ANNEXURE -VI

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

CHECKLIST FOR SUBMISSION OF FINAL RESEARCH PROJECT REPORT (RPP-III)

(For Guidelines Refer ANNEXURE – XI (F))

1. Institute Project Code
2. Investigators as approved in RPP-I, If any change attach IRC proceedings:
   Principal Investigator: **Dr. Jayanta Kumar Chamuah, Scientist, Veterinary Parasitology**
   National Research Centre on Mithun (ICAR), Jharnapani, Medziphema, Nagaland-797106
3. Any change in objectives and activities Yes/No
   (If yes, attach IRC proceedings)

4.	Date of Start & Date of Completion (Actual). If any extension granted enclose IRC proceedings			No
5.	Whether all objectives met		Yes	
6.	All activities completed		Yes	
7.	Salient achievements/major recommendations included		Yes	
8.	Annual Progress Reports (RPP-II) submitted	1st Year	Yes	
		2nd Year	Yes	
		3rd Year	NA	
		nth year	NA	
9.	Reprint of each of publication attached		Yes	
10.	Action for further pursuit of obtained results indicated		Yes	
11.	Report presented in Divisional seminar (enclose proceedings & action taken report)		Yes	
12.	Report presented in Institute seminar (enclose proceedings & action taken report)		Yes	
13.	IRC number in which the project was adopted		IRC No:	
14.	Any other Information		NA	

15. Signature:

Project Leader Co-PI I Co-PI Co-PI III Co-PI IV

Director, ICAR-NRC on Mithun

ANNEXURE - VII

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

FINAL RESEARCH PROJECT REPORT (RPP- III)

(For Guidelines Refer ANNEXURE – XI(G))

1. Institute Project Code

2. Project Title: **Studies on Ectoparasites of mithun *(Bos frontalis*)**

Key Words: **Mithun, Epidemiology, Ectoparasites, Efficacy, Drugs**

2. (a) Name of the Lead Institute: **NRC on Mithun (ICAR), Jharnapani, Nagaland-797106.**

(b) Name of Division/ Regional Centre/ Section: **Veterinary Parasitology, Animal Health**

3. (a) Name of the Collaborating Institute(s): NA

(b) Name of Division/ Regional Centre/ Section of Collaborating Institute(s)

4. Project Team(Name(s) and designation of PI, CC-PI and all project Co-PIs, with time spent)

S. No.	Name, designation and institute	Status in the project (PI/CC-PI/ Co-PI)	Time to be spent (%)	Work components assigned to individual scientist
1	Dr. Jayanta Kumar Chamuah	Principal Investigator	80%	Collection of ectoparasites, gross morphological study of ectoparasites, epidemiological study, Therapeutic trials against ectoparasites particularly against Rhipicephalus microplus
2	Dr. P.R Dutta	Co-PI	7. %	Assist in trial of acaricides against ectoparasites
3	Dr. A. Kumar	Co-PI	4%	Assist in collection of samples
4	Dr. K. Khate	Co-PI	4%	Assist in collection of samples
5	Dr. V. Singh	Co-PI	5%	Assist in collection of samples

5. Priority Area : Epidemiology of ectoparasites

6. Project Duration: Date of Start – **February, 2014**

Date of Completion –**January, 2016**

7. a. Objectives

i. Studies on epidemiology of ectoparasites of mithun.

ii. Evaluation of control measure against ectoparasites particularly of ***Rhipicephalus microplus***

8. Practical utility:

i. Immediate Benefits: To know different etiological agent of ectoparasites affecting mithun.

ii. Medium-term Benefits: Prevalence of ectoparasites in mithun and comparative efficacy of different chemical and herbal drugs which are available commercially

iii. Long-term Benefits:

This holistic approach will give scientist to formulate therapeutic and preventive strategy against ectoparasites parasites for **integrated** parasites control in near future.

9. Final Report on the Project (materials and methods used, results and discussion, objective wise achievements and conclusions)

Reports of *Amblyomma testudinarium* in mithuns (*Bos frontalis*):

Physical examination of semi-domesticated, free ranging mithuns (*Bos frontalis*) during the animal health check-up and treatment camp organized at Arunachal Pradesh, Nagaland, Manipur and Mizoram revealed presence of unusually large blood engorged ticks attaching to the dewlap and inner aspects of thighs as well as body surface of the mithun. On the basis of morphological study, the ticks were found indistinguishable from female *Amblyomma* species of ticks. The total tick was collected from seven animals including from Manipur (1), Nagaland (3), and Arunachal Pradesh (2) and Mizoram (1).

Materials and Methods

The present investigation formed a part of the animal health check-up and treatment at a camp organized in different parts of north eastern region. During the health check-up, three animals were found to have infestation with unusually large ticks on the skin of the dewlap and underside of the thigh. These ticks, three in numbers were carefully collected in specimen vial and brought alive to the laboratory. Two specimens engorged with blood were first examined under stereoscopic microscope and thereafter processed in 10% potassium hydroxide solution to study the morphological features for taxonomic identification with the help of available keys (Robinson, 1926; Sen and Fletcher, 1962; Yamaguti et al., 1971; Soulsby, 1982). The third fully engorged live tick was put in a tube and kept in a dark place at room temperature for egg laying.

