SYSTEMATIC STUDY ON ARTHROPOD INFESTATION IN SHEEP WITH SPECIAL REFERENCE TO TICKS

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CERTIFICATE

This is to certify that the thesis entitled **"SYSTEMATIC STUDY ON ARTHROPOD INFESTATION IN SHEEP WITH SPECIAL REFERENCE TO TICKS"** submitted by **Mrs. SUDHA RANI, R., ID. No. DVHK - 1413** in partial fulfillment of the requirements for the award of degree of **DOCTOR OF PHILOSOPHY** in **VETERINARY PARASITOLOGY** of the **Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar**, is a record of bonafide research work carried out by her during the period of her study in this University under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associate ship, fellowship or other similar titles.

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Affectionately Dedicated to

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LIST OF ABBREVATIONS

AIT	Adult immersion test
AIT - DD	Adult immersion test with differentiating dose
BAFCO	Bangalore Animal Food Corporation
BHC	Benzene hexa chloride
bp	Base pair
BW	Body weight
BLAST	Basic local alignment search tool
°C	Degree Celsius
cm	centimeter
DNA	Deoxyribonucleic acid
dNTP	2'-deoxyribonucleoside-5 triphosphate
EDTA	Ethylene diamine tetra acetic acid
ELISA	Enzyme linked immuno sorbent assay
et al.	et alia
F	Forward
FAO	Food and agricultural organization
Fig.	Figure
FL	Fiducial confedence limit
g	gravitation
g (s)	Gram (s)
Hb	Haemoglobin
hr (s)	Hour (s)
IFA	Indirect fluorescent antibody test
Kg	Kilo gram
LAMP	Loop-mediated isothermal amplification
LC50 / LC 99	Lethal concentration 50 / Lethal concentration 99

Ltr	Litre
LPT	Larval packet test
µl	Microlitre
µg	Microgram
mg	Milligram
min	Minutes
ml	Millilitre
NBAIR	Natinal Bureau of Agricultural Insect Resources.
NCBI	National centre for biotechnology information
NFW	Nuclease Free Water
No	Number
NTC	No Template Control
%	Per cent
p mol	Pico mole
PCR	Polymerase Chain Reaction
R	Reverse
RBC	Red Blood Cells
RFLP	Restriction fragment length polymorphism
RLB	Reverse line blot assay
rpm	Revolution per minute
sec	Seconds
spp	Species
Temp	Temperature
UV	Ultra Violet
V	Volts
X^2	Chi square

Introduction

I. INTRODUCTION

India's livestock sector is one of the largest in the world with a holding of 11.6 per cent of world livestock population, of which small ruminants contribute to 23.07 per cent. Small ruminants contribute to agrarian economy, especially in the arid / semi arid and mountainous areas where crop and dairy farming are not economical. Sheep and goat farming are one of the major animal husbandry activities practiced in India and worldwide. In India, the small ruminant population constitutes 39.11 per cent as a major source of income for the rural population, through milk, meat and manure. It also contributes to the national economy through the export of live animals, meat and by exporting the animal products particularly skin and wool. Sheep in India are mostly reared for wool, meat and manure which constitute important source of earnings particularly in southern India. Total wool production in India has increased from 27.5 million kg (1950-51) to 46.1 million kg (2012-13), with Rajasthan being the highest producer followed by Karnataka as per Basic Animal Husbandry & Fisheries Statistics, 2014.

Owing to their high fertility, short generation interval and adaptation to harsh environments, sheep and goats are considered as investment friendly and insurance particularly during seasons of crop failure and to meet seasonal purchases such as improved seed, fertilizer and medicine for rural households. In spite of the evolutionary adaptation to harsh environments, small ruminants are prone to ectoparasites when husbandry practices are unsatisfactory. The poor management, ineffective treatment, higher ambient temperature and warmer seasons favour the development of ectoparasites.

Ectoparasites are very common and widely distributed in all agro-ecological zones of tropical countries like India. Among the livestock, small ruminants are the most affected by ectoparasites of veterinary and medical importance, hindering their productivity. Skin problems caused by ticks, lice, keds and mange mites are among the major diseases of sheep causing serious economic loss to small farmers, the tanning industry and to the national economy as a whole, in the form of mortality, decreased production and reproduction. Further, skin diseases caused by ectoparasites cause serious down grading and rejection of skins and hides. Their role as vectors in viral and haemoprotozoan diseases is an additional concern.

Economic importance of ticks has long been recognized due to their ability to transmit diseases to humans and animals. Blood sucking by large numbers of ticks causes reduction in live weight and anaemia among animals, while their bites also reduce the quality of hides. However, major losses come by way of their ability to transmit protozoan (Theileriosis and Babesiosis), rickettsial (Anaplasmosis) and viral diseases.

There are quite a few methods available for controlling ticks, but every method has certain shortcomings. Synthetic acaricides such as arsenical compounds, chlorinated hydrocarbons, organophosphorous compounds, carbamates, BHC/cyclodines, amidines, macrocyclic lactones and pyrethroids are the most common ones adopted by the sheep owners for control of ticks in India. These chemicals are available over-the-counter and are applied indiscriminately over the infested animals at frequent intervals leading to the development of resistance in ticks, toxic manifestations in animals, human safety, residual effect on meat and milk and environmental hazards (FAO, 2004).

Failure of application of acaricides results in rapid multiplication of tick populations. Thereby sheep owners have reported treatment inefficiency of these chemicals in field conditions (Vatsya and Yadav, 2011). The broad-spectrum antiparasitic drug group *viz.*, Ivermectin, Moxidectin, Doramectin, Abamectin, *etc.*, have also been projected as a suitable alternative for topical application, however, these are not affordable to farmers, necessitating an alternate and effective means of control amidst the growing rate of insect resistance to synthetic insecticides.

The desire to minimize chemical pesticides and to offset rising prices of new pesticides is fostering the search for alternatives. As a whole, pest bio control agents are far more environment friendly than chemical pesticides. Biological pest control usually refers to the action of parasites, predators or pathogens on a pest population which reduces its numbers below a level causing economic burden.

Pathogens for biological control include bacteria, fungi and parasitic wasps. Entomopathogenic nematodes (EPNs) are widely distributed throughout the world and have been isolated from many types of natural and managed habitats in a wide variety of soils. In the last two decades EPNs have received much attention due mainly to their potential as biopesticides against insect pests, and agricultural pests. Therefore, the need of biological control of ticks gains importance and EPNs holds a promising future for tick control (Samish *et al*., 2004).

Entomopathogenic nematodes (EPN's) parasitize and kill insects and arachnids hence they are known as entomophilic or entomogenous. EPNs serve as vectors of bacteria,

which achieve quick kill of the target pest and thus have high potential capability in pest management.

EPNs of the genus Heterorhabditis (Rhabditida: Steinernematidae, Heterorhabditidae) are symbiotically associated with bacteria of genus *Xenorhabdus* and *Photorhabdus* (Enterobacteriacae). The infective juveniles of third stage is the only free living stage that persists in the soil in search of a susceptible arthropod host. Once the EPN enters the cuticle and or natural openings (i.e mouth, anus and spiracles), the Infective Juvenile's (IJs) release the symbiotic bacteria into the insect haemocoel, multiply and kill the host, usually within 24-48hrs.

Entomopathogenic nematodes feed on symbiotic bacteria and complete three generations in the host cadaver. As food resources are depleted, new IJs are produced and disperse in search of the new hosts. But tick mortality caused by EPNs seems to be due to the rapid proliferation of the nematode symbiotic bacteria within the ticks, since the nematodes do not go through their natural cycle within ticks. The fully engorged female ticks are most susceptible whereas the preimaginal stages are least sensitive.

Apart from EPNs, an ideal biological control agent should be effective against all the stages of the ticks and does not leave any residues in the environment as per the requirements of World Trade Organization. Compounds of plant origin constitute an important segment of such effort used against all the stages (adult, nymph and larva) of economically important tick species with encouraging results. The botanical pesticides are more ecofriendly, biodegradable and non-resistible when compared to synthetic pesticides (Rahman and Venkatesan 2008). Currently, technocrats have, therefore, diverted their

approach towards the development of environmentally safe, biodegradable and target specific botanical acaricides for combating the ectoparasites.

In order to prioritize future research on the development of improved control measures against tick and tick borne diseases, it is essential to define the prevalence of ticks and tick borne pathogens in target populations. The precise identification of these organisms is essential for understanding their epidemiology and classification. The methods traditionally used to detect and identify these haemoprotozoan parasites include microscopic examinations of thin blood smears and serological tests. In contrast to these conventional methods, the application of molecular techniques would allow direct, specific and sensitive detection of parasites and rapid simultaneous detection and differentiation of different species of haemoprotozoan parasites infecting a given animal.

Keeping these facts in view current study was undertaken with following objectives:

1. To systematically study the prevalence of arthropod parasites in sheep in organized and unorganized farms in different agroclimatic zones.
2. To evaluate the efficiency of commonly used acaricides in comparison with a newer compound for the control of ticks.
3. To evaluate phytoacaricides and EPNs in control of ticks.
4. To observe the vector potential of sheep ticks with reference to haemoprotozoan parasites.

Review of Literature

II. REVIEW OF LITERATURE

The literature pertaining to prevalence of arthropod infestation in sheep with special reference to ticks as vectors in sheep, their control methods *viz.*, chemical acaricides, phytoacaricides, entomopathogenic nematodes and vector potentiality of sheep ticks in disease transmission were reviewed and presented below.

2.1 Prevalence of arthropod infestation in sheep

The occurrence of ectoparasites in sheep flocks have been reported from most of the parts of India including Karnataka. The literature with respect to prevalence is reviewed under following subheadings

2.1.1 Prevalence of arthropods (fleas, lice, mite, nasal bots and flies) in India

Achar and Sreekantaiah (1934) studied the prevalence of *B.motasi* in sheep in Mysore, Karnataka and described the shape of *B.motasi* in the erythrocytes of sheep as pear-shaped forms in pair, arranged in acute angle with double chromatin mass. During this study, the presence of hard ticks, *Haemaphysalis bispinosa* was recorded on sheep positive for *B.motasi* was recorded.

Chhabra and Ruprah (1976) reported the incidence and biology of *Oestrus ovis* from slaughter house, at Hisar, Haryana. A total of 312 sheep and 64 goats were examined for *O.ovis* infection, out of which about 269 (86.21%) sheep and 35 (54.7%) goats were found to be positive for *O.ovis* infection. Highest infestation rate was found in sheep compared to goats. The lowest incidence of 56.7 per cent was recorded in younger animals up to six months of age and higher than average incidence in the older animals. During this

survey, highest incidence was found in December - February and lowest incidence in March – April. The maximum incidence of first instar larvae was found in the month of June – July and September – October and all stages larvae encountered throughout the year. Average larval burden recorded was 12 per animal during the survey.

Ahmed *et al.* (1977) studied an outbreak of heavy infestation of *Linognathus vituli* and *Damalinia ovis* in a flock of Bannur lambs of 2-6 months old, in Karnataka.The outbreak was recorded before shearing period when there was a growth of fleece in the flock.

Jagannath *et al.* (1989) studied incidence of *O.ovis* in sheep and goats at the Bangalore Animal Food Corporation (BAFCO) slaughter house, Bangalore. They examined 520 sheep, 186 lambs, 263 goats and 211 kids. They reported highest incidence of 89.2 per cent and 57.14 per cent and the lowest of 35.71 per cent and 16.66 per cent in sheep and goat respectively. The average larval burden per animal was 13 in sheep, 9.9 in goats, 5.3 in kids and 4.5 in lambs. They reported highest infestation rate during December followed by February and January. The lowest infestation rate was observed during August irrespective of age and species of animal studied.

D'Souza *et al*. (1992) reported the natural infestation of *O.ovis* in sheep.They found that with the oral administration of Rafoxanide at the rate of 7.5mg/kg weight as a single dose was successful in controlling the infestation of *O.ovis* in sheep.

Pathak (1992) studied the incidence of *O.ovis* infestation in sheep and goats at Bikaner, Rajasthan. He examined 334 sheep and 466 goats, out of which 312 (81.25%) sheep and 249 (53.4%) goats were found positive for *O.ovis* larvae. During the survey the

average larval burden found per animal was 10.86 and 9.26 in sheep and goat respectively. He also noted all three stages of larvae were found throughout the year, except the first instar larvae which were found maximum during June – July.

Kumar *et al*. (1994) surveyed about 1048 goats, 79.2 per cent of which were infested with *Bovicola caprae* and 37.98 per cent with *Linognathus africanus*. As many as 19.48 per cent goats had different species of ticks *viz*., *Boophilus microplus, Haemaphysalis montogomeri, Hyalomma brevipunctata, Rhipicephalus haemaphysaloides* and *Haemaphysalis bispinosa*. About 10.2 per cent goats were found infested with fleas of *Ctenocephalides* spp and 5.8 per cent with *Melophagus ovinus* infestation.

Narladkar *et al*. (1997) studied occurrence of *Oestrus ovis* infestation in Deccani sheep at Udgir in Maharashtra. They examined 50 sheep, out of which 3 (6%) adult sheep and 8 (16%) lambs were found positive for *Oestrus ovis*. They recorded the number of larvae per animal ranged from 30 to 142.

Shahardar *et al.* (2001) reported unusual recovery of larvae of *Oestrus ovis* from trachea and bronchi of Corriedale sheep in Srinagar for the first time in India.

Muraleedharan *et al.* (2004) assessed the efficacy of closantel and triclabendazole against the larvae of *Oestrus ovis* in sheep and goat. Closantel (15% oral solution) at 15mg/kg body weight was found 100 per cent effective in sheep (n=17) and goats (n=12).

Hiware (2006) studied the population dynamics of *Oestrus ovis* larvae causing myiasis in sheep from Aurangabad, India during March, 1998 to February, 1999. The parameters studied were percentage of incidence, intensity, density and index of infection.

The influence of different seasons was also recorded. The values for all the parameters were found higher during winter season followed by summer and rainy seasons.

Kassaye and Kebede (2010) conducted epidemiological study on mange mite, lice and sheep keds of small ruminants by screening 43,325 animals (22,337 sheep and 20,988 goats), from lowland, medium and high altitudes, during the dry and wet seasons. The prevalence of mange mite was 8.11 per cent (95% CI 4.2284-4.6179) and 0.95 per cent (95% CI 0.8303-1.0898) for goat and sheep, respectively. Goats were 9.17 (OR=9.17, 95% 7.92-10.61) times at higher risk of acquiring mange mite infestation than sheep. Statistically significant difference (X2 =121, P=0.0000) was observed during the dry (5.68%) and wet (3.48%) seasons which was associated with seasonal feed shortage and other stress factors. Animals in lowland were 2.63 (OR=2.6, 95% 2.15-3.20) times more exposed to mange mite infestation than the highland.

Godara *et al.* (2010) documented nasal oestrosis in a three year old, non descript goat, presented in lateral recumbency with clinical history of sneezing fits, laboured breathing, eroded mandibular lesions and bilaterally housing nasal bots. He also found that the nasal bots are in aberrant location (mandibles) in goat and discussed therapeutic management health significance.

Dixit *et al*. (2012) reported a two years-old domestic buck exhibiting symptoms of head pressing, scratching of frontal part of head, loss of appetite and sneezing fits. The larvae were collected and identified as *Oestrus ovis*.

Murthy *et al*. (2013) screened twenty sheep weighing 10–15 kg presented to veterinary dispensary, Ramanagara District, Karnataka with the history of alopecia on both ears and face from few days. Detailed examination revealed the presence of *Sarcoptes scabiei.*

Kour *et al*. (2015) reported the incidence of myiasis and bionomics of cyclorrhaphan flies in small ruminants of Jammu. The incidence of active maggot wound myiasis was 37.1 per cent. The larvae of *Lucilia cuprina* were recovered from both sheep and goats whereas *Calliphora erythrocephala* and *Phormia* spp. larvae were found only on sheep and *Hypoderma* spp. larvae only on goats. There were marked seasonal fluctuations in the occurrence of infestation rate of maggots with a significantly ($P<0.05$) maximum incidence (71.7%) in monsoon season.

Sudha rani *et al*. (2015) recorded flea infestations in 2 organized sheep farms in Karnataka. Out of 750 animals under study, more than 400 animals were flea infested showing severe anaemia with average haemoglobin levels of 4g/dl. The fleas recovered were identified as *Ctenocephalides felis felis* and *Ctenocephalides canis*. Dogs and cats in the farm premises were also found infested with *Ctenocephalides felis felis* and *Ctenocephalides canis*. A stringent treatment regime covering the host as well as the habitat drastically brought down the disease intensity.

Ashwini *et al*. (2017) recorded the prevalence of *Ctenocephalides felis felis*, *Ctenocephalides orientis* in sheep and goats in different parts of Karnataka.The study recorded the *in vitro* trials of efficacy of neem oil and citrus peel extracts on the adult fleas,

inducing 100 per cent mortality at a concentration of 0.75 per cent for 30mins where as the citrus peel extract induced 100 per cent mortality at 5 per cent concentration within 2mins.

Saleem *et al.* (2017) determined the prevalence of the parasite in sheep heads in so far less explored areas of Jammu region. The study found a high overall prevalence rate of 99.16 per cent indicating the poor hygienic measures and unscientific rearing of animals. On analyzing the results, the prevalence of infestation of *Oestrus ovis* in sheep of plain and kandi areas was found to be 98.3 per cent and 100 per cent respectively. When the results were analysed sex-wise, it was found that the prevalence was on higher side in males (100 percent) compared to female (98.2%) animals. Further, all age group animals were equally susceptible to infestations.

2.1.2 Prevalence of arthropods (fleas, lice, mite, nasal bots and flies): Other countries

Meleney *et al*. (1962) reported that over 99 per cent of the first instar were found in the nasal passage and less than 1 per cent in the frontal sinuses of sheep from Southwestern United States. In contrast only about one third of second instar and one sixth of third instar were found in nasal passage.

Ranatunga and Rajamahendran (1972) studied the occurrence of *Oestrus ovis* in goats on a dry zone farm in Ceylon.They recorded 30.2 per cent infestation rate in Jamunapari goats and 16.2 per cent in South Indian breed of goats.

Tenquist and Wright (1976) reported *Lucilia sericata* and *Calliphora stygia* as the two main species causing primary fly strike in New Zealand. Blowfly strike has long been recognised as a source of economic loss in the sheep industry. The only financial

assessment of losses attributable to fly strike was made by Miller (1934) who estimated $800000 at a time when New Zealand had 28.6 million sheep.

Horak and Butt (1977) studied the location wise distribution of *Oestrus ovis* larvae in sheep in South Africa. They recovered 43.9 per cent larvae from the median and dorsal turbinaes, 19.7 per cent larvae from the septum and nasal passage and 13.8 per cent larvae from maxillary, palatine, lachrymal sinuses and pharyngeal area.

Fagbemi (1982) studied about *Ctenocephalides felis strongylus* and its effect on the performance of sheep and goats. Fleas showed more affinity for sheep than goats. He reviewed the highly significant correlation between degree of flea infestation and lamb and kid mortality, which showed that *Ctenocephalides* infestation, should no longer be viewed as just an incidental finding when constraints to small ruminant production are considered in a husbandry system which allowed parasite build-up.

Kettle *et al*. (1984) screened the wool for sheep lice by collecting 1112 samples from sheep flock in New Zealand.Six per cent of the samples had more than 20 lice per 20 g of greasy wool, 3 per cent had 16-20, 4 per cent had 11-15, 9 per cent had 6-10 and 78 per cent had 5 or fewer lice per 20g of greasy wool. The recovered lice were identified as *Damalinia ovis*.

Pandey and Ouhelli (1984) studied the epidemiology of *Oestrus ovis* in sheep at Morocco. They recovered 59.2 per cent larvae from nasal passage, out of which 83.98 per cent were first instar, 10.76 per cent second and 5.26 per cent were third instar larvae. In the anterior part of frontal sinuses 34.7 per cent larvae were found out of which 22.65 per

cent, 33.20 per cent and 44.14 per cent were first, second and third instars respectively. While from the posterior part of frontal sinuses 6.1 per cent larvae were recovered, out of which 40 per cent, 60 per cent larvae were second and third instar respectively. There was not a single first instar larva found in this site.

Yilma and Dorchies (1991) studied the epidemiology of *Oestrus ovis* at southwest France. They recovered 27.1 per cent larvae from nasal passage out of which 91.95 per cent, 3.82 per cent and 4.23 per cent were first, second and third instar respectively. From middle meatus 58.4 per cent larvae were collected out of which 94.53 per cent 4.82 per cent and 0.65 per cent were first, second and third instar respectively. From conchae and sinuses 18.1 per cent larvae were recovered, out of which 76.36 per cent, 12.68 per cent and 10.98 per cent were first, second and third instars respectively.

Kusiluka *et al*. (1995) surveyed the prevalence of various ectoparasites infesting goats in Tanzania in 54 herds and reported 78 per cent, 66 per cent and 44 per cent infestation with ticks, fleas and lice, respectively. Of the 415 goats examined, 74.7 per cent, 63.1 per cent and 47.7 per cent were infested with ticks, fleas and lice, respectively. The species of ticks identified were *Rhipicephalus evertsi, R. appendiculatus* and *Amblyomma variegatum*. *Ctenocephalides felis* was the only species of flea recorded whereas; the louse species were *Damalinia caprae* and *Linognathus stenopsis*.

Qudoos *et al. (*1997) reported Sarcoptic mange mite infestation in sheep in Kalat district of Balochistan highland. Overall prevalence of *Sarcoptes scabiei* was recorded as 21 per cent.

Dorchies *et al.* (2000) conducted a slaughter house survey on a monthly basis to determine the prevalence and larval burden of *Oestrus ovis* in sheep and goats in Pezenas, South France. In this study, a total number of 1303 sheep and goat heads were observed randomly. Out of 631 sheep heads observed 274 heads (43.4%) were found positive for *Oestrus ovis* larvae and the prevalence rate varied from 14.3 per cent in February to 65 per cent in October. The mean number of larvae in infected sheep heads was found to be 10.86 with 9.24 L_1, 0.91 L_2 and 0.71 L_3. 191 goat heads (28.4%) were found positive out of 672 heads observed with prevalence rate varied from 6.25 per cent in September to 47.1 per cent in April. In infected goat heads, the mean parasitic burden was found to be 5.35 with 4.04 L_1, 0.73 L_2 and 0.58 L_3.

Caracappa *et al*. (2000) studied the epidemiology of ovine oestrosis by conducting a slaughter house survey at Messine (Sicily) from May 1996 to April 1998. A total of 841 sheep heads were examined on monthly basis. Out of the total number of heads observed 469 (55.8%) heads were infested by *Oestrus ovis* larvae. The mean larval burden for infected sheep was 9.4 larvae with an average of 3.9 L_1, 2.7 L_2 and 2.8 L_3. The proportions of larvae in each of different stages 8 were similar from January to September with higher percentage of L_1 in October to December, indicating a period of slowed development.

Yilma and Genet (2000) examined heads of 506 sheep and goats during a period of one year in central Ethopia.Of these, 380 (75.10 %) were found infested with different instars and prevalence in sheep and goats were 77.42 per cent and 72.87 per cent, respectively. Persistently high infection prevalence occurred throughout the year without any statistically discernible difference between study months. Relative humidity and

photoperiod revealed significant influence on monthly infection prevalence. The intensity of infection in sheep (12.74 ±1.15) was significantly higher than in goats (10.52 ± 0.65). Unlike in sheep, the larval burden in younger goats was significantly higher ($p < 0.001$) than the older ones, indicating the presence of age-based resistance to infection in caprine hosts.

Scala *et al.* (2001) reported the results of an epidemiological study of oestrosis in sheep of 6 months to 10 years in Sardinia, Italy. Out of 124 free ranging flocks examined, 100 per cent were found positive for *Oestrus ovis* larvae. The monthly prevalence ranged from 69 per cent in May to 100 per cent in June. The percentage of first, second and third stage larvae were found to be 82 per cent, 65 per cent, and 10 per cent respectively.

Bauer *et al*. (2002) conducted a survey to determine the seroprevalence of *Oestrus ovis* infection in southwest Germany from 1997 to 1998 by ELISA. Seventy six per cent of flocks had at least one seropositive animal and the seroprevalence of antioestrus antibodies was 50 per cent in sheep. Larger flocks (> 50 ewes) were more likely to be seropositive than smaller ones.

Alcaide *et al*. (2003) examined 477 adult sheep heads in southwest region of Spain for different larval stages of *Oestrus ovis* in nasal and sinus cavities. Out of 477 sheep heads, 339 heads were found positive with a prevalence of 71.1per cent. The mean larval burden was 18.54 larvae per head during the coldest months of the year in southwest Spain when larval burden reached its highest levels.

Razmi *et al.* (2003) conducted a prevalence study on ticks in *T. lestoquardi* infected sheep in an endemic area of Iran by screening 188 suspected cases of ovine theileriosis and found that 61.1, 33.42 and 33.42 per cent of the animals harboured *H.a.anatolicum, R. sanguineus* and *H. m. isaaci* respectively.

Kaal *et al.* (2006) made an epidemiological and clinical study of flea infestations of farm animals in northern Libya. Of 12,130 sheep examined from 124 flocks, 150 sheep were found to be infested with fleas, *Pulex irritans* from 50 different flocks. A significantly higher proportion of intensive farms had animals with flea infestation compared to semi-intensive farms. Fleas were not found in nomadic herds.

Yakhchali and Hosseine (2006) reported the prevalence of ectoparasites (ticks, mites, lice and fleas) in a herd of 1200 sheep in Iran for an year in which the highest number of ticks belonged to *Rhipicephalus bursa* (90.7%), followed by *Rhipicephalus sanguineus* (6.9%), *Boophilus annulatus* (2.4%), *Ornithodorus lahorensis* (2.6%). Whereas the lice infested were *Damalinia ovis* (58.8%), *Damalinia capre* (71.4%), *Haematopinus species* (76.6%), *Linognathus stenopsis* (36.1%), and *Linognathus ovillus* (29.4%). Out of 1200 sheep only two sheep (2.6%) were infested with *Sarcoptes scabiei.* The ectoparasite infestation was more in fall and winter than in spring and summer.

Sertse *et al*. (2007a) studied the prevalence of ectoparasites in sheep in Amhara state, Ethiopia, and the ectoparasites identified in sheep were *Damalinia ovis* (38.5%), *Melophagus ovinus* (12.5%), tick infestations (3.4%) and *Linognathus* spp (2.4%). The prevalence of *M.ovinus* and *D.ovis* infestations in sheep were significantly ($p \leq 0.05$) higher in the highlands than both lowlands and midlands, which were almost similar.

Yacob *et al.* (2008a) studied the ectoparasites prevalence in small ruminants in and around Wolaita Soddo (Southern Ethiopia). A total of 214 sheep and 102 goats of both sexes were examined for the presence of ectoparasites. The frequency of tick infestation was significantly more in sheep (31.78%) than in goats (18.63%). Whereas only one case of mange (*Demodex caprae*) in goat and pediculosis (*Damalinia ovis*) was observed in sheep with a relative high frequency (25.70%).

Rahbari *et al.* (2009) screened 289 cases of animal populations in Tehran, Iran. The highest prevalence of infestation was found in sheep and goat herds whilst chicken flocks infested with the lowest rate and cattle were infested moderately. They collected one hundred fifty six specimens of *Pulex irritans* from sheep, goats, cattle, chicken and human, which consisted of 92.8 per cent of all recovered fleas. Chickens infested by three species of fleas including *Pulex irritans* (84.6%), *Ctenocephalides canis* (12.9%) and *Ceratophillus gallinae* (2.5%). Among the two hundred and eighty nine cases the highest prevalence of infestation was found in sheep and goat followed by cattle and chicken.

Shoorijeh *et al*. (2009) examined 2002 sheep heads slaughtered at Fars abattoir, Shiraz, Southern Iran, between April 2006 and April 2007. Out of the total heads, 995 (49.7 %) were infested with *Oestrus ovis* larvae. A total of 6264 larvae were collected. The overall larval intensity per infested head was 6.3, with 3.9 in spring, 5.3 in summer, 5.9 in autum and 7.8 in winter. Prevalence ranged from 23.3per cent in spring to 80 per cent in winter. Increased infestation was observed in older animals.

Mulugeta (2010) reported an overall prevalence of 55.5 per cent and 58 per cent of ectoparasites in each examined 750 sheep and goats, respectively in three selected agro-

ecological sites of Tigray Region, Ethiopia. In the sheep population, the major ectoparasites identified were *Melophagus ovinus* (19.1%), tick infestations (16%), *Damalinia ovis* (15.3%), *Linognathus africanus* (11.5%), and *Ctenocephalides felis* (9%). Major ectoparasites identified in goats were tick infestations (29.7%), *L. africanus* (27.9%), *Sarcoptes scabiei var caprae* (12.5%), *C. felis* (11.1%), and *Demodex caprae* (6.8%).

Sarkar *et al*. (2010) reported in Gaibandha and mymensingh districts of Bangladesh that in Black Bengal goats, 91 (72.8%) out of 125 were infested with one or more species of ectoparasites. They were *Haemaphysalis bispinosa* (34.4%), *Boophilus microplus* (27.2%), *Rhipicephalus sanguineus* (7.2%), *Psoroptes cuniculi* (5.6%), *Damalinia caprae* (20.8%) and *Linognathus stenopsis* (18.4%). Overall mean parasitic burden was 2.36±1.49 per square inch of affected area. The highest parasitic burden was recorded in case of *L. stenopsis* (3.93±2.219), followed by *D.caprae* (3.00±2.424), *H.bispinosa* (2.32±1.278), *P.cuniculi* (2.00±1.414), *B.microplus* (1.59±1.098), and *R.sanguineus* (1.33±0.516). Significantly ($p<0.01$) higher prevalence of ectoparasites was recorded in the rainy season (90%), followed by winter (82.61%), and summer (53.06%). The ectoparasitic infestation was higher in case of kids (82%) and older goats (79.55%) than that of young (51.61%) goats. The female goats (77.63%) were more susceptible than male (65.31%) to ectoparasitic infestation.

Rony *et al*. (2010) in a study reported that, among 165 Black Bengal goats 114 (69.09%) were found to be infested with several species of ticks, lice and flea. The prevalence rate was highest in *Boophilus microplus* (45.45%) followed by *Rhipicephalus sanguineus* (31.51%), *Linognathus vituli* (25.45%), *Haemaphysalis bispinosa* (20%),

Haematopinus eurysternus (15.75%), *Damalinia caprae* (8.48%) and *Ctenocephalides canis* (4.84%). Young goats aged ≤ 6 months (75.86%) were more susceptible than adults aged > 6-24 months (65.51%) and older goats > 24 months (59.32%). In females, prevalence was recorded significaltly ($p<0.05$) higher than males. Animal with poor health was found to be significantly more vulnereable to such parasitic infestation than healthy animals. Prevalence of ectoparasites was significantly ($p<0.05$) higher in animals reared under free-range system than that of semi-intensive system. Prevalence was highest ($p<0.05$) in the summer (81.35%) followed by winter (62.96%) and rainy season (59.26%).

Barmon *et al.* (2010) recorded ectoparasites in sheep in Gaibandha district of Bangladesh. Overall mean parasitic burden was 3.1=1.0796 per square inch of heavily affected area. The highest parasitic burden was recorded in case of *Damalinia ovis* (5.2±1.5) followed by *Linognathus pedalis* (3.3±0.9), *Psoroptes ovis* (2.9±1.3). Significantly ($p<0.01$) higher prevalence of ectoparasites was recorded in the rainy season (87.9%), followed by winter (80%), and summer (71.4%). The ectoparasitic burden was higher in case of young (87.3%) and older (73.3%) than kid (71.4%). The female sheep were more susceptible than male.

Papadopolous *et al*. (2010) in Greece, reported that out of 450 sheep and goat heads examined, 246 (54%) were infected with *O. ovis* larvae. Goats (76%, 95% CI 68–82) were more commonly infected than sheep (38%, 95% CI 37–49). Larval stages were recovered during all months of the year from both host species, with an overall mean infection intensity of 8.7±13.1 (mean±SD). Seasonal variation in prevalence was much more pronounced for larval stage 1, than for total larvae. In sheep, infection was generally more

common and intense in early spring and autumn, whereas prevalence peaked in goats in winter. Both prevalence and larval intensity increased with age above 3–4 years in sheep, but not in goats.

Yagoob (2011) reported the prevalence of flea infestation in farm animals in Tabriz, Iran. One hundred forty nine specimens of *Pulex irritans* were collected from sheep, goats, cattle, chicken and human, which consisted of 91.2 per cent of all recovered fleas. Chickens infested by three species of fleas including *Pulex irritans* (81.7%), *Ctenocephalides canis* (11.2%) and *Ceratophillus gallinae* (2.1%). Three hundred and twenty five cases of animal and 239 cases of human infestation were recorded among the suspicious populations, the prevalence of infestation was more in sheep and goat flocks whilst chicken flocks with lowest rates and cattle were infested moderately.

Muller *et al.* (2011) studied infestation of fleas on livestock species in two small villages: Parhana and Khowari of Mansehera, Pakistan. During this study, 64 goats, 62 sheep and 71 chickens were examined. Fleas infestation rates in descending-order: goats (43.75%), chicken (30.98%), sheep (27.41%), were calculated. The three flea species belong to two different genera namely *Pulex irritans, Ctenocephalides felis felis* and *Ctenocephalides canis* were identified.

Asghar *et al*. (2011) surveyed about 2220 sheep and goats for scabies infection in modern Moisem slaughter houses. The total rate of infection by different species of mites in the examined animals reached to 63 (2.8 %). Scabies infection reached to 5.6 per cent and 6.5 per cent in native sheep and goats, respectively. *Psoroptes communis ovis* and *Psorergates ovis* mites were extracted from 7.9 per cent of the examined back lesions,

while *Psoroptes communis ovis* only were extracted from 3.2 per cent of the tail lesions of the affected animals.

Abebe *et al.* (2011) examined 991 small ruminants (600 sheep and 391 goats) for the presence of ectoparasites. Among them 310 (51.7 %) sheep and 233 (59.6%) goats were found infested with one or more ectoparasites. The overall prevalence for both host species was 54.8 per cent (n=543). The major identified ectoparasites in sheep were ticks (48%), sheep ked (6.7%) and lice (1.3%) and in goats were ticks (58.8%), lice (6.1%) and fleas (3.1%). The prevalence of ectoparasites infestation was significantly higher in small ruminants of the lowland and midland, small ruminants with poor body condition score, large flocks and mixed flocks than in their contemporaries within the same comparison category ($P < 0.001$).

Tadesse *et al.* (2011) studied the ectoparasitic infestation in sheep and goat skins to assess their skin defect on processed wet-blue (pickled) skins at Kombolcha tannery. A total of 240 cattle, 175 sheep, 66 goats, were used to study the prevalence of ectoprasites on live animals as well as 344 fresh goat pelts and pickled (wet-blue) goat skins were used to assess skin defects. Goats had high prevalence of *Sarcoptes scabiei* (30.3%) followed by *Linognathus stenopsis* (9.09%), *Amblyomma* spp (4.54%), *Ctenocephalides* spp (3.03%), *Bovicola capare* (1.51%) and *Demodex* spp (1.51%) in that order. Fresh goats pelts had an overall high prevalence of *Sarcoptes scabiei* (53.29%) followed by *Linognathus stenopsis* (9.88%), *Bovicola caprae* (2.08%) and *Demodex* (2.08%) spp.

Vazirianzadeh *et al*. (2011) collected a total of 65 ectoparasites from 10 animals (cows, buffaloes and sheep) in the small animal houses of Dezful area, south west Iran. Out

of 65 ectoparasites 12 were identified as *Hyalomma anatolicum* (Arachnida: Acari: Ixodidae) from cow, buffalo and sheep and 3 as *Rhipicephalus* spp. (Arachnida: Acari: Ixodidae). Only one *Linognathus* spp. (Insecta: Anoplura: Linognathidae) of lice was seen in sheep and about 49 of *Haematopinus tuberculatus* (Insecta: Anoplura: Haematopinidae) spp of lice was predominantly found in buffalo.

Emmanuel *et al*. (2012) compared the prevalence and mean intensities of ectoparasites of goats from two Nigerian regions and their epidemiological implications. Of 714 goats examined, prevalence of 77.9 per cent was recorded with infestation of mites, lice, ticks and fleas. The ectoparasites observed were *Rhipicephalus evertsi, Amblyomma variegatum*, *Psoroptes communis*, *Linognathus* spp, *Bovicola* spp and *Ctenocephalides felis*.

Tesfaye *et al.* (2012) estimated the prevalence of ectoparasites in small ruminants of Bahir Dar area of Northwest Ethopia. From the 395 (280 sheep and115 goats) small ruminant examined, 193 were positive for one or more type of ectoparasite with an overall prevalence of 48.9 per cent. Ectoparasites identified in sheep were: ticks (31.4%), fleas (13.2%), lice (3.8%), keds (1.8%) and mixed infections (4.6%) with total prevalence of 54.8 per cent; whereas, in goat ectoparasites encountered were: tick (12.2%), fleas (11.3%), lice (9.7%) and mixed infections (1.7%) with total prevalence of 34.9 per cent. *Ctenocephalides felis* was the most frequently observed flea species in both sheep and goats.

Linardi and Santos (2012) reviewed the characteristics which can be used for interspecific diagnosis and intraspecific variations found between the species

Ctenocephalides felis felis and *Ctenocephalides canis.* Data on distribution, hosts, prevalence and parasitological action are also presented as auxillary means for recognizing the species.

Radfar and Hajmohammadi (2012) conducted a study on 1964 goats from May 2007 to April 2008 in South-eastern part of Iran. Of the1964 goats, 289 (14.71%) were infected with *Przhevalskiana silenus* larvae. The infection was observed from July 2007 to February 2008 and the prevalence rate varied from 6.8 per cent in August to 41.8 per cent in February. The difference in the prevalence of the infection between males and females was not significant ($P>0.05$). The percentage of larvae in subcutaneous tissue of back and flanks was (71.25%) and (28.75%) respectively and this difference was significant ($P<0.05$).

Fentahun *et al.* (2012) conducted a study to determine the prevalence and to identify ecto-parasites on small ruminants. Among the 384 animals, the overall ectoparasites prevalence was 78.38 per cent. The most common ectoparasites encountered in the order of their predominance were lice (54.6%), flea (35.7), tick (20%), sheep ked (10.6%) and mite (7%). A significant difference (P<0.05%) was found in the prevalence of tick with age of small ruminants while it was relatively higher in adult (28.3%) than young (20.4%).

Zeryehun and Atomsa (2012) recorded the prevalence of ectoparasite as 34.1P per cent (148/434) accounting for 135 (43.9%) in sheep and 13 (10.2%) in goats. Among the external parasite identified, in sheep, *Damalinia ovis* (19.5%) was predominant followed by *Boophilus decoloratus* (14.9%) and *Linognathus ovillus* (11.1%), while in goats *Boophilus decoloratus* (4.4%) was the dominant external parasite followed by *Linognathus*

stenopsis (3.1%). The prevalence of *Amblyoma variegatum, Hyalomma* spp, *Damalinia ovis, Linognathus ovillus* and *Ctenocephalides* spp in sheep, and *Linognathus stenopsis* in goats was higher ($p<0.05$) in animal with poor than good body condition scores.

Zeryehun and Mengesha (2012) conducted a cross sectional stsudy on 350 goats (238 female and 112 male). Accordingly, the overall infestation rate of mange mites was 11.7 per cent (41 of 350). The highest level of infestation was observed in female adult goats (9.1 %) than in male adult goats (2.6%).

Ibe *et al*. (2012) performed a study in Nigeria on 714 goats. They reported an infestation an infestation prevalence of 77.9 per cent (66.3% for CRS goats, and 88.0% for MB goats. The ectoparasites prevalence species observed were, *Rhipicephalus evertsi, Amblyomma variegatum, Psoroptes communis, Linognathus* spp, *Bovicola* spp and *Ctenocephalidis felis*. Multiple infestations were high in both groups 67.5 per cent for CRS goats and 80 per cent for MB goats.

AL-Shebani *et al.* (2012) investigated the percentages of infestation of mange mites in sheep and reported that 186 of the sheep were infested with mites with an overall percentage of infestation 3.65 per cent. In this study four genera of mange mites were recorded that parasitized sheep: *Sarcoptes scabiei*, *Psoroptes ovis*, *Chorioptes* spp and *Chirodiscoides caviae* (fur mite) with a infection rate of 31.18, 52.15, 8.01 and 2.15 per cent respectively. In addition, the presence of mixed infestation such as *Sarcoptes* with *Psoroptes* 5.91 per cent and *Psoroptes* with *Chorioptes* 0.53 per cent. The prevalence of infestation was highest in sheep more than two years old (3.74%) and the lowest in sheep

with age less than two years old (3.40%). The prevalence of mange mites in male sheep was 3.93 per cent versus 3.59 per cent in females.

Elsaid *et al.* (2013) recorded a prevalence of 20.1 per cent and 35 per cent in sheep (322) and goats (132) respectively. Tick and mite infestation was high in summer in sheep, while fleas were the highest prevalence ectoparasites on goats (17.9%). The identification showed two different species of ticks (*Hyalomma anatolicum anatolicum* and *Rhipicephalus turanicus),* one species of mite (*Sarcoptes scabiei*), one species of lice (*Linognathus africanus*) and two species of fleas (*Pulex irritans and Ctenocephalides canis*).

Shibeshi *et al.* (2013) examined a total of 228 sheep and 155 goats of which 140 (61.40%) sheep and 90 (57.69%) goats were infested with various types of ectoparasites. The ectoparasites identified in both species of animals were ticks (24.74%), mange mites (15.36%), fleas (11.45%), lice (6.51%) and sheep ked (1.82%). Ticks were the most abundant ectoparasites recorded both in sheep and goats with a prevalence of 25.44 per cent and 23.72 per cent, respectively. The genera of ticks observed in both sheep and goats were *Amblyomma, Rhipicephalus* and *Boophilus* spp. in a decreasing order of prevalence. An overall prevalence of 15.87 per cent mange mites was observed in both sheep and goats with 13.16 per cent and 18.59 per cent, respectively. The identified species of mange mites found in both species of animals were *Sarcoptes scabiei, Psoroptes* and *Demodex* spp. in a decreasing order of prevalence. Sheep were found to be infested with two species of lice (*Linognathus* spp. and *Damalinia ovis*) while goats only with *Linognathus* spp. The fleas, *Ctenocephalides* spp. were detected in both sheep (12.28%) and goats (10.25%).

Mohamed *et al*. (2013) conducted a study on ectoparasites of small ruminants and reported that the sheep were infested by only two parasites (ticks and mites) while goats were infested by four different parasites (ticks, mites, fleas and lice). Out of 1600 sheep and 520 goats examined, 322 (20.1%) sheep and 182 (35%) goats were infested by one or more ectoparasites. The high number of sheep infested by ticks was (40.9%) and mites was (10.9%) during the summer season in Gahawat. Ticks were the most frequent ectoparasites on sheep (18.7%), while fleas were the highest number of ectoparasites on goats (17.9%).

Hanan (2013) screened about 480 sheep heads for the prevalence study of *Oestrus ovis*. Results indicated that 257 (53.54%) of the total examined sheep heads showed positive infestation. The highest infestation was detected in January (82.5%), while the lowest was in August (25.00%). Also, the first, second and third larval instars represented 3.02, 20.35, and 76.63 per cent from the total collected larvae, respectively. The study suggested the cold seasonal related *O. ovis* infestation in Jazan region.

Zangana *et al*. (2013) carried out an investigation into ectoparasites of sheep and goats. Among them majority of the animals (66.89%) were infested with one or more species of ectoparasites. Of the 720 (57.7%) and 753 (78.9%) sheep and goats, respectively were infested. Five different types of ectoparasites, ticks (46.7%, 34.9%), lice (3.8%, 33.8%), mites (7.1%, 0.1%), fleas (2.8%, 7.75%) and ked (1.2%, 4.5%) were identified in sheep and goats, respectively. Three species of fleas were found infesting both sheep and goats. Out of 106 fleas collected they were *Ctenocephalides felis felis* (47.2%), *Pulex irritans (*43.4%), and *Xenopsylla cheopis* (9.4%).

Obi *et al*. (2014) randomly screened 68 sheep and 92 goat in Uli, Nigeria.The overall infestation was; sheep (69.8%) and goat (70.7%). The prevalence of these ectoparasites were in sheep; ticks (17.0%), lice (25.8%), mites (15.0%) and fleas (42.2%), while in goats were; ticks (12.7%), lice (28.5%), mites (15.4%) and fleas (43.4%). In specific ectoparasite infestation, fleas (Ctenocephalides spp) were found to be higher in both sheep and goats by 42.2 and 43.4 per cent respectively. In comparison, there was no significant difference in flea infestation between the two ruminants. The age related infestation was more among 1–5 years sheep and goat with percentage infestation as 58.5 per cent and 54.0 per cent respectively. The predilection sites were mostly; ears (67), neck (57), back (51) and limb (48) in sheep, while in goats, they were; ears (83), neck (76), back (69), limbs (61), abdomen (56) and chest (52).

Jarso *et al.* (2014) screened a total of 1638 animals (685 sheep and 983 goats) in Kombolcha, north east Ethiopia. Out of these examined animals 460 (190 sheep and 270 goats) were found with skin lesion. Of a total 190 sheep with skin lesion 88.4 per cent were positive for one or more species of ectoparasites, out of 270 goats with skin lesion 100 per cent were infested with various ectoparasites. The major ectoparasites prevailing in the area and registered in this study were mange 2.7 per cent in sheep and 5.7 in goats, lice 21.7 per cent in sheep and 34 per cent in goats, flea 7.3 per cent in sheep and 8.2 per cent in goats and tick 7.35 in sheep and 13.7 per cent in goats.

Amare *et al.* (2013) found out that the prevalence of major ectoparasites of sheep and goats were associated with risk factors. Out of the 1230 clinically examined animals sheep (n=738) and goats (n=492), 331(44.9%) sheep and 214 (43.5%) goats were infested

with one or more ectoparasites. Ectoparasites identified in sheep were *Damalinia ovis* (30.9%), *Melophagus ovinus* (10.8%), ticks (3.9%), *Linognathus* species (3.1%) and flea (1.1%). Among goats, *Linognathus* species, ticks, flea and demodectic mange were identified with respective prevalence of 27, 17.7, 2.6 and 2.2 per cent.

Seyoum *et al.* (2015) studied the prevalence of ectoparasites *i.e.*, lice, ticks, *Ctenocephalides* spp, *Melophagus ovinus*, and *Demodex* spp. The infestation rates of ectoparasites with age and sex were significantly varied (P>0.05) in sheep but not in goats (P>0.05). Body condition score was not significantly associated (P>0.05) with ectoparasites infestation in both sheep and goats. In this study, only two cases due to Demodex species were recorded in sheep.

Yasine *et al*. (2015) reported that 97 (7.6 %) of sheep and 174 (13.8 %) goats were infested with one or more species of mites. In goats an overall prevalence of 10.3 per cent *Sarcoptes*, 2.8 per cent *Demodex* and 0.6 per cent *Psoroptes* were recorded whereas in sheep an overall prevalence of 3.5 per cent Sarcoptes, 2.1 per cent Demodex and 1.6 per cent Psoroptes were observed.

Adang *et al*. (2015) determined the prevalence of ectoparasites in sheep and goats in Gombe. Total of 312 animals comprising of 155 sheep were examined. The ectoparasites present on sheep were lice: *Damalinia ovis* (3.9%) ticks: *Rhipicephalus* spp (3.2%), *Amblyomma* spp (5.8%), *Boophilus* spp (6.5%) and fleas: *Ctenocephalides felis* (5.2%).

Sheep and goats (10 each) having cutaneous wounds were examined monthly (total 240 animals) for the presence / absence of maggots by Kour *et al*. (2015). The incidence

of active maggot wound myiasis was 37.1 per cent. The larvae of *Lucilia cuprina* were recovered from both sheep and goats whereas *Calliphora erythrocephala* and *Phormia* spp. larvae were found only in sheep and *Hypoderma* spp. larvae only in goats. There were marked seasonal fluctuations in the occurrence of infestation rate of maggots with a significantly ($P<0.05$) maximum incidence (71.7%) in monsoon season.

Ozdal *et al*. (2016) reviewed the prevalence of larval burden and some risk factors of ovine oestrosis in 328 heads of randomly selected sheep slaughtered in one year at Van, eastern Turkey. Of the total heads, 127 (38.71 %) were infested with *Oestrus ovis* (Linnaeus, 1761, Diptera: Oestridae) larvae. The prevalence of *Oestrus ovis* was 51.68 per cent in summer, 40.69 per cent in spring, 35.80 per cent in autumn, and 23.61 per cent in winter. The differences between seasons were statistically significant ($P>0.05$).

Mohammad (2016) screened 215 sheep and 87 goats in different regions of the middle and south of Iraq for a year. The intensity of 8.4 with 1533 ticks from sheep and count of 332 ticks from goats with intensity of 6.8 was recorded. Tick species recovered from sheep and their incidence rates were: *Rhipicephalus turanicus* (39%), *Hyalomma anatolicum* (28%), *R.(Boophilus) annulatus* (11%), *Hyalomma* spp. (9%), *H. turanicum* (6%), *H. excavatum* (6%) and *R. leporis* (1%) while the tick species recovered from goats and their incidence rates were: *R. turanicus* (64%), *H. anatolicum* (24%), *H. turanicum* (6%), and *Hyalomma* spp (6%).

Seid *et al.* (2016) made cross sectional study of small ruminant mange mites on a total of 324 sheep and 680 goats, to determine the prevalence of mange mites in sheep and goats, identifying the overall mange mite prevalence of 7.5 per cent (95 % CI 5.5–9.5) in

goats and 1.2 % (95 % CI 0.5–1.9) in sheep. The mites identified were *Sarcoptes* and *Demodex* in goats and *Sarcoptes* and *Psoroptes* in sheep. The prevalence of mange mites was significantly higher in goats than in sheep ($X^2 = 16.636$, $P = 0.0001$).

Mandado *et al.* (2016) screened a total of 300 small ruminants (155 goats and 145 sheep) for mange mite infestation. From these, 14 animals (9 goats with the prevalence of 5.8 per cent and 5 sheep with the prevalence of 3.45 per cent were positive for mange mites with the overall prevalence of 4.67 per cent. The species of mange mites identified in the current study were *Sarcoptes* (2.67%), *Demodex* (1.33%), and mixed (*Sarcoptes* and *Demodex)* 0.67 per cent. The prevalence of *Sarcoptes* was higher in goats and in lowland than in sheep and midlands and highlands.

2.1.2a Prevalence of Ticks: In India

Dhanda and Bhat (1968) described the tick species *Haemaphysalis (Allophysalias) garhwalensis* parasiting sheep and goat in Himalayan mountain range in Garhwal region, Uttar Pradesh, India.

Hiregoudar *et al.* (1977a) identified the ticks from Theileria carrier sheep in different sheep farms in Karnataka as *Haemaphysalis intermedia, Hyalomma marginatum isaaci, Rhipicephalus haemaphysaloides, Hyalomma hussaini, Hyalomma anatolicum anatolicum* and *Boophilus microplus*.

Kamble and Hiregoudar (1988) surveyed the prevalence of different species of ticks from cattle, buffaloes, sheep, goats, horses, asses and dogs in the Dharwad area, north Karnataka, India. A total of 8 ixodid species were recovered: *Haemaphysalis bispinosa,*

Haemaphysalis intermedia, Boophilus microplus, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum, Hyalomma marginatum isaaci and *Hyalomma hussaini.*

Jagannath and Lokesh (1988) studied the incidence of seasonal abundance of *Haemaphysalis intermedia, Hyalomma marginatum isaaci, Hyalomma hussaini* and *Rhipicephalus haemaphysaloides* in 1164 sheep and 372 goats in Kolar district, Karnataka.The preferred locations for each were, respectively, the ears, anal region and tail, anal region and crotch, and ears, crotch and brisket. *Haemaphysalis intermedia* was the commonest species, infesting 55-95 per cent of livestock throughout the year.

Saxena (1997) screened 127 rodents infested with *Haemaphysalis spinigera* and *Rhipicephalus ramachandrai* principal vector of Kyasanur Forest Disease (KFD) in South India; whereas the sheep in the same survey was infested with ixodid tick, *Haemaphysalis intermedia,* which is a vector of Bhanja virus.

Kumar *et al.* (2002a) studied the prevalence of ectoparasitic ticks from different localities of the Nilgiris district of Tamil Nadu state from various domestic animals. A total of 1232 adults and immature ticks were collected from domestic animals which comprised of the various species in the order of abundance *Boophilus microplus, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides* and *Rhipicephalus sanguineus.*

Ghosh *et al.* (2007) reported the status of tick distribution based on seasonal incidence in different hosts throughout the world, particularly in tropical and subtropical

countries including India, Pakistan, and Bangladesh where the major species of ticks found were *Boophilus*, *Haemaphysalis* and *Hyalomma* species.

Prakasan and Ramanai (2007) screened the domestic animals for the tick infestations with highest infestation rate in cow followed by goat, buffalo, dog, pig, cat and sheep in Kerala. The major ticks recorded were *Haemaphysalis, Rhipicephalus, Hyalomma, Nosomma and Amblyomma.*

Vathsala *et al*. (2008) made a survey on tick species distribution in sheep and goats in Tamil Nadu based on their predilection site and season of occurrence and found that *Hyalomma anatolicum anatolicum*, *Hyalomma marginatum isaaci, Rhipicephalus haemaphysaloides* and *Haemaphysalis bispinosa* were most abundant ticks found.The overall prevalence of these three species of ticks was found to be 64.66 per cent and 97.66 per cent in sheep and goats, respectively.The seasonal prevalence of ticks in sheep and goats were found to be 61.00 and 97.66 per cent in sheep and goats during January to March and 64.00 and 94.00 per cent in sheep and goats during October to November respectively.

Soundararajan *et al*. (2014) studied the seasonal activity of ticks in sheep and goat populations in Kanccheepuram district of Tamil Nadu. The most common species of ticks found were *Haemaphysalis bispinosa* followed by *Rhipicephalus haemaphysaloides, Hyalomma marginatum isaaci* and *Hyalomma anatolicum anatolicum* with mixed infestations. Although the infestations were found throughout the year, they were greater during the rainy season.

2.1.2b Prevalence of ticks: other countries

Eichler (1966) reported that the highest numbers of ticks were found in spring and summer in sheep in north Iraq with species of *Hyalomma detritum, Rhipicephalus bursa, Boophilus annulata, Dermacentor marginatum* and the number is less in winter.

Rahbari (1995) studied the tick population of sheep, goat and cattle in six villages of northwest of Iran. Out of the nine species of ixodid ticks recorded, *Ornithodorus lahorensis* was the only argasid tick, which infested sheep, goat and cattle. The prevalence of tick infestation on sampled animals was 55, 57 and 62 per cent in sheep, goat and cattle respectively. The predilection site of ticks per animal showed the largest number on head, axilla and groin. The seasonal distribution of tick indicated that hard ticks were apparently found throughout the year, but were more abundant in spring and least in autumn. Whereas, soft ticks were observed only during autumn up to late winter.

Yakhchali *et al*. (2006) reported the prevalence of ectoparasites (ticks, mites, lice and fleas) in a herd of 1200 sheep in Iran for an year in which the highest number of ticks belonged to *Rhipicephalus bursa* (90.7%), followed by *Rhipicephalus sanguineus* (6.9%), *Boophilus annulatus* (2.4%), *Ornithodorus lahorensis* (2.6%). The ectoparasite infestation was more in fall and winter than in spring and summer.

Nabian and Rahbari (2008) studied the distribution and ecological preferences of ticks of domestic animals in North Iran. A total of 1720 tick specimens were collected from cattle, sheep and goats from different localities of Iran. Fourteen tick species were identified as *Hyalomma anatolicum anatolicum* (5.23%), *Hyalomma marginatum* (20.34%), *Hyalomma detritum* (3.48%), *Haemaphysalis punctata* (12.79%), *Haemphysalis*

parva (0.58%), *Haemphysalis concinna* (0.58%), *Haemphysalis choldokovsky* (6.97%), *Ixodes ricinus* (2.32%), *Rhipicephalus sanguineus* (19.76%), *Rhipicephalus bursa* (4.65%), *Boophilus annulatus* (9.88%), *Dermacentor niveus* (6.39%), *Dermacentor marginatum* (1.74%) and *Ornithodoros lahorensis* (5.23%).

