Research Article

Molecular Prevalence of Theileriosis in Calf at Babylon, Iraq

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Abstract | *Theileria annulata,* a tick-borne hemoprotozoan parasite that result in a tropical theileriosis in bovine herds and causes significant economic losses in the cattle sector. We estimated and interpreted the frequency of theileriosis in the calf herds in Babylon Province. We carried out the study from July until the end of December 2022 in several regions of Babylon Province. Ninety jugular vein blood samples were collected. The calves ranged from <1 M to ≥4 M and both sexes were represented. Two ml of blood were placed in sterile, EDTA-treated tubes, and were sent to the parasitology lab in an ice pack. The blood was put in a deep freezer set at -20°C for DNA extraction. This genomic DNA purification was used following the instructions provided in the kit to extract the parasite DNA. The results revealed that the rates of infection of calves with *T. anullata* in Babylon Province were 36/90. The infection rate of *T. anullata* was recorded for females (47.5%) and males (34 %). Results showed that the highest infection rate (62.5%) was recorded at the <1 M, (39.1%), at 1-2 M, (20%) at 2-3 M, (22.7%) at the 3-4 M, and the lowest infection rate (14.3%) was recorded at the ≥4 M. The infection rate of calves with *T. anullata* (46.7%) was recorded in July. The highest infection rate (66.7%) was recorded in August and September; (33.3%) in October, and the lowest infection rate (13.3%) was recorded in November and December. There was a greater infection in female calves compared to males. Furthermore, there was an association between the disease and the surrounding temperature. These results serve as a starting point and make it easier to conduct future extensive epidemiological studies on tropical theileriosis in the calf herd on a national scale.

Keywords | Prevalence, Calf, Theileriosis, Molecular study

Received | January 24, 2024; **Accepted** | February 27, 2024; **Published** | April 16, 2024
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Citation | Al-Wasmee AKK, Hassone WS, Al-Janabi HT, Al-Jabory HAH (2024). Molecular prevalence of theileriosis in calf at Babylon, Iraq. Adv. Anim. Vet. Sci., 12(6):1061-1065.
DOI | https://dx.doi.org/10.17582/journal.aavs/2024/12.6.1061.1065
ISSN (Online) | 2307-8316

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INTRODUCTION

Bovine Tropical Theileriosis, also referred to as Theileriosis, is a tick-borne disease (TBD) that is ubiquitous around the world but is most prevalent in Southern Europe, Northern Africa, the Middle East, and Asia. *Theileria annulata*, a hemoprotozoan parasite, is the culprit. Geographically, it is also known as Mediterranean Theileriosis. Tropical bovine theileriosis, which has a major negative impact on animal productivity, especially in developing countries, poses a threat to about 250 million cattle (El-Damaty *et al.*, 2022).

When the right tick vectors are present, members of the family Theileridae (order Piroplasmida, genus *Theileria*) parasitize both wild and domestic animals, causing theileriosis (Kiara *et al.*, 2018). According to Valente *et al.* (2023), tropical bovine theileriosis is a serious illness spread by ticks. Due to immediate contact with infected ticks, which are more active in the summer and rainy seasons,

the newborn calves displayed a high-risk group (Singh *et al*., 2017). Calves born to dams who had received the cell culture vaccination for tropical theileriosis were likewise susceptible to the disease.

There is little information available on the hematological investigations on the disease in these native pure breed calves, despite theileriosis becoming a major and fatal condition in young calves (Singh *et al*., 2017). Strong evidence points to an intrauterine infection with *T. annulata*, and the infection appears to have started before conception (Sudan *et al*., 2012). *T. annulata* increases the degree of impermanence on a farm, reduces output, and restricts the growth plans of various breeds (Ullah *et al*., 2021).

PCR has been demonstrated to be a susceptible and accurate method for diagnosing bovine tropical theileriosis, especially in identifying samples that were negative on blood and lymph smears (Madkour *et al*., 2023). Thus, we aimed to estimate and interpret the frequency of theileriosis in calf herds of Babylon. They carried out the study from July until the end of December 2022 in several regions of Babylon.

MATERIALS AND METHODS

Period of study

The study was carried out in Babylon Province between July and the end of December 2022. Several cooling technologies inside the brans were used to control temperature and relative humidity including ventilation, outside cooling systems with heat exchangers, evaporation and desiccant systems were employed to manage the climatic conditions needed to raise the calves used for the study.