Results

Grossly, the live engorged ornate ticks were large, broad and prominent projecting mouth parts (Figure 1) and ticks were subsequently turned to earthy brown in old and preserved specimen (Figure 2). The ticks with long mouthparts measured 20 to 25 mm in length and 18 to 22 mm in width and weighed between 2.5 and 3.0 g. Body contour was broadly oval, narrower in front and widest in the region of spiracle. Posterior border of the engorged ticks was ridged indicating presence of festoons (Figure 2). Microscopic findings were: long mouth parts with palpal article 2 being longest and more than twice the length of article 3 (Figure 3); hypostomal dentition 4/4 with dental articles of the inner two files smaller than that of the outer files (Figure 4); basis capitulum rectangular dorsally with presence of two oval shaped large porose areas (Figure 5); scutum triangular with cone shaped narrow posterior angle, presence of diffuse golden brown ornamentation in the form of stripes on a pale ground coloration (Figure 6) and dark brown numerous punctuations of variable size; eyes distinct, large, pale and flat and slightly bulging beyond the contour of the scutum (Figure 7); 4 pairs of legs, tarsi abruptly attenuated, coxa I with two subequal spurs (Figure 8), the external being larger and coxa-IV with single broad rounded spur; spiracle plate triangular (Figure 9) and the anal groove posterior to the anus (Figure 10). Based upon these morphological findings the specimens were found indistinguishable from female *Amblyomma testudinarium*. The female tick which was kept in a tube died after 25 days of observation without laying eggs. Ecological and climatological factors

evidenced by the dense forested hilly environment with average temperature ranging from 10°-20°C in winter to 15°-30°C during summer months and abundant rainfall might be the contributing factors for the prevalence of this tick species in this region.

Ambylomma photographic plate

Figure 1

Figure 2

Figure 3

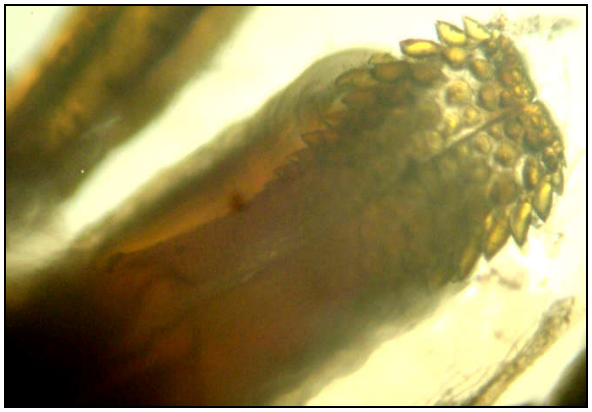


Figure 4

Figure 5

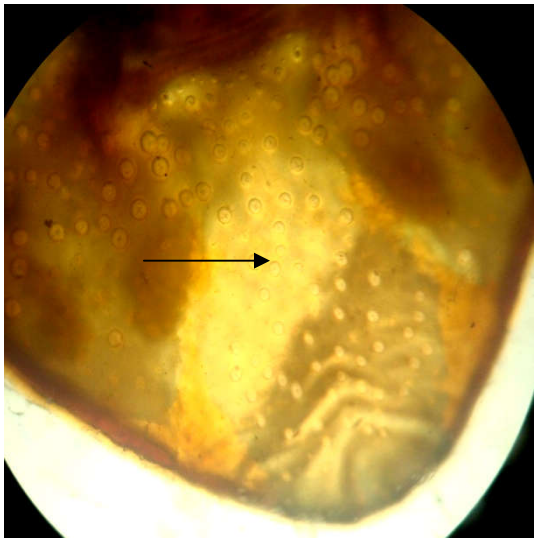


Figure 6

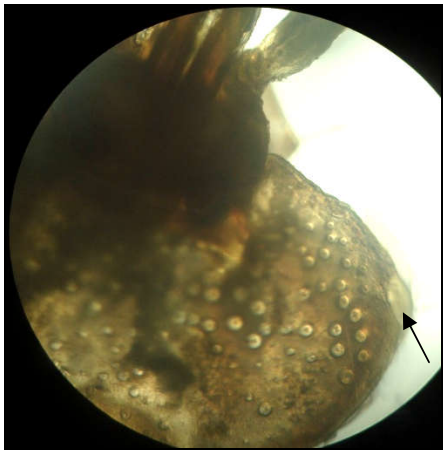


Figure 7

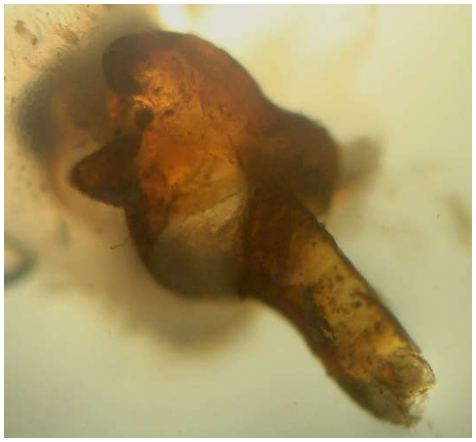


Figure 8

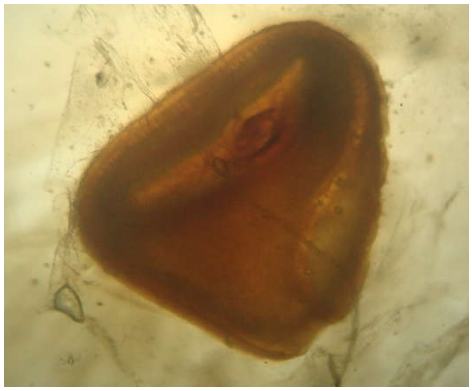


Figure 9

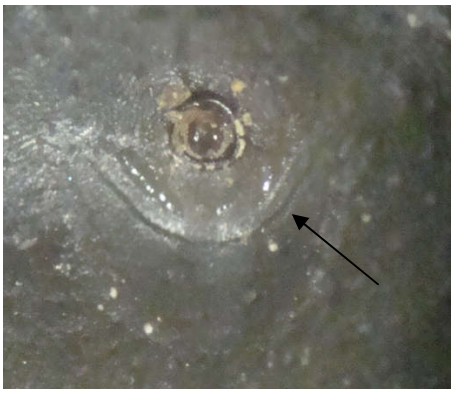


Figure 10

Figure legend

Figure-1: Dorsal and ventral view of live tick.
Figure-2: Posterior border showing festoons and colour change with development of dark markings in preserved tick.
Figure-3: Longirostrate with long palpal article 2.
Figure-4: Hypostomal dentition 4/4.
Figure-5: Rectangular basis capitulum and porose areas.
Figure-6: Triangular scutum showing ornamentation and punctuations.
Figure-7: Right eye
Figure-8: Coxa-I showing subequal spurs.
Figure-9: Triangular spiracle.
Figure-10: Anal groove posterior to the anus.

