaplasmosis (Bacterium *Anaplasma marginale*). There is an emergent need to control *Rhipicephalus (Boophilus) microplus* but this could not be done without knowing its prevalence, distribution etc.

Hence, prevalence study of *Rhipicephalus (Boophilus) microplus* on different indices has designed to investigate seasonal intensity of these ticks in India especially in U.P.

Uses of chemical base acaricides are the most common practices to control tick population. But persistent use of these acaricides produce resistance in many tick species (Harris *et al.*, 1988) thereby reducing the ability to control them. In some cases, residues of these chemicals has been found in areas where it was sprayed or applied and cause environmental hazards repeatedly (Nonga *et al.*, 2012).

The anti-tick drugs usually contain synthetic pyrethroids, organophosphates and amitraz (Gazim *et al.*, 2011). Although these acaricides are beneficial only when they are properly used, as their misuse has led to poisoning of humans and animals (FAO, 1998 and Zorloni *et al.*, 2010).

Ticks take nutrition from blood and lymph. They use their chelicerae and hypostome to penetrate into the host's skin. The feeding of *Ixodid* tick is slow. They get detached from the body when they become totally engorged with blood. It is important to characterize the tick resistance to conventional acaricides so that the current status of tick resistance in the area must be revealed out for effective tick control measures. Therefore keeping the above in view, the proposed study was designed to evaluate the status of tick resistance to acaricides in cattle and buffaloes in selected districts of U.P.

Biology of Ticks

All tick species feeds on the body of their hosts, they remain attached with the skin of host body with their mouthparts. The ticks slowly feed on blood because the body wall needs to grow before it can enlarge to take a large amount blood. Generally larvae feed 3 to 5 days to fully engorge with blood nymphs, male ticks take 4 to 8 days, and females 5 to 20 days. The ticks, when fully engorged with blood, detach from the host's skin and fall down to the ground. Male ticks do not drop to the ground, they feed enough for the continuous reproduction. *Ixodid* male ticks have active reproductive organs and do not need to feed on the time of nymphal moulting. Argasids ticks do not get attached to the skin whereas *ixodid* ticks have. Ticks find their hosts in many ways. Some ticks crawl onto vegetation to wait for their hosts to pass by. This behaviour is called questing. The questing behaviour is also seen in genera such as *Rhipicephalus*, *Hemaphysalis* and *Ixodes*. The ticks grasp onto the hosts using their front legs and then crawl over the skin to attach and feed. Adult ticks of the genera, *Amblyomma* and *Hyalomma* are active ticks, they find their hosts easily on the ground, they show sexophilic behaviour. Generally *Ixodid* tick species spend their entire life cycle in their host's nest and attach to their hosts skin. This is called endophilic or nidicolous behavior. Some tick species have domestic behaviour such as the dog tick, *Rhipicephalus sanguineus*, have adapted to living in houses and will feed on domestic animals.

In *ixodid* ticks, mating takes place on the body of the host, except with *ixodes* where it may also occur on the vegetation. Male ticks remain on the host and mates with many females whilst they are feeding. The females mate only once, before they are ready to engorge fully with blood. When they finally engorge, they detach from the host and

have enough sperm stored to fertilize all their eggs. A female tick lays 2000 to 20,000 eggs in a single batch.

Importance of ticks

The economic vitality of ticks has long been documented by many researchers. Diseases caused by ticks may adversely influence approximately 80% of cattle population. Additionally, they also lead to significant production losses in cattle annually. Globally, it is reported that ticks are second most popular vector of infectious pathogens to humans and animals after mosquitoes. Decline in blood biochemical parameters, blood trace components and levels of minerals in cattle population are consequences of tick infestation. A single female bloated tick imposes a daily loss of 1g of body weight, 0.5-2 ml of blood and 8.9 ml of milk (Minjauw and McLeod 2003). Haematological and sero-biochemical analysis are considered as authentic tools for diagnosis and may help to prognosis for useful therapy. Alterations in haematological and sero-biochemical parameters are the key indicators of severity of disease. In India, it is estimated that tick and tick born diseases causes loss of US \$498.7 million (more than 2000 corers) per annum. Further reports of over the past two decades suggested that, the occurrence of tick borne diseases has increased and created foremost public and animal health problems. Toxic substances released by some ticks into the host body cause ulceration of mouth, sweating of body and alopecia (Norval, 1983). Another condition reported in animals diagnosed with tick infestation is tick worry, whereby an animal, become weak and their resistance against disease become reduced, which results in a general economic loss because the animal does not grow and produce as it should (Drummond, 1983). In addition, ticks cause anaemia and injury to skin, hides, and teats and this further leads to formation of wounds, which predispose animals to secondary bacterial illness and the expansion of abscesses (Peter *et al.*, 2005).

Life Cycle of Ticks:

Ticks are versatile animals and show questing behaviour. Ticks live in outer environment, crawl onto vegetation rich area and do wait for their host. Genera *Rhipicephalus*, *Haemaphysalis* and *Ixodes* represent this kind of questing behaviour. Ticks use front legs to grab their host and crawl onto whole body to search the suitable place to

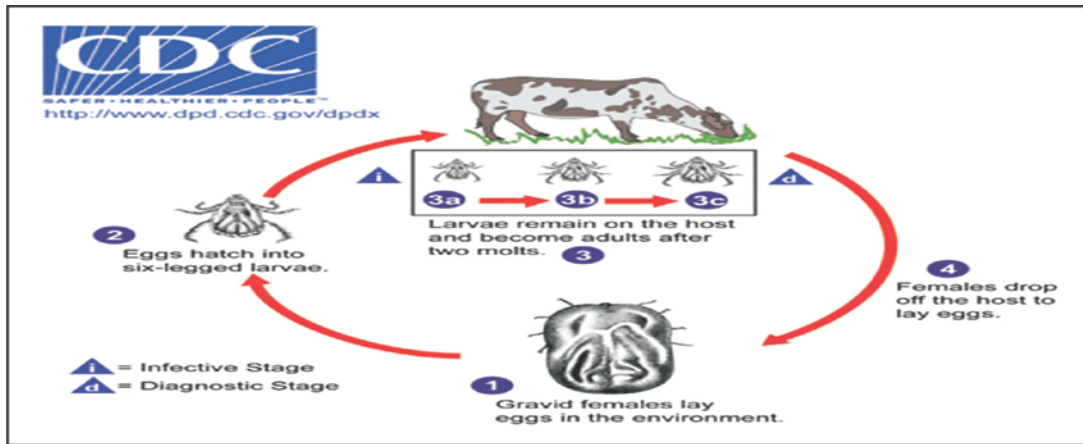
attach. Some ticks show endophilic or nidicolous type of behaviour. In this type of behaviour, parasites such as argasids and ixodes live their entire life in host nest. Another kind of behaviour has been observed i.e. domestic behaviour is exhibited by the genera *Rhipicephalus sanguineus*. They feed on pets that live in houses of humans.

Generally mating of ticks takes place on host but in case of Ixodes it may take place in vegetation as well. Male ticks attach to their host for mating with as many females as possible, where as females mate only once before they are ready to engorge fully with blood. During mating, male ticks transfer sac of sperm to the female and fully engorged female ticks with stored sperm detach from the body of host and fertilize their eggs.

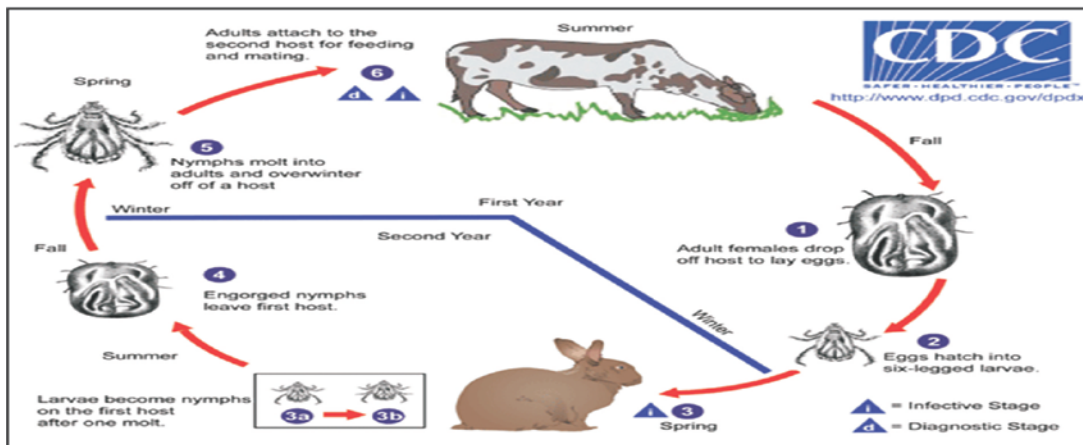
Ticks show one host, two host and three host life cycle. One host tick life cycle is rare type of life cycle. Genera *Boophilus* possess this type of life cycle. Eggs of ticks laid on soil and larvae crawl onto vegetation rich area to quest for a host. Larvae moult into nymph on the host body and both stages take nutrition in the form of blood from host and remain attached to the body of host until next moulting. Adults attach to the body of host and change their place for mating. All stages of ticks life feed on single host and this life cycle is very rapid, in case of *Rhipicephalus microplus*, the duration of the feeding is three weeks and for egg laying and larval development, they take two months.

In two host life cycle, larvae and nymph feed on same host while adults feed on another host. Rest of the life cycle is similar to the life cycle of three host life cycle. Two host life cycle is best shown by *Hyalomma detritum detritum* and *Rhipicephalus evertsi evertsi*.

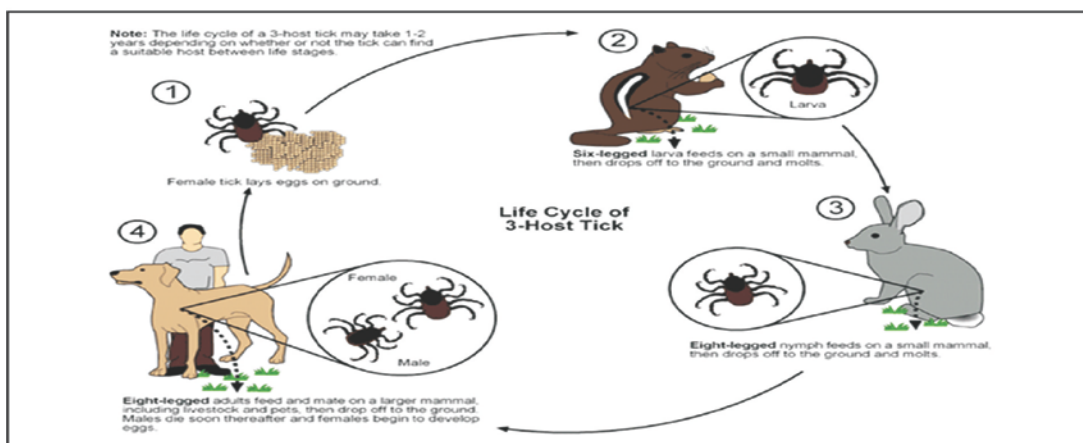
Three host life cycle is the common type of life cycle. Here all the stages of life cycle feed on different host. Larvae feed on individual host once and detach from the body of host. After detachment they hide themselves in soil or flora rich areas. Where they convert into nymph, these newly formed nymph feed once and undergo moulting to form either male or female tick. Female feed once and lay eggs. Female dies after depletion, male take number of feed, do mating then dies. Newly hatched ticks are soft until their outer body become hard. Three host life cycle is slow and take several months to year to complete.



A) Source: http://mcdinternational.org/trainings/malaria/english/dpdx5/HTML/Frames/S-Z/Ticks/body_Ticks_one_host_cycle



B) Source: http://mcdinternational.org/trainings/malaria/english/dpdx5/HTML/Frames/S-Z/Ticks/body_Ticks_two_host_cycle



C) Source: <https://extension.entm.purdue.edu/publichealth/insects/tick.html>

Plate 1.1. (A) One host tick life cycle (B) Two host tick life cycle (C) Three host tick life cycle

Table 1.1 Classification of *Rhipicephalus* sp.

Kingdom	Animalia
Phylum	Arthropoda
Class	Arachnida
Sub-class	Acari
Super-order	Parasitiformes
Order	Ixodida
Super-family	Ixodidea
Family	Ixodida
Genus	<i>Rhipicephalus</i>

Associated Diseases:

They transmit diseases such as Anaplasmosis, Babesiosis and Ehrlichiosis, all of which cause morbidity and mortality of animals. It is reported that Anaplasmosis is most widespread in South Africa with more than 99% of the total cattle production at risk (de Waal, 2000).

Prominent Tick Borne Diseases:

- Indian Tick Typhus (ITT)
- Bovine Anaplasmosis
- Crimean-Congo Haemorrhagic Fever (CCHF)
- Theileriosis
- Babesiosis
- Lyme Disease
- Kyasanur Forest Disease (Monkey disease)

Control/ Management of Ticks:

There are many available tools for tick control which consists of:

- Chemical control
- Phytochemical control
- Ethnoveterinary control

a) Chemical control:

Acaricides are used to control ticks since very long. The predictable acaricides are: Deltamethrin, Coumaphos, Chlorfenvinphos, Dieldrin, Cyfluthrin, Cyhalothrin, Cypermethrin Beugnet, Flumethrin, BHC/Cyclodines: Dieldrin Toxaphene Lindane Carbamate, Diazinon, Coumaphos, Diazinon, Dimethoate Cyclophos Trichlorfon, Chlorpyrifos Ethion, Chlorfenvinphos etc. These are used as dips, sprays and pour-ons as methods of application of these chemicals. Time and concentration dependent application of acaricides at various intervals is recommended (Garcia *et al.*, 2012).

Industrially synthetic compounds are accessible over the counter and are applied on infected animal at frequent gaps by the animal holder. On the basis of survey it is found that acaricides easily available in the market are: Butox, consist of deltamethrin and Butox which have the segment of cypermethrin are utilized more for the control of ticks. Deltamethrin acts on the parasite by contact and has strong affection towards lipoprotein. Consequently they enter the cuticles and bind the peripheral nerves ganglia and block the motor activity. This property of deltamethrin has been utilized for the treatment of ectoparasite contamination in domesticated animals with potential outcome (Anantachar *et al.*, 1990).

Casual application of acaricides with incorrect concentrations leads to the development of resistance in the ticks (FAO, 2004) and this fact is recently validated by an experiment conducted on Indian isolates of *R.(B.) microplus* and found that they were become resistant to OP compound “diazinon” when exposed to indiscriminate concentration of chemicals (Kumar *et al.*, 2011b).

Although owners of cattle have already reported treatment incompetence of these chemicals in field conditions whereas limited data on tick resistance to these chemicals are currently available (Kumar *et al.*, 2010 and Vatsya and Yadav, 2011).

b) Phytochemical control:

In search for low cost, safe and environment friendly plant based alternative, the efficacy of several plant extracts have been investigated for ticks control. Ghosh *et al.* (2011) evaluated the effect of *Acorouscalonus* against ticks, Magano and Mkolo (2007) evaluated the efficiencies of *Nicotianalavendula* oil, *Eucalyptus* sp. against ixodid ticks. It was found that they can provide effective ticks control option in the area where the chemical products are unavailable or unaffordable.

However further investigation is required to identify the specific compounds responsible for the effective ticks control.

c) Ethnoveterinary control:

In rural areas of the world, people perform ethnoveterinary practices to control ticks. During the present study, it was found that people use Geru, soil and Choona as alternative drug to control tick infestation and apply these materials on the body of cattle which they wash thoroughly after some time.

It is found that chemical control of the ticks is the most common method and has been used for the past several decades. These chemicals are very effective, however they possess several demerits being, like – non-biodegradable, high cost, residue in body and milk and most of all development of resistance to these drugs.

Acaricidal resistance:

The development of acaricide resistance refers to a chemical being ineffective to kill and control cattle ticks. This decreases the number of accessible useful acaricides in the market. The more often ticks are treated with these chemicals, the more likely they will gain resistance against it. The resistance is natural phenomenon, thus poses obstruction in effective tick control activity. The acaricidal resistance should be inspected time to time to check the status of development of resistance in ticks for a specific element. For this, the evaluation of acaricidal effects of chemicals should be investigated *in vitro* by following established protocols. The present study is designed to evaluate the acaricidal efficacy of deltamethrin available in market under the name of Butox.

To detect *R. (B.) microplus* populations resistant to acaricides, Food and Agriculture Organization (FAO) has recommended bioassays, Adult Immersion Test (AIT) and Larval Package Test (LPT), which can be done at low costs. Essentially, differences among these tests are the utilization of individual's different instars and their exposure time to treatment. Since more than 40 years, these methods, specially bioassays are being successfully used.

Ticks as documented are serious vectors of human and animal disease and act as potential vector for transmitting a greater variety of infectious organisms than any other blood-sucking arthropods. Ticks are responsible for broadcasting numerous protozoans, viral, bacterial and fungal pathogens. Ticks can be hazardous to domestic livestock and wildlife due to the blood loss that can occur when a large number of ticks feed. Secondary infections can occur at the feeding sites and diminish the value of livestock due to the damage to the hides Munderloh, U. G. *et al.*, (2011).

Significance of the study

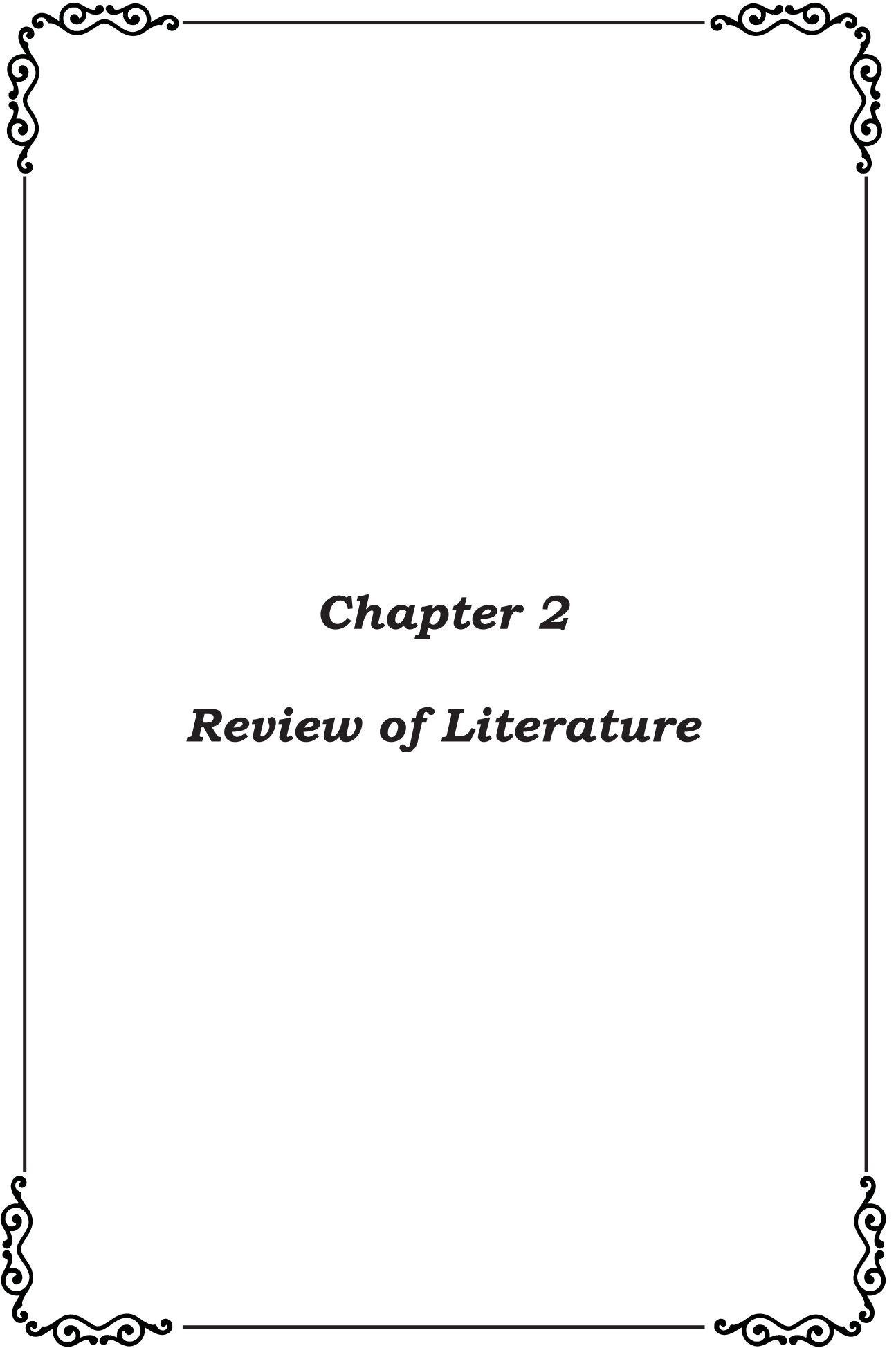
Uttar Pradesh is well known for its livestock, as it is situated in gangetic plane in North India. The environmental conditions of Northern India are suitable for the tick population growth. Now days, tick control has become a major challenge to animal rearers and researchers as there is lack in systematic survey on ixodid ticks in this area which aggravates this problem as well as control management system. Very few researchers have worked on the prevalence of tick species infesting in Uttar Pradesh. The development of resistance in ticks and the chemical footprint in meat, milk and hide has promoted researchers to find alternative ways to control ticks. Some of the researchers have revealed that rearers are using chemical acaricides in uncontrolled ways which are ecologically harmful as well as promoting resistance in ticks against these chemicals. The chemical acaricides also casts a huge economic burden on poor and marginal animal rearers. Though evaluation of resistance studies on these commercial chemicals have been done, studies are limited to research labs only. Therefore, it is necessary to evaluate tick resistance against these chemicals for better tick control management and find any alternative or better approach towards fighting these ecto-parasites. Epidemiological data on prevalence and distribution of ticks in Uttar Pradesh along with knowledge of tick resistance against commercial chemicals would form the basis for sustainable tick control

programme in the area. The results may be utilised for a proper decision making towards integrated tick control practices.

OBJECTIVES

The proposed study investigates the prevalence of ixodid ticks on animals in Uttar Pradesh and their resistance against commercial chemicals regularly used by farmers. The following objectives were proposed for the study-

1. To study the overall prevalence of *Rhipiciphalus (Boophilus) microplus* infesting cattle and buffaloes across selected districts of Uttar Pradesh.
2. To determine the differences in prevalence of ixodid ticks in livestock in reference to age, gender and breed of the host.
3. To study the seasonal prevalence of *Rhipiciphalus (Boophilus) microplus* in cattle and buffaloes identified from across various zones in the study area.
4. To assess the status of resistance of ixodid ticks against two popularly used commercial acaricides (cypermethrin and deltamethrin).



Chapter 2
Review of Literature

CHAPTER - 2

REVIEW OF LITERATURE

2.1. Prevalence of tick infestation in animals

Tripti et al., 2019 studied the prevalence of ixodid tick infestation on bovines of Dehradun district, Uttarakhand. A total of 490 bovines were examined on a random basis for nearly one year, from the months of July 2017 to June 2018. Overall tick infestation rate was found to be 66.32%. The month of September showed the highest prevalence (52.32%), while the lowest rate was reported in January (39.73%). Similarly, the season-wise prevalence was recorded highest in rainy season (61.29%), followed by summer (51.62%) and lowest in winter (41.09%). Age-wise tick infestation was observed highest in animals of less than 1 year age (60%), animals aged between 1 and 3 years (47.5%), and lowest in animals of age greater than 3 years (32.25%). Females had higher infestation rate than the males (66.67% and 45.2%, respectively).

Chisu et al., 2018 conducted a five-year survey of tick species and recognized tick-borne pathogen in Sardinia, Italy. Results revealed the presence of various circulating pathogens in Sardinian ticks. DNA of *Rickettsia* species was detected in 58 out of 1619 (4%) belonging to *D. marginatus*, *Ha. punctata*, *R. sanguineuss.l.*, *H. marginatum*, and *I. festai* species. Ehrlichia DNA was identified in 33 out of 1619 ticks (2%) belonging to *R. sanguineuss.*, *R. bursa*, and *Ha. punctata* species. A total of 61 out of 1619 (4%) ticks (*R. sanguineuss.*, *R. bursa*, *Ha. punctata*) tested positive for *Anaplasma spp.* *Coxiellaburnetii* was identified in 21 out of 1619 (1%) ticks belonging to *R. sanguineuss.l.*, *R. annulatus*, *R. bursa*, and *H. marginatum* species. Five *R. sanguineuss.* and one *R. bursa* ticks were found to be differently present.

Kaur et al., 2017 directed an overview on Epidemiology of ixodid ticks infesting cattle reared by farmers and rural people. A total of 2710 cattle were analyzed on random basis throughout the year, out of which 1602 cows were observed to be infested with ticks. The general predominance of tick pervasion in dairy cattle was watched (59.11%). An aggregate of 2042 ticks were gathered from the animal's body. The collected ixodid tick

species were recognized as *Rhipicephalus (Boophilus) microplus*, the most prevalent (59.74%) species followed by *Hyalomma anatolicum anatolicum* (26.80%) and *Haemaphysalis bispinosa* (18.80%). Male ticks were dominated in collected tick species.

Debbarma et al., 2017 studied the cattle of West Bengal, India to determine the prevalence of hard tick infestations in cattle from July, 2015 to June, 2016. The prevalence of hard tick infestation was studied in relation to sex and age of animals and seasonal changes in a year. Cattles from selected places were examined for the presence of ticks and in positive cases ticks were collected manually and identified on the basis of morphological characters. A total of 310 cattle were analyzed and out of which, 130 (41.93%) cattle were recognized to be infested with hard ticks and the prevalent species were *Rhipicephalus (Boophilus) sp.*, *Hyalomma sp.* and *Haemaphysalis sp.* of ticks. A significantly ($p < 0.01$) higher infestation was observed in female cattle (43.30%) than males (35.71%).

Abbasi et al., 2017 conducted epidemiological study of tick infestation in buffaloes of various regions of district Khairpur, Pakistan. A total of 720 water buffaloes from three tehsils (subdivisions) were selected randomly and examined from organized and unorganized dairy farms for tick infestation in district Khairpur, Pakistan. This epidemiological survey was conducted during April to September 2015. The overall mean population and preferred site of tick attachment to infested animals, in Gambat, Sobhodero, and Kot Diji tehsils, were observed on different body parts. The primary body area of infestation by ticks (head, thorax, abdomen, udder, and tail) ranged from highest in tail and udder part compared to lowest in the abdomen, head, and thorax. In all study areas, the infestation was higher ($p < 0.05$) in tail and udder than other parts of the body. In all the study areas, the overall highest population was found in the month of July. In addition, they also identified four new species of ticks (*Hyalomma anatolicum*, *H. anatolicum excavatum*, *Hyalomma Ixodes excavatum*, and *Ixodes ricinus*) in district Khairpur, Pakistan.

Singh, et al., 2017 studied the prevalence of ixodid ticks on buffaloes from eastern region of Lucknow, Uttar Pradesh. The present study was conducted to investigate the occurrence of ticks on buffalo. The survey was carried out at eastern region of Lucknow, Uttar Pradesh, India in February, 2016 to May 2016. The following genera of ticks belonging to the family ixodidae were recovered from buffaloes, *Hyalomma* and

Rhipicephalus (formerly *Boophilus*). A total of 300 buffaloes were recognized, out of which 160 were found to be infested with ticks. The overall prevalence of tick infestation in buffaloes was observed to be 53.33 per cent. Gender-wise prevalence of ticks showed that infestation was more prevalent in females (56.5%) as compared to male hosts (42.8%). Age-wise prevalence of ticks was also studied and it was observed that the highest prevalence of ticks was in adult (>3yrs) (23.7%) and lowest prevalence (45.7%) was recorded in young buffaloes (1-3yrs).