Rahbari *et al. (*2007) studied the geographical distribution and ecological preferences of *Haemaphysalis* in domestic animals in Iran. A total of 1,622 ixodid tick specimens were collected from 3 different zones. Among them, 108 (6.7%) *Haemaphysalis* ticks, consisting of six species, were identified; *H. punctata* (3.4%), *H. parva* (0.5%), *H. sulcata* (0.6%), *H. choldokovskyi* (1.7%), *H. concinna* (0.06%) and *Haemaphysalis* spp. (0.6%). *H. punctata* was the most abundant species, whereas *H. concinna* was the rarest species collected in humid and sub-humid zones on cattle, sheep and goats. *H. choldokovskyi* was principally collected from sheep and goats grazed in cold mountainous areas.

Razmi and Sarvi (2007) collected 953 ticks from 86 infested cattle during activating seasons of ticks. Nine species were identified: *Boophilus annulatus* (51.3%), *Rhipicephalus bursa* (16.8%), *Haemaphysalis punctat*a (6.3%), *Ixodes ricinus* (6.8%), *Hyalomma marginatum* (12.5%), *Hyalomma anatolicum excavatum* (5.2%), *Hyalomma asiaticum* (0.6%), *Hyalomma detritum* (0.2 %), and *Dermacentor spp*. (0.1%). The results showed that *Boophilus annulatus*, *Rhipicephalus bursa*, and *Hyalomma* species were dominant tick species in the surveyed area.

Sajid *et al.* (2008) screened randomly a total of 1050 cattle, 700 buffaloes, 1400 each of sheep, goats and 250 camels for the prevalence of tick infestation. The highest

(P=0.00) prevalence of tick infestation was found in cattle (n=789/1050; 75.1%) followed by goats (n=723/1400; 51.6%) and buffaloes (n=281/700; 40.08%). None of the examined camels and sheep was found infested with ticks. *Hyalomma anatolicum* was the most abundant followed by *Rhipicephalus sanguineus*.

Yacob *et al.* (2008b) studied the ectoparasites prevalent in small ruminants in and around Wolaita Soddo (Southern Ethiopia). A total of 214 sheep and 102 goats of both sexes (169 females and 147 males) divided in young and adult animals (72 and 244 respectively) were examined for the presence of ectoparasites or skin lesions. The overall ectoparasite prevalence was 55.7 per cent (147 infested sheep and 29 infested goats) and sheep were significantly more often infected than goats. Within the sheep population, the ectoparasite frequency was significantly higher in females than in males. The main ectoparasites identified in this geographic area were ticks (*Rhipicephalus, Boophilus* and *Amblyomma*) and fleas (*Ctenocephalides felis* and *C.canis* at a lesser extent). The tick infestation was significantly more frequently observed in sheep (31.78%) than in goats (18.63%) and was unaffected by the sex or the age.

Abunna *et al*. (2009) surveyed the prevalence of tick infestation in small ruminants of 328 goats and 40 sheep in Oromia region, Ethopia.The prevalence of tick infestation in goats and sheep was found to be 89.9 and 87.5 per cent respectively. In this survey, ten species of ticks grouped under four genera were identified. The most abundant species found in this survey were *Boophilus decoloratus* (60%), *Rhipicephalus pulchellus* (25.1%), and *Amblyomma gemma* (11%), *Hyalomma dromedarii* was the minor species observed on goat. The difference in the prevalence of tick infestation between sheep and goats was not

statistically significant (X^2 = 0.22, P = 0.63) but found to be statistically significant between male and females (X^2 = 9.8, P =0.03).

Barmon *et al.* (2010) recorded ectoparasites in sheep in Gaibandha district of Bangladesh. A total of 120 sheep were examined, among them 95 (79.2%) were infested with more than one species of ectoparasites. Among them three species were arachanids, *Haemaphysalis bispinosa* (16.7%), *Boophilus microplus* (12.5%), *Psoroptes ovis* (5.8%) and two species of lice (%).

Irshad *et al.* (2010) surveyed the prevalence of tick infestation and theileriosis in small ruminants of Islamabad and Kherimurat districts of Pakistan. A total of 662 animals (219 sheep and 443 goats) were screened for the presence of ticks. Of these, 95(43.37%) sheep and 184 (41.53%) goats were found infested with different species of ticks *viz.*, *Haemaphysalis, Ixodes, Amblyomma* and *Rhipicephalus* spp. *Rhipicephalus* spp were found to be the most abundant tick species infesting both in sheep and goats.

Nasiri *et al*. (2010) screened a total of 1095 sheep for tick infestation seasonally. Totally 864 hard ticks were collected and identified as *Hyalomma marginatum* (44.67%), *Hyalomma anatolicum* (43.17%), *Haemaphysalis asiaticum* (6.37%), *Hyalomma dromedarii* (5.55%), *Haemaphysalis sulcata* (0.24%). The highest seasonal activity was observed in spring (36.46%) and lowest in winter (11.57%).

Salim *et al*. (2010) screened about 83 hard ticks. The ticks were classified into three genera and 7 species including: *Hyalomma dromedarii* (55.92%), *Hyalomma marginatum* (13.20%), *Hyalomma anatolicum* (9.78%), *Hyalomma detritum* (4.98%), *Hyalomma*

asiaticum (3.94%), *Rhipicephalus sanguineus* (11.84%) and *Dermacentor marginatum* (0.34%). The highest seasonal activities occurred in summer. The prevalence of the Ixodidae ticks was more evident in plateaus area in Yazd Province.

Mustafa (2011) studied the distribution of ticks in sheep in three different zones in Sulaimani governate, by examining 2525 sheep. The prevalence rate of infested sheep in all zones was 11.8 per cent with prevalence of *Hyalomma anatolicum anatolicum, Hyalomma marginatum, Rhipicephalus turanicus* and *Rhipicephalus sanguineus.*

Tesfaye *et al.* (2012) examined a total of 395 small ruminants (280 sheep and 115 goats) for the presence of different ectoparasites. Among the 395 animals examined, 193 were positive for one or more type of ectoparasite with the highest prevalence of ticks followed by fleas, lice and keds. Ticks species were identified as *Rhipicephalus* with the highest proportion followed by *Amblyomma* and *Hyalomma* spp. The overall prevalence was significantly ($p<0.05$, OR=2.2) higher in sheep (54.8%) than goat (34.9%). Total ectoparasite prevalence was significantly ($P<0.05$) higher in young than adult small ruminants. Tick prevalence was markedly ($P<0.05$) higher in sheep than in goat.

Zakkyeh *et al. (*2012) reported the prevalence of ticks infesting sheep in Iran viz., *Rhipicephalus sanguineus* (82.35%), *Rhipicephalus bursa* (0.3%), *Ixodes ricinus* (15.2%), *Boophilus annulatus* (1.2%), *Haemaphysalis punctata* (0.3%) and *Haemaphysalis numidina* (0.6%) and the 55 per cent of tick specimens were PCR positive against the genome of *Theileria ovis.*

Abunna *et al.* (2012) made a survey to determine the prevalence of tick infestation in small ruminants of 121 goats and 330 sheep. The prevalence of tick infestation in goats and sheep was found to be 66.12 per cent and 80.7 per cent, respectively. Most abundant species found were *Amblyomma coherence* (35.25%), *Amblyomma gemma* (17.07%) and *Amblyomma lepidum* (18.48%), *Amblyomma variegatum* (*15.96%*), *Rhipicephalus evertsi evertsi* (2.44%) and *Boophilus decoloratus* (0.89%). *Boophilus decoloratus* is the minor species of tick observed on sheep and none of these ticks were recorded in goats in the study area of Ethopia.

Zangana *et al.* (2013) carried out an investigation in Duhok region of Iraq to study the ectoparsites of sheep (1248) and goats (954) in 110 flocks. Among them, majority of the animals (66.89%) were infested with one or more species of ectoparasites. Of which 720 (57.7%) and 753 (78.9%) sheep and goats, were infested respectively. The highest prevalence of five species of hard ticks (Ixodidae) was identified in both sheep and goats, namely *Hyalomma anatolicum anatolicun* (48.37%, 13.5%), *Hyalomma marginatum* (18.01%, 8.1%), *Rhipicephalus sanguineus* (21.09%, 39.93%), *Rhipicephalus turanicus* (16.8%, 49.54%) and *Haemaphysalis* spp. (2.5%, 0.9%).

Mohamed *et al*. (2013) screened 1600 sheep and 520 goats and found that 322 (20.1%) sheep and 182 (35%) of goats were infested with one or more ectoparasites. The high prevalence of sheep infested by ticks was 40.9 per cent while in mites was 10.9 per cent. While in goats was (9.3%) during the winter and spring season. Ticks were the most frequent ectoparasites on sheep (18.7%), while fleas were the highest prevalence ectoparasites on goats (17.9%). The identification showed two different species of tick

(*Hyalomma anatolicum anatolicum* and *Rhipicephalus turanicus*), one species of mites (*Sarcoptes scabiei*), one species of lice (*Linognathus africanus*) and two species of fleas (*Pulex irritans* and *Ctenocephalides canis*).

Riabi and Atarodi (2014) surveyed 612 ticks during summer, 6.4 per cent from the goats, 3.7 per cent from the sheep and 89.9 per cent from the cows in Khorasan Iran. 71.5 per cent of the ticks were from *Hyalomma anatolicum excavatum*, 25.6 per cent from *Hyalomma anatolicum anatolicum* and 2.7 per cent from *Hyalomma lusitanicum* spp. 57 per cent of the ticks were females and 43 per cent were males.

Gebreselama *et al.* (2014) reported the prevalence rate of 47 per cent in cattle and 40 per cent ectoparasite infestation in sheep with a widespread occurrence of major ectoparasites *i.e.*, ticks (*Amblyomma, Boophilus, Rhipicephalus* and *Hyalomma* spp), lice (*Damalina, Linognathus* spp) and mange mites (*Demodex* spp and *Psoroptes* spp).In this study the ticks and lice infestation in both cattle and sheep were higher in extensive farm system than intensive farm system at Bishoftu, Central Ethopia.

Sarani *et al.* (2014) screened 25 herds with 498 animals for the prevalence of ticks in Golestan, Iran. A total of 255 ticks was collected from a total of 219 ruminants including 44 sheep, 63 goats, 99 cows and 13 camels in two districts of the mountainous area of Golestan province, Azadshahr and Ramian. Five species of ixodid ticks were identified: *Rhipicephalus sanguineus* (66.5%), *Rhipicephalus bursa* (4.6%), *Hyalomma marginatum* (19.9%), *Hyalomma anatolicum* (6%) and *Hyalomma asiaticum* (4%).

Jafarbekloo *et al.* (2014) reported the occurrence of ticks in goats, cattle and sheep as 17, 15 and 26 per cent respectively. Totally three genera hard ticks including *Hyalomma, Rhipicephalus* and *Dermacentor* spp were recorded in these regions. *Hyalomma anatolicum*, *Hyalomma marginatum*, *Hyalomma asiaticum*, *Hyalomma detrinium*, *Rhipicephalus bursa*, *Rhipicephalus sanguineus*, *Dermacentor niveus*, and *Dermacentor marginatus*, were the tick species identified. *Hyalomma anatolicum anatolicum* and *Hyalomma asiaticum* were the most abundant species in the study area.

Desalegan *et al.* (2015) reported the prevalence of tick infestation in cattle, sheep and goats as 25.23, 10.1 and 10 per cent, respectively. The most abundant species found were *Boophilus decoloratus* (47.8%) followed by *Amblyomma variegatum* (28.4%), *Amblyomma gemma* (12.48%), *Rhipicephalus pulchellus* (9.3%) and, *Rhipicephalus evertsi evertsi* (2.02%). The difference in the prevalence of tick infestation between species and age was statistically significant ($X2$=25.143, P=0.000 and $X2$=21.806, P=0.000) respectively. But sex, breed, locality and body condition were not statistically significant (P>0.05).

Iqbal *et al. (*2015) studied the prevalence of tick infestation in 1200 sheep of various breeds, age and sex. *Hyalomma marginatum* and *Rhipicephalus(B) annulatus* were the predominant species. The prevalence was high during July and minimum (8%) during November and December, while the females were more heavily infested than males and younger animals were burdened than older ones.

Tamerat *et al.* (2016) screened a total of 400 small ruminants (244 Caprine and 156 Ovine) for the presence of ecto-parasite. There was 148 (37.0 %), in which caprine and

ovine had the prevalence of 34.8% and 40.4 per cent, respectively. The decending order of proportion of prevalence of ectoparasite in descending order were 17.2 per cent ticks, 11.5 per cent mite, 8 per cent lice, 7.2 per cent fleas and 0.5 per cent keds.

Riaz *et al.* (2017) studied the prevalence of tick infestation and reported 48.0% in small ruminants. Sheep was more infested with ticks (50.0%) compared to goats (43.6%). *Hyalomma anatolicum* (52.2 %) was the dominant tick species followed by *Rhipicephalus sanguineus (*17.4 %). The mixed infection was found in 30.4 per cent of small ruminants.

Rehman *et al*. (2017) screened 471 animals, including 179 cattle, 194 buffaloes, 80 goats and 18 sheep covering both semi-arid and arid agro-ecological zones in Pakisthan. In total of 3,807 ticks, representing four species were collected: *Hyalomma anatolicum* (n = 3,021), *Rhipicephalus microplus* (n = 715), *Hyalomma dromedari* (n = 41) and *Rhipicephalus turanicus* (n = 30). *Rhipicephalus microplus* was the predominant species in the semi-arid zone, whereas *Hyalomma anatolicum* was the most abundant species in the arid zone.

2.2 Efficacy Studies of Acaricides in Control of Ticks

Ticks and tick borne diseases are a major constraint to livestock health in many parts of the world including India where ticks are widely prevalent and losses caused by this tick to livestock is a combination of both direct and indirect effects. Direct effects on production include skin damage from tick bites (Biswas 2003), blood loss, toxicity from bites blood, reduced animal weight gain and milk production, whereas the indirect effects are related to the transmission of tick borne diseases like *Theileriosis, Babesiosis* and *Anaplasmosis.*

Acaricides have played a pivotal role in control of ticks but its indiscriminate and incessant use with improper concentrations has probably contributed to the development of resistance to most of the acaricides in several countries (FAO 2004). Large scale resistance to organophosphate (OP) compound, synthetic pyrethriods and formamidine has been experimementally validated against ticks in cattle, however the reports of development of acaricide resistance against ticks in sheep are limited.

Barnard *et al.* (1981) assessed the techniques for acaricide susceptibility in *Amblyomma americanum* by using bioassay method, filter paper residue, pipette and tea bag methods. Out of fourteen compounds used, Amitraz, permethrin, chlorofos and lindane in the order were most toxic based on LD_{50} values.

Heller – Haupt and Verma *(*1982) studied the effect of synthetic pyrethroids cypermethrin, deltamethrin and permethrin on African ticks *Rhipicephalus appendiculatus* and *Amblyomma hebraeum* using *in vitro* tests. They reported that the older nymphs were more susceptible than younger nymphs, but the difference in age-related susceptibility of the two species was less marked. The results showed that there is a significant and positive correlation between tick age and susceptibility to synthetic pyrethroids.

Rajeshwari *et al*. (1984) assessed the acaricidal efficacy of propetamphos at 0.035 per cent and 0.05 per cent on *Rhipicephalus sanguineus* in dogs and with 0.05 per cent in horses with *Otobius megnini* and with 0.05 per cent in cattle infested with mixed infestation of *Boophilus microplus* and *Hyalomma anatolicum anatolicum,* it was found that 0.05 per cent concentrations brought about complete mortality of ticks.

Synthetic pyrethrins against *Boophilus microplus* was evaluated by *in vitro* tests by Khan and Srivastava (1988a). The engorged females reared in laboratory was immersed for one minute in various concentrations of cyperkill (25% EC cypermethrin) and permasect (25% EC). *In vitro* trials against engorged females of *Boophilus microplus* revealed that cyperkill was more effective than permasect.

Basu *et al*. (1990) studied the efficacy of Neocidol application, inducing death within 24hrs against *Boophilus microplus, Hyalomma anatolicum anatolicum, Haemaphysalis bispinosa, Linognathus vituli, Haematopinus eurysternus* and *Haematopinus tuberculatus.* Following 11th day of first application, re infestation started indicating its diminished residual effect.

Abdul - Rahman *et al*. (1992) studied the acaricidal efficacy of Butox (Deltamethrin) against ectoparasites. A single application of the drug (25ppm) was found to be effective against *Rhipicephalus sanguineus,* 12.5 ppm for *Heterodoxus spiniger* and *Ctenocephalides felis felis*. Whereas weekly twice application of Butox (50ppm) required against *Sarcoptes scabiei*. Within 24 hrs of application of Butox, the ticks, lice and fleas were found dead on the animal and no live ectoparasites were observed on the treated animals up to 40 days post- treatment.

Srivastava *et al*. (1993) evaluated the efficacy of deltamethrin (BUTOX®) against ixodid ticks *Hyalomma anatolicum anatolicum* and *Boophilus microplus* by filter impregnation method at different concentrations and found that 0.2 per cent butox solution was the appropriate concentration to kill all stages of both species of ticks.

Efficacy of deltamethrin was evaluated by Bagherwal *et al.* (1994) against different stages of *Hyalomma anatolicum anatolicum. In vitro* tests conducted with concentrations of 25, 50 and 75ppm were 100 per cent effective against larvae and at 81.3,93.6, 98.2 per cent were effective against nymph and in case of adults it was 76.4,89.1 and 96.4 per cent effective. Hundred per cent inhibition of oviposition were found at 50 and 75 ppm concentrations.

Davey *et al.* (1998) reported the efficacy permethrin against strain of *Boophilus microplus* (*Canestrini*), the pyrethroid resistant (PR) strain was 5.4 times more resistant to permethrin than the pyrethroid susceptible (PS) strain, and the level of resistance increased in each successive generation of the PR strain, reaching a resistance factor (resistance factor) of 20.9 in the F7 generation. Thus, in only 5 generations the level of resistance in the PR strain was increased by 4-fold.

Talukdar *et al*. (1998) studied the efficacy of Deltamethrin (Butox) and diazinon (Neocidol) on animals infested naturally with *Haemaphysalis bispinosa.* Deltamethrin at 25 ppm concentration had 100, 84.66 and 72.86 per cent effect against larvae, nymphs and adults, respectively, while at 50 ppm concentration provided 100, 92.16 and 86.08 per cent control of these stages. Residual effect at these two concentrations was seen up to 18-20 days and 25-30 days, respectively.

Sangwan *et al.* (1988) compared the efficacy of some pesticides against *Hyalomma anatolicum anatolicum* ticks by *in vitro* assay. Commercially available pesticides were used *viz.,* oxinotiofos, amitraz, decamethrin, melathion and diazinon. Decamethrin was

most effective, oxinotiofos and diazinon found moderately while amitraz was least effective.

Biological efficacy of amitraz against the three host tick *Rhiphicephalus sanguineus* was evaluated by Shiva kumar *et al*. (2001). Filter paper method was used to evaluate the efficacy on engorged females and where as the tea bag method was followed. Amitraz at the concentrations of 150 ppm, 200 ppm, 250 ppm, 300 ppm and 350 ppm was found to be effective against all the stages. The efficacy of acaricides on *Boophilus microplus* was evaluated using chlorfenvifos / diclorofenil (0.05% / 0.015%), amitraz (0.025%), cypermethrin (0.015%), deltamethrin (0.0025%), coumaphos (0.05%). The efficacy of acaricides varied from 0.23 per cent to 100 per cent.

Souza *et al*. (2004) compared the efficacy of acaricides deltamethrin (0.0025%), cypermethrin (0.01%), amitraz (0.025%) and alphamethrin (0.05%) by using *in vitro* methods against *Boophilus microplus* at different immersion times. Efficacy of deltamethrin was found to be better when compared to other acaricides used in this study.

Vatsya and Das (2004) studied the acaricidal efficacy on the biological activities of female *Boophilus microplus.* Herbal acaricides namely pestoban and pestomar and chemical acaricides namely Butox (12.5% deltamethrin) and Sumicidin (20% fenvalerate) were used at 0.05 and 0.005 per cent concentrations. Engorged ticks at the concentration of 0.05 per cent of both chemical and herbal acaricides failed to produce the egg.

Bagherwal *et al. (*2005) studied the efficacy of Cypermethrin (Cyperol) against different stages of *Hyalomma anatolicum anatolicum*, by *in vitro* assay. It was observed

that the concentrations 92.3, 94.5 and 100 per cent were effective against nymphs and 88.3, 89.5 and 92.6 per cent effective against adults respectively.

Barre *et al.* (2008) conducted *in vitro* and *in vivo* on-animal efficacy trials, for the control of resistant *Rhipicephalus (B) microplus* using the modified larval packet tests (LPT) where it revealed up to 16.59-fold resistance to deltamethrin, and up to 5.86-fold resistance to amitraz.

Sajid *et al*. (2009) compared the efficacy of ivermectin and cypermethrin pour-on, for the treatment of *Hyalomma anatolicum anatolicum* infestations by *in vitro* and *in vivo* assays. The comparative quantitative assessment of tick burden was done on days 0, 5, 10, 15, and 20 after treatment using "finger counting." The results of the tick survival assay indicated both compounds were effective *in vitro* against *Hyalomma anatolicum anatolicum.* The transformed mean surviving ticks after 24 h post immersion, was 2.66 and zero in groups treated with the highest dilutions of IVM and CYM, respectively. At 15 days post-treatment, the CYM pour-on showed a higher *in vivo* efficacy (no surviving ticks) compared to IVM (mean of 20 surviving ticks).

Castro-Janer *et al.* (2010) studied the resistance of various tick populations to technical grade fipronil (95.3%) using the larvae immersion test (LIT) and the larval packet test (LPT), highlights the LIT as a more sensitive technique for the evaluation of fipronil resistance in *R. microplus* ticks.

Sharma *et al*. (2012) assessed the AIT with a discriminating dose to detect deltamethrin and cypermethrin resistance in the field isolates of *Rhipicephalus microplus.*

The overall prevalence of SP-resistant *Rhipicephalus microplus* among the sampled farms was 66.6 per cent (18/27). Out of these 18 areas, resistance to deltamethrin at level I was detected in 08 areas (resistance factor=2.0-4.9), at level II in 09 areas (RF=5.2-11.8), at level III in 01 area (RF=34.9) and at level IV in 01 area (RF=95.7). The resistance to cypermethrin was detected in 16 areas and level of resistance was detected at level I in 10 areas (RF=2.06-4.64) and at level II in 06 areas (RF=5.13-9.88).

Shyma *et al.* (2012) evaluated the acaricide resistance of *Hyalomma anatolicum* to deltamethrin, cypermethrin and diazinon. Results obtained by the "larval packet test" (LPT) showed a low grade resistance (level-I, RF <5) in the tick species to both deltamethrin and cypermethrin in ten areas and higher grade resistance (level-II, RF <25) to deltamethrin in one area, where intensive use of synthetic pyrethroids are practiced for tick control. Low grade resistance to diazinon (level I) was recorded in six areas where organophosphates compounds are extensively used for agricultural practices allowing increased exposure of the moulting instars of the ticks to these chemicals.

Shemshad *et al*. (2012) screened about 2638 sheep, 461 goats and 318 cattle of 38 herds in different geographical areas of Iran. The species collected 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%).

Ravindran *et al.* (2014) compared the acaricidal activity of commercially available preparations of cypermethrin (Clinar and Ectomin) and fenvalerate (Flytik and Ticomax,

20% E.C) against *Rhipicephalus* (*Boophilus*) *annulatus* using adult immersion test. Adult tick mortality was higher with Ectomin compared to Clinar. At the recommended dosage of 200 ppm Ectomin elicited 93.37 per cent inhibition of fecundity, while it was 91.7 per cent for Clinar. For fenvalerate, the recommended concentration was 1200 ppm at which Ticomax showed 86 per cent and Flytik produced 80.05 per cent inhibition of fecundity.

Shyma *et al.* (2015) evaluated the efficacy of deltamethrin, flumethrin, and fipronil against *Rhipicephalus (Boophilus) microplus* using Adult Immersion Test (AIT) and Larval Packet Test (LPT) using field strain for determination of 50 and 95 per cent lethal concentration of deltamethrin, flumethrin, and fipronil. Results obtained by the Adult Immersion Test showed that a low grade resistance (level I, RF > 5) has been developed against both deltamethrin and fipronil. However, deltamethrin by performing Larval Packet Test showed moderate grade resistance (level II, RF > 25).

Singh *et al.* (2015) estimated the efficacy of amitraz and cypermethrin against *Hyalomma anatolicum anatolicum* by larval packet test (LPT) with different concentrations of amitraz (125, 250, 500, 750 and 1,000 ppm) and cypermethrin (100, 200, 300, 400 and 500 ppm). The regression graphs of mean mortality of larval ticks indicated an increasing concentration of amitraz and cypermethrin for the estimation of LC_{95} values and were determined as 1,529.39 and 351.84 ppm.

Ibarra-Velarde *et al*. (2015) studied the efficacy of cypermethrin, zeta permethrin along with plant extracts using larval packet tests which revealed an efficacy of 100 per cent (Permethrin), 100 per cent (Zeta permethrin), and 98.03 per cent (Cypermethrin) using susceptible larvae, and an efficacy of 88.67 per cent (P), 91.51 per cent (C), and

99.27 per cent (Z) on triple-resistant larvae. Engorged females were explored to *in vitro* with the experimental products, and efficacy was as follows: 91.37 per cent (Z), 85.95 per cent (C), and 13.58 per cent (P). Adding plant extracts to a pyrethroid formulation led to dramatic increases of per cent reduction of both susceptible and resistant immature ticks in contrast to untreated larvae and susceptible adults.

2.3 Efficacy studies of Entomo -pathogenic nematodes in control of ticks

Samish and Glazer (1991) described that the EPNs viz, *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* killed engorged *Boophilus annulatus* ticks under laboratory conditions within 4 days post infestation by releasing symbiotic bacteria causing septecemia and death of insects after penetration.

Infectivity of EPNs (Steinernematidae and Heterorhabtidae) was studied by Samish and Glazer (1992) on female ticks of *Boophilus annulatus* and found exposing *Boophilus annulatus* to different concentrations of *Steinernema carpocapsae* infective juveniles in petridishes (50-10,000 nematodes per dish, 100 per cent of the ticks died within two days after inoculation and within four days of exposure after exposure of *Boophilus annulatus* with *Heterorhabditis bacteriophora* srain HP 88 at a concentration of 500 nematodes per dish.With the Mexican and all strains of *Steinernema carpocapsae* it was only 15 and 40 per cent mortality recorded respectively.

Glazer and Samish (1993) reviewed the suitability of engorged *Boophilus annulatus* tick as the hosts of nematode *Steinernema carpocapsae* after exposing to 2500 infective larvae and its symbiotic bacterium *Xenorhabdus nematophilus*. The results indicated that despite the high susceptibility of female *Boophilus annulatus* to nematode

infection and the establishment of the symbiotic bacteria, *Boophilus annulatus* was not a suitable host for the development of the nematode.

Mauleon *et al. (*1993) described the pathogenicity of 17 different isolates of EPNs (Steinernematidae and Heterorhabdtidae) for the ticks *Amblyomma variegatum, Boophilus annulatus* and *Boophilus microplus.* Eight strains of Steinernema and nine strains of Heterorhabditidae were placed in contact with each of the free living stages of three tick species where they found *Boophilus annulatus* was susceptible whereas *Amblyomma variegatum* and *Boophilus microplus* were resistant to all the nematodes.

Zhioua (1995) described the pathogencity of *Steinernema carpocapsae* and *Steinernema glaseri* against *Ixodes scapularis.* He found no difference in the timing of tick mortality and overall mortality after 17 days of infection. However, both species successfully produced infective juveniles when the tick body was slit before nematode infection. He even recorded that the mortality of infected ticks was greatest between 20 and 30˚C and was lower at 15˚C.

Kaya and Stock (1997) detailed the techniques in insect nematology and described the identification, classification, culture, quantification and preservation of EPN's and even their symbiotic bacteria.

Hill (1998) evaluated thirteen species or strains of EPNs of the genera *Steinernema* and *Heterorhabditis* by *in vitro* tests against unfed and engorged larvae, nymphs and adults of *Ixodes scapularis* and found that nematodes were pathogenic to engorged female ticks, but not to unfed or engorged larvae, nymphs, males and unfed females.

Interaction of Steinernematidae with *Dermacentor variabilis, Rhipicephalus sanguineus, Amblyomma maculatum and Amblyomma cajennense* was studied by Kocan *et al.* (1998). It was found that all species of ticks were susceptible to *Steinernema feliiae* or *S.riobravus,* to be attracted to kill and replete *A.americanum, S.riobravus* killed *D.variabilis* (96%), *R.sanguineus* (89%), *A.maculatum* (24%) and *A.cajennense* (88%) and *S.feltiae* killed *D.variabilis* (91%) and *R.sanguineus* (71%).They also reported that the nematode – exposed ticks were examined with light microscopy and were found to have entered ticks but didn't multiply or produce subsequent generations of infective juveniles.

The larval, nymphal and adult stages of the ticks of *Hyalomma excavatum, Rhipicephalus bursa* and *R.sanguineus* were assessed for their susceptibility to two strains of *S.carpocapsae* and three strains of *H.bacteriophora* in laboratory assays by Samish *et al*. (1999a). The preimaginal stages of ticks were found to be more resistant to the nematodes than the adult ticks which exhibited 80-100 per cent mortality. On unfed adult ticks *H.bacteriophora* IS-3 or IS-5 were found to be more virulent. They also found that not much difference between engorged ticks and unfed adults of *Rhipicephalus sanguineus* or *Hyalomma excavatum* in terms of mortality, whereas the effect on engorged males and unfed females of *Rhipicephalus bursa* were reported significant.

Samish *et al.* (2000a) reported the adult ticks mortality rate of *Boophilus annulatus*, *Hyalomma excavatum*, *R.bursa* and *R.sanguineus*. Mortality rates (LT 50 and LT90) for *Boophilus annulatus* engorged ticks was 0.8 – 5.0 days, whereas the unfed males died on 0.3 – 2.8 days after the unfed females. *Heterorhabditis* spp strain IS-3 and IS-5 appeared to be more pathogenic for ticks and killed ticks several days before other three nematode

strains. Major observation made was that the unfed adult ticks exposed to nematodes died within less time than adults to complete their pre feeding period after moulting.

Detailed study on biocontrol of ticks was made by Samish *et al.* (2000b) and found that EPNs were lethal to ticks even though they didn't use their normal propagation cycle within tick cadavers. They even found that *Boophilus annulatus* was more susceptible to EPNs than *Hyalomma excavatum, R.bursa* or *R.sanguineus.*

Factors affecting the virulence of EPNs to engorged female *Boophilus annulatus* ticks were studied by Glazer *et al.* (2001). Based on effect of exposure time on tick mortality, the quantity of nematodes that penetrate ticks and the rate of tick mortality after the injection of one, two or three nematodes found that the six hour exposure time of the ticks to heterorhabditid strains resulted in 80 per cent mortality, but only 20 or 65 per cent resulted against most steinernematids.

Samish and Glazer (2001) described the potential of EPNs for the control of ticks. It was observed that entomopathogenic steinernematid and heterorhabditid nematodes were increasingly used to control insect pests of economically important crops. Field and laboratory stimulation trials showed that ticks were also susceptible to these nemtaodes.

Samish *et al*. (2004) reported different aspects of pathogens such as bacteria, fungi, parasitoids, predators and EPNs for biological control of ticks and emphasized that EPNs are pathogenic to ticks and can potentially control them.

Alekseev *et al*.(2006) studied the effect of soil texture and moisture on the activity of EPN's against female *Boophilus annulatus* ticks.The activity and persistence of EPNs

viz, *Steinernema carpocapsae*, *Steinernema riobrave*, *Steinernema felitae*, *Steinernema carpocapsae* strain S-20 and heterorhabditid strains were observed.For nematodes, soil layer acts as a natural habitat .The arthropod pests and ticks prefer to stay on the soil surface and under stones or leaf litter and spend their life cycle in the humid environment of the soil upper layer where EPNs work on the ticks.

Ghosh *et al.* (2007) described the different aspects and future strategies in control of ticks and about tick vaccine, newer generation acaricides, herbal acaricides and emphasized endosymbiotic approach to control ticks.

Hanan *et al*. (2008) studied the quantitative and qualitative changes in heamolymph of *Hyalomma dromedarii* ticks by infecting it with four different EPNs viz, *Steinernema* spp. SII, *Steinernema carpocapsae* DD 136, *Heterorhabditis* sp.TWF and *Heterorhabditis bacteriophora* Hp88. The haemolymph was collected after 24, 48, 96 and 178 hrs intervals and proteins were separated in SDS-PAGE electrophoresis and found that tick mortality was related to marked changes in haemolymph (HL) proteins due to EPNs.

Carvalho *et al*. (2010) assessed the *in vitro* infection time of engorged female *Rhipicephalus (Boophilus) microplus* by the *Steinernema glaseri* CCA strain and they reported that the susceptibilities of ticks depended on the species of tick and development phase, species and strains of EPN and the time of exposure. The results showed that a 2hr exposure time was sufficient for the engorged *Rhipicephalus microplus* females to be infected by *Steinernema glaseri* CCA. They even observed that a minimum exposure of 24hr was necessary to bring about above 90% mortality.

James *et al.* (2010) evaluated the efficacy of different species of EPNs at different temperatures and when applied to wool by formulating in water with 8 per cent Tween 80 on *Bovicola ovis*. They found that *Steinernema carpocapsae, Steinernema riobrave* and *Steinernema feltiae* caused significantly higher lice mortality than *Heterorhabditis bacteriophora* at both 25 and 35˚C in wool assays. The mortality didn't differ among three Steinernematid species. Even though *Steinernema riobrave* was found most effective against *Bovicola ovis* when applied on body of sheep because of its greater tolerance to high temperature.

Monterio *et al. (*2010a) demonstrated that under laboratory conditions, *Heterorhabditis bacteriophora* HP 88 had a deleterious effect on the majority of the parameters of the non-parasitic phase of engorged *Rhipicephalus microplus* females, making this species a potential biological control agent of cattle ticks and they evaluated the effect at different concentrations (0, 75, 150, 300, 600 and 1200 EPNs/female). Treatment efficacy was higher than 90 per cent in all groups, reaching 99 per cent at a dosage of 1200 EPNs/female.

The effect of *H.bacteriophora* strain HP 88 on the biological parameters of the non-parasite phase of engorged females of *Rhipicephalus microplus* was studied under laboratory conditions by Monterio *et al*. (2010a). The treatment efficacy didn't reveal statistically significant differences in the female weight before oviposition and post oviposition period ($p \geq 0.05\%$) between the groups. Between the treated groups and the control group the nematode action caused a significant reduction ($p \leq 0.01\%$) in egg mass

weight, oviposition period, survival period , hatching percentage , per cent EPI and NI per cent between the treated groups and the control group.

Dose difference of *H.amazonensis RSC-5* on the biological parameters of engorged females of *Rhipicephalus (Boophilus) microplus* was evaluated by Monterio *et al*. (2010b). Between the treated and control group was no significant differences observed for the initial weight, pre-oviposition period, incubation period and NI. Where as there was a significant differences for the oviposition and survival period between all treated groups.

Monterio *et al.* (2010b) assessed the different doses of *Heterorhabditis amazonensis* RSC-5 on engorged females of *Rhipicephalus microplus*. The highest control rate of 67.8% per cent obtained at concentration of 300 EPN/tick.

Natural occurrence and distribution of entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) in Nepal for the first time was studied by Hari *et al.* (2010). In 276 soil samples, 29 EPN were recovered. Among the 29 positive samples collected, 22 samples (75.86%) contained steinernematidae and seven samples (24.14%) yielded heterorhabditis (*H.indica*) .Among Steinernematids, seven isolates were identified as one of four described species (*S.abbasi, S.cholashanense, S.feltiae* and *S.siamkayai*) where as 15 isolates did not fit any description. From the diverse habitats *Heterorhabditis indica* was isolated.

Monterio *et al*. (2012) evaluated the influence of infective juveniles of *Heterorhabditis bacteriophora* isolate HP88 at different exposure times on engorged Rhipicephalus female. They concluded that under laboratory conditions infective juveniles

of *Heterorhabditidis bacteriophora* HP88 interfere with majority of the biological parameters of the non-parasitic phase of engorged *Rhipicephalus microplus* female when the exposure time was greater than or equal to 24hr.

The action of *Heterorhabditis indica* (Rhabditida: Heterorhabditidae) strain *LPP1* on engorged females of *Rhipicephalus microplus* was studied by Silva *et al.* (2012). The action of the nematodes resulted in the percentage of control was above 97 per cent, reaching 100 per cent at concentrations of 3000 and 12,000 EPN s/female in all groups. They showed that *H.indica* LPP 1 was the one of the most virulent species under laboratory conditions.

The effect of infective juveniles of *H.bacteriphora* isolates HP88 on engorged female *Rhipicephalus microplus* was studied by Monteiro *et al.* (2012). Pre- oviposition period, egg mass weight and hatching percentage were observed during this study whereas there was no influence of time on pre –oviposition period ($P \geq 0.05$). There was a significant differences ($P \leq 0.05$) between the groups exposed for 24, 48 and 72hr and the control group with respect to the egg mass weight and 24 hours for hatchability.

The action of *Heterorhabditis indica* (Rhabditida : Heterorhabditidae) strain LPP1 on the reproductive biology of engorged females of *Rhipicephalus microplus* was studied by Silva *et al*.(2012).They found that the action of nematodes resulted in significant reduction in egg mass weight (mg), egg production index (%EPI) and hatching percentage (%) in all treatment groups.

The pathogenicity of five species of EPNs (Steinernema and Heterorhabditidae) to the ixodid tick *Dermacentor silvarum Olenev* was assessed by Yang *et al.* (2013) and they found all five species to have lethal effects on adults ticks. Among them, Hb E-6-7 and Sc BJ were highly pathogenic to female adult ticks, with 70 to 90 per cent mortality. Nematodes species were found to be lethal to unfed male ticks and there was a significant difference in mortality when compared to the control group ($P \leq 0.05$).

Monterio *et al*. (2014a) for the first time reported about the pathogenicity of *Heterorhabditis* spp on engorged females of *D.nitens*. Effect was evaluated at different concentrations of *H.bacteriphora* HP88 and *H.indica* LPP1 on partially engorged females of *D.nitens*. The *D.nitens* were divided into four groups with 10 females each and exposed to concentrations of 0.75, 300 and 1200 nematodes for each female. The mortality percentage was obtained at concentrations of 75, 300 and 1200 EPNs/female were 56.3, 89.3 and 98.8 and 77.5, 77.1 and 95 per cent for *H.bacteriophora* HP88 and *H.indica* LPP1.

EPNs strains in insect cadaver formulations were evaluated for control of *R. microplus* and compared the EPNs applied in cadavers of *Galleria mellonella* and *Tenebrio molitor* by Monteiro *et al*. (2014b). EPNs of genus *Heterorhabditis* caused significant reduction in the egg mass weight and hatchability percentage of larvae. There was a significant reduction in egg mass weight produced per female. *Steinernema carpocapsae* with two cadavers and *S.feltiae* SN applied with two, four and six cadavers caused a reduction in hatching percentage of larvae of *Rhipicephalus microplus*.

2.4 Efficacy studies of Phytoacaricides in tick control

The use of synthetic acaricides to target ticks of veterinary importance is becoming increasingly problematic. One alternative approach employs the bioactive attributes of plant –derived products which are more ecofriendly, biodegradable and non-resistible as compared to synthetic pesticides. The literature pertaining to control of ticks by phyto extracts was reviewed.

Khudrathulla and Jagannath (1998) assessed the *in vitro* acaricidal efficacy of aqueous extracts of 6 and 7 month old plants of *Stylosanthes scabra* against larval and nymphal stages of *Boophilus microplus, Haemaphysalis intermedia* and *Rhipicephalus sanguineus.* Significant variation ($P < 0.01$) in the mortality of larvae and nymphs atvarious concentrations of aqueous extract was observed. One hundred per cent mortality of *B. microplus* larvae was noticed in 3.0 per cent and above, whereas 6.0 per cent in *R. sanguineus.* However, highest percentage mortality of 56 ± 2 was observed in *H. intermedia* at six per cent concentration.

Kalakumar *et al. (*2000) compared the *In vitro* and *In vivo* trials on the acaricidal activity of custard seed oil [*Annona squamosa*], neem oil [*Azadirachta indica*] and pyrethrins against *Boophilus microplus, Hyalomma anatolicum anatolicum* and *Rhipicephalus haemaphysaloides*. Custard seed oil and pyrethrins were found 100 per cent efficacious, whereas, neem oil was only 60-75 per cent effective in animals infested with ticks. Oviposition was inhibited in all the tick species treated with custard seed oil, whereas females treated with neem oil oviposited.

Chungsamarnyart and Jansawan (2001) studied the acaricidal activity of Crude-extract of *Tamarindus indicus* L. on the engorged female cattle tick (*Boophilus microplus*) by dipping method. The mean of corrected mortality of ticks of these 4 crude-extracts of tamarind fruits were 56-70 per cent, 70-89 per cent and 77-99 per cent by no statistically significant different after dipping at 24 h, 48 h and 7 days, respectively.

Abdel-Shafy and Zayed (2002) studied the effects of the plant extract of neem seed (*Azadirachta indica*) on eggs, immature, and adult stages of *Hyalomma anatolicum excavatum* at concentrations of 1.6, 3.2, 6.4, and 12.8 per cent. Extract was found to have a significant effect on the hatching rate of eggs. It significantly increased the hatching rate during the first 7 days post-treatment (DPT) giving incompletely developed and dead larvae; however, it caused hatching failure at DPT 15.

Choudhary *et al*. (2004) assessed the *in vitro* effect of Nicotiana tabacum aqueous extract on engorged adult *Rhipicephalus haemaphysaloides,* where the ticks were treated with undiluted and 50% diluted aqueous extract of *Nicotiana tabacum.* It was observed that both undiluted and 50% aqueous dilution of tobacco extract adversely affected egg laying and production of larvae.

Abdel-Shafy *et al*. (2007) studied that diethyl ether, ethyl acetate and ethanol extracts of *Artemisia herba-alba* achieved the highest toxicity against larvae of *Hyalomma dromedarii* followed by *A. monosperma,* while hexane extract of *A. monosperma* gave the highest toxicity followed by *Mesembryanthemum forskalii* and *Artemisia herba-alba.* The effect of *Silybum marianum* extracts was temporary but *Peganum harmala* had a prolonged effect on *Boophilus annulatus* females. Petroleum ether, chloroform and ethyl acetate

extracts of *Peganum harmala* exhibited higher toxicity than *Silybum marianum*, while ethanol extracts showed the opposite. Larvae of *Hyalomma dromedarii* were most susceptible to *A. monosperma* and *H. tuberculatum* oils than adults of *A. Persicus.*

Muraleedharan *et al*. (2008) studied the acaricidal effect of eight aqueous herbal extract against ixodid ticks. The leaves of *Clerodendron inerme* and *Croton honplandianum* in goats could reduce infestation to 68.5 and 64.19 per cent respectively on day 9. In sheep, the maximum reduction percentage recorded with the extract of flowers of targets was 30.08 on day 6 and with cloves of *Allium sativum*, 30.02 on day 7 whereas maximum reduction percentage with leaves of *Leucas aspera* was 29.25 on day 8, *Parthenium hysterophorus*, 15.03 on day 5, *Synadenium grantii* 11.57 on day 4 and *Lantana camara* 3.47 on day 1 post treatment.

Muraleedharan *et al*. (2009) studied the effect of certain neem (*Azadirachta indica*) preparations against natural infestations of hard ticks, *Haemaphysalis* and *Hyalomma* spp on sheep and goats. Undiluted neem oil provided 100 per cent reduction of ticks on day 3 to 5 on sheep and 94.42 per cent on goats on day 4. Aqueous extract of 25g neem kernel processed in 100ml water had reduced tick counts to 84.86 per cent on day 10 on sheep. One per cent aqueous emulsion of Neemrich I 80-EC caused a reduction of 65.95 and 36.38 per cent of ticks on sheep and goats, respectively on day 6.

Choudhary *et al.* (2009) reported the *in vitro* toxicity of neem seed oil (*Azadirachta indica* A. Juss) against the larvae of a one-host tick, *Boophilus decoloratus* at 20, 40, 60, 80 and 100 per cent concentrations. They were found to kill all (100% mortality) the larvae after 27, 27, 27, 27 and 24 h respectively.

Zahir *et al*. (2010) reported the *in vitro* evaluation of crude hexane, chloroform, ethyl acetate, acetone, and methanol extracts of *Anisomeles malabarica (L.) R. Br., Gloriosa superba L., Psidium guajava L., Ricinus communis L,* and *Solanum trilobatum L* exhibited acaricidal and insecticidal activities against the adult of *Haemaphysalis bispinosa* (Acarina: Ixodidae) and *hematophagous* fly, *Hippobosca maculata* Leach (Diptera: Hippoboscidae). All plant extracts showed moderate toxic effect on parasites after 24 h of exposure; the complete inhibition (100%).

Habeeb *et al*. (2010) reviewed the use of crude plant extracts and its method of application for tick control, where they mentioned that the botanical pesticides had different properties which might vary from plant to plant as they were processed in various solvents or resins. They found the effect of these extracts against ticks such as reducing tick feeding, molting, fecundity and viability of eggs.

Muraleedharan *et al*. (2010) compared the efficacy of group and individual spraying of acaricides on tick infested sheep and goats. Two pyrethroid acaricides, 0.15% sumicidin and 0.20% butox were used during this study. The results depicted the percentage control of ticks for day 7, 14 and 21, with corresponding weekly efficacy score and total efficacy score was higher in individual spraying than group spraying.

Ghosh *et al*. (2011) studied the efficacy of *Acorus calamus* extract against *Rhiphicephalus (Boophilus) microplus.* Extract of *Acorus calamus* proved highly efficacious and induced 100 per cent final mortality within 14 DPT. The LC 85 value of the extract was determined as 11.26. The *in vivo* experiments confirmed the efficacy of extract up to 42 per cent and repeat application was required after seven DPT.

Kumar *et al*. (2011) evaluated the *in vitro* and *in vivo* acaricidal activity of pudina, arand and yellow kaner on ticks. Results indicated that all these extracts had similar *in vivo* adulticidal activity on tick population, where as *in vitro* results revealed that the mortality percentage was higher as the concentration of extract increased and the time interval progresses. Statistical analysis of data further revealed that mortality was significantly different ($P \leq 0.5$) at 4 and 24hrs interval in case of crude extract of arand, yellow kaner and pudina.

Zaman *et al*. (2012) evaluated the efficacy of combined aqueous herbal extracts of *Azadirachta indica* leaves, *Nicotiana tabacum* leaves, *Calotropis procera* flowers and *Trachyspermum ammi* seeds on ticks by adult immersion test, larval packet test, and ear bag method. The extract exhibited lethal effects on egg laying index (0.371404±0.00435), hatching (22.35%) and total larval motility at 50 mg/ml and reduced tick intensity on the infested animals.

Santhosh kumar *et al*. (2012) investigated the adulticidal and larvicidal activity of dried leaf hexane, ethyl acetate, acetone, and methanol extracts of *Nelumbo nucifera, Manilkara zapota, Ipomoea staphylina* and *Acalypha indica* against the adults of *Haemaphysalis bispinosa* (Acarina: Ixodidae).The per cent parasitic mortality observed in the crude leaf hexane, ethyl acetate, acetone, and methanol extracts of *N. nucifera* and *M.zapota, Ipomoea staphylina* and *Acalypha indica* against *H. bispinosa* were 80, 74, 72 and 100 respectively.

Ghosh *et al*. (2013) studied the acaricidal properties of *Ricinus communis* leaf extracts against organophosphate and pyrethroids resistant *Rhiphicephalus (Boophilus)*

microplus by *in vitro* assays. The extract significantly affected the mortality rate of ticks in dose dependent manner ranging from 35.0 to 95.0 per cent with an additional effect on reproductive physiology of ticks by inhibiting 36.4-63.1 per cent of oviposition.

Marimuthu *et al*. (2013) studied the acaricidal activity of synthesized Titanium oxide nanoparticles using *Calotropis gigantean* against larvae of *Rhipicephalus microplus* and adult *Haemaphysalis bispinosa.* The maximum efficacy was observed in the aqueous flower extract of *C.gigantean* and synthesised TiO2 NPs against *Rhipicephalus microplus*. They found that the synthesized Titanium oxide nano particles were highly stable and had a significant acaricidal activity against the larvae of *Rhipicephalus microplus* and adult *Haemaphysalis bispinosa.*

Shyma *et al*. (2014) studied the acaricidal properties of crude methanolic extract of *Datura stramonium, Azadirachta indica* and *Calotropis procera* leaves, *Allium sativum* cloves and *Carica papaya* seeds against cattle tick *Rhipicephalus (Boophilus) microplus* using *in vitro* studies i.e Adult immersion test and larval packet test. At the highest concentration of 100mg/ml, the adult tick mortality was 66.67, 73.33, 80.00 and 93.33 per cent for *C.procera, D.stramonium, A.sativum* and *C.papaya* extracts respectively and it was statistically significantly.Larvae exhibited significant mortality ($P \leq 0.001$) 55.2, 63.2, 71.8, 69.0 and 82.2 per cent at the highest concentration.

Nyahangare *et al. (*2015) studied about the practice of spraying of the plant extract on animals for the control of ecto-parasites. Almost 51 plant species were effectively used to control ticks and study its herbicidal effect, in which the plants *Cissus quadrangularis,*

Lippia javanica and *Psydrax livida* were found to be more effective when compared to other plant extracts in controlling ticks.

2.5 Vector potentiality of ticks in transmitting haemoprotozoan disease

Raghavachari and Reddy (1959) reported the acute theileriosis at Hyderabad in sheep, due to *Theileria ovis* and *Theileria hirci.* The former parasite was considered non-pathogenic because it did not produce any clinical symptoms of disease while the latter is associated with a definite and recognisable fatal disease.

Bhattacharayalu *et al. (*1975) made preliminary observations on the ability of *Hyalomma anatolicum anatolicum Koch*, which was normally found on cattle but occasionally infested sheep in northern India, to transmit Theileria infections in sheep. Larvae and nymphs were fed on the ears of infected sheep.

Prabhakar (1976) recorded the outbreak of Babesiosis at sheep breeding station, Suthatti (Belagavi), Karnataka, where three sheep were positive for mixed infection of *Babesia* and *Theileria*. *Haemaphysalis intermedia* ticks were found predominant on sheep.

Prabhakar and Hiregoudar (1977) recorded the presence of theilerial parasites in 799 sheep (56.1%) out of 1424 blood smears of sheep examined. They also found that about 70.1 per cent of exotic breeds, 64.2 per cent of cross breeds and 52.3 per cent of indigenous breeds of sheep were positive for theilerial parasites. Mixed infection of Anaplasma organisms in 22 per cent of sheep was observed.

Wall and Shearer (1977) compared the detection of *Theileria parva* sporoblasts in *Rhipicephalus appendiculatus* ticks by staining histological preparations of ticks salivary

glands with methyl green pyronin stain (MGP) and by PCR. The detection of infection by the PCR was greater than the results from methyl green pyronin stain. The PCR could detect the low infection and high infection prevalence and its intensity.

Walker *et al*. (1979) reported the prevalence of *Theileria parva* and *Theileria annulata* in whole salivary glands of *Rhipicephalus appendiculatus* and *Hyalomma anatolicum* subspecies respectively by staining with Feulgen stain. There was considerable variability in the rate and intensity of infection of these ticks with theilerial parasites and it was concluded that the method permited large samples (60 ticks per person per day) to be examined to overcome this variability when assessing infection quantitatively.

According to Walker *et al*. (1981) approximately 1.5 per cent of the ticks in the field collections of Kenya which were found to be infected with *Theileria* spp. It was considered that it would be feasible to survey field infection rates quantitatively by a combination of salivary gland staining and preparation of tick suspensions for microscopy.

Reid and Bell (1984) studied the infective rate of *Theileria annulata* at Hisar, India from salivary glands of tick, *Hyalomma anatolicum anatolicum*, using Giemsa-stained smears, methyl green-pyronin-stained preparations of whole glands, and electron microscopy. Nymphs which had engorged on *T. annulata* infected calves showed kinetes in the haemolymph from day 7 to day 14 post engorgement, when all ticks had completed moult.

Kirvar *et al*. (1998) studied the maturation and quantification of *Theileria lestoquardi* (*T. hirci*) parasites in unfed and partially fed adult *Hyalomma anatolicum*

anatolicum ticks. With Methyl Green Pyronin staining the greatest infection rate was seen in unfed ticks. *In vitro* infection of sheep peripheral blood mono nuclear cells demonstrated that infectivity peaked between 2 and 4 days of tick feeding whereas GUTS prepared from unfed ticks was not infective. The polymerase chain reaction (PCR) was both sensitive and specific, detecting *T. lestoquardi* DNA in unfed and partially fed ticks, with a maximum sensitivity of 0·022 infected acinus/tick in 2-day fed ticks.

Rao *et al.* (1991) studied the concurrent infection with *Theileria hirci, Anaplasma ovis* and *Ehrlichia ovina* in a sheep farm of Tamil Nadu. The sheep were heavily infested with *Hyalomma marginatum isaaci* ticks. Treatment of the sheep by injection of oxytetracycline and the use of an organophosphorus insecticide on the sheep and their housing greatly reduced the incidence of disease.

De kok *et al*. (1993) compared the detection of Theileria parasites in ticks by PCR and staining the salivary glands with methyl green pyronin stain. They found PCR to be sensitive when compared to staining techniques.

Muraleedharan *et al*. (1994) reported the prevalence of haemoprotozoan infection in small domestic ruminants. About 45 blood samples were examined at Veterinary Diagnostic Laboratory, Mysuru and only one (2.2%) had *B.motasi* infection.

Voigt *et al*. (1995) compared the methods for staining tick salivary glands for detection of *Theileria parva*. Staining with azure without hydrochloric acid hydrolysis was found to be the most reliable method and could be applied to field ticks. The capacity of the stains to allow detection of early stages of *T. parva* differed, but it became more reliable

during tick feeding as sporoblasts developed and matured. Geimsa's stain and Feulgen's stain followed by superimposition of Giemsa's stain were superior to other stains for the detection and quantification of immature salivary gland stages in feeding ticks.

Friedhoff *et al*. (1997) reviewed about *Babesia, Theileria,* and *Anaplasma* species infecting sheep and goats. *B. ovis* was the most important disease agent. It is transmitted by *Rhipicephalus bursa, Rhipicephalus turanicus, Hyalomma anatolicum excavatum,* and probably by *Rhipicephalus evertsi evertsi.*

Jianxun and Hong (1997) reported the severity of *T.hirci* infection in sheep and goats in West China with more severity of the disease in lambs and exotic adults than native adult animals where the morbidity ranged from 78 to 85, 41 and 9 per cent and mortality of 81.41, 62.5 and 65 per cent in lambs, exotic and native adult animals respectively.

Razmi *et al*. (2002) studied the prevalence of *Babesia* infection in sheep and goats in Iran. The study revealed that 26.1 per cent of sheep and 14.8 per cent of goats were infected with *Babesia* spp. In this study, five ixodid species were collected from sheep and goats. *Rhipicephalus sanguineus* and *Hyalomma marginatum,* were the most common species in sheep and goats. Other tick species encountered were *Dermacentor daghestanicus* in goats and *Hyalomma anatolicum, Hyalomma asiaticum* in sheep.

Razmi *et al*. (2006) studied the prevalence of ticks in theileriosis infected sheep in South Khorasan province of Iran and reported that *Rhipicephalus sanguineus* (50.5%) as the most prevalent tick species followed by *H. anatolicum* (48.5%) and *H. dromedarii* (0.89%).