Lice infestation in Mithun (*Bos frontalis*) of Nagaland:

The present investigation conducted to ascertain lice infestation in mithun revealed identification of *Linognathus vituli*, a sucking louse of cattle. One male mithun calf found to be positive out of 53 animals examined during the investigation.

Materials and Methods

The study was carried out in the hilly Porba village under Phek district of Nagaland, India. During the month of April, 2014 a total of 53 free ranging mithuns were examined for lice infestation. Entire body surface of the animals irrespective of age and sex was searched for the ectoparasites. Live specimens were collected in glass tubes containing 70% alcohol and thereafter processed in the laboratory by standard procedure using 10% potassium hydroxide solution. Morphological identification of the specimens was done with the help of available keys (Sen and Fletcher, 1962; HMSO, 1979).

Results

During the investigation one male calf was found to have infestation with lice on the ventral surface of the abdomen and inner aspect of thigh. All the collected specimens grossly looked similar in appearance. Morphological features of the specimens observed under microscope were elongated body with eyeless pointed head, 5-segmented antennae, membranous hollow and hairy

abdomen, 1 pair of spiracle dorsally on the mesothoracic region of fused thorax, first pair of legs smallest, tarsi with single claw, presence of thumb-like tibial pad and 6 pairs of abdominal spiracles without any paratergal plate.

Morphological findings agreed to the description of sucking louse, *Linognathus* (Sen and Fletcher, 1962; HMSO, 1979; Soulsby, 1982) and at species level it was presumptively identified as *Linognathus vituli* (Fig.1& 2). *L.vituli* is a sucking louse of bovines and it appears to be of common occurrence in India. Heavy infestation of young animals results irritation, annoyance, anaemia, loss of condition, emaciation and death (Sen and Fletcher, 1962). Present record of infestation in a calf also agrees to previous opinion of common occurrence of this louse species in young bovines. However, least number of animal infestation (1/53 = 1.89%) observed in the present study conducted during April month might be due to seasonality of lice which are common in winter.

Conclusion

Identification of *L.vituli* in the present study forms a baseline information on lice infestation in mithun. Further studies would be required to ascertain possible occurrence of *D.bovis* and *H.eurysternus*, the other bovine species of lice found in India.

Morphological study of *Linognathus vituli*

Fig 1: Anterior portion of *Linognathus vituli*

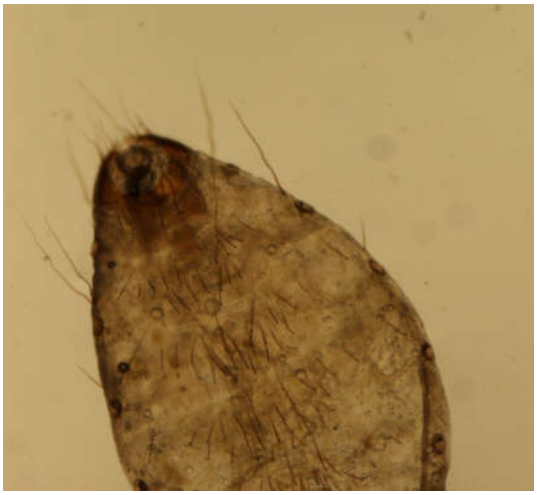


Fig 2: Posterior portion of *L. vituli*

Molecular identification of Ixodid ticks of mithun (*Bos frontalis*) from Nagaland

An animal health check-up and treatment camp during the year 2015 for the free ranging mithun (*Bos frontalis*) organized at Khonoma and Jotsoma villages of Kohima district, Nagaland showed Ixodid tick infestation on these animals, with the ticks attached to the dewlap and inner aspects of the thighs. Based on their morphological features the ticks were identified to belong to the genus *Ixodes*. However, identification of these ticks to *Ixodes ovatus* and *I. acutitarsus* was made by the sequence analysis of the internal transcribed spacer-2 region of these ticks. The present study showed both *I. ovatus* and *I. acutitatarsus* are prevalent on the mithun in this area and is the first report on the occurrence of *I. ovatus* on the mithun.

Materials and Methods

Live specimens of gross ticks were collected from the dewlap and inner aspects of thighs of the mithun from Jotsoma (n=1 animal) and Khonoma (n=1 animal) villages with the help of thumb forceps in plastic vials containing 70% alcohol and thereafter processed in the laboratory by standard procedures using 10% potassium hydroxide solution (HMSO, 1979; Soulsby, 1986). Based on the available morphological keys on the surrounding anal grooves anteriorly, long palpi and absence of festoons these were identified as *Ixodes* ticks. For the accurate identification of the ticks to the species level, internal transcribed spacer-2 region of some of the tick specimens was PCR amplified using the following primers 5'-CTGCGAGACTTGGTGTGAAT-3' and 5'-TATGCTTAAGTTCAGCGGGT-3' flanking 5.8S and 28S rRNA genes (Poucher *et al*. 1999). Genomic DNA of the six tick specimens was isolated with a commercial genomic DNA isolation kit (Qiagen, Germany) and was used as a template for PCR amplification of the ITS-2 region of these ticks. The PCR conditions followed for the amplification of the ITS-2 were initial denaturation at 95°C for 5 min ; 35 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 2 min and one cycle at 72°C for 5 min. The PCR products were purified using gel extraction kit (QIAquick Gel Extraction Kit, Qiagen, Germany) and custom sequenced at the University of Delhi. The sequence analysis and similarity searches were performed with the basic local alignment search tool (BLAST), NCBI. Specific identification of the ticks was made by sequence comparison with available sequences of the corresponding species in the GenBank database.