Rehman et al., 2017 highlighted the distribution of ticks infesting ruminants and risk factors associated with high tick prevalence in livestock farms in the semi-arid and arid agro-ecological zones of Pakistan. Total, 3,807 ticks representing four species were collected: *Hyalomma anatolicum* (n = 3,021), *Rhipicephalus microplus* (n = 715), *Hyalomma dromedarii* (n = 41) and *Rhipicephalus turanicus* (n = 30). The latter species is reported for the first time from the study area. *Rhipicephalus microplus* was the predominant species in the semi-arid zone, where as *H. anatolicum* was the most abundant species in the arid zone. The overall proportion of tick-infested ruminants was 78.3% (369/471). It was highest in cattle (89.9%), followed by buffaloes (81.4%), goats (60.0%) and sheep (11.1%). The median tick burden significantly differed among animal species and was highest in cattle (median 58), followed by buffaloes (median 38), goats (median 19) and sheep (median 4.5). Female animals had significantly higher tick burdens than males and, in large ruminants; older animals carried more ticks than younger animals. The intensity of infestation was significantly lower in indigenous animals compared to exotic and crossbred cows. Analysis of questionnaire data revealed that the absence of rural poultry, not using any acaricides, traditional rural housing systems and grazing were potential risk factors associated with a higher tick prevalence in livestock farms.

Patel et al., 2015 conducted the study in which they investigated the intensity and seasonal ups and downs of Ixodid ticks on buffaloes of western Uttar Pradesh, India. The overall prevalence of ticks during the study period was found to be 51.81%. During the study of age-wise tick infestation, overall maximum percentage of positive cases (74.17%) were noticed in the group I (up to 1 year) followed by 60.93 % in group II (1–3 years) and minimum tick infestation (36.33%) was observed in group III (> 3 years) (Graph 3). During the study period, ixodid ticks — *Hyalomma anatolicum anatolicum* and *Boophilus microplus* were recorded both in pure and mixed infestation in different seasons.

Kaur et al., 2015 studied the prevalence of Ixodid ticks infesting cattle and their control using plant extracts. The present study was conducted to investigate the occurrence of ticks on cattle. The survey was carried out between April, 2014 to March, 2015 period across various locations in Lucknow, U.P, India. A total of 2150 cattle were examined on random basis throughout the year, out of which 1262 cattle were found infested with ticks. On seasonal investigation, highest percentage of tick infestation was found in rainy season (68.08%) followed by summer (59.85%) and winter (48.70%). A higher prevalence was observed in female cattle (63.63%) than males (50.84%). Ticks are commonly controlled by using conventional synthetic acaricides.

Musa et al., 2014 conducted a study during June to December 2009 using standard parasitological procedures to determine the prevalence of tick infestation among cattle of different breeds in Maiduguri, North Eastern Nigeria. The tick species identified were *Boophilus microplus*, *Amblyomma variegatum*, *Hyalomma* spp., *Rhipicephalus sanguineus* and *Ornithodoros* spp. Of the 205 cattle examined, 63.4% (95% CI: 56.8-70.0) were tick infested. Males had a non – significantly ($p>0.05$) higher infestation rate of 63.4% (56.7-71.70) compared with the females (60.9%) (46.8-75.0). Younger animals aged ≤ 3 years had a significantly ($P<0.05$) higher prevalence of 85.4% (74.6-96.2) as compared with the adults aged $> 3-7$ years 55.8% (46.3-65.3) and older animals >7 years 35.0% (22.9-47.1).

Joseph et al., 2014 surveyed tick infestation of cattle at four selected grazing sites in the Tropics. Survey of tick infestation of cattle was studied at different communities of Nsukka district, Enugu State, Nigeria from November 2010 to October, 2011. The prevalence of ticks in relation to age, sex, breed, weight, season of the year and different body parts of the host was studied. A total of 139 cattle were examined, of which 123 (88.49%) cattle were tick infested. Four species of tick were identified namely *Rhipicephalus (Boophilus) microplus*, *Amblyomma variegatum*, *Rhipicephalus (Boophilus) annulatus* and *Amblyomma maculatum*. The range of tick burden was 1-8 per four square inch of heavily infested area of Nsukka district. Mean tick burden was high in case of *R. (B.) microplus* (2.93 ± 0.210), followed by *A. variegatum* (2.01 ± 0.15), *R. (B.) annulatus* (0.74 ± 0.08) and *A. maculatum* (0.25 ± 0.04). Prevalence of tick infestation was significantly ($p<0.01$) higher in dry season (32.16%) than in wet season (11.72%). Prevalence of ticks by body weight is significantly ($P<0.01$) higher in cattle with 299kg

body weight (41.10%) than in the cattle with > 299 kg body weight (27.15%). It was concluded that *R. (B.) microplus* is the main tick species identified which was a threat to the cattle population in Nsukka district, irrespective of age, sex, breed of the animal, season of the year and weight of the animal of the study area.

Moghaddam et al., 2014 worked on prevalence of cattle ticks in Nur Country and prepared a list of tick fauna. This investigation was carried out on 150 head of cattle ticks of rural areas of Nur city which is located in Mazandaran province during spring and summer season of 2011. A total number of 1563 ticks were isolated and examined in which ixodes *Ricinus* (51%) and *Boophilus annulets* (49%) were found to be prevalent.

Chillar et al., 2014 investigated some Hard Ticks (Acari: Ixodidae) infesting domestic buffalo and cattle from Haryana, India. A cross-sectional study was conducted to determine the diversity and prevalence of ticks infesting domestic cattle and buffalo from Haryana, state of India. From 10 populations, overall 17 collections had been done during the survey period during August 2011 to January 2013. Out of a number of 662 animal surveyed, 309 animals were found to be infested with ticks from the three genera of family Ixodidae and a total of 867 ticks were collected and recognized.

Mashebe et al., 2014 worked to study the occurrence of 7 tick-borne disease of livestock in Zambezi. The large population of wildlife in the tropics provides a reservoir of ticks and infective microbes that spread to domestic animals during grazing in the same environment. Ticks infesting livestock transmit numerous kinds of viruses, fungi, bacteria and protozoa in livestock, wildlife and human hosts causing poor health and socio-economic losses to their respective hosts. This review work focused on the current situation of ticks infesting livestock in Zambezi region, typical ticks of livestock, and common genera of ticks infesting livestock in Zambezi region.

Chhillar et al., 2014 conducted an investigation on some Hard Ticks (Acari: Ixodidae) infesting domestic buffalo and cattle from Haryana, India to determine the diversity and intensity of hard tick species infesting domestic buffalo and cattle from Haryana, India. A total of 17 collections were performed from 10 allopatric populations during the study period of August 2011 to January 2013. Out of a total number of 662 animals surveyed, 309 animals were found infested with ticks from three different genera

of family, Ixodidae and a total of 867 ticks were collected and identified. The genera recorded were *Hyalomma*, *Rhipicephalus* and *Dermacentor*. Tick species identified of the three genera were *Hyalomma anatolicum anatolicum*, *Hyalomma anatolicum excavatum*, *Rhipicephalus sanguineus*, *Rhipicephalus (Boophilus) microplus*, *Rhipicephalus (Boophilus) decoloratus* and *Dermacentor* spp.

Anderson et al., 2013 studied tick infestation patterns in free ranging African buffalo. The effects of host innate immunity and niche segregation among tick species and to assess niche segregation, evaluated attachment site preferences and tested for correlations between abundances of different tick species. They also investigated and identified the host factors which drive variability in tick abundance, measured age; body condition, reproductive and immune status in all hosts, and examined their effects on tick burdens.

Singh et al., 2013 worked on epidemiology of ticks in cattle population of many agro-climatic zones of Punjab, state of India. A total of 4459 cattle of all age groups and sex were examined from eighteen districts of five major agro-climatic zones of Punjab state, The overall prevalence of ixodid ticks, *Rhipicephalus microplus (R. microplus)*, *Hyalomma anatolicum anatolicum (H. a. anatolicum)* and mixed infestation were recorded to be 58.06%, 50.16%, 11.34% and 3.45%, respectively. Among the various agro-climatic zones highest prevalence rate of *R. microplus* and *H. a. anatolicum* were recorded in sub-mountain undulating region (79.36%) and western region (20.40%), respectively indicating that *R. microplus* prefers a hot & humid whereas arid & semi arid condition suit better to *H. a. anatolicum*. The overall tick prevalence was recorded highest in monsoon season (83.74%), followed by summer (69.01%) and winters (31.64%) and a significant variation ($P < 0.01$) was also observed: whereas, maximum prevalence of *R. microplus* and *H. a. anatolicum* were recorded in monsoon (72.42%) and summers (18.06%), respectively.

Khan et al., 2013 observed tick infestation rate in buffalo and cattles in many regions of Khyber Pakhtunkhwa, Pakistan. An active surveillance study was conducted to estimate the tick infestation rate and its geographical distribution in relation to climate change along altitudinal gradients in Khyber Pakhtunkhwa, Pakistan. A total of 1223 (48.35%) cattle and 1306 (51.65%) buffaloes were examined in the study at different

altitudes in the same season and tick infestation rate was observed. Cumulative ticks infestation rate was found significantly higher (34.79%) in hot arid plane area than that of (18.63%) in cold hilly area ($p>0.05$). The overall tick infestation rate in cattle (33.36%) was significantly higher than that of buffaloes (22.58%).

Shubber et al., 2013 studied ixodid Ticks of Water Buffalo *Bubalus bubalis* in the Middle and South of Iraq. A total of 188 adult buffaloes were inspected for ticks at 23 collection sites in 9 provinces in the middle and south of Iraq. The overall infestation rate of ixodid ticks was recorded to be 41.5%. It is 38.5% for middle provinces; Baghdad, Babylon, Wasit, Diwaniya, Al-Najaf Al Ashraf, Al-Muthana and 45.6% for south provinces; Missan, ThiQar and Basra. The species of ticks recovered and their infestation rates were recorded *Hyalomma anatolicum* (38.99%), *H. scupense* (17.09%), *H. excavatum* (13.78%), *H. turanicum* (13.04%), *Hyalomma sp.* (6.77%), *H. dromedarii* (6.15%), *Rhipicephalus turanicus* (2.83%), *R. (Boophilus) annulatus* (0.86), and *Haemaphysalis sulcata* (0.49%). Reporting of *H. sulcata* constituted a new host record. The impact of marsh desiccation during the past years on the tick fauna of buffaloes was also discussed.

Aktas et al., 2012 have done a survey of ticks feeding on cattle and prevalence of tick-borne pathogens in the Black Sea region of Turkey. Their reports suggested that most prevalent tick species in the humid climatic zone was *Ixodes ricinus* and infection rates were recorded at 95% confidence intervals (CI). Results of sequencing suggested that *Babesia spp.* has similarity with the unnamed *Babesia sp.* Kashi 1 and 2 and CS58 *Anaplasma/Ehrlichia sp.* were 98% and 100% identical to *Ehrlichiacanis* and *Ehrlichia sp.* Omatjenne strain, respectively.

Shemshad et al., 2012 studied the distribution of hard tick infestation in livestock in boeen Zahra & Takistan countries of Qazvin province from April 2010 to September 2010. Nearly about 2638 sheep, 461 goats & 318 cattle of 38 Herds in different geographical areas where studied for ticks infestation and the composition in different topographic areas were found to be different. *Hyalomma* species was found to be the most prevalent in these areas.

Shemshad et al., 2012 conducted survey of hard ticks (Acari: Ixodidae) on cattle, sheep and goats in Boeen Zahra and Takistan counties, Iran. The species compositions collected from Boeen Zahra were *Haemaphysalis concinna* (0.63%), *Haemaphysalis sulcata* (12.66%), *Hyalomma anatolicum* (3.80%), *Hyalomma asiaticum* (3.16%), *Hyalomma detritum* (5.70%), *Hyalomma dromedarii* (28.48%), *Hyalomma marginatum* (13.29%), *Hyalomma schulzei* (1.89%), *Rhipicephalus bursa* (3.16%) and *Rhipicephalus sanguineus* (3.16%). In Takistan the tick infesting the livestock were *Hyalomma dromedarii* (9.86%), *Hyalomma marginatum* (13.29%), *Hyalomma schulzei* (1.89%) and *Rhipicephalus sanguineus* (3.16%), respectively. Hard ticks compositions in different topographic areas were different. *Hyalomma* species is found to be the most prevalent species.

Jawale et al., 2012 surveyed the prevalence of hard tick species (Acari: Ixodidae) on cattle in Sinner Diary farms at Nasik District, India. A total of 1364 (958 cow and 305 buffaloes) post acaricide treated animals were selected and examined for the prevalence of tick infestation. Prominently *Boophilus*, *Amblyomma* and *Hyalomma* spp. were found on cows and buffaloes. Age and sex of host play non-significant role in prevalence of tick infestation, while breed of the host and status are important parameters found to be significantly affecting tick prevalence.

Obadiah et al., 2012 studied tick infestation in cattle in Zaria Abattoir, Nigeria. In their study, they observed four species of ticks, collected from three breeds of cattle, *Amblyomma variegatum* 27 (22.5%), the commonest, followed by *Boophilus decoloratus*, 21 (17.5%), *Hyalomma* sp. (6.7%) and *Rhipicephalus sanguineus* (3.3%), were found to be the prevalent.

Kabir et al., 2011 investigated tick infestation in cattle at Chittagong District, Bangladesh. Epidemiology of the tick infestation was studied in cattle at different sub districts of Chittagong District from November, 2008 to May, 2009 to know the prevalence of ticks in relation to age, sex, breed, management of cattle, season of the year, topography of the area and different body parts of the host. A total number of 380 cattle were examined, of which 138 (36.31%) cattle were found infested. Three species of ticks were identified namely, *Boophilus microplus*, *Rhipicephalus sanguineus* and

Haemaphysalis bispinosa. The range of tick burden was 1 to 7 per four square inch in the heavily infested area of Chittagong District. Field grazing (41.96%) cattle were more susceptible ($p < 0.01$) to tick infestation than the stall-feeding (24.8%) animals. Prevalence of tick infestation was significantly ($p < 0.01$) higher in summer (41.66%) season followed by winter (31.5%) season. Prevalence of tick infestation was significantly ($p < 0.01$) higher in hilly areas (44.44%) followed by plain areas (30.27%). It was concluded that *B. microplus* is the main tick species identified and threatening to the cattle population in Chittagong District irrespective of age, sex, breed of the animal and seasons of the year and topography of the study area.

Tsai et al., 2011 conducted first systematic survey of tick infestation of cattle in Taiwan with species identification of these ectoparasites by morphology. Cattle (40%) of the 15 enrolled farms were infested by ticks, and ticks were collected from 133 (4.5%) cattle among the total of 2950 cattle. A total of 783 ticks were collected, including 714 (91.2%) adults, 64 (8.2%) nymphs, and 5 (0.6%) larvae. Of the 714 adults ticks, 706 (98.9%) were identified as 8 (1.1%) were *Recipicplus haemaphysaloides*.

Tessema et al., 2010 studied the prevalence of ticks on local and crossbred cattle in and around Asella town, South East Ethiopia. The prevalence of bovine tick species in and around Asella town was studied over a period of eight months from October 2007 to May 2008. Adult ticks were collected from 384 local and crossbred cattle which were kept under extensive management system and at different agro-ecological zones. A total of 6298 adult ticks were collected from half body part of cattle, and were identified to genera and species level. Five tick species of four genera were identified, in which two species belong to genus *Amblyomma*, and one species each in the genus *Boophilus*, *Rhipicephalus* and *Hyalomma*. Of all the total ticks collected, *Amblyomma*, *Rhipicephalus*, *Boophilus* and *Hyalomma* constituted 60.1%, 22%, 15.4% and 2.5%, respectively. The tick species encountered were *A. variegatum* (48.2%), *Rhipicephalus evertsi-evertsi* (22%) *Boophilus decoloratus* (15.4%), *A. cohaerence* (11.9%) and *Hyalomma marginatum rufipes* (2.5%).

Salih et al., 2008 conducted a Preliminary survey of ticks (Acari: Ixodidae) on cattle in Central Equatoria State, Southern Sudan. They conducted a survey on four tick genera and ten species were identified. The tick species identified were *Amblyomma lepidum*, *Amblyomma variegatum*, *Boophilusa nnulatus*, *Boophilusdecoloratus*,

Hyalomma marginatum rufipes, *Hyalomma truncatum*, *Rhipicephalus appendiculatus*, *Rhipicephalus evertsievertsi*, *Rhipicephalus praetextatus* and *Rhipicephalus sanguineus* group. The highest number of ticks was collected in October during the rainy season. A finding of great significance was that *R. appendiculatus*, vector of East Coast fever, has now firmly established itself throughout the year with possible implications for cattle production in Central Equatoria State.

Rodri'guez-Vivas et al., 2006 conducted a study on prevalence and potential risk factors for amitraz resistance in *Boophilus microplusticks* in cattle farms in the State of Yucatan, Mexico. A cross-sectional study was carried out in order to investigate the possible association between management risk factors and the prevalence of *B. microplus* ticks resistant to amitraz in Yucatan, Mexico. Ninety-eight cattle farms were randomly selected and a sample of 20–30 engorged *B. microplus* females was collected from at least 10 cattle from each farm. Collected ticks were taken to the laboratory where the larval progeny were obtained. The modified larval immersion test was used to detect ticks resistant to amitraz. A discriminating dose of 0.0002% amitraz was used.

Aktas et al., 2004 investigated a total of 2388 cattle and 442 animal sheds from two areas (Elazig and Malatya) endemic for Turkey. They were assessed for *Hyalomma* tick. A total of 4581 of 7455 *Hyalomma* ticks were collected from cattle. All the ticks collected from shelters were *Hyalomma anatolicum anatolicum*.

Gitau et al., 1997 studied serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a District, Kenya. One hundred and fifty farms were studied from three administrative sub locations in each of the five AEZs. Prevalence of serum antibodies to three tick-borne parasites, that is *T. parva*, *T. mutans* and *B. bigemina*, were determined using the enzyme-linked immune sorbent assay (ELISA) technique. Antibody prevalence values differed across the AEZs. The ranges of means for the prevalence were: *T. parva* (1&72%), *T. mutans* (1 S-28%) and *B. bigemina* (12-49%).

2.2. Acaricide resistance in animals:

Surbhi et al., 2018 studied efficacy of commercial acaricides against *Ornithodoros savignyi* ticks collected from Haryana, a Gaushala at Madhogarh village of Mahendergarh district in Haryana was found heavily infested with *Ornithodoros savignyi*.

Laboratory standardized adult immersion test was employed for efficacy trials at different concentration of deltamethrin following 10-minute protocol and 14 days as observation period. The ticks were adjudged dead on the basis of their immobility. Probit analysis of the data generated showed LC50 and LC95 values to be 60.95 ppm and 370.68 ppm, respectively. In addition, the ticks were also employed to adult immersion test with discriminating dose (AIT-DD). Results of the test revealed 86.67%, 60% and 86.67% survivability against cypermethrin, amitraz and fipronil, respectively at the country specific discriminating dose suggested for *Rhipicephalus (Boophilus) microplus* indicating a need for the separate discriminating dose for *Ornithodoros* sticks.

Petermann et al., 2016 conducted a survey of cattle tick, *Rhipicephalus (Boophilus) microplus*, resistance to amitraz and deltamethrin in New Caledonia. The evolution of tick resistance to acaricides in New Caledonia was followed in two ways: through two large scale surveys in 1998 and 2014 and through the routine analysis of samples of ticks with suspected resistance. The results of the two approaches were qualitatively similar though analysis of ticks with suspected resistance gave higher frequencies of resistance, as expected of a biased sample. Resistance tests using a larval packet test have been conducted since 1993 for deltamethrin and 2003 for amitraz. Deltamethrin was used in country-wide control from 1986 to 2003 and amitraz since 1996. This study analyzed the variation of resistance parameters such as lethal concentration 50 (LC50) and the resistance factor over 21 years for deltamethrin and LC99 over 11 years for amitraz. There was an increase in deltamethrin's annual mean LC50 from 1993 to 2004 when it reached 2.9 g/l and then a progressive decrease, reaching a level consistently below 1 g/l since 2007. Even though breeders stopped using deltamethrin in New Caledonia in 2003, the percentage of susceptible strains has remained below 30% since 1998. Amitraz's LC99 mean increased significantly from 0.31 g/l in 2003 to 2.96 g/l in 2014. Whereas all tested strains in 2003 were susceptible, only 40% of strains tested at the request of farmers were susceptible in 2014. The recent territory-wide survey showed that 76.7% of strains are still susceptible. This study established that resistance to amitraz has developed slowly in New Caledonia. A reversion phenomenon may have occurred concerning deltamethrin resistance, visible through the decrease of LC50 mean and the decreased proportion of very resistant strains; however the proportion

of susceptible strains remains at a low level and there is anecdotal evidence that high resistance can re-emerge rapidly.

Ramírez et al., 2016 observed LC50 of carvacrol and salicylic acid analogues with acaricide activity in larvae and adult ticks of *Rhipicephalus (Boophilus) microplus*. In this research the analogues, carvacrol and salicylic acid were evaluated in larvae of *R. (B.) microplus* with data obtained in Larval Packet Test (LPT) and Larval Immersion Test (LIT). A lethal concentration 50 (LC50) was assessed. The most potent compounds were evaluated in the adult ticks since there are no reports of evaluation in the life state of the parasite. From all the tested compounds, the ethyl 2-methoxybenzoate ($91.82 \pm 1.66\%$, $0.91\mu\text{mol/mL}$) and ethyl 2, 5-dihydroxybenzoate ($89.14 \pm 1.61\%$, $2.04\mu\text{mol/mL}$) showed the highest percentage of mortality and the lowest LC50. They were found to be the best candidates for a study in vivo.

Nava et al., 2015 deliberated strategic applications of long acting acaricides against *Rhipicephalus (Boophilus) microplus* in North Western Argentina, with an analysis of tick distribution among cattle. Strategic applications of long-acting acaricides for the control of *Rhipicephalus (Boophilus) microplus* in North Western Argentina were evaluated for one year. In addition, tick distribution among cattle was analyzed to evaluate if partial selective treatment or culling them all proportion of most heavily infested animals were feasible options to control *R. (B.) microplus*. Two different treatment schemes based on two applications of Fluazuron and one application of 3.15% ivermectin were performed. Treatment were made in late winter and spring so as to act on the small 1st spring generation of *R. (B.) microplus*, in order to preclude the rise of the larger autumn generation. The overall treatment effect was positively significant in both schemes. The number of ticks observed in the control group was significantly higher than in the treated groups on all post-treatment counts. Group 2 exhibited more than 80% of efficacy almost throughout the study period, whereas Group 1 exhibited an efficacy percentage higher than 80% in September, October, December, February, April and May, but not in November (73.4%), January (58.3%), March (45.2%) or June (53.4%). Absolute control was observed in Group 2 in the counts of September and October, and in Group 1 in the count of February. The control strategies evaluated in this work provide an acceptable control level with only three applications of acaricides at the same time; they prevent the occurrence of the autumn peak of tick burdens, which is characteristic of

R.(B.) microplus in North Western Argentina. Tick distribution was markedly aggregated in all counts. Although ticks were not distributed evenly among calves, the individual composition of the most heavily infested group was not consistent throughout the study period. In addition, the level of aggregation varied with tick abundance.

Kaushal et al., 2014 investigated the vertical distribution of ticks of domestic animals and their public health importance in Nilgiri Hills and adjoining areas of Tamil Nadu state (India) A study has been undertaken during 2008–2010 on the distribution and abundance of ticks of domestic animals in seven different biotopes. A total of 3,008 domestic animals were examined in areas ranging from an altitude of 300 to 2200 meters above mean sea level (MSL) of which 1,335 (44.5%) animals were having tick infestation. A total of 6,012 adult and immature ticks belonging to 12 species (11 ixodid and one argasid) were collected. Eleven tick species were collected from Kallar area situated downhill eastern slopes of the Nilgiris followed by Burliar area (7 species) located at higher altitudes. From Masinagudi area near to dense forests and scrub jungles, five species were recorded. However at higher elevations on the hills, Udhagamandalam area, only one species was recorded. Among various tick species recorded in the study, *Boophilus microplus* was distributed in almost all areas surveyed followed by *Haemaphysalis spinigera* and *Rhipicephalus sanguineus*. The factors governing their distribution and epidemiological significance in the transmission of various tick-borne diseases of public health importance are discussed.

Abbas et al., 2014 studied acaricide resistance in cattle ticks and actions to its management. Cattle ticks are an important constraint on livestock production, particularly in tropical and subtropical areas. Use of synthetic acaricides is the primary method of tick control; therefore, it would be imperative to develop strategies to preserve the efficacy of existing acaricides. This paper summarizes the status of acaricide resistance in cattle ticks from different parts of the world and reviews modes of action of currently used acaricides, mechanism of resistance development, contributory factors for the development and spread of resistance, management of resistant strains and strategies to prolong the effect of the available acaricides. Application of vaccines, chemical base, plant base acaricides and educating villagers about recommended tick control practices are discussed, along with the mixing of currently available options for the management of medicinal resistance and ultimately the control of cattle ticks.

Singh et al., 2014 evaluated amitraz resistance through Adult Immersion Test (AIT) in *Boophilus microplus* from Punjab. The results of amitraz suggested that the LC50 (95% CI) and LC95 (95% CI) values were recorded as 332.52 ppm (305.06–362.44) and 1646.93 ppm (1383.97–1959.84), respectively and the resistance factor (RF) was 13.17 which belong level II resistance status.

Eshetu et al. 2013 done an experiment on 180 engorged adult female ticks (ninety ticks of each *A. gemma* and *R. pulchellus* Species) were immersed in amitraz 0.025% or diazinon 0.06% at field recommended concentration (treated groups), or in distilled water (Control groups) for 10 min and then incubated at 27=1⁰c for 7 days. The oviposition response of each tick species in both groups was followed. The mean mass of eggs laid by each *A. gemma* and *R. pulchellus* in the treated group and those of untreated groups was compared to estimate the efficacy of each tested acaricide. Amitraz at 0.025% was found to have significantly higher ($P < 0.005$) overall mean percent oviposition control ($C\% = 95.47$) of *A. Gemma* and *R. pulchellus* than diazinon 0.06% ($C\% = 80.9$). At this recommended concentration, both amitraz 0.025% and diazinon 0.06% were less efficient in *R. pulchellus* oviposition control ($C\% = 90.94$ for amitraz and $C\% = 71.41$ for diazinon) than *A. gemma* oviposition control ($C\% = 100$ for amitraz and $C\% = 88.85$ for diazinon). The results of their study suggested that amitraz at field recommended concentration provides better efficient in controlling *R. pulchellus* and *A. gemma* oviposition inhibition than diazinon.

Sharma et al., 2012 examined cypermethrin and deltamethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from different six agro-climatic regions of India. A cross sectional study was designed to evaluate the prevalence of synthetic pyrethroids (SP) resistance in *Rhipicephalus (Boophilus) microplus* in India. Twenty-seven areas located in six agro-climatic regions were selected for the collection of engorged ticks using two stages stratified sampling procedure. Adult immersion test (AIT) and larval packet test (LPT) were optimized using laboratory reared susceptible line of *R.(B.) microplus* (IVRI-I) for determination of 95% lethal concentration(LC95) of deltamethrin (29.6 ppm in AIT and 35.5 ppm in LPT) and cypermethrin (349.1 ppm in AIT and 350.7 ppm in LPT). The AIT with a discriminating dose ($2 \times LC95$) was used to detect deltamethrin and cypermethrin resistance in the field isolates of *R. (B.) microplus*.

