Theileria in cattle: treatment experiment

Theileria is a vector borne protozoan parasite that causes theileriosis which is a fatal disease for cows. It is a disease of tropics. *Theileria* poses a serious challenge to the exotic crossbred cattle population. *T. annulata* and *T. parva* are considered to be the most pathogenic species of theileria. Tropical theileriosis is one of the most prevalent diseases of cattle caused by *T. annulata* . It is transmitted through Ixodid tick of genus *Hyalomma* . Occurrence influenced by the abundance and distribution of the main vector and the presence of cattle, the main host. In recent years, the effects of global weather change on the epidemiology of vector borne diseases like malaria have started receiving increasing attention [363]. Theileria, like malaria, is likely to be sensitive to climate changes.

Diagnosis based on clinical signs is not trustful in many cases because other parasitic diseases have clinical signs similar to those of tropical theileriosis. The diagnosis of acute theileriosis cases is mainly based on microscopic examination of Giemsa stained blood smears. Blood smears are rapid and inexpensive technique. Giemsa staining of the blood smear is the common method for identification and characterization of the piroplasms.

Theileriosis has serious economic impact in view of mortality and reduced milk yield. In India theileriosis has been reported from Punjab, Haryana, Gujarat etc. geographical regions. Earlier studies suggested that Theileria did not occur in Himalayan region. *Theileria* is usually thought as disease of warmer climate and here not been reported in Uttarakhand to the best of our knowledge. Dehradun district of Uttarakhand is also located at the foothills of Himalaya. With the aim to assess the spread of theileria in Uttarakhand this

chapter highlights the problem of theileriosis and its season wise prevalence rate in crossbred cattle in Dehradun district of Uttarakhand, India.

For the preliminary study the reference population with sample size of 58 crossbred, was selected from the Graphic Era University herd and two different blocks (Raipur and Subhashnagar) of Dehradun district, Uttarakhand, India. From 58 cows, 18 Holstein Frisian (HF) cross bred in Graphic Era University herd and 40 cross bred cows from Raipur and Subhashnagar block were examined. Sampling was done during the summer months. Clinical examination was performed for all the animals. The signs of theileria infection were observed and recorded. Then blood samples have been collected from the ear vein of the animals and thin blood smears were prepared. The blood smears were examined microscopically using Giemsa stain to detect the occurrence of *Theileria* .

3. 2. 2 Experimental Study

The study was conducted during the January 2012 to March 2014. Total 301 animals were observed during the whole study at Graphic Era University, Dehradun district, Uttarakhand. Clinical examination was performed for all the animals. Blood Samples was collected during the months March to December 2012. Screening of the animals was done by blood smears examination. All the sampling was done as per ethical standards by a Veterinarian. After blood smear examination animal declared free from *Theileria* were retained for further study and rest microscopically positive animals were designated as diseased group.

3. 2. 2. 1 Clinical Examination: –

Clinical manifestation of theileriosis recorded in all examined cross bred cattle during each season i. e. spring, summer, rainy and winter season.

3. 2. 2. 2 Blood Examination: –

Collection of blood samples: –

The blood samples were collected from different locations of Dehradun district (Raipur, Subhashnagar, Ajabpur and Selaqui region) Uttarakhand, India (Fig 3. 1) in different seasons from March to December 2012 for detection of *Theileria* in cross bred cattle. Blood sample from ear vein of each examined animal were collected for detection of *Theileria* piroplasms. The thin blood smears were prepared from blood collected from ear vein [364].

Processing Of Samples:

For whole study laboratory at Department of Biotechnology, Graphic Era University, Dehradun, Uttarakhand was used in order to conduct, smear examination and polymerase chain reaction test.

Microscopic Examination:

The dried blood smears were stained as described by Soulsby [376].

Giemsa’s staining technique

1. The dried blood film was flooded with methyl alcohol for 10 min. The slides were washed gently with tap water to remove alcohol.

2. Fixed slides were stained in working dilution of Giemsa’s stain (1: 10) for 30 min.
3. The slides were washed with tap water and dried in air.

A drop of immersion oil was placed on the smear and slide was examined under oil immersion lens of microscope for the presence of blood protozoan and morphology of red blood cells. Morphological characteristics of *Theileria* were identified [376, 377]. *Theileria annulata* occur more commonly as round oval or ring form with diameter of 0. 5-1. 5 micrometer.

3. 3. 2 Results of Experimental Study:

The study was carried out in cross bred cattle in different location of Dehradun district, Uttarakhand, India during the year Jan 2011- Mar 2014. A total of 301 cattle were observed during the study to find out the occurrence of *Theileria* and find out the prevalence rate in different seasons in Dehradun district of Uttarakhand.

1. Prevalence of theileriosis in different location of Dehradun district and in different season.

Prevalence of theileriosis at different location of Dehradun district in different seasons was shown in table 3. 3.