Altay *et al.* (2007a) studied the prevalence of theileriosis in small ruminants by microscopic examination (ME) and polymerase chain reaction (PCR).Piroplasms of *Theileria* spp. were detected in 18.29 per cent (120/656) of sheep and 2.88 per cent (4/139) of goats by ME. *T. ovis* was detected in 58.79 per cent (398/677) of sheep and 11.27 per cent (16/142) of goats by PCR, whereas *T. lestoquardi* was not detected in the same animals.

Altay *et al.* (2007b) surveyed *Theileria* and *Babesia* species in 920 apparently healthy small ruminants in eastern Turkey, as well as parasite genetic diversity, was investigated using a specifically designed reverse line blot (RLB) assay. The survey indicated a high prevalence of piroplasm infections in small ruminants (38.36%). Prevalence of *Theileria* spp was 36.08 per cent. Prevalence of *B. ovis* was 5.43 per cent. The most abundant *Theileria* species identified was *T. ovis* (34.56%) followed by *Theileia* spp. MK (1.30%) and *Theileria* spp. OT3 (0.43%).

Biu *et al*. (2009) examined two hundred giemsa stained thin blood smears of sheep, and recorded a prevalence of *Babesia ovis* in 15 (7.5%).

Bami *et al*. (2010) identified *Theileria* species causing ovine theileriosis in sheep blood by using both nested PCR and PCR –RFLP which were sensitive and specific to identify *Theileria lestoquardi* and *Theileria ovis* species. Out of 220 blood samples from sheep 60 per cent (132.220) were positive for *Theileria* spp by nested PCR compared with 22.27 per cent (49.220) by microscopic examination where as the RFLP –PCR out of 132 positive blood samples, 53.3 per cent (73.132) were positive for *Theileria lestoquardi* and 44.7 per cent (59.132) were positive for *Theileria ovis* .

Dehkordi *et al*. (2010) screened 154 blood samples collected from animals at Iran for haemoptrotozoan parasites by microscopy and by PCR. Out of 154 blood smears examined 38(24.67%) and 40 (26%) samples were infected by *Babesia* and *Theileria* respectively. The mixed infections occurred in four (2.6%) samples. The results of the PCR assays showed 9 (5.85%), 81(53%) and 18 (11.7%) were distinguished as *Babesia*, *Theileria* and mixed infection, respectively.

Rashid *et al*. (2010) examined 310 blood samples from sheep of Livestock experimentation station, Qadirabad, Pakistan and surrounding areas. The samples were examined microscopically and 30 (9.67%) were positive for babesiosis.

A total of 100 ticks of *Hyalomma anatolicum anatolicum* species collected from sheep with signs of theileriosis were subjected to PCR by Namavari *et al.* (2011), where 59 per cent of tick samples were positive for Theileriosis.Female ticks (67.3%) had a higher infection rate compared to the male ones (51.8%).

Durrani *et al*. (2011) screened about 200 whole blood samples and 100 samples of ticks from 20 flocks of sheep from different localities of district Lahore for the prevalence of Theileria species during spring and summer seasons in 2007. On microscopic examination 44/200 (22%) samples were positive for Theileria, while 70/200 (35%) blood samples were found positive for Theileria species by PCR, out of which 79 per cent were positive for *T. ovis* and 21 per cent for *T. lestoquardi*. Out of 100 tick samples the prevalence of *Hyalomma* spp was highest (45%) followed by *Rhipicephalus* spp (41%) and *Boophilus* spp (14%). The prevalence of *T. ovis* was 65.8 per cent (27/41) as compared to

66.6 per cent (30/45) for *T. lestoquardi* in *Rhipicephalus* and *Hyalomma* spp ticks, respectively.

Vidya *et al*. (2011) reported the incidence of *Babesia ovis* in goat in Bidar, Karnataka with clinical signs of pyrexia of 104°F, reduced haemoglobin of 8.8g/dl, PCV of 32 per cent and TLC of 11600/µl.

Zakkyeh *et al*. (2012) for the first time attempted molecular detection of *Theileria ovis* in *Rhipicephalus sanguineus* ticks. In about 323 ticks collected from 102 animals (88 sheep, 12 goats and 2 cattle), eleven (55%) tick specimens were PCR positive against genome of *Theileria ovis.*

Fakhar *et al.* (2012) determined the prevalence of the Babesiosis infection in domestic animals in Kurdistan Province of Iran.From a total of 9,111 collected samples, 2,642 were sheep and 6,469 were cattle. *Babesia* spp was detected in 1,359 (51.4%) out of sheep samples and 136 (2.1%) out of cattle samples by direct examination of blood smear. Altogether, the prevalence rate of Babesiosis was 16.4% (n = 1,495) in both animal groups. *Babesia ovis* and *Babesia bigemina* were the most prevalent species found in sheep and cattle, respectively.

Ebrahimi *et al*. (2012) surveyed the frequency of Babesia, during two seasons (spring and summer 2010). Blood samples of 300 sheep from different area of Piranshahr were screened. They reported that 41.6 per cent (125 sample) was infected by Babesia. The infection of *Babesia ovis* and *Babesia motasi* was 94.4 per cent (118/125) and 5.6 per cent

(7/125) respectively. This study indicated that frequency of infection babesia in sheep of Piranshahr was high.

Fadly *et al*. (2012) examined the blood samples from 800 animals (300 cattle, 200 buffaloes and 300 sheep) aged from < one year till > 3 years in period of April 2011 to March 2012 by Geimsa staining. It was found that 130 out of 300 (43.3%), 40 out of 200 (20 %) and 117 out of 300 (39%) cattle, buffaloes and sheep were positive for haemoprotozoan infection by microscopic examination. Then the incidence of *Babesia* spp. in cattle, buffaloes and sheep by blood film examination were 19.33 per cent, 9 per cent and 17 per cent. While the incidence of *Theileria* spp. in cattle, buffaloes and sheep by blood examination were 23 per cent, 10 per cent and 20 per cent. Mixed infection by *Babesia* and *Theileria* spp were 1, 1 and 2 per cent in cattle, buffaloes and sheep.

Rashidi and Razmi (2012) surveyed ninety sheep to identify *Theileria* spp infections of both sheep and ticks. *Theileria ovis*, *Theileria lestoquardi*, and mixed infection were detected in 63/90 (70%), 5/90 (5.5%), and 6/90 (6.6%) of samples, respectively.

Razmi and Yaghfoori (2013) conducted a study on prevalence of ticks in theileriosis infected sheep in south of Khorasan Razavi Province, Iran by examining 169 ixodid ticks and found that *Rhipicephalus turanicus* (91.7%) as the most prevalent tick species followed by *H. a. anatolicum* (4.7%) and *H. m. turanicum* (3.5%). They also reported that *R. turanicus* as the vector for *T. ovis* and *T. lestoquardi* in sheep.

Tahamtan *et al*. (2013) screened 300 sheep for the presence of pathogens in blood using Giemsa staining. Ticks were collected and six tick species were identified as: *Hyalomma anatolicum, Hyalomma asiaticum, Hyalomma marginatum, Rhipicephalus turanicus, Rhipicephalus bursa* and *Rhipicephalus sanguineus. Rhipicephalus* spp was the most frequent tick (51.87%) and *Rhipicephalus sanguineus* was the minor species (0.58%). In blood smears examination, *Theileria* was the only parasite which was seen with 2% frequency. None of the stained salivary glands had positive reaction to Methyl Green Pyronin that showed, positive animals for the presence of *Theileria*, infected by another ticks previously.

Ijaz *et al*. (2013) studied the prevalence of babesiosis in sheep and goat in Lahore and its peri-urban areas. A total of 620 blood samples (n=243 sheep; n=377 goats) were collected and examined microscopically. Babesia infection was found in 57(23.46%) sheep and 51(13.53%) goats. Haemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs) and thrombocytes were found to be significantly decreased ($P \leq 0.05$). while there was no effect on other blood parameters.

Hasan *et al.* (2013) screened 32 blood samples (sixteen from ewes and sixteen from rams) were randomly sampled monthly from January 2010 to December 2010 in Syria. A total of 384 sheep, thin blood smear- based diagnostic methods were used to assess the presence of Babesia in sheep. The study revealed that 22.1 per cent of sheep were infected with Babesia. Seasonally, the prevalence of Babesia infection in sheep reached highest level in July (37.5%), while reached the lowest level in January and February (12.5%).

Detection of naturally infected ticks by different species of *Babesia* and *Theileria* species was studied by Abdigoudarzi (2013). Totally 21 tick samples were detected to be infected with *Theileria lestoquadri* and *Babesia ovis* when subjected to PCR Ticks *Hyalomma anatolicum anatolicum* and *Rhipicephalus turanicus* were infected with *Theileria lestoquadri* whereas the *Hyalomma detritum* was infected with *Theileria lestoquadri.*

Razmi and Yaghfoori (2013) made a molecular surveillance of *Theileria ovis, Theileria lestoquadri* and *Theileria annulata* infection in sheep blood and tick vectors. About 150 blood samples were collected from 30 different flocks of sheep in which 18.6% of blood smears were positive for *Theileria* spp i.e *T.ovis* (58.6%) and *T.lestquadri* or *T.annulata* (6.6%).Total of 169 Ixodid ticks were collected in which most prevalent were *Rhipicephalus turanicus* (91%) followed by *Hyalomma anatolicum anatolicum* (4.7%) and *Hyalomma marginatum turanicum* (3.5%).

Haghi *et al*. (2013) screened 220 sheep and goats from 22 flocks in different regions of Iran. Standard PCR and semi nested- PCR was performed to differentiate genus of *Theileria* and *Babesia*, also identify the species of *Babesia*. Out of 220 blood samples (160 sheep and 60 goats), 34 cases (15.4 %) showed *Babesia* infection using microscopic examination. Whereas, 11 cases (5%) were found positive for *Babesia* spp using standard PCR. Also, two positive cases were showed mixed infection with *Theileria* spp. In addition, two microscopic negative samples were positive by PCR assay for *Babesia*.Using semi nested PCR, *Babesia ovis* (n=10) and *B. motasi* (n=1) were detected.

Shahzad *et al.* (2013) determined the prevalence and molecular diagnosis of *B. ovis* and *T. ovis* in Lohi sheep. Thirty two (16%), 48 (24%) and 26 (13%) were the number of animals found positive for *B. ovis*, *T. ovis* and for mixed infection with both parasites, respectively, through microscopy. Sixty eight (34%), 73 (37%) and 42 (21%) were the number of animals found positive for *B. ovis*, *T. ovis* and for mixed infection with both parasites, respectively by PCR.

Kumsa *et al*. (2014) studied the prevalence of Theleriosis in ticks collected from cattle and sheep. About 1,246 adult ticks and 264 nymphs were collected. The study showed infestation of 85.4 per cent in Cattle and 77.8 per cent in sheep with adult ticks. Overall, eight tick species, belonging to three genera (*Amblyomma, Rhipicephalus, Hyalomma*), were identified and *Amblyomma cohaerens* (n=577), *Rhipicephalus evertsi evertsi* (n=290), *Rhipicephalus (Boophilus) decoloratus* (n=287), and *Amblyomma variegatum* (n=85) were the more prevalent species.

Abdallal *et al*. (2014) screened 450 adult sheep from different localities in the North West area of Libya for detection of blood parasite infections. Out of 450, 107 (23.78%) and 47 (10.44%) sheep were found infected with *B. ovis* and *T. ovis* respectively. A significant higher prevalence of *B. ovis* infection among sheep was reported in spring 33 (30%), followed by summer 31(28%), autumn 17(15.5%) and winter 26 (21.67%). While for *T. ovis* infection, there was a significant higher prevalence in spring 20 (18%), followed by summer 12 (11%), autumn 5 (4%) and winter 10 (9.9%).

Noaman *et al.* (2014) screened a total of 87 blood samples collected from sheep in Al-Kut province in the east of Iraq to detect *Theileria* species. Regions of Theileria SSU

rRNA genes were amplified to determine *T.ovis* and *T. annulata* and 30KDa gene to fix *T.lestoquardi*. PCR analysis showed that 63 per cent, 71.2 per cent and 48.2 per cent of examined blood samples were positive for *T. ovis, T. annulata* and *T. lestoquardi* respectively. In addition, 45 per cent were recorded as a mixed infection.

Nasser *et al*. (2014) studied the carriers of pathogenic protozoa such as *Theileria* and *Babesia* in Ixodid ticks. About 219 ticks were collected from 150 sheep suffering from fever and anaemia in which 152 *Rhipicephalus sanguineus*, 13 *Rhipicephalus bursa* and 54 *Hyalomma anatolicum anatolicum* ticks. Salivary gland DNA was extracted and subjected to PCR. PCR revealed that 37 out of 152 *Rhipicephalus sanguineus* (24/34%) were positive for *Theileria ovis* whereas none of the *Rhipicephalus bursa* was positive. Five out of 54 *Hyalomma anatolicum antolicum* (9.25%) were positive for *Theileria lestoquadri.*

Jalali *et al*. (2014) assessed the status of ovine theileria infection in sheep by microscopic examination of blood smears which revealed 69.7 per cent (83/119) infection with *theileria* spp whereas by RFLP-PCR it was 89 per cent (106/119) positive for Theileria using Hind II. In enzymatic digestion of PCR products by Vsp I, 91.5 per cent (97/106) of *Theileria ovis* was detected, while the mixed infection of *Theileria ovis, Theileria annulata, Theileria lestoquardi* were found in 9 samples analyzed with an nested PCR-RFLP method by HpaII enzyme digestion.

Esmaeilnejad *et al*. (2014) studied the prevalence of *Babesia ovis* infection in adult *Rhipicephalus bursa* and small ruminants. Specific *B. ovis* fragment was detected in 67

animals (16.7%), of which 52 animals (18.6%) were sheep and 15 animals (12.2%) goats ($P< 0.05$).

Ayidin *et al.* (2015) studied the distribution of *Theileria* and *Babesia* species via microscopic examination and reverse line blotting (RLB) techniques in sheep and goats in the Black Sea region of Turkey. About 1,128 blood samples (869 sheep and 259 goats) were collected from sheep and goats in different provinces of Turkey. A total of 38 animals (3.37 %) including 34 sheep (3.91 %) and 4 goats (1.54 %) were found to be positive for *Theileria* spp. piroplasms in microscopic examination of smears while *Babesia* spp. piroplasm could not be detected. Infection rates were 34.64 % in sheep, 10.04 per cent in goats; totally 28.99 per cent for *Theileria ovis* while 0.58 per cent in sheep and totally 0.44 per cent for *Babesia ovis*.

Khamesipour *et al*. (2015) screened the blood samples of sheep by PCR to detect Babesia DNA in the blood samples whereby an amplified band size of 428 bp was considered positive for *Babesia* spp. The results indicated that 7.10 per cent (n= 155), 6.56 per cent (n= 122) and 0.00 per cent (n= 95) of the blood samples from cattle, camel and sheep were positive for Babesia DNA, respectively.

Saeed *et al.* (2015) determined the prevalence of *Theileria lestoquardi* from 165 ruminants, five (3%) out of 165 samples were positive for *T.lestoquardi* by PCR,produced 730 base pairs DNA fragment, through PCR amplification of 18S SSU rRNA gene, specific for *T. lestoquardi*.

Tiwari *et al.* (2015) studied the theileria infection in salivary glands of ticks by methyl green pyronin stain. Among total 60 adult semi engorged *H.a.anatolicum* ticks, 8.3 per cent (5/60) were found positive for *Theileria* sporoblast by methyl green pyronin stain.

Hageb *et al.* (2016) investigated the status of ovine Theileria infection in sheep at Giza governorate, Egypt. A total of 347 sheep blood samples (240 from different flocks and 107 from slaughter houses all over the governorate) were screened. Geimsa stained blood smears by microscopy showed that 15.56 per cent (54/347) were infected with *Theileria* spp. PCR applied for amplification of a fragment of the 18S ribosomal DNA on 5 positive and 10 microscopically negative samples, 40 per cent (6/15) of examined samples were infected.

Rjeibi (2016) studied the prevalence of *Theileria* and *Babesia* species in sheep with Giemsa stained blood smear examination and polymerase chain reaction to identify the different piroplasms in 270 sheep from three Tunisian bioclimatic zones (north, centre, and south). The overall infection in Giemsa-stained blood smear for *Babesia* spp and *Theileria* spp was 2.9 per cent (8/270) and 4.8 per cent (13/270) respectively. The PCR results showed that sheep were more often infected by *Theileria ovis* than *Babesia ovis* with an overall prevalence of 16.3 per cent (44/270) and 7.8 per cent (21/270) respectively ($P = 0.01$). The molecular prevalence of *Babesia ovis* was significantly higher in females than in males ($P < 0.05$).

Mamatha *et al.* (2017) described the first report of *Theileria luwenshuni* in India and its molecular characterisation by 18S rRNA gene based phylogenetic analysis. During the study microscopic examination revealed the presence of *Theileria* spp. in 53 sheep and

32 goats. Theileria genus specific PCR targeting amplification of 1098 bp region on 18S rRNA gene showed the presence of *Theileria* spp. in 103 sheep and 45 goats. The ticks collected from sheep and goats during this study were identified as *Haemaphysalis kutchensis, Haemaphysalis intermedia, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides* and *Rhipicephalus sanguineus*.

Bello *et al.* (2017) screened two hundred and fifty (250) blood samples from apparently healthy sheep of various age groups of both sexes for the presence of haemoprotozoan parasites. An overall 18.40 per cent prevalence rate of haemoparasitic infection was recorded. *Anaplasma* (9.60%), *Babesia* (5.20%), *Theileria* (2.80%) and *Trypanosoma* (0.80%) species were the genera of haemoparasites encountered in the infected sheep.

Hussein *et al.* (2017) conducted a survey by screening blood samples from sheep by Geimsa staining in Qena province of upper Egypt. Out of 130 examined samples, 15 (11.53%) were infected with *Babesia motasi*, 13 (10.0%) infected with *Babesia ovis* and 10 (7.69%) infected with *Theileria ovis* Six (4.61%) hosts had mixed infections with *B. motasi* and *T. ovis*; 3 (2.3%) with *B. motasi* and *B. ovis*; 2 (1.53%) with *B. motasi, B. ovis* and *T. ovis* and one (0.76%) was infected with *Toxoplasma* sp. and *T. ovis*. The total prevalence of infections with *B. motasi, B. ovis, T. ovis* and *Toxoplasma* spp. were 20.0, 13.83, 14.6 and 0.76 per cent, respectively.

Bilgic (2017) studied the prevalence of haemoprotozoan parasites from blood samples ($n = 1979$) collected from sheep ($n = 1727$) and goats ($n = 252$) in Turkey by polymerase chain reaction and the reverse line blotting (RLB) assay. Overall, 84 and 74.43

per cent of the small ruminants sampled were identified as hosting one or more pathogen(s) by species-specific PCR and RLB respectively. A high prevalence of mixed infection was evident, with PCR and RLB approaches indicating that 52.24 and 35.42 per cent of animals were co-infected with multiple species, respectively.

Phipps (2016) reported that polymerase chain reaction-reverse line blot assay indicated the presence of *Theileria* spp in blood samples from five sheep in Great Britian. Subsequent testing with a pan-piroplasm PCR of a larger panel of blood samples detected a piroplasm amplicon in 19 of 21 sheep submitted from the affected flock. Automated sequencing confirmed that these amplicons shared 99–100 per cent identity with *T. luwenshuni*.

Materials and Methods

III. MATERIALS AND METHODS

3.1 Prevalence of arthropod parasites in sheep in different places of Karnataka

3.1.1 Study area

Different organised and unorganised sheep farms representing eight districts from seven agroclimatic zones in Karnataka were screened (Table.1). The arthropods were collected by hand picking or by using hair brush/ comb. Collection of arthropods were done in different seasons, *viz.*, rainy season (south west monsoon: June, July, August, September: north west monsoon: October, November and December), winter season (January, February) and summer season (March,April and May) during 2015-16 from different locations of Karnataka state.The different agroclimatic zones of Karnataka were covered during this study, wherein sheep were screened in the four different seasons to know the prevalence of ectoparasites in sheep by considering different variables like season, breed, farm management practices, and site preference.

3.1.2 Collection, preservation, clearing of arthropods

For the collection of ectoparasites, the selected animals were thoroughly investigated by close inspection, palpation by parting the hairs. Ticks, fleas, keds and lice were collected from different parts of the body of the sheep by hand picking. When required, small hair brush dipped in ethanol was used for the collection of ticks and fine black comb was used for the collection of fleas and lice. Insect traps were used to collect flies. Bots were collected from the sheds early in the morning which were sneezed out during night time confinement.

Adequate precautions were taken to preserve the mouth parts and appendages of the ectoparasites during collection. To collect mites, skin scrapings from the affected areas were collected and the skin scrapings were examined by adding 10 per cent potassium hydroxide (Hendrix and Robinson, 2006). Ticks, fleas and lice were preserved in 70 per cent ethanol in clean, well-stopper glass vials. Flies collected by using traps were dry preserved.

3.1.3 Procedure for clearing

Liquefied phenol was prepared by diluting pure phenol (Carbolic acid) crystals by adding enough absolute (100%) ethyl alcohol to form a saturated solution with an about ½ by volume crystals in the bottom of the container. The phenol which was liquefied was placed into a cavity slide and the specimen's *viz.*, unfed ticks, fleas, lice were transferred to it and were left in the solution until they were cleared. The adult ticks, skin scrapings and the posterior spiracles of bots / maggots were cleared by boiling it with 10 per cent potassium hydroxide until cleared properly.

Equal portion of phenol – balsam was used for making permanent slides. This solution was prepared when required and used freshly. A small amount of phenol balsam mixture was prepared by mixing equal parts of (V/V) of liquefied phenol and canada balsam of the consistency normally used for making slides. The mixture was prepared one day in advance of use and not more than 3 weeks, since it becomes thicker and darker.

3.1.4 Slide preparation

Permanent slides were prepared by placing a drop of phenol-balsam mixture on a microscopic slide. The tick, fleas, wings of flies, posterior spiracles and lice from the

phenol solution were then mounted on a glass slide with cover slip. Then it was properly labelled with details such as species, date and place of collection and kept for drying.

3.1.5 Identification based on morphology

The arthropods were identified according to the keys and descriptions given by Ferris (1951), Roberts (1952), Hoogstraal (1956), Soulsby (1982), Sharif (1928), Danda (2007) and Geeverghese *et al.*2011).

Table 1. Particulars of the study area

Sl. No.	AgroclimaticZone	Study area (Un Organised farms)	Study area (Organised farms)
1	North eastern transition	Bidar.	-
2	Northern dry zone	Bellary, Athani (Belgaum district)	Sheep Breeding and Training Centre, Athani, Belgaum (D).
3	Central dry zone	Chitradurga, Davangere, Tumkur, Tiptur	Sheep Breeding and Training Centre (SBTC), Khudapura, Challakere. Livestock Research & Information Centre (LRIC), Konehally, Tiptur.
4	Eastern dry zone	Tumkur, Anekal, Bangalore(U)	Livestock Research &Information Centre (LRIC), Nagamangala, Instructional Livestock Farm Complex (ILFC),Bangalore.
5	Southern transition zone	Shivamoga, Hassan.	Instructional Livestock Farm Complex (ILFC) at Veterinary college. Shivamoga, ILFC, Veterinary College, Hassan
6	Southern dry zone	Malavalli, Nagamangala.	Bandur Sheep Breeding and Training Centre, Dhangur, Malavalli, Mandya.
7	Northern transition zone	Hukkeri, Haveri, Belgaum	Sheep Breeding and Training Centre, Guttala, Haveri (T& D).

The different study places in this study either from organised and unorganized farms from different agroclimatic zones belonged to arid and semi arid regions as follows:

Table 2: Particulars of Arid and Semi arid region

Sl. No.	Agroclimatic Zone	UnOrganised	Organised
1	Arid	Bidar, Athani, Haveri.	Sheep Breeding and Training Centre, Athani, Belgaum (D). Sheep Breeding and Training Centre, Guttala, Haveri (T& D).
2	Semi arid	Bellary, Chitradurga, Davangere, Tumkur, Tiptur, Anekal, Bangalore (U), Shivamoga, Hassan, Malavalli.	SBTC- Challakere. LRIC- Konehally, Tiptur. LRIC- Nagamangala, ILFC- BangaloreILFC- Shivamoga, ILFC- Hassan, BSBTCB - Dhangur, Malavalli, Mandya.

Ticks being the major vectors of haemoprotozoan parasites in sheep, different methods are in practice through out country to control tick infestation in sheep. The common methods of practice in control of tick infestation is by chemical acaricides and in combat of chemical acaricde resistance new methods were emerged by use of phytoacaricides and Entomopathogenic neamtodes. Hence the *in vitro* efficacy of commonly used acaricides (cypermethrin, deltamethrin and amitraz), phytoacaricide extracts (*Carica papaya* seeds, *Ricinus communis* leaves) and two important species of Entomopathogenic nematodes *viz.*, *Heterorhabditis indica* and *Steinernema abbasi* were evaluated in this study.

3.2 Efficacy of acaricides on ticks

3.2.1 Selection of acaricides

The commonly used acaricides at the organised and unorganised farms in Karnataka were selected *viz.*, Amitraz (Ridd* Petcare), Deltamethrin (Butox, Intervet Pvt

limited) and Cypermethrin (Tickicide* Brilliant Biopharma limited). Amitraz was used at the concentration of 0.1, 0.2, 0.3 and 0.4 per cent (Rajkhowa *et al*.,2005), whereas deltamethrin and cypermethrin were used at the concentrations of 0.05, 0.1, 0.2, 0.3 and 0.4 per cent (Khan and Srivatsava, 1988b; Bagherwal *et al*., 1994).

3.2.2a Collection of ticks

The fully engorged female ticks from Bangalore, Bellary, Belgaum, Chitradurga, Davanagere and Mandya were collected from the animals in unorganised farms of Bellary, Belgaum, Chitradurga, Davangere and Mandya. The collected ticks viz., *Haemaphysalis kutchensis, Haemaphysalis intermedia, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* were subjected to *in vitro* studies. Engorged female ticks were collected from sheep of the above mentioned regions where the acaricides commonly used included were *viz*., cypermethrin, deltamethrin and amitraz for control of ticks.

3.2.2b Collection of reference susceptible tick population

The sheep which were naturally infested with engorged female ticks of spp *Haemaphysalis kutchensis, Haemaphysalis intermedia, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* which were not exposed earlier for any acaricidal treatment (based on earlier treatment records) were collected from farms in Bangalore, Bellary, Belgaum, Chitradurga, Davanagere, and Mandya. These ticks were used as the standard to assess the susceptibility / resistance status of the ticks collected in the present study. They were later confirmed to be susceptible to the recommended concentrations of deltamethrin, cypermethrin and amitraz.

Plate 1. Different species of ticks collected for *In vitro* assays

3.2.3 *In vitro* efficacy study of acaricides on ticks

The *in vitro* evaluation of the efficacy of acaricides *viz.*, Amitraz (Ridd* Pet care), Deltamethrin (Butox, Intervet Pvt limited) and Cypermethrin (Tickicide* Brilliant biopharma limited) was done on larval stages as per FAO (1984) by larval packet test (LPT) and adult immersion test with a discriminating dose (AIT-DD) against engorged females ticks as recommended by FAO, (1999) with slight modifications in the present study.

3.2.4 Preparations of the acaricides

Commercially available selected acaricides were purchased from local pharmaceuticals. The diluent solution was prepared by mixing trichloroethylene and sterile olive oil in ratio 2:1. The different concentrations were made by dilution of appropriate volume of acaricide with diluents.

3.2.5 *In vitro* efficacy study on larval stages of ticks by larval packet test (LPT)

After identification of different species of engorged ticks, they were held individually at 28±1˚C and 85±5 per cent relative humidity in labelled glass tubes for oviposition. The eggs laid were separated and allowed to hatch to larvae under similar

conditions of incubation in glass tubes closed with cotton plug. Twelve to fifteen day old larvae were taken for evaluation of efficacy of acaricides in this technique. Larvae were taken from the glass tubes by means of a paint brush.

3.2.5 (a) Larval packet test procedure:

1. The selected acaricides were dissolved in the diluent solution to prepare different concentrations for testing against different species of tick larvae.
2. Briefly, 0.7 ml of different concentrations of acaricides *viz*., Amitraz (0.1%, 0.2%, 0.3% and 0.4%), Cypermethrin (0.05%, 0.1%, 0.2%, 0.3% and 0.4%) and Deltamethrin (0.05%, 0.1%, 0.2%, 0.3% and 0.4%) were streaked by using micropipette to impregnate to the Whatman No.1 filter paper of 11cm diameter. The aqueous solution of acaricide was dried by keeping the filter paper for 30 min in the incubator at 37˚C. The filter papers were then folded in half diagonally and sealed on sides with adhesive tapes, forming an open ended to place tick larvae.
3. After insertion of approximately 100 larvae, the open end of each packets were closed and kept in desiccators placed in BOD incubator maintained at 28±1˚C and 85±5 per cent RH for 24hrs.
4. The packets were removed after 24hrs and larval mortality was calculated. For each concentration of acaricide the test was conducted in triplicate along with control groups.
5. Then the mortality rate of larvae was calculated by dividing the dead larvae by total larvae of each packet, the larvae which were moving were considered as live. One

control packet was prepared for each concentration of acaricide by impregnated it with diluents solution only.

6. The larval mortality in Larval Packet Test at a given testing dose was calculated with the formula.

$$\textbf{\% Mortality} = \frac{\textbf{Dead larvae}}{\textbf{Total larvae}}$$

7. The larval mortality data was subjected to probit analysis for calculating LC_{50} (lethal concentration to 50 per cent of tick larvae tested), LC_{99} (lethal concentration to 99 per cent of tick larvae tested) values along with 95 per cent confidence lines and slope of probit lines. The data was analysed by computer program software based on Finney (1971).

8. Resistance factor (RF) was obtained by comparing the LC_{50} of field strains with the LC_{50} of reference susceptible strain.

Plate 2: (a) Eggs laid by engorged ticks (b) 12-15 days old tick larva for LPT (c) Larval packet for LPT

3.2.5b *In vitro* efficacy study on adult stages by Adult immersion technique modified with a discriminating dose (AIT-DD)

The adult immersion test with a discriminating dose (AIT-DD) as per FAO, 1999 was used to study resistance if present.

1. The selected acaricides were diluted for recommended doses with the help of distilled water.
2. To the 100 ml plastic containers with screw cap lids, 20 ml of the diluted acaricides was added. About 20 ml of distilled water was added to another container as a control with proper labelling.
3. Ten adult engorged female clean ticks collected within 48 hrs of the test were kept in each of these containers.
4. These adult engorged female ticks were immersed in the different acaricidal solutions and control for 30minutes at 25˚C.
5. The acaricide solutions were poured off after 30 minutes into a safe storage container and the ticks were dried gently on the filter paper.
6. Then individual ticks were kept separately in small plastic petri dishes with proper labelling.
7. Petri dishes were kept in a desiccator containing saturated potassium chloride at the bottom for seven days.
8. Oviposition activity of these ticks was observed for a period of about seven days.
9. After seven days the number of ticks that laid eggs were counted.

Calculation of resistance

Ticks immersed in water which laid eggs after 7days were taken as controls. Ticks that were treated with acaricide and still laid eggs were considered resistant. Ticks that were treated with acaricide and did not lay eggs were taken as susceptible. The percentage resistance was calculated as follows

$$\text{Resistance (\%)} = (Nt/Nw) \times 100$$

Where Nt= Number of treated ticks laying eggs

Nw= Number of untreated ticks laying eggs.

3.2.6 The discriminating dose for AIT-DD of the selected acaricides as per FAO, 1999 is as follows:

Acaricide	Suggested discriminating dose (DD)
Amitraz	2.5g/ltr
Deltamethrin	0.075g/ltr
Cypermethrin	0.05g/ltr

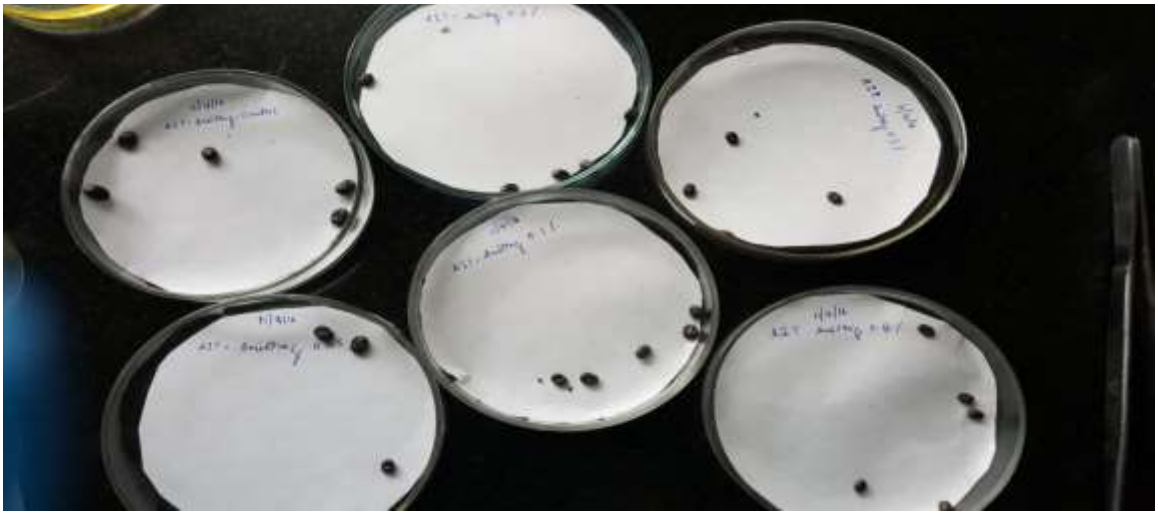


Plate 3: Adult / female engorged ticks set for AIT-DD assay.

3.3 *In vitro* Efficacy of Entomopathogenic Nematodes (EPNs) on ticks

In recent research studies, EPNs have been a promising method in control of ticks in livestock. Hence, the EPNs were evaluated under laboratory conditions for the control of sheep ticks in this study by bioassay, wherein the mortality of ticks was recorded after 24hrs of application of EPNs on engorged ticks. Further, the effect was also evaluated by reproductive biology assay to check the effectiveness of EPNs on engorged tick by recording the weight of engorgrd ticks before oviposition, egg mass weight, hatching percentage, egg production index and effectiveness of EPNs in percenatge control.

3.3.1 Procurement of Entomopathogenic Nematodes (EPNs)

The two species of EPN's *viz.*, *Heterorhabditis indica* and *Steinernema abbasi* for the study were procured from the ICAR - National Bureau of Agricultural Insect Resources (NBAIR), Bangalore and were used against the engorged female's ticks collected from naturally infested sheep flocks of Bangalore, Belgaum, Bellary, Chitradurga, Davanagere and Mandya regions.

3.3.2 EPNs dose preparation

3.3.2(a) Calculation of concentration of EPNs by dilution method of counting

The stock solution of each EPN was mixed thoroughly to make a homogenous suspension. A volume of 50 µl from the thoroughly mixed diluted suspension was drawn and transferred to a watch glass and counted under stereo zoom microscope. This process was repeated at least five times to check the accuracy. The average of five values was taken as mean IJs/50 µl sample. Later this was further multiplied by the dilution factor (20) to get the total number of nematode IJs per ml (Woodring and Kaya, 1988).

IJs per ml = Average IJs/50 µl × 20 (dilution factor)

3.3.2(b) The calculation of EPNs dose

Five doses of each EPN at a concentration of 500, 1000, 2000, 4000, 6000 IJs/1.5ml aqueous suspension were prepared using stock solution of 10000IJs/ml as per the guidelines of NBAIR, as detailed here with

a. 500IJs dose = 500 x1000µl/ 10000 = 50µl

= 50 µl stock solution + 1450 µl distilled water.

= 500 µl IJs/1.5ml

b. 1000IJs dose = 1000X 1000 µl / 10000= 100µl

= 100 µl stock solution + 1400 µl distilled water.

=1000 µl IJs/1.5ml

c. 2000 IJs dose = 2000 X 1000µl / 10000 = 200µl

= 200µl stock solution + 1300 µl distilled water.

= 2000µl IJs/1.5ml

d. 4000 IJs dose = 4000 X 1000µl / 10000 = 400µl

= 400 µl stock solution + 1100 µl distilled water.

= 4000µl IJs/1.5ml

e. 6000IJs dose = 6000 X 1000µl /10000 = 600µl

= 600 µl stock solution + 900 µl distilled water

= 6000 µl IJs/1.5ml

3.3.3 Bioassay experiments for *H.indica* against engorged female ticks.

3.3.3 (a) Procedure

The experiment was carried out as described by Silva *et al*. (2012). A total of 144 engorged females were divided into six groups, each containing 24 engorged ticks with statistically similar weights ($P \geq 0.05$) each carrying out the experiment in four replicates for each dose of EPNs so that each group consisted of 4 ticks along with a control group of 4 ticks. The experiment was carried out in petri dishes of 9 cm in diameter containing 15g of sterilized and as substrate.

About twenty one petridishes were taken and numbered from 1 to 20 for treatment group and no. 21 for control group. 15g of sterilized sand was placed at the bottom of the petridishes. Then 1450 µl of distilled water and 50 µl of EPN with a concentration of 10000 IJs/ml was added to the petridishes numbered 1 to 4 to get final concentration of 500 IJs / petridish of 1.5ml suspension. Similarly, 1400 µl of distilled water and 100 µl of EPN with a concentration of 10000 IJs/ petridish was added to petridishes numbered from 5 to 8 to get final concentration of 1000 IJs / petridsh of 1.5 ml suspension. 1300µl of distilled water and 200µl of EPN with a concentration of 10000IJs/ petridish was added to petridishes numbered from 9 to 12 to get final concentration of 2000IJs/ petridish of 1.5 ml suspension. 1100µl of distilled water and 400µl of EPN with a concentration of 10000IJs/ petridish was added to petridishes numbered from 13-16 to get final concentration of 4000IJs / petridish of 1.5 ml suspension. 900 µl of distilled water and 600 µl of EPN with a concentration of 10000IJs/ petridish was added to petridish numbered from 17 to 20 to get final concentration of 6000IJs/ petridish of 1.5 ml suspension. Finally 1.5 ml of distilled water was added to control petridishes numbered 21 and EPNs were not added to the control

group. Engorged female *R.sanguineus* ticks of similar weight were placed in all the treatment groups and control group petridish.Later the petridishes were covered by lid and the EPN strain, dose, date, time was labelled on the respective petridish lids.

The petridishes were incubated at 27±1°C and RH≥ 80 per cent ±10 per cent in dark. To maintain the optimum moisture 1.0 ml of distilled water was added to each petridish daily for the optimum activity of nematodes. The experiment was carried out in four replicates for each dose of *H.indica.*

Similar procedure was followed for the bioassay experiments using *H.indica* against *R.haemaphysaloides, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.* The parameters observed in this bioassay test were per cent mortality and egg mass laid. The engorged ticks were observed for mortality for every 24hrs interval, till the complete mortality in treatment groups based on the visual observation such as absence of leg reflex and changes in coloration of the external surface of the tick. After complete mortality of ticks in the treatment group, the egg masses were removed, weighed and transferred to sterile test tubes.

The observations recorded were statistically analysed for the experiments. Means of all experiments were used to compare the efficacy of treatments. Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range test ($P>0.05$) for separation of means. LC_{50} values were calculated according to Finny, (1971).

3.3.4 Bioassay experiments with *Steinernema abbasi* against engorged female ticks

The experiment was carried out as described by Silva *et al*. (2012). A total of 180 engorged females were divided into six groups, each containing 24 engorged ticks with statistically similar weights ($P \geq 0.05$) each carrying out the experiment in four replicates for each dose of EPNs so that each group consisted of 4 ticks along with a control group of 4 ticks. The experiment was carried out in petri dishes of 9cm in diameter containing 15g of sterilized sand as substrate.

About twenty one petridishes were taken and numbered from 1 to 20 for treatment group and no.21 for control group. 15g of sterilized sand was placed at the bottom of the petridishes. Then 1450 µl of distilled water and 50 µl of EPN with a concentration of 10000IJs/ml was added to the petridishes numbered 1 to 4 to get final concentration of 500 IJs / petridish of 1.5ml suspension. Similarly 1400 µl of distilled water and 100 µl of EPN with a concentration of 10000IJs/ petridish was added to petridishes numbered from 5 to 8 to get final concentration of 1000 IJs / petridsh of 1.5ml suspension. 1300µl of distilled water and 200µl of EPN with a concentration of 10000IJs/ petridish was added to petridishes numbered from 9 to 12 to get final concentration of 2000IJs/ petridish of 1.5ml suspension. 1100µl of distilled water and 400µl of EPN with a concentration of 10000IJs/ petridish was added to petridishes numbered from 13-16 to get final concentration of 4000IJs / petridish of 1.5ml suspension. 900 µl of distilled water and 1100 µl of EPN with a concentration of 10000IJs/ petridish was added to petridishes numbered from 17 to 20 to get final concentration of 6000IJs/ petridish of 1.5ml suspension. Finally 1.5ml of distilled water was added to control petridish numbered 21 and EPNs were not added to the control group. Engorged female *R.sanguineus* ticks of similar weight were placed in all the

treatment groups and control group petridish. Later the petridishes were covered by lid and the EPN strain, dose, date, time was labelled on the respective petridish lids.

The petridishes were incubated at 27±1°C and RH≥ 80% ±10% in dark. To maintain the optimum moisture 1.0 ml of distilled water was added to each petridish daily for the optimum activity of nematodes. The experiment was carried out in four replicates for each concentration of *S.abbasi*.Similar procedure was followed for the bioassay experiments using *S.abbasi* against *R.haemaphysaloides, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.* The parameters observed in this bioassay test were per cent mortality and egg mass laid.

The engorged ticks were observed for mortality for every 24hrs interval, till the complete mortality in treatment groups based on the visual observation such as absence of leg reflex and changes in coloration of the external surface of the tick. After complete mortality of ticks in the treatment group, the egg masses were removed, weighed and transferred to sterilised test tubes.

The observations recorded were statistically analysed for the experiments. Means of all experiments were used to compare the efficacy of treatments. Per cent insect mortality data were analysed by multifactor ANOVA followed by Duncan's multiple range test ($P>0.05$) for separation of means. LC_{50} values were calculated according to Finny, (1971).

3.3.5 *In vitro* Efficacy of EPNs on reproductive biology of ticks

WhatmanNo 1 filter paper, petridish with 11cm diameter, micropipettes, micro pipette tips, test tubes, stereomicroscope, egg counter, distilled water and desicators were the materials used.

3.3.5a *In vitro* efficacy of *Heterorhabtidis indica* against *R. haemaphysaloides*

The experiment was carried out as described by (Monteiro *et al*. 2010a). A total of 180 engorged females were divided into six groups, each containing 24 engorged ticks with statistically similar weights ($P \geq 0.05$) for the experiment in four replicates for each dose of EPNs so that each group consisted of 4 ticks along with a control group of 4 ticks. The experiment was carried out in petri dishes of 9cm in diameter containing 15g of sterilized sand as substrate.

About twenty one petridishes were taken and numbered from 1 to 20 for treatment group and no.21 for control group. 15g of sterilized sand was placed at the bottom of the petridishes .Then 1450 µl of distilled water and 50 µl of EPN with a concentration of 10000IJs/ml was added to the petridish numbered 1 to 4 to get final concentration of 500 IJs / petridish of 1.5ml suspension .Similarly 1400 µl of distilled water and 100 µl of EPN with a concentration of 10000IJs/ petridish was added to petridish numbered from 5 to 8 to get final concentration of 1000 IJs / petridsh of 1.5ml suspension. 1300 µl of distilled water and 200 µl of EPN with a concentration of 10000IJs/ petridish was added to petridish numbered from 9 to 12 to get final concentration of 2000IJs/ petridish of 1.5ml suspension. 600µl of distilled water and 400µl of EPN with a concentration of 10000IJs/ petridish was added to petridish numbered from 13-16 to get final concentration of 4000IJs / petridish of

1.5ml suspension. 900 µl of distilled water and 600 µl of EPN with a concentration of 10000IJs/ petridish was added to petridish numbered from 17 to 20 to get final concentration of 6000IJs/ petridish of 1.5ml suspension. Finally 1.5ml of distilled water was added to control petridish numbered 21 and EPNs were not added to the control group. Engorged female *R.haemaphysaloides* ticks of similar weight were placed in all the treatment groups and control group petridish.Later the petridishes were covered by lid and the EPN strain, dose, date, time was labelled on the respective petridish lids.

The petridishes were incubated at 27±1°C and RH≥ 80% ±10% in dark. To maintain the optimum moisture 1.0ml distilled water was added to each petridish daily for the optimum activity of nematodes. The experiment was carried out in four replicates for each concentration of *H.indica.* The engorged ticks were checked for mortality for every 24hrs interval till the complete mortality in treatment groups based on the visual observation such as absence of leg reflex and changes in coloration of the external surface of the tick.

3.3.5b Observations on reproductive biology of ticks

After 48 hrs, on observation the ticks that were alive in each treatment were collected and placed separately in glass vials covered with muslin cloth and proper labelling. Later it was monitored continuously for the following parameters *i.e*., egg mass weight (mg), hatching percentage (%) and the values were used to calculate the egg production index and efficacy of treatments. The egg production index (% EPI) was calculated according to the formula proposed by Bennett (1974). EPI = egg mass weight × 100/female weight before oviposition. Index of estimated reproduction (ER) was calculated first later the percentage control was calculated. Index of estimated reproduction

(ER) was calculated by using formula= (egg mass weight/ final weight) X per cent EC X 20000 (Drummond *et al.* 1973). The percentage of control of treatments, through offspring inhibition was obtained according to Drummond *et al*. (1973), formula of per cent control = (control group ER – treatment group ER /control group ER) X 100. The statistical analysis was performed using Biostat version 5.0. The data in percentages were transformed into √arcsin x. Similar procedure was followed for the *in vitro* efficacy test of *H.indica* against *R.sanguineus, R.haemaphysaloides, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.*

3.3.6 *In vitro* efficacy of *Steinernema abbasi* against engorged female ticks

In vitro efficacy test was carried out for each dose of *S.abbasi* against *H.a.anatolicum, H.m.isaaci, H.bispinosa, H.intermedia, H.kutchensis, R.haemaphysaloides* and *R.sanguineus* as describedin the section 3.3.5.

Plate 4: *In vitro* efficacy of EPNs on reproductive biology of ticks

3.4 *In vitro* efficacy of Phytoacaricides in control of ticks

The herbal plants selected for the study were *Ricinus communis* plant leaves and *Carica papaya* seed extracts against *H.a.anatolicum, H.m.isaaci, H.bispinosa, H.intermedia, H.kutchensis, R.haemaphysaloides* and *R.sanguineus.*

3.4.1 Collection of *Ricinus communis* plants and *Carica papaya* seeds

The *Ricinus communis* plant leaves were collected from different farmer's field plots in different places of Karnataka.The plant was identified by a botanist and used for further *in vitro* study. The papaya seeds were collected from different fruit stalls in Bangalore.

3.4.2 Preparation of plant leaf extract / seeds extract

The *Carica papaya* seeds were collected from different fruit stalls located in Bangalore. The papaya seeds were cleaned, washed with distilled water and dried in shade at room temperature for about 2 weeks. Dried materials were coarsely powdered by using an electric mixer. Extraction was carried out by maceration wherein 100g of the powdered material was taken and dissolved in 200 ml methanol for two to three days and at the end, the material was filtered and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator. Finally, the concentrated extract was lyophilized to remove traces of methanol by freeze dry system. The extract was preserved in airtight container till usage. The extract was mixed with distilled water to prepare dilutions of 6.25, 12.5, 25, 50 and 100mg/ml that were used for the testing of ticks by adult immersion test (AIT) and larval packet test (LPT).

The *Ricinus communis* plant leaves were collected from different farmer's field plots in different places of Karnataka. The collected plants leaves were identified cleaned and shade dried. After drying the leaves, they were coarsely powdered in a mixer.

About 100 g of the coarsely powdered material was mixed with 200ml of 95 per cent ethanol at room temperature. The extraction process continued for three days and at the end, the material was filtered and the filtrate was concentrated under reduced pressure using a rotary vacuum evaporator. Crude extract was further partitioned using 100 ml of hexane, where the soluble hexane extract and the insoluble extract was formed. The insoluble extract was macerated with 100 ml of chloroform, where it formed chloroform soluble and insoluble form, further this insoluble form was partitioned between 50ml n-butanol and50 ml water. Then, the water soluble extract along with the n-butanol soluble extract was preserved in an air tight bottle and kept in dark till usage.

3.4.3 Collection of ticks

The ticks were collected from different unorganised sheep farms from different places like Bangalore, Belgaum, Bellary, Chitradurga, Davangere and Mandya, they were identified based on the keys given by (Sharif,1928, Geevarghese and Danda, 1987). The collected ticks were put in vials and labelled with date of collection and place. The vials with ticks were wrapped in cotton net cloth for oxygen supply and transported to the laboratory. At the laboratory the species of the ticks were identified.

3.4.4 *In vitro* efficacy of the *Ricinus communis* extract on ticks

In vitro effect of ethanolic extract of *Ricinus communis* was evaluated on ticks collected from naturally infested sheep. Serial dilution of the extract was done using

distilled water at concentrations of 6.25, 12.5, 25, 50,100,150 and 200mg/ml that was used for the testing of ticks by adult immersion test (AIT) and larval packet test (LPT).

3.4.4 (a) Larval Packet Test

The larval packet test (LPT) was done as per FAO (1984) guidelines with minor modifications. After making different dilutions of 6.25, 12.5, 25, 50 and 100mg/ml. Briefly, 0.7 ml of ethanolic crude plant extract was used to impregnate 3.75- cm by 8.5-cm Whatman filter paper rectangles. The compound was dried by keeping the filter paper for 30 min in incubator at 37 °C. The rectangles were folded in half and sealed on the sides with adhesive tapes, forming an open-ended packet to place tick larvae. After the insertion of approximately 100 larvae (12 days old), the top of each packet was sealed with adhesive tape, and the packets were transferred to desiccators and then placed in BOD incubator at 28±1 °C and 85±5 % RH. The packets were removed after 24 h, and larval mortality was calculated. Control group was treated with 0.7 ml of distilled water similar to the above mentioned procedure. Statistical analysis of data was expressed as the mean±SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. A value of $P<0.05$ was considered significant.

Similar procedure of larval packet test was followed for the *in vitro* efficacy test of *Ricinus communis* ethanolic extract against 12 days old larvae of *R. sanguineus, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.*

3.4.4 (b) Adult Immersion Test.

The AIT was conducted according to FAO (1984) guidelines. The identified ticks were washed in distilled water and dried on an absorbent paper. A total of 560 adult

engorged female ticks were used for the present study. Out of this, about 80 ticks were used, three replications for each concentration, and in each replication 5 ticks / petridish were taken for the study. All the ticks were immersed for two minutes in different concentrations of the extract while control ticks were immersed in distilled water, later the ticks were transferred to the test petridishes padded with Whatmann filter paper no.1. Every 24 hrs the mortality of ticks was recorded.

After 48hrs, the ticks that were alive were transferred to glass vials covered with muslin cloth and kept in desiccators having 85±2 per cent relative humidity and placed in BOD incubator at 28±2 °C. These ticks were observed for oviposition and death up to 15 days. The per cent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded in comparison with the control. The eggs were incubated in the same condition, and the percentage of hatched eggs was estimated visually. The index of egg laying and percentage inhibition of fecundity were calculated with the formula whereinIndex of egg laying (IE) =mean weight of eggs laid(g) / weight of females(g) and per cent inhibition of egg laying = (IE control group – IE treated group / IE control group) × 100 respectively (Sabatini *et al.* 2001).

This procedure was followed to test the *in vitro* efficacy of the ethanolic extract of *R.communis* against adult ticks of species against against *R.sanguineus, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.*

3.4.5 *In vitro* efficacy of the *Carica papaya* extract on ticks.

In vitro effect of methanolic extract of *Carica papaya* seeds was evaluated on different ticks species collected from naturally infested sheep. Serial dilution of the extract

was done using distilled water at concentrations of 6.25, 12.5, 25, 50 and 100mg/ml that were used for the testing of ticks by adult immersion test (AIT) and larval packet test (LPT).

3.4.5(a) Larval Packet Test

The larval packet test (LPT) was conducted according to FAO (1984) guidelines with minor modifications. After making different dilutions of 6.25, 12.5,25,50 and 100 mg/ml. Briefly, 0.7 ml of methanolic crude plant extract was used to impregnate 3.75- cm by 8.5-cm Whatman filter paper rectangles. The compound was dried by keeping the filter paper for 30 min in incubator at 37 °C. The rectangles were folded in half and sealed on the sides with adhesive tapes, forming an open-ended packet to place tick larvae. After the insertion of approximately 100 larvae (12 days old), the top of each packet was sealed with adhesive tape, and the packets were placed in a desiccator placed in BOD incubator maintained at 28±1 °C and 85±5 % RH. The packets were removed after 24 h, and larval mortality was calculated. Control group was treated with 0.7 ml of distilled water similar to the above mentioned procedure. Statistical analysis of data was expressed as the mean±SEM. Groups were compared using one-way ANOVA for repeated measurements using SPSS software. A value of $P<0.05$ was considered significant. Similar procedure of larval packet test was followed for the *in vitro* efficacy test of *Carica papaya* seed methanolic extract against 12days old larvae of *R.sanguineus, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.*

3.4.5 (b) Adult Immersion Test

The AIT was conducted according to FAO (1984) guidelines. The identified ticks were washed in distilled water and dried on an absorbent paper. A total of 560 adult

engorged female ticks were used for the present study. A total of 80 ticks were used with three replications for each concentration and in each replication 5 ticks / petridish were included. All the ticks were immersed for two minutes in different concentrations of the extract while control ticks were immersed in distilled water, later the ticks were transferred to the test petridishes padded with Whatmann filter paper no.1. Every 24 hrs the mortality of ticks was recorded.

After 48hrs, ticks that were live were transferred to glass vials covered with muslin cloth and kept in desiccators having 85±2 per cent relative humidity and placed in BOD incubator at 28±2 °C. These ticks were observed for oviposition and death up to 15 days. The percent adult tick mortality and the weight of the eggs laid by the treated ticks were recorded in comparison with the control. The eggs were incubated in the same condition and the percentage of hatched eggs was estimated visually. The index of egg laying and percentage inhibition of fecundity was calculated with the formula wherein Index of egg laying (IE) = mean weight of eggs laid (g) / weight of females (g) and per cent inhibition of egg laying = (IE control group – IE treated group / IE control group) X 100 respectively (Sabatini *et al*., 2001).

Similar procedure was followed to test the *In vitro* efficacy of the methanolic extract of *Carica papaya* seeds against adult ticks of species against *R.sanguineus, H.bispinosa, H.intermedia, H.kutchensis, H.a.anatolicum* and *H.m.isaaci.*

3.5 Vector potential of sheep ticks with reference to haemoprotozoan parasites

Tick - borne hemoprotozoan parasites including *Theileria* and *Babesia* spp is a major problem in small ruminants especially in tropical and sub tropical regions of the

world. They cause significant economic losses affecting the international trade of animals. Generally, the diagnosis of ovine piroplasmosis is based on morphological examination of blood smears and clinical symptoms, however a negative result does not rule out the possibility of infection. Besides, animals which recover from acute infection become carriers of the haemo-parasite in course of time making diagnosis difficult.

The detection of haemoprotozoan parasites in the vector for assessing the infection rate in vectors will help to curtail the risk of Theileriosis and Babesiosis in small ruminants. Hence, in this study the tick tissues *viz.*, salivary gland, mid gut and ovaries were stained by methyl green pyronin and giemsa stain to detect parasite infection. Apart from staining techniques in diagnosis of the haemoprotozoan diseases, the application of molecular techniques including conventional PCR, Reverse Transcriptase – PCR, Nested PCR, Semi nested or Reverse line blot would allow direct, specific and sensitive detection and simultaneous detection and differentiation of different pathogens.

