Materials and Methods
MATERIALS AND METHODS

The experimental materials used and methods employed during the course of this investigation entitled "Studies on the Ectoparasites (Ticks and Mites) of cows in Rohailkhand area" (U.P.) adjoining areas of Bareily and Badaun, Saharanpur and Rampur, Etah and Aligarh was carried out mostly in the campus of IVRI Itzatnagar, Bareily. It involved the breeds of cows such as, N.D.(Nondiscript), Sahival, Hariyana, Red Sindhi, Gir, M(Mewati), Nimari and D (Deoni) and their crosses spread over different months, seasons and years. The animals involved for the purpose from organized farms, unorganized farms, and individual farmers from villages.

The work involved not only the assaying of the seasonal distribution of ectoparasites and their impact on growth. Changes in composition of blood and weight losses, but also formulation and designing of control measures preferably out of the available indigenous materials traditionally credited for sparse acaricidal properties. The impact of the ectoparasitic infestation of animals on
their feed consumption and utilization were also studied to assess the metabolic disorders if any, created by such infestation.

3.1 GEOGRAPHICAL LOCATION AND CLIMATE

This research station, IVRI Itzatnagar, Bareily. The year was divided into 3 seasons (Tata et al. 1987) viz., winter (November-February), summer (March- June) and Rainy (July- October). The characteristic environment of this place had ambient temperature in (°C) which range from 2.60- 28.20, 11.00- 40.00 and 15.20- 35.20 during winter, summer and rainy seasons, respectively. The relative humidity in % in the morning and noon were 71- 96 and 33- 91 during winter, 49- 91 and 17- 79 during summer and 80- 95 and 37- 79 during rains. The data so generated were classified and statistically analyzed to study the season wise distribution of reproductive events mentioned earlier by Snedecor and Cochran (1976) and Saxena et.al. (1983). The geographical location is as under:

Latitude - 79.24
Longitude - 28.20 North
Altitude - 152.24 meters (Above the mean sea level)
PHASE- 1

3.2 FIELD INVESTIGATION

The field studies extending over a period of two years and eight months i.e. March 2004 to October 2006 covered the following areas of Bareily and Badaun, Saharanpur and Rampur, Etah and Aligarh district of Rohailkhand area.

a) Rural Areas:
   1. Badaun
   2. Saharanpur
   3. Rampur
   4. Etah
   5. Aligarh

b) Institutions
   1. IVRI, Itzatnagar, Bareily dairy farm.

The rural areas covered under the present study are characterized by intensive, multiple and mixed type of indigenous cows.

3.3 SELECTION OF COWS

The animals studied were maintained under intensive semiintensive and farmer’s conditions of management. All the
animals of villages were more facilitated for grazing and roaming freely than the farm animals. The animals of farms were kept in sheds and were served with stall feeding and watering.

The different breeds of cows and their calves, such as N.D. (Nondiscript), Sahival, Hariana, Red Sindhi, Gir, M (Mewati), Nimari and Deoni are selected from the adjoining areas of Rohailkhand. The number of selected cows and their calves are 1081 and 456 respectively. A group of cows from strategic of the rural areas and from IVRI dairy farm were selected for the purpose. Due care was taken to select animals with almost physiological status in each category. Where as the animals in the rural areas were maintained in the open environment to emulate their natural habitat, the farm animals were maintained in the experimental sheds with convenient arrangements for individual feeding.

3.4 SURVEY OF ECTOPARASITES

After selecting the villages referred to above and the households within the villages the intensity of infestation by ectoparasites viz. ticks and mites were ascertained on different classes of cattle during survey period. Keeping in view the socio-psychological situation of rural community the survey work was carried out preferably during morning and evening hours in
presence of the owners. The number of infested and non-infested animals with a particular household was recorded. One care was taken during the visible ectoparasites. Data were collected separately for adults and calves. The survey under reference covered all dairy cows. The investigations were replicated to seasonal fluctuations.

3.5 INFLUENCE OF WEATHER

To understand the impact of weather, the year was divided into three seasons of four months each on the basis of atmospheric temperature, rainfall, viz.