In Subhashnagar the overall prevalence on the basis of blood smear examination was 36. 5% (30/82). In spring season the prevalence rate was 13. 3 % (2/15). In summer season the prevalence rate was 32 % (8/25). In rainy season the prevalence rate was 71. 8% (18/26). In winter season the prevalence rate was 12. 5% (2/16).

In Raipur the overall prevalence on the basis of blood smear examination was 33. 3% (24/72). In spring season the prevalence rate was 14. 2% (1/7). In summer season the prevalence rate was 26. 8 % (11/41). In rainy season the prevalence rate was 64. 7% (11/17). In winter season the prevalence rate was 14. 2% (1/7).

In Selaqui the overall prevalence on the basis of blood smear examination was 16. 6% (12/72). In spring season the prevalence rate was 5. 5 % (1/18). In summer season the prevalence rate was 18. 1 % (2/11). In rainy season the prevalence rate was 25% (8/32). In winter season the prevalence rate was 9% (1/11).

In Ajabpur the overall prevalence on the basis of blood smear examination was 15. 5% (9/58). In spring season the prevalence rate was 1% (1/10). In summer season the prevalence rate was 28 % (7/25). In rainy season the prevalence rate was 35. 7% (5/14). In winter season no sample was found positive for *Theileria* .

In other private herd the overall prevalence on the basis of blood smear examination was 17. 6% (3/17). The *Theileria* positive sample was found only in rainy season with 3 % (3/10) prevalence rate.

The prevalence rate in different locations of Dehradun district was shown in figure3. 4. The highest prevalence was found in Subhashnagar with 36. 5% prevalence rate followed by in Raipur with 33. 3 % prevalence rate.

3. 4 Discussion:

The genesis of this problem emerged from the dairy herd maintained at Graphic Era University (GEU) Dehradun, Uttarakhand. From few cows in Graphic Era University herd the number of cross bred is over 50 now. These cows suffered due to lack of higher management skill, heat stress and they has been getting ill during summer. Therefore of this reason the suggestion has made to breed native cows. So, Sindhi cows and calves have brought from Rajasthan in the month of March 2010. These cows have been added to dairy herd of GEU without quarantine. This results in death of 8 high yielding cross-bred cattle. No veterinary treatment has been able to save these cows. So the Biotech department of GEU has started examining the problem. Microscopic examination of blood revealed presence of theileria which has also confirmed from Indian Veterinary Research Institute (IVRI), Bareilly. Then the problem has taken as a subject of research. We carried out our study in cross bred cattle in Dehradun district, Uttarakhand during summer, rainy, winter and spring season. Since then we have been diagnosing theileria all around the Dehradun, the capital of Uttarakhand. The cows fall sick on the onset of summer. In this connection one of the technicians has sent to IVRI for training in blood examination. It has reviewed that *Theileria* has not been prevalenting in the Himalayan region of Uttarakhand however this disease is much prevalent in herds of Punjab, Haryana, and Gujarat etc. However addition of native cattle from Rajasthan causes diseases.

Microscopically we have examined a total of 301 cattle by using Giemsa’s stained blood smear method. Giemsa’s-stained blood films contained *Theileria* piroplasms, including cocci, rod, stick, comma, fusiform, racquet-shaped, signet-ring, and pear-shaped forms with diameter of 0. 5-1. 5

micrometer [15]. The ring form has found to be the most common in present study. Microscopic examination of blood smears have revealed 27. 2% overall prevalence of theileriosis. Season wise 9. 0% cows has found positive for theileria as per blood smear in spring season, 19. 6% has found positive in summer season, 45. 4% has found positive in rainy season and 8. 8% has found positive in winter season. Highest numbers of positive cases has obtained in rainy season which corresponds to months between July and October when the THI has also found high i. e. above 80. Similar observations have observed in previous reports [365, 366]. High incidences of tropical theileriosis in cross bred cattle were reported during summer and monsoon and identified tick vector as *Hyalomma* species [367].

All the infected animals during field and experimental study have showed clinical manifestations ranging from mild to severe reactions. The clinical signs included high fever, swelling of submandibular and sub scapular lymph nodes, weakness, increased respiration and pulse, anorexia, anaemia and loss of condition [113]. The first symptom of fever was manifested by the animal in theileriosis [368]. In sub-acute and chronic forms of the *Theileria annulata* infection the symptoms observed in animal were swelling in lymph node, haemorrhages on visible mucous membranes, reduced milk yield, anaemia and jaundice [37].

We have reported higher incidence of theileriosis in the hot and humid month and this is in agreement with other workers [62, 108, 111]. The highest abundance of the ticks has reported in the month of July [239] whereas *Hyalomma* sp. of ticks is most abundant in June [369].

After reporting this study the industrialist making dairy with 200 cows and has started making dairy from Bangalore because in Haryana and Punjab cows are infected. Sindhi cows infected with ticks but are not affected with *Theileria* . Pure Sahiwal has not infected with Theileria [12]. Cross bred cattle suffer from Theileria but local cows do not suffer [12, 370, 371]. We have first time observed this disease in hilly region.