3.5.1 Detection of haemoprotozoan parasites in the vector by staining methods

3.5.1(a) Collection of ticks:

Fully engorged ticks were collected randomly from organised and unorganised sheep farms from different parts of the Karnataka. The ticks were collected in clean glass vials covered with muslin cloth and identified before dissection. About 300 ticks were collected, washed with distilled water and cleaned with an absorbent paper then it was dissected to remove the salivary glands, mid gut and ovaries and was stained with Methyl green pyronin stain and geimsa stain.

3.5.1(b) Dissection of ticks

Dissection of ticks and its tissues was done as per the method described by Edward *et al.* (2009) with minor modifications. The cleaned ticks were held in between thumb and forefingers with the dorsal side up and dissected with a sharp blade from the posterior border proceeding anteriorly expose the viscera. Dissected ticks in phosphate buffered saline were examined under a dissecting microscope. Paired salivary glands visible anteriorly on either side of trachea were removed carefully with a teasing needle and transferred on to the microscopic slides for further examination. The gut had brownish strands in the central area and the ovaries were visible as a bunch of grapes towards the posterior part which were separated and spread on different glass slides in phosphate buffered saline.

3.5.1(c) Staining of tick tissues by Methyl green pyronin stain.

1. The salivary glands of dissected ticks were taken on the microscopic slides and were fixed by dipping the slide in Cornoy's fixative for 5min.
2. The slides were then rinsed in 70% alcohol for 2min followed by distilled water for 2min and then air dried.
3. They were next immersed in Methyl green pyronin (Hi media) staining solution for 7 min and rinsed in distilled water and air dried.
4. Lastly when completely dried, the slides were dipped briefly in xylene before mounting in DPX.

In the similar manner the gut and ovaries were also stained to know the intensity of haemoprtozoan parasitic infection in the ticks (Tahamtan *et al*., 2013).

3.5.1(d) Staining of the salivary glands with Geimsa's stain

1. The salivary glands of dissected ticks were taken on the microscopic slides and were fixed by dipping the slide in Cornoy's fixative for 5min.
2. Then the salivary glands were air dried and rinsed for 30-45 sec in methanol.
3. The smear was stained by giemsa stain diluted in ratio of 1:2 with distilled water for 40 minutes and washed with tap water and air dried (Zajac *et al*., 2012).

3.5.2 Molecular detection of Haemoprotozoan parasites in ticks.

The blood samples from sheep were screened randomly from organised and unorganised farms of Karnataka.The blood samples were drawn by a syringe from the jugular vein of the sheep and collected into labelled EDTA vacutainers and simultaneously ticks were also collected from the same blood collected animals and were placed into plastic containers with the lids having holes on it for aeration till brought to the laboratory.

From the collected blood samples thin blood smears were prepared and fixed with methanol for 5 min and stained with Giemsa stain diluted in ratio of 1:2 with phosphate buffered saline (PBS), pH 7.2 for 45 minutes, later washed with distilled water and air dry the slides and then examined for the presence of piroplasms under oil immersion lens (100X) of microscope. (Radica RXLr-3).

3.5.2(a) Tick examination

The ticks were collected and species were identified by using keys (Sharif 1928, Walker *et al*., 2003, Geeverghese *et al*., 2011).The collected ticks were grouped into pool of five ticks according to their species .Then, the salivary glands of each tick pool were

dissected out in 0.8% saline solution under stereo microscope .Then, the salivary gland samples were kept in 70% ethanol at -20˚C until they were used for PCR. The total DNA was extracted from the EDTA blood and tick samples using Qiagen blood tissue DNA mini kit according to the manufacturer's protocol and it was stored at -20˚C till subjected for PCR.

3.5.2(b) Extraction of tick salivary gland genomic DNA

Materials required:

a. Microcentrifuge tubes (Tarsons).
b. Micropipettes (Tarsons).
c. Microcentrifuge (Tarsons).
d. DNA extraction kit.

The genomic DNA of adult ticks salivary glands were extracted as per the standard protocol using the DNeasy Blood and Tissue Kit procured from Indus bio solutions, Bangalore. Qiagen Germany (Manufacturers).

List of materials provided in the kit

DNeasy Mini Spin Column	50 Nos
Collection tubes (2ml)	150 Nos
Buffer ATL (Tissue lysis buffer)	10ml
Buffer AL (Lysis buffer)	12ml
Buffer AW1 (Concentrate)	19ml
Buffer AW 2 (Concentrate)	13ml
Buffer AE	22ml
Proteinase K	1.25ml

3.5.2 (c) Procedure for extraction of DNA

The genomic DNA was extracted from the 25 mg of tick salivary gland (5-6 ticks) using the "DNeasy® Blood & Tissue Kit" (Qiagen, Germany) as per the protocol with slight modification. The salivary glands of 5-6 same species ticks were dissected and pooled, the pooled salivary gland sample was used for DNA extraction.

Disruption and homogenization of tick salivary gland

Tick salivary gland stored in 70 per cent ethanol in room temperature were taken and were snap freezed in liquid N_2 (-196°C) in a prechilled pestle and crushed to get fine powdered tick salivary gland which was utilized for homogenization in concurring steps.

- 25 mg of pooled tick salivary gland was added to a 1.5 ml microcentrifuge tube containing 80 µl PBS and homogenized with a tissue homogenizer and 100 µl of ATL buffer was added.
- Add 20 µl of proteinase K, and mix by vortexing and incubated at 56 °C until the tick salivary gland completely lysed (1-3 hrs). The tubes were vortexed occasionally during incubation to disperse the sample.
- Brief centrifugation of the 1.5 ml microcentrifuge tube was done to remove drops on the inside of the lid.
- Add 200 µl of Buffer AL to the sample, and mixed by pulse-vortexing for 15 s, and incubated at 70 °C for 10 mins. Later brief centrifugation of the 1.5 ml microcentrifuge tube was done to remove drops from inside of the lid.

- Add 200 µl ethanol (96-100%) to the sample and mixed again by pulse vortexing for 15 sec. After mixing, brief centrifugation of the 1.5 ml microcentrifuge tube was done for removing the drops from the inside of the lid.
- The collected mixture from step 5 was carefully applied to the QIAamp Spin Column (in a 2 ml collection tube) without wetting the rim, cap was closed and centrifuged at 6000 x *g* (8000 rpm) for 1 min. The QIAamp Spin Column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded.
- The QIAamp Spin Column was carefully opened and add 500 µl buffer AW1 without wetting the rim, cap was closed and centrifuged at 6000 x g (8000 rpm) for 1 min. QIAamp Spin Column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded.
- The QIAamp Spin Column was carefully opened and add 500 µl buffer AW2 without wetting the rim, cap was closed and centrifuged at full speed (20,000 x g; 14,000 rpm) for 3 min. To eliminate any chance of possible Buffer AW2 carryover, step 9a was performed and then continued with step 10.
- The QIAamp Spin Column was placed in a new 2 ml collection tube and the collection tube with the filtrate was discarded. Centrifugation was done at 20,000 x g (14,000 rpm) for 3 min.
- The QIAamp Spin Column was placed in a new, autoclaved 1.5 ml microcentrifuge tube and the collection tube containing the filtrate was discarded. The QIAamp Spin Column was carefully opened and 200 µl buffer AE was added. Incubation at room temperature (15–25 °C) for 1 min was done and then centrifuged at 6000 x g (8000 rpm) for 1 min

to obtain a final DNA yield of volume 200 µl and DNA was stored at -20 °C until further use.

To know the prevalence of haemo protozoan parasites in the host (sheep) blood, the blood samples were collected and thin blood smears were prepared from the whole blood within three to four hrs of collection as described by Benjamin (1998) by using clean, grease free slides and air dried. After preparation of smears, the blood samples were stored at -20ºC until DNA extraction was carried out.

3.5.3a Staining of Blood smears by Geimsa method

- Blood smears were fixed in methanol for 30 sec to 1 min, air dried and placed on the staining rack.
- Diluted Geimsa stain (1:9) was added and allowed to act for 30 min and washed with distilled water.
- Stained smears were drained in a drain rack and dried at room temperature.
- Dried smears were examined under 100X oil immersion objective of a compound microscope

3.5.3b Extraction of genomic DNA from the blood sample collected from sheep.

The genomic DNA was extracted from the 200 µl of venous blood using the "QIAamp DNA minikit" (Qiagen, Germany) as per the protocol described by the manufacturer with slight modification.

- Pipetted 20µl proteinase K into a 1.5ml microcentrifuge tube. Add 50-100µl of anticoagulant treated blood. Adjust volume to 220 µl with PBS.
- Added 200µl buffer AL to the above and mixed thoroughly by vortexing for 15 sec. And incubated at 56 ˚C for 10 min.
- Added 200µl of ethanol (100%) to the above and mixed thoroughly by vortexing for 15 secs.
- Pipetted out the whole mixture into a DNeasy Mini Spin column placed in a 2ml collection tube. It was centrifuged at 6000Xg (8000 rpm) for 1 min. Discarded the flow through and collection tube.
- Placed the spin column in a new 2ml collection tube and added 500µl of buffer AW1. It was centrifuged for 1 min at 6000Xg (8000rpm). The flow- through and collection tube was discarded.
- The spin column was placed in a new 2ml collection tube and 500µl of buffer AW2 was added. It was centrifuged for 1 min at 6000Xg (8000rpm). The flow- through and collection tube was discarded.
- Transferred the spin column into a new 2ml micro centrifuge tube.
- Eluted the DNA by adding 50µl of buffer AE to the centre of the spin column membrane. It was incubated for 1min at room temperature (15-25˚C) and centrifuged for 1 min at 6000g (8000rpm).
- Later the extracted DNA was stored at -20˚C.

3.5.4 DNA confirmation by agarose gel electrophoresis.

Equipment.

a. Weighing balance (KERN).

b. Horizontal electrophoresis apparatus with power pack (GENETIX).

c. Microwave oven (LG).

d. Gel documentation unit (DNR MiniLumi)

e. Agarose (Himedia).

Tris – borate EDTA buffer (TBE buffer) (10X, pH8)

Tris base	10.8 g
Boric acid	5.5 g
EDTA (0.5 M pH 8)	4ml
Distilled water	up to 100ml

The stock was sterilized by autoclaving before use. To make 1X TBE buffer, 10X buffer and distilled water was added in the ratio of 1:20. To make 0.5X TBE take 25ml of 10X TBE and makeup the volume to 500ml.

Gel loading dye (6X)

Bromophenol blue	:	0.25 % (w/v)
Xylene cyanol	:	0.25 % (w/v)
Sucrose	:	40% (w/v) in distilled water

Ethidium bromide (10mg/ml)

Ethidiumbromide	:	100mg
Double distilled water	:	10ml

The suspension was stirred to ensure that the dye had dissolved. The container was then wrapped in aluminium foil.

Preparation of 0.8 % gel for checking genomic DNA

About 0.8g of agarose (analytical grade) was dissolved in 95ml of distilled water and 5ml of 10X TBE buffer and melted in micro-oven for 2 min until a velar uniform suspension was obtained. Prior to casting the gel, the molten agarose was allowed to cool to about 50°C, after which 2ml of Ethidium bromide was added and mixed thoroughly. Gel was cast on the gel casting tray fitted with with acrylic comb and left for setting. The acrylic comb was carefully removed after the gel was set. The tray with gel was submerged in an electrophoresis tank containing 0.5X TBE buffer.

5µlof DNA to be analyzed was mixed with 2µl of 6X DNA loading dye and charged into wells. Electrophoresis was carried until the tracking dye just reached the end of gel. Following the electrophoresis, DNA bands were visualized and the images were captured by using Gel Doc unit (DNA MiniLumi).

3.5.5 Polymerase Chain Reaction (PCR)

The DNA extracted from the salivary gland was subjected to PCR by using the genus and species specific primers targeting the 18S ribosomal RNA gene sequence of *Theileria* and SS U RNA gene of *Babesia* spp.

Reagents

a. 10X Tag buffer (Genei).

b. dNTP (deoxy nucleotide triphosphate mix) (Genei).

c. Tag DNA polymerase 1unit/ml (Genei).

d. Sterilised nuclease free water (NFW) (Himedia)

e. Primers (BIOSERVE)

F. Template DNA extracted from ticks salivary gland and host blood.

g. 100 bp DNA ladder (HIMEDIA)

Nested PCR amplification of 18S Ribosomal RNA gene of *Theileria* spp and conventional PCR amplification of SS U RNA gene of *Babesia* spp was done by using genus and species specific primers. The published genus and species specific primers were synthesized by Bioserve Biotechnologies (India) Pvt.Ltd, Hyderabad. The details of primers and their base sequences are detailed below (Table 3 & 4). The primers obtained were reconstituted in nuclease free water as per the requirement and stored at -20˚C.

Table 3: Primer sequence of 18 S rRNA gene of *Theileria* species.

Target Parasite	Nucleotide sequence	Product size (bp)	Reference
Theleria genus specific	F: AGTTTCTGACCTATCAG R: TTGCCTTAAACTTCCTTG	1098bp	Allsopp *et al*. (1993)
Theleria ovis	F1: CACAGGGAGGTAGTGACAAG R2: AAGAATTTCACCTATGACAG F2: AAGAATTTCACCTATGACAG R2: TTGCTTTTGCTCCTTTACGAG	426bp 237bp	Razmi *et al*. (2013)
Theileria luwenshuni	F1: CATGGATAACCGTGCTAATT R1: ATCGTCTCGATCCCCTAACT F2: GGTAGGGTATTGGCCTACCGG R2: TCATCCGGATAATACAAGT	388bp	Peng *et al*. (2015)
Theileria lestoquadri	F1: CACAGGGAGGTAGTGACAAG R2: AAGAATTTCACCTATGACAG F2: AAGAATTTCACCTATGACAG R2: ATTGCTTGTGTCCCTCCG	426bp 235bp	Razmi *et al*. (2013)

Table 4: Primer sequence of ssurRNA gene of *Babesia* species

Target parasite	Nucleotide sequence	Product size	Reference
Babesia genus specific	F1: GTCTTGTAATTGGAATGATGG R1: CCAAAGACTTTGATTTCTCTC	350bp	Aktas *et al*. (2005)
Babesia ovis	F1: TGGGCAGGACCTTGGTTCTTCT R2: CCGCGTAGCGCCGGCTAAATA	549bp	Aktas *et al*. (2005)
Babesia motasi	F1: TAAACCAATTTGTTGGT R2: TCTGCCCAGGGTTTAAGTCGG	294bp	Peng *et al*. (2015)

Table 5. Compositions of PCR mix for species specific amplification of *Theileria* and *Babesia* species.

Contents	**Quantity**
Master mix	12.5 µl
Red dye	2.5 µl
Forward primer	2 µl
Reverse primer	2 µl
Template	2 µl
NFW to make final volume	4 µl
Total volume	**25µl**

The amplification reactions were carried out in 0.2ml PCR tubes using a programmable thermal cycler using the following cycling conditions with slight modification (Table.6).

Table 6: PCR conditions for the amplification of 18S rRNA gene of *Theileria* and ssurRNA *Babesia*.

Target parasite	Initial denaturation	Denaturation	Annealing	Extension	Final extension	Cycles
Theileria genus	94 °C -3 min	94 °C - 30secs.	56 °C -1 min.	72 °C - 1min	72 °C-5min	40
T.luwenshuni						
1set primers	94 °C - 5 min	94 °C - 30secs	55 °C -1 min	72 °C-10min	72 °C-1min	35
2set primers	94 °C - 3 min	94 °C - 30secs	55 °C -1 min	72 °C-10min	72 °C-1min	
T.ovis						
1set primers	94 °C - 5 min	94 °C -45secs	55 °C -45sec	72 °C - 5min	72 °C-1min	36
2set primers	94 °C - 5 min	94 °C -45secs	55 °C -45 sec	72 °C - 5min	72 °C-1min	
T.lestoquadri						
1set primers	94 °C - 5 min	94 °C -45secs	55 °C -45 sec	72 °C - 5min	72 °C-1min	35
2set primers	94 °C - 5 min	94 °C - 45secs	55 °C -45 sec	72 °C -5min	72 °C-1min	
Babesia genus	94 °C - 5 min	94 °C –1 min	55 °C – 1min	72 °C - 1min	72 °C- 10min	30
B. ovis	94 °C - 3 min	94 °C –1 min	60 °C – 1min	72 °C - 5min	72 °C - 1min	30
B.motasi	94 °C - 3 min	94 °C –30 sec	55 °C – 1min	72 °C - 1min	72 °C- 10min	35

3.5.5 (a) Nested PCR for Theileria species

After completion of first round of PCR with first set of primers, 5µl of amplified product along with 6X gel loading dye were subjected to electrophoresis in 1.5 per cent agarose gel and 100 bp DNA ladder was used as marker and visualised using ethidium bromide and a UV-illuminator visible bands at 426 bp for Theileira species were produced in the first round of PCR. The images were captured using gel documentation system. In the second round, second set of primers mentioned in Table.3 were used to detect *T.ovis* and *T.luwenshuni*. The second round PCR products were also separated using electrophoresis on a 1.5 per cent agarose gel to determine the presence of specific bands for *T.ovis* (237bp) and *T.luwenshuni* (388bp).

3.5.5 (b) Conventional PCR for Babesia species:

Conventional PCR was done with genus specific and species specific primers. After completion of PCR, 5µl of amplified product along with 6X gel loading dye were subjected to electrophoresis in 0.8% agarose gel and 100bp DNA ladder was used as marker and visualised using ethidium bromide and a UV-illuminator visible bands at 350bp for specific Babesia species and the images were captured using gel documentation system.

3.5.6 Sequencing of PCR products

The PCR products were sent to Bioserve. Ltd. Hyderabad for sequencing, where PCR products were sequenced in both forward and reverse directions. Sequences were then checked for homology by using bioinformatics tool BLAST (Basic local alignment search tool) from NCBI (National centre for Biotechnology information) server.

Results

IV. RESULTS

4.1 Prevalence of arthropod parasites in sheep

During this study, 1817 sheep from organised farms and 2133 sheep from flocks in different parts of Karnataka were screened for arthropod infestation by considering different variables such as season, breed, age, sex, farm management and agro climatic conditions. The different ectoparasites collected were identified and are listed in the Table 7. Out of 1817 sheep, 20.7 per cent (377/1817) of the population was infested in organised farms, whereas 43.7 per cent (933/2133) of the population was infested with ectoparasites in other unorganised sheep flocks in Karnataka.

In this study, of the organised sheep farms screened, the prevalence of ectoparasites was higher in females with 23.3 per cent (353/1515) than males with 7.90 per cent (24/302). Prevalence of ectoparasites was the highest in rainy 29 per cent (177/608) followed by winter with 21 per cent (130/613) and summer season 11.7 per cent (70/596). Among the age groups, hogget animals were heavily infested with 24.5 per cent (235/957) followed by adults comprising 16.9 per cent (119/702) and in lambs 14.5 (23/158). Based on the farm management it was the semi intensive farm with 21.7 per cent (350/1606) animals which were heavily infested with ectoparasites than intensive animals of 12.7 per cent (27/211). In arid regions, 37.10 per cent of sheep (128/345) were heavily infested with ectoparasites than the sheep in semi-arid region with 16.9 per cent (249/1472).

In unorganised sheep flocks, the prevalence of ectoparasites was slightly higher in females of 49.2 per cent (893/1813) than males with 12.5 per cent (40/320). Prevalence of ectoparasites was highest in rainy season 61.76 per cent (441/714) followed by summer

with 52.7 per cent (378/725) and winter season with 42.48 per cent (294/692). Among the age groups, hoggets were heavily infested comprising 52.27 per cent (528/1010)) followed by adults with 39 per cent (374/960).

With regard to farm management, semi intensive farm had heavy infestation with 49.2 per cent (893/1813) that were heavily infested with ectoparasites than intensive animals with 13.2 per cent (30/227). The sheep in arid regions were heavily infested with ectoparasites with 48.48 per cent (305/629) than the sheep in semi arid region with 41.7 per cent (628/1504). Among the breeds either in organised and un-organised farms; the non-descriptive breeds were heavily infested than descriptive breeds.

Among all ectoparasites, ticks were the most frequently found species of ectoparasites. A total of 3233 ticks (326 from organised farms and 2907 from un-organised farms) were collected and identified as *Haemaphysalis kutchensis, Haemaphysalis intermedia, Haemaphysalis bispinosa, Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci*. The highest number of ticks belonged to *Haemaphysalis* spp followed by *Rhiphicephalus* spp, *Hyalomma* spp and only 4 numbers of *Amblyomma* spp. Majority of the ticks were found infested in the ears, near eyelids, axillae, around perineum, anal and tail region.

The ticks belonging to four genera and eight species were oberved. Out of 3233 ticks collected from both organised and unorganised sheep farms, 1532 ticks were found to be *Haemaphysalis* spp, followed by *Rhipicephalus* spp (936), *Hyalomma* spp (645) and *Amblyomma* spp (4).

Lice were also encountered in sheep from both organised and un-organised farms. Among 1817 sheep in organised farms about 58 were infested with *Damalinia ovis* and 36 were infested with *Linognathus stenopsis.* In unorganised farms 201 animals were infested with lice in which 103 were infested with *Damalinia ovis* and 98 sheep with *Linognathus stenopsis.* Lice were found in high numbers in winter, in which adults were found heavily infested than young ones.

About 149 animals in un-organised flocks and 94 animals in organised farms were infested with *Sarcoptes scabiei var ovis* mites which were observed in hoggets and adults during winter season followed by post monsoon. *Ctenocephalides orientis* and *Ctenocephalides felis felis* were the fleas found to infest 44 and 95 animals from organised and unorganised farms. Adult sheep were heavily infested followed by young ones. Flea infestation was more wide spread in post monsoon and winter. In this study it was observed that the females were heavily infested with ectoparasites than males.

The nasal bots were collected from the farms of both organised and unorganised flocks. The prevalence was high in winter season in the LRIC, Konehally, ILFC, KVAFSU an organised farm, whereas in unorganised farms it was reported in Belgaum, Chitradurga and Davangere. Fly traps placed in the farms could trap *Tabanus striatus, Musca domestica, Haematopota pluvialis, Chrysomya megacephala* flies.

Table 7. Different ectoparasites collected from different places of Karnataka

Ectoparasite	Species name	Places of collection	
		Organised farms	**Un organised farms**
Fleas	*Ctenocephalides orientis.*	ILFC - Hassan. LRIC- Nagamangala, ILFC- KVAFSU,	Tumkur, Bellary, Chitradurga, Davangere, Mandya, Belgaum, Bidar, Bangalore ®.
	Ctenocephalides felis felis.	LRIC-Nagamangala.	Chitradurga, Davangere, Bangalore ®.
Lice	*Damalinia ovis.*	BSBT-Mandya, SBTC- Chitradurga, LRIC- Nagamangala, ILFC- KVAFSU, SBTC- Athani	Tumkur, Davangere, Chitradurga.
	Linognathus stenopsis.	BSBT- Mandya, ILFC - Hassan, SBTC- Chitradurga, ILFC- KVAFSU.	Tumkur, Bellary, Mandya, Davangere, Belguam, Bidar
Mite	*Sarcoptes scabiei var ovis.*	SBTC-Chitradurga LRIC-Nagamangala, SBTC- Athani.	Tumkur, Bellary, Davangere, Mandya, Chitradurga, Bidar.
Flies	*Haematopota pluvialis.*	LRIC-Nagamangala	-----
	Musca domestica.	LRIC-Nagamangala, SBTC- Guttal, BSBT-Mandya, SBTC-Athani	Bellary, Belgaum, Chitradurga, Davangere.
	Chrysomya megacephala.	Athani	
	Tabanus striatus	---	Bellary, Davangere
Nasal bots	*Oestrus ovis*.	I ILFC- Konehally, BSBT- Mandya.	Belgaum, Chitradurga, Davangere.

Table 8. Ticks identified from organised and unorganised sheep farms from ten districts of Karnataka state

Sl. No.	Farm name Unorganised	Tick spp	No of ticks collected	Sl. No.	Farm Organised	Tick spp	No of ticks
1	Mandya	*H.bispinosa*	110	1	ILFC, KVAFSU	*H.kutchensis*	13
		H.intermedia	98				
		H.kutchensis	79				
		R.sanguineus	38				
		R.haemaphysaloides	43				
2	Davangere	*Hy.anatolicum.anatolicun*	98	2	ILFC, Hassan	*H.bispinosa*	20
		Hy.marginatum.isaaci	94			*H.intermedia*	14
		R.haemaphysaloides	54				
		R.sanguineus	40				
3	Hassan	*H.bispinosa*	*62*	3	ILFC, Shivamoga	*H.bispinosa*	10
		R.haemaphysaloides	*40*				
4	Bellary	*H.bispinosa*	85	4	SBTC, Guttal	*H.bispinosa*	13
		H.intermedia	63			*R.haemaphysaloides*	15
		H.kutchensis	60			*H.anatolicum.anatolicum*	15
		R.haemaphysaloides	46				
		R.sanguineus	64				
5	Shivamoga	*H.bispinosa*	46	5	LRIC, Konehally	*H.kutchensis*	15
		R.haemaphysaloides	40			*R.haemaphysaloides*	6
6	Belgaum	*H.bispinosa*	60	6	SBTC, Khudapura Chitradurga	*H.bispinosa*	15
		H.intermedia	78			*H.intermedia*	18
		R.haemaphysaloides	48			*H.kutchensis*	29
		R.sanguineus	36			*R.haemaphysaloides*	25
		H.a.anatolicum	62			*R.sanguineus*	10
		H.marginatum.isaaci	12				
7	Bidar	*H.bispinosa*	72	7	LRIC Nagamangala	*H.kutchensis*	11
		H.intermedia	68			*H.bispinosa*	4
		R.haemaphysaloides	12				
		R.sanguineus	59				
		H.anatolicum.anatolicum	123				
		H.marginatum.issaci	126				
		Amblyomma americanum	04				
8	Hessaraghatta Bangalore	*H.kutchensis*	120	8	BSBT, Dhanagur, Mandya	*H.bispinosa*	4
		R.haemaphysaloides	78			*H.kutchensis*	22
		R.sanguineus	41			*H intermedia*	8
9	Chitradurga	*H.intermedia*	102			*R.haemaphysaloides*	10
		H.kutchensis	70				
		R.haemaphysaloides	118				
		R.sanguineus	10				
		H.a.anatolicum	79				
10	Tumkur	*H.bispinosa*	90	9	SBTC, Athani	*H.bispinosa*	13
		H.kutchensis	180			*H.a.anatolicum*	20
		R.haemaphysaloides	71			*H.marginatum isaaci*	16
		R.sanguineus	32				
	Total						

Table 9. Common predilection sites of arthropod infestation in sheep.

Ectoparasite	Species name	Site of predilection
Ticks	*Haemaphysalis bispinosa* *Haemaphysalis intermedia* *Haemaphysalis kutchensis*	Ear pinna, eye & ear axis, neck
	Hyalomma .a.anatolicum	Anus, tail, vulva
	Hyalomma.m.isaaci	Vulva, anus
	Rhipicephalus sanguineus *Rhipicephalus haemaphysaloides*	Anus, tail, vulva
	Amblyomma americanum	Anus, vulva
Fleas	*Ctenocephalides orientis* *Ctenocephalides felis felis*	Limbs
Lice	*Damalinia ovis* *Linognathus stenopsis*	Ear, neck, abdominal region, hind quarter, tail region
Mite	*Sarcoptes var scabiei ovis*	Ear, face, neck, hind quarter, ventral abdomen, hind legs
Flies	*Haematopota pluvialis, Musca domestica, Chrysomya megacephala, Tabanus striatus*	Collected in farm premises
Nasal bots	*Oestrus ovis*	Collected in farm premises

Table 10. Overall prevalence of ectoparasites in sheep in Karnataka

Parasites Identified	Organised farms			Unorganised farms		
	Number examined	Number infested	% infestation	Number examined	Number infested	% infestation
Ticks	1817	145	7.98	2133	533	30
Lice		94	5.17		201	9.4
Fleas		44	2.42		95	4.45
Mites		94	5.17		149	6.98
Total	1817	377	20.7	2133	933	43.74
$\chi 2$ - 234, df -1						

Note:*Significant at $p<0.05$

Table 11. Prevalence of ectoparasites in sheep of different age groups

Age	Organised farms			Unorganised farms		
	Number examined	Number infested	(%)	Number examined	Number infested	(%)
Kids	158	23	14.5	155	53	34.19
Hogget	957	235	24.5	1010	528	52.27
Adult	702	119	16.9	968	374	38.6
Total	1817	377	20.7	2133	933	43.7
	$\chi 2$ - 18.27, df- 2			**$\chi 2$ - 44.34, df – 2**		

Note: *Significant at $p<0.05$

Table 12. Prevalence of ectoparasites in sheep based on agroclimatic zones

Weather	Organised farms			Unorganised farms		
	Number examined	Number infested	% infestation	Number examined	Number infested	% infestation
Arid	345	128	37.01	629	305	48.48
Semi arid	1472	249	16.91	1504	628	41.7
Total	1817	377	20.7	2133	933	43.7
	$\chi 2$ - 69.27, df -1			**$\chi 2$ - 8.17, df – 1**		

Note: *Significant at $p<0.05$

Table 13. Prevalence of ectoparasites in sheep based in sheep based on breed

Breed	Organised farms			Unorganised farms		
	Number examined	Number infested	% infestation	Number examined	Number infested	% infestation
Descriptive	1817	377	20.7	-	-	-
Non descriptive	-	-	-	1595	933	43.7
Total	1817	377	20.7	2133	933	43.7
	$\chi 2$ - 511.7, df 1					

Note: *Significant at $p<0.05$

Table 14. Prevalence of ectoparasites in sheep based on Sex wise

Sex	Organised farms			Unorganised farms		
	Number examined	Number infested	% infestation	Number examined	Number infested	% infestation
Female	1515	353	23.3	1813	893	49.2
Male	302	24	7.9	320	40	12.5
Total	1817	377	20.7	2133	933	43.7
	$\chi 2$ - 36.10, df 1			**$\chi 2$ - 149.3, df 1**		

Note: *Significant at $p<0.05$

Table 15. Prevalence of ectoparasites in sheep based in different season

Seasons	Organised farms			Unorganised farms		
	Number examined	Number infested	% infestation	Number examined	Number infested	% infestation
Winter	613	130	21.2	692	294	42.48
Rainy	608	177	29.11	714	441	61
Summer	596	70	11.7	725	378	52.7
Total	1817	377	20.7	2133	933	43.7
	$\chi 2$ - 37.4, df 2			**$\chi 2$ - 50.22, df 2**		

Note: Significant at $p<0.05$

Table 16. Prevalence of ectoparasites in sheep based on farm management practice

Farm management	Organised farms			Unorganised farms		
	Number examined	Number infested	% infestation	Number examined	Number infested	% infestation
Semi intensive	1606	350	21.7	1813	893	49.2
Intensive	211	27	12.7	227	30	13.2
Total	1817	377	20.7	2133	933	43.7
	$\chi 2$ - 9.18, df 1			**$\chi 2$ - 105.8, df 1**		

Note: Significant at $p<0.05$

Table 17. Flies and nasal bots collected in organised farm premises

	Organised farm	Number recovered	Species identified
Flies	LRIC, Nagamangala	12	*Musca domestica (10)* *Haematopota pluvialis (2)*
	SBTC, Guttal	15	*Musca domestica (15)*
	BSBT, Dhangur, Mandya	10	*Musca domestica (10)*
	SBTC, Athani	17	*Chrysomya megacephala (10)* *Musca domestica (7)*
Nasal bots	LRIC, Konehally, Tiptur	11	*Oestrus ovis*
	BSBT, Dhangur, Mandya	8	*Oestrus ovis*

Table 18. Flies and nasal bots collected in unorganised farm premises

	Un organised farm	Number recovered	Species identified
Flies	Davangere	28	*Musca domestica* (28) *Tabanus striatus* (16)
	Bellary	10	*Musca domestica* (10) *Tabanus striatus* (10)
	Belgaum	10 08	*Musca domestica* (10) *Chrysomya megacephala* (8)
	Chitradurga	10 11	*Chrysomya megacephala* (10) *Musca domestica* (11)
Nasal bots	Belgaum	15	*Oestrus ovis*
	Chitradurga	08	*Oestrus ovis*
	Davangere	11	*Oestrus ovis*

Fig. 1. Prevalence of ectoparasites in sheep of different age groups in organised farms

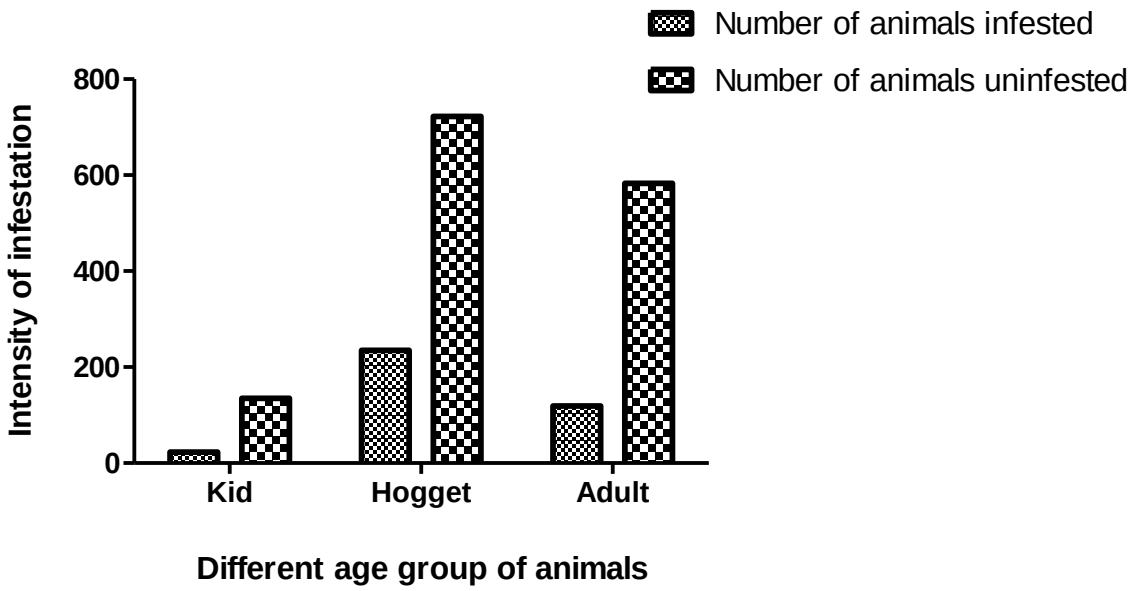


Fig. 2. Prevalence of ectoparasites in sheep of different age groups in unorganised farms

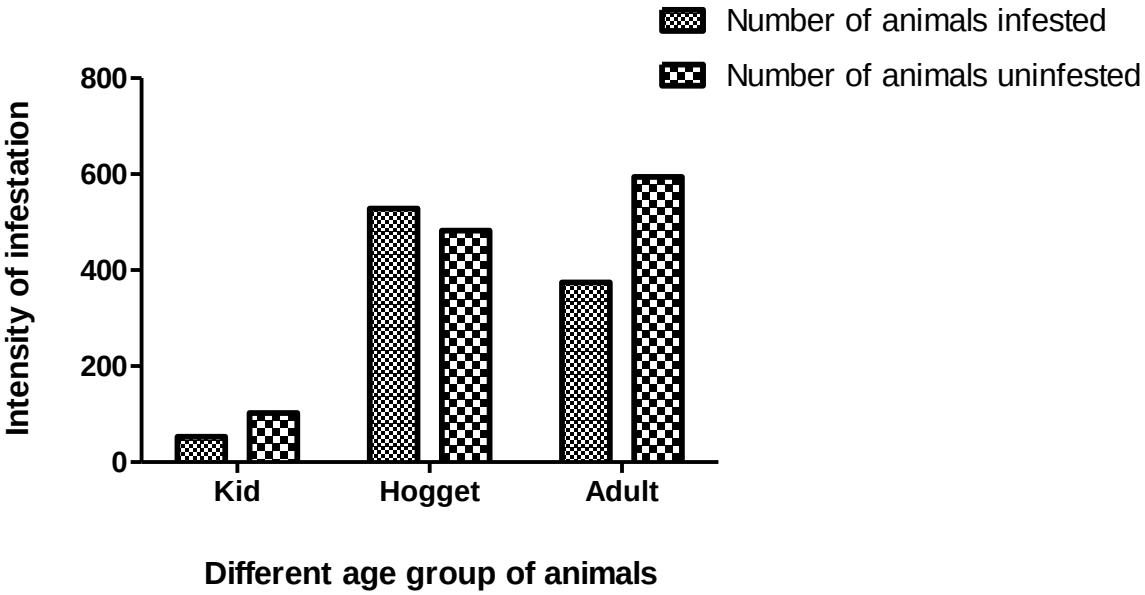


Fig. 3. Prevalence of ectoparasites in sheep based on agroclimatic zones in organised farms

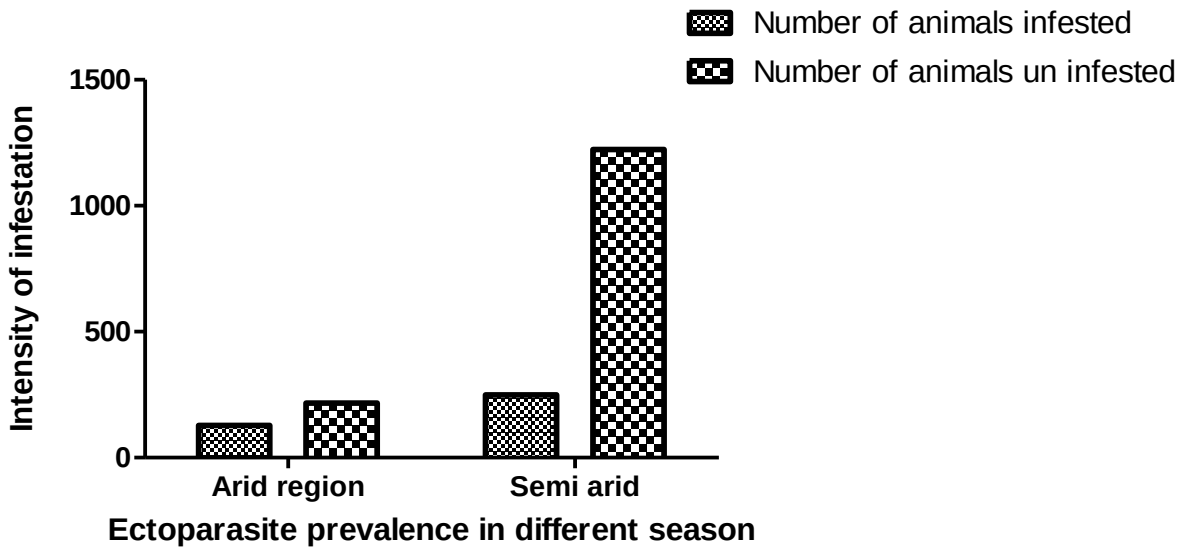


Fig. 4. Prevalence of ectoparasites in sheep based on agroclimatic zones in organised farms

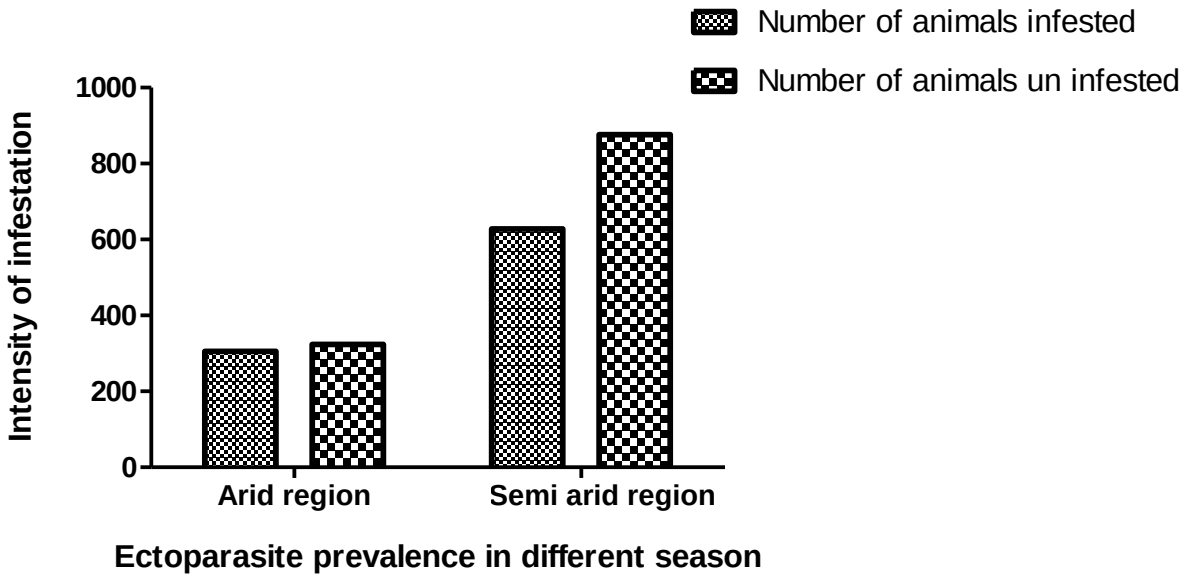


Fig. 5. Prevalence of ectoparasites in sheep based on Sex wise in organised farms.

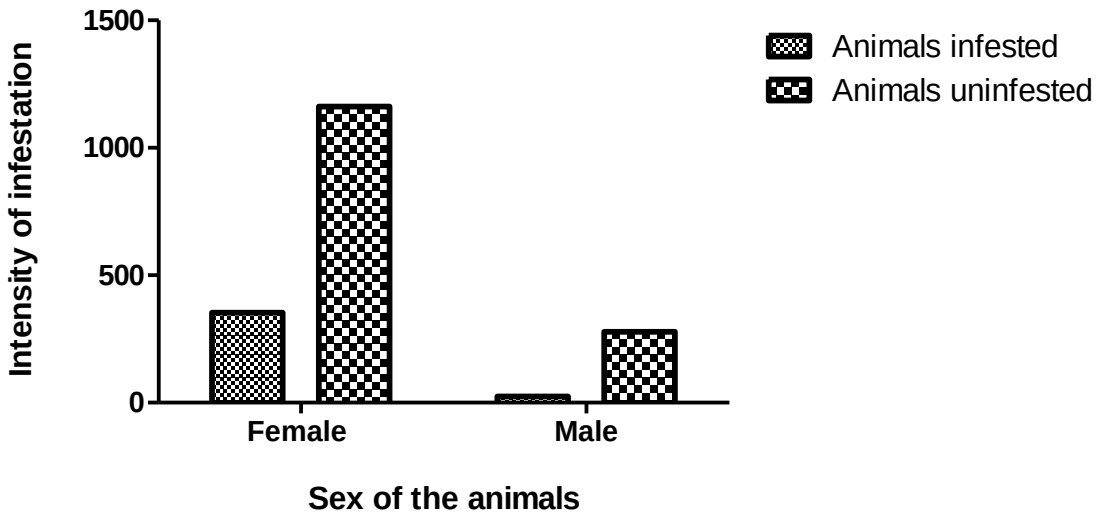


Fig. 6. Prevalence of ectoparasites in sheep based on Sex wise in unorganised farms.

Fig. 7. Prevalence of ectoparasites in sheep based in different seasons in organised farms

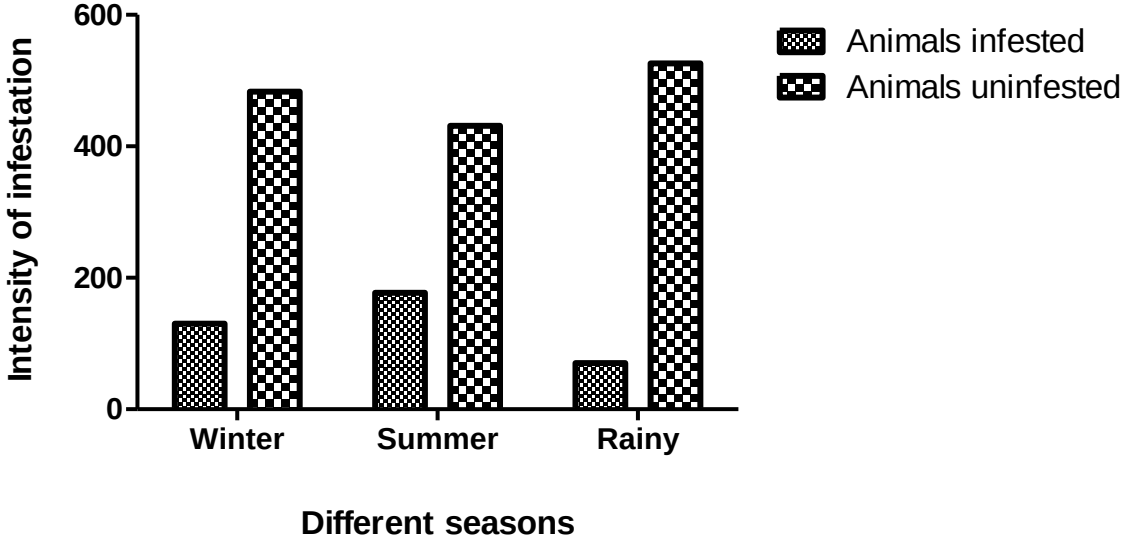


Fig. 8. Prevalence of ectoparasites in sheep based in different seasons in unorganised farms

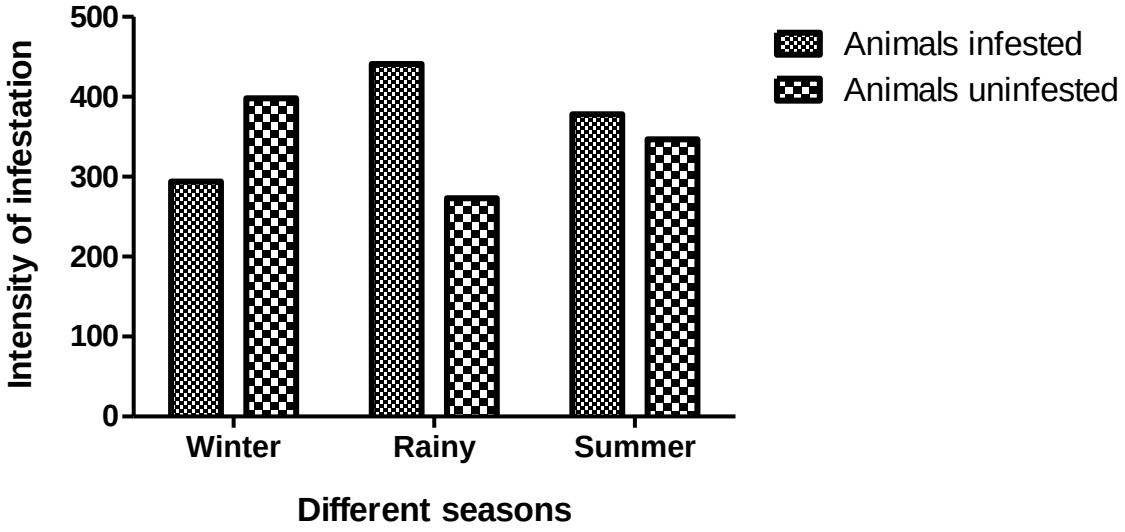


Fig. 9. Prevalence of ectoparasites in sheep based on farm management practice in organised farms.

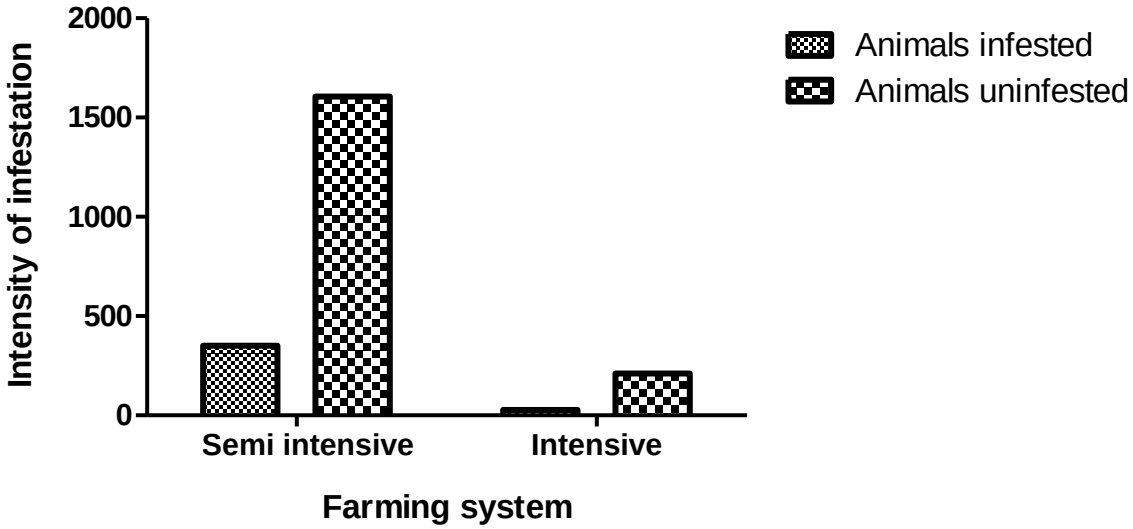
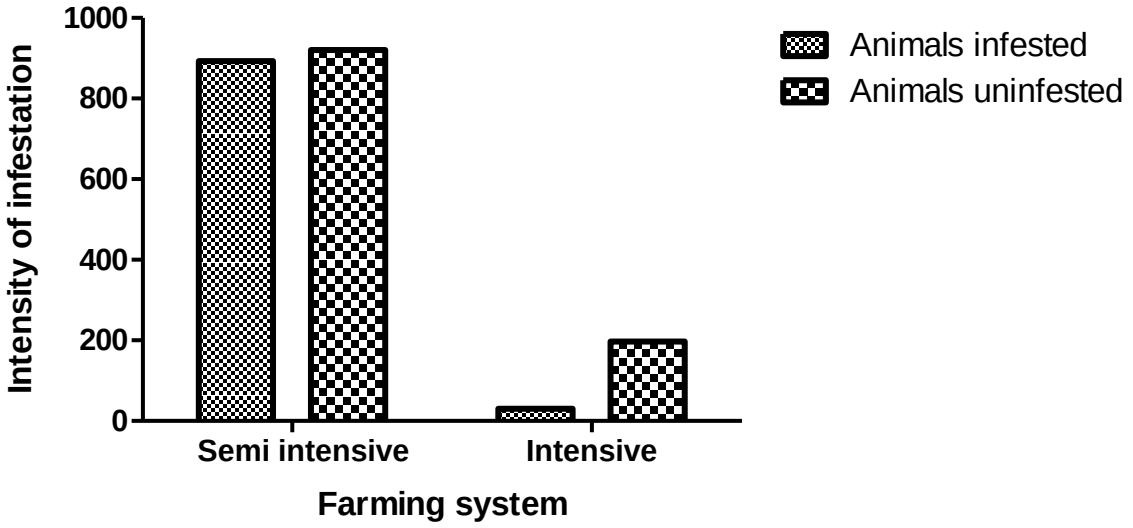


Fig. 10. Prevalence of ectoparasites in sheep based on farm management practice in unorganised farms



4.2 Evaluation of the efficacy of chemical acaricides in control of ticks

4.2.1 Larval packet test

The results of the *in vitro* trials by larval packet test (LPT) on larval stages of different species of ticks *i.e., H.bispinosa, H.kutchensis, H.intermedia, R.sanguineus, R.haemaphysaloides*, *H.a.anatolicum* and *H.m.isaaci* at different concentration of amitraz (0.1%, 0.2%, 0.3%, 0.4%) deltamethrin and cypermethrin (0.05%, 0.1%, 0.2%, 0.3%, 0.4%) was recorded. After 24hrs of exposure, 100 per cent mortality rate was recorded at higher concentrations of deltamethrin and cypermethrin (0.3%), whereas amitraz at lower concentration at 0.2 per cent induced 100 per cent mortality.

In the larval packet test 100 per cent mortality against species of ticks *i.e., H.bispinosa, H.kutchensis, H.intermedia, R.sanguineus, R.haemaphysaloides*, *H.a.anatolicum* and *H.m.isaaci* was recorded at 0.4 per cent concentration of deltamethrin whereas cypermethrin induced 100 per cent mortality at 0.3 per cent. Amitraz at concentration of 0.2 per cent induced 100 per cent mortality against all species of ticks. The details of larval mortality after 24 hrs intervals are presented in the Table 19 to 25.

4.2.2 Adult immersion test with discriminating dose (AIT-DD)

The results of adult immersion test with discriminating dose for amitraz, deltamethrin and cypermethrin at the discriminating doses of 2.5g/ltr, 0.075 g/ltr, 0.05 g/ltr respectively have also been recorded. The adult immersion test with discriminating doses (AIT-DD) was conducted in triplicates and results were recorded on the average of three replicates against the ticks species *Hyalomma* spp, *Haemaphysalis* spp and *Rhipicephalus* spp. The AIT-DD revealed resistance to cypermethrin and deltamethrin but was found susceptible to amitraz (Table 27 to 33)

Table 19. Efficacy of acaricides against larvae of *H. bispinosa* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	41	41	41
b. 0.1	100	56	56	56
c. 0.2	100	73	73	73
d. 0.3	100	92	92	92
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	60	60	60
b. 0.1	100	68	68	68
c. 0.2	100	81	81	81
d. 0.3	100	100	100	100
Amitraz				
a.0.1	96	96	96	96
b.0.2	100	100	100	100

Table 20. Efficacy of acaricides against larvae of *H.intermedia* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	42	42	42
b. 0.1	100	58	58	58
c. 0.2	100	78	78	78
d. 0.3	100	93	93	93
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	61	61	61
b. 0.1	100	65	65	65
c. 0.2	100	80	80	80
d. 0.3	100	100	100	100
Amitraz				
a.0.1	94	94	94	94
b.0.2	100	100	100	100

Table 21. Efficacy of acaricides against larvae of *H. kutchensis* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	40	40	40
b. 0.1	100	55	55	55
c. 0.2	100	70	70	70
d. 0.3	100	85	85	85
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	59	59	59
b. 0.1	100	62	62	62
c. 0.2	100	83	83	83
d. 0.3	100	100	100	100
Amitraz				
a.0.1	100	95	95	95
b.0.2	100	100	100	100

Table 22. Efficacy of acaricides against larvae of *H.a.anatolicum* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	52	52	52
b. 0.1	100	69	69	69
c. 0.2	100	87	87	87
d. 0.3	100	95	95	95
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	76	76	76
b. 0.1	100	86	86	86
c. 0.2	100	94	94	94
d. 0.3	100	100	100	100
Amitraz				
a.0.1	100	96	96	96
b.0.2	100	100	100	100

Table 23. Efficacy of acaricides against larvae of *H.m.isaaci* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	54	54	54
b. 0.1	100	79	79	79
c. 0.2	100	89	89	89
d. 0.3	100	95	95	95
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	75	75	75
b. 0.1	100	85	85	85
c. 0.2	100	92	92	92
d. 0.3	100	100	100	100
Amitraz				
a.0.1	100	90	90	90
b.0.2	100	100	100	100

Table 24. Efficacy of acaricides against larvae of *R.sanguineus* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	63	63	63
b. 0.1	100	77	77	77
c. 0.2	100	89	89	89
d. 0.3	100	93	93	93
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	80	80	80
b. 0.1	100	90	90	90
c. 0.2	100	96	96	96
d. 0.3	100	100	100	100
Amitraz				
a.0.1	100	92	92	92
b.0.2	100	100	100	100

Table 25. Efficacy of acaricides against larvae of *R.haemaphysaloides* by LPT

Concentration % of acaricide	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality (%)	Percentage efficacy (%)
Control	100	100	0	0
Deltamethrin				
a. 0.05	100	65	65	65
b. 0.1	100	75	75	75
c. 0.2	100	86	86	86
d. 0.3	100	94	94	94
e.0.4	100	100	100	100
Cypermethrin				
a. 0.05	100	81	81	81
b. 0.1	100	92	92	92
c. 0.2	100	96	96	96
d. 0.3	100	100	100	100
Amitraz				
a.0.1	100	98	94	94
b.0.2	100	100	100	100

Table 26: LC $_{50}$ and LC $_{95}$ fiducial limit, mortality slope, chi square and resistance factor against deltamethrin, cypermethrin and amitraz as determined by LPT against different species of sheep ticks collected from Karnataka, India

Acaricides	Tick spp	LC_{50}	95%FL	LC_{99}	95%FL	Slope±SE	$\chi 2$	RF	RL
Deltamethrin	Reference population for Deltamethrin	36.23	10.06-57.32	194.02	140.02-248.96	1.86±0.66	7.04	-	-
	H.bispinosa	40.34	4.71-42.21	88.98	51.10- 115.57	2.14±0.47	20.54	8.10	I
	H.kutchensis	42.19	5.14-42.11	89.24	52.68-118.98	2.60±0.56	21.34	7.89	I
	H.intermedia	44.36	5.20- 42.13	89.40	52.20-118.04	2.68±0.56	24.24	2.54	I
	H.a.anatolicum	49.18	5.04-44.64	89.46	51.45- 115.21	2.25±0.51	19.22	6.20	I
	H.marginatum.isaaci	48.16	5.02-44.26	89.04	51.21-116.68	2.22±0.49	18.90	8.78	I
	R.haemaphysaloides	59.30	4.04-42.75	85.84	46.36-112.62	2.18±0.51	18.38	7.40	I
	R.sanguineus	62.13	5.23-44.64	86.34	49.94-114.56	2.20±0.86	18.92	6.09	I
Cypermethrin	Reference population for Cypermethrin	12.39	10.03-57.32	87.14	54.13-167.99	2.44±0.14	18.19	-	-
	H.bispinosa	24.24	6.17-48.91	90.13	54.01- 125.70	2.07±0.46	19.63	2.96	I
	H.kutchensis	23.34	4.71-42.12	88.98	51.10-115.57	2.14±0.47	20.54	3.34	I
	H.intermedia	24.18	5.04-44.64	89.46	51.45-115.21	2.25±0.51	19.22	2.45	I
	H.a.anatolicum	29.01	7.20-49.01	92.32	59.45- 123.21	2.20±0.47	21.42	2.36	I
	H.m.isaaci	28.16	6.18-46.78	90.46	56.31-119.21	2.01±0.31	19.89	1.89	I
	R.haemaphysaloides	36.73	9.34- 55.67	99.43	102.36223.20	6.33±0.56	26.92	2.67	I
	R.sanguineus	34.17	5.39-52.90	95.01	97.26-203.24	5.46±0.46	21.95	1.82	I
Amitraz	Reference population for Amitraz	2.56	2.51-4.74	4.19	2.61-7.48	4.13±0.124	14.46	-	-
	H.bispinosa	1.61	0.85-1.87	2.44	0.24-2.90	1.55±0.31	24.3	1.02	S
	H.kutchensis	1.63	0.64-1.91	2.49	2.26-3.15	1.48±0.33	19.37	1.13	S
	H.intermedia	1.66	1.28-1.85	2.42	2.27-2.68	2.02±0.30	42.95	1.04	S
	H.a.anatolicum	1.90	1.62-2.05	2.53	2.36-2.85	2.02±0.30	42.95	1.07	S
	H.marginatum.isaaci	1.86	1.61-2.00	2.48	2.33-2.73	2.05±0.28	53.54	1.15	S
	R.haemaphysaloides	1.70	1.61-1.91	2.37	2.18-2.75	1.92±0.37	27.09	1.14	S
	R.sanguineus	1.71	1.56-1.81	2.28	2.20-2.39	2.23±0.19	33.6	1.04	S

Note: LC_{50}: median lethal concentration; 95% FL: 95% fiducial limit; SE: standard error; X^2 – Chi square; RF- resistant factor; RL- resistance level; S – susceptibility.

