Studies on prevalence of theileriosis and assessment of its risk factors among cattle in and around Udaipur, Rajasthan

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Abstract
Objective: To study the prevalence of theileriosis in cattle in Udaipur, Rajasthan.

Material and Method: The animals were screened for Theileriosis from May 2019 to April 2020 in the Udaipur region irrespective of age, sex and breed. Animals showing clinical manifestations were screened and subjected to blood smear examination.

Result: A total of 836 blood samples were collected from cattle. The overall prevalence of theileriosis was 67.22% whereas the prevalence of mixed haemoproteozoan infection was 18.54%, and 14.23% were negative for any haemoprotozoan infection. The age-wise analysis revealed the highest prevalence in older cattle (>5years) as compared to that in younger cattle of 1-2 years. Sex-wise prevalence study revealed that females were more affected by the infection. The prevalence was higher in crossbred cattle as compared to indigenous cattle during the present study. The seasonal analysis revealed the highest prevalence in the summer season followed by rainy, spring and winter. During the study period, the highest prevalence was recorded in May and lowest prevalence in December.

Conclusion: We concluded that non-descript breed and younger animals were less prone to Haemoproteozoan infection whereas vice versa for older and crossbred. Extra care and managerial practices required to avoid economic losses due to high Haemoproteozoan prevalence in summer and monsoon.

Keywords: prevalence, theileriosis, cattle, blood smear examination

Introduction
The livestock sector plays a critical role in the welfare of India's rural population by employing about 8.8% of the population in India with vast livestock resources and income to the momentous rural population. It contributes 4.11% to GDP and 25.6% of total Agriculture GDP and employs eight per cent of the labour force. The livestock sector aids in India's economy, with 70% population owing livestock for generating additional employment through milk, meat, wool and egg production [1]. According to the 19th livestock census, Rajasthan state possesses 57732 thousand numbers of livestock animals which account for about 11 per cent of the total livestock population of India and is the second leading milk-producing state but the major constraints in the livestock sector are the parasitic infections, among which the haemoproteozoans, Tick-Borne Diseases and ectoparasitic infestations are of prime importance. Globally, ticks and tick-borne diseases (TTBDs) continue to be a major constraint on profitable livestock production and productivity. Globally, ticks are second to mosquitoes as a vector of infectious pathogens of humans and animals. They transmit diseases like Babesiosis, Theileriosis, Anaplasmosis, KFD and many more that are considered as a major constraint on livestock productivity [2].

Bovine tropical theileriosis (Theileria annulata infection) is a tick-borne disease whose causative pathogen (Theileria annulata) is transmitted by Hyalomma ticks. There is a paucity of literature regarding the accurate diagnosis and control of this protozoan disease in Rajasthan with few reports from the Southern part of the state. Therefore, the present investigation was undertaken to study the prevalence of Theileria annulata in cattle in Udaipur.
Material and Methods
The present study was conducted by a collection of the blood samples from May 2019 to April 2020 for one year in different villages of Vallabhnagar tehsil of Udaipur district in Southern Rajasthan. The animals were screened for Theileriosis in the Udaipur region irrespective of age, sex, breed showing clinical manifestations of pyrexia and swollen lymph nodes. Animals were monitored for additional clinical signs like salivation, lacrimation, emaciation, loss of appetite, depression, loss of production, diarrhoea, pale mucus membrane, anorexia, nasal discharge and in severe cases jaundice etc. The clinical signs exhibited and findings were recorded systematically for clinical characterization of the disease. The blood samples of the animals were collected individually from the jugular vein in EDTA coated vacutainers. Animals were randomly selected from a total population of ruminants present in and around the Udaipur district. Tick samples were also collected along with blood from affected animals.

Method for Smear preparation: Routinely blood samples in EDTA vacutainers were collected randomly from different locations/villages and farms of Udaipur (Rajasthan) from cattle showing clinical manifestations. The dried smears were examined by Giemsa staining technique.[3]

Blood smear examination: A thin layer smear was prepared from the blood of cattle randomly selected and fixed and stained with methanol and Giemsa dye respectively. Giemsa-stained blood smears were examined for the presence of haemoparasites and identified as per characters.[3].

Result and Discussion
The animals suspected of Theileriosis were investigated in and around Udaipur based on visible clinical manifestations and the presence of tick infestation. The age, breed, sex, season and month of blood collection etc. from the animals were recorded regularly.