Results

ITS-2 region of the ticks collected from Khonoma village on PCR amplification resulted in a PCR product of 900 bp whereas *Ixodes* ticks collected from Jotsoma village amplified a product of 1000 bp. Sequence analysis of the ITS-2 of the *Ixodes* tick from Khonoma region showed 100% similarity with *Ixodes ovatus* (acession no. AB280550) whereas ticks from Jotsoma region showed 95% similarity with *I. acutitarsus* (accession no. AB105168). Therefore, ticks collected from Khonoma village were assigned to *I. ovatus* and those from the Jotsoma village to *I. acutitarsus*. Based on the morphological features of each tick specimen and subsequent sequence analysis it was thus confirmed that both *I. ovatus* and *I. acutitarsus* are infesting the mithun in these areas.

The present finding is the first report on *I. ovatus* infestation on mithun, along with the infestation of *I. acuitarsus* being recorded by ITS-2 sequencing.

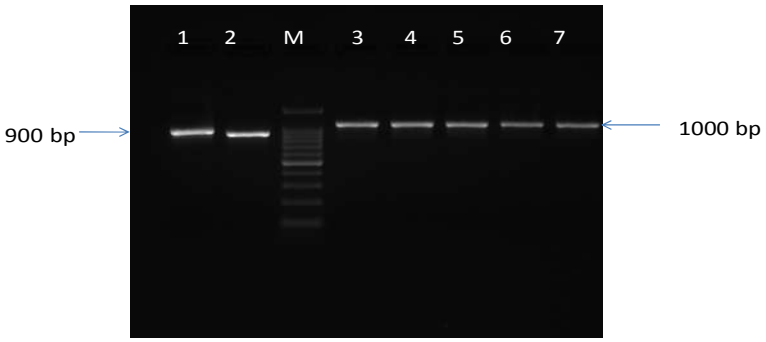


Fig. 1 PCR amplification of ITS-2 region of *Ixodes ovatus* (lanes 1,2) and *I. acutitarsus* (lanes 3-7). Lane M 100 bp DNA ladder marker

Fig: Gross view of *Ixodes ovatus* and microscopic view of *I. acutitarsus*

Molecular identification of Leech:

The unidentified leech *of* mithun (*Bos frontalis*), were recovered from nasal cavity and thoroughly washed in order to remove dirt and debris and inject 70% alcohol for gross morphological observation. For genomic study, after washing the gross leech with normal saline, they were preserved in 70% ethanol and stored at -20°C until used for extraction of DNA. Genomic DNA was extracted from individual leech particularly from the portion of sucker in order to avoid host blood using a commercial genomic DNA extraction kit (Qiagen blood and tissue kit, Germany) according to the manufacturer's instructions. The DNA was measured in Nanodrop Spectrophotometer at the absorbance of 260/280. Genetic marker used in this study is Cytochrome I oxidase (CO1) as a molecular probe.

For the amplification of **700** bp fragment of Cytochrome C oxidase (CO1) using primers LCO1490(F):5'GGTCAACAAATCATAAAGATATTGG3'andHCO2198(R):5' TAAACTTCAGGGTGACCAAAAAATCA3'.Each 25 µl reaction consisted of 16.37 µl H2O, 2.5 µl 10 mM MgCl2, 0.5 µl dNTP, 0.13 µl DreamTaq DNA Polymerase (5 u/µl) (Fermentas, Thermo Scientific Inc.) and 1.25 µl of each of the two primers. An initial denaturation at 94°C for 3 min was followed by 35 cycles (denaturation at 95°C for 1 min, annealing at 49°C for 1 min and extended at 72°C for 1 min) and final extension at 72°C for 10 min. The PCR products were checked for size on 1% agarose gel and visualized by ethidium bromide staining. The PCR

products were purified using gel extraction kit following the manufacturer's protocol and sent for custom sequencing.

Fig: Leech recovered from nasal cavity of mithun

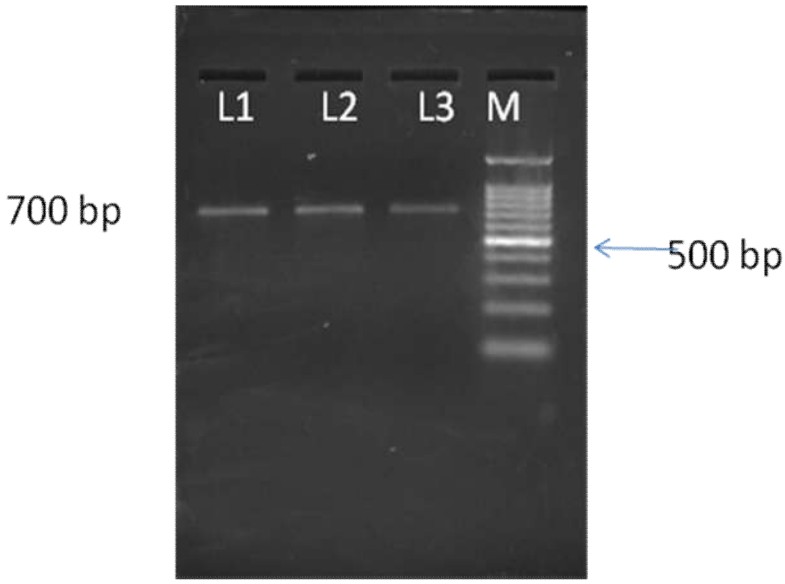


Fig: PCR amplification of Cytochrome Oxidase 1 of Leech

Incidence of *Rhipicephalus microplus* infestation in mithun (*Bos frontalis*) and evaluation of therapeutic efficacy of some herbal acaricides (on 7 days interval):

Present study on the tick infestation on mithun was undertaken at Jharnapani and Porba mithun farms of the National Research Centre on Mithun (semi-intensive) and the results are

compared with free ranging condition of North Eastern Region. The ixodid tick of the genus *Rhipicephalus microplus* was found to be the most prominent tick infesting mithun in this region. A pilot study was undertaken to evaluate the efficacy of some herbal acaricides and compare these results with two most commonly used synthetic acaricides.