** The mortality was recorded in larvae of tick spp of *Haemaphysalis* spp, *Rhipicephalus* spp and *Hyalomma* spp against Deltamethrin, Cypermethrin and Amitraz. After 24hrs of treatment, LC_{50} and LC_{90} lethal concentration that kills 50% and 90% of exposed larvae respectively, FL-Fiducial confidence limits, SE-standard error, χ^2 –chi-square value, P<0.0001highly significant.The lowest LC $_{50}$ values for larvae were observed for Amitraz ranging from 1.61 to1.90, which had a highest acaricidal effect for the larval control of *H.bispinosa, H.kutchensis, H.intermedia, R. sanguineus, R. haemaphysaloides*, *H.a.anatolicum* and *H.m.isaaci*.

Fig. 11. Efficacy of acaricides against larvae of *Haemaphysalis bispinosa* by larval packet test.

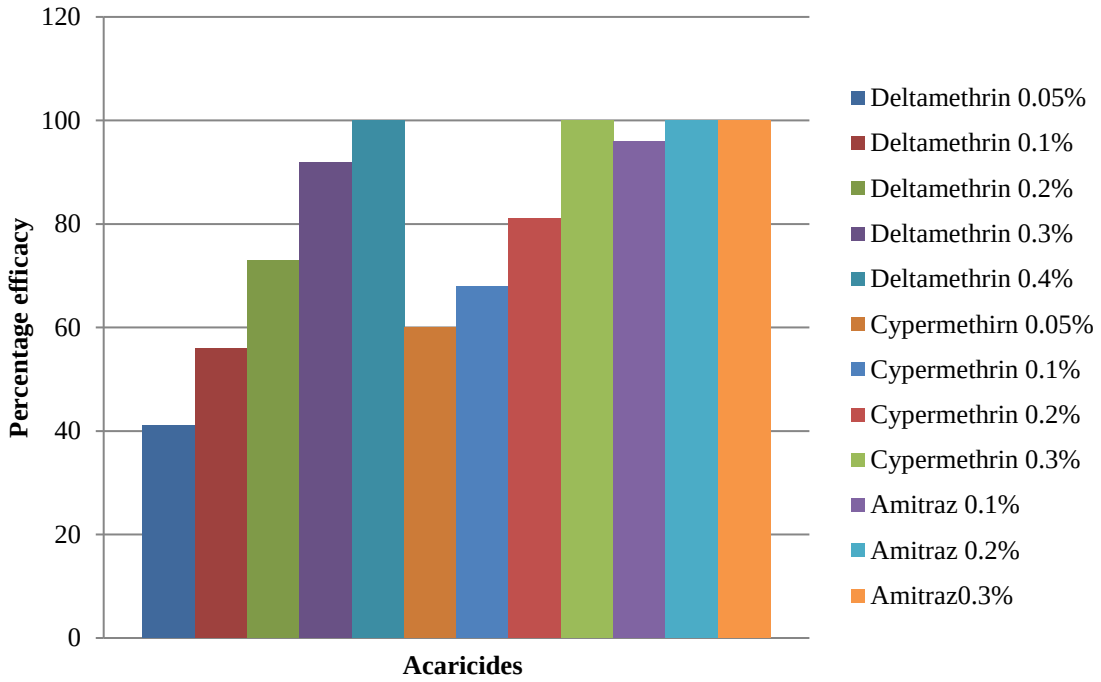


Figure 12. Efficacy of acaricides against larvae of *Haemaphysalis intermedia* by larval packet test.

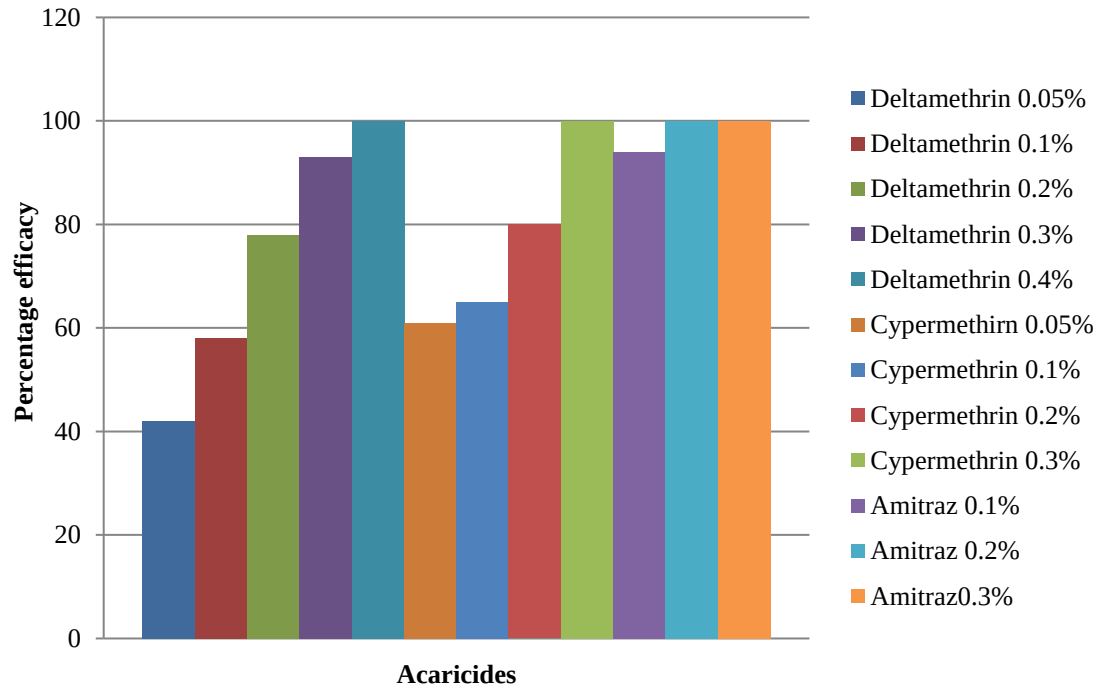


Figure 13. Efficacy of acaricides against larvae of *Haemaphysalis kutchensis*

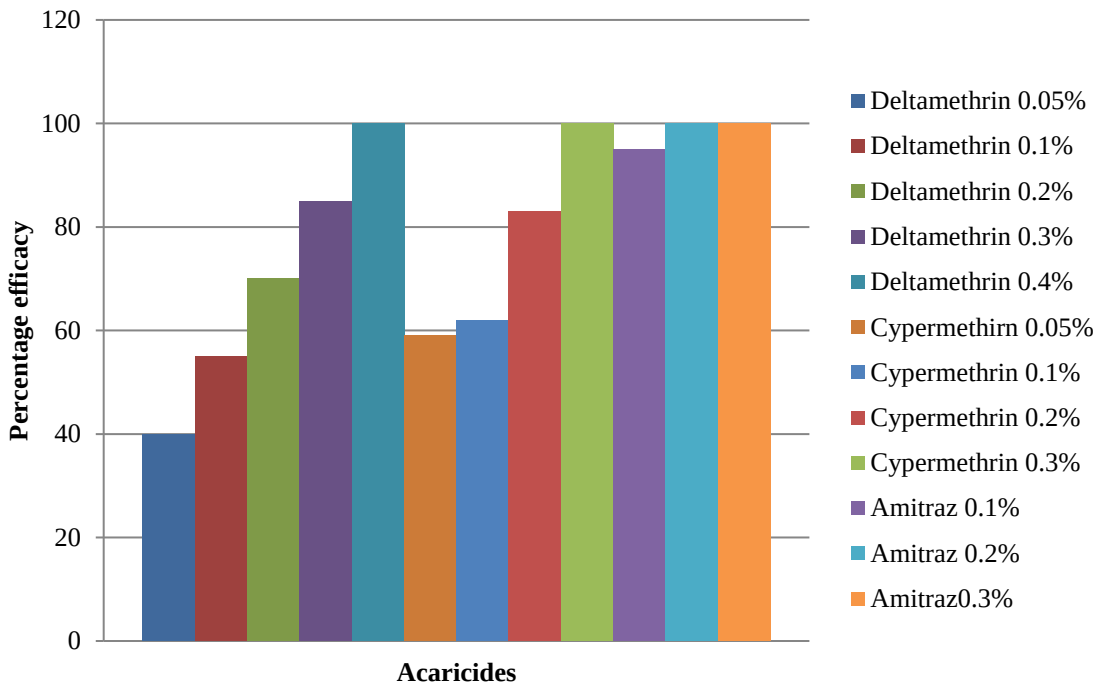


Figure 14. Efficacy of acaricides against larvae of *Hyalomma anatolicum anatolicum*

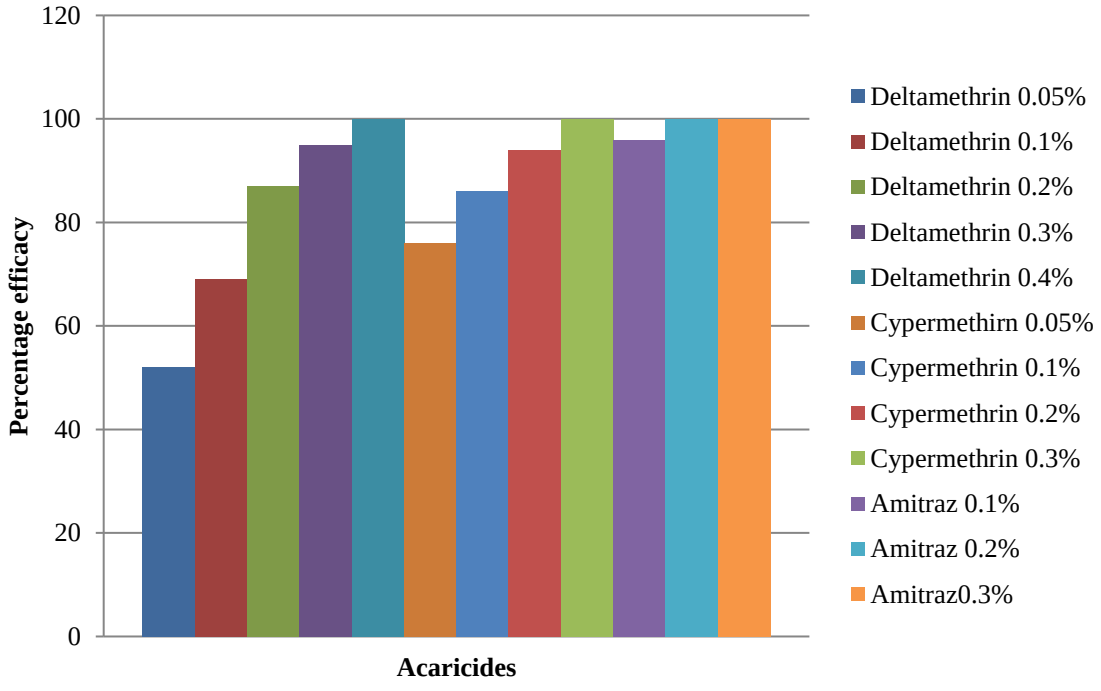


Figure 15. Efficacy of acaricides against larvae of *Hyalomma marginatum isaaci*

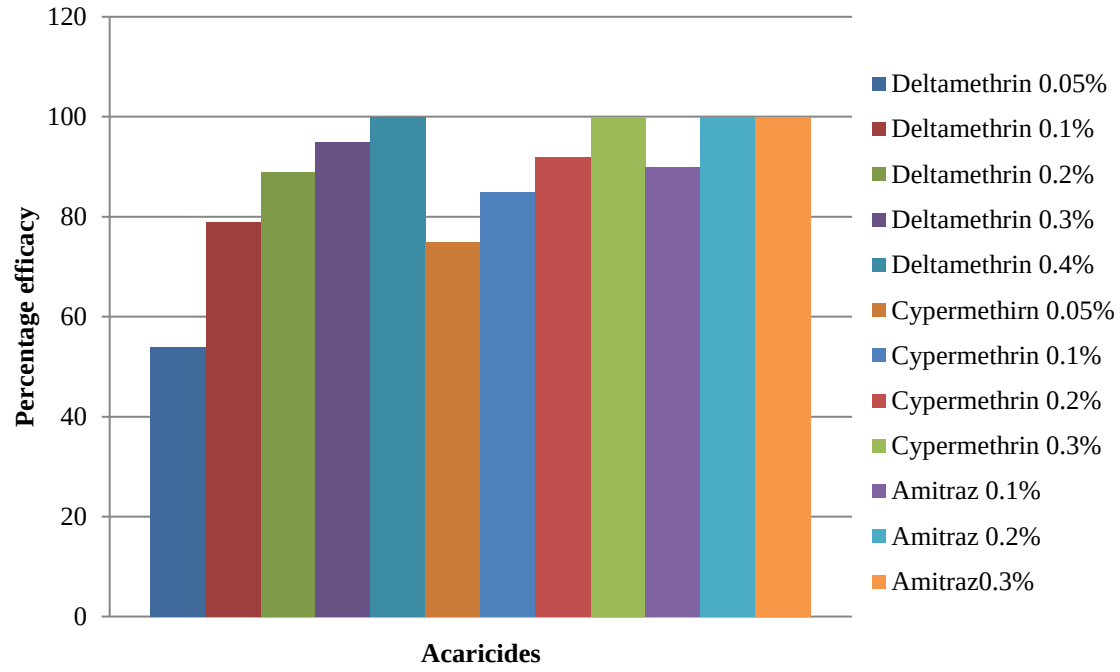


Figure 16. Efficacy of acaricides against larvae of *Rhipicephalus sanguineus*

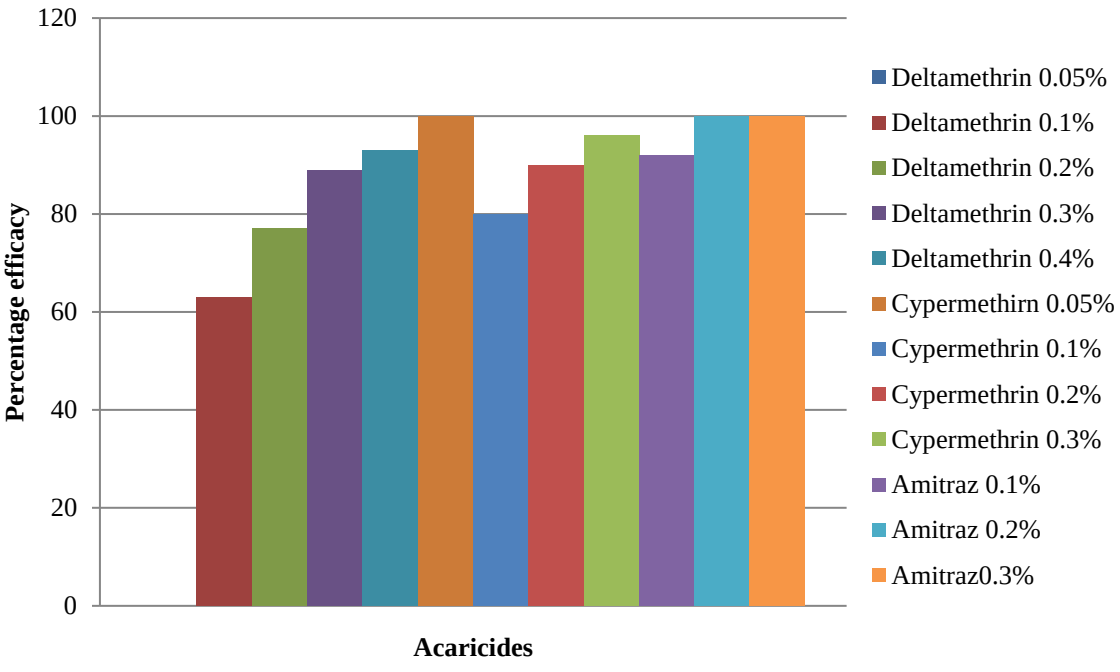


Figure 17. Efficacy of acaricides against larvae of *Rhipicephalus haemaphysaloides* by larval packet test

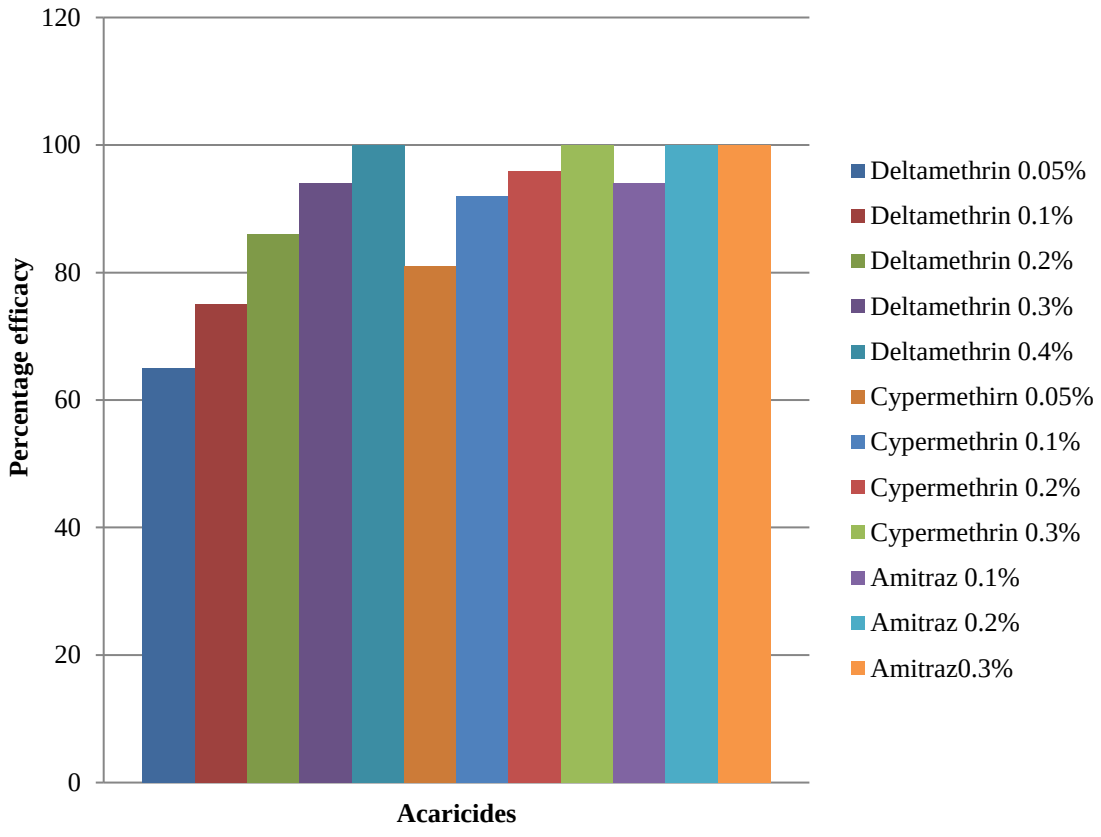


Table 27. Efficacy of acaricides on engorged *H.bispinosa* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	2	20
Deltamethrin	0.075g/ltr	10	30 min	5	50
Amitraz	2.5g/ltr	10	30 min	0	-

Table 28. Efficacy of acaricides on engorged *H.intermedia* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	2	20
Deltamethrin	0.075g/ltr	10	30 min	6	60
Amitraz	2.5g/ltr	10	30 min	0	

Table 29. Efficacy of acaricides on engorged *H.kutchensis* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	2	20
Deltamethrin	0.075g/ltr	10	30 min	5	50
Amitraz	2.5g/ltr	10	30 min	0	-

Table 30. Efficacy of acaricides on engorged *H.a.anatolicum* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	3	30
Deltamethrin	0.075g/ltr	10	30 min	6	60
Amitraz	2.5g/ltr	10	30 min	0	-

Table 31. Efficacy of acaricides on engorged *H.m.isaaci* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	4	40
Deltamethrin	0.075g/ltr	10	30 min	6	60
Amitraz	2.5g/ltr	10	30 min	0	-

Table 32. Efficacy of acaricides on engorged *R.haemaphysaloides* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	3	30
Deltamethrin	0.075g/ltr	10	30 min	7	70
Amitraz	2.5g/ltr	10	30 min	0	-

Table 33. Efficacy of acaricides on engorged *R.sanguineus* by AIT-DD

Concentration % of acaricide	Discriminating doses recomended	Number of engorged females tested	Exposure time	No of ticks laying eggs	Percentage resistance
Control	-	10	30 min	10	0
Cypermethrin	0.05g/ltr	10	30min	2	20
Deltamethrin	0.075g/ltr	10	30 min	7	70
Amitraz	2.5g/ltr	10	30 min	0	-

4.3 *In vitro* efficacy of Entomopathogenic nematodes to control ticks

The present study was conducted to evaluate the *in vitro* efficacy of two entomopathogenic nematodes (EPNs), *Steinernema abbasi* and *Heterorhabditis indica* procured from National Bureau of Agricultural Insect Resources (NBAIR), Bangalore which were used against the engorged female ticks of *Haemaphysalis, Hyalomma* and *Rhipicephalus* collected from naturally infested sheep of Bangalore, Belguam, Bellary, Chitradurga, Davanagere and Mandya regions.

4.3.1 Bioassay experiments for *Heterorhabditis indica* against engorged female ticks in 9cm petri dish

Efficacy of the EPNs was assessed at a concentration of 500, 1000, 2000, 4000 and 6000 infective juveniles (IJs) / petri dish on above mentioned species of ticks under laboratory conditions. The bio efficacy was tested on the basis of per cent mortality of ticks at every 24hrs interval. As shown in table 34- 45 based on the mortality per cent age, the ticks were found to be susceptible to *Heterorhabditis indica,* when compared to *Steinernema abbasi* under laboratory conditions. However, the degree of susceptibility of ticks to nematodes increased according to the doses of their infective juveniles as well as the exposure period. Also, a positive correlation was found between the doses of infective juveniles (IJ's) applied and time of tick percentage mortality for both species of entomopathogenic nematodes.

Bio assay of *H.indica* at a concentration of 500, 1000, 2000, 4000 and 6000 IJ's / petri dish on engorged females of *Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* was done.

The tick mortality was recorded by observing the visual observations such as absence of leg reflex, changes in coloration of external surface of the tick and the presence of EPN inside the tick after mortality. The maximum 100 per cent mortality was induced by *H.indica* against different species of ticks *i.e* at 2000IJs concentration.

4.3.2 Mortality induced by *Heterorhabditis indica* against engorged *Haemaphysalis* ticks

Bioassay of *H.indica* at a concentration of 500, 1000, 2000, 4000 and 6000IJs on engorged females was recorded, where the tick mean per cent mortality of 34.39, 47.36,52.32, 87.67 and 90.64 per cent was recorded at 24hrs against *Haemaphysalis bispinosa* whereas it was 32.93, 50.26, 55.69, 80.96 and 95.56 per cent against *Haemaphysalis intermedia* and 36.93, 48.36, 55.34, 78.92 per cent against *Haemaphysalis kutchensis*. The tick mortality of 100 per cent was found first after 72 hrs in all the *Haemaphyalis* spp of ticks at all concentrations of EPN IJs/petridish. There was no mortality observed in control group. The details of the mean per cent mortality at every 24 hrs intervals are presented in the table 34, 35 and 36.

4.3.3 Mortality induced by *Heterorhabditis indica* against engorged *Hyalomma* ticks.

Bioassay of *H.indica* at a concentration of 500, 1000, 2000, 4000 and 6000IJs on engorged females was recorded. Tick mean per cent mortality of 23.75, 42.93, 80.24, 78.34 and 90 per cent was recorded at 24hrs against *Hyalomma anatolicum anatolicum* whereas it was 20.23, 54.03, 88.46, 92.34 and 90 per cent against *Hyalomma marginatum isaaci*. The tick mortality of 100 per cent was found after 72 hrs in all spp of ticks at 2000IJs/petridish. There was no mortality observed in control group. The details of the mean per cent mortality at every 24hours intervals are presented in the table 37 and 38.

4.3.4 Mortality induced by *Heterorhabitidis indica* against engorged *Rhipicephalus* ticks

Bioassay of *H.indica* at a concentration of 500, 1000, 2000, 4000 and 6000IJs on engorged females was recorded. Tick mean per cent mortality of 26.76, 48.13, 56.26, 68.23 and 92.45 per cent was recorded at 24hrs against *R.haemaphysaloides* whereas it was 20.67, 40.13, 54.26, 92.23 and 95.28 per cent against *R.sanguineus*.

The tick mortality of 100 per cent was found after 72hrs in both species of *Rhipicephalus* spp of ticks at 2000IJs/petridish. There was no mortality observed in control group. The detail of the mean per cent mortality at every 24hrs intervals is presented in the table 39 and 40.

4.3.5 Bioassay experiments for *Steinernema abbasi* against engorged female ticks in 9cm petri dish.

Bio assay of *S.abbasi* at a concentration of 500, 1000, 2000, 4000, 6000 and 8000 IJ's / petri dish on engorged females of *Haemaphysalis bispinosa*, *Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* was done.The tick mortality was recorded by observing the visual observations such as absence of leg reflex and changes in coloration of external surface of the tick. Bioassay of *S.abbasi* at a concentration of 500, 1000, 2000, 4000 and 6000IJs on engorged females was recorded. There was no tick mortality till 96hrs. The maximum 100 per cent mortality was induced by *S.abbasi* against all species of ticks in between 6000- 8000IJs concentration.

4.3.6 Mortality induced by *Steinernema abbasi* against engorged *Haemaphysalis* ticks.

Bioassay of *S.abbasi* at a concentration of 500, 1000, 2000, 4000, 6000IJs and 8000IJs on engorged females was recorded. Tick mean per cent mortality of 6.25, 8.38, 16.82, 20.04, 24.04 and 29.12 per cent was recorded at 120 hrs against *H.bispinosa* whereas it was 0, 7.14, 17.10, 22.04, 25.04 and 30.24 per cent against *H.intermedia.* Against *H.kutchensis* it was 4.45 per cent 7.54, 18.20, 23.60, 28.70 and 32.46 per cent. The tick mortality of 100 per cent was found after 216 hrs at EPN concentration of 6000IJs/petridish. There was no mortality observed in control group. The detail of the mean per cent mortality at every 24 hrs intervals is presented in the table 41, 42 and 43.

4.3.7 Mortality induced by *Steinernema abbasi* against engorged *Hyalomma* ticks.

Bioassay of *S.abbasi* at a concentration of 500, 1000, 2000, 4000, 6000 IJs and 8000IJs on engorged females was recorded. Tick mean per cent mortality of 0, 9, 18, 23 and 38 per cent was recorded at 24 hrs against *H.anatolicum anatolicum* whereas it was 6, 10, 20, 35 and 56 per cent against *H.m.issaci.* The tick mortality of 100 per cent was found after 72 hrs at EPN concentration of 8000IJs/petridish. There was no mortality observed in control group. The details of the mean per cent mortality at every 24 hrs intervals are presented in the table 44 & 45.

4.3.8 Mortality induced by *Steinernema abbasi* against engorged *Rhipicepahlus* ticks

Bioassay of *S.abbasi* at a concentration of 500, 1000, 2000, 4000, 6000IJs and 8000IJs on engorged females was recorded. Tick mean per cent mortality of 0, 6, 9, 16, 21 and 42 per cent was recorded at 24 hrs against *R.sanguineus* whereas it was 0, 7, 10, 20,

44 and 56 per cent against *H.m.isaaci*. The tick mortality of 100 per cent was found after 216 hrs at EPN concentration of 8000IJs/petridish. There was no mortality observed in control group. The details of the mean per cent mortality at every 24 hrs intervals are presented in the table 46 and 47.

4.3.9 Comparison of two species of EPN against engorged female tick spp of *Haemaphysalis, Hyalomma* and *Rhipicephalus* spp in 9cm petri dish.

Heterorhabditis indica was more effective as it caused 100 per cent mortality within 72 hr at all concentrations against engorged female tick spp of *Haemaphysalis, Hyalomma* and *Rhipicephalus* in contrast to 50 to 100 per cent mortality by *S.abbasi* at 214hrs at 8000IJs concentration against *Haemaphysalis, Hyalomma* and *Rhipicephalus* spp of ticks. All species of ticks in this study after 72hrs of incubation with *H.indica* showed lesser LC_{50} values compared to the same species of ticks incubated with *S.abbasi* showing higher values of LC_{50}. *H.indica* was found more effective than *S.abbasi* against engorged ticks in this study.

The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *H.bispinosa* ticks after 72hrs of incubation with *H.indica* showed a LC_{50} of 48.34 IJs/petridish (95% Fiducial limit; 13.78-169.57), whereas *S.abbasi* showed LC_{50} of 1255.44.IJs/petridish (95% Fiducial limit; 742.74-2122.06). The calculated values of LC_{50} are presented in Table 48.

The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *H.intermedia* ticks after 72hrs of incubation with *H.indica* showed a LC_{50} of 205.68 IJs/petridish (95% Fiducial limit; 90.33-468.34), whereas *S.abbasi* showed LC_{50}

of 650.18IJs/petridish (95% Fiducial limit; 352.98-1197.60). The calculated values of LC_{50} are presented in Table 49.

The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *H.kutchensis* ticks after 72hrs of incubation with *H.indica* showed a LC_{50} of 164.40 IJs/petridish (95% Fiducial limit; 72.06-375.09), whereas *S.abbasi* showed LC_{50} of 757.22IJs/petridish (95% Fiducial limit; 425.16-1348.62). The calculated values of LC_{50} are presented in Table 50.

The calculated values of LC_{50} are presented in Table 51. The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *H.a.anatolicum* ticks after 72 hrs of incubation with *H.indica* showed a LC_{50} of 193.28 IJs/petridish (95% Fiducial limit; 94.58-394.97), whereas *S.abbasi* showed LC_{50} of 1120.4IJs/petridish (95% Fiducial limit; 665.35-1886.77).

The calculated values of LC_{50} are presented in Table 52. The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *H.m.issaci* ticks after 72 hrs of incubation with *H.indica* showed a LC_{50} of 71.99 IJs/petridish (95% Fiducial limit; 20.63-251.21), whereas *S.abbasi* showed LC_{50} of 839.02IJs/petridish (95% Fiducial limit; 498.83- 1411.22).

The calculated values of LC_{50} are presented in Table 53. The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *R.haemaphysaloides* ticks after 72 hrs of incubation with *H.indica* showed a LC_{50} of 48.34 IJs/petridish (95% Fiducial limit; 13.78-169.57), whereas *S.abbasi* showed LC_{50} of 818.88IJs/petridish (95% Fiducial limit; 759.98 - 2226.51).

Table 34: Bioassay of *Heterorhabditis indica* against *Haemaphysalis bispinosa* tick

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	**2**	**3**
500	34.39	68.28	100
1000	47.36	86.29	100
2000	52.32	94.34	100
4000	87.67	100	-
6000	90.64	100	-
Control	00	00	02
P value	<0.0001		

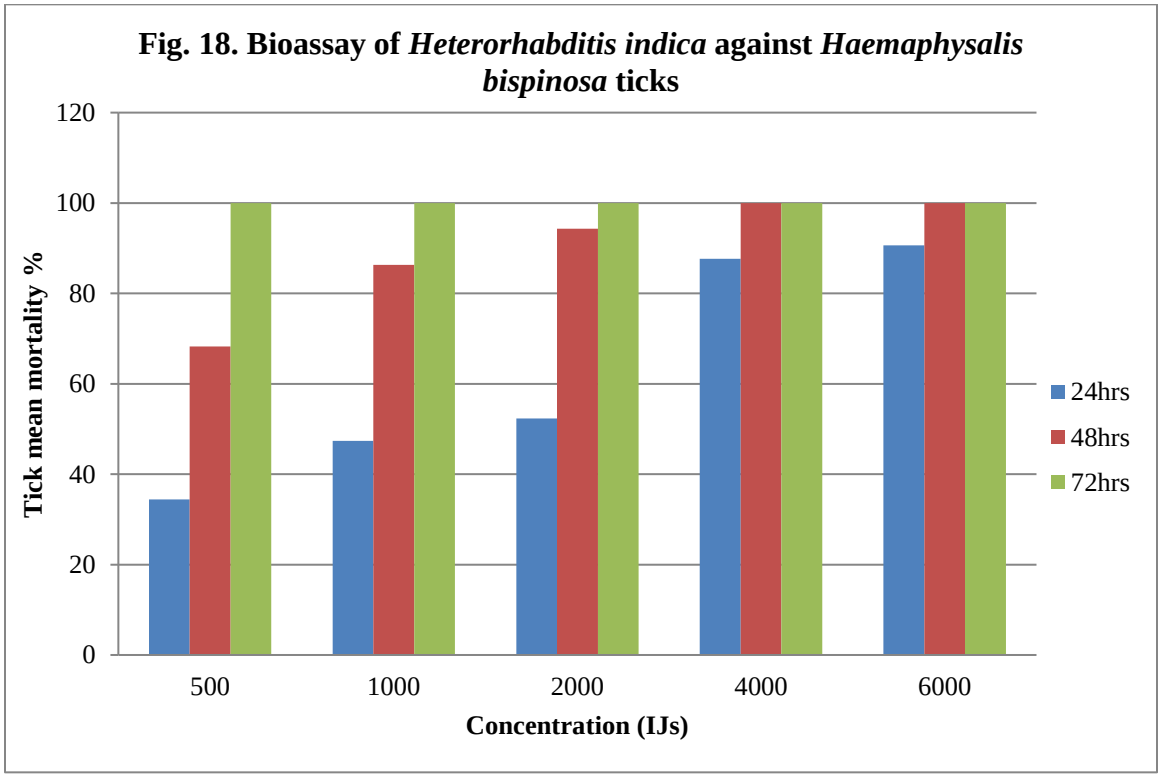


Table 35: Bioassay of *Heterorhabditis indica* against *Haemaphysalis intermedia* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	**2**	**3**
500	32.93	52.14	100
1000	50.26	68.18	100
2000	55.69	89.18	100
4000	80.96	100	-
6000	95.56	100	-
Control	00	00	02
P value	<0.0001		

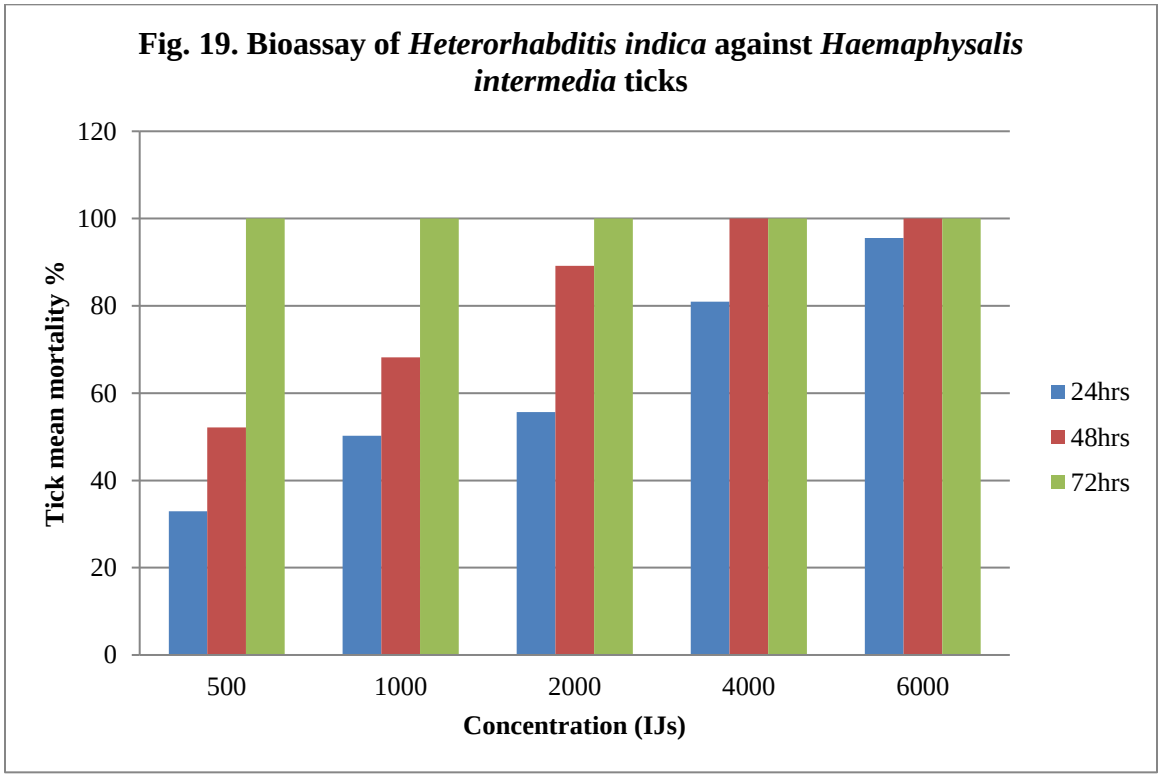


Table 36: Bioassay of *Heterorhabditis indica* against *Haemaphysalis kutchensis* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	**2**	**3**
500	36.39	69.18	100
1000	48.36	88.92	100
2000	55.34	96.31	100
4000	78.92	100	-
6000	90	100	-
Control	00	00	02
P value	<0.0001		

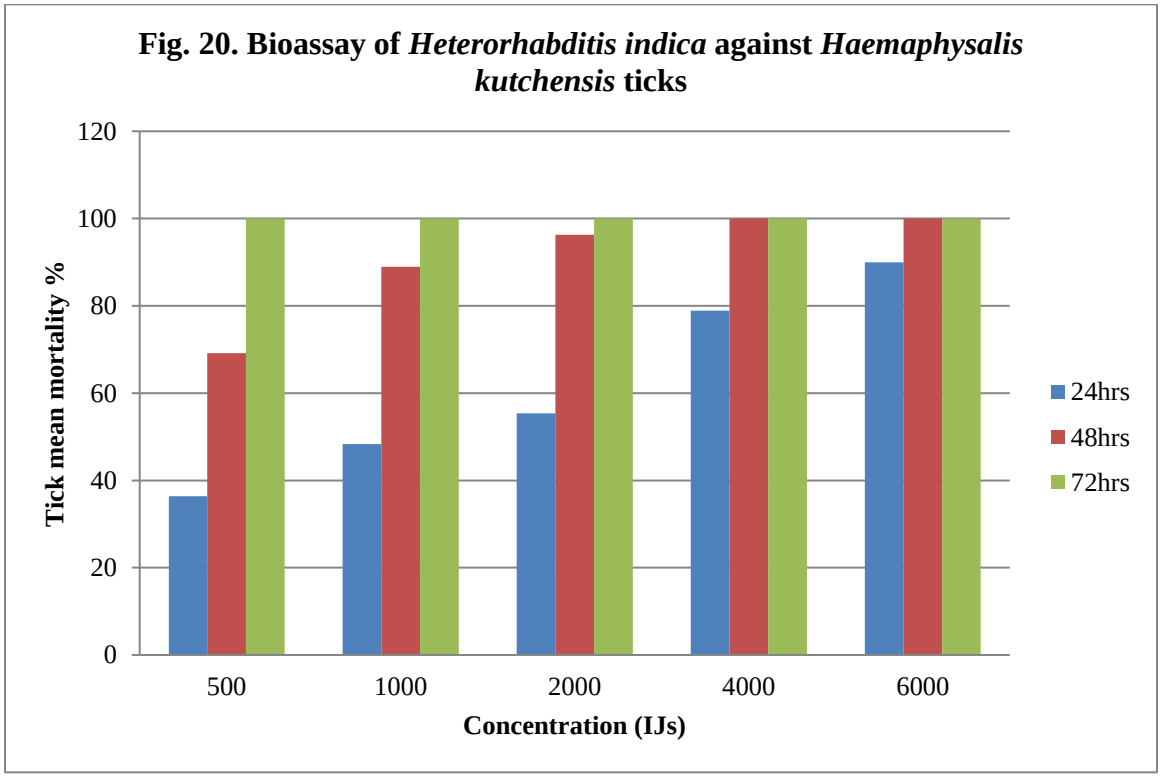


Table 37: Bioassay of *Heterorhabditis indica* against *Hyalomma anatolicum anatolicum* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	**2**	**3**
500IJs	23.75	48.75	100
1000IJs	42.93	85.15	100
2000IJs	80.24	100	-
4000IJs	78.34	100	-
6000IJs	90.0	100	-
Control	00	00	00
P value	<0.0001		

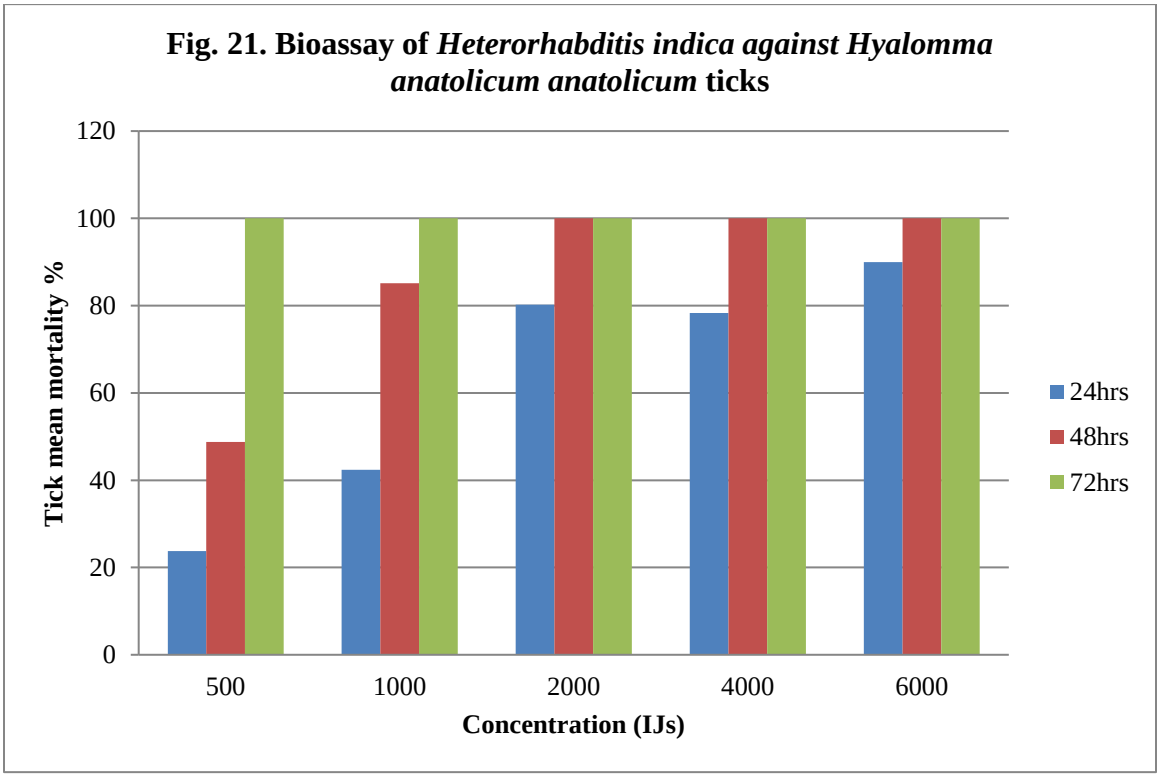


Table 38: Bioassay of *Heterorhabditis indica* against *Hyalomma marginatumi isaaci* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	**2**	**3**
500	20.23	45.43	100
1000	54.03	92.18	100
2000	88.46	100	100
4000	92.34	100	-
6000	90	100	-
Control	00	00	00
P value	<0.0001		

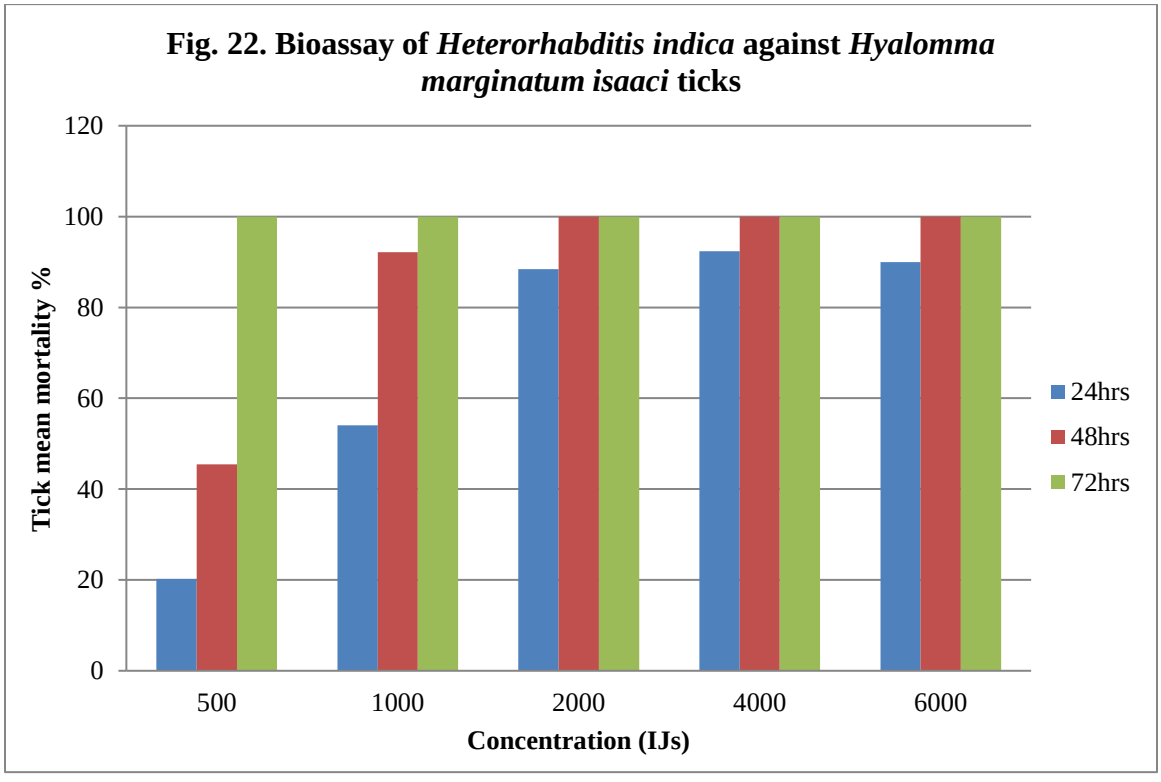


Table 39: Bioassay of *Heterorhabditis indica* against *Rhipicephalus haemaphysaloides* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	2	3
500	26.76	50.29	100
1000	48.13	89.59	100
2000	56.26	95.25	100
4000	68.23	100	-
6000	92.45	100	-
Control	00	00	00
P value	<0.0001		

Fig. 23. Bioassay of *Heterorhabditis indica* against *Rhipicephalus haemaphysaloides* ticks

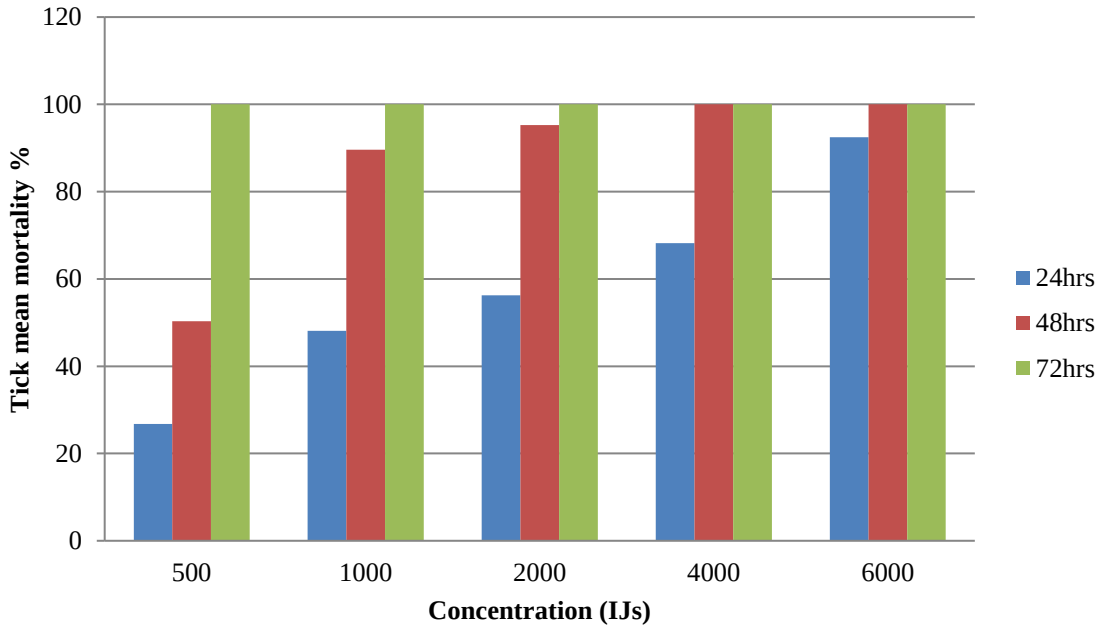


Table 40: Bioassay of *Heterorhabditis indica* against *Rhipicephalus sanguineus* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)		
	1	2	3
500	20.67	47.15	100
1000	40.13	80.15	100
2000	54.26	94.70	100
4000	95.28	100	-
6000	92.23	100	-
Control	00	00	00
P value	<0.0001		

Fig. 24. Bioassay of *Heterorhabditis indica* against *Rhipicephalus sanguineus* ticks

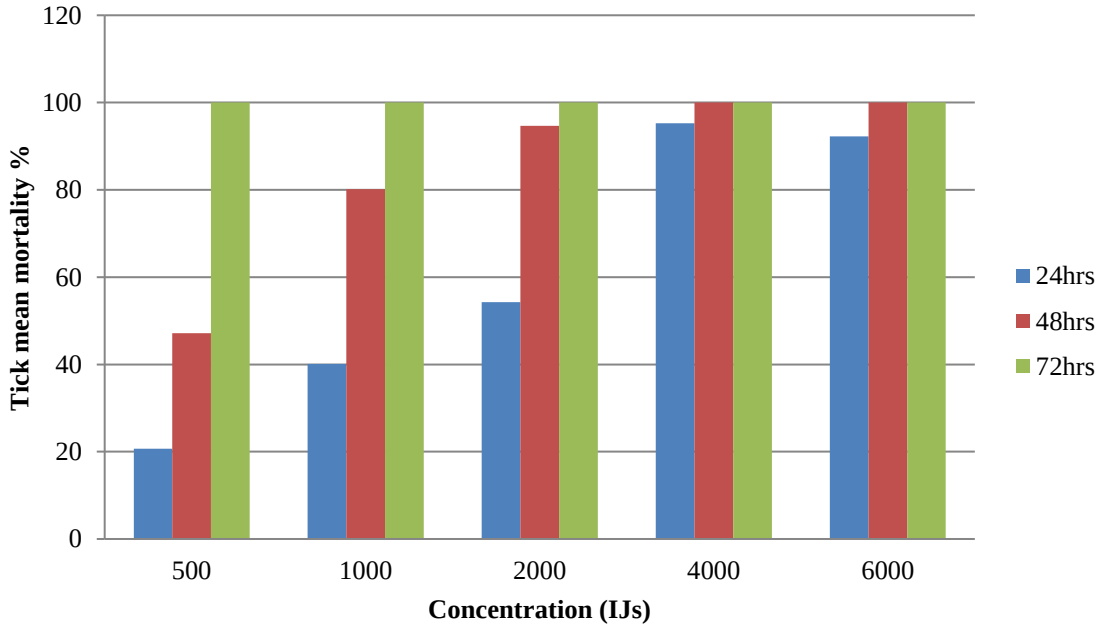


Table 41: Bioassay of *Steinernema abbasi* against *Haemaphysalis bispinosa* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)				
	5	**6**	**7**	**8**	**9**
500	6.25	24.06	31.14	38.69	58.98
1000	8.38	28.93	42.04	58.94	65.01
2000	16.82	34.02	54.02	63.21	76.81
4000	20.04	37.89	62.10	80.01	88.91
6000	24.04	41.92	64.13	92.91	100
8000	29.12	68.01	100	-	-
Control	00	00	00	00	02
P value	<0.0001				

Fig. 25. Bio assay of *Steinernema abbasi* against *Haemaphysalis bispinosa* ticks