In the present study, the overall prevalence for Theileria sp. by blood smear examination in cattle with clinical symptoms was recorded to be 562 (67.22%) out of 836 samples screened whereas 155 (18.54%) were positive for other mixed haemoprotozoan infection like Anaplasma and Babesia species and remaining 119 samples (14.23%) were found negative for any type of haemoparasitic infection as shown in Table 1.

| Host | Total number of cattle Screened with symptoms | Number of cattle Positive for Theileria | Percentage | Number of cattle Positive for mixed haemoprotozoan infections | Percentage | Negative | Percentage |
|------|-----------------------------------------------|----------------------------------------|------------|---------------------------------------------------------------|------------|----------|------------|
| Cattle | 836 | 562 | 67.22 | 155 | 18.54 | 119 | 14.23 |

The prevalence of Theileria annulata infection in clinical cases of cattle at different age groups by blood smear examination method has been presented in Table 2. In the present study, the highest prevalence of 269 (78.19%) was recorded in older cattle (>5 years) as compared to younger cattle of 1-2 years with 129 (58.90%) prevalence whereas the prevalence in cattle of <1 year and 2-5 years age groups were 91 (59.86%) and 73 (60.33%) respectively. Statistically, the variability in different age groups was found to be significant at both 0.01% and 0.05% level of significance by chi-square analysis. The present study indicates that age has a high effect on the prevalence of Theileria annulata infection and there is a relation between infection and age factor of animals.

| Age | The total number of animals screened | Number of animals positive for Theileria | Number of animals negative for Theileria | Total (Percentage) |
|-----|-------------------------------------|----------------------------------------|----------------------------------------|-------------------|
| <1 year | 152 | 91 | 61 | 59.86 |
| 1-2 year | 219 | 129 | 90 | 58.90 |
| 2-5 year | 121 | 73 | 48 | 60.33 |
| >5 year | 344 | 269 | 75 | 78.19 |
| Total | 836 | 562 | 274 | 67.22 |

The chi-square statistic is 32.0237. The $p$-value is <0.00001. The result is significant at $P<0.01$

The sex-wise prevalence of Theileria annulata infection in clinical cases was also studied by blood smear examination as shown in Table 3. A total of 836 animals were screened having clinical symptoms, out of which 384 were male and 452 were females. Out of 384 males and 452 females, 205 (53.38%) male and 357 (78.98%) females respectively were positive for the infection and it was found that variability in both sexes was significant at both 0.01% and 0.05% level of significance. It indicates that sex also has a relation with the prevalence rate of Theileria annulata infection. The present study findings are in accordance with a study.[5].

| Breed | Total number of cattle screened | Number of cattle positives for Theileria | Number of cattle negatives for Theileria | Total (Percentage) |
|-------|--------------------------------|----------------------------------------|----------------------------------------|-------------------|
| Male | 384 | 205 | 179 | 53.38 |
| Female | 452 | 357 | 95 | 78.98 |
| Total | 836 | 562 | 274 | 67.22 |

The chi-square statistic is 61.7395. The $p$-value is <0.00001. The result is significant at $P<0.01$

The chi-square statistic is 61.7395. The $p$-value is <0.00001. The result is significant at $P<0.05$
The breed-wise prevalence of *Theileria annulata* infection in clinical cases of cattle by blood smear examination has been tabulated in the Table 4. In the present study, breeds were grouped under crossbred and non-descript cattle. Out of a total of 558 crossbreds and 278 non-descript cattle, 417 (74.73%) and 145 (52.15%) were found positive for *Theileria annulata* respectively. The results showed that crossbred was more susceptible than non-descript cattle.

**Table 4: Breed-wise Prevalence of clinical cases of Theileriosis by blood smear examination**

| Breed          | The total number of animals screened | Number of animals positive for *Theileria* | Number of animals negative for *Theileria* | Total (Percentage) |
|----------------|-------------------------------------|------------------------------------------|------------------------------------------|--------------------|
| Non-descript   | 278                                 | 145                                      | 133                                      | 51.25              |
| Cross Bred     | 558                                 | 417                                      | 141                                      | 74.73              |
| Total          | 836                                 | 562                                      | 274                                      | 67.22              |

The chi-square statistic is 42.9115. The *p*-value is <0.00001. The result is significant at *P* < 0.01

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The month-wise prevalence of *Theileria annulata* infection in clinical cases by blood smear method for the study period of 12 months (May 2019-April 2020) has been presented in Table 5. During the study period, out of 836 animals, the highest prevalence was recorded in May with 67 (80.72%) positive cases followed by September with 64 (80.00%). June with 108 (76.59%), July 122 (74.39%) and October 38 (71.69%). In August it was 75 (63.02%) whereas in April, February, March, November, January and December the number of positives was 31 (58.49%), 14 (50.00%), 12 (42.85%), 12 (41.37%), 09 (40.90%) and 10 (27.77%) respectively. The lowest prevalence was observed in December with 10 (27.77%). The variability of the prevalence of Theileriosis in different months was found to be significant (*P* < 0.05) indicating that the effect of different months on the prevalence of *Theileria annulata* infection. The present study is in agreement with the study [1].