Neto Laercio et al., 2011 carried out study on applied molecular genetic approaches for identifying the basis of variation in resistance to tick infestation in cattle. In recent years there has been renewed interest in the adaptation of cattle to challenging environments, largely driven by advances in genomic methods. The current interest in tick resistance is understandable given the major production and welfare implications of tick infestation in tropical and subtropical zones where around 70% of beef cattle are located. Heritability for tick burden in cattle has been shown to range about 0.30, which is enough to result in the success of some programs of selection for resistance of ticks in animals. Gene expression studies strongly indicate that both immune and non-immune mechanisms are associated with tick resistance in cattle. Recent quantitative-trait mapping studies have identified chromosome segments and single nucleotide polymorphisms associated with tick burden, but no causal variant has been identified so far. Most of the genetic markers identified for tick burden explain a relatively small proportion of the variance, which is typical of markers for quantitative traits. This leads to the conclusion that panels of multiple markers for tick resistance rather than a single marker will most likely be developed, possibly involving specific panels for zebu or taurine breeds, which could be used for future selection and breeding programs in cattle.

Lovis, et al., 2011 conducted a study to determine acaricide resistance in *Rhipicephalus (Boophilus) microplus* Brazilian field population and found that lethal concentrations inducing 50% and 90% mortality and their respective 95% CI are presented for the 17 field strains in comparison with the reference strains.

Lovis et al., 2011 highlighted a study in vitro test to evaluate the resistance level against acaricides of the cattle tick, *Rhipicephalus (Boophilus) microplus*, they found the LTT and LPT dose–response curves for the susceptible, Munoz, and the resistant, Ultimo, *R. microplus* strains. The LTT produced results covering the whole range of mortality from 0 to 100% for both susceptible and resistant *R. microplus* strains for all compounds. Using the LPT, the complete dose–response range from 0 to 100% was obtained for both *R. microplus* strains for all compounds except for coumaphos tested on the resistant Ultimo strain. For coumaphos, 67% mortality was obtained at 3381 mg/m² (36,224 ppm), the highest dose tested for the susceptible Munoz strain, testing doses from 0.0015 to 100 mg/m² with the LTT provided a complete dose–response curve for all the compounds while the interval to obtain similar results with the LPT ranged from 0.05 to 845 mg/m².

Dose–response mortality data obtained with the LTT showed very low dispersion for SP and PYZ, while the highest variability was observed with ivermectin and amitraz.

Garcia, et al., 2011 evaluated the acaricidal effect of *Targets minuta* Linnaeus (*Asterraceae*) essential oil. They identified the qualitative and quantitative analyses of a commercial sample of essential oil extract on ticks that was obtained from *targets minuta*. Their results demonstrated that the *T. minuta* essential oil had over 95% efficacy against four species of ticks at a concentration of 20%. Their results suggest that the essential oil of *T. minuta* could be used as an environmentally friendly acaricide.

Perez et al., 2010 dose-mortality regressions, lethal concentration (LC), their confidence intervals and slope by probit analysis. Resistance ratios (RR) were determined in the three investigated populations at the LC50 and LC99 estimates. The LUADY (RR50: 2.04 and 2.29, RR99: 267 and 3.55), SPN (RR50: 3.55 and 3.68, RR99: 8.19–11.06) and SFDO (RR50: 6.84 and 8.59, RR99: 54.17 and 87.86) ticks had significantly higher LC50/LC99 than the reference susceptible Dutch strain, demonstrating resistance in the field-collected populations. Furthermore, there was significant difference between LC50/LC99 of the SFDO, SPN and LUADY tick populations, which indicates not only the presence of resistant population, but also different levels of resistance to ivermectin in the field populations studied. There was no difference observed at the neither LC50 nor LC99 estimates at two different times of collection from any of the three populations they have studied.

Castro et al., 2010 conducted an experiment on *Rhiphcephalus (Boophilus) microplus* by use of fipronil, a type of phenyl pyrazolic insecticide which had been used as an important alternative to control acaricide-resistant ticks. Fipronil (95.3%) was primarily evaluated using the larval packet test (LPT), four populations were evaluated with the LPT, and two populations displayed lower RR50, while other populations displayed higher RR50 than those determined by the LPT. They reported the first case of fipronil resistance in Brazil and highlighted that the LPT as a more sensitive technique for the evaluation of fipronil resistance in *R.(B) microplus* ticks.

Castro et al., 2009 conducted an experiment on in vitro type of chemical on ticks and checked the LPT and AIT test and calculated LC50 and LC99.9 with the help of

fipronil. In spite of the highest LC50 variability, all variables determined for AIT were appropriate to discriminate both strains, AIT and LIT had more sensitivity than LPT, with larger resistance factors. It was used two times LC99.9 as discriminating doses (DCs) following FAO suggestion. The mortality rates recorded using by AIT, LIT and LPT were: 6.96_105 ppm, 343.26 ppm and 5.7_103 ppm, respectively and their respective resistant factors were: 202.4, 5.36 and 1.52.

Jonsson *et al.*, 2007 studied engorged adult female ticks that were immersed in one of a series of dilutions of commercial acaricides in water and then incubated at room temperature for 7 days. Data on ovipositions were collected, after seven days treated with acaricides, probit analysis was conducted. Substantial differences in both LC50 and LC99 for the susceptible strain was found. For amitraz, the discriminating concentration recommended by FAO was 0.25% but there estimates ranged from 0.46% -9000%, for cypermethrin, the recommended DD was 0.0050%, with there estimate ranging from 0.00022% two 0.74%.

Rajput *et al.*, 2006 studied the medical and economic importance of ticks. They reviewed that Ticks cause great economic losses to livestock, and adversely affect livestock host in several ways. Anaemia is a direct response of ticks infection as potential vector for blood protozoa and helminth parasites. Sucking of bloods by plenty of ticks causes reduced weight and blood loss among live stocks while their piercing on hides cause reduced quality hide. However, potential losses caused by ticks are due to their ability to transmit parasite world-wide.

George *et al.*, 2004 conducted an experiment during World War II provided superior alternative products. Most of the major groups of organic pesticides are represented as the list of chemicals used to control ticks on cattle. Unfortunately, the successive evolution of resistance of ticks to acaricides in each chemical group with the concomitant reduction in the usefulness of a group of acaricides is a major reason for the diversity of acaricides. Whether a producer chooses a traditional method for treating cattle with an acaricides or uses a new method, he must recognize the benefits, limitations and potential problems with each application method and product. Simulation models and research were the basis of recommendations for tick control strategies advocating approaches that reduced reliance on acaricides. These recommendations for controlling

ticks on cattle are in harmony with recommendation for reducing the rate of selection for acaricide resistance. There is a need to transfer knowledge about tick control and resistance mitigation strategies to cattle producers.

Sabatini *et al.*, 2001 conducted laboratory tests on larvae and adult of the cattle tick, *Boophilus micropilus* to determine the toxicity of the macrocyclic lactone acaricides (MLs). Technical and commercial MLs were used in larval packet test (LPT), larval immersion test (LIT) and adult immersion test (AIT). In LIT and AIT the toxicity of MLs was much higher than for LPT. In the AIT, diluting the injectable formulation of MLs in water was effective as dilution in ethanol Triton X -100. LC50, LC99.9 and 95 % confidence limit were determined so that a discrimination dose (DD) could be set for larval and adult tests in order to diagnose potential resistance to MLs in field sample of the tick. These DDs are for Australian strain of Bm and may not be suitable for other strains until further work is carried out.



Chapter 3

***Prevalence of Rhiphicephalus
(Boophilus) microplus
infesting cattle and buffaloes
across selected districts of
Uttar Pradesh***

CHAPTER - 3

Prevalence of *Rhipicephalus (Boophilus) microplus* infesting cattle and buffaloes across selected districts of Uttar Pradesh

Ticks are usual in all agro ecological zones of the country. The main impact of the ticks is the diseases causing agent and a vector that has adverse effects on skin and hide (Byu, 2005).

3.1 Introduction

More than 89 species of ticks are prevalent, as has been recorded from all over the world significant to human beings and live stock. Tick species should be controlled to meet world need for animal protein in terms of livestock production (Drummond, 2007).

The capacity to transmit diseases has been widely reported due to *Boophilus*, *Rhipicephalus* and *Argas*. A number of researchers reported the prevalence of ticks in various parts of the country, Panjab (Singh and Rath, 2013), Kerala (Prakasan and Ramani, 2007), Uttaranchal (Vatsya *et al.*,2008), Haryana (Chhillar *et al.*,2014), Tamilnadu (Kumar and Balkrisnan, 2002), South India (Jagganath and Lokesh 1998).

From management practices, awareness and education of farmers, agroclimatic conditions, association and life style of different species has been attributed to prevalence of ticks in different areas of India and this distribution pattern is prime basis of evolving strategies and in prevention of diseases caused by ticks. Therefore the study has been designed to identify and determine the prevalence of ixodid ticks in livestock, thereby keeping in view the socio economic importance and lack of available data on the prevalence of ticks in these regions.

3.2 Materials and Methods

3.2.1 Sample collection

A thorough investigation was performed for collection of *Rhipicephalus (Boophilus) microplus* from the organized and unorganized cattle & buffalo farms of different districts

in Uttar Pradesh in different seasons of the year. Animals were categorized based on the age, sex and breed which were carefully examined for the presence of ticks from different parts of body and are taken to laboratory in desiccators.

3.2.2 Study duration

This survey study was framed from January 2015 to March, 2017. Data of survey was recorded and analysed. Whole examination was conducted in three seasons i.e. summer (March to June), winter (November to February) and rainy (July to October) season in both years.

3.2.3 Study Area

The study area, Uttar Pradesh, India was divided into five zones i.e. North, East, West, South and Central zone for the epidemiological studies and the same is hereby depicted in Table 3.2.1 and Figure 3.2.1.



Figure 3.1 Location Map of Study Area: Uttar Pradesh (Source: Census of India, 2011)

Table 3.1 Zones and selected sites of Uttar Pradesh

S. No.	Zones	Areas covered
1.	North	Basti, Bastidehat, Saray.
2.	East	Varanasi, Bhagwanpur, Anantpur, Deorai.
3.	West	Moradabad, Angwanpur, Bareilly dehat, Amroha, Premnagar
4.	South	Etawah, Jaswantnagar, Badpura, Agupur.
5.	Central	Lucknow, Kakori, Rehmanikhera, Barabanki, Hardoi.

3.2.4 Selected animals for the study: Cattle and Buffaloes

A thorough investigation of prevalence of ticks in Cattle and Buffaloes was conducted on the animals of genus *Bos* and genus *Bubalus*.

Table 3.2 Scientific classification of the genus *Bos* (Parker and Haswell, 1962):

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Subclass	Theria
Infraclass	Eutheria
Order	Cetartiodactyla
Family	Bovidae
Subfamily	Bovinae
Genus	<i>Bos</i>

Table 3.3 Classification of the genus *Bubalus*

Kingdom	Animalia
Phylum	Chordata
Class	Mammalia
Order	Artiodactyla
Family	Bovidae
Subfamily	Bovinae
Genus	<i>Bubalu</i>

3.2.5 Host Sex

The sex of the host was taken as one of the variable to study the differences in prevalence of tick infestation. Hence, while surveying both the male and female cattle and buffaloes were included in the sample.

3.2.6 Season-wise Prevalence

The epidemiological survey was conducted in the study area for two years: 2015- 2017 in three seasons namely summer, rainy and winter.

3.2.7 Management practices

The rearing practices of animals can be done in three types:

1. Extensive
2. Semi-Intensive/extensive
3. Intensive

The rearing practice in which animals are reared totally on grazing feed is known as extensive rearing practice. The rearing practice in which animals partially depend on grazing as well as indoor feeds is termed as semi intensive rearing. The rearing practice in which the animals totally depend on indoor feed is called as intensive practice.

3.2.8 Host Breed

Cattle breeds inclusive of Sahiwal, Local Zebu, exotic Holstein Friesian, and cross breed were taken as sample hosts for studying the prevalence of tick infestation. Bhadavari, Mehsana, Murrah, Surti, and cross breed were the buffalo breeds, in which the tick infestation was studied.

3.2.9 Prevalence with predilection site wise

Ticks attachment was observed in different body parts of cattle and buffalo namely ear, dewlap, flank, udder, perineum, groin, external genitalia.

3.2.10 Host Age

On the basis of animal age, animals were divided into three groups:

- Group I: < 2 years
- Group II: 2-8 years
- Group III: >8 years

3.2.11 Collection of Test Organism

Adult ticks were collected from different body parts (ear, dewlap, flank, udder, perineum, groin, external genitalia) of the cattle and buffalo. Un-engorged, semi-engorged, fully-engorged ticks were collected from the animal's skin using blunt forceps and during the collection of ticks from animal's skin, care was taken, so that the mouthparts of the ticks are not damaged. The procedure of collection of ticks from the animal's skin was adopted as per the procedure devised by Soulsby, 1982.

3.2.12 Preservation of Sample

All the collected ticks were transferred and preserved in 70% alcohol in glass vials. Glass vials were labelled and permanent mounting was done.

3.2.13 Mounting of ticks

Permanent mounting of ticks was done by using standard protocol as standardised by Walker *et al.*, 2003 (Figure 3.2).

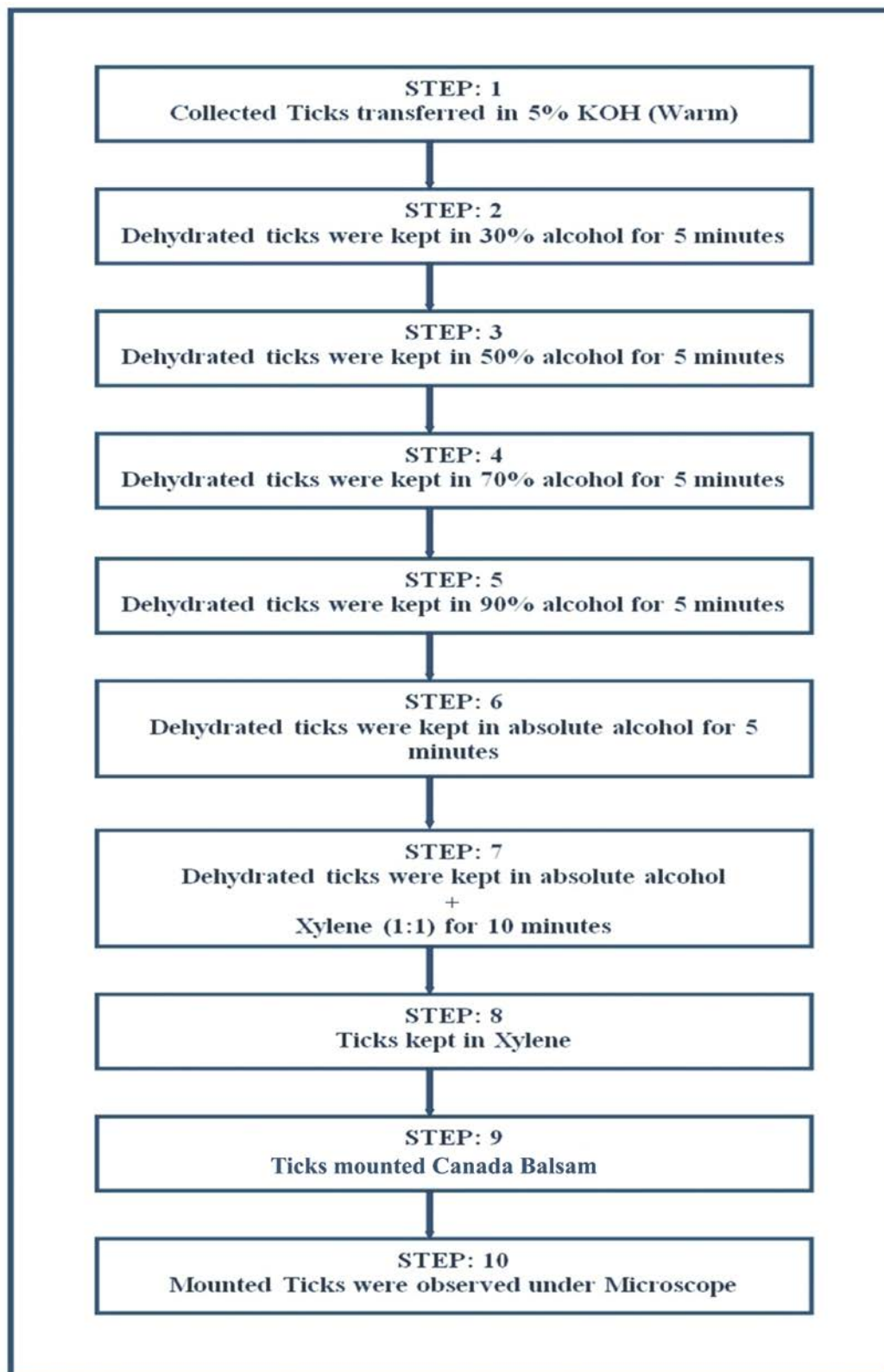


Figure 3.2 Procedure adopted in mounting of ticks (Protocol standardised by Walker *et al.*, 2003)

3.2.14 Identification of ticks

According to the description given by Walker *et al.*, 2003, ticks were identified using a stereoscopic microscope.

Percentage of infestation: The percentage of animals infested due to ticks were calculated using the formula-

$$P = \frac{\text{No. of infested Animals during specified period}}{\text{Total Animals Surveyed}} \times 100$$

(P = % infestation)

3.2.15 Statistical analysis and tools

Chi-Square test (χ^2) analysis was conducted to determine the significant association of all the independent variables with tick infestation.

3.3 RESULTS AND DISCUSSION

The current study was designed to generate an epidemiological data of tick infestation in cattle and buffaloes across various parameters during the period of June 2015 to May 2017. The data was collected from different districts of Uttar Pradesh, India.

3.3.1 Prevalence of Tick Infestation in Cattle and Buffaloes

During the study period between 2015-16, a total of 2540 cattle were observed and of which 1545 cattle were found infested with ticks and during 2016-17, 2865 cattle were examined and of which 1790 cattle were found infected with ticks. Total prevalence was found to be 60.82% in the year 2015-16. (Table 3.4, Figure 3.3) and 62.47% prevalence was recorded for the year 2016-17. Overall prevalence of ticks in cattle is depicted in table 3.4 & figure 3.3. A total of 4722 (2262 ticks in 2015-16 and 2460 ticks in 2016-17) field isolates were collected from various body parts of animals during the survey period. During the research period, identification of ticks was done and tick species identified were *Rhipicephalus microplus*, *Hyalomma anatolicum anatolicum*, *Haemaphysalis bispinosa*. Among the tick species identified during 2015-16. *Rhipicephalus microplus*

was found to be the most prevalent (Prevalence=56.01%, M:F= 1.42:1) tick species. *Hyalomma anatolicum anatolicum* was the second most abundant species among the identified species. (Prevalence= 26.08%, M:F= 1.83:1), followed by *Haemaphysalis bispinosa* (Prevalence=17.9%, M:F= 1.31:1) (Table 3.5, Figure 3.4). During the study period, 2016-17, *Rhipicephalus (Boophilus) microplus* was identified to be the most prevalent tick species (Prevalence= 57.64%, M:F= 1.34:1) followed by *Hyalomma anatolicum anatolicum* (Prevalence= 28.57%, M:F= 1.35:1) and *Haemaphysalis bispinosa* (Prevalence= 1.78%, M:F= 1.51:1) (Table 3.6, Fig. 3.5).

Among buffaloes surveyed, a total of 2152 animals were observed in 2015- 16, and of which, 1080 animals were found infested with field isolates and during 2016- 17, 1275 buffaloes were found infested of a total of 2360. Overall abundance was found to be 50.18%. During 2015- 16 and 54.02% prevalence was found in the study period during 2016- 17. During 2015-16 *Hyalomma anatolicum anatolicum* (56.02%) was found to be the most prevalent tick species followed by *Rhipicephalus (Boophilus) microplus* (43.97%) (Table 3.7, Figure 3.6) and in the year 2016-17 *Hyalomma anatolicum anatolicum* (58.97%) was identified to be the most prevalent tick species followed by *Rhipicephalus (Boophilus) microplus* (41.02%) (Table 3.8, Figure 3.7). It is also evident from the data that the collected ticks from the animals were dominated by male tick species.

Table 3.4 Overall Prevalence of ticks in surveyed Animals

Year	Total Animals Surveyed		Infected Animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
2015-16	2540	2152	1545	1080	60.82	50.81
2016-17	2865	2360	1790	1275	62.47	54.02

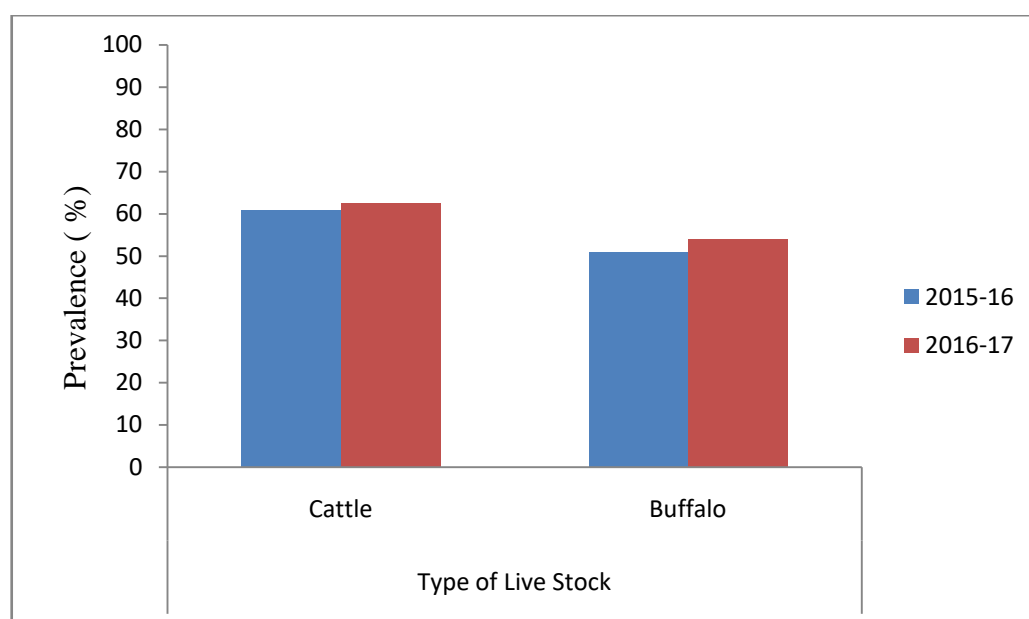


Figure 3.3 Overall prevalence of ixodid ticks in surveyed animals

Table 3.5 Sex wise prevalence of different tick species in the cattle study during (2015-16)

Tick species	Male ticks	Female ticks	M:F ratio	Total count of tick species	Prevalence Percentage (%)
<i>Rhipicephalus (Boophilus) microplus</i>	745	522	1.42:1	1267	56.01
<i>Hyalomma anatolicum anatolicum</i>	382	208	1.83:1	590	26.08
<i>Haemaphysalis bispinosa</i>	230	175	1.31:1	405	17.9
Total	1357	905		2262	

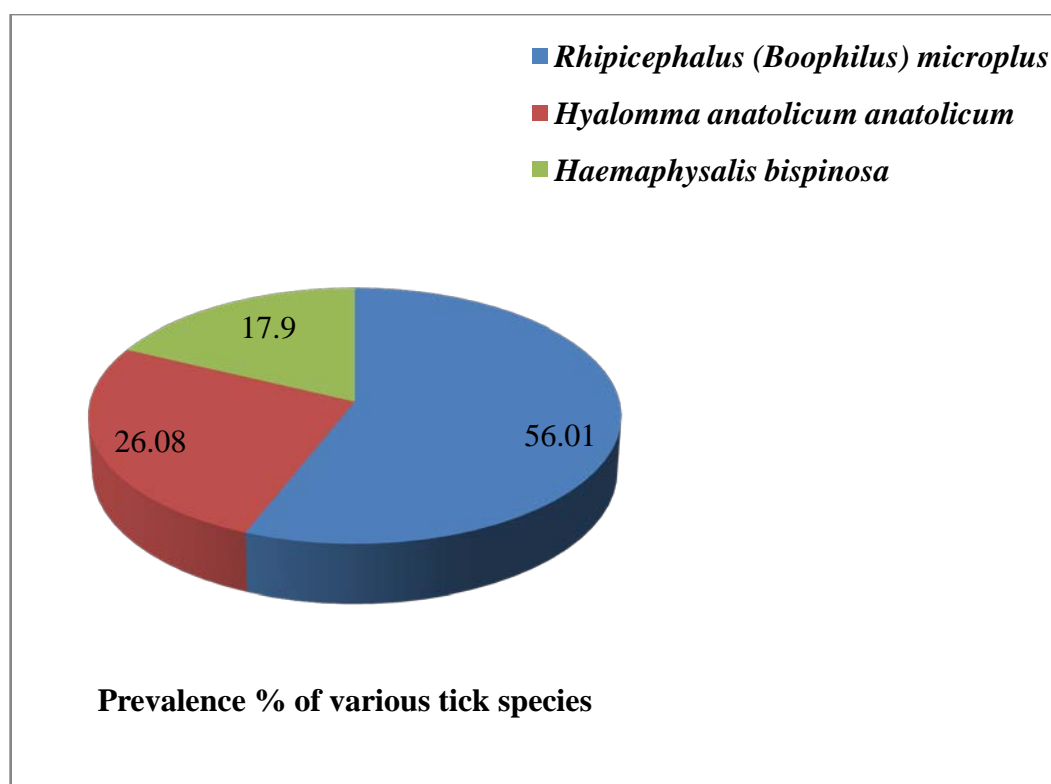


Figure 3.4 Distribution of different tick species in the cattle during study in (2015-16)

Table 3.6 Sex wise prevalence of different tick species in the cattle during study in the (2016-17)

Tick species	Male ticks	Female ticks	Male: Female ratio	Total count of tick species	Prevalence Percentage (%)
<i>Rhipicephalus (Boophilus) microplus</i>	815	605	1.34:1	1418	57.64
<i>Hyalomma anatolicum anatolicum</i>	405	298	1.35:1	703	28.57
<i>Haemaphysalis bispinosa</i>	205	134	1.52:1	339	13.78
Total	1425	1037		2460	

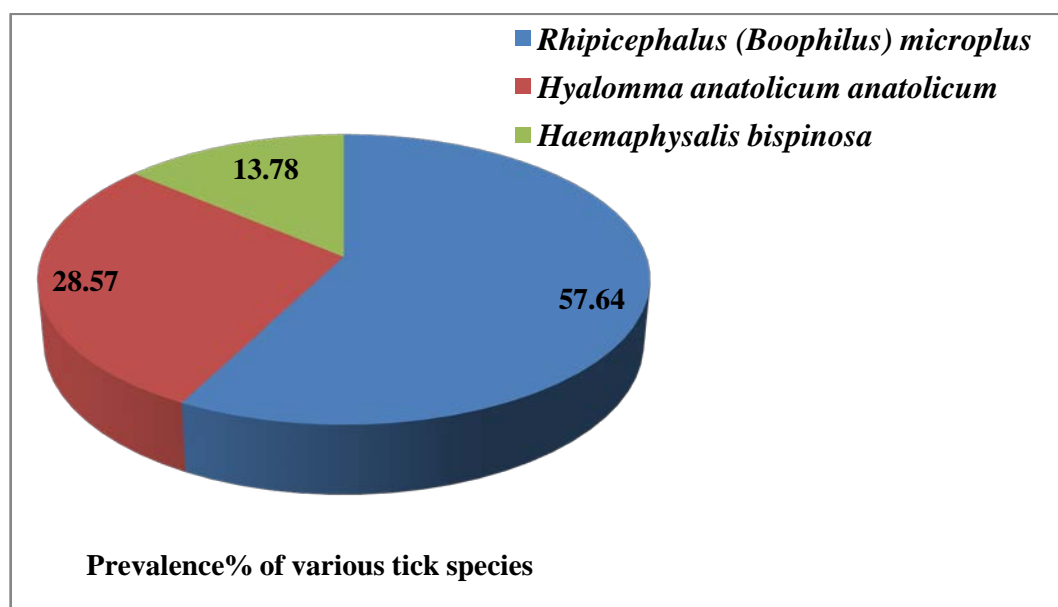


Figure 3.5 Distribution of different tick species in the cattle during study (2016-17)