Calves are fed on milk and green hay and have access to clean, fresh water, and are not contaminated by dung, feedstuff, or additional environmental pollutants.

Natural lighting source, regular rodenticide and insecticide disinfection were used in the calf housing, and the absence of any treatment program for the calves employed in this study.

Blood collection

90 animals of both sexes between the ages of one month and four months had their jugular vein blood samples collected. Two milliliters of blood had to be drawn into sterile, EDTA-treated tubes (Thermo Fisher Scientific, Spain), moved quickly with an ice pack, and then frozen at -20˚C to extract the DNA.

DNA extraction

The parasite DNA was extracted using the Geneaid (Korea) genomic DNA isolation kit as directed by the product's instructions. 200 µl of frozen blood was used as the initial substance for the first stage of the DNA extraction process. The final DNA product was NanoDrop, Thermo Scientific/ UK which was found to be both excellent quality and quantity.

PCR

T. annulata was identified using the rRNA gene as a molecular target primers: F: ATTGCTTGTGTCCCTCTGGG and R: TCCACCAACTAAGAACGGCC. The 18S rRNA gene was used to identify *T. annulata* (620 bp), (Hailemariam *et al*., 2017). For the PCR, the 20µl reaction mixture included 10µl of the green master mix, 1µl of each upstream and downstream primer, 2µl of the DNA template, 5.5µl of water for molecular usage, and 0.5µl of MgCl2. 72ºC for 5 minutes was used for the one-cycle initial denaturation, 39-cycle main denaturation, annealing, and main extension, and one cycle for a final extension. The thermocycler settings were 95ºC for 5 minutes (Denaturation 95ºC for 35s, Annealing 57ºC for 35s, and Extension72ºC for the 40s). 0.5 µg/ml ethidium bromide was used with 2% agarose gel for the electrophoresis. After that, a UV imager was used to study the bands.

Statistical analysis

Analysis was done using SPSS (Statistical Package for Social Sciences) version 26. The association between infection rate and each of the animals was detected using the Chi-square test. Probability values of P <0.05 were considered statistically significant (SAS, 2012)

RESULTS AND DISCUSSION

Prevalence study

Total rates of infection of calves with *T. anullata*

The total infection rate of calves with *T. anullata* from July to the end of December 2022 in different regions of Babylon Province was (36/90) Table 1.

Table 1: Total rates of infection of calves with *T. anullata* at Babylon Province.

No. of samples examined	No. of positive samples
90	36

Infection rate of *T. anullata* according to sex

Females recorded 47.5% while males recorded 34% as shown in Table 2.

Infection rates of *T. anullata* according to age groups

Results showed that the highest infection rate 62.5% was recorded at <1 M, 39.1% at 1-2 M, 20% at 2-3 M age,

22.7% at 3-4 M age, while lowest infection rate 14.3% was recorded at ≥4 M Table 3.

Table 2: Infection rate of *T. anullata* according to sex.

Sex	No. of samples examined	No. of infected calves	(%)
Female	40	19	47.5
Males	50	17	34
Total	90	36	40
P-value	0.193931		
X^2	1.687500 NS		

NS: Non-significant differences at (P≤0.05).

Table 3: Infection rates of *T. anullata* according to age groups.

Age group	No. of samples	No. of infected calves	(%)
<1 M	24	15	62.5
1-2 M	23	9	39.1
2-3 M	20	4	20
3-4 M	22	5	22.7
≥4 M	21	3	14.3
Total	90	36	32.7
P-value	0.003291*		
X^2	15.805652		

*: Significant differences at (P≤0.05).

Infection rate of *T. anullata* according to months of study

July recorded 46.7% infection rates. The highest infection rate 66.7% was recorded in August and September, 33.3% in October, while the lowest infection rate 13.3% was recorded in November and December as shown in Table 4.

Table 4: Infection rate of *T. anullata* according to months of study.

Month	No. of samples	No. of infected calves	(%)
July	15	7	46.7
August	15	10	66.7
September	15	10	66.7
October	15	5	33.3
November	15	2	13.3
December	15	2	13.3
Total	90	36	40
P-Value	0.002556*		
X^2	18.333333		

*: Significant differences at (P≤0.05).