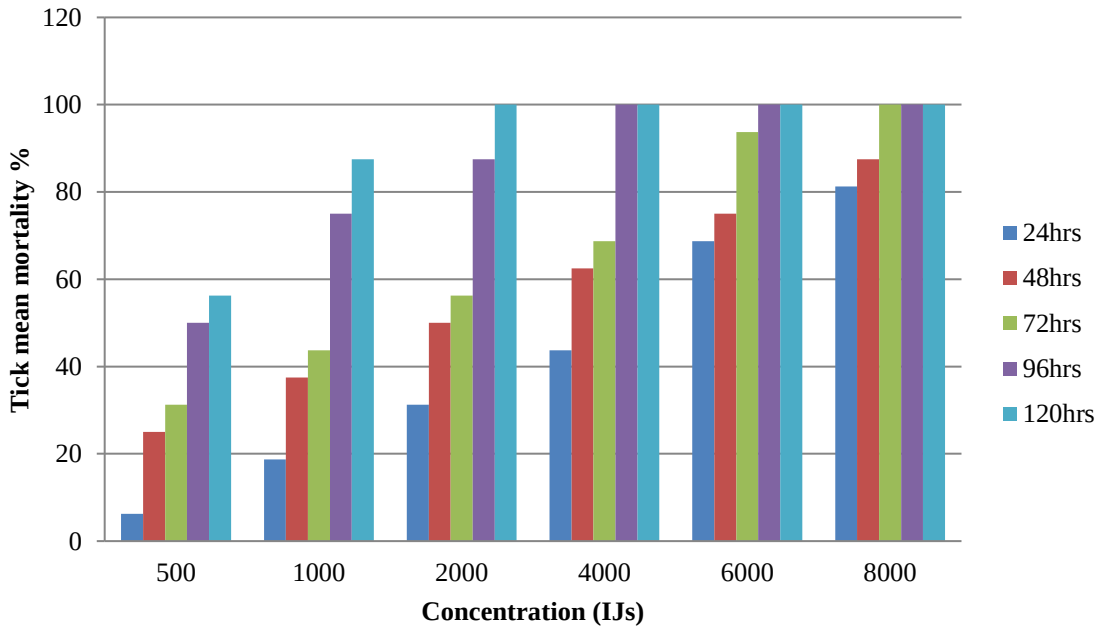


Table 42: Bioassay of *Steinernema abbasi* against *Haemaphysalis intermedia* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)				
	5	**6**	**7**	**8**	**9**
500	0	22.60	30.03	36.96	56.80
1000	7.14	26.83	40.40	57.49	64.10
2000	17.10	35.10	56.20	64.65	78.18
4000	22.04	38.68	64.01	82.01	90
6000	25.04	42.24	65.18	93.40	100
8000	30.24	68	100	-	-
Control	00	00	00	00	02
P value	<0.0001				

Fig. 26 .Bio assay of *Steinernema abbasi* against *Heamaphysalis intermedia* ticks

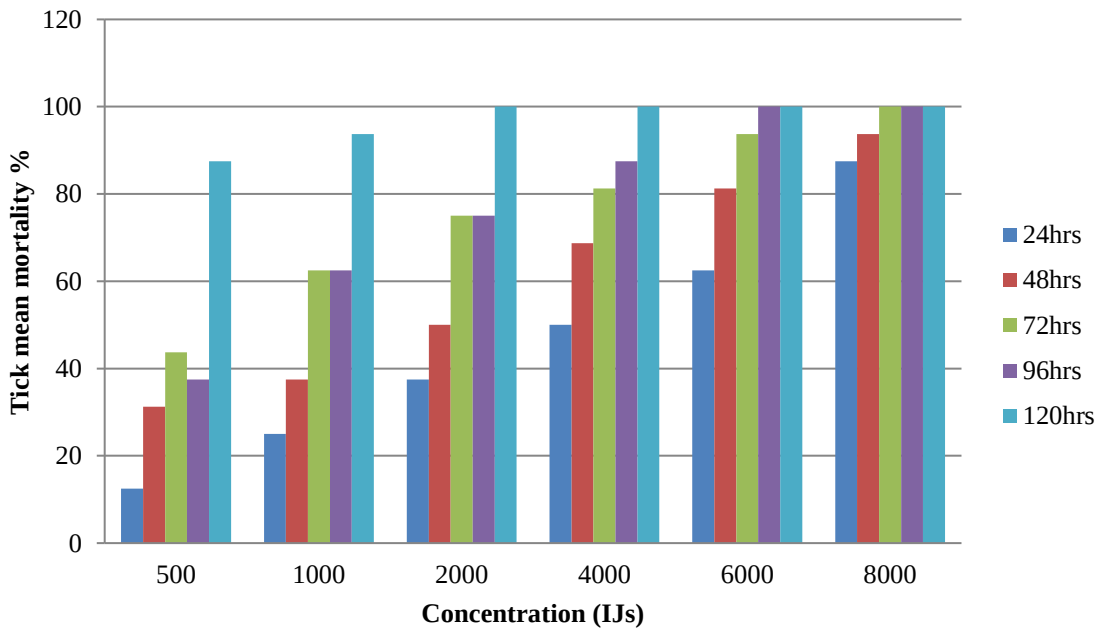


Table 43: Bioassay of *Steinernema abbasi* against *Haemaphysalis kutchensis* ticks

EPN Conc IJs/petridish	Mean per cent mortality of at different intervals (days)				
	1	**2**	**3**	**4**	**5**
500	4.45	22.16	32.04	37.64	59.0
1000	7.54	27.63	41.40	54.32	68.01
2000	18.20	36.20	56.0	66.56	79.18
4000	23.60	40.20	65.01	84.01	90
6000	28.70	43.01	66.48	94.50	100
8000	32.46	69.24	100	-	
Control	00	00	00	00	00
P value	<0.0001				

Fig. 27. Bio assay of *Steinernema abbasi* against *Haemaphysalis kutchensis* ticks

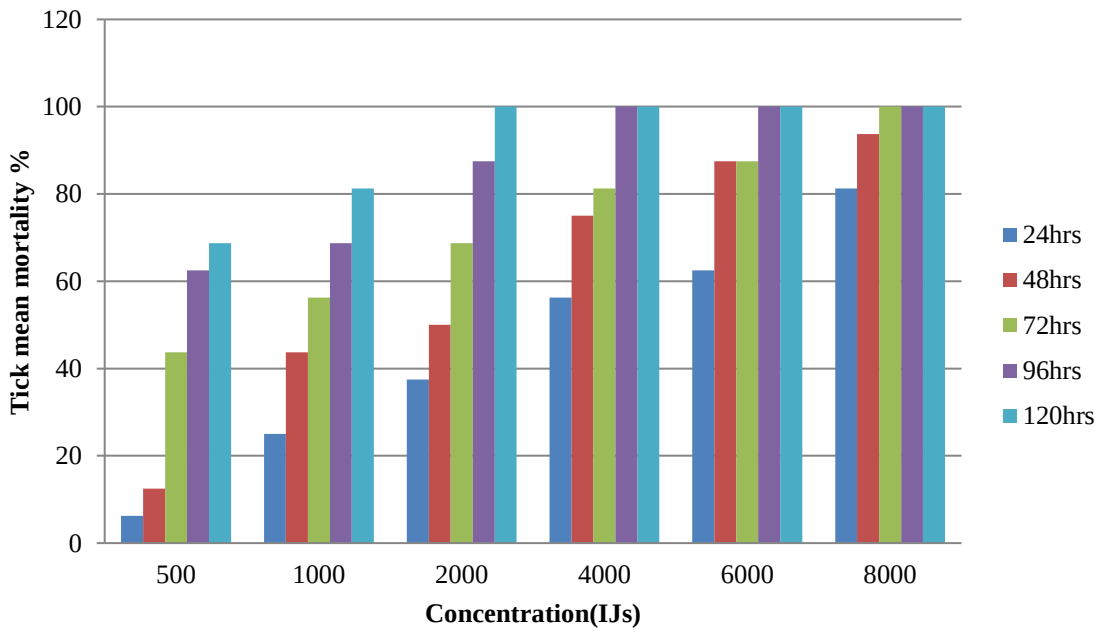


Table 44: Bioassay of *Steinernema abbasi* against *Hyalomma anatolicum anatolicum* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)				
	1	**2**	**3**	**4**	**5**
500	0	26	34	42	62
1000	9	32	45	69	73
2000	18	38	60	74	80
4000	23	40	68	90	96
6000	32	48	72	100	-
8000	38	52	100	-	
Control	00	00	00	00	02
P value	<0.0001				

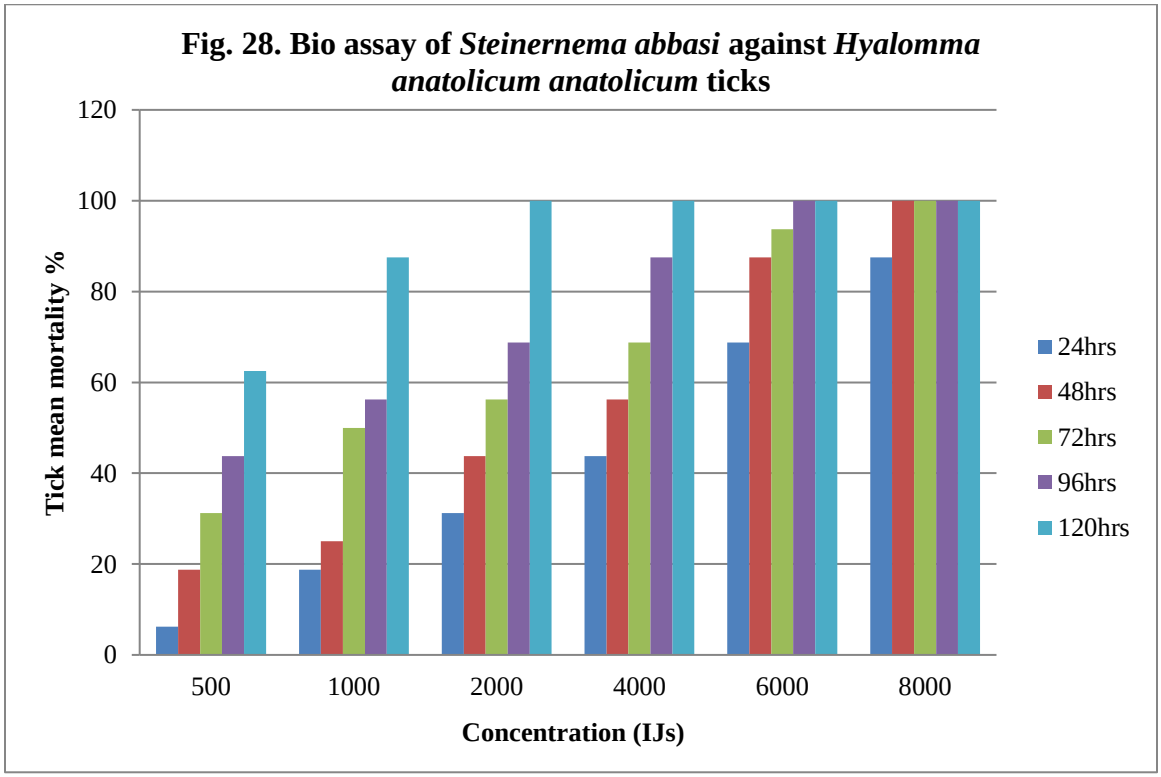


Table 45: Bioassay of *Steinernema abbasi* against *Hyalomma marginatum isaaci* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)				
	1	**2**	**3**	**4**	**5**
500	6	24	30	44	68
1000	10	30	40	70	75
2000	20	40	64	78	85
4000	25	48	72	90	94
6000	35	52	75	100	
8000	56	80	100	-	
Control	00	00	00	00	02
P value	<0.0001				

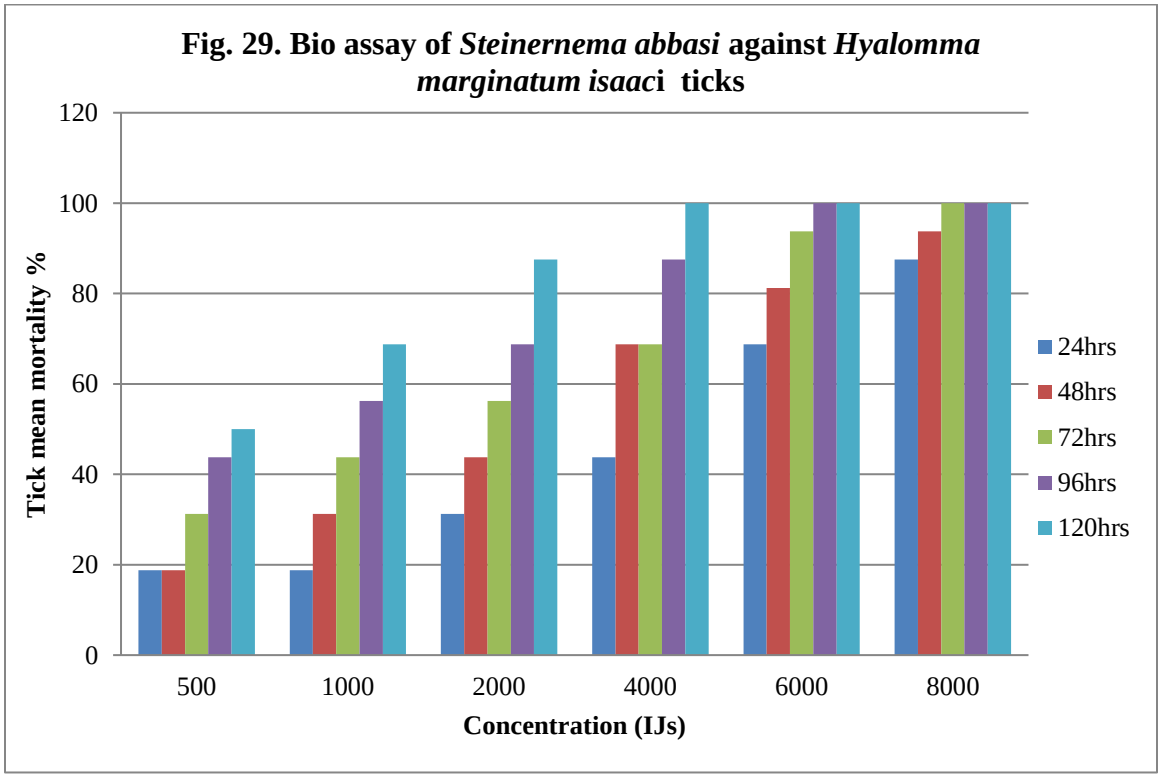


Table 46: Bioassay of *Steinernema abbasi* against *Rhipicephalus sanguineus* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different intervals (days)				
	4	**5**	**6**	**7**	**8**
500	00	19	30	44	74
1000	6	28	49	75	82
2000	9	33	67	80	95
4000	16	42	80	96	100
6000	21	50	84	100	-
8000	42	80	100	-	
Control	00	00	00	00	03
P value	<0.0001				

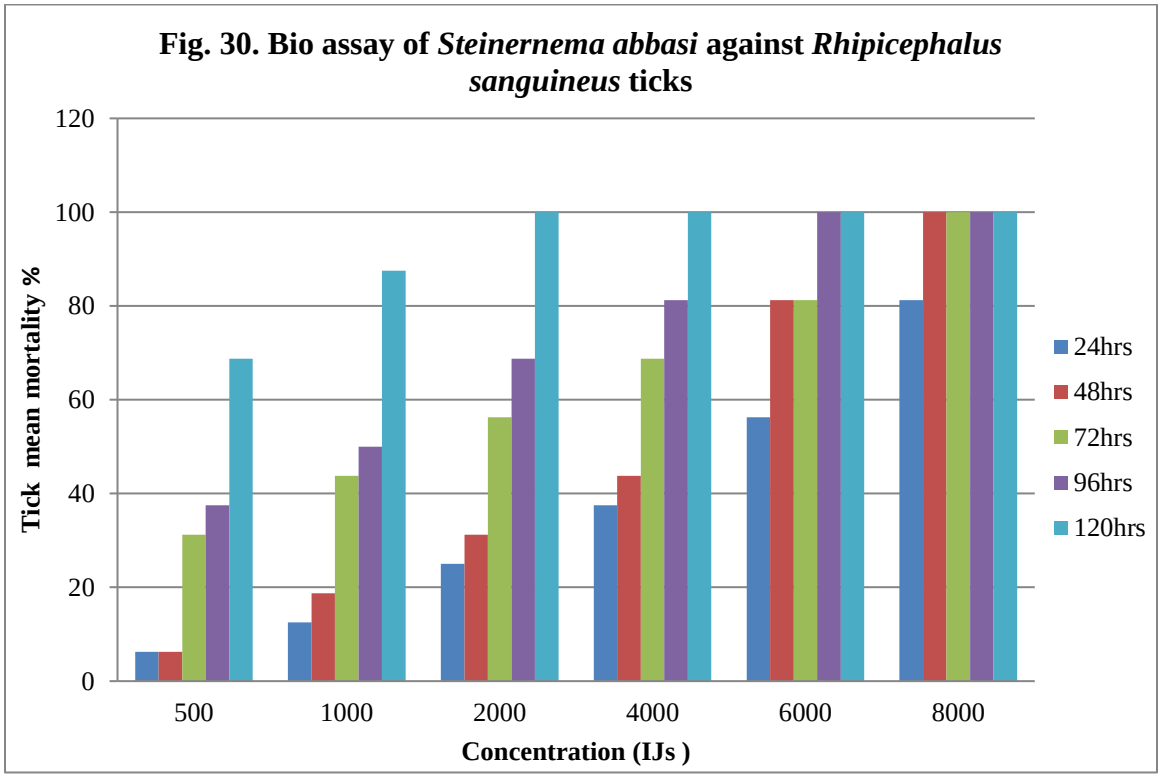


Table 47: Bio assay of *Steinernema abbasi* against *Rhipicephalus haemaphysaloides* ticks

EPN Conc IJs/petridish	Mean per cent mortality at different time interval (days)				
	4	**5**	**6**	**7**	**8**
500	00	14	28	42	70
1000	7	29	50	73	85
2000	10	35	69	84	92
4000	20	50	90	100	-
6000	44	76	100	-	-
8000	56	100	-	-	
Control	00	00	00	00	00
P value	<0.0001				

Fig. 31. Bio assay of *Steinernema abbasi* against *Rhipicephalus haemaphysaloides* ticks

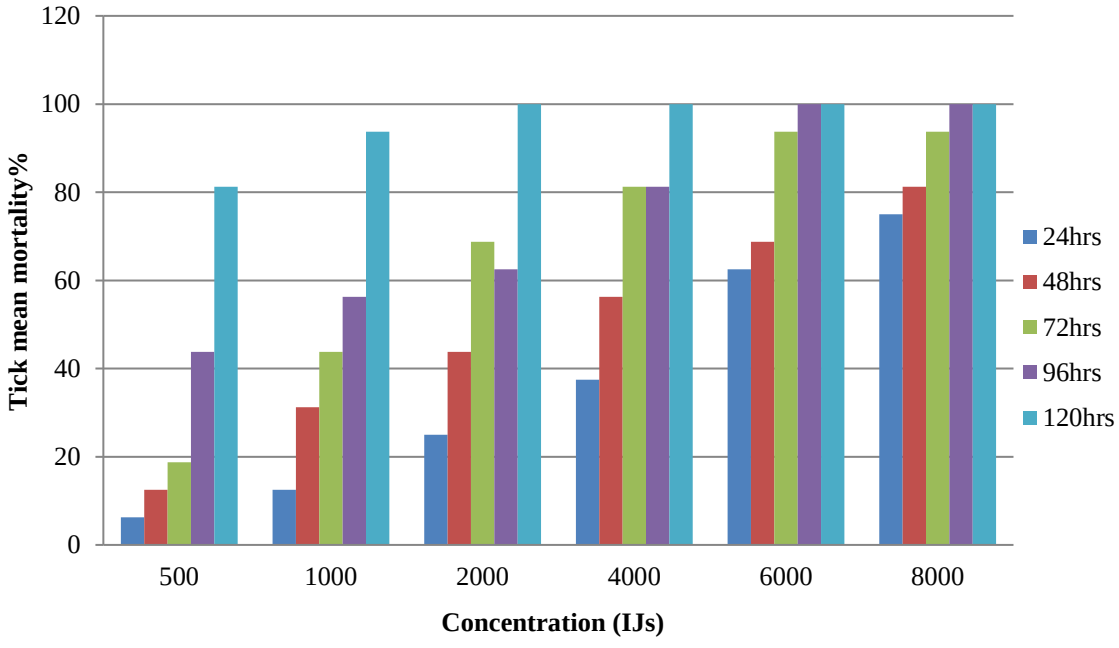


Table 48. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indicia* and *Steinernema abbasi* of EPN species against *Haemaphysalis bispinosa* ticks.

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
	24hrs	1300.81	759.98-226.51		24hrs	3789.61	2360.08-6085.27
	48hrs	575.58	372.19-890.13		48hrs	1835.82	995.83-3384.34
H.indica	72hrs	48.34	13.78-169.57	*S.abbasi*	72hrs	1255.44	742.74-2122.06
					96hrs	480.64	281.19-846.77
					120hrs	448.02	98.33-672.76

Table 49. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indica and Steinernema abbasi* EPN species against *Haemaphysalis intermedia* ticks.

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
	24hrs	1227.75	715.52-2106.7		24hrs	3596.11	2580.08-6085.27
	48hrs	433.65	212.62-884.48		48hrs	1396.61	772.67-2524.39
H.indica	72hrs	205.68	90.33-468.344	*S.abbasi*	72hrs	650.18	352.98-1197.60
					96hrs	428.25	219.29-1164.80
					120hrs	193.28	94.58-394.97

Table 50. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indica* and *Steinernema abbasi* of EPN species against *Haemaphysalis kutchensis* ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
	24hrs	995.52	509.98-1943.32		24hrs	3088.36	1962.01-4861.31
	48hrs	227.20	91.33-565.19		48hrs	1763.36	1107.52-2807.5
H.indica	72hrs	164.40	72.06-375.09	*S.abbasi*	72hrs	757.22	425.16-1348.62
					96hrs	338.18	168.84-677.36
					120hrs	48.34	13.78-169.57

Table 51. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indica and Steinernema abbasi* of EPN species against *Hyalomma anatolicum anatolicum* ticks

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation period	LC50	Fiducial limit (95%)
	24hrs	847.33	493.34-1455.31		24hrs	3789.69	1962.01 - 4861.31
	48hrs	393.77	183.08-846.93		48hrs	2285.23	1382.82 - 3776.56
H.indica	72hrs	193.28	94.58 – 394.97	*S.abbasi*	72hrs	1120.43	665.35 - 1886.77
					96hrs	710.66	351.52 - 1436.75
					120hrs	383.39	237.71 - 618.33

Table 52. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indica and Steinernema abbasi* of EPN species against *Hyalomma marginatum isaaci* ticks.

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation Period	LC50	Fiducial limit (95%)
	24hrs	867.53	480.42 – 1566.6		24hrs	3234.69	2136.51-4897.35
	48hrs	187.19	86.59 – 404.66		48hrs	1918.47	1195.50-3078.66
H.indica	72hrs	71.99	20.63 – 251.210	*S.abbasi*	72hrs	839.02	498.83-1411.22
					96hrs	710.66	351.52 -1436.75
					120hrs	517.79	306.02-876.12

Table 53. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indica and Steinernema abbasi* of EPN species against *Rhipicephalus haemaphysaloides* ticks.

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation Period	LC50	Fiducial limit (95%)
	24hrs	719.52	365.51– 1416.42		24hrs	2969.91	2969.91-8149.80
	48hrs	217.50	92.35 – 512.21		48hrs	1577.22	1968.20-4317.20
H.indica	72hrs	48.34	13.78 – 169.57	*S.abbasi*	72hrs	818.88	759.98-2226.51
					96hrs	566.72	20.63-251.21
					120hrs	193.28	94.58-394.97

Table 54. LC_{50} values calculated from dose response bio assays conducted with *Heterorhabditis indica and Steinernema abbasi* of EPN species against *Rhipicephalus sanguineus* ticks.

Nematode spp	Incubation period (hrs)	LC 50 (IJs)	Fiducial limit (95%)	Nematode Spp	Incubation Period	LC50	Fiducial limit (95%)
H.indica	24hrs	984.02	527.73 – 1834.85	*S.abbasi*	24hrs	4919.77	2969.91-8149.80
	48hrs	478.95	236.75 – 968.93		48hrs	2914.98	1968.20-4317.20
	72hrs	193.28	94.58 – 394.97		72hrs	1300.81	759.98-2226.51
					96hrs	71.99	20.63-251.21

The calculated values of LC_{50} are presented in Table 54. The increase in exposure time resulted in reduction of the values of LC_{50} at all exposure time. *R.sanguineus* ticks after 72 hrs of incubation with *H.indica* showed a LC_{50} of 193.28 IJs/petridish (95% Fiducial limit; 94.58-394.97), whereas *S.abbasi* showed LC_{50} of 1300.81IJs/petridish (95% Fiducial limit; 759.98- 2226.51).

In order to study the effect of EPN on reproductive potential of ticks, the ticks were exposed to different concentrations of Infective Juveniles *viz.*, 500, 1000, 2000, 4000 and 6000IJs. Later the reproductive biology of ticks *viz*., egg mass weight, hatching percentage, egg production index and percentage control (Table 55 to 68) was recorded.

4.3.10 Reproductive biology assay against engorged female ticks

4.3.10.1 *In vitro* Efficacy of *Steinernema abbasi* against engorged *Haemaphysalis, Hyalomma* and *Rhipicephalus* spp of ticks

Steinernema abbasi against *H.bispinosa* caused a reduction of egg mass weight with significant reduction ($P<0.05$) between the treated groups where the weight ranged from 16.1mg to 1.1mg and the control group 65.69mg. Egg production indexes (EPI) were observed to treatments 500, 1000, 2000, 4000 IJ's and 6000IJ's of 6.44, 4.13, 2.18, 1.12 and 0.36 per cent (6000IJ's) differing significantly ($P<0.05$) from control group. Percentage control was recorded at 6000IJ's. (T.63)

Against *H.intermedia, Steinernema abbasi* caused a reduction of egg mass weight with significant reduction ($P<0.05$) between the treated groups where in the weight ranged from 16.58 mg to 1.31 mg and the control group 71.36 mg. Egg production index (EPI)

were observed to treatments 500, 1000, 2000,4000 and 6000 IJ's between of 11.23, 8.54, 4.98, 1.74 and 0.70 per cent (6000IJ's) differing significantly (P<0.05) from control group. Percentage control was recorded at 6000IJ's. (T.62)

Steinernema abbasi against *H.kutchensis* caused a reduction of egg mass weight with significant reduction (p<0.05) between the treated groups where in the weight ranged from 16.02 mg to 0.96 mg and the control group with 69.32 mg. Egg production index (EPI) were observed to treatments 500, 1000, 2000, 4000 and 6000 IJ's between of 12.06, 11.58, 2.18, 5.73, 1.55 and 0.66 per cent (6000IJ's) differing significantly (P<0.05) from control group. Percentage control was recorded at 6000IJ's. (T.64)

Steinernema abbasi against *H.a.anatolicum* caused a reduction of egg mass weight with significant reduction (P<0.05) between the treated groups where in the weight ranged from 17.12 to 1.5mg and the control group with 71.87mg. Egg production index (EPI) were observed to treatments 500, 1000, 2000, 4000 and 6000 IJ's between of 12.05, 9.16, 1.42, 5.01, 2.13 and 1.06 per cent (6000IJ's) differing significantly (P<0.05) from control group. Percentage control was recorded at 6000IJ's. (T.65)

Steinernema abbasi against *H.marginatum.issaci* caused a reduction of egg mass weight with significant reduction differences (P<0.05) between the treated groups where the weight ranged from 17.81mg to 0.35mg and the control group 67.85mg. Egg production index (EPI) were observed to treatments 500, 1000, 2000, 4000 and 6000 IJ's between of 500, 1000, 2000 and 6000 IJ's with values between 6.88, 4.23, 1.95, 0.87 and 0.13 per cent (6000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at concentration of 6000IJ's. (T.66)

Steinernema abbasi against *Rhipicephalus sanguineus* caused a reduction of egg mass weight with significant reduction (P<0.05) between the treated groups where in the weight ranged from 14.56mg to 1.1mg and the control group with 65.66mg. Egg production index (EPI) were observed to treatments 500, 1000, 2000, 4000 and 6000 IJ's between 5.58, 4.28, 2.19, 1.12 and 0.36 per cent (6000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at concentration of 6000IJ's. (T 68)

Steinernema abbasi against *Rhipicephalus haemaphysaloides* caused a reduction ofegg mass weight with significant reduction (P<0.05) between the treated groups where in the weight ranged from 15.2mg to 0.50mg and the control group 67.0mg. Egg production index (EPI) were observed to treatments 500, 1000, 2000, 4000 and 6000 IJ's between 5.0, 3.28, 2.78, 1.02 and 0.0 per cent (6000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at concentration of 6000IJ's. (T 67). Besides decreasing the egg mass of females in the treated groups, the exposure to *Steinernema abbasi* the tick spp led to the production of infertile eggs, since the hatching percentage of all treated groups showed significant differences (P<0.05) compared to control group.

4.3.10.2 Efficacy of *Heterorhabditis indica* against engorged *Heamaphysalis, Hyalomma,* and *Rhipicephalus* spp of ticks

Heterorhabditis indica against *Haemaphysalis bispinosa* caused a reduction of egg mass weight with significant reduction differences (P<0.05) between the treated groups where the weight ranged from 2.47mg to 0.12mg and the control group 73.03mg. Egg production index (EPI) were observed to treatments 500, 1000 and 2000 IJ's between 1.84,

0.25 and 0.0 per cent (2000IJ's) differing significantly (P<0.05) from control group.100 percentage control was recorded at 2000IJ's.(T. 55)

Heterorhabditis indica against *Haemaphysalis intermedia* caused a reduction of egg mass weight with significant reduction differences (P<0.05) between the treated groups where the weight ranged from 2.57mg to 0.17mg and the control group 67.85mg. Egg production index (EPI) were observed to treatments 500, 1000 and 2000 IJ's between 1.95, 0.87 and 0.13 per cent (2000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at 2000IJ's. (T.56)

Heterorhabditis indica against *Haemaphysalis kutchensis* caused a reduction of egg mass weight with significant reduction differences (P<0.05) between the treated groups where the weight ranged from 2.90mg to 0.47mg and the control group 65.66mg. Egg production index (EPI) were observed to treatments 500, 1000 and 2000 IJ's between 2.19,1.12 and 0.36 per cent (2000IJ's) differing significantly (P<0.05) from control group. 100 Percentage control was recorded at 2000IJ's. (T.57)

Heterorhabditis indica against *Hyalomma anatolicum anatolicum* caused a reduction of egg mass weight with significant reduction (P<0.05) between the treated groups where the weight ranged from 1.87 mg to 0.036 mg and the control group 64.5 mg. Egg production index (EPI) was observed to treatments 500, 1000 and 2000 IJ's between 1.42, 0.48and 0.027 per cent (2000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at 2000IJ's. (T.58)

Heterorhabditis indica against *Hyalomma marginatum isaaci* caused a reduction of egg mass weight with significant reduction (P<0.05) between the treated groups where the weight ranged from 1.44mg to 0.16mg and the control group 67.85mg. Egg production index (EPI) was observed to treatments 500, 1000 and 2000 IJ's between 01.09, 0.17 and 0.0 per cent (2000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at 2000IJ's. (T.59)

Heterorhabditis indica against *Rhipicephalus sanguineus* caused a reduction of egg mass weight with significant reduction differences (P<0.05) between the treated groups where the weight ranged from 2.0mg to 0.0mg and the control group 67.0mg. Egg production index (EPI) observed to treatments with 500, 1000 and 2000 IJ's between 2.47, 0.80 and 0.0 per cent (2000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at 2000IJ's. (T.60)

Heterorhabditis indica against *Rhipicephalus haemaphysaloides* caused a reduction ofegg mass weight with significant reduction differences (P<0.05) between the treated groups where the weight ranged from 13.16 mg to 0.50mg and the control group 67.85mg. Egg production index (EPI) observed to treatments 500, 1000 and 2000IJ's between 2.78, 1.02 and 0.0 per cent (2000IJ's) differing significantly (P<0.05) from control group. 100 percentage control was recorded at 2000IJ's. (T.61)

Table 55: Effect of *Heterorhabditis indica* on reproductive biology of *H.bispinosa* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	133.67±0.63	73.03±0.97	50.0±1.56	54.5±2.12	-
500	133.66±0.23	2.47±1.68	13.4±0.07	1.84±0.62	98.01
1000	133.46±0.14	0.34±0.06	2.12±0.005	0.25±0.024	99.0
2000	133.14±0.45	0.12±0.04	0.0±0.0	0.0±0.0	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at 5% significance.

Table 56: Effect of *Heterorhabditis indica* on reproductive biology of *H. intermedia* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	135.7±0.49	67.85±0.38	66.75±0.34	51.66±0.28	-
500	129.48±0.91	2.57±1.05	25.88±0.24	1.95±0.79	98.18
1000	132.40±1.07	1.15±0.33	19.54±0.08	0.87±0.26	99.33
2000	129.76±0.90	0.175±0.22	5.74±0.009	0.13±0.061	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at 5% significance.

Table 57: Effect of *Heterorhabditis indica* on reproductive biology of *H. kutchensis* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	132.10±0.41	65.66±0.37	63.36±0.11	49.71±0.44	-
500	132.35±20.42	2.9±1.19	24.96±2.08	2.19±0.91	97.73
1000	132.57±0.51	1.48±0.62	14.31±2.62	1.12±0.47	99.25
2000	132.01±0.29	0.47±0.22	0.11±0.07	0.36±0.17	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at 5% significance.

Table 58. Effect of *Heterorhabditis indica* on reproductive biology of *H.a.anatolicum* ticks

Conc of EPN / petridish	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	132.2^{a} ± 0.67	64.5^{a}±19.1	53.02^{a}±2.02	15.8±4.14	-
500	132.4^{a}±0.71	1.87^{b}±1.19	22.5^{b}±0.48	1.42±0.89	95.05
1000	129.76^{a}±1.71	0.63^{b}±.41	4.61±0.08	0.48±0.31	98.39
2000	129.48^{a}±0.95	0.036±0.06	0.0±0.0	0.027±0.023	100

(n): Samplesize, Means followed by equal letters in the same column for same nematode don't differ statistically at 5% significance.

Table 59. Effect of *Heterorhabditis indica* on reproductive biology of *H.m.isaaci* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	131.8±0.83	67.85±22.01	44.54±3.62	51.6±2.40	-
500	132.04±0.23	1.44±0.48	19.54±1.39	1.09±0.18	98.18
1000	132.12±0.20	0.23±0.13	2.02±1.59	0.17±0.05	99.33
2000	131.47±0.39	0.16±0.27	0.0±0.0	0.0±0.0	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at 5% significance.

Table 60: Effect of *Heterorhabditis indica* on reproductive biology of *R. sanguineus* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	131.6±2.43	67±0.51	62.12±0.39	50.67±0.42	-
500	130±0.85	2.0±1.17	22.8±3.11	2.47±0.18	97.03
1000	133.3±1.8	0.94±0.20	16.11±0.60	0.80±0.14	99.17
2000	129.76±0.47	0.0±0.0	0.0±0.0	0.0±0.0	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at 5% significance.

Table 61: Effect of *Heterorhabditis indica* on reproductive biology of *R. haemaphysaloides* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	132.23±3.4	72.30	68.78	50.67	-
500	131.62±1.09	13.16±0.78	20.70±1.5	2.78±0.56	97.5
1000	132.4±1.09	1.76±0.51	10.19±0.63	1.02±0.18	99
2000	130.36±1.8	0.50±0.1	0.0066±0.005	0.0 ± 0.0	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at $P<0.05$ significance.

Table 62. Effect of *Steinernema abbasi* on reproductive biology of *H. intermedia* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	140.52±	71.36±0.87	60.098±0.39	50.7±0.76	-
500	142.32±0.29	16.58±2.55	60.32±2.79	11.23±1.44	84.33
1000	143.25±0.64	14.68±2.08	34.09±1.51	8.54±0.89	94.0
2000	142.81±0.19	7.45±1.28	40.43±3.81	4.98±0.44	96.88
4000	141.88±0.71	3.96±0.31	28.04±3.08	1.74±0.22	98.80
6000	142.26±0.21	1.31±0.24	20.33±1.07	0.70±0.10	99.25

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at $P<0.05$ significance.

Table 63. Effect of *Steinernema abbasi* on reproductive biology of *H. bispinosa* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	132.57±0.51	65.69±0.19	56.02±1.01	49.12±0.18	-
500	132.26±0.46	16.1±2.99	50.9±1.32	6.44±2.26	88.88
1000	132.35±0.42	12.34±2.43	61.03±1.07	4.13±1.86	90.90
2000	132.6±0.45	6.01±1.19	55.76±0.65	2.18±0.89	95.05
4000	132.10±0.29	3.25±0.62	47.05±0.24	1.12±0.47	98.39
6000	131.7±0.41	1.1±0.22	18.54±0.03	0.36±0.17	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at $P<0.05$ significance.

Table 64. Effect of *Steinernema abbasi* on reproductive biology of *H. kutchensis* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	142.55±0.29	69.32±0.40	65.77±0.53	48.63±0.34	-
500	142.89±0.64	16.02±0.92	64.65±2.66	12.06±0.60	84.70
1000	143.67±0.19	1.024±2.18	36.05±2.79	11.58±1.53	92.50
2000	142.06±0.71	8.03±0.77	33.85±1.32	5.73±1.38	96.83
4000	142.44±0.21	2.23±0.25	25.3±1.11	1.55±0.79	99.21
6000	142.37±0.17	0.96±0.075	23.5±1.01	0.66±0.12	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at P<0.05 significance.

Table 65: Effect of *Steinernema abbasi* on reproductive biology of *H.a.anatolicum* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	141.19±0.56	71.87±0.68	52.46±1.91	50.17±0.46	-
500	141.67±0.37	17.12±0.36	54.2±2.39	12.05±1.61	87.10
1000	141.55±0.60	13.02±1.87	43.63±1.66	9.16±1.23	93.15
2000	141.40±0.54	7.02±1.31	32.88±1.04	5.01±0.96	96.39
4000	141.52±0.49	3.02±0.73	22.99±0.89	2.13±0.34	98.74
6000	142.08±0.63	1.5±0.32	18.4±0.56	1.06±0.14	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at P<0.05 significance.

Table 66: Effect of *Steinernema abbasi* on reproductive biology of *H. m. isaaci* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	133.35±0.57	67.85±0.38	44.54±0.23	52.32±0.28	-
500	131.68±0.70	17.81±3.44	45.69±3.17	6.88±2.59	88.54
1000	131.78±0.66	11.34±2.34	38.66±7.6	4.23±1.76	95.37
2000	131.78±0.29	5.23±1.05	25.88±3.6	1.95±0.79	98.18
4000	132.51±0.63	2.0±0.34	16.32±1.39	0.87±0.26	99.33
6000	131.59±0.69	0.35±0.08	2.02±0.13	0.13±0.061	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at P<0.05 significance.

Table 67: Effect of *Steinernema abbasi* on reproductive biology of *R.haemaphysaloides* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	131.6±2.43	67±0.51	66.27±3.0	52.67±1.10	-
500	130±2.30	15.2±3.08	41.04±3.67	5.0±2.01	84
1000	132.11±1.94	9.72±2.13	20.86±2.21	3.28±1.20	96
2000	131.89±1.82	14.16±0.84	19.73±1.5	2.78±0.56	97.5
4000	132.4±2.2	1.96±0.41	9.10±0.63	1.02±0.18	99
6000	130.36±1.8	0.50±0.10	0.0066±0.005	0.0±0.0	100

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at $P<0.05$ significance.

Table 68: Effect of *Steinernema abbasi* on reproductive biology of *R.sanguineus* ticks

Conc of EPN	Female wt before oviposition	Egg mass weight (mg)	Hatching percentage (%)	Egg production index (EPI%)	Percentage of control
0	132.10±0.41	65.66±0.37	62.27±	49.7±0.44	-
500	132.26±0.46	14.56±2.88	42.92±7.32	5.58±2.17	80
1000	132.6±0.45	12.34±2.43	29.37±4.21	4.28±1.82	95.26
2000	131.24±0.42	6.01±1.19	26.82±2.08	2.19±0.91	97.73
4000	132.57±0.51	3.25±0.62	16.86±2.16	1.12±0.47	99.21
6000	132.04±0.29	1.1±0.22	6.6±	0.36±0.17	99.00

(n): Sample size, Means followed by equal letters in the same column for same nematode don't differ statistically at $P<0.05$ significance.

4.4 *In vitro* efficacy of phytoacaricides in control of ticks

Crude methanolic extract of *Carica papaya* seeds and ethanolic extract *of Ricinus communis* extract was used against *Haemaphysalis, Hyalomma and Rhipicephalus* ticks to check the *in vitro* efficacy of extracts in control of ticks by larval packet test (LPT) and adult immersion test (AIT). The results of adult immersion test using the crude methanolic extracts of *Carica papaya* seeds and *Ricinus communis* are shown in the Table no 69 to 74. The efficacy of crude extracts of both herbs against *Haemaphysalis, Hyalomma* and *Rhipicephalus* ticks was assessed by estimating the per cent adult mortality (cut-off date for mortality observation was fixed at 15days of post treatment and egg mass laid up to this day was considered under the study) , reproductive index , inhibition of oviposition and larval mortality by larval packet test.

The results of the *in vitro* trials by larval packet test (LPT) on larval stages of different species of ticks three genera *i.e., Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma annatolicum anatolicum* and *Hyalomma marginatum isaaci* at different concentration of *Ricinus communis* (,6.25, 12.5, 25, 50,100,150 and 200mg/ml) and *Carica papaya* seeds (6.25, 12.5, 25, 50 and 100 mg/ml) was recorded (Table 69 to 74). After 24 hrs of exposure, 100 per cent mortality rate was recorded at 100 mg/ml concentration of *Carica papaya* and at higher concentration of *Ricinus communis* at 200 mg/ml against all species of ticks in this study. The details of larval mortality after 24 hrs intervals are presented in the Table 69 to 74.

The percent mortality caused by the methanolic extracts of *Carica papaya* and *Ricinus communis* was recorded. The results of the *in vitro* trials by larval packet test (LPT) on larval stages of different species of ticks i.e *Haemaphysalis* spp, *Rhipicephalus* spp and *Hyalomma* spp at different concentration of *Ricinus communis* (6.25, 12.5, 25, 50 100,150 and 200mg/ml) and *Carica papaya* seeds (6.25, 12.5, 25, 50 and 100mg/ml) was recorded. After 24 hrs of exposure, 100 per cent mortality rate was recorded at higher concentrations (200mg/ml) of *Ricinus communis* whereas the *Carica papaya* seed extract at lower concentrations (100mg/ml) induced 100% mortality.

Methanolic extract of *Carica papaya* seeds induced 100 per cent larval mortality of *H.bispinosa* , *H.intermedia*, *H.kutchensis*, *H.a.anatolicum* and *H.m.isaaci* at concentration of 100mg/ml (Table 69-71). Ethanolic extract of *Ricinus communis* seeds induced 100 per cent larval mortality of *H.bispinosa, H.intermedia*, *H.kutchensis*, *H.a.anatolicum, H.m.isaaci* at concentration of 200 mg/ml(Table 72-74).

The per cent adult mortality caused by the methanolic extract of *Carica papaya* varied from 25.57 to 100mg/ml when tested at concentrations of 6.25 to 100mg/ml against *H.bispinosa, H.intermedia* and *H.kutchensis,* whereas against *Hyalomma* ticks the mean adult mortality varied from 20 to 100mg/ml. The mean mortality for *Rhipicephalus* ticks varied from 21 to 100mg/ml. Mortality at higher concentration was significantly higher than that of lower concentrations. At highest concentration, egg laying was completly blocked and percentage inhibition of fecundity was more than 90. (T.75)

The per cent adult mortality caused by the ethanolic extract of *Ricinus communis* varied from 6.67 to 70.35 mg/ml when tested at concentrations of 6.25 to 100mg/ml against

H.bispinosa, H.intermedia and *H.kutchensis,* whereas against *Hyalomma* ticks the mean adult mortality varied from 15.25 to 72.45mg/ml. The mean mortality for *Rhipicephalus* ticks varied from 7.67 to 80mg/ml. Mortality at higher concentration was not significant than lower concentrations. At highest concentration, egg laying was not completely blocked and percentage inhibition of fecundity was less than 90. (T.76)

Table 69: *In vitro* efficacy of *Carica papaya* seed extract on *Haemaphysalis* ticks by Larval packet test

Concentration % of Carica papaya	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality	Percentage efficacy
H.bispinosa				
Control	100	0	0	0
6.25	100	6	6	6
12.5	100	24	24	24
25	100	45	45	45
50	100	85	85	85
100	100	100	100	100
H.intermedia				
Control	100	0	0	0
6.25	100	5	5	5
12.5	100	26	26	26
25	100	50	50	50
50	100	80	80	80
100	100	100	100	100
H.kutchensis				
Control	100			
6.25	100	9	9	9
12.5	100	28	28	28
25	100	46	46	46
50	100	82	82	82
100	100	100	100	100

Table 70: *In vitro* efficacy of *Carica papaya* seed extract on *Hyalomma* ticks by Larval packet test

Concentration % of Carica papaya	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality	Percentage efficacy
H.a.anatolicum				
Control	100	0	0	0
6.25	100	6	6	6
12.5	100	20	20	20
25	100	46	46	46
50	100	78	78	78
100	100	100	100	100
H.m.issaci				
Control	100	0	0	0
6.25	100	5	5	5
12.5	100	24	24	24
25	100	45	45	45
50	100	75	75	75
100	100	100	100	100

Table 71: *In vitro* efficacy of *Carica papaya* extract on *Rhipicephalus* ticks by Larval packet test

Concentration % of Carica papaya	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality	Percentage efficacy
R.sanguineus				
Control	100	0	0	0
6.25	100	10	10	10
12.5	100	26	26	26
25	100	48	48	48
50	100	70	70	70
100	100	100	100	100
R.haemaphysaloides				
Control	100	0	0	0
6.25	100	5	5	5
12.5	100	24	24	24
25	100	45	45	45
50	100	65	65	65
100	100	100	100	100

Table 72: *In vitro* efficacy of *Ricinus communis* extract on *Haemaphysalis* ticks by Larval packet test

Concentration % of Ricinus communis	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality	Percentage efficacy
H.bispinosa				
Control	100	0	0	0
6.25	100	5	5	5
12.5	100	16	16	16
25	100	35	35	35
50	100	65	65	65
100	100	80	80	80
150	100	92	92	92
200	100	100	100	100
H.intermedia				
Control	100	0	0	0
6.25	100	8	8	8
12.5	100	15	15	15
25	100	33	33	33
50	100	68	68	68
100	100	85	85	85
150	100	94	94	94
200	100	100	100	100
H.kutchensis				
Control	100	0	0	0
6.25	100	9	9	9
12.5	100	16	16	16
25	100	32	32	32
50	100	70	70	70
100	100	82	82	82
150	100	92	92	92
200	100	100	100	100

Table 73: *In vitro* efficacy of *Ricinus communis* extract on *Hyalomma* ticks by Larval packet test

Concentration % of *Ricinus Communis*	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality	Percentage efficacy
H.a.anatolicum				
Control	100	0	0	0
6.25	100	6	6	6
12.5	100	20	20	20
25	100	40	40	40
50	100	60	60	60
100	100	72	72	72
150	100	87	87	87
200	100	100	100	100
H.m.isaaci				
Control	100	0	0	0
6.25	100	5	5	5
12.5	100	24	24	24
25	100	45	45	45
50	100	65	65	65
100	100	75	75	75
150	100	89	89	89
200	100	100	100	100

Table 74: *In vitro* efficacy of *Ricinus communis* extract on *Rhipicephalus* ticks by Larval packet test

Concentration % of *Ricinus Communis*	Number of larvae	Number of larvae dead after 24hrs	Percentage mortality	Percentage efficacy
R.sanguineus				
Control	100	0	0	0
6.25	100	10	10	10
12.5	100	26	26	26
25	100	48	48	48
50	100	70	70	70
100	100	80	80	80
125	100	90	90	90
200	100	100	100	100
R.haemaphysaloides				
Control	100	0	0	0
6.25	100	5	5	5
12.5	100	24	24	24
25	100	45	45	45
50	100	65	65	65
100	100	72	72	72
125	100	85	85	85
200	100	100	100	100

Table 75. Results of adult immersion test by Methanolic extract of *Carica papaya* against common ticks infesting sheep.

Tick species	Conc mg/ml	Mean Tick weight	Mean adult mortality	Mean Egg mass	Reproductive index	Inhibition of egg laying
H.bispinosa	Control	134.28±12.2	0.0	48.5±0.004	0.43±0.40	-
	6.25	135.7±0.49	26.67±6.67	34.3±0.002	0.182±0.012	36.7
	12.5	129.48±0.91	43.33±11.54	23.3±0.001	0.149±0.06	44.2
	25	132.40±1.07	68.79±13.23	20.1±0.001	0.132±0.03	54.4
	50	129.76±0.90	72.43±	18.9±0.001	0.112±0.01	90.3
	100	135.7±0.49	100±0.0	0.0	0.0±0.00	100
H.intermedia	Control	133.26±9.8	0.0	44.54± 0.04	0.39±0.01	-
	6.25	133.35±0.57	25.57±5.57	30.15±0.003	0.035±0.01	34.3
	12.5	131.68±0.70	42.20±12.22	24.0±0.002	0.028±0.02	53.3
	25	131.78±0.66	69.82±6.60	20.1±0.001	0.026±0.02	95.1
	50	131.78±0.29	70.23±6.67	16.60±0.001	0.019±0.03	100
	100	132.51±0.63	100±0.0	0.0	0.0±0.0	
H.kutchensis	Control	134.64±6.8	0.0	46.56±0.002	0.48±0.21	-
	6.25	132.57±0.51	27.27±6.67	34.3±0.002	0.273±0.016	19.1
	12.5	132.26±0.46	43.45±13.33	23.3±0.001	0.198±0.009	39.4
	25	132.35±0.42	67.89±13.33	20.1±0.001	0.178±0.12	48.1
	50	132.6±0.45	73.36±6.62	18.9±0.001	0.152±0.006	45.1
	100	132.10±0.29	100±0.0	0.0	0.0±0.0	100
H.a.anatolicum	Control	133.34±9.2	0.0	49.2±0.006	0.43±0.01	-
	6.25	135.7±0.49	20±10.2	36.2±0.004	0.062±0.002	44.1
	12.5	129.48±0.91	38.32±10.55	28.7±0.002	0.058±0.002	53.1
	25	132.40±1.07	66.53±13.33	24.3±0.001	0.052±0.001	65.1
	50	129.76±0.90	75.25±11.55	15.45±0.001	0.042±0.001	80.1
	100	135.7±0.49	100±0.O	0.0	0.0	100
H.m.isaaci	Control	142.78±11.20	0.0	50.20±0.006	0.46±0.003	-
	6.25	140.52±	20.24±6.67	52.20±0.006	0.159±0.003	44.1
	12.5	142.32±0.29	32.32±11.26	34.43±0.004	0.153±0.004	56.4
	25	143.25±0.64	64.37±15.56	28.7±0.001	0.140±0.003	83.1
	50	142.81±0.19	79.39±7.62	29.3±0.001	0.128±0.001	95.1
	100	141.88±0.71	100±0.0	0.0	0.119±0.009	100
R.haemaphysaloides	Control	142.45±14.46	0.0	50.2±0.006	0.42±0.004	-
	6.25	141.19±0.56	21.13±6.27	36.68±0.006	0.243±0.18	32.13
	12.5	141.67±0.37	48.9±12.35	34.3±0.004	0.172±0.009	43.13
	25	141.55±0.60	65.67±13.53	23.3±0.002	0.156±0.012	80.0
	50	141.40±0.54	72.45±7.67	20.1±0.001	0.139±0.06	94.21
	100	141.52±0.49	100±0.0	0.0	0.0±0.0	100
R.sanguineus	Control	1412.76±11.46	0.0	51.12±0.009	0.420±	-
	6.25	142.55±0.29	22.23±7.33	54.2±0.008	0.273±0.16	33.33
	12.5	142.89±0.64	49.27±15.67	36.23±0.004	0.198±0.009	55.3
	25	143.67±0.19	68.16±14.92	20.01±0.002	0.178±0.012	73.33
	50	142.06±0.71	78.56±6.67	16.11±0.001	0.149±0.06	93.33
	100	142.44±0.21	100±0.0	0.0	0.0±0.0	100

Table 76. Results of adult immersion test by Ethanolic extract of *Ricinus communis* against common ticks infesting sheep.

Tick species	Conc mg/ml	Tick weight	Mean mortality	Mean Egg mass	Reproductive index	Inhibition of egg laying (%)
H.bispinosa	Control	131.30±0.83	0.0	58±0.004	0.580±0.39	-
	6.25	132.23±0.54	6.76±0.76	42±0.003	0.215±0.016	60.16
	12.5	132.50±0.34	13.33±6.67	38±0.001	0.198±0.009	63.51
	25	132.40±0.23	26.67±6.67	26.7±0.0.01	0.187±0.012	65.50
	50	132.16±0.03	43.33±11.20	27.3±0.004	0.149±0.06	70.61
	100	134.2±0.0	68.79±3.0	26±0.005	0.132±0.03	73.15
H.intermedia	Control	133.33	0.0	48.9±0.004	0.520±0.27	-
	6.25	136.7±0.40	8.16±4.67	40.3±0.003	0.420±0.013	62.45
	12.5	135.48±0.90	15.23±6.34	38.2±0.001	0.437±0.013	68.45
	25	132.40±0.07	29.47±6.67	29.10±0.001	0.430±0.016	70.05
	50	134.76±0.90	45.62±11.32	23.0±0.001	0.410±0.040	70.50
	100	135.7±0.49	70.35±11.55	21.6±0.004	0.401±0.046	75.23
H.kutchensis	Control	132.34	0.0	45±0.004	0.526±0.27	-
	6.25	135.7±0.49	8.86±4.60	65±0.003	0.432±0.013	62.45
	12.5	133.48±0.91	15.33±6.34	60±0.001	0.427±0.013	68.45
	25	132.40±1.07	29.30±6.60	45±0.001	0.420±0.016	70.05
	50	129.76±0.90	47.32±11.55	32±0.001	0.402±0.040	70.50
	100	135.7±0.49	68.48±11.55	25±0.004	0.385±0.046	75.23
H.a.anatolicum	Control	135.7±0.49	0	54.02±0.005	0.436	-
	6	139.48±0.91	15.67±6.67	54±0.001	0.111±0.01	68.76
	12.5	132.40±1.07	21.13±8.76	49.2±0.001	0.101±0.03	68.94
	25	139.76±0.90	48.98±12.38	42±0.001	0.100±0.03	72.31
	50	135.7±0.49	65.67±11.02	32±0.01	0.064±0.01	75.24
	100	135.7±0.49	72.45±15.65	22±0.01	0.054±0.01	76.38
H.m.isaaci	Control	136.48±0.91	0	49.10±0.003	0.490±0.019	-
	6.25	135.40±1.07	16.70±7.76	54±0.001	0.100±0.01	58.26
	12.5	134.76±0.90	20.30±6.67	59±0.001	0.99±0.03	65.0
	25	135.7±0.49	45.18±12.56	42±0.001	0.99±0.03	68.67
	50	135.7±0.49	69.10±13.33	30±0.01	0.60±0.01	72.24
	100	133.48±0.91	70. 5±15.67	21±0.01	0.044±0.01	72.42
R.haemaphysaloides	Control	132.40±1.07	0	43.15±0.004	0.510±0.021	-
	6.25	134.26±0.90	7.67±2.46	40±0.003	0.392±0.027	43.8
	12.5	134.17±0.49	13.33±7.67	46.3±0.001	0.337±0.023	64.0
	25	134.71±0.49	26.67±11.56	38.12±.001	0.330±0.023	70.2
	50	134.58±0.91	60.17±13.46	29.64±0.0106	0.312±0.016	74.2
	100	134.42±1.07	75.87±11.0	28.76±0.004	0.329±0.040	80.0
R.sanguineus	Control	132.60±0.90	0	43.53±0.006	0.500±0.020	-
	6.25	134.27±0.59	9.20±5.67	50±0.003	0.410±0.030	43.68
	12.5	133.7±039	15.53±6.70	30±0.001	0.347±0.028	50.4
	25	134.48±0.81	28.50±6.67	30.43±.001	0.330±0.023	63.2
	50	132.40±1.03	62.0±12.55	29.18±0.010	0.322±0.012	69.0
	100	134.76±0.80	80±12.5	19.29±0.004	0.350±0.45	71.7

4.5 Vector potentiality of ticks in disease transmission

4.5.1 Parasitological method of diagnosis

4.5.1.1 Detection of Theileria parasites from blood of sheep in organised farms by staining method

During this study, in organised farms out of 85 samples examined by microscopy, only 14 blood samples (16.4%) were found positive only for *Theileria* by giemsa stain.