Table 3.7 Sex wise prevalence in buffaloes of different tick species in the Year (2015- 16)

Tick species	Male ticks	Female ticks	M:F ratio	Total count of tick species	Prevalence Percentage (%)
<i>Rhipicephalus (Boophilus) microplus</i>	506	312	1.62:1	818	43.97
<i>Hyalomma anatolicum anatolicum</i>	586	456	1.28:1	1042	56.02
Total	1092	768		1860	

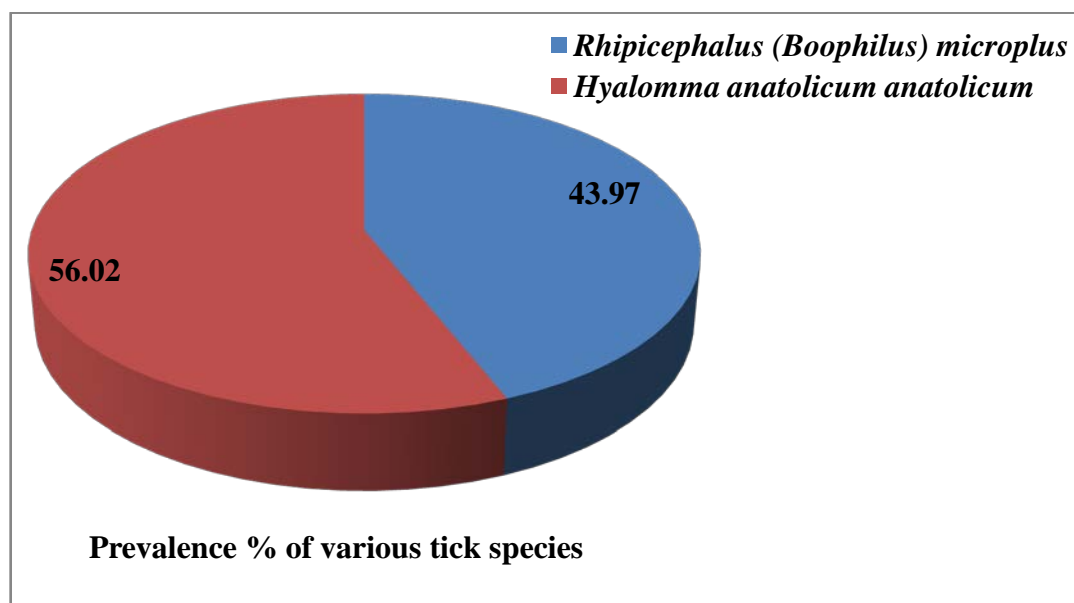


Figure 3.6 Distribution of different tick species in the buffaloes during study (2015-16)

Table 3.8 Sex wise prevalence in buffaloes of different tick species in the Year (2016- 17)

Tick species	Male ticks	Female ticks	M:F ratio	Total count of tick species	Prevalence Percentage (%)
<i>Rhipicephalus (Boophilus) microplus</i>	520	440	1.18:1	960	41.02
<i>Hyalomma anatolicum anatolicum</i>	705	675	1.04:1	1380	58.97
Total	1225	1115		2340	

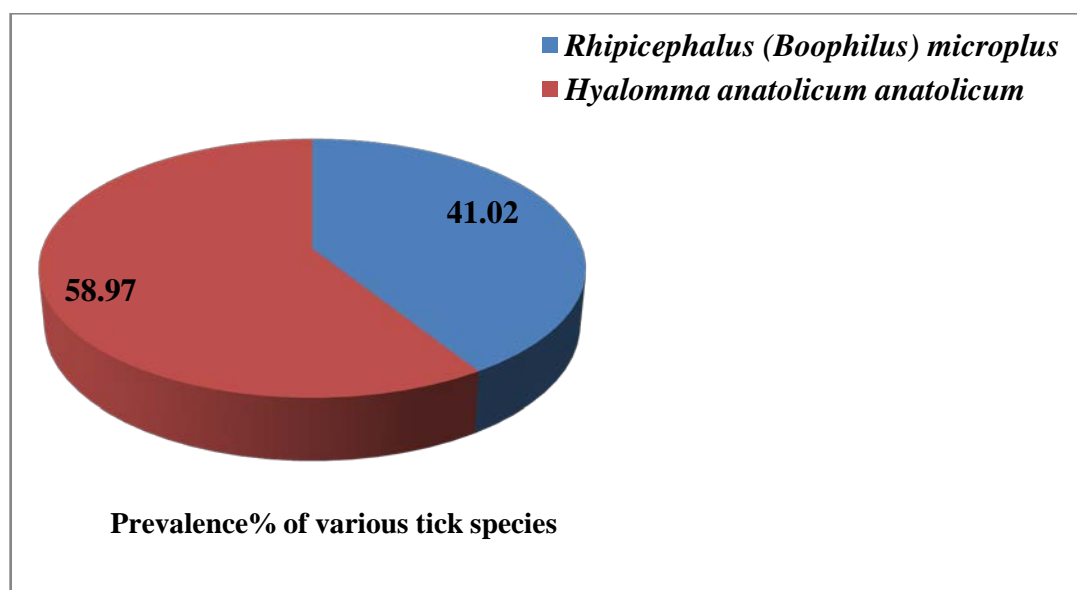


Figure 3.7 Distribution of different tick species in the buffaloes during study (2016-17)

Wide dispersion of tick species is observed in the animals (cattle and buffaloes) across various regions under study. During the entire study period, more than half of the examined cattle and buffaloes were infested with ixodid tick species. Many researchers worked on the prevalence of ixodid ticks in district of Uttar Pradesh and other districts of Uttar Pradesh, i.e. at Lucknow (Singh and Mishra 2017), in Mathura (Patel *et al.*, 2013), in Azamgarh (Ali and Singh 2013). The findings of the present study were found to be at par with the findings of the study conducted by Kaur, *et al.*, 2017, who worked on epidemiological survey on ixodid ticks in Lucknow district of Uttar Pradesh, in which 2710 cattle were surveyed and of which it was found that 1602 cattle were infested with ticks. Overall prevalence of tick infestation was observed to be 59.11%. The same study also reported that 300 buffaloes were examined and of which 160 buffaloes were found infested with ticks. Overall prevalence was observed to be 53.33%. Studies on the prevalence of tick infestation in livestock was also carried out of India and other countries too, i.e. in Tamilnadu (Kumar *et al.*, 2014), in Panjab (Singh *et al.*, 2013), in Pakistan (Rehman *et al.*, 2017), in Iran (Moghaddam *et al.*, 2014). Several factors influence the increase in tick infestation in animals such as lack of awareness about harmful effect of tick infestation among animals in rural areas farmers, use of poor livestock practices etc.

Rhipicephalus (Boophilus) microplus is a common tick species infesting livestock also is reported in buffaloes by Patel *et al.*, 2015 and Abbasi *et al.*, 2017. Similar tick prevalence studies in buffaloes were reported by Singh *et al.*, 2017 who observed *Hyalomma anatolicum anatolicum* is more prevalent tick species in buffaloes. Some other researchers found in their study that *Haemaphysalis bispinosa* species infest cattle, buffalo, goat and sheep (Latha *et al.*, 2004; Saxena, 2004 and Mamun *et al.*, 2010).

Among all the species identified, as per the sex and presence of tick on the animals body, it is observed that male ticks are higher than female ticks. Tadesse *et al.*, 2012 reported similar findings where in it was highlighted that male ticks are greater in no. on the host body in comparison to female ticks and this may due to the reason that engorged female ticks fall on the ground for egg laying, while male ticks remain on the body of animals.

3.3.2 Factors Affecting Prevalence of Tick Infestation the Cattle and Buffaloes:

3.3.2.1 Host Age

For the infestation of tick species on the body of livestock, host age is one of important factors (Manan *et al.*, 2007). During study period (2015-16 and 2016-17) data was recorded according to the age of cattle and buffalo for recording the prevalence of tick infestation rate on animal's body. In age group I (< 2years) highest prevalence was recorded in cattle and buffalo (78.11% in cattle and 70.68% in young calves of buffaloes) in the year 2015-16. In the year 2016-17, highest prevalence was recorded in young calves of animals (80.78% in cattle and 72.3% in buffaloes). The tick infestation in the cattle and buffaloes in the age group II (2-8 years) also was recorded and found that the rate of infestation is 65.73% in cattle and 62.17% in buffaloes during the year 2015-16. In the year 2016-17, the infestation was recorded to be 66.23% in cattle and 64.93% in buffaloes. The data clearly indicates least tick infestation in the III age group, (51.66% in cattle and in buffaloes 38.52%) in year 2015-16. In 2016 -17, tick prevalence was recorded to be 46.48% in cattle, 42.95% in buffaloes (Table 3.9 & 3.10 Figure 3.8 & 3.9). A significant variation was found among the different age group cattle and buffaloes ($\chi^2=106.33$, $p\leq 0.05$), ($\chi^2=151.79$, $p\leq 0.05$), respectively.

Table 3.9 Prevalence of Ticks with respect to age of cattle and buffaloes in the year (2015-16)

Range of Age	Total Surveyed Animals		Infected Animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
< 2 years	425	290	332	205	78.11	70.68
2-8years	855	668	562	415	65.73	62.17
> 8years	1260	1194	651	460	51.66	38.52
Total	2540	2152	1545	1080	$\chi^2=106.33$ P= 8.16E-24 (≤ 0.05 Sig.)	$\chi^2=151.79$ P= 1.09E-33 (≤ 0.05 Sig.)

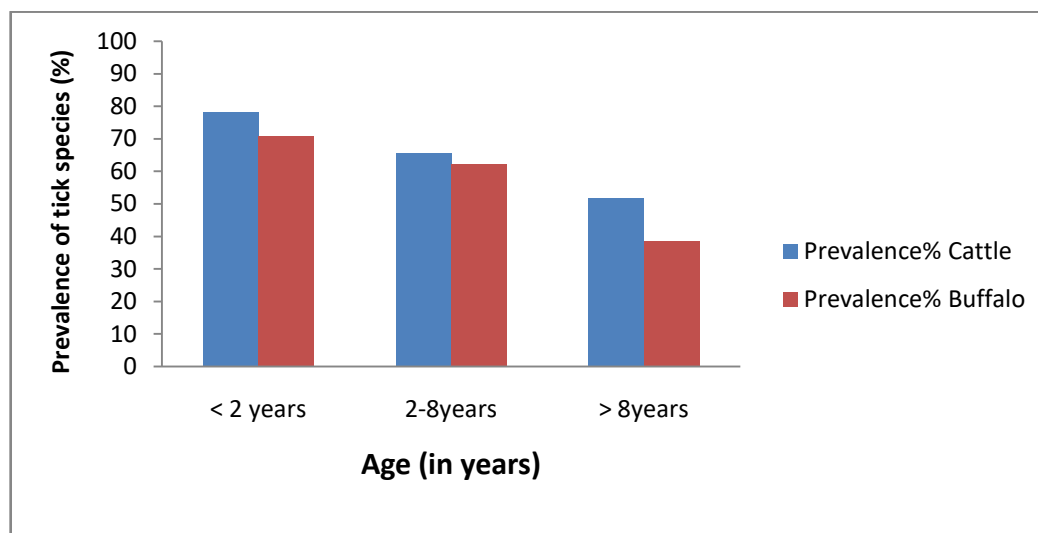


Figure 3.8 Prevalence of Ticks with respect to age of cattle and buffaloes in the year (2015-16)

Table 3.10 Prevalence of Ticks with respect to age of cattle and buffaloes in the year (2016-17)

Range of Age	Total Surveyed Animals		Infected Animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
<2 years	557	365	450	264	80.78	72.3
2-8years	770	810	510	526	66.23	64.93
> 8years	1538	1185	715	509	46.48	42.95
Total	2865	2360	1790	1275	$\chi^2=224.3$	$\chi^2=146.11$

0
P= 1.97E-49 (≤ 0.05 Sig.)
P= 1.87E-32 (≤ 0.05 Sig.)

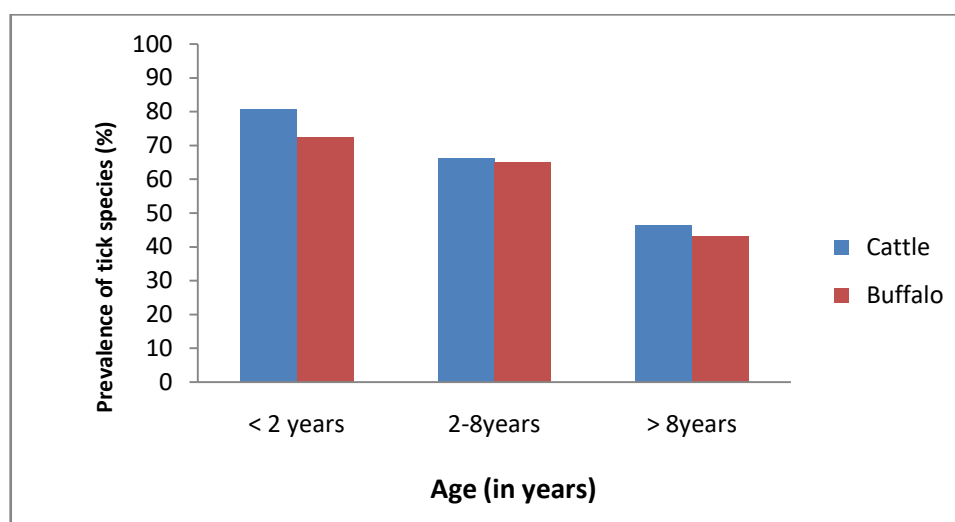


Figure 3.9 Prevalence of Ticks with respect to age of cattle and buffaloes in the year (2016-17)

Present study concludes that the young animals are very prone to infestation of ticks. Similar results were reported by many researchers in their study, Patel *et al.*, 2015 observed that infection rate was high in calves (<1 year), the infection rate was observed to be 80.20%. Khan *et al.*, 2013 reported that young calves of buffalo and cattle have more tick infestation, Manan *et al.*, 2007 also reported similar results in their study and this may be due to the reason that weak immune system in young calves increases the infestation rate of ticks.

3.3.2.2 Host Sex

Host sex also is one of the important factors which influence the prevalence of the tick infestation in cattle and buffaloes. In this study, during 2015-16, 1035 male and 1505 female cattle were analyzed, out of which, 425 male and 1120 female cattle were infested with the ticks. In case of buffaloes 830 male and 1322 female buffaloes were examined out of which 370 male and 710 female buffaloes were found to be infected with ticks. From the data, it is clearly evident that high tick infestation rate is prevalent in females in both cattle and buffaloes (74.41% and 53.70% respectively). In 2016-17, 975 male and 1890 female cattle were identified, out of which 375 male and 1415 female cattle were found to be infested with ticks. 950 male and 1410 female buffaloes were examined and out of which 445 male and 830 female buffaloes were found to be infected with ixodid ticks. High prevalence rate was recorded in female cattle and buffaloes (74.86%, 58.86% respectively). Prevalence of infestation was found to be higher in females than in the males (in the year 2015-16 $\chi^2= 286.35$, $P < 0.05$ and in 2016-17 $\chi^2= 263.65$, $P < 0.05$ in the cattle) (in the year 2015-16 $\chi^2= 16.99$, $P < 0.05$ and in the year 2016-17 $\chi^2= 33.3$, $P < 0.05$ in buffaloes) (Table 3.11 & 3.12 & Figure 3.10 & 3.11).

Table 3.11 Prevalence of ticks with respect to gender of cattle and buffaloes (2015-16)

Gender of Animals	Total Animals Surveyed		Infected Animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Male	1035	830	425	370	41.06	44.57
Female	1505	1322	1120	710	74.41	53.7
TOTAL	2540	2152	1545	1080	$\chi^2=286$.35, p=3.10 E-64 (≤ 0.05 Sig.)	$\chi^2=16.99$, p=3.75-05 (≤ 0.05 Sig.)

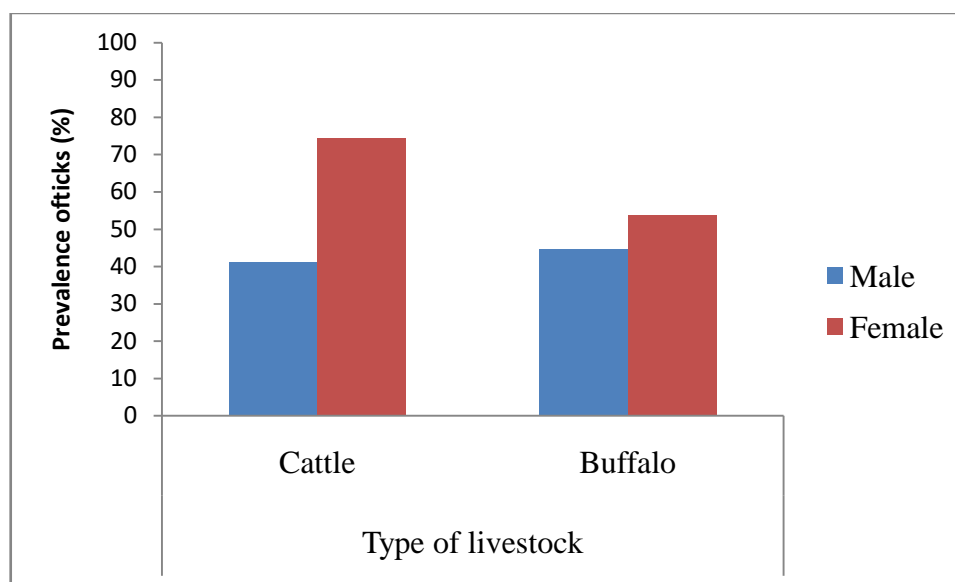


Figure 3.10 Prevalence of ticks with respect to gender of cattle and buffaloes (2015-16)

Table 3.12 Prevalence of ticks with respect to gender of cattle and buffaloes (2016-17)

Gender of Animals	Total Animals Surveyed		Infected Animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Male	975	950	375	445	38.46	46.84
Female	1890	1410	1415	830	74.86	58.86
TOTAL	2865	2360	1790	1275	$\chi^2=363.65$ p=4.52E-81 (≤ 0.05 Sig.)	$\chi^2=33.03$ p=9.06E-09 (≤ 0.05 Sig.)

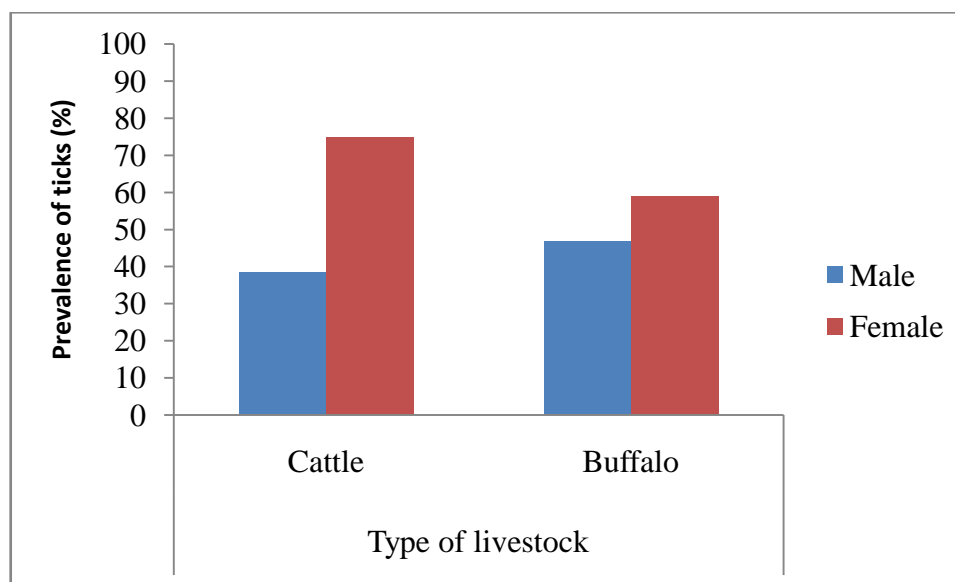


Figure 3.11 Prevalence of ticks with respect to gender of cattle and buffaloes (2016-17)

Higher tick infestation rate in female cattle was reported by Kaur *et al.*, 2017, Mamun *et al.*, 2010 also reported in their study that female animals are more prone to tick infestation, they also observed that female animals were 2.61% more susceptible than male animals. Kabir *et al.*, (2011) reported in their research that higher tick infestation rate in female animals is may be due to hormonal effects and contrary to the results of the present research, Atif *et al.*, (2012) and Singh and Rath (2013) reported in their study conducted at Panjab, that higher tick infestation is observed in male cattle than female. They also highlighted that higher tick infestation in male animals may be due to their neglect by farmers in the state.

3.3.2.3 Season- Wise Prevalence of Tick Infestation in Cattle and Buffaloes:

Season is also one of the important factors for tick infestation in both cattle and buffaloes. Tick Prevalence was recorded during three seasons rainy, summer and winter, (Rainy= July to October), (Summer= March to June), and (Winter= November to February). The results of two consecutive years (2015-16 and 2016-17) are given in the Table 11 & 12 and Figure 11 & 12. In the year 2015-16, during the study period, a total of 890, 835 and 815 cattle were examined, out of these 703, 450 and 392 cattle were infected with the ticks, in rainy summer and winter seasons respectively. Significantly higher tick prevalence was observed during rainy season (78.98%) followed by summer season (53.89%) and winter season (48.09%). During the study period, 2016-17, prevalence of ticks was recorded higher during rainy season (73.92%) followed by summer (56.97%) and winter (55.25%). In the buffaloes during survey period 2015-16, a total of 813, 685 and 654 buffaloes were identified and out of these 560, 300 and 220 buffaloes were found to be infested with the ticks in rainy, summer and winter seasons respectively. The prevalence of ticks was recorded significantly higher in rainy season (68.88%) followed by summer (43.79%) and winter (33.63%). Significant differences across seasons was evident from the data in both cattle and buffaloes ($\chi^2=195.47$, $p \leq 0.05$ significant, $\chi^2=196.47$, $p \leq 0.05$, respectively). In year, 2016-17, tick infestation was recorded significantly higher in both the animals, cattle and buffaloes in rainy season (67.95%) followed by summer (52.86%) and winter (41.1%) ($\chi^2= 89.06$, $p < 0.05$ significant, $\chi^2= 116.70$, $p < 0.05$, respectively) (Table 3.13 & 3.14, Figure 3.12 & 3.13).

Table 3.13 Prevalence of ticks with respect to season year (2015- 16)

Season	Total animals surveyed		Infected animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Rainy (Jul to Oct)	890	813	703	560	78.98	68.88
Summer (Mar to Jun)	835	685	450	300	53.89	43.79
Winter (Nov to Feb)	815	654	392	220	48.09	33.63
Total	2540	2152	1545	1080	60.82	50.18

$\chi^2=195.47$ $\chi^2=196.47$
 $P=3.58 \times 10^{-43}$ $P=2.17 \times 10^{-43}$
(≤ 0.05 Sig.)
(≤ 0.05 Sig.)

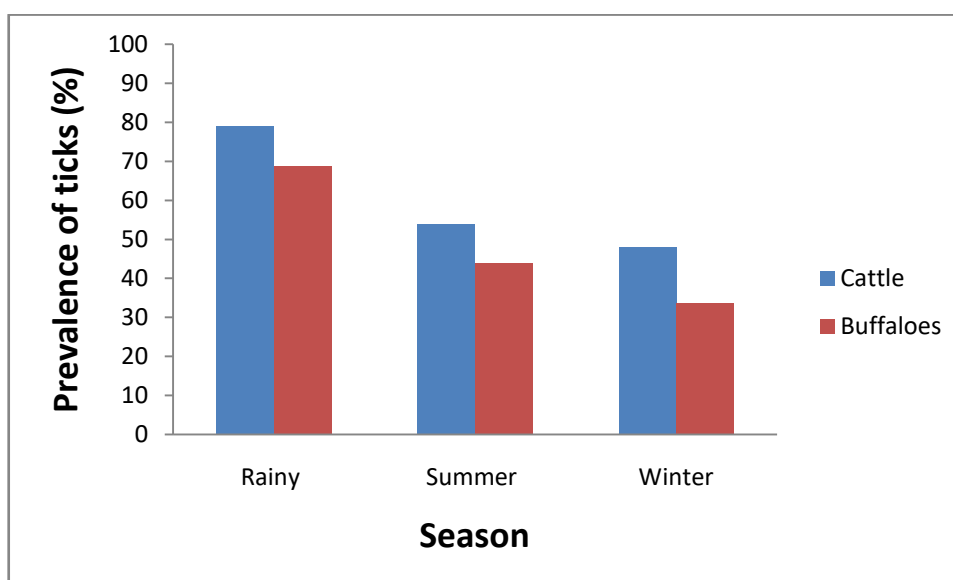


Figure 3.12 Prevalence of ticks on cattle and buffaloes with respect to season year (2015- 16)

Table 3.14 Prevalence of ticks with respect to season (2016- 17)

Season	Total animals surveyed		Infected animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Rainy (Jul to Oct)	1020	802	754	545	73.92	67.95
Summer (Mar to Jun)	960	760	547	402	56.97	52.86
Winter (Nov to Feb)	885	798	489	328	55.25	41.1
Total	2865	2360	1790	1275	62.47	54.02
					$\chi^2=89.0$ 6 P=4.58 E-20 (≤ 0.05 Sig.)	$\chi^2=116.70$ P=4.57E-26 (≤ 0.05 Sig.)

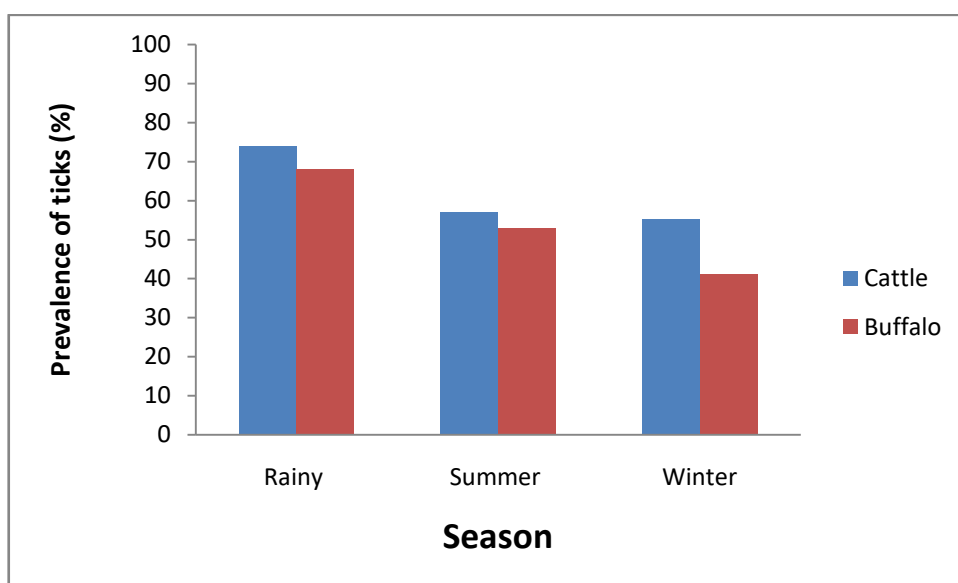


Figure 3.13 Prevalence of ticks in cattle and buffaloes with respect to season (2016- 17)

Studies on seasonal variations in tick infestation were conducted and reported by many researchers and the results of the present research are at par with the result recorded by the study of Kaur *et al.*, 2017 in Lucknow district of Uttar Pradesh. Seasonal variation in tick infestation in cattle, in three seasons was conducted and higher infestation was found during rainy season (rainy season (73.55%) followed by summer (57.05) and winter (46.27%)); Vatsya *et al.*, (2008), Atif *et al.*, (2012), Patel *et al.*, (2013) have also recorded higher tick infestation in rainy season. Higher tick infestation in rainy season is may be due to humidity and low temperature, and these two factors may be influencing tick infestation. In contrary to the findings of the present research, Mamun *et al.*, (2010) reported higher tick infestation in winter season and Islam *et al.*, (2006) have observed higher tick infestation rate in summer season. Changes in geographical area, soil composition, temperature and humidity variation, the prevalence of tick infestation in animals is found to be varied.