Materials and Methods

External body parts of 96 and 29 mithun, irrespective of the age and sex of the animal, reared at Jharnapani and Porba mithun farms, respectively were examined for occurrence of ticks in the pre-monsoon, monsoon and post-monsoon seasons. For identification purpose, the ticks were handpicked and collected in a sterilized plastic vial, labeled and brought to the laboratory. Ticks were fixed in hot water for a few minutes followed by dipping in 10% formalin containing 1-2 drops of glycerin and kept for 24-48 hrs. They were washed thoroughly in running tap water for 6 hrs and processed as per the method of HMSO (1979). Processed ticks were then mounted in DPX and dried in a BOD incubator at 38^{o}C. Thereafter, the ticks were examined under a microscope and their taxonomy was studied using the key of HMSO (1979).

To find out the efficacy of some drugs including herbal ones, a total of 24 animals were divided into six groups with four animals in each group in a completely randomized design. Each group was treated separately with crude neem (*Azadirachta indica*) (500 g / litre and 1 kg / litre), crude tobacco (*Nicotina tobacum*) (100 g / litre) and cypermethrin (1ml / litre) as body spray, whereas ivermectin (1ml / 50 kg body weight) was used subcutaneously. The four tick infested animals were maintained without treatment as a control group. The plant leaves which were used as crude herbal extracts were initially collected as fresh leaves, sun dried and homogenized to powder by grinding. The powder was solubilized in distilled water as per the mentioned dose for 72 hrs. Thereafter, the extract was strained and used as a spray on the tick infested animals. Efficacy of various treatments was analyzed at 0, 7, 14 and 21st day post-treatment. The percentage efficacy of the drug was expressed by percentage of reduction of nymphal stages of ticks per square inch of affected animals. The data were analysed as per the procedure of Snedechor and Cochran (1989).

Results

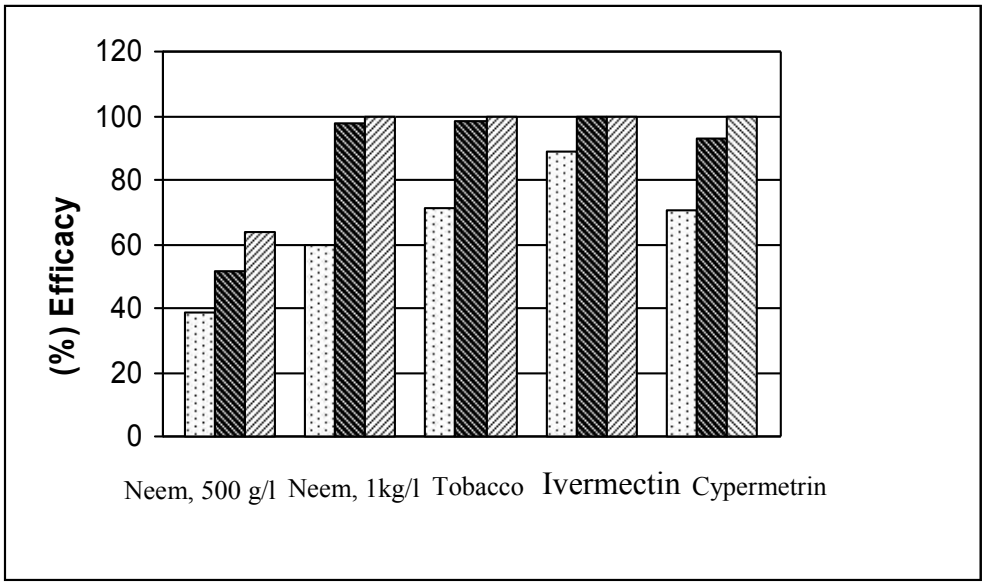
In the present investigation, Jharnapani farm had 20.47% and Porba farm 36.04% tick infestation with *R. microplus*, where animals were extensively reared in semi-intensive conditions. In free range conditions, data were collected from different places of Nagaland including Khomi, Mussolomi and Thetsumi villages of Phek district and also from Khuwangleng village of Champhai district of Mizoram. In the free range condition, total percentage of infestation with *R. microplus* was found to be 54.9%, out of 71 animals examined. Based on these results, it was observed that free range condition had higher percentage of tick infestion than in a semi-intensive condition that may be attributed to the practice of frequent medication done to the farm animals in semi-intensive condition. In the present investigation, *R. microplus* was prevalent throughout the year but with highest incidence in the monsoon season.

In semi-intensive condition at Jharnapani farm, the incidence of *R. microplus* was found to be highest in monsoon (28.42%), followed by pre-monsoon (13.72%) and lowest in post-monsoon season (8%), respectively. Similarly, in Porba farm also, its incidence was found to be highest in monsoon (45.6%), followed by post-monsoon (32%) and pre-monsoon (31.8%), respectively. The incidence of *R. microplus* was found to be lowest in Jharnapani compared to Porba farm. As a whole, lowest prevalence of ticks might be due to periodic acaricide treatment and better system of management.

The percentage efficacy of the drug was expressed by percentage of reduction of nymphal stages of ticks per square inch of affected animals. In the treatment group, all the drugs showed high efficacy except herbal extract of neem @ 500g / litre on 21st day of treatment. On 7th day of treatment, ivermectin showed highest efficacy (88.70%), followed by tobacco (71.28%) and of cypermethrin (70.22%), respectively. However, neem showed 38.93% and 59.55% at the dose rate of 500g / litre and 1kg / litre, respectively. As against the effect on 14th day post-treatment, ivermectin showed cent percent efficacy followed by tobacco (98.01%), neem @1kg /litre of 97.79% and cypermethrin (93.12%), respectively. However, neem @ 500g / litre showed only 51.32% efficacy on 14th day of treatment. On 21st day of treatment, all the compounds used against the tick showed cent percent efficacy, except neem @ 500g / lit showing 63.71 % efficacy (Fig.1).The control group did not show any variation in terms of reduction of ticks during the course of study.