(a) Summer- (March- June)
(b) Rainy- (July- October)
(c) Winter- (November- February)

The weather observations including average temperature, relative humidity and rainfall were recorded on the meteorological station of the institute of Agriculture and Technological University of IVRI, Itztanagar, Bareily. This was regularly done during the whole experimental period from March 2004 to 2006.
3.6 COLLECTION METHOD OF ECTOPARASITES

The ecto-parasites viz., ticks and mites were collected from cattle by hand picking method. The parasites were removed from various parts of the body of host animals using a pair of fine forceps. Care was taken to ensure that the capitulum of tick and mites is not left embedded in the host skin and hypostoma remained intact. In the calves, a swab of chloroform was used to anaesthetize the ticks and mites before being picked up.

3.7 PRESERVATION AND PREPARATION OF SLIDES

Ectoparasites at their various growth stages viz., the adults, nymphs and larvae were collected and preserved in 70% alcohol (Mishra et al., 1974) in small glass sample bottles provided with a cork. Prior to examination, excess alcohol was removed by placing the specimen on some soft absorbent paper.

Representative specimen of ticks and mites from each collection were placed in 10% KOH followed by thorough washing with distilled water. The washed specimen were dehydrated by placing them in alcohol bearing ascending order of concentration i.e. 30%, 50%, 70%, 90% and 100% of absolute alcohol for 15 to 30 minutes each. The dehydrated material was put in equal ratio of
clove oil + acetic acid for 30 minutes followed by a treatment in clove alone for another 30 minutes. The specimen thus cleared were mounted and identified following the methods the ectoparasites were examined under the binocular dissecting microscope (Misra et al., 1974).

3.8 EXAMINATION AND IDENTIFICATION

Prepared slides were examined as such under a binocular microscope using direct light and identified as adults, nymphs and larvae of ticks and mites. The identification for ectoparasites was based on their external morphology. Preliminary identification of ticks was done making use of the keys given by Dhanda and Raja (1974); Ghalsasi and Dhanda (1974) and Dhanda et al. (1977). Mites were identified by their external characters as described by Soulsby (1968) and Putalunda et al. (2004).

3.9 LONGEVITY AND SURVIVAL

The longevity of male and female ticks and their larval farms was also studied by keeping them separate glass tubes, without food, covered from the day of their food deprivation to the day of death. The survival of the collected ticks was not based only on their starvation factor also on the varied climatic conditions such
as humidity and lower temperature. In winters, the survival period increases in the relatively high humidity and lower temperature. In winters as the climatic temperature drops rapidly, the metabolic activities of ticks reduce gradually in order to maintain the protective adjustment with reduction or loss of water. It is observed minutely that the summer seasons play the major hazardous role on the survival of the ticks because of the increase in temperature and wind velocity, and the decrease in humidity of the wind.

3.10 BIONOMICS OF THE ECTOPARASITES

The study of biology of parasites could be possible only in case of ticks, lice and some varieties of flies. For the study of tick biology in special, adult female ticks were removed from the infected animals at different stages of engorgement, weighed and kept in separate muslin cloth covered gloss tube to determine the durations of egg laying (Oviposition) and the number of eggs laid in the tubes, during the previous day were counted under the compound microscope in both low and high magnification. They were allowed to develop and produce larval farms at the ambient temperature and humidity of the farms and IVRI, Itzatnagar, Bareily.

The biology of ticks and mites was studied on both the groups of animals viz. adults and calves. The host was observed carefully
to record the complete life cycle of ticks and mites such as eggs and larval nymphs. Some of the ticks and mites, ready for oviposition, were picked up and placed in glass-wares to find out the accurate count of deposited eggs and other ticks and mites were observed on the bodies of the groups of host (adults and calves). The ticks present on natural hosts glued their eggs with cementing materials on these hair shafts of protected part of the host’s body. The eggs hatched timely and the tiny light yellowish or reddish younglet emerged were alike to adults in shape but smaller in size. Nymphs then mounted several times (averaging once on a week) and gradually achieved the adults size. In most cases of adult mite the average life span decoded was few weeks.