3.3.2.4 Management Practices: Rearing System

Three types of rearing system are generally followed, which includes extensive, semi intensive and intensive rearing system. Two types of rearing system (semi intensive and intensive) are found to be popularly used by farmers in the study area. Rearing mostly were using intensive type of rearing for calves (<2 years), weak and diseased animals. In intensively reared cattle and buffaloes higher prevalence of tick infestation was recorded (75.00%, 50.59%, respectively) in the year 2015-16. In animals which were reared in semi- intensive rearing system, prevalence of tick infestation was recorded in cattle (53.26%) and in buffaloes (47.93%). However variations in prevalence of tick infestation in cattle and buffaloes was found significant ($\chi^2=114.31$, $P\leq 0.05$, $\chi^2=1.50$, $P\leq 0.05$ respectively) (Table 3.15 & Figure 3.14). In the year 2016-17, prevalence of tick infestation was observed, significantly higher ($\chi^2=109.84$, $P\leq 0.05$, $\chi^2=10.67$, $P\leq 0.05$) in intensively reared cattle and in the buffaloes (76.37% and 57.73%, respectively). Semi-intensively reared animals showed variation in the prevalence of tick infestation in cattle and buffaloes (56.01% and 51%, respectively) (Table 3.16 & Figure 3.15).

Table 3.15 Prevalence of ticks with respect to rearing practices cattle and buffaloes (2015-16)

Rearing system	Total animals surveyed		Infected animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Semi-intensive	1656	1233	882	591	53.26	47.93
Intensive	884	919	663	465	75	50.59
Total	2540	2152	1545	1080	$\chi^2=114.31$ p=1.12E-26 (≤ 0.05 Sig.)	$\chi^2=1.50$ p=0.221 (≥ 0.05 Non-Sig.)

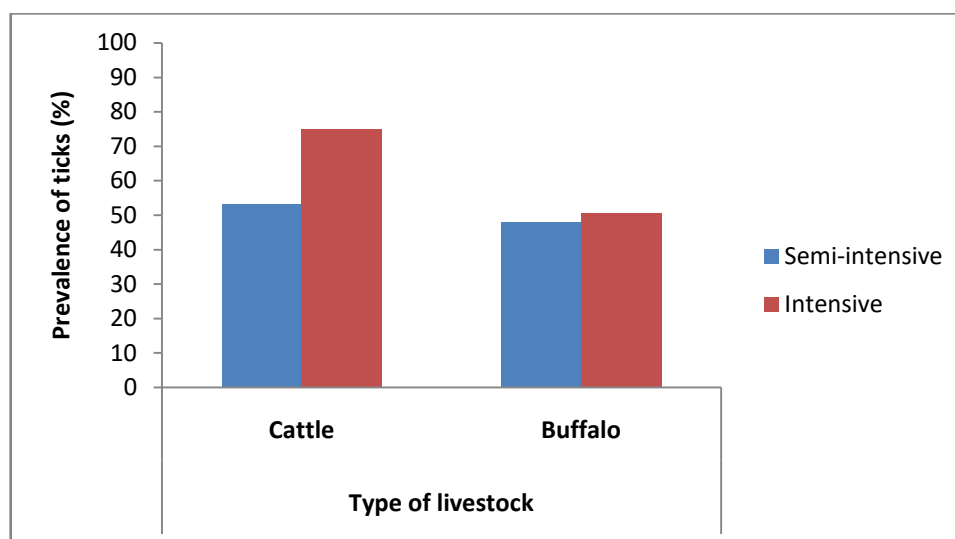


Figure 3.14 Prevalence of ticks with respect to rearing practices (2015-16)

Table 3.16 Prevalence of ticks with respect to rearing practices of cattle and buffaloes (2016-17)

Rearing system	Total animals surveyed		Infected animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Semi-intensive	1955	1300	1095	663	56.01	51
Intensive	910	1060	695	612	76.37	57.73
Total	2865	2360	1790	1275	$\chi^2=109.84$ p=1.06 E-25 (≤ 0.05 Sig.)	$\chi^2=10.67$, p=0.011 (≤ 0.05 Sig.)

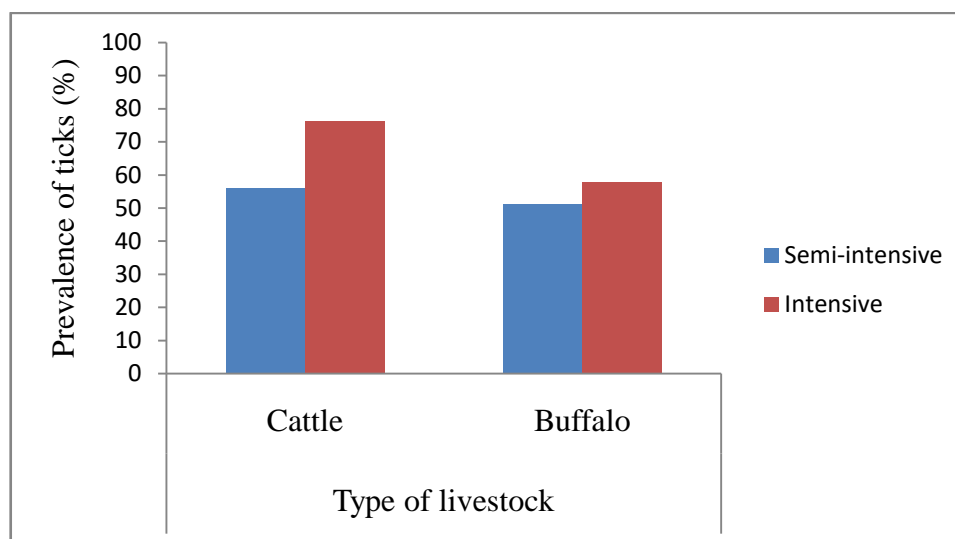


Figure 3.15 Prevalence of ticks with respect to rearing practices (2016-17)

In the present study, it was found that intensively reared animals are more prone to tick infestation in comparison to other rearing practices. The animals which are reared in the semi- intensive and extensive rearing system, depends on outdoor feeding like grazing, and during which ticks may be fed by the birds from the body of animals. High prevalence of tick infestation in extensive rearing system was reported by Tiki and Addis, 2011.

3.3.2.5 Host Breed

Different animal breeds were identified which includes local breeds Sahiwal, Local Zebu (cattle breeds) along with pure exotic breed, Holstein Friesian, Bhadavari, Mehsana, Murrah, Surti (buffalo breeds) and cross between cross and exotic breeds. On the basis of origin and physical characteristics, the breeds of cattle were determined. During the study period (2015-16) 1237, 705 and 598 cattle and 1015, 650 and 487 buffaloes were examined (Local, cross and exotic, respectively) out of which 531, 423 and 474 cattle, 385, 260 and 324 buffaloes were found to be infested with ticks, prevalence of ticks was recorded in 42.92% local cattle breed, 60% cross breed and 79.43% in exotic breed cattle and among breeds of buffaloes, tick infestation was recorded in 37.93%, 40% and 40.27% local, cross, exotic, respectively (Table 3.17, Figure 3.16). Significant differences in both the animals was recorded across breeds in tick infestation, $\chi^2= 223.85$, $p\leq 0.05$, $\chi^2=386.32$, $p\leq 0.05$ cattle and buffaloes, respectively. As per the data recorded in the year, 2016-17, 1565, 680 and 620 cattle and 1315, 407 and 638 buffaloes were examined (Local, cross and exotic, respectively) and out of which, 719, 490 and 581 cattle, 626, 205 and 444 buffaloes were found to be infested with ticks (Table 3.18, Figure 3.17). In cattle, tick infestation was recorded in 45.94%, 72% and 93.7% local, cross and exotic breeds, respectively. In buffaloes tick infestation was found in 47.6%, 50.36% and 69.59% local, cross and exotic breeds, respectively. Infestation of ticks was observed significantly higher in both the i.e. cattle and buffaloes, respectively ($\chi^2= 467.13$, $p\leq 0.05$ significant, $\chi^2=367.62$, $p\leq 0.05$ significant).

Table 3.17 Prevalence of ticks with respect to breed of cattle and buffaloes (2015-16)

Breed of animal	Total animals surveyed		Infected animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Local	1237	1015	531	385	42.92	37.93
Cross	705	650	423	260	60	40
Exotic	598	487	475	435	79.43	40.27
Total	2540	2152	1545	1080	$\chi^2=223.8$ 5, P=2.46E- 49, (\leq 0.05 Sig.)	$\chi^2=386.32$ P= 1.29E-84 (\leq 0.05 Sig.)

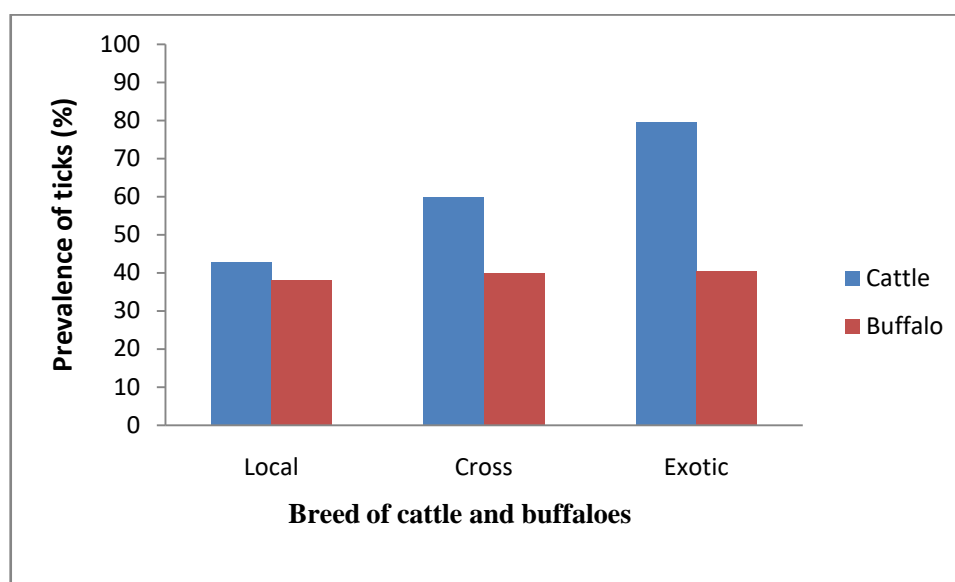


Figure 3.16 Prevalence of ticks with respect to breed of cattle and buffaloes (2015-16)

Table 3.18 Prevalence of ticks with respect to breed of cattle and buffaloes (2016-17)

Breed of animal	Total animals surveyed		Infected animals		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes	Cattle	Buffaloes
Local	1565	1315	719	626	45.94	47.6
Cross	680	407	490	205	72	50.36
Exotic	620	638	581	444	93.7	69.59
Total	2865	2360	1790	1275	$\chi^2=467.13$ P= 3.67E-102 (\leq 0.05 Sig.)	$\chi^2=368.62$ P= 1.34E-78 (\leq 0.05 Sig.)

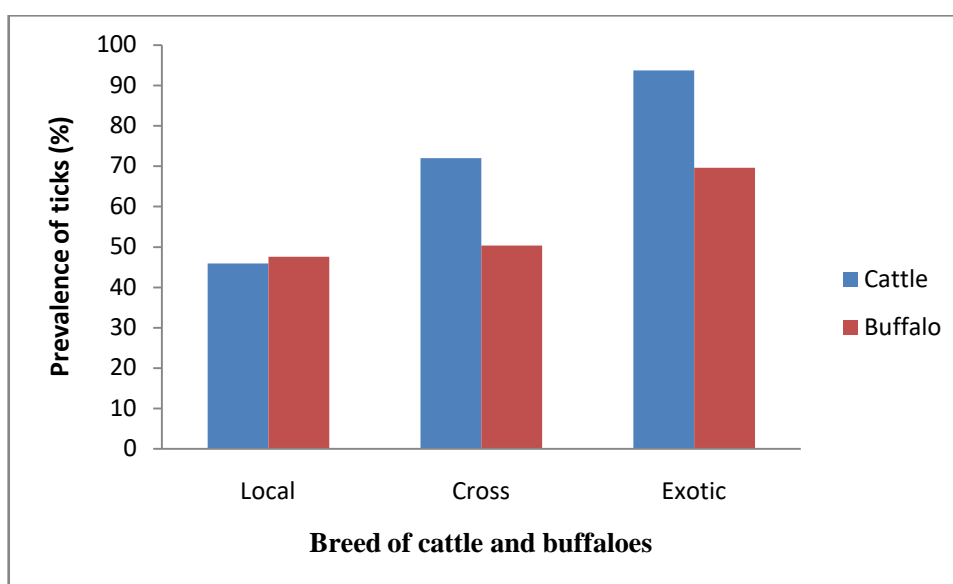


Figure 3.17 Prevalence of ticks with respect to breed of cattle and buffaloes (2016-17)

In the above finding of two years (2015-16, 2016-17) high infestation of ticks was found in exotic animals followed by the local and cross breeds. Similar type of studies has been conducted by Tessema and Gashaw (2010) and Asmaa *et al.*, (2014) and Dilpreet *et al.*,(2017) and recorded similar findings. It was also established in one of the study that local breed, *B. indicus* (Brahman) cattle, develops resistance against ticks up to 99% (Utech *et al.*, (1978)). Contradictory to this, tick infestation was found to be significantly higher ($p < 0.01$) in local animals as compared to cross breed as reported by Kabir *et al.*,(2011) due to lack of supplementary feeding for local breeds. Variation in different management system and scanty knowledge of tick control measures might have been attributed to such variation in the prevalence study.

3.3.2.6 Predilection Site Wise Prevalence of Tick Infestation in Cattle and Buffaloes

The main aim of the present study was to identify the correlation among the exodid ticks in animals with respect to specific attachment site. During study period (2015-16) tick load in animals varied from high on flank (19.67% in cattle and 19.01% in buffalo) followed by dewlap, perineum, groin, genitalia, ear and udder (Table 3.19 & Figure 3.18). In the year 2016-17 the tick burden in cattle was recorded as follows, higher in flank (16.22%) followed by udder, perineum, dewlap, groin, genitalia and ear. Tick load recorded in buffaloes was high on flank (16.22%) followed by dewlap, genitalia, udder, ear, groin and perineum (Table 3.20 & Figure 3.19).

Table 3.19 Tick attachment site on the body of cattle and buffaloes (2015-16)

Body Part	Ticks collected		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes
Ear	250	237	11.05	10.12
Dewlap	415	418	18.34	17.68
Flank	472	445	20.86	19.01
Udder	215	205	9.5	8.76
Perineum	392	365	17.32	15.59
Groin	225	350	9.94	14.95
Genitalia	290	320	12.82	13.67
Total Ticks Collected	2262	2340		

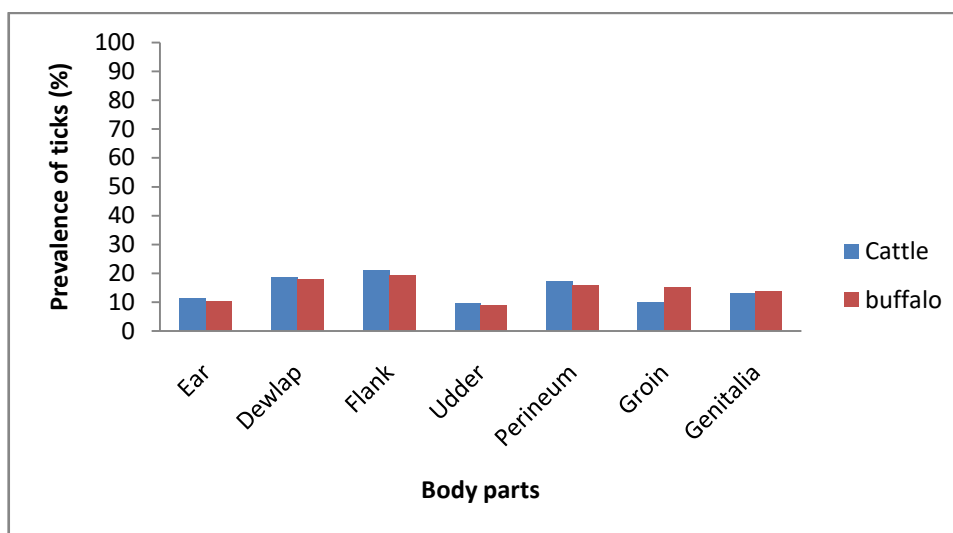


Figure 3.18 Tick attachment site on the body of cattle and buffaloes (2015-16)

Table 3.20 Tick attachment site on the body of cattle and buffaloes (2016-17)

Body Part	Tick collected		Prevalence Percentage (%)	
	Cattle	Buffaloes	Cattle	Buffaloes
Ear	320	312	11.28	13.22
Dewlap	425	385	14.99	16.31
Flank	460	415	16.22	17.58
Udder	450	340	15.87	14.4
Perineum	430	280	15.16	11.86
Groin	400	283	14.1	11.99
Genitalia	350	345	12.34	14.61
Total Ticks Collected	2835	2360		

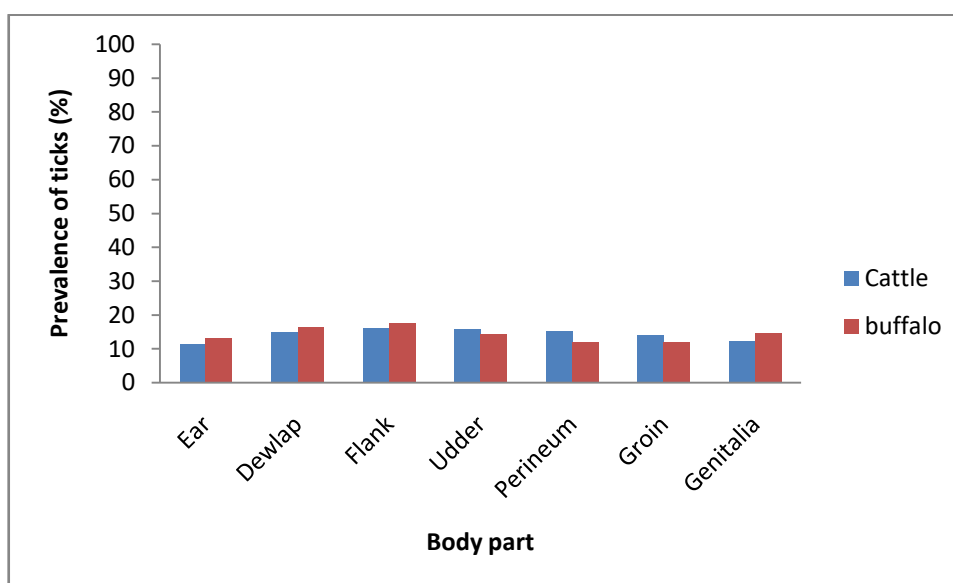


Figure 3.19 Tick attachment site on the body of cattle and buffaloes (2016-17)

Other researchers also reported similar type of findings where in high tick burden was observed on dewlap and flank region of animal body (Okello-Onen *et al.*,1999). Factors such as time and season accessibility for grooming have also been considered that determined the attachment site of ticks (Chandler and Read, 1994). Correspondingly, it has been reported that thinner skins and shorter hairs that is the area with rich blood supply as the area which is highly prone for tick infestation, (Pegram *et al.*,1981).

3.3.2.7 Zones of study area

To assure the effect of local conditions in different zones and to know the prevalence of tick burden, the result of two years (2015-16 and 2016-17) has been presented in the Table 3.21 & Figure 3.20 and Table 3.22 & Figure 3.21. The data was taken from five zones of Uttar Pradesh. During the year, 2015-16, amongst the cattle, north zone (71.57%) has been found to have the highest prevalence in animals followed by South (60.19%), East (60%) West (56.51%) and least in central zone (56.49%). In the buffaloes North zone (67.39%) have highest prevalence of ticks followed by East (51.31%), West (45.56%), Central (43.62%) and least in South zone (42.55%). Zone wise prevalence of ticks during 2016-17 was found to be almost similar, wherein highest prevalence was recorded in the North zone (71.92%) followed by West (70.90%), Central (66.12%), South (63.44%) and least in East zone (60.51%). In the buffaloes in same year, tick prevalence was recorded highest in North zone (68.08%) followed by Central (59.53%), East (52.17%), West (47.64%) and least in South zone (44.08%).

Table 3.21 Prevalence of ticks with respect to different zones in the study area (2015-16)

S. No.	Zones in the study area	Total Cattle Infected Prevalence (%)	Surveyed/ Cattle/ Total surveyed/ buffaloes/ Infected Prevalence Percentage (%)
1	North	475/340/71.57%	460/310/67.39%
2	East	560/336/60.00%	380/195/51.31%
3	West	430/243/56.51%	395/180/45.54%
4	South	505/304/60.19%	470/200/42.55%
5	Central	570/322/56.49%	447/195/43.62%

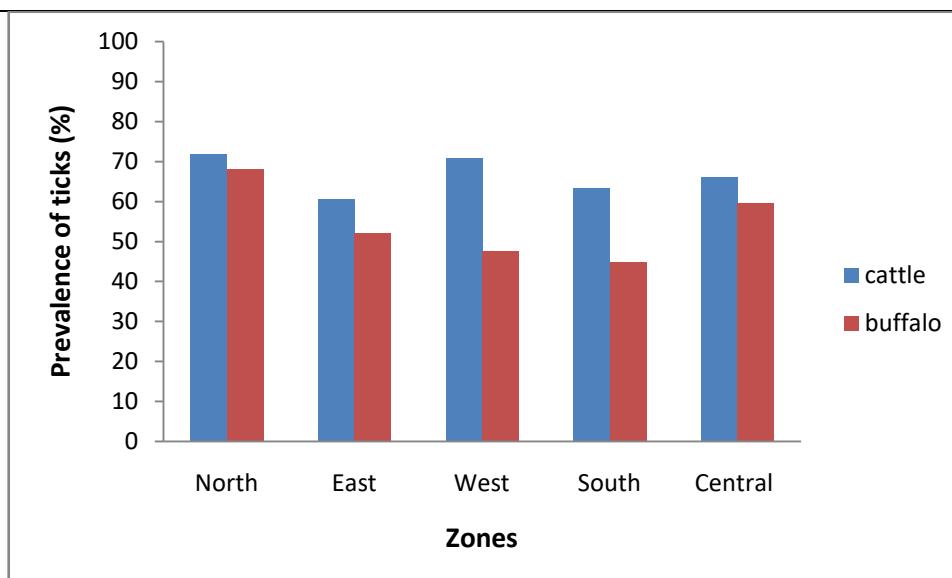


Figure 3.20 Prevalence of ticks with respect to different zones in the study area (2015-16)

Table 3.22 Prevalence of ticks with respect to different zones in the study area (2016-17)

S. No.	Zones in the study area	Total Cattle Infected Prevalence (%)	Surveyed/ Cattle/ Cattle/ Prevalence (%)	Total Buffalo Infected Prevalence (%)	Surveyed/ buffalo/ buffalo/ Percentage (%)
1	North	570/410/71.92%		470/320/68.08%	
2	East	580/351/60.51%		460/240/52.17%	
3	West	550/340/70.90%		510/243/47.64%	
4	South	610/387/63.44%		490/216/44.08%	
5	Central	555/302/66.12%		430/256/59.53%	

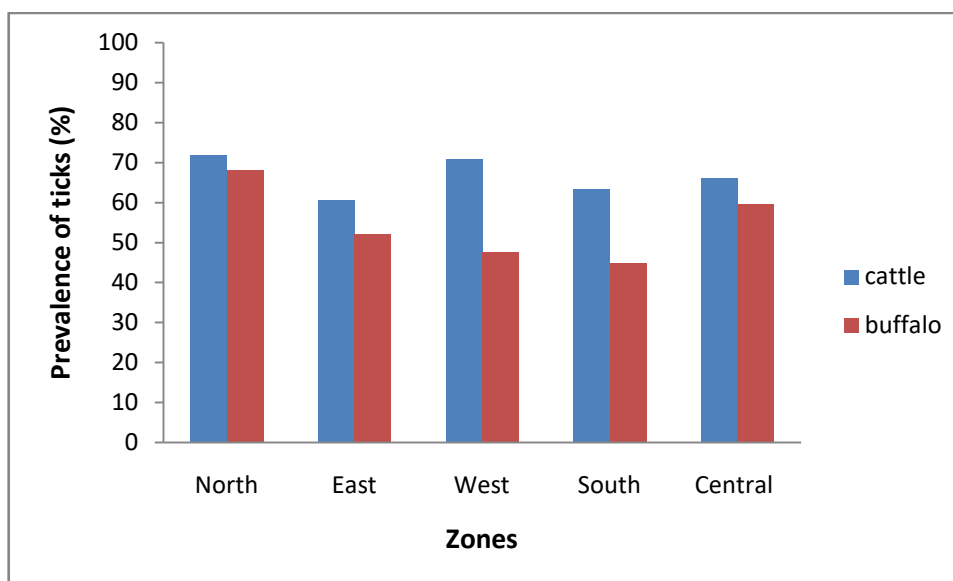


Figure 3.21 Prevalence of ticks with respect to different zones in the study area (2016-17)

In context of tick infestation, from the present study it can be deduced from the data collected from different zones of study area, the differences in the prevalence might be considered due to the differences in housing and the place where animals were kept or the use of acaricides and management practices that has been taken in to account to rear the animals. Hence these attributes play a very significant role in tick infestation with any parasites (ecto and endo parasites).

3.4 Conclusion

It is very important to analyze the economic loss encountered due to tick infestation. It is very important to collect different information regarding distribution and dynamics of tick species. Ticks are ecto-parasites and cause huge economic loss by transmitting tick born diseases which ultimately affect the quality of hide and skin causing loss in livestock population. Therefore the present study, epidemiological investigation of infestation of ticks in animals was conducted in Uttar Pradesh state of India. The study also focused that high tick load on cattle has been paid scanty attention by the rearers in the area. So the present study contributes to understanding of tick epidemiology.

The prevention measures are necessary for tick and tick born diseases and tick distribution pattern on different attachment sites on the host body should also be focused for proper analysis of tick born diseases in the livestock. The study gives a better idea for formulating prevention and control programs to decrease the tick infestation and tick born diseases in animals.