Among various treatment groups, neem at lower concentration was found to be least effective even after 21 days, whereas at higher concentration it gave the results comparable with other compounds. Ivermectin was found to be equally effective on 7th and 14th day of treatment Cypermethrin and ivermectin had a significantly higher efficacy on nymph reduction ($p<0.05$) than neem at its lower concentration. However, tobacco was found to be significantly effective on 14th and 21^{ist} day of treatment. Furthermore, among the herbal drugs tobacco (100 g / litre) was found to have significantly higher efficacy ($p<0.05$) than neem in nymph reduction on 14th and 21^{ist} day. From this study, it may be concluded that tobacco in the herbal category and ivermectin as a synthetic acaricide are the most effective acaricides against *R. microplus* infestation in mithun.

The study on other herbal acaricides and chemical is on progress.



*Bars I, II and III represent 7th, 14th and 21^{ist} day of observation in each treatment group exposed to different acaricides.

Fig. 1. Efficacy of different compounds on *R. microphilus* infeststion in mithun.

Acaricidal efficacy of ectoparasiticides against tick in mithun (*Bos frontalis*) (on three interval)

A pilot study was undertaken with herbal extracts of Lemon grass oil and Leachia oil to study the effect of these plant extracts on the survival of the Rhipicephalus microplus on mithun. Two synthetic acaricides viz. Nayflee and Extick were also used to compare the efficacy of these acaricides against *R. microplus*. Among herbal drugs, lemon grass oil showed moderate efficacy where as Nayflee showed cent percent efficacy against nymphal stages of tick.

Materials and methods:

To find out the efficacy of some drugs including herbal ones, a total of 24 animals were divided into six groups with four animals in each group in a completely randomized design. Each group was treated separately with Deltrin, Nayflee, Extick, Lemon grass oil, and Leachia oil as body spray. The four tick infested animals were maintained without treatment as a control group. The oils were prepared as per conventional procedure. Efficacy of various treatments was analyzed at 0, 3, 6, 9 and 12^{th} day post-treatment. The percentage efficacy of the drug was expressed by percentage of reduction of nymphal stages of ticks per square inch of affected animals. The data were analysed as per the procedure of Snedechor and Cochran (1989).

Results:

The percentage efficacy of the drug was expressed by percentage of reduction of nymphal stages of ticks per square inch of affected animals. Out of all drugs experimented, only Nayflee showed cent percent efficacy on the third day of treatment. In the treatment trials, percentage of efficacy was noticed highest in Nayflee followed by Extick, Lemon grass, Deltrin and leachia oils. As a whole, Leachia showed no significant efficacy in terms of reduction of nymphal stages of ticks. However, lemon grass oil showed moderate efficacy against tick and 98% efficacy was noticed in 12 days of treatment. Among all drugs, significant efficacy was recorded than that of other drugs. The control group did not show any variation in terms of reduction of percentage of nymphal stages of tick in the course of study.

Sl. No	Group	Treated with	No. of nymph/tick present on day of treatment				
			0 day	3rd day	6th day	9th day	12th day
1	A	Deltrin	20.25^{ab}±1.45	11.5^{c}±1.50 (43.20%)	3.75^{ab}±0.75 (81.48%)	1.75^{a}±0.48 (91.35%)	1.25^{a}±0.48 (93.82%)
2	B	Nayflee	16.5^{a}±1.56	0.00^{a}±0.00	0.0^{a}±0.00	0.0^{a}±0.0	0.0^{a}±0.0
3	C	Extick	15.5^{a}±3.22	5.50^{b} ± 1.56 (64.51%)	1.0^{a}±0.41 (93.54%)	0.25^{a}±0.25 (98.38%)	0.25^{a}±0.29 (98.38%)
4	D	Lemon grass oil	25.5^{b}±2.32	13.5^{cd}±1.71 (47.05%)	6.5^{b}±0.87 (74.50%)	1.75^{a}±1.18 (93.13%)	0.5^{a}±0.50 (98%)
5	E	Pipper extract	20.25^{ab}±2.56	16.75^{de}±2.75 (17.25%)	13.50^{c}±2.53 (33.33%)	13.25^{b}±2.66 (34.56%)	13.0^{b}±1.78 (35.80%)
6	F	Control group	21.75^{ab}±0.75	21.5^{e}±1.32	20.75^{d}±0.1.25	21.0^{c}±0.91	21.25^{c}±0.63
P value			0.045	<0.001	<0.001	<0.001	<0.001

Table: Treatment trial in mithun with different ectoparasiticides against tick infestation

Photograph gallery of ticks and their treatment

Fig: Adult mithun infested with *Rhipicephalus microplus*

Fig: Cultured *R. microplus*

Fig: Seed tick of *R. microplus*

Fig : Tick collection from mithun heifer s/c

Fig: Treatment through spray against tick

Fig: Treatment with Ivemectin @0.200mg/kg bw

Fig: Photograph of animal after treatment

Fig: Photograph of animal after treatment

Fig: Photograph of animal after treatment

Activities at Field level

Fig: Treatment at Khonoma village

Fig: Examination at Topopfisu village

Fig: Examination of animals at Porba village

Fig: Health check up camp at Lossami village

Fig: Treatment at Mani village, Arunachal Pradesh Fig: Treatment at Abango village, Roing, AP

Conclusion

1. Reports of *Ambylomma testudinarium* from different parts of north eastern region.
2. Molecular identification of Ixodid ticks of mithun using internal transcribed spacer-2 as a molecular marker.
3. First time reports of *I. ovatus* in mithun from Jotsoma area of Kohima district of Nagaland.
4. Reports of *I. acutitarsus* in mithun from Khonoma area of Kohima district of Nagaland.
5. Acaricidal evaluation of efficacy of chemical and herbal drugs against *R. microplus.*
6. The chemical drug was found to be higher efficacy than that of herbal drugs.