Molecular study

The current study results revealed the occurrence of *T. annulata* in the confirmed samples of blood from the calves. *T. annulata* is a tick-borne pathogen that causes a massive economic and health impact in calf herds, contributing to significant death rates and damage to efficiency as show in Figure 1 (Gomes *et al*., 2013).

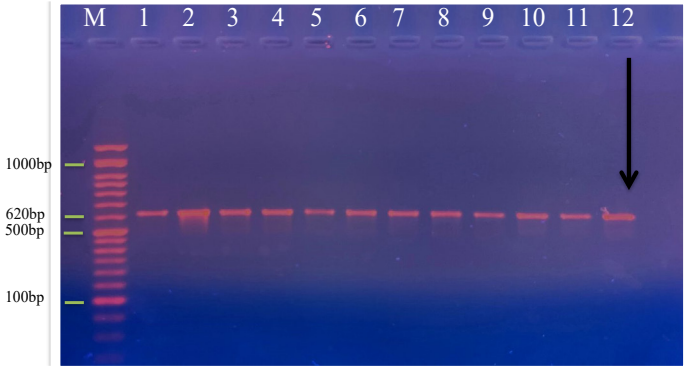

Figure 1: Agarose gel electrophoresis PCR product of 18S ribosomal RNA (18SrRNA) in *Theileia annulata*. Where; M: Marker (1000-100bp), lane (1-12).

In the current study, many epidemiological aspects of a herd of calves of both sexes in Babylon Province were examined. This demonstrated a greater prevalence of female *T. annulata* infection (47.5%) compared to male infection (34%). Although there were more males than females in this study, this contradicts Valente *et al*. (2023), who found that females were more likely to be infected with *T. annulata* than males were, and Calleja-Bueno *et al*. (2017), who found that newly younger calves are less likely to be exposed to infectious and parasitic diseases.

Moreover, in this study, the possibility of infection with *T. annulata* in calf decreases suggestively with increasing age. Results showed that the highest infection rate (62.5%) was documented at <1 M, 39.1% at 1-2 M, 20% at 2-3 M, 22.7% at 3-4 M, and the lowest 14.3% infection rate was documented at ≥4 M.

Furthermore, the infection rate of *T. annullata* in the present study was recorded according to months of the year: in July, was 46.7%; in August and September, the highest infection rate was 66.7%; in October was 33.3%; and the lowest infection rate was 13.3% in November and December. This variation in infection in this study month may be due to the spread of tick-borne diseases and higher temperatures (Moumouni *et al*., 2015).

According to particular studies on many classes of ticks, habitats with higher temperatures also result in more eggs being produced and eggs that are more likely to hatch (Esteves *et al*., 2015). In these cases, both the pre-oviposition phase and the oviposition time are shortened.

The findings from this analysis were in line with other research because PCR is an additional specialized approach

(Alhaboubi *et al*., 2017). Due to the different structural characteristics of piroplasms, False-negative detection of theileriosis by blood smear testing is common (Edith *et al*., 2018; Farooq *et al*., 2019).

To find out the parasite's prevalence in an enormous herd of cattle while disregarding the restrictions of blood smear testing, PCR-based molecular diagnostics may be done (Faraj *et al*., 2019; Al-Abedi and Al-Amery, 2021).

CONCLUSIONS AND RECOMMENDATIONS

There was a greater infection was established in female calves compared to males. Additionally, there had been a connection between the temperature of the environment and disease. These data will serve as a starting point for future large-scale epidemiological investigations on temperate theileriosis in calf herds.

ACKNOWLEDGMENT

The authors are thankful to the staff researcher for their support and for providing the facilities during the sample processing.

NOVELTY STATEMENT

The majority of this study has been devoted to the identification and measurement of T. annulata DNA in blood samples using molecular PCR technique. Ideas differ over the best objectives to employ, and the inadequacy of diagnostic tools make it challenging to compare labs.

AUTHOR'S CONTRIBUTION

Every author made an equal contribution.

Ethical statement

The writers took into account every ethical concern, such as presentation originality, plagiarism, and duplicate submission.

Conflict of interest

The authors have declared no conflict of interest.

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