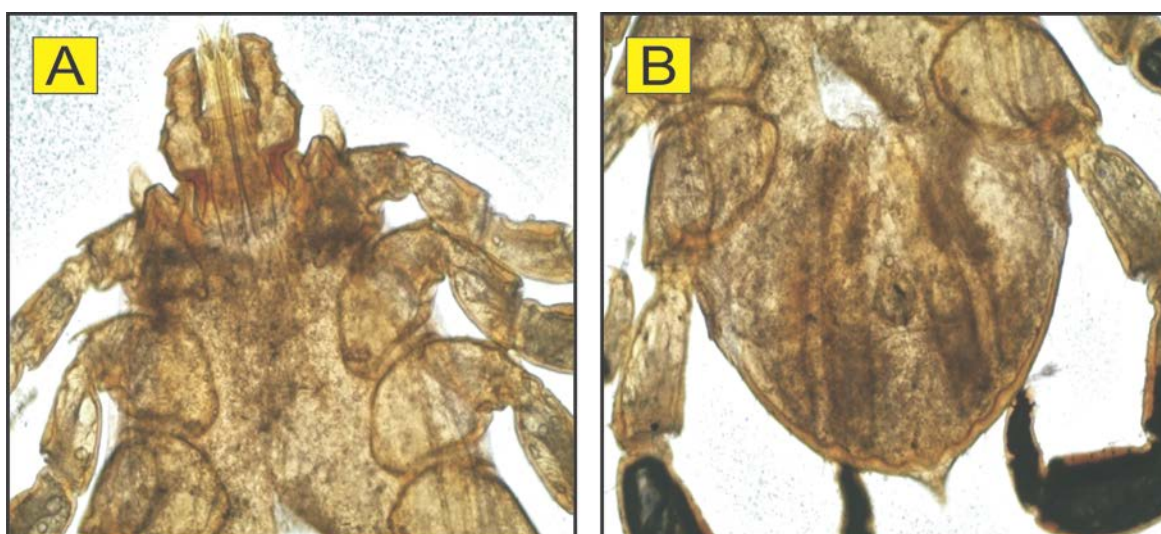


Plate -3.1 A-B: *Rhipicephalus (Boophilus) microplus*, Anterior and Posterior region



Plate. 3.2 A-B: *Hyalomma anatolicum anatolicum*, Anterior and Posterior region

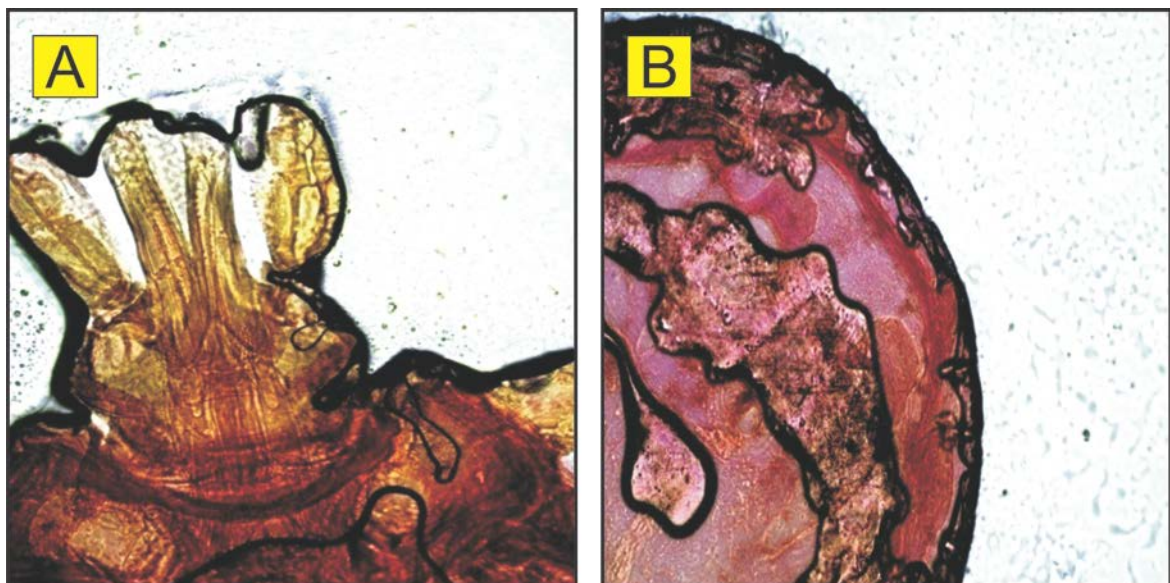


Plate 3.3 A-B: *Haemaphysalis bispinosa*, Anterior and Posterior region

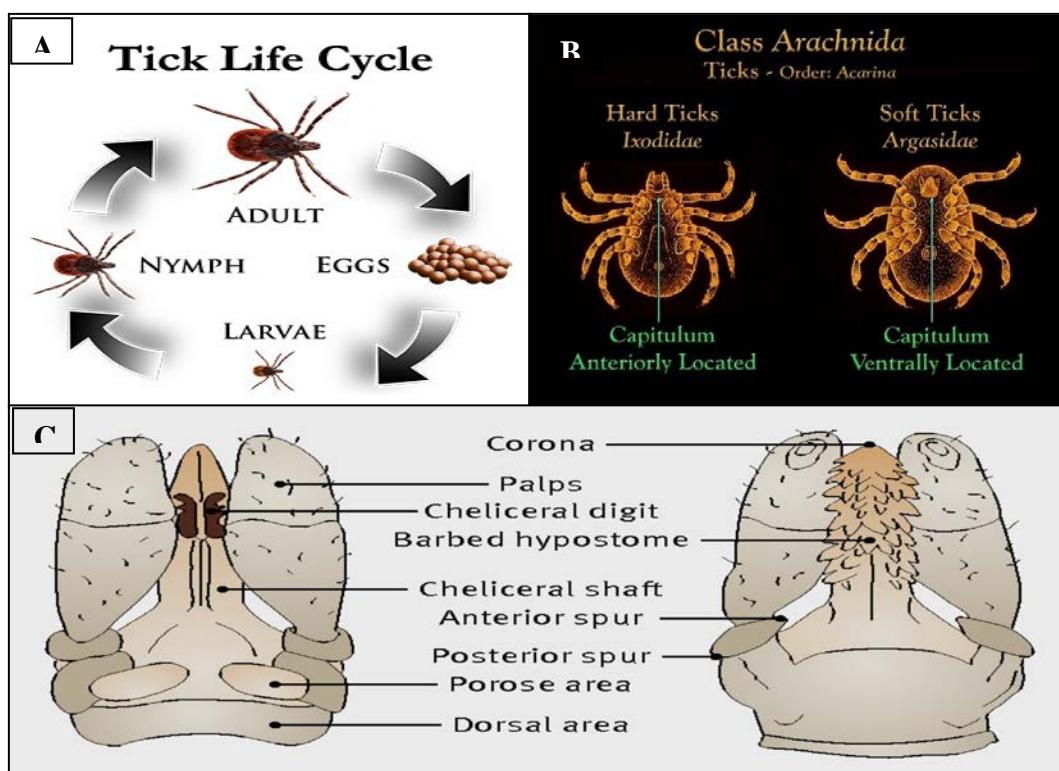


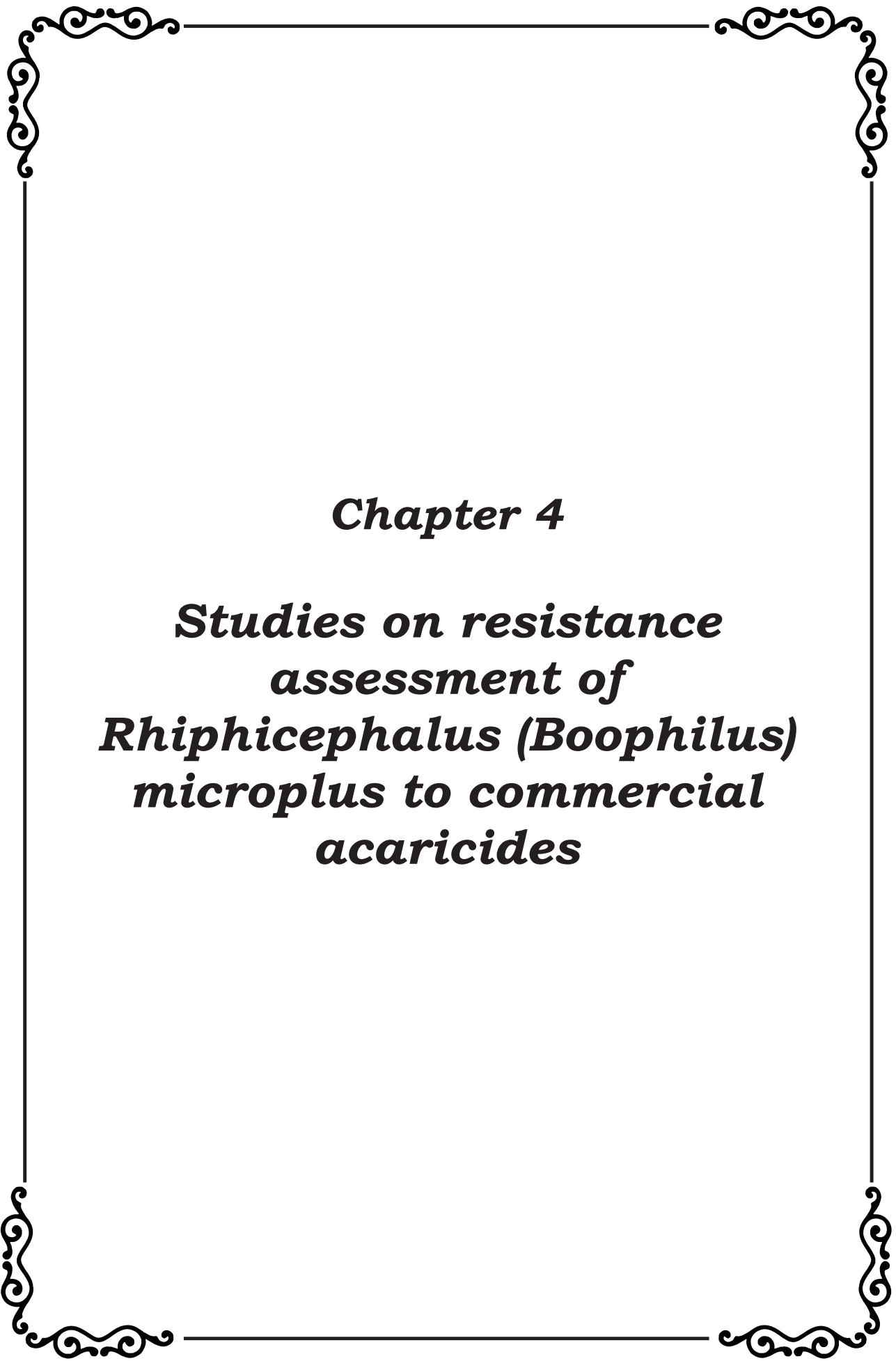
Plate 3.4 (A) Stage of Ticks (B) Hard and Soft Ticks (C) Mouth Parts of Ixodid Ticks

Source. (a) <http://www.northwestmvcd.org/Northwestmvcd/Ticks.html> (b) http://www.publicdomainfiles.com/show_file.php?id=13518466816048 (c)

<https://www.bmj.com/content/347/bmj.f7123>



Plate 3.5 Different animals (Cattle and Buffaloes) body parts affected with Ticks



Chapter 4

***Studies on resistance
assessment of
Rhiphicephalus (Boophilus)
microplus to commercial
acaricides***

CHAPTER - 4

Studies on resistance assessment of *Rhipicephalus (Boophilus) microplus* to commercial acaricides

Rhipicephalus (Boophilus) microplus is a worldwide tick species generally found in tropical and southern areas. It causes large scale economic losses in the livestock. It also leads to weight loss, blood loss, hair loss, irritation, reduced quality of hides, reduction in milk production, less meat production, dejection of immune functioning and transmission of pathogens (Alim *et al.*, 2012 and Valente *et al.*, 2014).

4.1 INTRODUCTION

Commercially available synthetic chemicals in India are – pyrethroids, cypermethrin and deltamethrin; These are predominating acaricides which are used for tick controlling system and regular use of these acaricides leads to increased resistance (Sharma *et al.*, 2012 and FAO, 2004).

Resistance has significantly limited the chemicals use that were earlier used and developed resistance progressively, for e.g. organophosphates, pyrethroids and carbamates, chlorinated hydrocarbons, arsenic etc. The fate of remaining acaricides is a matter of long concern and discussion as resistance is eventually affecting the use of acaricides (Nari and Hansen, 1999).

The development of resistance of *Rhipicephalus (Boophilus) microplus* to arsenic, was first reported in Australia in 1937 by Newton; The increasing tick resistance in animals against all the acaricides which are available in the stores, had discouraged the livestock holders. Many researchers have conducted studies on acaricides and problem of resistance due to them (Wharten and Roulston, 1970).

Some ethnoveterinary plants are also used for the removal of ticks from animals body. In recent research, Sharma *et al.*, 2012 and Kumar *et al.*, 2011 have reported resistance to diazinon (organophosphate compound) and synthetic pyrethroids,

deltamethrin, cypermethrin and the same were experimentally validated in field isolates of *Rhipicephalus (Boophilus) microplus*.

4.1.1 COMMON TERMS

A) Resistance

According to FAO (2004), “The ability of a parasite strain to survive or/ and to multiply despite the administration and exposure to drug given in recommended dose or above it” is generally considered as a failure of a drug to inhibit parasitism. In other words definition of resistance is a shift in the stimulus susceptibility to a drug (Corley *et al.*, 2013 and Sanguster, 2001).

a) *Acquired resistance*

Acquired resistance results from continuous heritable reduction in sensitivity to drug along with the time (Chapman 1997). There is a direct relationship between degree of resistance and concentration of the drug. Tick resistance increases by continuous high exposure of a drug.

b) *Cross- Resistance*

Cross resistance is the resistance to various acaricides with a similar mode of action. A vital pattern of cross resistance was seen between two organophosphates (diazinon and coumaphos) and one carbamate (carbaryl) acaricides in various strains of *Rhipicephalus (Boophilus) microplus* (Li *et al.*,2005).

c) *Multiple Resistance*

Multiple resistance means, in any tick species resistance is shown by more than one drug whether they have different mode of action. Foil *et al.*,2004 concluded in his research, a significant occurrence of multiple resistance in southern cattle ticks, different classes of acaricides including organophosphates, formamidines (amitraz) and chlorinated hydrocarbons (DDT).

This study was designed to generate data to know status of acaricides resistance in common animal tick species, *Rhipicephalus (Boophilus) microplus* collected from five zones of Uttar Pradesh, state of India.

4.2 MATERIALS AND METHODS

4.2.1 Study Area and Sample Collection

During the study period, a survey was conducted for collection of engorge female ticks (*Rhipicephalus (Boophilus) microplus*) in many rural, semi-urban and urban areas from sheds of dairy animals and farms, which were having cross bred cattle as well as buffaloes from different zones of Uttar Pradesh (table 3.1). In this state of India many small and large livestock holders generally use semi-intensive and intensive rearing system to rear the animals, farmers depends on agriculture and animal husbandry for their livelihood. During survey, a questionnaire was prepared to collect data on frequency, method and type which were used by the livestock owners and to know about potency of acaricides.

4.2.2 Collection of Engorged Female Ticks of *Rhipicephalus (Boophilus) microplus*

Collection of ticks was done through random sampling method in different zones of Uttar Pradesh state of India. Collected engorged female tick species *Rhipicephalus (Boophilus) microplus* was kept in separate vials, for exchange of moisture and air, mouth of vials was closed with muslin cloth and then brought to the Parasitology Laboratory, Department of Applied Animal Sciences, B.B.A. University, Lucknow. Vials were put in humidified dessicator with 10% KCL and dessicator was kept at $28^{\circ} \pm 1^{\circ}$ in incubator.

4.2.3 Maintenance of Life Stages of Ticks in Laboratory up to Larval Stages for Larval Packet Test (LPT)

4.2.3.1 Stages of larval formation

(a) **Egg Laying:** After 7-8 days, the ticks started laying eggs in many stocks up to 24-25 days.

(b) Egg Hatching: After egg laying, eggs, were hatched in to larvae in 6-7 days.

4.2.3.2 Acaricides used for the test

1. Cypermass (Cypermethrin E. C. 25%)
2. Butox (Deltamethrin E. C. 1.25%)

4.2.3.3 Preparation of Acaricides for Bioassay

For the experimental bioassay, i.e. Larval Packet Test and Adult Immersion Test, stock solutions of 10,000 ppm in distilled water was prepared with the Cypermass (Cypermethrin E.C. 25%) and Butox (Deltamethrin E. C. 1.25%). Different desired concentrations of the acaricides (25, 50, 75, 100 and 125 ppm) were prepared in distilled water from the stock solutions and tested against the tick species *Rhipicephalus (Boophilus) microplus*.(Anantachar *et al.*, 1992)

4.2.3.4 BIOASSAY

4.2.3.4.1 Larval Packet Test (LPT)

Larval packet test was conducted, as per the standard procedure designed by the Food and Agricultural Organization (FAO 2004). The test was used to analyse the potency of acaricides. Larval Packet Test was firstly reported by Stone and Hydock (1962). Castro-Janer *et al.* (2009) and Miller *et al.*(2001) also described this method. Packets were prepared using filter paper no. 1. Packets were impregnated in 2 ml of each concentration of chemicals and were dried. After drying the packets, 50 larvae were put in each packet. Five replicates of each concentration were prepared. Mouths of packets were sealed with the tape. Packets were kept in dessicator and the dessicator was kept in BOD incubator at $28^{\circ} \pm 1^{\circ}$ maintained with relative humidity of $85 \pm 5\%$. Mortality of larvae was recorded every day.

Mortality = Total no. of tick larvae in packet – Live tick larvae

(%) Percentage mortality was recorded using the given formula -

$$(\%) \text{ mortality} = \frac{\text{No. of dead larvae}}{\text{Total no. of larvae}} \times 100$$

4.2.3.4.1a DATA ANALYSIS

a) Probit Analysis (Calculation of LC 50 and LC 95)

For the evaluation of bioassay in living organism, probit analysis method was used; Determination of dose response data on tick larvae was analysed using two chemicals (cypermethrin and deltamethrin). Binary response (death and survival of the tick larvae) was calculated after treatment of cypermethrin and deltamethrin. Calculation of LC 50 and LC 95 values was determined by probit analysis method (Finney, 1962).

Among the groups, the differences in mean values of data was analysed by Student's t- test (Snedecor and Cochran, 1968).

b) REFERENCE FOR ACARICIDE SUSCEPTIBLE TICKS

Data of the reference for the present study was taken from the literature (Shyama *et al.*, 2013) and observation of resistance status in ticks was used as the standard.

4.3a RESULTS AND DISCUSSION

Present study was designed to determine any development of acaricide resistance in the tick *Rhipicephalus (Boophilus) microplus* and to calculate quantum of acaricidal resistance of any kind of changes in tick species. During survey period high tick infestation was recorded in rainy season. Results of survey study revealed high tick infestation in the livestock. Animal holders also reported that, less attention is paid to the problem of tick infestation in animals. In this study it also has been reported that in rural areas, farmers, and livestock holders generally use acaricides for tick removal from animal's body. Cypermethrin and deltamethrin were found to be commonly used acaricides by the farmers/ animal holders, which are easily available in the market.

Larval Packet Test was conducted to study the evaluation of anti-tick activity of the acaricides/ chemicals. LPT test was completed with in 5-6 weeks. Two commercial acaricides, Cypermass (cypermethrin E.C. 25%) and Butox (deltamethrin E.C. 1.25%) were used for the purpose. The stock solutions (10,000 ppm) of cypermethrin and deltamethrin in working concentrations were prepared with distilled water.

Table 4.1 Mean % mortality and LC50/ LC 95 value on exposure to cypermethrin and deltamethrin after 24 hr

Concentration	Mean % mortality on exposure to cypermethrin after 24 hr	Mean % mortality on exposure to deltamethrin after 24 hr
25ppm	47%	54%
50ppm	60%	63%
75ppm	69%	71%
100ppm	77%	81%
125ppm	84%	87%
LC 50	29.847ppm	25.106 ppm
LC95	422.458 ppm	328.031 ppm
P Value	P =.0010	P =.0013
	(≤ 0.05 Significant)	(≤ 0.05 Significant)

Larval Packet test was performed as per standards designed by Food and Agriculture Organization (2004). Tick mortality data was analysed through probit analysis (Finney, 1962). LC50 and LC95 values of cypermethrin and deltamethrin were calculated. The LC50 and LC95 values of cypermethrin for the ticks collected from different zones of Uttar Pradesh were 29.847ppm and 422.458 ppm, respectively and that of deltamethrin were 25.106 ppm and 328.031 ppm respectively (Table 4.1). Regression graph was also plotted for both the chemicals and regression equation and R² values were also obtained (Figure 4.1 & Figure 4.2).

In the Larval Packet Test 100 % tick mortality reference IVRI I line was observed. The results revealed that LC50 and LC95 values of cypermethrin were 242.2 ppm and 350.7 ppm, while in case of deltamethrin the LC50 and LC95 values were 11.8 ppm and

35.5 ppm, respectively (Shyama *et al.*, 2013). Result showed a large difference in the LC50 and LC95 values of chemicals. Present study also indicated development of resistance in the ticks collected from Uttar Pradesh region.

Similar findings were discussed in the study of Kaur, D. *et al.*, (2017), where a large difference in the development of acaricide resistance in the ticks was found. The LC50 and LC95 values with respect to deltamethrin were recorded to be 58.8 ppm and 234.42ppm, respectively and in case of cypermethrin they were found to be 165.95ppm and 7244.35 ppm, respectively.

Many other researchers also reported the status of acaricide resistance, (Sharma *et al.*, 2012 and Kumar *et al.*, 2011) and the literature cites a big difference in the LC50 values of these acaricides against reference lines of *Rhipicephalus (Boophilus) microplus*.

The study of Shyama *et al.*, (2013), showed that acaricidal resistance is comparatively higher in one host tick *viz.* *Rhipicephalus (Boophilus) microplus*, in comparison to other three host ticks, *Hyalomma anatolicum anatolicum*. They found higher fraction of presence in one host ticks, which has sustained the chemical challenges since that species had shorter life cycle and specificity of host in domestic animals.

Many variations in the LC50 and LC95 values are highlighted in the given literature, which clearly suggests that there is an urgent need for the generation of base line data of respective tick species of the country. The results of this study will prove to be useful in assessment of resistance status of acaricides in ticks.

The increased level of acaricide resistance in the tick species, found in overall prevalence is due to popular tick control methods used in organised and unorganised animal farms in the study area. Rearers generally used pyrethroids in cross breed animals. Approximately 85% cross breed cattles were treated with these chemicals or frequent use of these acaricides for tick control is very popular.

4.4a CONCLUSION

The aim of the present study is to determine the status of acaricide resistance of field isolates from the study area against two common acaricides, i.e cypermethrin and deltamethrin. Available literature cited the values of LC50 and LC95 is far less in case of susceptible tick line available than in the results observed from tick isolates from Uttar Pradesh state of India.

Goncalves *et al.*,(2007) and Stratton and Corke (1982) suggested that the continuous use of organic solvents increases adsorption of compounds over the surface area of target biological materials and also increase penetration of active ingredients of the acaricide across the exoskeleton. According to Sharma *et al.*, (2012), the effect of different solvents on the biology of reference IVRI-I line of *Rhipiciphalus (Boophilus) microplus* had been evaluated and the safety levels of acetone and methanol were observed up to the level of 50%.

The increment of immunity in domestic animals is also an important factor for tick control. Use of vaccines and ethno-veterinary plants in place of acaricides is another method to reduce the use of acaricides.

According to O' Kelly and Seifert (1969), nutritional aspect is an important factor in resistance management. Defective T-cells, important for immune response also gets affected by protein energy deficiency.

Continuous monitoring of animal ticks for the cause of resistance against various classes of chemical acaricides, has a great role in the management of resistance and making a correct choice of an effective drug. Use of synthetic and plant based acaricides, vaccines in combinations and also educating the farmers by providing appropriate knowledge about tick control practices for controlling animal ticks will be beneficial.

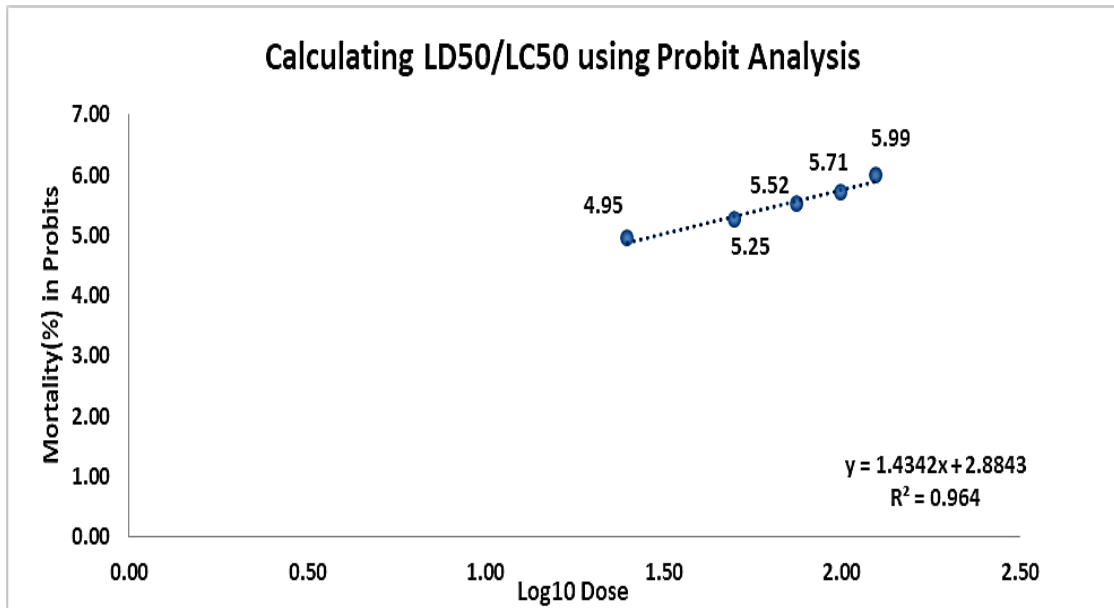


Figure 4.1 Probit mortality x log concentration plots from *Rhipicephalus (Boophilus) microplus* larvae submitted to Larval Packet test with cypermethrin

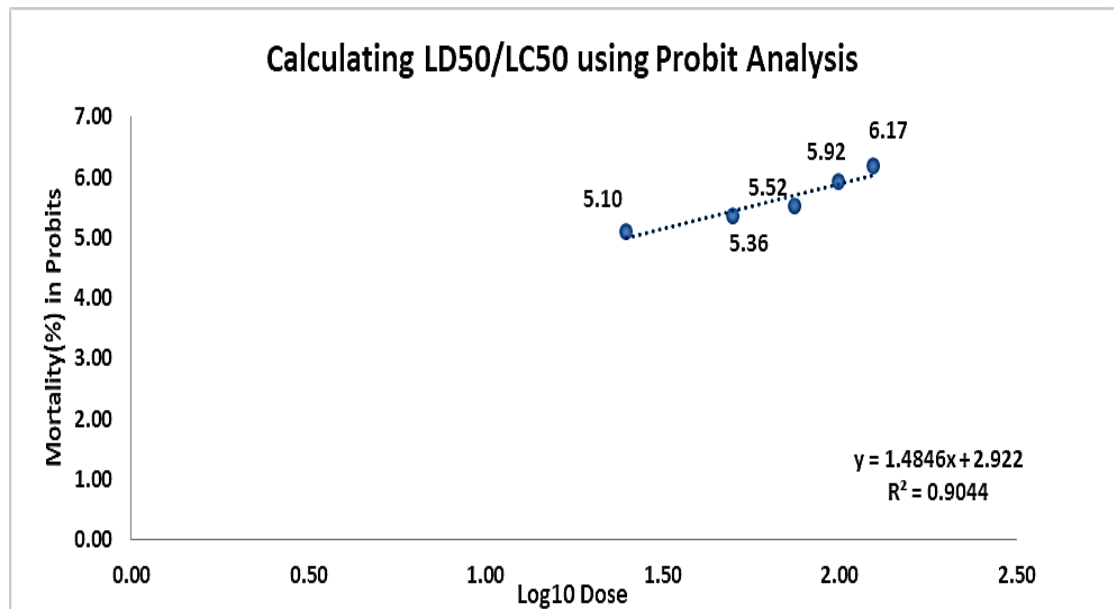


Figure 4.2 Probit mortality x log concentration plots from *Rhipicephalus (Boophilus) microplus* larvae submitted to Larval Packet test with deltamethrin

4.2.3.4.2 Adult Immersion Test

Detection of resistance levels against deltamethrin in *Rhipicephalus (Boophilus) microplus* collected from different regions of Uttar Pradesh was carried out using the adult immersion test. Cypermethrin compound was obtained from cypermass 25 EC commercial poisoning by using the procedure standardised by FAO 2004.