10. Financial Implications (` in Lakhs)

11.1 Expenditure on

(a) Manpower:

(b) Research/Recurring Contingencies

(c) Non-Recurring Cost (Including cost of equipment): NA

(d) Any Other Expenditure Incurred: NA

11.2 Total Expenditure:

11. Cumulative Output

a. Special attainments/innovations: Survey and prevalence of ectoparasites in mithun.
b. List of Publications (one copy each to be submitted if not already submitted)

i. Research papers:

1. J.K. Chamuah, P.R. Dutta, Ved Prakash, O.K. Raina, A. Sakhrie, D. Borkotoky, P. Perumal, R. Neog and C. Rajkhowa (2014). Comparative efficacy of some plant extracts

on *Rhipicephalus* (*Boophilus*) *microplus* infestation on mithun (*Bos frontalis*) in the north east, *Journal of Veterinary Parasitology,* 27(1):5-7.

2. J.K. Chamuah, D. Borkotoky, P.C. Sarmah, S. Lama and P.R.Dutta (2014) .Lice infestation in Mithun (*Bos frontalis*) of Nagaland. Veterinary Research International 2(4): 103-104

3. J. K. Chamuah, K. Bhattacharjee, P. C. Sarmah, O. K. Raina, S. Mukherjee & C. Rajkhowa (2015). Report of *Amblyomma testudinarium* in mithuns (*Bos frontalis*) from eastern Mizoram (India) J Parasit Dis. DOI 10.1007/s12639-015-0654-4

ii. Reports/Manuals : **(As a contributor)**

1. Hands-On Training on Augmentation of Health and Production Through Biotechnological and Bioinformatics Tools

2. **Training manual** on "Scientific Mithun Husbandry for Improving Livelihood" 2016. *Edited by:* M. H. Khan, Perumal P, N. Haque, Saroj Toppo, S. Mukherjee, Naresh Prasad, Vidya Singh, Jayanta Chamuah, Akhilesh Kumar, S. B. Hazarika and Papiya Sinha.page 120.

iii. Working and Concept Papers: Nil

iv. Popular articles:

Parasitic Diseases of mithun (*Bos frontalis*) and their suggested control measure by J.K. Chamuah. 2016. "Scientific Mithun Husbandry for Improving Livelihood" 2016.

v. Books/Book Chapters : Nil

vi. Extension Bulletins: NA

c. Intellectual Property Generation :NA

d. Presentation in Workshop/Seminars/Symposia/Conferences:
(relevant to the project in which scientists have participated)

e. Details of technology developed
(Crop-based; Animal-based, including vaccines; Biological – biofertilizer, biopesticide, etc;
IT based – database, software; Any other – please specify)

f. Trainings/demonstrations organized (Contributed):

- Imparted Hands-On Training on Augmentation of Health and Production Through Biotechnological and Bioinformatics Tools" from 21st to 30th March, 2015 to the B.Tech students from SETAM (Nagaland University).

g. Training received: NA

H.Any other relevant information : NA

12. (a) Extent of achievement of objectives and outputs earmarked as per RPP-I

Objective wise	Activity	Envisaged output of monitorable target(s)	Output achieved	Extent of Achievement (%)
1. Studies on epidemiology of ectoparasites of mithun.	Collection of ectoparasites	Percentage s of infected animals for ectoparasites	Incidence of ectoparasites	90%
	Gross morphological study of ectoparasites	Morphological identification of tick and lice	Identification of ticks and lice	95%
	Identification of gross ectoparasites followed by molecular identification	PCR amplification of marker genes of Ixodid in order to confirm molecular identity	Molecular identification of Ixodid ticks.	90%
Evaluation of control measure against ectoparasites particularly of against *R. microplu*	Therapeutic trial against *R. microplus*	Evaluation of Therapeutic efficacy of available Chemical and herbal drugs against *R. microplus*	- To preparing of effective treatment schedule against ectoparasites.	90%

(b) Reasons of shortfall, if any : NA

13. Efforts made for commercialization/technology transfer: NA

14. (a) How the output is proposed to be utilized?

The knowledge of epidemiology will be utilized for making of preventive strategy against ectoparasites and also it knowledge will be very essential for development of control measures against ticks.

(b) How it will help in knowledge creation

The proposed project was end up with the generation of base line data on ticks and lice along with evaluation of acaricidal efficacy of chemical and herbal drugs which will help in formulation of control strategy against the ticks.

15. Expected benefits and economic impact(if any)

The proposed research project was highlighting the different ectoparasites parasites harboured by the mithun with their incidence, along with their evaluation of efficacy against chemical and herbal drugs. This study will help the mithun farmers to take proper preventive strategy against ectoparasites.

16. Future line of research work/other identifiable problems :

The genetic diversity existing in the tick populations will be elucidated to find out phylogenetic and evolutionary relationship among hard ticks. Therefore, characterization of the ectoparasites by molecular tools will establish an accurate database on the existence of different ectoparasites of their strains / hybrid genotypes in this ruminant in north east. Above all, development of reliable biopesticide may be attempted as a ecofriendly and substitute to toxic groups.