4.5.1.2 Detection of Haemoprotozoan parasites from blood of sheep by molecular method

Among 85 blood samples, 14 blood samples which were positive for theileriosis by blood smear examination were subjected to PCR using genus specific primers of theileria, all fourteen samples were found positive for genus specific theileria primers with amplification at 1098 bp and was later subjected to PCR with theileria species specific primers , in which twelve samples were amplified for *Theileria luwenshuni* species with an amplification of 388 bp by nested PCR and two samples were positive for *Theileria ovis* with an amplification of 237 bp semi-nested PCR.(Fig 37-38)

Among the 71 blood smear negative samples, 11 samples were found positive for Theileriosis by PCR against theielria genus specific primer, which later when subjected to nested PCR and semi nested PCR showed positive for *Theileria luwenshuni* (9) and *Theileria ovis* (2) (Table 77).

5.1.1.3 Detection of Haemoprotozoan parasites from ticks infested on sheep by staining and molecular method.

The tick tissues i.e salivary gland, mid gut and ovaries were stained by giemsa (59) and Methyl green pyronin stain (59) in which eight ticks was found positive for Theileria by giemsa stain and seven ticks were found positive for theileria by Methyl green pyronin stain (Table 80 to 81).

About 94 salivary gland of tick spp *H.bispinosa, H.kutchensis, H.intermedia, R.sanguineus, R.haemaphysaloides, H.a.anatolicum* and *H.m.isaaci* was dissected and the DNA was extracted from it by using Qiagen mini kit and subjected for PCR, in which 13 samples DNA of *H.kutchensis* (9) was amplified for *Theileria luwenshuni* by nested PCR whereas *H.a.anatolicum* (4) amplified to *T.ovis* and none of the samples DNA of *H.bispinosa* (17), *H.intermedia* (14), *R.heamaphysaloides* (18) *R.sanguineus* (5) and *Hyalomma marginatum isaaci*(5) showed amplification for haemoprotozoan parasites by PCR (Table 78-79).

4.5.2 Detection of Haemoprotozoan parasites from blood of sheep in unorganised farms by staining and molecular method.

In unorganised farms, a total of 100 blood samples were stained with Giemsa and examined for haemoprotozoan parasites. Out of 100 samples, 64 revealed Theileria organisms (T.82). All 64 samples positive for Theileriosis by blood smear examination was subjected to PCR with Theileria species primers. Out of 64 blood smear positive samples, 14 samples were found positive for *Theileria ovis* with amplification of 237bp, whereas 50 samples were found positive for *Theileria luwenshuni* with amplification of 388 bp.Apart

from the 64 blood smear positive samples, other 36 samples which were negative by blood smear subjected to PCR against *Theileria* and *Babesia* species specific primers, 18 samples were found positive for *Theileria luwenshuni* spp with amplification of 388 bp by Nested PCR and nine samples amplified for *T.ovis* with 237 bp amplicon. Remaining 4 blood samples amplified with Babesia species specific primers and was found positive for *Babesia ovis* with an amplification of 549 bp by species specific PCR (Table 82).

4.5.2.1 Detection of haemoprotozoan parasites from ticks infested on sheep by staining and molecular method in unorganised farms.

The tick tissues, 200 each were stained with giemsa and methyl green pyronin in which 20 ticks were found positive for haemoprotozoa by geimsa stain. Whereas 31 were found positive for theileriosis by methyl green pyronin stain. None of them were positive for *babesiosis* by giemsa and methyl green pyronin stain (T. 85-86). A tick was interpreted as positive for heamoprotozoan infection by methyl green pyronin stain only if any one of the three tick tissues *i.e.,* salivary gland / mid gut / ovaries revealed any of the parasitic stages. In the mid gut the presence of parasitic stages was indicated by hypertrophy of infected epithelial cells and vacuolations in the cell cytoplasm and in infected tick ovaries on methyl green pyronin staining, the oocytes shows blue coloured spherical masses denoting the developmental stages of the haemoprotozoan parasites, but none of the ovaries and midgut showed the parasitic stages in this study. In the present study the infected salivary glands showed bluish green mass (Plate5) which is an indicative of haemoprotozoan parasitic infection. Whereas in geimsa stain, the infected salivary gland showed enlarged acini and black dots as parasites indicative of haemoprotozoan infection (Plate 6).

The DNA was extracted from salivary glands of 315 ticks in the un-organised farm animals. In which 49 DNA samples comprising of *Haemaphysalis kutchensis* (34) amplified at 388bp positive for *Theileria luwenshuni* by nested PCR and *Hyalomma anatolicum anatolicum* (15) amplified for *T.ovis* whereas other DNA samples of *Haemaphysalis bispinosa, Haemaphysalis intermedia, Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus, Hyalomma marginatum isaaci* were found negative for haemoprotozoan parasite.(T:83-84)

4.5.3 Phylogenetic analysis of *T.luwenshuni* isolates recovered in this study

The phylogenetic analysis of this study showed that *T. luwenshuni* KVAFSU isolates 1, 2 and KVAFSU isolate 3 shared >99.9 per cent nucleotide sequence identity with *T. luwenshuni* isolates of accession numbers KU554730, KU234526, KU247949, KC769996 and KJ850935. The isolates that were identified as *T. luwenshuni* during this study were different from other *Theileria* spp. *viz., T. ovis, T. lestoquardi* but showed 99.99 per cent homology with *T. orientalis, T.buffeli* and *T. sergenti* complex.

4.5.4 Phylogenetic analysis of *T.ovis* isolates recovered in this study

The phylogenetic analysis revealed that *T. ovis* KVAFSU isolate shared 100 per cent nucleotide sequence identity with *T. ovis* isolate from China, Iran, Turkey, France deposited in GenBank.

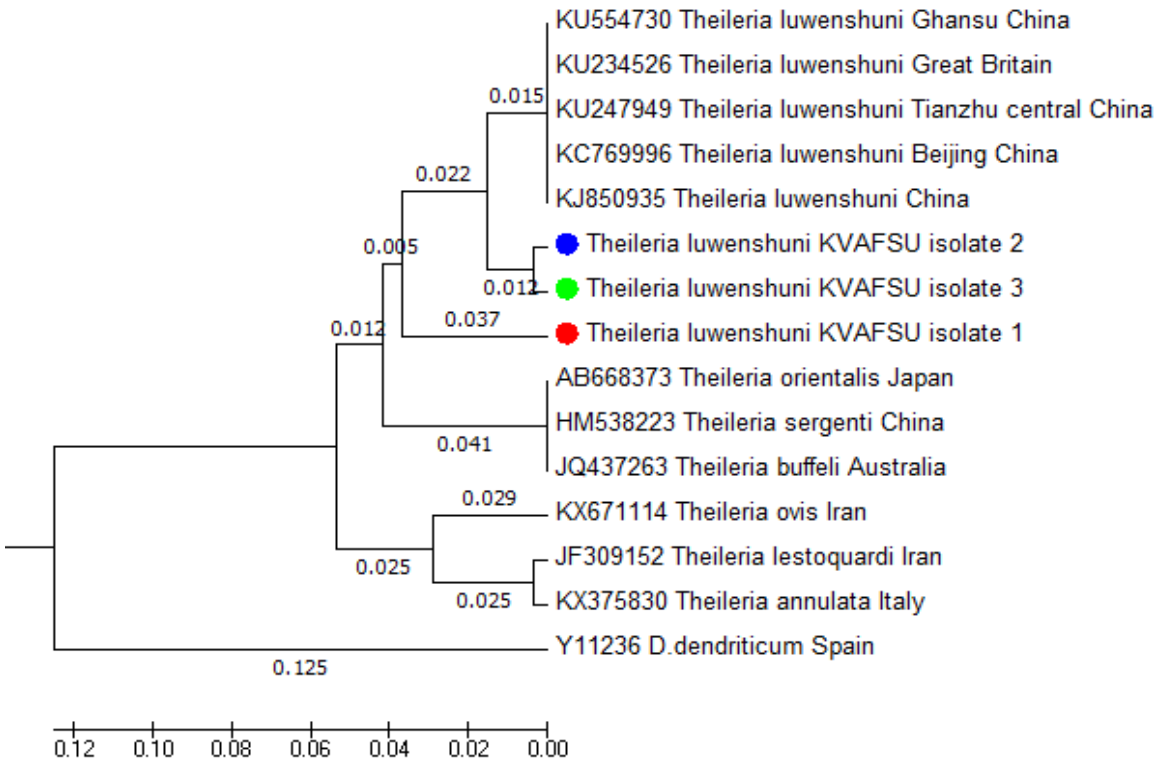


Fig. 32. Phylogenetic tree of *Theileria luwenshuni* isolates recovered in this study.

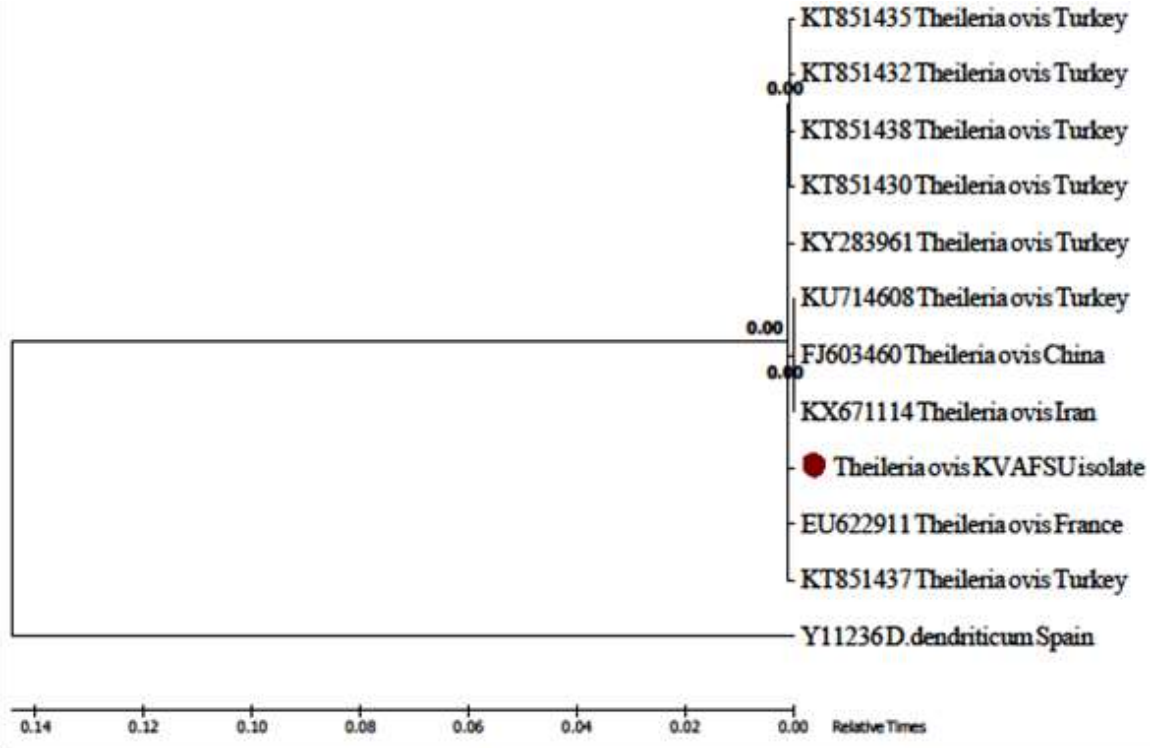


Fig. 33. Phylogenetic tree of *Theileria ovis* isolates recovered in this study.

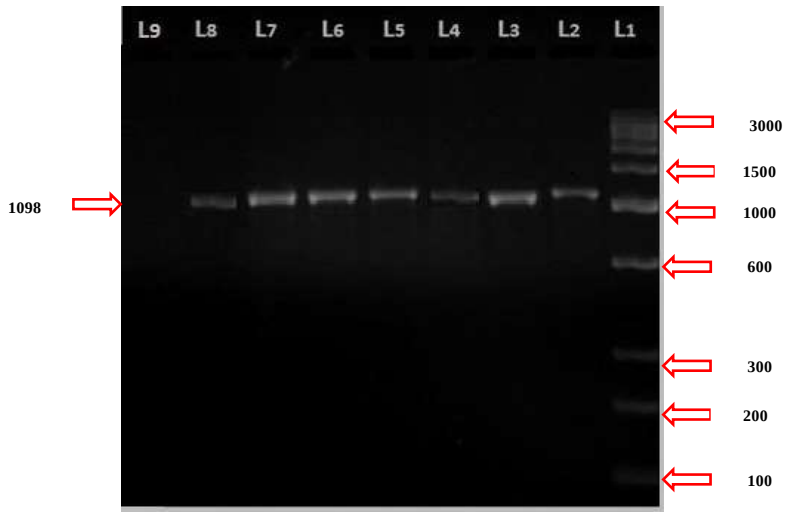


Fig. 34. Gel showing amplification of *Theileria* genus specific amplicon at 1098 bp

Lane 1 – 3.0 kb molecular weight DNA ladder

Lane 2 –Sample from Mandya

Lane 3 - Positive Control

Lane 4- Sample from Chitradurga

Lane 5- Sample from Bangalore

Lane 6- Sample from Belgaum

Lane 7- Sample from Athani

Lane 8- Sample from Tumkur

Lane 9 - Negative Control

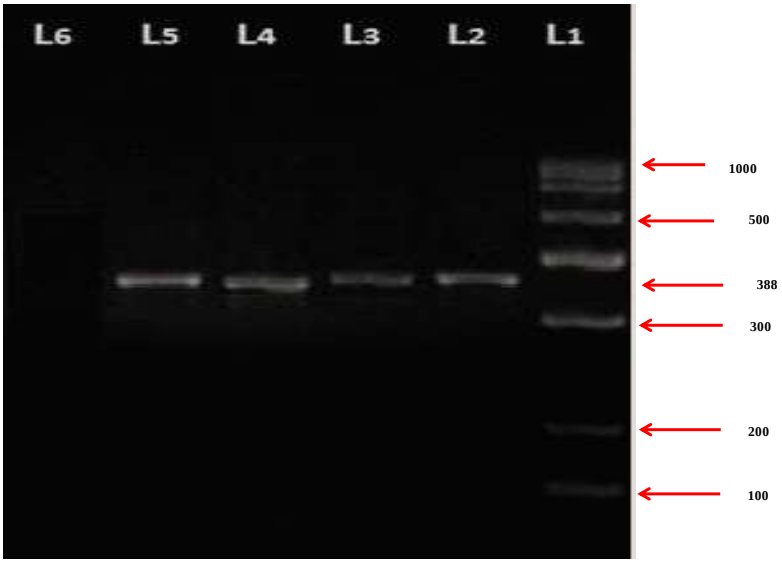


Fig. 35. Amplification of *Theileria luwenshuni* species specific gene of 388bp by using 2nd set primers by Nested PCR.

Lane 1 - 1 kb molecular weight DNA ladder

Lane 2 –Samples from Bangalore

Lane 3 –Samples from Tumkur

Lane 4- Samples from Mandya

Lane 5- Positive Control

Lane 6- Negative Control

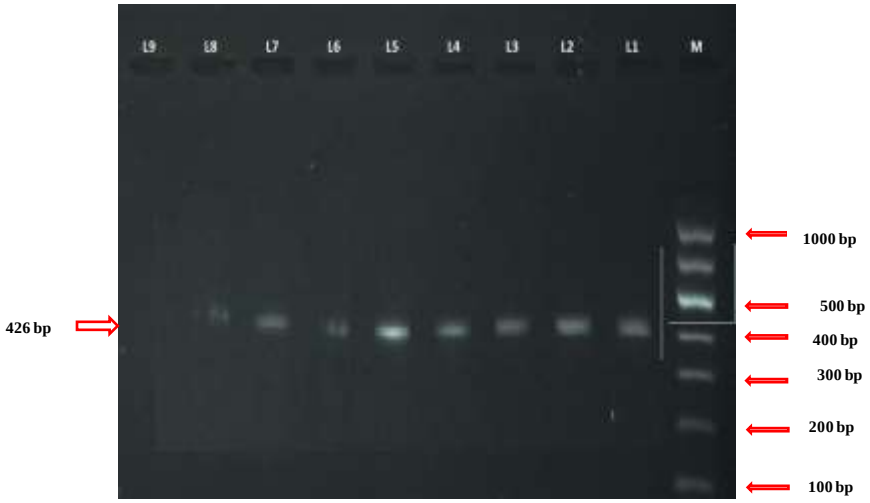


Fig. 36. Amplification of *Theileria ovis* species specific gene of 426bp by using 1st of primers by Semi nested PCR.

Lane 1 - 1 kb molecular weight DNA ladder

Lane 2 -samplefrom Mandya

Lane 3 - sample from Chitradurga

Lane 4 - sample from Belgaum.

Lane 5 - Positive Control

Lane 6 - sample from Bangalore

Lane 7 - sample from Athani

Lane 8 - sample from Tumkur

Lane 9 - Negative Control

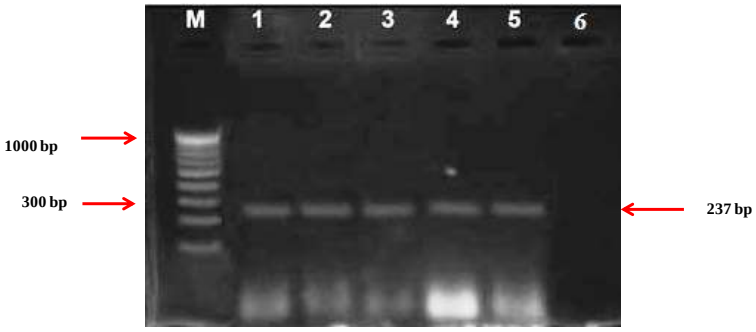


Fig. 37. Amplification of *Theileria ovis* species specific gene at 237bp by using 2nd set of primers amplicon by Semi nested PCR

Lane 1 - 1 kb molecular weight DNA ladder

Lane 2 –Sample from Bangalore

Lane 3 –Sample from Belgaum.

Lane 4- Sample from Davangere

Lane 5- Positive Control

Lane 6- Negative Control

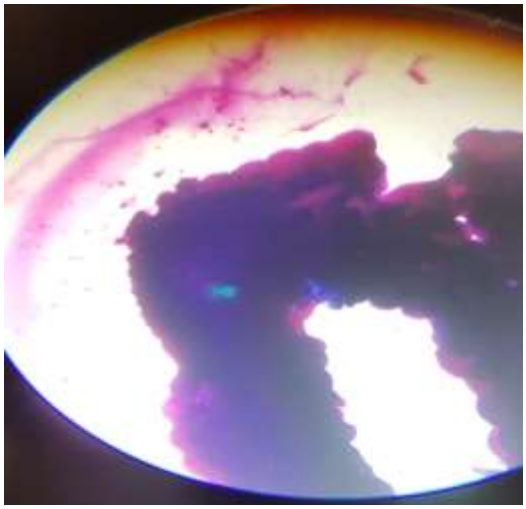


Plate 5: Infected tick salivary gland: Bluish green mass

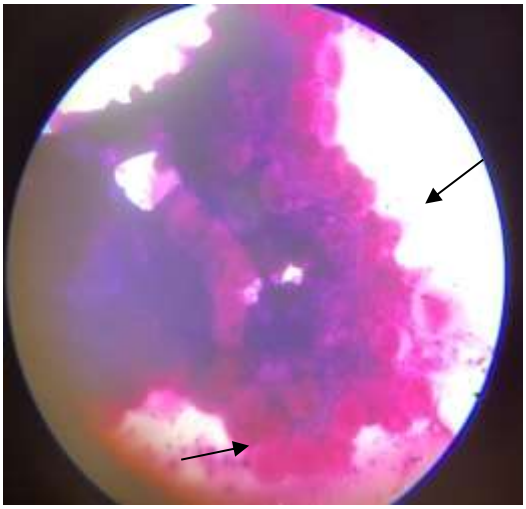
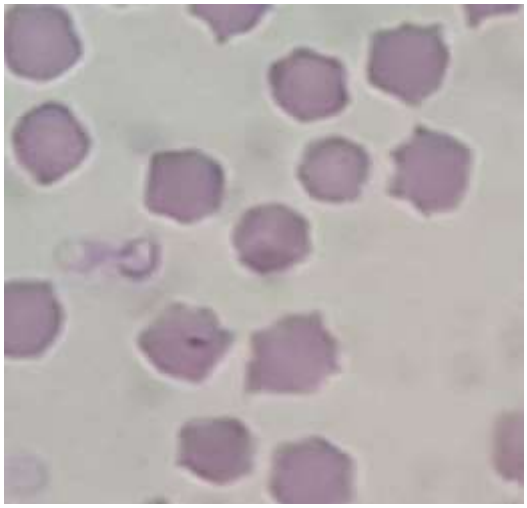


Plate 6: Infected tick salivary gland: Hypertrophied acini & dot shaped parsaites



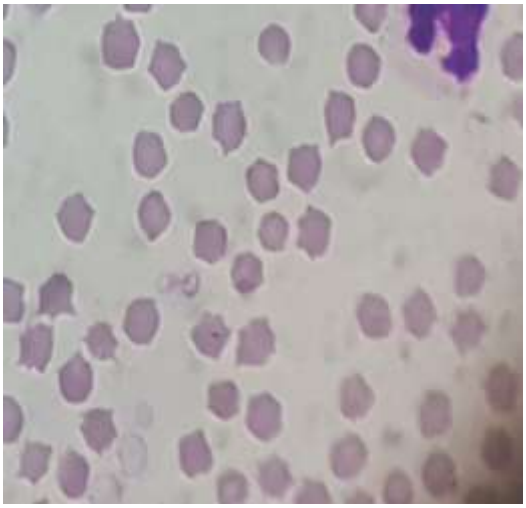
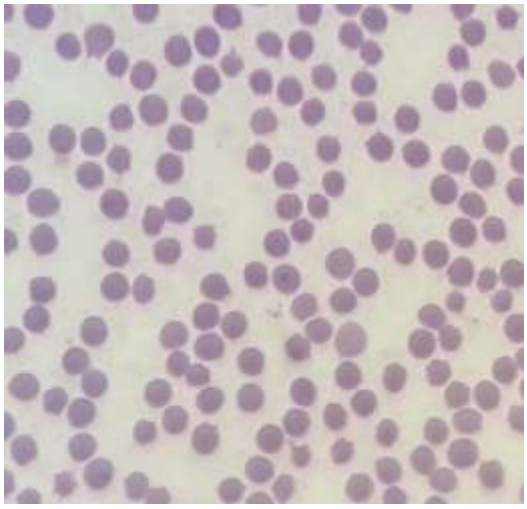


Plate 7-8. Geimsa stained blood smears with Theileria parasites



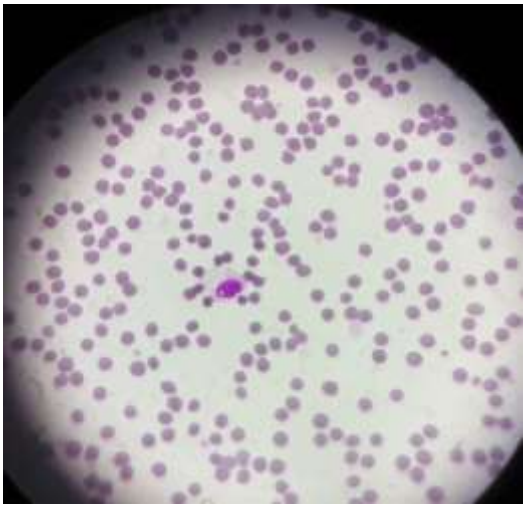


Plate 9-10. Geimsa stained blood smears with Theileria parasites

Table 77. Prevalence of Theileria parasites based on microscopy and PCR in sheep under organised farms

Sl. No.	One farm was considered as one flock	Microscopy				Polymerase Chain Reaction							
		Healthy / Suspected	Tick infested	No of samples positive	No of samples negative	Positive for *T.luwenshuni*		Positive for *T.ovis*		Positive for *B.ovis*	Total no of samples positive	Total no of samples negative	Total no of samples
						+ ve by blood smear	-ve blood smear	+ ve blood smear	-ve blood samples				
1	KVAFSU, B'lore	5	5	3	7	2	1	1	-	-	4	6	10
2	Hassan	5	5	0	10	-	-	-	-	-	-	10	10
3	Konehally, Tiptur	5	5	0	10	-	-	-	-	-	-	10	10
4	Shivamogaa	5	0	0	05		-	-	-	-	-	05	05
5	Guttala, Haveri	5	5	2	08	2	2	-	1	-	5	05	10
6	Nagamangala	5	5	0	10	-	-	-	-	-	-	10	10
7	Athani	5	5	2	08	2	2	-	-	-	4	06	10
8	Chitradurga	5	5	4	06	3	2	1	1	-	7	03	10
9	Dhanagur, Mandya	5	5	3	07	3	2	-	-	-	5	05	10
	Total	45	40	14	71	12	9	2	2	0	25	60	85

Table 78. Prevalence of Theileria parasites in vectors by PCR in sheep under organised farms

Sl. No.	Organised farms	Name of the tick spp														Total no examined	Total no Positive
		H. bispinosa		*H. intermedia*		*H. kutchensis*		*H.a. anatolicum*		*H. marginatum isaaci*		*R. sanguineus*		*R. haemaphysaloides*			
		**	*	**	*	**	*	**	*	**	*	**	*	**	*		
1	KVAFSU, B'lore	-	-	-	-	05	2	-	-	-	-	-	-	-	-	05	2
2	Hassan	02	-	05	-	-	-	-	-	-	-	-	-	-	-	07	-
3	Konehally		-	-		05	-	-	-	-	-	-	-	03	-	08	-
4	Shivamogaa	03	-	-	-	-	-	-	-	-	-	-	-			03	-
5	Guttala	03	-	-	-	-	-	05	2	-	-	-	-	05	-	13	2
6	Nagamangala		-	-	-	05	-	-	-		-	-	-	-	-	05	-
7	Athani	03	-	-	-	-	-	05	2	05	-		-	-	-	13	2
8	Chitradurga	03	-	05	-	05	3	-	-	-	-	05	-	05	-	23	3
9	Dhanagur, Mandya	03	-	04	-	05	4	-	-	-	-	-	-	05	-	17	4
	Total	17	-	14	-	25	9	10	4	05	-	05	-	18	-	94	13

Note: ** ---Number examined * ---Number positive

Table 79. Prevalence of Theileria parasites in vectors by PCR in sheep from organised farms

Sl. No.	Tick spp	No examined	Positive for Theileria parasites			Total positive
			T.ovis	*T.luwenshuni*	*Babesia spp*	
1	*Haemaphysalis bispinosa*	17	-	-	-	-
2	*Haemaphysalis intermedia*	14	-	-	-	-
3	*Haemaphysalis kutchensis*	25	-	9	-	9
4	*Hyalomma anatolicum anatolicum*	10	4	-	-	4
5	*Hyalomma marginatum isaaci*	05	-	-	-	-
6	*Rhipicephalus sanguineus*	05	-	-	-	-
7	*Rhipicephalus haemaphysaloides*	18	-	-	-	-
	Total	94	4	9		13

Table 80. Prevalence of Theileria parasites in vectors by Methyl green pyronin staining method in sheep under organised farms

Sl. No.	Organised farms	Tick species															
		H.bispinosa		*H.intermedia*		*H.kutchensis*		H.*a.anatolicum*		*H.m.isaaci*		*R.sanguineus*		*R.haemaphysaloides*		Total	
		**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*
1	KVAFSU, Bangalore	-	-	-	-	3	1	-	-	-	-	-	-	-	-	3	1
2	Hassan	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	
3	Konehally	-	-		-	2	-	-	-	-	-	-	-	3	-	5	
4	Shivamoga	2	-	2	-	-	-	-	-	-	-	-	-	-	-	4	
5	Guttala	3	-	-	-	-	-	3	2	-	-	-	-	3	-	9	2
6	Nagamangala	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Athani	3	-	-	-	-	-	-		3	-	-	-	-	-	6	-
8	Chitradurga	3	-	3	-	4	2	-	-	-	-	3	-	3	-	16	2
9	Dhanagur, Mandya	4	-	3	-	4	2	-	-	-	-	-	-	3	-	14	2
		18	-	8	-	13	5	3	2	3	0	2	-	12	-	59	7

Note: ** ---Number examined * ---Number positive

Table 81. Prevalence of Theileria parasites in vectors by Geimsa staining method in sheep under organised farms

Sl. No.	Organised farms	Tick spp															
		H.bispinosa		*H.intermedia*		*H.kutchensis*		H.*a.anatolicum*		*H.m.isaaci*		*R.sanguineus*		*R.haemaphysaloides*		Total	
		**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*
1	KVAFSU, Bangalore	-	-	-	-	2	-	-	-	-	-	-	-	-	-	3	-
2	Hassan	3	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-
3	Konehally	-	-		-	2	-	-	-	-	-	-	-	3	-	5	-
4	Shivamog	2	-	2	-	-	-	-	-	-	-	-	-	-	-	4	-
5	Guttala	3	-	-	-	-	2	3	2	-	-	-	-	3	-	10	4
6	Nagamangala	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
7	Athani	3	-	-	-	-	-	-		3	-	-	-	-	-	6	-
8	Chitradurga	3	-	3	-	3	2	-	-	-	-	2	0	3	-	15	2
9	Dhanagur, Mandya	4	-	3	-	3	2	-	-	-	-	-	-	3	-	13	2
		17	-	8	-	10	6	3	2	3	0	2	0	12	-	59	8

Note: ** ---Number examined * ---Number positive

Table 82. Prevalence of Haemoprotozoan parasites by microscopy and PCR from sheep blood from unorganised farms

Sl. No.	One sheep flock examined in each Organised farms	Microscopy					Polymerase Chain Reaction					
		Healthy / Suspected	Tick infested	Total no of samples	No of samples positive	No samples negative	Positive for *T.luwenshuni*	Positive for *T.ovis*	No of samples negative by microscopy	Microscopy negative samples on PCR amplified for *T.luwenshuni and T.ovis*		
							+ ve by blood smear	+ ve blood smear		*B.ovis*	*T.luwenshuni*	*T.ovis*
1	Hessaraghatta B'lore	5	5	10	7	3	4	3	3	-	1	-
2	Mandya	5	5	10	8	2	6	2	2	-	3	1
3	Davangere	5	5	10	7	3	4	2	3	1	2	1
4	Hassan	5	5	10	8	2	6	2	2	-	1	
5	Bellary	5	5	10	8	2	4	2	2	1	1	2
6	Shivmoga	5	5	10	7	3	5	2	3	-	1	1
7	Belgaum	5	5	10	8	2	4	2	2	1	2	1
8	Bidar	5	5	10	8	2	4	2	2	1	2	1
9	Chitradurga	5	5	10	9	1	5	4	1	-	3	1
10	Tumkur	5	5	10	9	1	6	3	1	-	2	1
	Total	50	50	100	64	36	50	14	36	4	18	9

Table 83. Farmwise Prevalence of Haemoprotozoan parasites in vectors by PCR in sheep from unorganised farms

Sl. No.	Organised farms	Name of the tick spp															
		H.bispinosa		***H.intermedia***		***H.kutchensis***		**H.*a.anatolicum***		***H.m.isaaci***		***R.sanguineus***		***R.haemaphysaloides***		**Total**	
		**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*
1	Hessaraghatta, B'lore	-	-	-	-	6	2	-	-	-	-	6	-	5	-	20	2
2	Mandya	9	-	8	-	8	6	-	-	-	-	7	-	8	-	30	6
3	Davangere	-	-	-	-	-	-	8	3	8	-	7	-	7	-	30	3
4	Hassan	8	-	5	-	-	-	-	-	-	-	-	-	7	-	35	
5	Bellary	7	-	6	-	6	6	-	-	-	-	7	-	7	-	35	6
6	Shivmoga	5	-	-	-	-	-	-	-	-	-	-	-	7	-	10	-
7	Belgaum	6	-	7	-	-	-	8	4	8	-	7	-	8	-	55	4
8	Bidar	5	-	8	-	6	7	8	4	8	-	-	-	7	-	40	11
9	Chitradurga	-	-	8	-	6	7	8	4	8	-	7	-	7	-	40	11
10	Tumkur	7	-	-	-	6	6	-	-	-	-	7	-	7	-	20	6
		47	-	42	-	44	34	32	15	32	-	48	-	70	-	315	49

Note: ** ---Number examined * ---Number positive

Table 84. Prevalence ofTheileria parasites in vectors by PCR in sheep from unorganised farms

Sl. No.	Tick spp	No examined	Positive for Theileria parasites			Total positive
			T.ovis	***T.luwenshunii***	***Babesia spp***	
1	*Haemaphysalis bispinosa*	47	-	-	-	-
2	*Haemaphysalis intermedia*	42	-	-	-	-
3	*Haemaphysalis kutchensis*	44	-	34	-	34
4	*Hyalomma anatolicum anatolicum*	32	15	-	-	15
5	*Hyalomma marginatum isaaci*	32	-	-	-	-
6	*Rhipicephalus sanguineus*	48	-	-	-	-
7	*Rhipicephalus haemaphysaloides*	70	-	-	-	-
	Total	315	15	34	-	49

Table 85. Prevalence of Theileria parasites in vectors by Methyl green pyronin staining method in sheep under unorganised farms

Sl. No.	Organised farms	Name of the Tick spp															
		H.bispinosa		*H.intermedia*		*H.kutchensis*		H.*a.anatolicum*		*H.m.isaaci*		*R.sanguineus*		*R.haemaphysaloides*		**Total**	
		**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*
1	Hessaraghatta, Bangalore	-	-	-	-	5	2	-	-	-	-	2	-	5	-	12	2
2	Mandya	5	-	5	-	5	4	-	-	-	-	3	-	5	-	23	4
3	Davangere	-	-	-	-	-	-	5	3	5	-	2	-	5	-	17	3
4	Hassan	5	-	5	-	-	-	-	-	-	-	-	-	5	-	15	-
5	Bellary	5	-	5	-	5	4	-	-	-	-	3	-	5	-	23	4
6	Shivamoga	5	-	-	-	-	-	-	-	-	-	-	-	5	-	10	-
7	Belgaum	5	-	5	-	-	-	5	2	5	-	3	-	5	-	25	2
8	Bidar	5	-	5	-	5	4	5	2	5	-	-	-	5	-	30	6
9	Chitradurga	-	-	5	-	5	4	5	3	5	-	3	-	5	-	28	7
10	Tumkur	5	-	-	-	5	3	-	-	-	-	3	-	5	-	18	3
		35	-	30	-	30	21	20	10	20	-	19	-	50	-	200	31

Note: ** ---Number examined * ---Number positive

Table 86. Prevalence of Theileria parasites in vectors by Geimsa staining method in sheep from unorganised farms

Sl. No.	Organised farms	Tick spp															
		H.bispinosa		*H.intermedia*		*H.kutchensis*		H.*a.anatolicum*		*H.m.isaaci*		*R.sanguineus*		*R.haemaphysaloides*		**Total**	
		**	*	**	*	**	*	**	*	**	*	**	*	**	*	**	*
1	Hessaraghatta Bangalore	-	-	-	-	5	2	-	-	-	-	2	-	5	-	12	2
2	Mandya	5	-	5	-	5	2	-	-	-	-	2	-	5	-	22	2
3	Davangere	-	-	-	-	-	-	5	1	5	-	2	-	5	-	17	1
4	Hassan	5	-	5	-	-	-	-	-	-	-	-	-	5	-	15	-
5	Bellary	5	-	5	-	5	3	-	-	-	-	2	-	5	-	22	3
6	Shivamoga	5	-	-	-	-	-	-	-	-	-	-	-	5	-	10	-
7	Belgaum	5	-	5	-	-		5	1	5	-	3	-	5	-	28	1
8	Bidar	5	-	5	-	5	3	5	1	5	-	-	-	5	-	30	4
9	Chitradurga	5	-	5	-	5	3	5	1	5	-	2	-	5	-	27	4
10	Tumkur	5	-	-	-	5	3	-	-	-	-	2	-	5	-	17	3
		35	2	30	-	30	16	20	4	20	-	15	-	50	-	200	20

Note: ** ---Number examined * ---Number positive

Discussion

V. DISCUSSION

The ectoparasites have a variety of direct and indirect effects on small ruminant production (Wall and Shearer, 2001). Ticks, mites, lice and keds are the most common and important parasites because of their disease transmission, blood feeding habit and skin damage in most of the livestock population. Ectoparasites are considered as annoying pests because of their movement over the skin (Sarkar *et al.*, 2010). In small ruminants, the ectoparasites cause blood loss and heavy infestations may result in severe anaemia. Ectoparasites play an important role as vectors in transmission of various diseases of humans, pets and wild animals. They are able to transmit different pathogens of medical and / or veterinary relevance like viruses, bacteria, protozoa, and helminths (Ahmed *et al.*, 2007). They are the most important vectors of protozoan, bacterial, viral and rickettsial diseases (Radostits *et al.*, 2007).

Ectoparasitic infestation causes decreased production, and lowered reproduction and even mortality in small ruminants. The serious skin defects lead to down grading of quality and rejection of skin (Bayu, 2005; Sertse and Wossene, 2007a; Yebegashet *et al*., 2010). Even the parasitic mites, lice and keds are considered as a potential threat and pose a serious economic problem to the development of sheep production and the tanning industry in the country and need control intervention (Sertse and Wossene, 2007b; Yebegashet *et al*., 2010). As a result, feeding and digestion is hampered that may lead to retarded growth, loss of weight and reduced milk and meat production. The infested animals bite and rub the affected area leading to skin abrasion. Finally, myiasis and other secondary infections may occur which might lead to death of the animals (Soulsby, 1982).

5.1 Prevalence of Ectoparasite infestation in sheep

In the present study, the prevalence of ectoparasitic infestations in sheep in organised farms was found to be 20.7 per cent whereas in unorganised farms it was 43.7 per cent. The findings are in agreement with Yacob *et al.*(2008b), Malugeta *et al*.(2010), Hailu *et al*.(2010) , Shibeshi *et al*.(2013) and Bedada *et al*.(2015).Comparatively the overall prevalence of ectoparasites was high in unorganised farms than organised farms.The higher infestation rates might be attributed to various factors including favourable climatic conditions, malnutrition, especially during long dry season, poor husbandry system, poor awareness of farmers to the effects of ectoparasites and inadequate animal health services in un organised sheep flocks (Mekonnen, Hussein *et al*. 2001; Mekonnen *et al*. 2007) .

The higher prevalence of ectoparasites was in females than males, which is in agreement with Chakrabarti *et al*. (1994), Mohammad Yakhchali and Ali Hosseine (2006), Obi *et al*., (2014), Soundarrajan *et al*., (2014), Meseret *et al*., (2014), This might be due to some hormonal influences. Moreover, physiological factors during pregnancy and lactation could have made the female animals more susceptible to infection.

Overall prevalence of ectoparasitic infestation was higher in hogget's followed by adult animals than younger ones which is in agreement with Yakhchali and Ali Hosseine (2006), Sarkar (2010) and Soundararajan (2014). This could be due to incapability of hoggets, adults and debilitated or emaciated animals to groom and lick themselves and presence of weak defence mechanism. This could also be due to climatic variations and prevailing differences in that geographical area besides less developed immune system or

exhausted immune system. On the contrary, Abdul Manan *et al.* (2007), Obi *et al*. (2014) reported the high intensity of ectoparasites in younger animals due to their reduced immunity and high exposure to ectoparasites with overcrowding, warm and humid environment which aids in spread of ectoparasites.

Prevalence rate based on the farm conditions, in this study indicated that the animals in semi intensive farming system were more prone to ectoparasitic infestation wherein 21.7 per cent prevalence was observed in organised and 49.2 per cent in unorganised farms whereas in intensive management it was found to be 12.7 per cent in organised farm and 13.2 per cent in unorganised farms. These findings are similar to report of Rabbi (2006) where highest ectoparasitic infestation in semi intensive aystem (59.7%) followed by extensive system (33.5%) and intensive (8.27%) was recorded. Even Rony *et al*. (2010) and Sounadarrajan *et al*. (2014) reported high ectoparasitic in semi intensive system. The reason for higher prevalence of ectoparasites in semi intensive sytem might be due to free range movement of animals, unhygienic farm management in pens and shed. The non-descriptive breeds were more prone to ectoparasitic infestation (43.7%) than the descriptive breeds with 20.7 per cent infestation which is in agreement with Meseret *et al*. (2014) and Soundararajan *et al*. (2014). Most of the non-descriptive or local breeds were reared under semi intensive farming which were susceptible to ectoparasitic infestations than the descriptive breeds reared under intensive farming system where they are not exposed to ectoparasitic infestation. The results are in agreement with the findings of Rony *et al.* (2010), Abadi *et al.* (2010), Kabir *et al*. (2011), Meseret *et al*. (2014) who recorded higher ectoparasitic infestation in small ruminants maintained under semi intensive or extensive system.

Seasonal fluctuation of the year had a significant ($P<0.05$) effect on the prevalence of the ectoparasitic infestation, a relatively higher infestation with ectoparasites was observed in rainy season (29%) followed by winter (21%) and summer season with 11.7 per cent in organised farms which is similar to the findings of Yeasmin *et al.*(2014) who reported the intensity of ectoparasitic infestation in sheep with 85 per cent in summer followed by 75 per cent in winter and 55 per cent in rainy season.Whereas in unorganised farms the ectoparasitic infestation was high in rainy season with 61.7 per cent (441/714) followed by summer season with 52.1 per cent (378/725) and winter season 42.4 per cent (294/692). Seasonal prevalence of ectoparasites was reported by different authors *viz.* Mushi *et al*. (1996), Latha *et al.* (2004), Brito *et al*.(2005), Yakhchali and Hosseine (2006) and Sarkar *et al.*(2010) where the prevalence of ectoparasites was higher in rainy season followed by winter or summer season .

Among the ectoparasites, the ticks has the highest intensity followed by lice, mites, fleas and flies. Among all the ectoparasites screened ticks recorded 7.98 per cent in organised farms and 30 per cent in unorganised farms. This was followed by lice with percentage of 5.17 in organised and 9.4 in unorganised farms whereas the intensity of mites was 5.17 and 6.98 per cent in organised and unorganised farms respectively. Low infestation of fleas *i.e.,* 2.42 and 4.45 per cent in organised and unorganised farms was reported. In this study ticks were seen in a high intensity between March and June *i.e.,* in summer. The highest prevalence in summer season may be due to high humidity with an ambient temperature and overcrowding of sheep in farms in close contact increases the probability of contamination. The variation in tick prevalence in different areas can be attributed to a variety of factors like geoclimatic conditions, association and rearing

practices of different species of animals, awareness/ education of the farmers and farm managemental practices (Khan *et al*., 1993). The high prevalence rate during the hot months (May-July) may be attributed to hot and humid season prevalent during these months as tick infestation is influenced by temperature, rainfall and relative humidity (Gosh *et al*., 2007).

Out of 3233 ticks collected from organised and unorganised sheep flocks, 52.27 per cent (1532) belonged to the genus *Haemaphysalis* spp followed by 28.95 per cent (936) *Rhipicephalus* spp, 645 belong to *Hyalomma* spp and only 4 ticks of *Amblyomma* spp. Many research workers from India have reported the prevalence of ticks in small ruminants which they include Hiregoudar (1977a) from Karnataka, Miranpuri and Singh (1978) from Assam, Jagannath *et al.* (1973), Kamble *et al*. (1988) and Kumar *et al*. (2002) from Karnataka and Latha *et al*. (2004) from TamilNadu. In the present study also the ixodid ticks were found to be commonly prevalent in sheep in different parts of Karnataka state.

The higher rate of tick infestation in sheep (80.90%) during this study is in line with the findings of Irshad *et al*. (2010) who reported high tick infestation in sheep (43.37%) from Islamabad, Pakistan whereas in TamilNadu, Vathsala *et al*. (2008) reported high tick infestation in sheep (64.66%).

The higher prevalence of Ixodid ticks viz., *Haemaphysalis* spp. is in accordance with findings of Ramanujachari and Alwar (1954) who has reported the high prevalence of *H. bispinosa* (90%) followed by *Hyalomma aegypticum* in sheep in Madras state. Hiregoudar and Prabhakar (1977) reported the highest prevalence of *H.intermedia* followed by *R.haemaphysaloides, R.sanguineus, Hyalomma marginatum isaaci,*

Hyalomma anatolicum anatolicum and Boophilus microplus in sheep from Karnataka. Jagannath and Lokesh (1988) has recorded highest prevalence of *H. intermedia* (>70%) along with *R. haemaphysaloides* followed by *Hyalomma marginatum isaaci, Hyalomma hussaini* spp of ticks in sheep from different taluks of Kolar districts in Karnataka. This indicated that the prevalence of ticks has not declined over years and require continous efforts to control them.

Latha *et al.* (2004) in Tamilnadu had reported the highest prevalence of *H. bispinosa* throughout the state followed by *R. haemaphysaloides, H.m.isaaci* and *H.a.anatolicum.* Soundarrajan *et al*. (2014) reported that *H. bispinosa* (100%) was most common followed by *H. m. isaaci* (7.29%), *R. haemaphysaloides* (3.13%) and *H.a.anatolicum* (2.08%) in small ruminants in Tamilnadu.

In the study of Irshad *et al*. (2010) reported highest prevalence of *Rhiphicephalus* spp (73.36% and 73.68%) and lower prevalence of *Haemaphysalis* spp (23.36% and 26.31%) in sheep respectively in Islamabad, Pakistan. Nasiri *et al*. (2010) reported the highest prevalence of *Hyalomma marginatum isaaci (*44.67%), *Hyalomma anatolicum anatolicum* (43.17%), whereas *R. sanguineus* (82.35%) and *R. turanicus* were found to be prevalent in Iran (Zakkyeh *et al*., 2012; Razmi and Yaghfoori *et al*., 2013) and *R. turanicus* in Northern Tunisia (Ghirbi *et al.*, 2013), Mustafa *et al.,* (2011) from Sulaimani reported the highest prevalence of *Hyalomma anatolicum anatolicum, Hyalomma marginatum issaci* and *R.sanguineus.*

Zangana *et al*. (2013) reported the highest prevalence of *Hyalomma anatolicum anatolicum* (48.37%) followed by *H.marginatum* (18.01%), *R.sanguineus* (21.09%) and

Haemaphysalis spp (2.5%)in Iraq. Riaz *et al.* (2017) from Pakisthan reported the highest prevalence of *Hyalomma anatolicum anatolicum* (52.2%) and *R.sanguineus* (17.4%).These findings revealed that prevalence of species is based on the different agroclimatic conditions in the respective regions.

In the present study, infestation rate of *Sarcoptes scabiei var ovis* was 7 and 5.17 per cent in unorganised and organised farms. Intensity was more in winter season with lesions observed on head, neck and face. Many authors have reported the prevalence of *Sarcoptes scabei* mites *viz*., Alvi and Khan, 1963; Chineme *et al*., 1979; Chakrabarti *et al*., (1994) and Neog *et al*., (1992) they found that the prevalence of infection was higher in young and female animals, and more common in winter season. Whereas Nadalian *et al.* (1989) reported that the rate of infestation with *Sarcoptes scabiei var ovis* was 13.2 per cent in central parts of Iran. Qudoos *et al. (*1997) reported sarcoptic mite infestation in sheep in Kalat district of Balochistan highland. Overall prevalence of *Sarcoptes scabiei var ovis* was recorded as 21 per cent with severe intensity of lesions observed on ears, face, shoulder, loin, on the back and upper side of the body by Fentahun *et al.* (2012) of Gondar region in Ethopia. Murthy *et al.* (2013) reported clinical cases of sheep infected with Sarcoptic mange in Ramanagara in Karnataka. Seid*et al.* (2016) reported 0.3 per cent of mite infestation in sheep in Eastern Amhara region, Ethiopia. More recently Mamatha *et al*. (2018) reported sarcoptic mange in sheep in Karnataka.

Heavy infestation of lice of 5.17 and 9.4 per cent was observed in organised and un organised farms in this study with *Linognathus stenopsis* followed by *Damalinia ovis* in the present study. Ahmed *et al*. (1977) reported the incidence of heavy infestation of

Linognathus vituli and *Damalinia ovis* in Bannur lambs in Karnataka. Similarly Yakhchali and Hosseine (2006) from Iran, Sertse *et al*. (2007), Yacob *et al.* (2008) from Ethopia, Barmon *et al*. (2010), Zeeyehun and Atomsa *et al.* (2012), Amare *et al*. (2014), Sarkar *et al*. (2010) from Bangladesh, Zangana *et al.* (2013) from Iraq reported high infestation of *Linognathus stenopsis* followed by *Damalinia ovis.*

Heavy flea infestation of *Ctenocephalides orientis* and *Ctenocephalides felis* in adults was reported from organised and un organised farms in this study during post monsoon and in winter season which is in agreement with Muraleedharan and Paramsivaiah (1993) who observed a severe infestation with *Ctenocephalides felis orientis* in a flock of 179 sheep, 52 lambs, 18 goats and 15 kids belonging to the Regional Research Station, Tiptur, Karnataka state and also as per the study of Ashwini *et al*.(2017) the prevalence of *C.orientis* was more in sheep in southern parts of Karnataka state. How ever heavy prevalence of *C.felis* and *C.canis* was reported in sheep by Yakhchali and Hosseine (2006), Yagoob (2011), Sudha Rani (2015) and Obi (2014).

The highest degree of flea infestation was seen in farms practicing an intensive management system. Housing plays an important role in the development of fleas since it enables eggs to develop in litter containing organic matter with many hosts available on emergence. The condition allows manure to accumulate in animal houses results in increased warmth and humidity which favour's the proliferation of fleas (Dipelu and Ayoade, 1982; Obasaju and Otesile, 1980) and the abundance of organic matter provides nutrition and protection for the developing larvae.

The nasal bots were collected from both organised and unorganised farms. Chhabra and Ruprah (1976) reported the incidence of *Oestrus ovis* from a slaughter house study at Hisar, Haryana. Horak and Butt (1977) reported the distribution of *Oestrus ovis* larvae in sheep in South Africa. *Oestrus ovis* in sheep and goats at the Bangalore Animal food Corporation (BAFCO) slaughter house, Bangalore was reported by Jagannath *et al.,* (1989). The incidence of *Oestrus ovis* infestation in sheep and goats at Bikaner, Rajasthan was reported by Pathak (1992). *Oestrus ovis* infestation in Deccani sheep at Udgir in Maharashtra was reported by Narladkar *et al*. (1997).

Pandey and Ouhelli (1984) reported prevalence of *Oestrus ovis* in sheep at Morocco. Yilma and Dorchies (1991) reported of *Oestrus ovis* at southwest France. *Oestrus ovis* infestation in sheep and goats in a sheep farm of Konehally, Karnataka was reported by Muraleedharan *et al*. (1999). Dorchies *et al.* (2000) conducted a slaughter house survey on a monthly basis to determine the prevalence and larval burden of *Oestrus ovis* in sheep and goats in Pezenas, South France. Shahardar *et al.* (2001) reported the recovery of larvae of *Oestrus ovis* from trachea and bronchi of Corriedale sheep in Srinagar for the first time in India.

Alcaide *et al*. (2003) examined 477 adult sheep heads in southwest region of Spain for different larval stages of *Oestrus ovis* in nasal and sinus cavities. Dixit *et al*. (2012) reported the presence of *Oestrus ovis* larvae in a 2 years-old domestic buck exhibiting symptoms of head pressing, scratching of frontal part of head, loss of appetite and sneezing fits. Saleem *et al.* (2017) determined the prevalence of the *Oestrus ovis* in sheep heads of Jammu region.

Based on the agroclimatic zones the ectoparasitic infestation in the present study was more in arid regions with 37.01 and 48.48 per cent in organised and unorganised farms whereas in semi-arid regions the infestation was 16.91 and 41.7 per cent in organised and unorganised farms. The high intensity of ectoparasites in arid regions in this study might be attributed to differences in husbandry practices. Further, the semi-arid zone is located at a higher elevation and has low annual mean temperatures (minimum and maximum) compared to the arid zone. Rehman *et al*. (2017) had also made similar observations.

5.2 *In vitro* Efficacy Studies of acaricides in control of ticks.

Ticks and tick borne diseases are a major constraint in livestock health in many parts of the world including India where ticks are widely prevalent and losses caused by this tick to livestock is a combination of both direct and indirect effects. Direct effects on production include skin damage from tick bites (Biswas 2003), blood loss, toxicity from bites, decreased animal weight gain and milk production, whereas the indirect effects are related to the transmission of tick borne diseases like theileriosis, babesiosis and anaplasmosis.

Acaricides have played a pivotal role in control of ticks but its indiscriminate and incessant use with improper concentrations has probably contributed to the development of resistance to most of the acaricides in several countries (FAO 2004). Large scale resistance to organophosphorous (OP) compounds, synthetic pyrethriods and formamidine has been reported against ticks in cattle however the reports of development of acaricide resistance against ticks in sheep are limited.

5.2.1 *In vitro* Efficacy on larval stages of ticks

5.2.1.1 Larval packet test

Most of research in knowing the efficacy of acaricides in control of ticks is reported against *Boophilus microplus* followed by *Hyalomma anatolicum anatolicum* and a few reports against *Rhipicephalus sanguineus* and *Rhipicephalus haemaphysaloides* in cattle by the larval packet test. Hence as a reference base line, these published reports were considered while interpretation of the results against different species of ticks used in this study against deltamethrin, cypermethrin and amitraz.

In the present study 100 per cent mortality was observed in the larvae of different spp of ticks *i.e., Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* at 0.2 per cent of amitraz, 0.3 per cent of cypermethrin and 0.4 per cent of deltamethrin. The LC_{50} values for the same was recorded, among which amitraz showed the lowest LC_{50} values from 1.61 to 1.90 and had the highest acaricidal effect followed by cypermethrin showing LC_{50} values ranging from 36.7 to 24.1 and deltamethrin with highest values ranging from 62.13 to 40.3 showing lesser acaricidal effect in control of ticks. The resistance factor was obtained by comparing the LC_{50} of field strains with the LC_{50} of reference susceptible strain. Thus RF values obtained from LPT revealed that the ticks were resistant to deltamethrin and cypermethrin whereas found susceptible to amitraz.

The resistance of deltamethrin and cypermethrin against all species of ticks in this study was in agreement with different authors *viz.,* Mavi *et al.* (2007) reported the high

LC_{50} values of cypermethrin as 0.00349 per cent which is almost 4.5 times higher than the reference susceptible strain indicative of development of resistance against *B.microplus.* Barre *et al.* (2008) reported up to 16.59 fold resistance to deltamethrin against *Rhipicephalus microplus* using the modified larval packet tests. Vatsya and Yadav (2011), Sharma *et al*. (2012) reported the development of delatmethrin and cypermethrin resistance against *R.microplus* from the ticks collected from six agro climatic regions of India. Similarly, Shyma *et al*. (2015) detected cypermethrin resistance in ticks from Fatehbad district of Haryana and Jyotimol *et al*. (2014) reported the development of low level deltamethrin resistance in *R.microplus* in Thumburmuzhi and Vithura regions of Kerala. Even Ghosh *et al.* (2014) reported the development of resistance in the tick populations of various places of Bihar against deltamethrin and cypermethrin.

Recently Kumar *et al.* (2017) by LPT reported highest LC_{50} values for both deltamethrin and cypermethrin against *R.microplus* ticks collected from different parts of Andhra Pradesh whereas LPT indicated that the *R. (B.) microplus* ticks collected from different parts of Andhra Pradesh were totally susceptible to amitraz. Similarly Kaur *et al.* (2017) reported the development of resistance to deltamethrin and cypermethrin against *R.microplus* with high LC_{50} values.