The regression graphs of probit mortality of ticks plotted against log value of concentration of chemicals were utilized for the assessment of slope of mortality and lethal concentration for 50% (LC50).

Resistance to deltamethrin was analysed on the basis of data, on mortality, reproductive index, egg mass weight and percentage inhibition of oviposition resistance.

Deltamethrin and cypermethrin resistance status were analysed to reveal the extent of resistance and further will help in formulating tick control strategy in the region

4.2.3.4.2a Collection and preparation of the sample for the test

Laboratory standardized adult immersion test was employed at different concentrations of cypermethrin and deltamethrin, following 10-minute protocol and 14 days as observation period. In addition, adult soft ticks were also used in adult immersion test with discriminating dose (AIT-DD) of cypermethrin and deltamethrin. The ticks of control group were dipped in distilled water. The ticks were adjudged dead on the basis of their immobility.

4.2.3.4.2b Acaricides

The commercially available preparations of deltamethrin (Butox 1.25% EC, Intervet) was used at the concentration of 30, 35, 70, 140 and 280 ppm in distilled water from stock solution. Other acaricides used was cypermethrin (cypermass 25 ec). The country specific discriminating dose in India for cypermethrin, amitraz and fipronil is 698.2 ppm, 975.4 ppm and 12.4 ppm, respectively.

4.2.3.4.2c Statistical analysis

Dose response data was analysed by probit method using Microsoft excel software. Line regression curve of adult mortality is plotted against various values of drug concentration to observe LC50 and LC95 values of cypermethrin and deltamethrin. R² value was also calculated.

4.3b RESULTS AND DISCUSSION

Prevalence of resistance in *Rhipicephalus (Boophilus) microplus* tick against cypermethrin and deltamethrin was studied. The ticks collected were identified as *Rhipicephalus (Boophilus) microplus* based on its morphological features such as presence of short palps, indistinct eyes, anals and accessory adanal plates with slender yellow slightly beaded legs.

4.3b1 Doses / concentration

Probit analysis of the data generated showed LC50 and LC95 values of deltamethrin to be 30, 35, 70, 140, 280 ppm, respectively. Tick mortality was found to increase with the increasing concentration of acaricides. Steep slopes of dose response curve and high value of goodness of fit (R²) indicated that field collected *Rhipicephalus (Boophilus) microplus* population is resistant to deltamethrin and cypermethrin (Godara, R., 2014 and Surbhi, S. *et al.*, 2018). The results are given in the table 4.2.

Table 4.2 Mean % mortality and LC50/ LC 95 value on exposure to cypermethrin and deltamethrin after 24 hr. for Adult Immersion Test

Concentration	Mean % mortality on exposure to cypermethrin after 24 hr	Mean % mortality on exposure to deltamethrin after 24 hr
30ppm	16%	24%
35ppm	28%	40%
70ppm	60%	64%
140ppm	72%	80%
280ppm	84%	88%
LC 50	73.242 ppm	54.553 ppm
LC95	522.221 ppm	436.056 ppm
P Value	P – 8.77E-07 (≤ 0.05 Significant)	P – 5.03E-06 (≤ 0.05 Significant)

Adult Immersion test was performed as per the procedure standardised by Food and Agriculture Organization (2004). Tick mortality data was analysed using probit analysis (Finney, 1962). LC50 and LC95 values of cypermethrin and deltamethrin were calculated. The LC50 and LC95 values of cypermethrin for the collected ticks were found to be 73.242 ppm and 522.221 ppm, respectively. On the other hand, the values of LC50 and LC95 values deltamethrin were 54.553 ppm and 436.056 ppm, respectively (Table 4.2). Regression graph was also plotted for both the chemicals and regression equation R^2 values were also obtained, (Figure 4.3 & Figure 4.4).

Adult immersion test (AIT) and larval packet test (LPT) were modified using susceptible tick line of *R.(B.) microplus* (IVRI-I) for a dissolve of 95% lethal concentration (LC95) of cypermethrin (350.7 ppm in LPT and 349.1 ppm in AIT) and deltamethrin (35.5 ppm in LPT and 29.6 ppm in AIT). The AIT with a discriminating dose (DD) ($2 \times LC95$) was used to perceive cypermethrin and deltamethrin resistance in the field isolates of *R.(B.) microplus*, (Sharma, A.K. *et al.*, 2012).

The Adult Immersion Test, is a standardized test to determine the level of resistance and resistant factors (RF) in the range of 1.2–95.7 and it is used in many researches of the total eighteen isolates, three were considered as susceptible (RF < 1.4), five isolates at level I (RF = 1.5–<5), eight at level II (RF = 5.1–<25), and one isolate each at level III (RF = 26–<40) and level IV (RF = >41). The esterase enzyme ratio and survival percent of tick isolates were determined significantly ($p < 0.001$) and is found related with correlation coefficient (r) in esterase activity. The correlation of observation (R^2) for esterase activity shows that 73.3% and 55.3% data points of field isolates were extremely near the correlation lines. The incidence of mutation in the field isolates having high resistance factor proposed that target site inattentiveness and improved esterase activity is the possible mechanism of resistance to deltamethrin in the field isolates of *R. (B.) microplus*, (Kumar Rinesh, *et al.*, 2012).

Many variations in the LC50 and LC95 values in given literature, suggests that there is an urgent need for the generation of base line data of respective tick species of the country. The results of this study will prove to be beneficial for the assessment of resistance status of acaricides in ticks.

The increased level of acaricide resistance in the tick species, found in overall prevalence is due to popular tick control method used in organised and unorganised animal farms in the study area. Rearers generally used pyrethroids in cross breed animals. Near about 85% cross breed cattles were treated with these chemicals or acaricides for tick control by many individuals.

4.4b CONCLUSION

The aim of the proposed study is to conclude the status of acaricide resistance of field isolates from the study region against two common acaricides, i.e. cypermethrin and deltamethrin. Available literature referred estimation of LC50 and LC95 is far less in case of susceptible tick line available than in the results observed from tick isolates from state of India i.e. Uttar Pradesh.

Stratton and Corke (1982) and Goncalves *et al.*, (2007) considered that the ordinary utilization of natural solvents builds adsorption of compound over the surface region of

target organic materials and furthermore increment entrance of dynamic elements of the acaricide over the exoskeleton. In accordance to the study conducted by Sharma *et al.*, (2012), the impact of different solvents on the biology of reference IVRI-I line of *Rhipicephalus (Boophilus) microplus* had been assessed and the safety levels of acetone and methanol was seen up to the level of 50%.

The addition of insusceptibility in livestock is additionally an essential factor for tick control. Use of ethno-veterinary and vaccines instead of acaricides is another strategy to reduce the use of commercial chemicals.

Continuous exposure of different drugs against tick infestation leads to many possibilities to identify various effective drug concentrations and duration of exposure. Further frequent monitoring of these drugs may give new insights to tick drug resistance mechanism and immunological alteration during the treatment of animals with various acaricides.

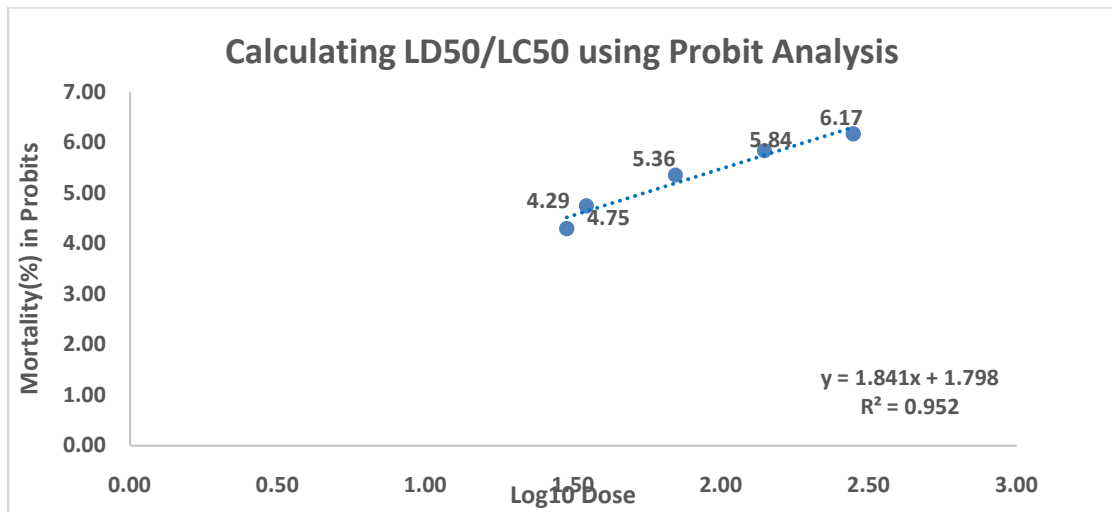


Figure 4.3 Probit mortality x log concentration plots from *Rhipicephalus (Boophilus) microplus* Larvae introduced to Adult Immersion Test with deltamethrin

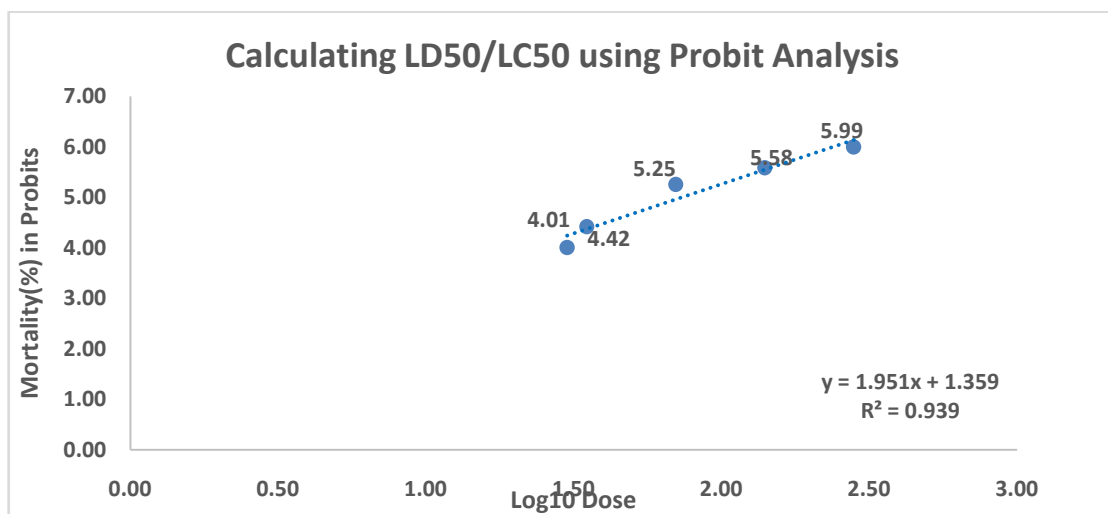
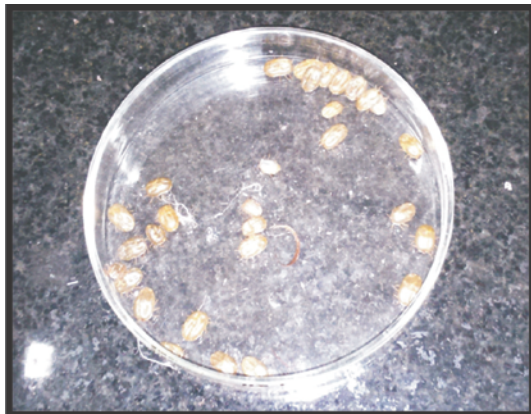


Figure 4.4 Probit mortality x log concentration plots from *Rhipicephalus (Boophilus) microplus* Larvae introduced to Adult Immersion Test with cypermethrin



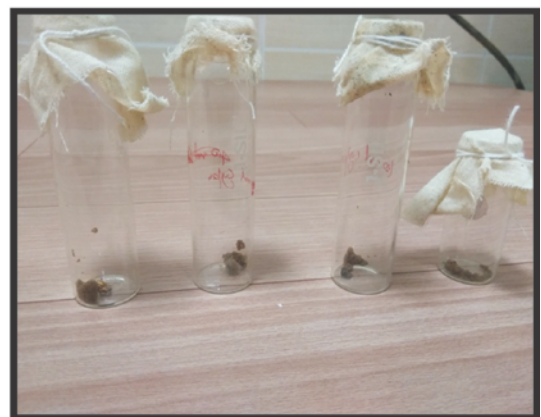
A



B



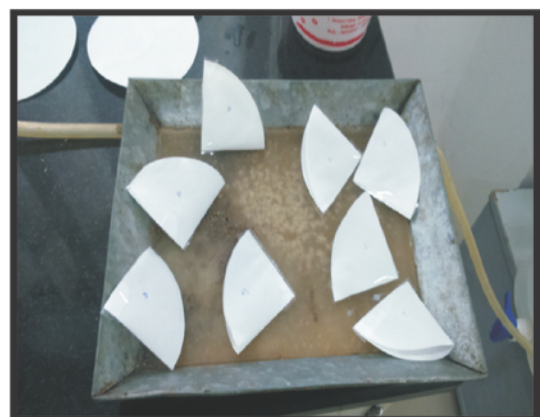
C



D



E



F

Plate.4.1A. Collected engorge ticks, B. Preparation for storing in desiccator,C. Ticks stored in desiccator in the lab conditions, D. Tick laid eggs, E. Preparation of different concentration of chemicals, F. Packets for LPT



References

References

- Abbas, R. Z., Colwell, D.D., Iqbal, Z. and Khan, A. 2014. Acaricidal drug resistance in poultry red mite (*Dermanyssus gallinae*) and approaches to its management. *Worlds Poult. Sci. J.*, 70: 113- 124.
- 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.
- Aktas, M., Dumanli, N. and Angin, M. 2004. Cattle infestation by *Hylomma* ticks and prevalence Specices in the east of Turkey. *Vet. Parasitol.*, 119: 1-8.
- Aktas, M., Altay, K., Ozubek, S., & Dumanli, N. 2012. A survey of ixodid ticks feeding on cattle and prevalence of tick-borne pathogens in the Black Sea region of Turkey. *Veterinary parasitology*, 187(3-4), 567-571.
- Aktas, A., & Dirikolu, M. H. 2003. The effect of stacking sequence of carbon epoxy composite laminates on pinned-joint strength. *Composite Structures*, 62(1), 107-111.
- Alexander, G.I., Reason G.K. and Clark, C.H. 1984. The development of the Australian Friesian Sahiwal: A Tick resistant dairy breed. *Wild. Anim. Review*. 51: 27-34.
- Ali, S. Z. and Singh, R. P. 2013. Observation on the Prevalance of Habitat of Full-Fed Ixodid Ticks in Azamgarh District Of Eastern Uttar Pradesh *Indian J. Sci. Res.* 4(2): 193-194.
- Alim M. A., Das, S., Roy, K., Masduzzaman, M., Sikder, S., Hassan, M.M., SSiddiki, A.Z. and Hossain, M.A. 2012. Prevalence of hemoprotozoan disease in cattle population of Chittagong division, Bangladesh. *Pak. Vet.J.*, 32: 221- 224.

- Anantachar M., Maurya, N.L., Guruswamy, T. 1992. Effect of Butox (Deltamethrin) Against *Hylomma anatolicum anatolicum*. Karnataka J. Agric. Sci., 5(1): 79- 80.
- Anderson, K., Ezenwa, V. O., & Jolles, A. E. 2013. Tick infestation patterns in free ranging African buffalo (*Syncercus caffer*): effects of host innate immunity and niche segregation among tick species. International Journal for Parasitology: Parasites and Wildlife, 2, 1-9.
- Antherden, L.M. 1967. Text book of Pharmaceutical Chemistry, 8th edition Oxford University Press, London, pp. 813- 814.
- Asmaa, N.M., El Bably, M.A. and Shokier, K.A. 2014. Studies on prevalence, risk indicators and control options for tick infestation in ruminants. J. Basic and Applied Sci., 3: 68-73.
- Atif, F.A., Khan, M.S., Iqbal, H.J., Ali, Z, Ullah, S. 2012. Prevalence of cattle tick infestation in three districts of the Punjab, Pakistan. Pak J Sci; 64:49.
- Bayu, K. 2005. Standard Veterinary Laboratory diagnostic manual. Vol. III. MOA Addis Abada.
- Castro-Janer, E., Rifran, L., Piaggio, J., Gil, A., Miller, R.J. and Schumaker, T.T.S. 2009. In vitro tests to establish LC50 and discriminations for fipronil against *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) and their characterization. Vet.Parasitol., 162:120-128.
- Chandler, C. and Read, P. 1994. Introduction to parasitology, John Weakly and Sons Inc. UK., 10:882.
- Chapman H.D. 1997. Biochemical genetic and applied aspects of drug resistance in *Eimeria* parasites of the fowl. Avian.Patho., 26: 221- 244.

- Chhillar, S., Singh, J.C. and Kaur, H. 2014. Investigations on some hard ticks (Acari: Ixodidae) infesting domestic buffalo and cattle from Haryana. India. J. Entomology and Zoological studies. 2(4):99-104.
- Chisu, V., Foxi, C., & Masala, G. 2018. First molecular detection of the human pathogen *Rickettsia raoultii* and other spotted fever group rickettsiae in Ixodid ticks from wild and domestic mammals. Parasitology research, 117(11), 3421-3429.
- Corley, S.W., Jonsson, N.N., Piper, E.K., Cutullé, C., Stear, M.J., Seddon, J.M. 2013. Mutation in the RmAOR gene is associated with amitraz resistance in the cattle tick *Rhipicephalus microplus*. Proc. Natl. Acad. Sci. USA.
- Das, K. and Srivastava, S.K. 2002. Bovine tick, *Boophilus microplus* canestrini infestation in Rural Chhattisgarh, J. Appl. Zoo. Res., 13 (2&3): 247-248.
- Das S.S. 1994. Prevalence of ixodid tick infestation on farm animals in Pantnagar, tarai of Uttar Pradesh. J Parasitol Appl Anim Biol 3: 71-73.
- Debbarma, A., Pandit, S., Jas, R., Baidya, S., Mandal, S. C., & Jana, P. S. 2018. Prevalence of hard tick infestations in cattle of West Bengal, India. Biological Rhythm Research, 49(5), 655-662.
- De Waal, D. T. 2000. Anaplasmosis control and diagnosis in South Africa. Annals of the New York Academy of Sciences, 916(1), 474-483.
- Drerup- Ekker, K. 1980. Investigations in ticks infestation of farm animals in Egypt. Inaug. Dissert. 1-90.
- Drummond, R.O. 2007 Tick born livestock diseases and their vector. *Word Animal Review*; htm Ethiopia, FAO Consultancy report 9 June- 24 July 1979 FAO, Rome (Italy).

- Drummond, R.C. 1983. Tick borne livestock diseases and their vectors Wild animal review (FAO), 36:26-33.
- Elango, G. and Rahuman, A.A. 2010. Evaluation of medicinal plants against ticks and fluke, Parasitol. Res., 108(3): 513- 519.
- Eyo, J. E., Ekeh, F. N., Ivoke, N., Atama, C. I., Onah, I. E., Ezenwaji, N. E., & Ikele, C. B. 2014. Survey of tick infestation of cattle at four selected grazing sites in the tropics. Global Veterinaria, 12(4), 479-486.
- Eshetu, E., Dinede, G., Lakew, M., & Tolosa, T. 2013. In-vitro efficacy evaluation of amitraz 0.025% and diazinon 0.06% against *Rhipicephalus pulchellus* and *Amblyomma gemma* in Borena pastoral area, Southern rangeland of Ethiopia. Journal of Parasitology and Vector Biology, 5(6), 72-76.
- FAO. 2004. Resistance management and integrated parasite control in ruminants. Guidelines. Animal Production and Health Division, pp 25–77.
- FAO and IDF. 2011. Guide to good dairy farming practice. Animal Production and Health Guidelines.No. 8.Rome pp. 1- 34.
- Feseha, B. 1997. Species composition and distribution of Ixodid ticks in Eastern Harerghiea, Ethiopia.Agr. Sci. 16:37-51.
- Finney, D.J. 1962. Probit Analysis- A statistical treatment of the response curve. Cambridge University Press, Cambridge, pp. 1- 318.
- Foil, L.D. *et al.* 2004. Factors that influence the prevalence of acaricide resistance and tick- born diseases.Vet. Parasitol.,125: 163- 181.

- Garcia, M., Hunka W and Chauvez, A. 1989. Body weight, tick burden (*Boophilus microplus*), physiological parameters and reproductive efficiency of crossbred Zebu cattle. *Acta. Vet. Scand.*, 30: 347- 353.
- Garcia-Manero, G. 2012. Myelodysplastic syndromes: 2012 update on diagnosis, risk-stratification, and management. *American journal of hematology*, 87(7), 692-701.
- George, J.E., Pound, J.M. and Davey, R.B. 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. *Parasitology*, 129: S353-S366.
- Ghosh, S., Azhahianambi, P. 2007. Laboratory rearing of *Theileria annulata*-free *Hyalomma anatolicum anatolicum* ticks. *Exp. Appl. Acarol.*, 43:137–146.
- Ghosh, S., Bansal, G.C., Gupta, S.C., Ray, D., Khan, M.Q. 2007. Irshad, H., Shahiduzzaman, M., Seitzer, U., Ahmed, J.S., Status of tick distribution in Bangladesh, India and Pakistan. *Parasitol. Res.*, 101 (Suppl 2):207–216.
- Ghosh S. Azhahianambi P, Fue nte de la. 2006. Control of ticks of ruminants, with special emphasis on livestock farming system in India: Present and future possibilities for integrated control- a review. *Exp Appl Acorl* 40:49-66.
- Ghosh Chaudhuri, R., & Paria, S. 2011. Core/shell nanoparticles: classes, properties, synthesis mechanisms, characterization, and applications. *Chemical reviews*, 112(4), 2373-2433.
- Ghose, S. and Nagar, G. 2014. Problem of ticks and tick- borne diseases in India with special emphasis on progress in tick control research: A Review. *J. Vector Born Dis.*, 51: 259- 270.
- Gill, H. S., & Gill, B. S. 1977. Qualitative district-wise distribution of adult ixodid ticks in the Punjab state. *Ixodid Ticks of Domestic Animals in the Punjab State*, 2-14.

- Gitau, G. K., Perry, B. D., Katende, J. M., McDermott, J. J., Morzaria, S. P., & Young, A. S. 1997. The prevalence of serum antibodies to tick-borne infections in cattle in smallholder dairy farms in Murang'a District, Kenya; a cross-sectional study. *Preventive Veterinary Medicine*, 30(2), 95-107.
- Goncalves, K. Toigo, E., *et al.* 2007. Effects of solvents and surfactant agents on the female and larvae of cattle tick *boophilus microplus*. *Vet.Parasitol.*,100 (6): 1267-70.
- Hadis, M., Lulu M., Mekonnen, y. and Asfaw, T. 2003. Field trials on the repellent activity of four plant products against mainly *Manson ia* population in Western Ethiopia. *Phytother. Res.*, 17:202-205.
- Harris, A. S., Croall, D. E., & Morrow, J. S. 1988. The calmodulin-binding site in alpha-fodrin is near the calcium-dependent protease-I cleavage site. *Journal of Biological Chemistry*, 263(30), 15754-15761.
- Haseeb, A.K. 2001. Studies on *Babesia canis* and ticks of dogs. M.Sc (Hons.) Thesis, Univ. of Agric (CVS), Faisalabad.
- Hernandez, R., Guerrero, F.D. George, J.E. and Wagner, G.G. 2002. Allele frequency and gene expression of a putative cardoxylesterase encoding gene in a kpyrethroid resistant strain of the tick, *Boophilus microplus microplus*, *Insect Biochem, Mol, Biol.*, 32: 1009-1016.
- Inokuma, H., Kerlin, R.L., Kemp, D.H. and Willadsen. P. 1993. Effects of cattle tick (*Boophilus microplus*) infection on the bovine immune system. *Vet. Parasitol.*, 47:107-118.
- Islam, M.K., Alim, M.A., Tsuji, N., Mondal, M.M. 2006. An investigation into the distribution, host preference and population density of ixodid ticks affecting domestic animals in Bangladesh. *Trop. Anim. Health Prod.*, 38(6): 485-490.

- Islam, F.M.S. 1989. Incidence of parasites of buffaloes in some areas of Bangladesh. MS thesis, Submitted to the Department of Parasitology, Bangladesh Agricultural University, Mymensingh.
- Jabbar, A., Abbas, T., Sandhu, Z., Saddiqi, H. A. Qamar.M.F. and Gasser, R.B. 2015. Tick-borne diseases of bovines in Pakistan: Major scope for future research and improved control. *Parasite. Vectors*, 8:283.
- Jagganath, M.S. and Lokesh, V.V. 1988. Incidence of ixodid ticks of sheep and goat in Kolar district. *Indian J.Of Animal Science*, 58: 72-76.
- Jagganath, M.S., Alwar, V.S. and Lalitha, C.M. 1973. Ixodid ticks of domestic stock in Tamil Nadu. *Indian Journal Ani. Sci.*, 43: 119- 124.
- Jagannath M.S., Muraleedharan, K., Hiregoudar, L.S. 1979. On the prevalence of ixodid ticks of cattle in Bangalore, *Indian Journal Ani.Sci.*, 890- 894.
- Jawale C.S. *et al.* 2012. Prevalence of ixodid ticks in post acaricide treated cattle and buffaloes at Sinner district Nashik (M.S) India. *Trends Parasitol. Res.*, 1(1): 2319-3158.
- Johnston, L.A.Y. and Hydock, K.P. 1969. The effect of cattle tick (*Boophilus microplus*) on production of Brahman- cross and British- breed cattle in Northern Australia. *Aust. Vet. J.*, 45: 175.
- Jonsson, N.N. and Matschoss, A.L. 1998. Attitudes and practices of Queensland dairy farmers to the control of the cattle tick, *Boophilus micropus*, *Aust. Vet. J.*,76: 746-751.
- Jonsson, N. N., Miller, R. J., & Robertson, J. L. 2007. Critical evaluation of the modified-adult immersion test with discriminating dose bioassay for *Boophilus microplus* using American and Australian isolates. *Veterinary Parasitology*, 146(3-4), 307-315.

- Jongejan F. and Uilenberg G. 2004. The global importance of ticks. *Parasitology* 129, S3-S14.
- Kabir, MHB., Mondal, MMH., Eliyas, M., Hashem, MA., Manan, MA., Debnath, NC, Miazhi, OF., Mohiuddin, C., Kashem, MA., Islam, MR., Elah, MF. 2011. An epidemiological survey on investigation of tick infestation in cattle at Chittagong district, Bangladesh. *Afr. J. Microbiol. Res.* 5(4):346-352.
- Kamble C.K.; Hiregoudar L.S. 1988. Prevalence of different species of ticks on domestic animals in dharwad area of karnataka india. *Mysore Journal of Agricultural Sciences* 22(3): 331-333.
- Kaur, D., Jaiswal, K., & Mishra, S. 2017. Studies on evaluation of resistance in common ixodid tick: A preliminary study. *Int. J. Fauna Biolog. Studies*, 4(3), 112-115.
- Kaur, D., Jaiswal, K. and Mishra, S. 2015. Studies on Prevalence of Ixodid Ticks Infesting Cattle and Their control by Plant Extracts. *IOSR Journal of Pharmacy and Biological*, 10 (6): 01-11.
- Kaushal J, K., Balakrishanan, N., Rakesh, K. and Gill, K.S. (2002). Prevalence of Ixodid ticks in Nilgiri district of Tamil Nadu State (India). National Institute of Communicable Diseases. 22- Sham Nath Marg, Delhi, India. *J. Commu. Dis.* 34(2): 124-127.
- Khan, A., Mushtaq, M. H., Ahmad, M., & Tipu, Y. 2013. Tick infestation rate in cattle and buffalo in different areas of Khyber Pakhtunkhwa, Pakistan. *Journal of Veterinary & Animal Sciences (Lahore)*, 3(1/2), 31-35.
- Keiser, M.N. 1987. Ethiopia, report on tick taxonomy and biology. AG: DP/ETH/83/23 Consultant Report, FAO of the United Nations, pp: 92.