17. Signature of PI : **Jayanta Kumar Chamuah**, Scientist, Veterinary Parasitology

18. CC-PI(s), all Co-PIs

Co-PI I Co-PI II Co-PI III Co-PI IV

15. Signature of Head of Division

16. Observations of PME Cell based on Evaluation of Research Project after Completion

17. Signature (with comments if any along with rating of the project in the scale of 1 to 10 on the overall quality of the work) of JD (R)/ Director ☐

ANNEXURE - VIII

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI(H))

PROFORMA FOR RESEARCH PERFORMANCE EVALUATION OF INDIVIDUAL SCIENTIST

1. Institute Project Code *

2. Evaluation by PI on the contribution of the team in the project including self

S. No.	Name	Status in the project (PI/CC-PI/Co-PI)	*Rating in the scale of 1 to 10
	Dr. Jayanta Kumar Chamuah	PI	9
	Dr. Vidya Singh	Co-PI	7
	Dr. Akhilesh Kumar	Co-PI	7
	Dr. Kobu Khate	Co-PI	7
	Dr. P.R Dutta	Co-PI	8

3. Signature of PI

* Individual scientists participating in the project would be assessed for their performance through an appraisal system in a scale of 1 to 10 for each of the following attributes:

S. No.	Criteria	Marks
1.	Percentage of the assigned activity completed	**40**
2.	Quality of the completed activity	**10**
3.	Authenticity/reliability of the data generated	**10**
4.	Enthusiasm and sincerity to work	**10**
5.	Inferences made	**10**
6.	Collaboration and cooperation demonstrated in performing the task at hand	**10**
7.	Amenability to scientific/academic/laboratory discipline	**10**
	Total Score	**100**

ANNEXURE - IX

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI(I))

PROFORMA FOR EVALUATION OF A RESEARCH PROJECT AFTER COMPLETION BY PI

1. Institute Project Code

2. Evaluation research project after completion by PI

S. No.	Criteria	Methodology	Marks (output)	Self Evaluation by PI
1.	Achievements Against approved and stipulated outputs under project	**Qualitative and quantitative assessment of objectives and stipulated outputs under the project will be carried out**	**75**	
		a) Activity Input /Projected Output/ Output Achieved	35	**35**
		b) Extent to which standard design methodology, experimental designs, test procedures, analytical methods followed	10	**10**
		c) Does the data justify the conclusions?	05	**5**
		d) Innovativeness and creating of new knowledge	10	**7**
		e) Additional outputs over those stipulated under the project	05	**5**
		f) Creation of linkages for commercialization of technology developed under the project	05	**0**
		g) Is scientific input commensurate to output (manpower, Financial input and time duration)?	05	**5**
2.	Publication/ awards	Assessment will be done in respect of: Research papers; Reports/Manuals; Working and Concept Papers; Books/Book Chapters/Bulletins. Quality of publication (s) and Awards /Scientific recognitions received	**10**	**10**
3.	Additional facilities created	Facilities created in terms of laboratory. Research set-up, instrumentation, etc. during the project.	**05**	**5**
4.	Human Resource Development (Scientific and Technical)	Scientist trained in different areas	**05**	**3**
5.	Revenue generated under the project/ avenues created for	Resources and revenues generated	**05**	**0**

	revenue generation				
6.	Product/Process/Technology/ IPR / commercial value of the technology developed	Details to be provided on a) Products b) Process c) Technology d) IPR e) Registration of the varieties		**10**	**7**
7.	Quality of available documents of the project duly authenticated	Research Project Files, Data, Reports etc.		**05**	**5**
Total Marks				**115**	**94**
8.	Timelines of execution of the project	Marks will be deducted if extension sought over the approved project duration beyond recorded and officially granted extension with recorded reasons	Marks to be deducted		
		Up to 5%	01		
		Up to 10%	02		
		Up to 30 %	03		
		Beyond 30 %	05		
Net Score: Score obtained to be counted out of 100 to compensate for activities not relevant to the project				**100**	

However, looking into the requirements of different research institutes and disciplines, IRC may modify the indicators, their weights and total scores. The time gap for assessment of different indicators may also be decided by IRC

3. Signature of PI

ANNEXURE - X

INDIAN COUNCIL OF AGRICULTURAL RESEARCH

(For Guidelines Refer ANNEXURE – XI (J))

PROFORMA FOR EVALUATION OF A RESEARCH PROJECT AFTER COMPLETION BY EVALUATION COMMITTEE

1. Institute Project Code

2. Evaluation research project after completion by Evaluation Committee

S. No.	Criteria	Methodology	Marks (output)	Evaluation by Evaluation Committee
1.	Achievements Against approved and stipulated outputs under project	**Qualitative and quantitative assessment of objectives and stipulated outputs under the project will be carried out** a) Activity Input /Projected Output/ Output Achieved b) Extent to which standard design methodology, experimental designs, test procedures, analytical methods followed c) Does the data justify the conclusions? d) Innovativeness and creating of new knowledge e) Additional outputs over those stipulated under the project f) Creation of linkages for commercialization of technology developed under the project g) Is scientific input commensurate to output (manpower, Financial input and time duration)?	**75** 35 10 05 10 05 05 05	
2.	Publication/ awards	Assessment will be done in respect of: Research papers; Reports/Manuals; Working and Concept Papers; Books/Book Chapters/Bulletins. Quality of publication (s) and Awards /Scientific recognitions received	**10**	
3.	Additional facilities created	Facilities created in terms of laboratory. Research set-up, instrumentation, etc. during the project.	**05**	
4.	Human Resource Development (Scientific and Technical)	Scientist trained in different areas	**05**	
5.	Revenue generated under the project/	Resources and revenues generated	**05**	

	avenues created for revenue generation				
6.	Product/Process/Technology/ IPR / commercial value of the technology developed	Details to be provided on a) Products b) Process c) Technology d) IPR e) Registration of the varieties		**10**	
7.	Quality of available documents of the project duly authenticated	Research Project Files, Data, Reports etc.		**05**	
Total Marks				**115**	
8.	Timelines of execution of the project	Marks will be deducted if extension sought over the approved project duration beyond recorded and officially granted extension with recorded reasons	Marks to be deducted		
		Up to 5%	01		
		Up to 10%	02		
		Up to 30 %	03		
		Beyond 30 %	05		
Net Score: Score obtained to be counted out of 100 to compensate for activities not relevant to the project				**100**	

4. Signature of Evaluation Committee