Although there are many reports available on acaricidal resistance in one host tick i.e *R.microplus*, few studies had shown the presence of resistance in *Hyalomma anatolicum anatolicum* in India and abroad *viz.*,Sangwan *et al.* (1993), Shyma *et al*. (2012), Singh *et al.* (2013a) and Singh *et al*. (2015).

The efficacy of cypermethirn and delatmethrin against three host tick i.e *H.a.anatolicum* was reported by different authors *viz*., Sangwan *et al. (*1998) in an *in vitro* study reported that among commercially available pesticides *viz*., Oxinotiofos, amitraz, decamethrin, melathion and diazinon against *Hyalomma anatolicum anatolicum*, amitraz was least effective when compared to other acaricides. Bagherwal *et al. (*2005) studied the efficacy of cypermethrin (Cyperol) against different stages of *Hyalomma anatolicum anatolicum*. In the *in vitro* assay it was observed that the concentrations of 92.3, 94.5 and 100 per cent were effective against nymphs and 88.3, 89.5 and 92.6 per cent effective against adults respectively. Shyma *et al.* (2012) reported a low grade resistance (level-I, RF <5) to deltamethrin and cypermethrin by LPT against *Hyalomma anatolicum anatolicum*, where organophosphate compounds are extensively used for agricultural practices allowing increased exposure of the moulting instars of the ticks to these chemicals.

Singh *et al.* (2013a) reported a resistance of level I against cypermethrin whereas, a comparatively higher resistance level (II) was recorded against amitraz by LPT against *H. a. anatolicum* collected from Banaskantha district, Gujarat (India). Singh *et al.* (2013a) reported a resistance factor of 5.18, 4.52 and 8.96 against amitraz, cypermethrin and deltamethrin, respectively against *H. anatolicum.anatolicum* collected from Muktsar, Punjab. Singh *et al.* (2015) reported a resistant level I against cypermethrin with LC_{95} values of 351.84 ppm and with higher resistance level II against amitraz with LC_{95} value of 1,529.39 against *Hyalomma anatolicum anatolicum* in Banaskantha, Gujarat.

Effectiveness of amitraz against all species of ticks in the present study finds its agreemen with the report of Gono *et al*. (2014) who reported that the LPT proved to be a suitable test to evaluate the susceptibility of *R.microplus* field populations to amitraz in Mazowe distric of Zimbabwe. Santos *et al.* (2013) reported among different field isolate of *Rhipicephalus (Boophilus) microplus* only one population was susceptible to amitraz by LPT, presenting a RR of 1.9. Using the same technique, the other populations presented RRs in between 92.9 and 3445.8 and were considered resistant by larval immersion test (LIT) and syringe immersion test (SIT).

A wide variation in the LC_{50} values of the acaricides against reference lines of *R.microplus* and other tick species has been attributed to the use of different reference tick lines and different types of bio assay (AIT or LPT) (FAO,2004). Further the variations in the development of resistance in different tick populations to both synthetic pyrethroids tested might be due to the repeated usage of the same acaricide, improper application strategy of acaricide, in addition to the existing host fauna and local environmental conditions suitable for survivability of ticks. Acaricidal development also varies with strain specific difference and genetic tolerance of ticks.

The LC_{50} values was less when compared to cypermethrin and deltamethrin. The main reason for no development of amitraz resistance might due to the less exposure of the ticks to amitraz comparatively to synthetic pyrethroids *viz.*, cypermethrin and deltamethrin. However, further tests with ticks from other areas of the country need to be carried out in order to gain a clear picture of the susceptibility of the different tick species to amitraz, and to determine the natural variability in the LC50s and LC99s for this test.

5.2.1.2 *In vitro* Efficacy on adult engorged female ticks by AIT-DD

Different assays have been developed by many workers around the world for detection of acaricidal resistance but FAO, 2004 recommended LPT as standard test and AIT for quick results. Both LPT and AIT have been used to detect resistance in *R. (B) microplus* populations in different parts of the world. As per review of literature many research workers carried out AIT with full dose-response data (Sabatini *et al.* 2001; Jonsson *et al.* 2007; Singh *et al*. 2015) in which ticks can be exposed to several doses of acaricides in order to establish the doses which induce 50 per cent or 90 per cent mortality and compare them to a susceptible reference strain to determine the corresponding resistance ratios (RR50 and RR90).

Lovis *et al.* (2013) stated that resistance status of tick populations could be determined by exposing ticks to a unique dose based on the data of a susceptible reference strain and survival to this discriminating dose (DD) is considered as an indicator of resistance (FAO, 2004). Potential DD were calculated as 2× LC99 of the susceptible strains (Jonshon *et al*. 2007). Many of the researchers used technical grade acaricides for conducting bioassay (Sabatini *et al*. 2001; Singh *et al*. 2014;) but there are also references recommending use of commercial acaricides by many workers (Jonson *et al*. 2007; Singh *et al*. 2013a; Pradeep *et al*. 2010; Mathivathani *et al*. 2011). Hence, in the present study AIT-DD bioassay was carried out with commercial acaricides which are commonly used in field conditions in Karnataka.

In the present study all species of ticks were found to be susceptible to amitraz by adult immersion test with discriminating dose (AIT-DD) causing inhibition of egg laying

whereas cypermethrin and deltamethrin treated ticks produced eggs and resistance was observed in *Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* ticks.

These findings are in accordance with Pradeep *et al.* (2010) who conducted adult immersion test with recommended DD as per FAO, (1999) for cypermethrin, deltamethrin and amitraz against *Rhipicephalus sanguineus* using commercial formulations in and around Bangalore region. In this study the authors found 30 per cent resistance to both cypermethrin and deltamethrin whereas amitraz did not indicate any resistance.

Singh *et al*. (2010) carried out AIT with recommended DD as per FAO, 2004 for cypermethrin and deltamethrin using commercial formulations against *R.(B.)microplus* in Ludhiana where they found high frequency of resistance against cypermethrin and deltamethrin *viz*; 93.33 and 96.67 per cent, respectively. Mathivathani *et al*. (2011) also reported 64.72 per cent resistance to deltamethrin in *R. sanguineus* ticks in Chennai by AIT with DD. The development of acaricide resistance by different species of ticks reported by various workers across the globe could be due to number of factors such as frequency of acaricide used, pattern of life cycle of prevalent ticks and obviously the method employed for detection of acaricide resistance.

5.3 *In vitro* Efficacy studies of Entomopathogeneic nematodes in control of ticks

Effective and safer alternative methods to chemical control are the entomopathogenic nematodes (EPNs). Entomopathogenic nematodes have been isolated from many types of natural and managed habitats in a wide variety of soils are being

employed successfully throughout the world to control insect pests (Grewal *et al*., 2001; Dolinski *et al*., 2006).

Entomopathogenic nematodes (EPN) of the genus *Steinerrnema* and *Heterorhabditis* are symbiotically associated with bacteria of the genus *Xenorhabdus* and *Photorhabdus* (Enterobacteriacae), respectively. From the last two decades much attention is being given for use of biopesticides as an alternate approach. Many researchers have suggested that nematodes were effective in controlling ticks. Different species of ixodid tick and argasid species were shown to be susceptible to nematodes, where in adults are apparently being more susceptible (Samish, 2000a).

Most of the studies have been directed towards the control of *R. annulatus* (Samish and Glazer, 1992; Samish and Glazer, 2000a; Samish and Glazer *et al*., 2001) and *R. (B) microplus* (Vasconcelos *et al.,* 2004; Monterio *et al*., 2010a; Carvalho *et al*., 2010; Monteiro *et al.,* 2012).

The present study was conducted to assess the bio efficacy of two EPNs *i.e., Steinernema abbasi* and *Heterorhabditis indica* on engorged female tick of *Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* by bioassay experiment and reproductive biology assay.

5.3.1 Efficacy of *Heterorhabditis indica* against engorged female ticks by bioassay experiment and reproductive bioassay

Entomopathogenic nematodes are used to control different insect pests successfully in various locations around the world (Grewal *et al*., 2001; Dolinski *et al*., 2006), and more

recently research work is being conducted on the use of EPNs to control ticks (Samish *et al.,* 2008). Most of the research studies have been directed toward the control of *R. microplus* (Vasconcelos *et al.,* 2004; Reis-Menine *et al.,* 2008; Molina-Ochoa *et al.,* 2009; Monteiro *et al.,* 2010b; Carvalho *et al*., 2010; Monteiro *et al.,* 2012) and *Rhipicephalus annulatus* (Say, 1921) (Samish and Glazer, 1991; Samish and Glazer, 1992; Samish *et al.,* 2000; Glazer *et al*., 2001; Alekseev *et al.,* 2006, Samish *et al.,* 2008) and also against *R.sanguineus, R.haemaphysaloides* by Hussain *et al*. (2015)

In this study two EPN species i.e *Heterorhabditis indica* and *Steinernema abbasi* were tested against sheep ticks i.e *Haemaphysalis bispinosa, Haemaphysalis intermedia, Haemaphysalis kutchensis, Hyalomma anatolicum anatolicum, Hyalomma marginatum isaaci, Rhipicephalus haemaphysaloides* and *Rhipicephalus sanguineus* by bioassay test. It was found that among two EPNs used, *Heterorhabditis indica* were more effective against all species of ticks than S*teinernema abbasi* suggesting that EPN efficiency is greatly influenced by its dose and is in agreement with other reports (Mauleon *et al*., 1993, Hassanain *et al*., 1997, EL-Sadawy and Habeeb 1998, Hill *et al*.,1998, Samisha *et al.*,2000a, Glazer *et al.,* 2001, Samish *et al*., 2008, Silva *et al*.,2012, Hussain *et al*.,2015)

In the present bio assay study increasing concentration of *H.indica* resulted in inducing 100 per cent mortality within 48 to 72 hrs against all species of ticks *i.e., Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci.* Whereas *Steinernema abbasi* induced 100 per cent mortality against all species of ticks by 120 hrs.

Among the two EPN species tested, *Heterorhabditis indica* was most virulent with minimum values of LC_{50} at 48 hrs of incubation and induced 100% mortality Whereas *S.abbasi* showed higher LC_{50} values at 48 hrs of incubation.Many workers have used these bio assays to test the efficacy of EPN against various insect pests (Ricci *et al*., 1996; Sims *et al*., 1992; Bhatnagar *et al.,* 2004; Grewal *et al*., 2001).

Apart from the bio assay test, reproductive potentiality of EPN against ticks with different parameters *i.e.,* egg mass weight, hatching per cent, egg production index and percentage of control was recorded. In the present study it was observed that the reduction in the number of eggs produced by engorged females at the highest concentrations of *H.indica* at 2000 EPNs/petridish and at 6000 EPNs /petdridish concentration of *S.abbasi* along with reduced hatchability, reduced egg production index and percentage control. Similar findings of reduction in egg mass along with other parameters was also reported against *H. bacteriophora* HP88 (Monteiro *et al.,* 2010a) and *H.indica LPP1* (Silva *et al*., 2012) tested against engorged females of *R. microplus.* Similar results were reported by Freitas-Ribeiro *et al.* (2009) and Monterio *et al. (*2014b) in tests using two strains of *S. carpocapsae* on engorged females of *D. nitens*.

The reduction in viability of eggs produced by partially engorged females was observed because the numbers for the hatching percentage of the treated groups were lower than the controls. Similar results were reported by Monteiro *et al.* (2010a,b) and Silva *et al.* (2012) by using *H. bacteriophora* HP88 and *H. indica* LPP1, respectively, where the hatching percentages of the treated groups were lower than the control.

The findings of the present study i.e 100 per cent mortality, significant reduction in the egg production and no hatchability are in agreement with Silva *et al*., (2012) who reported 99.7 to 100 per cent mortality of *R.microplus* at 1500, 3000 and 6000 EPNs/ petridish by 48hrs with a significant reduction ($p<0.05$) in egg mass weight 0.1 to 2.2 mg and hatching percentage 0 to 5 per cent with *H.indica* . Similarly, Hussain *et al*. (2015) reported efficacy of *Heterorhabditis indica* inducing 100 per cent mortality within 48 -72 hrs at all three concentrations *viz.*,1250, 2500 and 5000 infective juveniles (IJs) / Petri dish. Significant reduction in the egg production i.e. 0.1-14.1mg in *R. haemaphysaloides* at all three concentrations whereas 255.25 mg of eggs were produced by untreated control ticks.

Further, in this study the results showed low values for the EPI of females in treated groups are in agreement with Freitas Ribeerio *et al*.(2009), Silva *et al*.(2012), Monteiro *et al*.(2014).The increase in concentration of the EPNs might be attributed to the increased penetration of infective juveniles into the haemocoel which contributes to the establishment of the infection process which aids EPNs to overcome the host's immune barriers (Dowds and Peters,2002). So there by the increase in concentration of EPNs resulted in decreased values for all parameters evaluated. However further confirmation studies are needed on histology of structures and organs related to oviposition process and evaluation of lipid and protein profile in eggs from treated females.

5.3.2 *In vitro* efficacy of *Steinernema abbasi against* engorged female ticks by bioassay and reproductive biology assay

Most of the research has been done in knowing the efficacy of *Heterorhabditis indica* in control of agricultural pests and few against ticks. Many researchers have been

reported *Heterorhabditis indica* as an virulent species of EPN in control of pests and ticks. There are no reports about the efficacy of *Steinernema abbasi* in the control of ticks. Hence, *S.abbasi* was used in this study to know its efficacy against different tick species i.e *Haemaphysalis bispinosa. Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* in the present bio assay study increasing concentration of *Steinernema abbasi* resulted in inducing 100 per cent mortality in about 72 hrs to 120 hrs against all species of ticks used in this study.

Due to lack of literature on the efficacy of *Steinernema abbasi* against ticks, the findings of this study are compared with that of *S.abbasi i*n control of insects and pests of plants. Pokrel *et al*. (2016) assessed efficacy *Steinernema siamkayai* (CD1) and *S. abbasi* (CS1) against third instar of *Chiloloba acuta*. Mortality of *C. acuta* exposed to series of increase dose of two nematodes strains was analysed at two days intervals upto 14th days after the inoculation by time dose mortality regression. Between these strains, *S.abbasi* was found more effective (LD_{50} 44.9IJs/ml) compared to *S.siamkayai* (LD_{50} 98.1IJ/ml) after 14^{th} days. At initial days both strains had high LD_{50} value and it was gradually decreased with increase time.

Kumari *et al.* (2017) assessed the virulence of five strains of *Steinernema abbasi* and two *Heterorhabditis indica* against *Spodoptera litura* in four doses (5, 10, 20 and 40IJs/insect larvae) under *in vitro* conditions. The significant mortality in larvae started at 5IJs/insect larvae. However, nearly 50 per cent mortality of the larvae was observed at an inoculum level of 10IJs per larva after 24h. Among all the isolates of EPNs, *S. abbasi*

isolate HAR-EPNSa-3 was highly virulent against *S. litura.* In case of *H. indica,* isolate HAR-EPN-Hi-2 was highly virulent and HAR-EPN-Hi-1 recorded the least virulence against *S. litura.* Laboratory studies revealed that *S. abbasi* in combination with *H. indica* had more virulence than *S. abbasi* and *H. indica* when applied alone.

Archana *et al.* (2017) in a study evaluated the survival of EPN species *Steinernema feltiae*, *Heterorhabditis indica, S. carpocapsae, S. glaseri* and *S. abbasi* in poultry manure and also their efficacy against different developmental stages of house fly. It was reported that infective juveniles of *S. feltiae, H. indica,* and *S. carpocapsae* showed higher survivability in poultry manure, whereas *S. glasseri* and *S. abassi* were susceptible to manure. After 24h exposure of poultry manure to EPNs, effectiveness was 100 per cetn but their reproductive capacity decreased presumably due to effect of toxic effect of manure contents on the EPNs. Survivals of all five EPNs were drastically reduced with increase in exposing time.

During the efficacy studies of the EPN in controlling ticks, ticks were kept on a soil surface inoculated with EPNs/petridish.Ticks were killed when placed on the soil surface which inoculated with the nematode species (Mauleon *et al*., 1993, Hassanian *et al*., 1997, EL-Sadawy andHabeeb, 1998). This finding is in agreement with the present study where two nematodes species killed the ticks when placed on the inoculated sandy soil. This wet environment was suitable for both nematodes and ticks. EL-Sadawy and Habeeb, (1998) succeeded in applying EPN in control of engorged females of *H. dromedarii* in sandy soil under laboratory conditions. While feeding on a host, ticks were resistant to nematodes except on moist feeding sites (Samish *et al*., 1999). However, these nematodes were highly

sensitive to ultraviolet light and desiccation and EPN was most efficacious in soil or other protected environments (Kaaya and Gaugler 1993).

5.4 *In vitro* Efficacy of Phytoacaricides in control of ticks.

There are various methods to control ticks, but every method of tick control had certain shortcomings. Chemical control with acaricides was considered as one of the best methods, but it was shown that ticks developed resistance against a range of acaricides (Garf *et al*., 2004) and these acaricides were toxic and costly. Problems of acaricide resistance, chemical residues in food and the environment and the unsuitability of tick resistant cattle for all production systems made the current situation unsatisfactory. The development of new acaricides is a long and expensive process, which reinforces the need for alternative approaches to control tick infestations (Garf *et al*., 2004).

Currently, researchers have therefore, diverted their approach towards the development of environmentally safe, biodegradable and target specific botanical acaricides for combating these ectoparasites. The botanical pesticides are more ecofriendly, biodegradable as compared to synthetic pesticides (Rahuman and Venkatesan 2008).

Amongst the natural products plant extracts and essential oils have been shown to have significant activity against economically important tick species (Borges *et al.*,2003; Pereira and Famdas 2006; Fernandes and Freitas 2007; Kamaraj *et al*., 2010; de Douza Chagas *et al*., 2012; Juliet *et al*., 2012; Sunil *et al*., 2013) including resistant species (Gosh *et al.*, 2014, 2013). Moreover, these botanicals were found to contain a mixture of active substances which can delay or prevent the development of resistance to herbal products (Wang *et al*., 2007). Hence two phytoacaricides i.e *Carica papaya* seeds and *Ricinus*

communis leaves extract were used in the present study to know its efficacy in control of ticks.

Most of the researchers have studied the efficacy of *Carica papaya* seeds and *Ricinus communis* leaf extract against *Boophilus microplus* and this has been used to compare the results against other species of ticks used in this study.

The results of the *in vitro* trials by larval packet test (LPT) on larval stages of different species of ticks *i.e., Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* at different concentrations of *Ricinus communis* (6.25, 12.5, 25, 50 and 100mg/ml) and *Carica papaya* seeds (6.25, 12.5, 25, 50 and 100mg/ml) was recorded .Wherein after 24 hrs of exposure, 100 per cent mortality was recorded at 100 mg/ml concentration of *Carica papaya* and at higher concentration of *Ricinus communis* at 200 mg/ml against all species of ticks in this study.

The per cent adult mortality caused by the methanolic extract of *Carica papaya* and *Ricinus communis* was recorded at concentrations of 6.25 to 100mg/ml against *H.bispinosa, H.intermedia* and *H.kutchensis, Hyalomma* and *Rhipicephalus spp*. Mortality at higher concentration was significantly higher than at lower concentrations. At highest concentration, egg laying was completely blocked and percentage inhibition of fecundity was more than 90 in *Carica papaya* extract where as it was not the same with *Ricinus communis* extract mortality at higher concentration which was not significantly higher than

at lower concentrations. At highest concentration, egg laying was not completely blocked and percentage inhibition of fecundity was less than 90.

Carica papaya seed extract was found to be more virulent against all species of ticks in this study was also reported by Shyma *et al*. (2014) where they reported that the *C.papaya* extracts by larval packet test showed significant mortality ($P<0.001$) at the highest concentration of 82.2 per cent. In the adult immersion test it was found that the highest concentration (100 mg/ml) induced 93.33 per cent adult tick mortality with *C.papaya* extracts. Inhibition of fecundity of treated groups differed significantly from the control and was concentration dependent.

C. papaya is considered as a well-known antiparasitic remedy (Heinrich *et al*. 1992). The matured leaves are widely used to treat splenomegaly and malaria (Adjanohoun *et al.*1996). To expel round worms in humans, seeds of this plants are given with honey to humans in India (Kapoor 1990), and an infusion of the raw seeds is taken for intestinal parasites in Martinique (Longuefosse and Nossin 1996). Even the petroleum ether extract of the seed rind showed antimalarial activity; which is an indication of the presence of highly active compounds in this plant (Bhat and Surolia 2001). Stepek *et al.* (2007) in their study found that the extracts of this plant exhibit a potent anthelmintic effect as cystein proteinases enzymes attack on the protective cuticle of the worm. Further, Calzada *et al.* (2007) reported very good anti-trichomonal activity. Different enzymes present in *C. papaya* extract including proteolytic enzymes as (papain, chymopapain, peptidases A and B, and lysozyme) having anti-inflammatory, anticoagulant, analgesic, bactericidal, anthelmentic, and hemolytic properties were isolated by Pendzhiev (2002). These enzymes

are capable of decomposing the proteins of polypeptides and amino acids and dissolving necrotized cells (while not affecting intact ones).

The leaf extract of *Carica papaya* was found to achieve a 100 per cent mortality rate of *Anopheles* mosquito larvae at the concentration of 0.06 mg/ml. A much higher concentration (0.10 mg/ml) was required to achieve the same mortality rate for Culex larvae but the lethal strength of the solution decreased with time of storage, losing its larvicidal property on the 18th day of storage (Okolie,2006). Rawani *et al*. (2012) reported great anti-mosquito potential of *C. papaya,* including larvicidal, pupicidal, of filaria and malaria, respectively. Phytochemical analysis of methanol extract of *C. papaya* stems showed the presenceof flavonoids, tannins, alkaloids, carbohydrates, andtriterpenes (Khaled Nabih Rashed *et al*. 2013). Meltem *et al.* (2005) also reported high triterpene content in the *C. papaya* extracts, and these compounds being Cycloartane-typetriterpene glycosides isolated from Astragalus oleifolius showed notable growth inhibitory activity against *Leishmania donovani*. It was also found to have adulticidal activity, smoke toxicity, and repellent activities against *Culex quinquefasciatus* and *Anopheles stephensi*, the vectors of filaria and malaria, respectively.

Phytochemical analysis of methanol extract of *C. papaya* stems has shown the presence of flavonoids, tannins, alkaloids, carbohydrates and triterpene (Khaled Nabih Rashed *et al.* 2013). Meltem *et al.* (2005) also reported high triterpene content in the *C. papaya* extracts and these compounds contained Cycloartane-type triterpene glycosides isolated from *Astragalus oleifolius* which showed notable growth inhibitory activity against *Leishmania donovani.*

Plant based acaricides have been found to exhibit adulticidal effect on ticks (Ribeiro *et al*., 2007, 2008; Ravindran *et al.*, 2011; Elango and Rahuman, 2011; Ghosh *et al.*, 2011).Besides ticks, Okonkwo and Okoye (1992) showed the efficacy of *R.communis* leaf powder against *Callosobruchus maculatus*, a stored grain pest while Babu *et al.* (1989) and Salas and Hernandez (1985) reported the efficacy of Ricinus oil against *C.chinensis* and *Acanthoscelides obtectus*, respectively. Under semi-field condition, Tounou *et al.* (2011) reported significant mortality of adults and larvae of *Plutella xylostella* L. (Lepidoptera: Plutellidae) treated with aqueous extract of seed kernel and oil extracts of *R.communis*. Using aqueous extracts from leaves of *R. communis*, Aouinty *et al*.(2006) reported high larvicidal activity against 2nd and 4th instar larvae of four mosquito species, *Culex pipiens* (L.), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen).

The per cent adult mortality caused by the ethanolic extract of *Ricinus communis* varied from 26.67 to 100 mg/ml when tested at concentrations of 0.0 to 70.35 mg/ml against *H.bispinosa , H.intermedia* and *H.kutchensis*, whereas against *Hyalomma* spp the mean adult mortality varied from 0 to 72.45 mg/ml. The mean mortality for *Rhipicephalus* spp varied from 0 to 80 mg/ml. Mortality at higher concentration was not significantly higher than at lower concentrations. At highest concentration, egg laying was not completely blocked and percentage inhibition of fecundity was less than 90.

Not many studies have been reported with regard to *Ricinus communis* leaf extracts in control of ticks except Zahir *et al*. (2009) and Ghosh *et al*. (2013). Zahir *et al.* (2009) reported high acaricidal activity of *R. communis* leaf extracts against the larvae of *R. (B.)*

microplus and calculated LC_{90} as 1829.94 ppm. Zahir *et al.* (2010) reported mortality of *Haemaphysalis bispinosa* in the range of 77.0 ± 2.1–100.0 ± 0.0 per cent when treated with *R. communis* leaf extracts prepared in hexane, chloroform, ethyl acetate, acetone and methanol. Arnosti *et al. (*2011) reported inhibition in the development and maturation of oocytes of dog tick, *R. sanguineus* treated with ricinoleic acid esters in *R. communis* oil.

Ghosh *et al.* (2013) reported that the *Ricinus communis* leaf extract at 6-10 per cent concentration was highly efficacious and caused significant ($p<0.001$) mortality of ticks in the range of 55.0±9.6 – 95:0±5.0. The highest percentage of ticks died when treated at 10 per cent concentration and the survived ticks laid fewer eggs and the RI values (0.171±0.0) was significantly different ($p<0.001$) from the control ticks (0.464±0.01). Although the ticks were treated with 2.5 per cent concentration, the mortality was comparatively low (35-45%), the RI values significantly differed at 0.05-0.001 levels. The extract significantly affects the mortality rate of ticks in dose dependent manner ranging from 35.0 5.0 to 95.0 5.0 per cent with an additional effect on reproductive physiology of ticks by inhibiting 36.4-63.1 per cent of oviposition.

Apart from *Ricinus communis* and *Carica papaya* extracts other herbicides have also been effective in control of ticks and pests. Choudhary *et al.* (2004) conducted *in vitro* study with *Nicotiana tobaccum* against *R. haemaphysaloides*. Further, Vatsya *et al.* (2004) reported *in vitro* tickicidial action of few herbal plants against *Boophilus microplus*. Srivastava *et al.* (2008) studied efficacy of *Azadirachta indica* extracts against *Boophilus microplus.* Magadum *et al.* (2009) studied comparative efficacy of *Annona squamosa* and *Azadirachta indica* extracts against *Boophilus microplus.* Pirali - Kheirabadi *et al*. (2010)

studied *Lavandula angustifolia* essential oil as a novel and promising natural candidate for tick *Rhipicephalus annulatus* control. Kumar *et al*. (2011) have performed *in vivo* and *in vitro* study on acaricidal effect of arand yellow kaner and pudina crude extracts on goat ticks. Pirali - Kheirabadi *et al*. (2010) reported *in vitro* assessment of the acaricidal properties of *Artemisia annua* and *Zataria multiflora* essential oils to control cattle ticks.

The plants for medicinal purposes have been used are important components in traditional Indian therapeutic measures since time immemorial. Natural botanical compounds are residue less, flora and fauna friendly, biodegradable, contain a range of chemically active ingredients which can intervene in all biological processes of the insects, thus interrupting their life cycle and dispersal, and are accepted as an integrated part of ethno-veterinary practices (Habeeb 2010; Zaman *et al.* 2012). As per our knowledge, no reports are available on the effect of selected plants from the Indian subcontinent. The plants materials included in this study were selected on the basis of their reported acaricidal activities, frequency of usage in traditional Indian veterinary medicine, ease of availability, and cost of their application. Among the natural products, plant extracts have shown significant activity against all the stages of *R. (B.) microplus* tick species.

However, methanolic extract of *R.communis* leaf had strong acaricidal activity (90%) against the tick at 200mg/ml concentration. In line with this finding, Kumar *et al.* (2011) and Ghosh *et al*. (2013) have reported that similar methanolic extract of the plant has potent activity on *R. decoloratus* even at lower concentrations. Moreover, previous phytochemical studies reported that the crude methanol extract of *R. communis* leaves was positive for saponins, flavonoids, glycosides, fixed oils and fats, while negative for

alkaloids, carbohydrate, tannins and phenolic compound and proteins and amino acids (Kumar *et al*., 2011).

Reports on *Carica papaya* and *Ricinus communis* extract efficacy against ticks are limited, whereas other phytoacaricides were reported by different authors against different tick species. Khudrathulla *et al*. (1998) reported the herbicidal effect of leguminous plant-*Stylosanthes scabra* in control of larval and nymphal stages of *Boophilus microplus*, *Haemaphysalis intermedia* and *Rhipicephalus sanguineus* ticks.

Muraleedharan *et al*. (2009) reported that the undiluted neem oil provided 100 per cent reduction of ticks *i.e.,* on *Haemaphysalis* and *Hyalomma* spp. on sheep and goats on day 3 to 5 on sheep.Aqueous extract of 25g neem kernel processed in 100ml water reduced tick counts to 84.86 per cent on day 10 in sheep .One per cent aqueous emulsion of Neemrich I 80-EC recorded a reduction of 65.95 per cent of ticks in sheep on day 6.

Santhosh kumar *et al*. (2012) investigated the adulticidal and larvicidal activity of dried leaf hexane, ethyl acetate, acetone, and methanol extracts of *Nelumbo nucifera, Manilkara zapota, Ipomoea staphylina* and *Acalypha indica* against the adults of *Haemaphysalis bispinosa* (Acarina: Ixodidae).The per cent parasitic mortality observed in the crude leaf hexane, ethyl acetate, acetone and methanol extracts of *N. nucifera* and *M. zapotaIpomoea staphylina* and *Acalypha indica* against *H. bispinosa* were 80, 74, 72, and 100.

Marimuthu *et al*. (2013) studied the acaricidal activity of synthesized Titanium oxide nanoparticles using *Calotropis gigantea* against larvae of *Rhiphicephalus microplus*

and adult *Haemaphysalis bispinosa.*The maximum efficacy was observed in the aqueous flower extract of *C.gigantea* and synthesised TiO2 NPs against *Rhipicephalus microplus (*LC_{50} they found that the synthesized Titanium oxide nano particles were highly stable and had significant acaricidal activity against the larvae of *Rhipicephalus microplus* and adult *Haemaphysalis bispinosa.*

5.5 Parasitological method: Microscopic examination

5.5.1 Diagnosis of haemoprotozoan parasites by examination of Geimsa stained blood smear

During this study, in organised farms out of 85 sheep blood samples examined by microscopy, about 14 blood samples (16.4%) were found positive only for *Theileria* by Geimsa stain. Whereas in unorganised farms the prevalence rate of Theileriosis was 64 per cent by giemsa staining method.

Many authors have reported the prevalence of haemoprotozoan parasites by microscopic examination of Geimsa stained blood smears especially theileriosis in India and abroad: Lewis *et al*. (1981) from Britain reported 40 per cent of prevalence; Razmi *et al.* (2003) from Iran reported 43 per cent; Bami *et al*. (2010) from eastern half of Iran reported 22.27 (49.22/220) per cent; Durrani *et al*. (2011) from Lahore reported 22 (44/200) per cent; Prabhakar and Hiregoudar, (1977) from Karnataka reported 56.1 per cent of prevalence. During this study the overall prevalence was found to be higher which is in agreement with Jalali *et al*. (2014) who reported higher prevalence of 69.7 (83/119) per cent in sheep from Southern Iran. Altay *et al*. (2007a) reported 18.29 per cent in sheep from Turkey, Irshad *et al*., (2010) recorded 7.36 per cent in sheep from Islamabad

(Pakistan). Naz *et al.* (2012) reported 13.9 from sheep in Lahore district of Pakistan respectively and Mamatha *et al*. (2017) from Karnataka.

Lower intensity of *B.ovis* was reported by authors *viz*., Ziapour *et al*.(2011) and Fakhar *et al*. (2012) in Iran (16.03%, 15.4%), Rjeibi *et al*. (2016) , Yeruham *et al*.(1992) and Savini *et al*.,(1999) who recorded a lower rate (2.9%, 5.56%, 10.0%) whereas contrary to these findings Razmi *et al*.(2003) recorded a higher rate of *B.ovis* of 23.5% from Iran, Vidya *et al*. (2011) from Bidar reported *B.ovis* in a clinical case from goats, Abdallal *et al.*,(2014) .

Many authors have reported the staining of cattle ticks to know the vector potentiality of ticks in disease transmission by Methyl green pyronin *viz.*,Rejitha *et al.* (2009), Haque *et al.*(2010) and Tiwari *et al*.(2015) whereas geimsa staining was done according to Cowdry and Ham (1930) and Zajac *et al*.(2012) same procedure is followed for staining of sheep ticks in this study.Under unorganised farms the tick tissues were stained with Giemsa (200 ticks) and Methyl green pyronin (200 ticks), in which 20 (1%) of ticks were positive found for theileriosis by Geimsa stain.Whereas 31 (15.5%) ticks out of 200 ticks were found positive for theileriosis by methyl green pyronin stain.None of them were positive for babesiosis by giemsa and methyl green pyronin stain. In organised farms 22 per cent of the ticks were positive for theileriosis by Methyl gree pyronin stain and 5 per cent of the ticks by geimsa stain.

A tick is considered as positive for heamoprotozoan infection if any one of the three tissues of tick i.e salivary gland, mid gut, ovaries revealed positive parasitic stages. In

infescted salivary glands we can observe enlarged acini, hypertrophied with pink acinar cells cytoplasm and, even bluish green mass was also indicative of parasite.

In the mid gut cells the presence of parasitic stages was indicated by hypertrophy of infected epithelial cells and vacuolations in the cell cytoplasm. In ovaries the oocytes will appear as blue coloured spherical masses denoting the development stages of the parasites, but none of the ovaries showed the parasitic stages in this study.

In this study bluish green mass in salivary gland was indicative of presence of haemoprotozoan infection by methyl green pyronin staining method which was in agreement with many authors who reported the prevalence of blood parasites and detection of their vectors using methyl green pyronin and giemsa staining from abroad viz., Walker *et al*. (1979); Irvin *et al.* (1981); Buscher and Tangus (1986); Walker *et al*.(1983) ; Kirvar *et al*.(1998); Watt *et al*.(1997); Kirvar *et al*.(2000) for epidemiological studies.

However, in India only limited work has been carried out in some parts of the country which was in agreement to findings of this study viz., Haryana (Sangwan *et al*.1988), Tripura (Das and Sharma 1991), Uttar Pradesh (Das and Ray 2003) ,Georges *et al.* (2001), Rejitha *et al.* (2009) from Kerala, Rashid*et al.* (2009) and Punjab (Haque *et al.* 2010) in contrary to the findingsTahamtan *et al.*(2013) from Varamin, Iran has reported that none of the stained salivary glands had positive reaction to methyl green pyronin that was positive for presence of *Theileria*, infected by another ticks previously.It seems that the life cycle of Theileria was not completed in the ticks because of less feeding period.

In geimsa staining method, the infected salivary gland will have enlarged or hypertrophied acini with enlarged sporoblast, sometimes with developmental stages of protozoa sporozoites appear in pyriform shape or dot form which can be seen in acini of salivary gland and ovaries, and midgut.In this study only 5 per cent of ticks showed dot form of parasites along with hypertrophied acini indicating positive for haemoprotozoan infection by geimsa staining method which is in agreement with Cowdry and Ham (1930), Martin *et al.*(1964), Purnell and Joyner (1968); Voigt *et al*.,(1995) and Abdigoudarzi (2013) .

5.5.2 Detection of haemoprotozoan parasites in host blood by molecular method in unorganised farms

In organised farms by semi nested and nested PCR about 24.7 per cent of blood samples revealed the presence for *T.luwenshuni* with an amplicon of 388 bp and 4.7 per cent amplified for *T.ovis* at 237 bp whereas in unorganised farms 72 per cent of blood samples were positive for *T.luwenshunii,* 19 per cent positive for *T.ovis*. About 4 per cent were positive for *Babesia ovis* by conventional PCR with a amplification at 549bp which is in agreement with Durrani *et al. (*2011), Iqbal *et al*. (2011), Naz *et a*l. (2012), Atlay *et al*. (2005), Kim *et al*. (2007), Aktas *et al*. (2005) and Razmi *et al*. (2003).

The results of highest prevalence of *T.luwenshuni* is in agreement with the epidemiological studies conducted by Yin *et al*., (2007) who has reported widespread distribution of *T.luwenshuni* in sheep in north western China by PCR. Yin *et al.* (2008) detected and differentiated *T. luwenshuni* and *T. uilenbergi* by using 18S rRNA species specific primers and found that out of 100 samples, 27 and 18 per cent samples were

positive for *T. luwenshuni* and *T. uilenbergi* with an expected product of 389 and 388 bp respectively by PCR in Gansu province of China. Whereas Ge *et al*. (2012) reported the prevalence of *T. luwenshuni* in 26 per cent of sheep by screening 100 blood samples by nested PCR in China.

Similarly, Li *et al.* (2014) reported the highest prevalence of *T. luwenshuni* (96.8%) in small ruminants in China. Zhang *et al.* (2014) detected and differentiated *Theileria* spp. infecting small ruminants in China by multiplex PCR with 10^{-3}per cent sensitivity and 100 per cent specificity by targeting 5.8S rRNA gene for *T. luwenshuni* (303 bp) and *T. ovis* (530 bp), the 18S rRNA gene for *T. uilenbergi* (884bp) and reported the prevalence rate of 67, 24, and zero per cent respectively.

Yang *et al*. (2014) conducted FRET qPCR and sequencing to detect *T. luwenshuni* in sheep and goats in China. Chen *et al*. (2014) reported *T. luwenshuni* in sheep and hedgehogs in Central China and Li *et al*. (2015) reported that *T. luwenshuni* and *Theileria* spp. which were frequently found in Gansu cervids like sika deer, roe deer and red deer and have also concluded that *T. luwenshuni* is a multi-host parasite which can also infect hedgehogs, mongolian gazelle, sheep and goats. *T. lestoquardi* (*T. hirci*) and *T. ovis* have been reported previously in India (Levine, 1973).

In the present study, *Theileria ovis* is reported in a lesser intensity. Theileria infection in small ruminants has been reported due to infection of *Theileria ovis* and *T.lestoquardi* by Heiderpour *et al.* (2009); Heiderpour *et al.*(2010); Yaghfoori *et al*. (2013) from Iran and Durrani *et al*. (2011) from Pakistan. In contrary to the findings Sayin *et al*. (2009) revealed higher *T. ovis* infection 64.2 per cent and 12.4 per cent in sheep and goats

in Turkey. Rjeibi *et al*. (2014) reported higher rate of *T. ovis* in sheep as compared to goats during a study in Africa. The difference of infection rate could be due to difference of climatic conditions and genetic resistance in different breeds against theileriosis.

Altay *et al*. (2012) revealed higher prevalence of *T. ovis* (18.9%) compared to other Theileria species infection in Turkey. Yaghfoori *et al.* (2013) reported higher prevalence of *T. ovis* (43%) than *T. lestoquardi* (3%) in sheep in Iran which is in agreement to present study. Even Heiderpour *et al.* (2009) reported 87.5 per cent *T. lestoquardi* and 12.5 per cent *T. ovis* infection in Iran. 54.8 per cent and 40.2 per cent prevalence of *T. lestoquardi* and *T. ovis* respectively revealed in sheep in Iran by Zaeemi *et al.* (2011). Riaz *et al*. (2017) in Pakistan reported the prevalence of *T.ovis* at 14 per cent and *T.lestoquadri* at 9.5 per cent.

Others authors *viz*., Zaeemi *et al*. (2011) also detected 40.2 per cent of *T.ovis* and 54.8 per cent of *T.lestoquardi* in sheep from western Iran reported high prevalence of *T.ovis* in Turkey by Aktas *et al*., (2005) and in China by Ge *et al.* (2012).

In the present study, PCR has showed higher sensitivity for detection of *Theileria* infection by detecting organisms in samples where no organisms were found by microscopy. Hence, PCR was found to be very sensitive, rapid and reliable method for detection of *Theileria* infection in both carrier animals and in animals with mixed infection (Atlay *et al*., 2005; 2008). In the present study, 18S rRNA gene was targeted, because the previous studies indicated that 18S rRNA gene are more conserved amongst protozoans and hence they act as specific target for the detection of *Theileria* spp (Yin *et al.* 2008).

5.5.3 Detection of haemoprotozoan parasites from vectors by molecular method from organised farms

The salivary gland of tick spp *Haemaphysalis, Rhipicephalus* and *Hyalomma* from organised farms was dissected and the DNA was extracted from it by using Qiagen mini kit and subjected for PCR, in which from organised farms, the ticks *H.kutchensis* (9) were amplified for *Theileria luwenshuni* by nested PCR and *H.a.anatolicum* 4 samples for *T.ovis* whereas other species of ticks did not amplify for none of the haemoprotozoan parasites. The DNA of salivary gland from ticks was extracted from 315 collected ticks in the unorganised farm animals. Among which *H.kutchensis* (34) showed amplification at 388bp positive for *T.luwenshuni* by nested PCR and *H.a.anatolicum* (15) showed amplification at 237 bp for *T.ovis* whereas other species of ticks were found negative for haemoprotozoan parasites.

Some of the authors from India have reported prevalence of *Haemaphysalis* and *Rhipicephalus* ticks in theileria infected sheep flock and are in accordance with findings of Ramanujachari and Alwar (1954) who has reported the high prevalence of *H. bispinosa* (90%) followed by *Hyalomma aegypticum* in sheep infected with *T. hirci* in Madras state. Hiregoudar and Prabhakar (1977a) reported the prevalence of *H. intermedia* followed by *R.haemaphysaloides* in *Theileria* carrier sheep from Karnataka. Jagannath and Lokesh (1988) has recorded prevalence of *H. intermedia* (˃70%) along with *R. haemaphysaloides* on examination of ticks in sheep (1164) and goats (372) from Chintamani, Bangarpet, Sidlaghatta, Gudibanda, Malur and Bhagepalli taluqs of Kolar districts in Karnataka.In a recent study in Karnataka by Mamatha *et al.,* (2017) out of five tick species identified

during their study, *H. kutchensis* was found to be the most predominant tick in sheep (51.44%) followed by *R. haemaphysaloides,* suggested that these ticks may play a important role as a vector in transmission of *T. luwenshuni.* In this study the highest number of *Haemaphysalis kutchensis* ticks salivary gland DNA showed amplification for *T.luwenshuni* and was found to be a potential vector in transmission of *T.luwenshuni* in sheep.

In this study around 15 *Hyalomma anatolicum anatolicum* ticks salivary gland DNA showed amplification for *Theileria ovis.* In this study less prevalence of *T.ovis* was noticed in host blood and in vectors. Even though *Hyalomma anatolicum anatolicum* has been confirmed as vector for transmission of *T.ovis* in this study, large number of *T.ovis* positive cases with high intensity should be considered for futher confirmation of *Hyalomma anatolicum anatolicum* in disease transmission as in many circumstances the severeity of infection might be very less, the life cycle of the theileria might have not completed in collected ticks because of their shortage in feeding period.

Previously the vectors for *T. ovis* has been reported as *H. anatolicum* (Bhattacharyulu *et al.,* 1972); *Rhipicephalus* spp. and *H. anatolicum* in India (Sisodia, 1981); *Rhipicephalus evertsi* in South Africa (Jansen and Neitz, 1956), *R. bursa* in Turkey (Sayin *et al*., 2009), *Rhipicephalus spp.,* in Pakistan (Durrani *et al*., 2011), *R. sanguineus* and *Rhipicephalus turanicus* in Iran (Zakkyeh *et al*., 2012; Razmi and Yaghfoori, 2013).

In this study only larval and nymphal stage of ticks of species *Rhiphicephalus sanguineus, Rhipicephalus haemaphysaloides* and *Haemaphysalis intermedia* were found

on babesia infected sheep where the salivary gland DNA from nymphal stages did not show amplification for babesia parasites.

In contrary to this finding, in India Achar and Sreekantaiah (1934) reported the presence of hard ticks, *Haemaphysalis bispinosa* on sheep positive for *B. motasi*. Jagannath *et al*. (1974) identified the ticks from sheep suffering from *B. motasi* infection were *H. intermedia,* the known vector for this infection. *Babesia* infection was recorded from Sheep Breeding Station, Suthatti (Belgavi dt.) where the *H.intermedia* ticks were found predominant on sheep (Prabhakar, 1976).

In India, vectors for *T. hirci* have been reported as *H. bispinosa*, *Hyalomma aegyptium*, *R. haemaphysaloides* and *H. a. anatolicum* (Ramanujachari and Alwar, 1954; Raghvachari and Reddy, 1959; Sisodia and Goutham, 1983); *R. bursa* in Serbia (Dschunkovsky and Urodschevich, 1924). During this study *T. lestoquardi* (*T. hirci*) which have been reported previously in India (Levine, 1985) were not detected by PCR in host blood. This might be due to non-existence of these species in the study areas. Absence of *T.hirci* in host blood might be the reason we could not confirm the presence of parasite species in the vectors.

5.5.4 Phylogenetic analysis

In the present study, the phylogenetic analysis results showed that *T. luwenshuni* (Karnataka) isolates obtained during this study were genetically similar amongst each other and were in the same clade as *T. luwenshuni* isolates deposited in the GenBank (Accession numbers KU247949, KJ850935, KC769996, KU554730, KU234526). Therefore, the

nucleotide sequence analysis results further confirmed that the isolates of the present study as *T. luwenshuni.*

Whereas the phylogenetic analysis results of *T.ovis* (Karnataka) isolates obtained during this study were genetically similar amongst each other and were in the same clade as *T.ovis* isolates deposited in the GenBank (Accession numbers KT 851435, KT851432, KT851438, KT851430, KT283961, KT14608, FJ603460 and KX671114). Therefore, the nucleotide sequence analysis results further confirmed that the isolates of the present study as *T. ovis.*

Summary

VI. SUMMARY

The present study was conducted to know the prevalence and distribution of arthropods infesting sheep in different farms of Karnataka by considering season, breed, management system, agroclimatic zone and site preference. Different managemental methods of controlling ticks in sheep farms were evaluated including chemical acaricides, phytoacaricides and entomopathogenic nematodes. The vector potentiality of ticks in disease transmission was identified by staining methods and molecular methods.

In the present study, the prevalence of ectoparasite infestations in sheep in organised farms was found to be 20.7 per cent whereas in unorganised farms it was 43.7 per cent. Comparatively the overall prevalence of ectoparasites was high in unorganised farms than organised farms. The prevalence of ectoparasites was higher in females than males. Hogget's followed by adult animals were more infested than young ones. The prevalence of ectoparasites was high in non descriptive breeds maintained in semi intensive system than descriptive breeds and in animals at intensive system. Season wise the prevalence was high in rainy season followed by summer and winter in unorganised farms but in organised farms, prevalence was high in summer season followed by rainy and winter season.

Among the ectoparasites, ticks were more prevalent followed by mites, lice, fleas, nasal bots and flies. The morphological identification of ticks were identified as *Haemaphysalis bispinosa, Haemaphysalis kutchensis*, *Haemaphysalis intermedia*, *Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus*, *Hyalomma anatolicum anatolicum*, *Hyalomma marginatum issaci* and *Amblyomma americanum.* The intensity of *Haemaphysalis spp* was more followed by *Rhipicephalus* and *Hyalomma* spp. Mites were

identified as *Sarcoptes scabie var ovis*, lice as *Damalinia ovis* and *Linognathus stenopsis.* The fleas identified in this study were *Ctenocephalides felis orientis* and *Ctenocephalides felis felis.* Flies were identified as *Haematopota pluvialis, Chrysomya megacephala, Tabanus striatus.* Larvae of *Oestrus ovis* was also reported during this study.

The evaluation of three commonly used acaricides was conducted against the tick species *Haemaphysalis bispinosa, Haemaphysalis kutchensis*, *Haemaphysalis intermedia*, *Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus*, *Hyalomma anatolicum anatolicum* and *Hyalomma marginatum issaci.* The commonly used synthetic pyrethroids, cypermethrin, deltamethrin (0.05%, 0.1%, 0.2%, 0.3% and 0.4%) and amitraz (0.1, 0.2% and 0.3% concentration) by larval packet test (LPT) and adult immersion test with differentiating dose (AIT-DD).

The following results were revealed by LPT, the higher concentration of cypermethrin(0.3%) and deltamethrin (0.4%) resulted in 100 per cent mortality against all tick species whereas the amitraz was able to induce 100 per cent mortality at a lesser concentration of 0.1 per cent against all species of ticks.The LC_{50} values for the same was recorded, among which amitraz showed the lowest LC_{50} values from 1.61 to 1.90 which had a highest acaricidal effect whereas the cypermethrin had a LC_{50} values ranging from 36.7 to 23.31 and deltamethrin with highest values ranging from 62.13 to 40.3 showing lesser acaricidal effect in control of ticks.

In another *in vitro* test i.e adult immersion test with discriminating doses (AIT-DD) which was done to know the status of resistance development against acaricides revealed 50 to 70 per cent resistance to deltamethrin at 0.075 g/ltr and 20- 30 per cent resistanceto cypermethrin at 0.05 g/ltr whereas amitraz was found to be more effective followed by

cypermethrin and then deltamethrin.Amitraz did not show any resistance to all the species of ticks used in the present study.

The efficacy of entomopathogenic nematodes (EPNs) viz., *Heterorhabditis indica, Steinernema abbasi* in biocontrol of ticks was undertaken. EPN efficacy was assessed at a concentration of 500, 1000, 2000,4000, 6000 and 8000 infective juveniles (IJs) / petri dish in triplicates on *Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus haemaphysaloides, Rhipicephalus sanguineus*, *Hyalomma anatolicum anatolicum* and *Hyalomma marginatum isaaci* species.

In the present bio assay study *H.indica* induced 100 per cent mortality against all species of ticks *i.e., Haemaphysalis bispinosa, Haemaphysalis kutchensis, Haemaphysalis intermedia, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides, Hyalomma anatolicum anatolicum* and *Hyalomma marginatum issaci*, at 500IJs concentration / petridish at 72hrs and the increasing concentration (2000IJs) of *H.indica* resulted in inducing 100 per cent mortality within 48hrs and thereby causing a reduction of egg mass weight, hatching per cent, egg production index per cent with significant reduction difference ($P<0.05$) between the treated and control group.

Whereas *Steinernema abbasi* resulted in inducing 100 per cent mortality in about 192 hrs to 216 hrs against all species of ticks thereby causing a reduction of egg mass weight, hatching per cent, egg production index per cent with significant reduction difference ($p<0.05$) between the treated and control group. The LC_{50} and LC_{90} values indicated that *H.indica* was more virulent against all species of engorged ticks when compared to *S.abbasi.*

Evaluation of *In vitro* efficacy of phytoacaricides in control of ticks was done on both larval and adult stages of ticks. The per cent adult mortality caused by the methanolic extract of *Carica papaya* varied from 25.29 to 100mg/ml when tested at concentrations of 6.25 to 100mg/ml against *H.bispinosa, H.intermedia* and *H.kutchensis,* whereas against *Hyalomma* spp the mean adult mortality varied from 20 to 100mg/ml. The mean mortality for *Rhipicephalus* spp varied from 21 to 100mg/ml. Mortality at higher concentration was significantly higher than at lower concentrations. At highest concentration, egg laying was completely blocked and percentage inhibition of fecundity was more than 90.

The per cent adult mortality caused by the ethanolic extract of *Ricinus communis* varied from 26.67 to 100mg/ml when tested at concentrations of 0.0 to 70.35mg/ml against *H.bispinosa, H.intermedia* and *H.kutchensis,* whereas against *Hyalomma* spp the mean adult mortality varied from 0 to 72.45 mg/ml. The mean mortality for *Rhipicephalus* spp varied from 0 to 80 mg/ml. Mortality at higher concentration was not significantly higher than at lower concentrations. At highest concentration, egg laying was not completely blocked and percentage inhibition of fecundity was less than 90.

Prevalence of haemoprotozoan parasites from host blood and vectors were done during this study, in organised farms out of 85 sheep blood samples examined by microscopy, about 14 blood samples (16.4%) were found positive only for Theileria by geimsa stain. Whereas in unorganised farms the prevalence rate of Theileriosis was 64 per cent by giemsa staining method.

The tick tissues *i.e.,* salivary gland, mid gut and ovaries were stained by Geimsa (59) and Methyl green pyronin stain (59) in which only eight was positive for Theileria by Geimsa stain and seven ticks were found positive for theileria by Methyl green pyronin

stain whereas in the unorganised farms 400 tick tissues were stained with Geimsa (200 ticks) and Methyl green pyronin (200 ticks), in which 20 ticks were positive found positive for Theileria by Geimsa stain. Whereas 31 ticks out of 200 ticks were found positive for theileriosis. None of them were positive for *babesiosis* by Geimsa stain and methyl green pyronin.

In organised farms by semi nested and nested PCR about 24.7 per cent of blood samples was amplified for *T.luwenshuni* at 388bp and 4.7% amplified for *T.ovis* at 237 bp whereas in unorganised farms 72 per cent of blood samples were positive for *T.luwenshuni*, 19 per cent positive for *T.ovis*. About 4 per cent was positive for *Babesia ovis* by conventional PCR.

The salivary gland of tick spp *Haemaphysalis, Rhipicephalus* and *Hyalomma* was dissected and the DNA was extracted from it by using Qiagen mini kit and subjected for PCR, in which from organised farms, 13% of *Haemaphysalis* species of ticks *H.kutchensis* (9) amplified for *Theileria luwenshuni* by nested PCR and *H.a.anatolicum* amplified for *T.ovis* whereas other none of the other species of ticks in the study *viz., Haemaphysalis intermedia, Haemaphysalis bispinosa, Rhipicephalus sanguineus, Rhipicephalus haemaphysaloides* and *Hyalomma marginatumm isaaci* spp did not amplify for haemoprotozoan parasites. Whereas in unorganised farm animals 17 per cent of *Haemaphysalis* spp of ticks *viz., H. kutchensis* (34) showed amplification at 388bp for *T. luwenshuni* by nested PCR and *Hyalomma anatolicum anatolicum* (15) showed amplification for *T. ovis* by semi nested PCR. The highest number of *H. kutchensis* ticks showing amplification for *T. luwenshuni* was considered as vector for disease tranasmission of *T. luwenshuni* in sheep in this study.

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Abstract

VIII. ABSTRACT

A systematic study on arthropod infestation in sheep in Karnataka based on season, breed, management system, agroclimatic zone and site preference was undertaken in nine organized and ten unorganised farms. The prevalence of ectoparasite infestations was 20.7% and 43.7% in organised and unorganized farms. Among all the ectoparasites, prevalence of ticks was highest followed by lice, mites, fleas, nasal bots and flies. The ticks were found resistant to deltamethrin, cypermethrin and were susceptible to amitraz using LPT and AIT-DD test. Among the two EPNs assessed, *H.indica* was found to be more virulent than *S.abbasi*. Among the phytoacaricides, *Carica papaya seed* extract was found to be more virulent than *Ricinus communis* leaves against ticks by LPT and AIT. By microscopy the prevalence rate of *Theileria* spp was 16.4% and 64% in organized and unorganized farms respectively. By PCR 24.7 % were positive for *T.luwenshuni* and 4.7% for *T.ovis* in organised farms. Also 72 % were positive for *T.luwenshuni*, 19 % positive for *T.ovis* and 4 % positive for *B.ovis* in unorganised farms. Staining of tick tissues with Geimsa revealed 13.5% and 10% of parasitic infection in organized and unorganized farms respectively. Similarly, MGP stain revealed 11.86% and 15.55% infection in organized and unorganized farms. Using PCR, the prevalence of *T.luwenshuni* in *H.Kutchensis* was 36% and of *T.ovis* in *H.a.anatolicum* was 4% in organised farms. Prevalence of *T.luwenshuni* and *T.ovis* was 77% in *H.kutchensis* and 47 % in *H. a. anatolicum* in unorganised farms. *H.kutchensis* ticks was considered as potential vector in disease transmission of *T.luwenshuni* and *T.ovis* in sheep in Karnataka.

Key words: Arthropods prevalence, sheep farms, acaricides, phytoacaricides, EPNs, vector potentiality.