- Kettle, D.S. 1995. Medical and Veterinary Entomology, 2nd edition. UK: CAB International, pp. 420-460.
- Kim C.M., Kim M.S., Park M.S., Park J.H., Chae J.S. 2003. Identification of Ehrlichia chaffeensis, Anaplasma phagocytophilum, and A. bovis in Haemaphysalis longicornis and Ixodes persulcatus ticks from Korea. Vector- Borne Zoon. Dis. 3(1), 17-26.
- Kumar, A., Singh, S., Mahour, K., Vihan, V.S. 2010. Status of synthetic drugs against ticks in organized and farmer goat flock. J. Adv. Lab. Res. Biol. 1 (1), 68–71.
- Kumar, R. 1996. Studies on tick infestations in cattle and buffaloes. M.V.Sc. Thesis submitted to C.S.A., University of Agriculture and Technology, Kanpur, pp 1–178.
- Kumar, K., and Balakrishnan, N. 2002. Prevalence of ixodid ticks in nilgiri district of Tamil nadu state (India). J. Commun. Dis., 34: 124-127.
- Kumar, K., Balakrishnan, N., & Sharma, A. K. 2014. Studies on the vertical distribution of ticks of domestic animals and their public health importance in Nilgiri Hills and adjoining areas of Tamil Nadu State (India). International Journal of Zoology, 2014.
- Kumar, S., Paul, S., Sharma, A.K., Kumar, R., Tewari, S.S., Chaudhuri, P., Ray, D.D., Rawat, A.K.S., Ghosh, S. 2011b. Diazinon resistant status in *Rhipicephalus (Boophilus) microplus* collected from different agro climatic zones of India. Vet. Parasitol. 181, 274–281.
- Kumar, R., Nagar, G., Sharma, A. K., Kumar, S., Ray, D. D., Chaudhuri, P., & Ghosh, S. 2013. Survey of pyrethroids resistance in Indian isolates of *Rhipicephalus (Boophilus) microplus*: identification of C190A mutation in the domain II of the para-sodium channel gene. Acta tropica, 125(2), 237-245.

- Koney , E.B.M., Walker, A. R., *et al.* 1994. Seasonal prevalence of ticks and their association with dermatophilosis in cattle on the Accra plains of Ghana, *Rev. Elev. Med. Vet. Pays. Trop.*, 47: 163-167.
- Korenberg, E. I., *et al.* 2001. The prevalence of *Borrelia burgdorferi* in *Ixodes persulcatus* and *I. ricinus* ticks in the zone of their sympatry. *Folia Parasitologica*. 48: 63- 68.
- Latha, B.R., Aiyasami, S.S., Pattabiraman, G., Sivaraman, T. and Rajavelu, G. 2004. Seasonal activity of ticks on small ruminants in Tamil Nadu State, India. *Trop. Anim. Health Prod.*, 36: 121-133.
- Loomis, E.C. 1986. Ectoparasites of cattle. *Vet. Clin. North America*, 2: 299–321.
- Lwande, W., dakala, A.J.N., Hassanali, A., Moreka, L., Nyandat, E., Ndungu, M., Amiani, H., Gitu, P.M., Malonza, M.M., and Punyua, D.K. 1999. Gynandropsis gynandra essential oil and its constituents as ticks (*Rhipicephalus appendiculatus*) repellents . *Phytochemistry*, 50: 401-405.
- Latif, A.A and Pegram, R.G. 1992. Naturally acquired host-resistance in tick control in Africa. *Insect Sci. & Appl.*, 13: 505-513.
- Li, A.Y., Davey, R.B. and George, J.E. 2005. Carbaryl resistance in Mexican strains of the southern cattle tick (Acari: Ixodidae). *J. Econ. Entomol.*, 98: 552-556.
- Lovis, L., Reggi, J., Berggoetz, M., Betschart, B. and Sager, H. 2013. Determination of acaricide resistance in *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) field populations of Argentina, South Africa, and Australia with the larval tarsal test. *J. Med. Entomol.*, 50: 326-335.
- Lovis, L., Perret, J. L., Bouvier, J., Fellay, J. M., Kaminsky, R., Betschart, B., & Sager, H. 2011. A new in vitro test to evaluate the resistance level against acaricides of the cattle tick, *Rhipicephalus (Boophilus) microplus*. *Veterinary parasitology*, 182(2-4), 269-280.

- Madder, M., Thys, E., Achi, L., Toure. A. and De Deken, R. 2011. *Rhipicephalus (Boophilus) microplus*: A most successful invasive tick species in West-Africa. *Exp. Appl. Acarol.*, 53: 139-145.
- Magano, S. R., Thembo, K.M., Ndlovu, S. M. and Makhubela N. F. H. 2007. The antitick properties of the root extracts of *Senna italica* subsp. *Arachoides*. *African J. Biotech.*, 7(4) 476-481.
- Mahmood, A. 1994. Effect of theleriosis on various blood parameters and total serum protein in exotic and cross-bred cattle in and around Lahore M.Sc (Hons.)Thesis, Univ. of Agric. (CVS), Faisalabad.
- Maske, *et al.* 2012. Prevalence of *Schistosoma nasale* infection in bovines from eastern Vidharbha (Maharashtra) vis-à-vis infection in *Indoplanorbis exustus*. *Article 12 (26)*: 140-143.
- Mashebe, P., Lyaku, J. R., & Mause, F. 2014. Occurrence of ticks and tick-borne diseases of livestock in Zambezi region: a review. *Journal of Agricultural Science*, 6(2), 142.
- Mamun M. A., A, Begum, N., Shahadat, H.M. and Mondal, M. H. 2010. Ectoparasites of buffaloes (*Balbulus balbulis*) in Kurigram district of Bangladesh. *J. Bangladesh Agril. Univ.* 8(1): 61-66.
- Manan, A., Khan, Z., Ahmad, B., Abdullah. 2007. Prevalence and identification of ixodid tick genera in frontier region Peshawar. *J Agric Biol Sci* 2(1):21–25.
- McLeod R, Kristjanson P. 1999. Tick cost: economic impact of ticks and TBD to livestock in Africa, Asia and Australia. International Livestock Research Institute (ILRI), Nairobi, Kenya.[http:// www.esys.com.au](http://www.esys.com.au) and <http://www.cgiar.org/ilri>.
- Miller, R.J., George, J.E., Guerrero, F., Carpenter, L., Welch, J.B. 2001. Characterization of acaricide resistance in *Rhipicephalus san-guineus* (Latreille) (Acari: Ixodidae) collected from the Corozal Army Veterinary Quarantine Center, Panama. *J. Med. Entomol.* 38,298–302.

- Minjauw, B., McLeod, A. 2013. Tick-borne diseases and poverty: The impact of ticks and tick-borne diseases on the livelihood of small scale and marginal livestock owners in India and eastern and southern Africa. Research Report, DFID-AHP. UK: Centre for Tropical Veterinary Medicine, University of Edinburg ; p. 116.
- Miranpuri, G.S. and Naithani, R.C. 1978. A Checklist of Indian ticks(Ixodoidea: Acarina) Indian Veterinary Research Institute, Izatnagar, Bareilly.
- Misra, S.C. 1984. A note on the incidence and control of ixodid ticks at Bhubaneswar. Cheiron 13(1):5-8.
- Moghaddam, A. G., Seyed, M. R., Rasouli, M., Hosseinzade, S., Darvishi, M. M., Rakhshanpour, A., & Rahimi, M. T. 2014. Survey on cattle ticks in Nur, north of Iran. Asian Pacific journal of tropical biomedicine, 4(3), 209-212.
- Mooring, M.S., *et al.* 1996. Grooming in imphala: Role of oral grooming in removal of ticks and effects of ticks in increasing grooming rate. Pysiol. And Behav., 59: 965-971.
- Munderloh, U. G. 2011. Emerging and reemerging tickborne infections: genetic manipulation of intracellular tick-borne pathogens. Critical needs and gaps in understanding Prevention, amelioration, and Resolution of lyme and Other tick-borne.
- Musa, H. I., Jajere, S. M., Adamu, N. B., Atsanda, N. N., Lawal, J. R., Adamu, S. G., & Lawal, E. K. 2014. Prevalence of tick infestation in different breeds of cattle in Maiduguri, northeastern Nigeria. Bangladesh Journal of Veterinary Medicine, 12(2), 161-166.
- Narahashi, T. 1971. Mode of action of pyrethroids. Bulletin of the World Health Organization, 44(1-2-3), p.337.

- Nari, A., & Hansen, J. W. 1999. Resistance of ecto-and endoparasites: current and future solutions. 67th General Session of the International Committee. Paris, France, May, 17, 509-529.
- Nava, S., Mangold, A. J., Canevari, J. T., & Guglielmone, A. A. 2015. Strategic applications of long-acting acaricides against *Rhipicephalus (Boophilus) microplus* in northwestern Argentina, with an analysis of tick distribution among cattle. *Veterinary parasitology*, 208(3-4), 225-230.
- Neto, L. R. P., Jonsson, N. N., Michael, J. D., & Barendse, W. 2011. Molecular genetic approaches for identifying the basis of variation in resistance to tick infestation in cattle. *Veterinary parasitology*, 180(3-4), 165-172.
- Newton, L.G. 1967. Acaricide resistance and cattle tick control. *Australian Vet. J.*, 42: 389-394.
- Norval, R. A. I., Fivaz, B. H., Lawrence, J. A., & Brown, A. F. 1984. Epidemiology of tick-borne diseases of cattle in Zimbabwe. II. Anaplasmosis. *Tropical Animal Health and Production*, 16(2), 63-70.
- Obadiah, H. I., & Shekaro, A. 2012. Survey of tick infestation in cattle in Zaria abattoir, Nigeria. *Journal of Veterinary Advances*, 2(2), 81-87.
- Okello-Onen, J., Hassan, S. M., & Suliman, E. 1999. Taxonomy of African ticks: an identification manual. International Centre of Insect Physiology and Ecology (ICIPE).
- O'Kelly, J.C., Seifert, G.W. 1969. Relationships between resistance to *Boophilus microplus*, nutritional status and blood composition in Shorthorn X Hereford cattle. *Aust. J. Biol. Sci.* 22, 1497-1506.
- Parker, T.Z. and Hawell, W. A. 1962. Text Book of Zoology. Vol 2 Vertebrates, 7th Edition. Revised by Marshall, A.J. Published London.

- Parola, P. and Raoult, D. 2001. Ticks and tick borne bacterial diseases in humans: An emerging infectious threat .Clin. Infect. Dis., 32:897-928.
- Patel, G., Shanker, D., Jaiswal, A. K., Sudan, V., & Verma, S. K. 2015. Prevalence and seasonal variation in ixodid ticks on buffaloes of Mathura District, Uttar Pradesh, India. International Buffalo Information Center (IBIC) BUFFALO BULLETIN, 34(1), 21.
- Patel, G., Shanker, D., Jaiswal, A.K., Sudan, V. and Verma, S.K. 2013. Prevalence and seasonal variation in ixodid ticks on cattle of Mathura district, Uttar Pradesh. J. Parasit. Dis., Oct; 37(2): 173–176.
- Papadopoulos, B., Morel, P.C., *et al.* 1996. Ticks of domestic animals in the Macedonis region of Greece. Vet. Parasitol., 63: 25-40.
- Perez-Cogollo, L. C., Rodriguez-Vivas, R. I., Ramirez-Cruz, G. T., & Miller, R. J. 2010. First report of the cattle tick *Rhipicephalus microplus* resistant to ivermectin in Mexico. Veterinary parasitology, 168(1-2), 165-169.
- Peter, R.J., Van den, Bossche, P., Penzhorn, B.L. and Sharp, B. 2005. Tick, fly and mosquito control-lessons from the past, solutions for the future. Vet Parasitol132 (3): 205-15.
- Pegram, R., Hogstral, H. and Wassef, H. 1981. Distribution, ecology and host relationship of species infesting livestock. Bull. Entomol. Res., 71: 339-359.
- Pengram, R.G. and Oosterwijk, G.P.M. 1990. The effect of *Amblyomma variegatum* on live weight gain of cattle in Zambia. Med. Vet. Entomol., 4: 327-330.
- Petermann, J., Cauquil, L., Hurlin, J. C., Gaia, H., & Hüe, T. 2016. Survey of cattle tick, *Rhipicephalus (Boophilus) microplus*, resistance to amitraz and deltamethrin in New Caledonia. Veterinary parasitology, 217, 64-70.

- Poonam, S., & Suman, M. 2017. The prevalence of ixodid ticks on buffaloes from eastern region of Lucknow, Uttar Pradesh. *Asian Journal of Animal Science*, 12(1), 38-42.
- Prakasan, K. and Ramani, N. 2007. Tick parasites of domestic animals of Kerela, South Asia. *Asian J.Of Ani. Vet. Adv.* 2(2): 74-80.
- Pratap, G., Misra, S. C., & Panda, M. R. 1991. A note on the incidence of ectoparasites on black Bengal goats at Bhubaneswar. *Indian Veterinary Journal*, 68(1), 92-94.
- Raizada, R. N., & Nagar, S. K. 1979. Observations on the environmental stress on distribution and abundance of *Boophilus microplus* (Acarina: Ixodidae) and its preference for host and feeding sites in Uttar Pradesh [India]. *Indian Journal of Animal Sciences*.
- Ramaprabha, M. and Vasantha, K. 2012. Phytochemical and antibacterial activity of *Calotropis* (Ait.)R.Br. flowers. *Int. J. Pharma & Biosciences*, 3(1): 1-6.
- Ramírez, C., Ibarra, F., Pérez, H. I., Manjarrez, N., Salgado, H. J., & Ortega, L. 2016. Assessment and determination of LC50 of carvacrol and salicylic acid analogues with acaricide activity in larvae and adult ticks of *Rhipicephalus* (*Boophilus*) *microplus*. *Parasite epidemiology and control*, 1(2), 72-77.
- Rajput, Z.I., Hu, S., Chen, W. *et al.* 2006. Importance of ticks and their chemical and immunological control in livestock. *J. Zhejiang Univ. Sci. B.*, 7(11): 912-921.
- 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 the semi-arid and arid agro-ecological zones of Pakistan. *Parasites & vectors*, 10(1), 190.

- Riek, R.F. 1962. Studies on the reactions of animals to infestation with ticks. VI Resistance of cattle to infestation with the tick *Boophilus microplus*. Aust. J. Agr. Res., 13: 532-551.
- Roberts, J.A. 1968b. Resistance of cattle to the tick *Boophilus microplus* (Canestrini). J. Parasite against which resistance is manifest. J. Parasitol., 54: 667-673.
- Robson, J., Robb, J.M. and Al- Wahayyib, T. 1968. Ticks (Ixodoidea) of domestic animals in Iraq. J. Med. Entomol., 5: 27-31.
- Rodriguez-Vivas, R. I., Rodriguez-Arevalo, F., Alonso-Díaz, M. A., Fragoso-Sanchez, H., Santamaria, V. M., & Rosario-Cruz, R. 2006. Prevalence and potential risk factors for amitraz resistance in *Boophilus microplus* ticks in cattle farms in the State of Yucatan, Mexico. Preventive veterinary medicine, 75(3-4), 280-286.
- Rony, S.A., Mondal, M. M. H., Begum, N., Islam, M. A. and Affroze, S. 2010. Epidemiology of Ectoparasitic Infestations In Cattle at Bhawal Forest Area, Gazipur. Bangl. J. Vet. Med., 8(1): 27 – 33.
- Sabatini, G. A., Kemp, D. H., Hughes, S., Nari, A. and Hansen, J. 2001 .Tests to determine LC50 and discriminating doses for macrocyclic lactones against the cattle tick, *Boophilus microplus*. Vet. Parasitol., 95(1): 53-62.
- Sajid, M. S., Iqbal, Z., Khan, M. N., Muhammad, G., & Khan, M. K. 2009. Prevalence and associated risk factors for bovine tick infestation in two districts of lower Punjab, Pakistan. Preventive veterinary medicine, 92(4), 386-391.
- Salih, D. A. Julia, H., Hassan. S. M., El-Hussain, A.M. and Jongejan, F. 2008. Preliminary survey of ticks (Acari: Ixodidae) on cattle in Central Equatorial state. Southern Sudan. Onderstepoort J. Vet. Res., 75(1): 47-53.
- Sangster, N.C. 2001. Managing parasiticide resistance. Vet. Parasitol., 98: 89-109.

- Sanjay, K., Prasad, K.D. and Deb, A.R. 2007. Seasonal Prevalence of different ectoparasites infecting cattle and buffaloes. *J. Res.*, 16(1): 159-163.
- Saxena, V.K. 1997. Ixodid ticks infesting rodents and sheep in diverse biotopes of South India. *J. Parasitol.*, 83: 766-767
- Seifert, G.W. 1971. Variations between and within breeds of cattle in resistance to field infestation of the cattle tick *Boophilus microplus*. *Aust. Res.*, 22: 159-168.
- Seyoum, Z. 2005. Distribution of host parasite relationship of ixodids ticks in Eastern Amhara. Ethiopia. *Ethi. Vet. J.*, 9(1): 9-17.
- Sharif, M. 1928. A revision of the Indian ixodidae with special reference to the collections in the Indian Museum. *Rec. Indian Mus.*, 30: 217-344.
- Sharma, A. K., Kumar, R., Kumar, S., Nagar, G., Singh, N. K., Rawat, S. S., & Ghosh, S. 2012. Deltamethrin and cypermethrin resistance status of *Rhipicephalus (Boophilus) microplus* collected from six agro-climatic regions of India. *Veterinary Parasitology*, 188(3-4), 337-345.
- Shaw, R.D. 1979. Culture of an organophosphorus resistant strain of *Boophilus microplus* (Canestrini) and assessment of its resistance spectrum. *Bull. Entomol. Res.*, 56(4): 398-405.
- Shaw, R.D. 1970. Tick control on domestic animals. *Tropical Science*. 12: 29-36.
- Shemshad, M., Shemshad, K., Sedaghat, M. M., Shokri, M., Barmaki, A., Baniardalani, M., & Rafinejad, J. 2012. First survey of hard ticks (Acari: Ixodidae) on cattle, sheep and goats in Boeen Zahra and Takistan counties, Iran. *Asian Pacific journal of tropical biomedicine*, 2(6), 489-492.

- Shokeen, P., Anand, P., Krishna, Y.M. and Tandon, V. 2008. Antidiabetic activity of 50% ethanolic extract of *Ricinus communis* and its purified fractions. Food Chem. Toxicol., 46: 3458-3466.
- Shubber, H. W. K., Mohammed, M. K., & Al-Hassani, N. A. W. 2016. Ixodid Ticks of Water Buffalo *Bubalus bubalis* in the Middle and South of Iraq. Adv. Biores., 4 (3): 58-63. Bull. Iraq nat. Hist. Mus, 14(1), 43-50.
- Shyama, K.P. Kumar. S., Sangwan. A.K. Sharma, A.K., Nagar, G., Ray. D.D. and Ghosh, S. 2013. Acaricide resistance status of *Rhipicephalus (Boophilus) microplus* and *Hyalomma anatolicum* collected from Haryana. Indian J. Animal Sci., 83(6): 591-594.
- Singh, A., and Chhabra., R.C. 1973. Incidence of arthropod pests of domestieated animals and bird. Ind. J. Anim. Sci., 43: 393-397.
- Singh, N.K. and Rath, S.S. 2013. Epidemiology of ixodid ticks in cattle population of various agroclimatic zones of Punjab. India. Asian Pac. J. Trop. Med., 6(12): 947-951.
- Singh, N.K. and Rath, S.S. 2014. Esterase mediated resistance against synthetic pyethroids in field populations of *Rhipicephalus (Boophilus) microplus* (Acari: Ixodidae) in Punjab districts of India. Vet. Parasitol., 204(3-4): 330-338.
- Singh, N. K., Saini, S. P. S., Singh, H., Sharma, S. K., & Rath, S. S. 2017. In vitro assessment of the acaricidal activity of Piper longum, Piper nigrum, and Zingiber officinale extracts against *Hyalomma anatolicum* ticks. Experimental and Applied Acarology, 71(3), 303-317.
- Sivasankar, V., & Rao, P. R. 1984. Tick fauna of Andhra Pradesh. Livestock Adv. Bangalore, 9, 29-33.

- Snedecor, G.W. and Cochran, W.G. 1968. Statistical methods. Oxford and IBH, Calcutta, India, pp. 1-593.
- Soulsby, E.J.L. 1982. Helminths, Arthropodes and Protozoa of domesticated animals, 7th edition the ELBS and Baillieve, Tindal cass LTD. London, pp. 766.
- Stone, B.F. and Haydock, P. 1962. A method for measuring the acaricide susceptibility of the cattle tick *Boophilus microplus* (Can.). Bull. Entomol. Res., 53: 563-578.
- Stratton, G.W. and Corke, C.T. 1982. Toxicity of the insecticide permethrin and some degradation products towards algae and cyanobacteria. Enviro. Pollut., 29: 71-80.
- Surbhi, S. G., Sangwan, A. K., & Sangwan, N. 2018. Determination of efficacy of commercial acaricides against *Ornithodoros savignyi* ticks collected from Haryana.
- Solomon G, Night M, Kaaya G.P. 2001. Seasonal variation of ticks on calves at Sabetha, Western Shewa zone. Ethiopia Vet. J. 7(1&2):17-30.
- Sri Balaji, N 1 and Vikrama Chakravarthi, P. 2010. Ethnoveterinary Practices in India – A Review. Veterinary World Vol. 3(12):549-551.
- Srivastava, R., Ghosh, S., Mandal, D.B., Azhahianambi, P., Singhal, P.S., Pandey, N.N., Swarup, D. 2008. Efficacy of *Azadirachta indica* extracts against *Boophilus microplus*. Parasitol. Res. 104, 149– 153. Trapido, H., Govardhanan, M.K., Rajagopalan, P.K. and Rebello, M.J. 1964. Tick ectoparasites on monkeys in the KFD area of Shimoga distrit, Mysore state, India. Am. J. Trop. Med. Hyg., 13: 736-772.
- Tadesse, F., Abadfaji, G., Girma, S., kumsa, B. and Tariku, J. 2012. Identification of tick species and their preferred site on cattle's body in and around Mizan Teferi, Southwestern Ethiopia. Journal of Veterinary Medicine and Animal health, 4(1): 1-5.

- Tsai, Y. L., Chan, J. P. W., Chen, S. K., Hsieh, J. C., & Chuang, S. T. 2011. Survey of species of ticks infesting cattle in Taiwan. 37(2), 74-82.
- Tessema T., Gashaw A. 2010. Prevalence of ticks on local and crossbreed cattle in and around Asela Town, South East, Ethiopia, Amber Animal Health Department, East Gojam, Ethiopia Vet. J. 14(2):79- 89.
- Tiki, B. and Addis, M. 2011. Distribution of ixodid ticks on cattle in and around Holeta town. Ethiopia. Global Veterinaria. 7(6): 527-531.
- Tripti Negi & Kusum Arunachalam. 2019. Study on prevalence of ixodid tick infestation on bovines of Dehradun district, Uttarakhand, Biological Rhythm Research.
- Utech, K.B.W., Wharton, R.H. and Kerr. J.D. 1978. Resistance to *Boophilus microplus* (Canestrini) in different Breeds of cattle. Aus. J. Agr. Res., 29: 885-895.
- Valente, P.P., Amorim, J. M., Castilho, R.O., Leite. R.C., Ribeiro, M.F. 2014. In vitro acaricidal efficacy of plant extracts from Brazilian flora and isolated substances against *Rhipicephalus microplus* (Acari: ixodidae). Parasitol. Res., 133: 417-423.
- Vatsya, S., Yadav, C.L., Kumar, R.R. and Garg, R. 2007. Seasonal activity of *Boophilus microplus* on large ruminants at an organized livestock farm. J. Vet. Parasitol., 21(2): 125-128.
- Vatsya, S., Yadav, C.L., Kumar, R.R., Garg, R. 2008. Prevalence of ixodid ticks on bovines in the foothills of Uttarakhand state: a preliminary report. Indian J. Anim Sci 78(1):40-42.
- Vatsya, S., & Yadav, C. L. 2011. Evaluation of acaricide resistance mechanisms in field populations of *Rhipicephalus (Boophilus) microplus* collected from India. International journal of acarology, 37(5), 405-410.

- Walker, A.R., Bouattour, A., Camicas, J.L., Estrada, Pena, A, Horak, I.G., Latif, A.A., Pegram, R.G., Preston, P.M. 2003. Ticks of domestic animals in Africa: a guide to identification of species. Biosci Rep.pp 1–221.
- Weston, D.P., *et al.* 2013. Multiple origins of pyrethroid insecticide resistance across the species complex of a non target aquatic crustacean, *Hyadellaazteca*. *Proc. Natl. Acad. Sci. USA*, 110: 16532- 16537.
- Wasihun, P. And Doda, D., 2013. Study on prevalence and identification of ticks in Humbo district, Southern Nations, Nationalities, and people's region (SNNPR), Ethiopia. *Journal of Veterinary Medicine and Animal health*, 5(3): 73-80.
- Wharton , R.H. and Roulston, W.J. 1970. Resistance of ticks to chemicals. *Annual Review of Entomol.*,15: 381- 403.
- Whitehead, G. B. 1958. A review of insecticide resistance in the blue tick, *Boophilus decoloratus* Koch in South Africa. *Indian journal of malariology*, 12, 427-432.
- Wiladson, P. & Kemp, D.H. 1988.Vaccination with 'Concealed' Antigens for tick control. *Parasitology today*, 4:196-198.
- Wu, K. O., & Wang, X. G. 1987. A survey of Ixodidae in domestic animals in Anhui Province, China. *Chin J Vet Med*, 13, 2-6.
- Yacob H.T., Ataklty H, Kumsa B. 2008. Major ectoparasites of cattle in and around Mekelle, northern Ethiopia, *Entomology Research*, 38(2): 126-130.
- Zahir A.A., Rahuman A.A., Bagavan A., Santhosh kumar T., Mohamed R.R., Kamaraj C., Rajakumar G., Elango G., Jayaseelan C., Marimuthu S. 2010. Evaluation of botanical extracts against *Haemaphysalis bispinosa* Neumann and *Hippoboscamaculata* Leach. *Parasitology Research* 10(7): 585–592.

- Zheng HY, Zhou LF, *et al.* 2012. Seasonal abundance and activity of the hard tick *Haemaphysalis longicornis* (Acari: Ixodidae) in north China. *Experimental and Applied Acarology* 5(6): 133–141.
- Zumpt, F. 1958. A preliminary survey of the distribution and host-specificity of ticks (Ixodoidea) in the Bechuanaland Protectorate. *Bulletin of Entomological Research*, 49(2), 201-223.



Appendix

PAPERS PUBLISHED IN JOURNALS

S. No.	Title of the paper	Authors	Journal Name/Volume (Issue)/Page Number	Year
1.	Prevalence of Ticks in Cattle and Buffaloes: a Detailed Investigation at Barabanki District, Uttar Pradesh, India	Neeraj Kumar, Suman Mishra and Kamal Jaiswal	International Journal of Research and Analytical Reviews (IJRAR) Volume 6, Issue 1, E-ISSN 2348-1269	2019
2.	Acaricide Resistance Status of <i>Rhipicephalus (Boophilus) microplus</i> Against Cypermethrin and Deltamethrin in Uttar Pradesh	Neeraj Kumar, Suman Mishra and Kamal Jaiswal	International Journal of Research and Analytical Reviews (IJRAR) Volume 6, Issue 1, E-ISSN 2348-1269	2019