**Table 5: Month-wise prevalence of clinical cases of Theileriosis by blood smear examination**

| Month          | The total number of animals screened | Number of animals positive for *Theileria* | Number of animals negative for *Theileria* | Total (Percentage) |
|----------------|-------------------------------------|------------------------------------------|------------------------------------------|--------------------|
| May            | 83                                  | 67                                       | 16                                       | 80.72              |
| June           | 141                                 | 108                                      | 33                                       | 76.59              |
| July           | 164                                 | 122                                      | 42                                       | 74.39              |
| August         | 119                                 | 75                                       | 44                                       | 63.02              |
| September      | 80                                  | 64                                       | 16                                       | 80.00              |
| October        | 53                                  | 38                                       | 15                                       | 71.69              |
| November       | 29                                  | 12                                       | 17                                       | 41.37              |
| December       | 36                                  | 10                                       | 26                                       | 27.77              |
| January        | 22                                  | 09                                       | 13                                       | 40.90              |
| February       | 28                                  | 14                                       | 14                                       | 50.00              |
| March          | 28                                  | 12                                       | 16                                       | 42.85              |
| April          | 53                                  | 31                                       | 22                                       | 58.49              |
| Total          | 836                                 | 562                                      | 274                                      | 67.22              |

The chi-square statistic is 23.50. The *p*-value is <0.00001. The result is significant at *P* < 0.05

Season plays a very important role in the population dynamics of ticks and theileriosis. The prevalence of *Theileria annulata* infection in cattle of clinical cases by blood smear method has been analyzed season-wise and is presented in Table 6. The prevalence of *Theileria annulata* infection in summer, rainy/monsoon, winter and spring seasons were 297 (76.54%), 177 (70.23%), 45 (39.13%) and 43 (53.08%) respectively out of 388, 252, 115 and 81 cattle screened respectively. The prevalence was highest in the summer season followed by rainy, spring and winter. Statistical analysis revealed that there was a significant effect of season. The findings are in agreement with [7] where the authors have recorded the highest prevalence of *T. annulata* (45%) in the summer season.

**Table 6: Season wise prevalence of clinical cases of Theileriosis by blood smear examination**

| Season                      | Total number of cattle screened | Number of cattle positives for *Theileria* | Number of cattle negatives for *Theileria* | Total (Percentage) |
|-----------------------------|---------------------------------|------------------------------------------|------------------------------------------|--------------------|
| Summer (May – July 2019)    | 388                             | 297                                      | 91                                       | 76.54              |
| Rainy/Monsoon (August – October 2019) | 252                             | 177                                      | 75                                       | 70.23              |
| Winter (November 2019-February 2020) | 115                             | 45                                       | 70                                       | 39.13              |
| Spring (March-April 2020)   | 81                              | 43                                       | 38                                       | 53.08              |
| Total                       | 836                             | 562                                      | 274                                      | 67.22              |

The chi-square statistic is 61.3156. The *p*-value is <0.00001. The result is significant at *P* < 0.05

Variability of the prevalence of Theileriosis between different season was found to be significant (*P* < 0.05). The Chi-Square value is 9.22.

In the present study prevalence of 76.54% of *T. annulata* was noted during the summer season which is due to the hot and humid climate after the rainy season at Udaipur. The animals undergo stress during the post-monsoon season and are thus prone to infection. Secondly, the incidence of *Hyalomma anatolicum anatolicum* is more in this season and might have spread the disease. The agro-ecological and geo-climatic conditions of this region are highly favourable for the growth and multiplication of
natural vectors of these haemoprotozoan diseases. The findings of the present study are in agreement with other studies [8, 9, 10]. Prevalence largely depends on the distribution and density of reservoir host-vector and season [11]. Differences among results may be variation in the geographical area, the climatic condition of the experimental area, method of study and tick infestation. The lower prevalence in the present study is in agreement with the findings of Tuli et al [12] which may be due to the low sensitivity of microscope and time of blood collection. In the present study sex wise variation is due to hormonal changes and in age group it is due to inverse age resistance and immune response of host. The cross bred is more susceptible than local animals and season wise it is more after rainy season due to multiplication intensity of vectors in that season.

Conclusion
The present study revealed that the infection status of various haemoparasites was very high. The prevalence of haemoprotozoan parasite of cattle at Udaipur district, Rajasthan shows high susceptibility for haemoprotozoan infection. Further study is warranted to determine the economic losses due to protozoan infections and to develop effective control measures against them. It is further suggested that bio-control measures with effective agents would help in minimize the infection and help in optimum growth and productivity of cattle in the region.

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