**PHASE- II**

**3.11 CONTROL MEASURES**

Completed the survey part of the Arthropodan parasites affecting cows of the intended region for the incidences of infestation and studying the intensity aspects on infection, it was considered necessary to carry out some treatment trails against them using a few well known recent insecticides used for treatment of infected animals are represented below in the form of the variable formulation.
The anti-parasitic preparation as detailed again were applied to various parts of the animal's body in pre-specified concentration. Due care was taken to reach all the vulnerable parts of the body by hand dusting while maintaining precautions to avoid the orifices. The frequency of dusting twice per week was resorted to.

3.11.1 Ticks control-

In order to combat the infestations of ticks in common, the following insecticides/ acaricides were used as dusts wettable powders and washes.

(i) As dusts:

Since, ticks are very dynamic ectoparasites that infect animals and develop easy resistance to various acaricides applied as dust, it was only imperative therefore, to design a suitable form of the acaricides that could render relatively a lasting impact. As such, the preparation were tried in liquid form selection and grouping of animals.

The 3%, 5%, 10%, 15% and 25% concentration of the following acaricides using sieved ash as the filter were used:

1- DDT (5%)- Chlorinated hydrocarbons,
2- Malathion (5%)- Organo-phosphorus compound (O.O dimethyl, S- 1, 2) bis (ethoxycarbonyl)

3- Aldrin (10%)- Chlorinated hydrocarbon (ethylphosphodithioate) (1:2:3:4:10:10 a) hexachlor-1:4- 4a:5:8a-hexa-hexahydro-: 4-endo -5:8 – denethano- natholine.

(ii) As wettable Powders:

The five concentrations of the same acaricides used as the formulations of wettable powder for the proper treatment of ticks infestations. These powders are used as solutions made by mixing into water the powders in different amounts. The used acaricides are DDT (25%, 30% and 35%), Malathion (25%, 30% and 40%) and Aldrin (30%, 35% and 40%) in the concentration.

(iii) As washes:

The four acaricides/insecticides used within these concentrations i.e. 0.025%, 0.05% and 0.1% of DDT; 0.75%, 1.0% and 2.0% of Malathion; 0.5%, 1.0% and 2.5% of Aldrin and 0.1% and 0.3% of Sevlon solution were prepared in water as follows:

1- DDT (25% EC) as emulsifiable concentrate.

2- Malathion (50%) EC) as emulsifiable concentrate.

3- Aldrin (30%EC) as emulsifiable concentrate.

4- Sevlon (50% EC).
The known number of animals infested with ticks were exposed to their treatment, while dusts and granules were thoroughly rubbed on the body of the animals with the help of palms and fingers. By securing the animal, the wash was administered on the animal body with the help of wash-soaked cotton swabs, applied especially over the affected parts. The survival of ticks was checked up on the subsequent day to record the acarcidal/insecticidal efficiency. The efficacy of the residual effect of the drugs was, however, checked on the 10th, 20th and 30th days subsequent to the treatment in terms of these parasites reappeared on the treated hosts.

3.11.2 Mites control:

Suitable dilutions were obtained by adding the required quantity of the acaricide to a measured quantity of ordinary water with through stirring of the mixture before actual application using a hand compression sprayer. Separate group of animals were tried for each dilution. Due care was taken to spray such animals of a specific group for the same number of days with known quantity of aqueous solution of the acaricide in such a way as to ensure complete contact of the preparation with mites present on animal body. As soon as body coat got dried, the animals were allowed grazing together with untreated
animals during the day but were tethered back separately in sheds during the night. Counting of mites was executed daily during morning and evening over a period of 40 days. Live and dead parasitic counts were recorded separately.

3.11.3 Lotions and creams:

1. Sulphur + Vasline
2. Himax
3. Ascabiol

These were administered repeatedly on the affected parts of the infested animals at an interval of and their efficiency recorded in terms of recovery and normalization of the administered part.