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Prevalence with risk factors associated with *Theileria annulata* in cattle of Babylon province, Iraq

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Abstract

Theileriosis is one of the most important tick-borne haemoprotozoan diseases affecting cattle and causing significant economic losses worldwide, particularly in tropical and subtropical regions. The present study aimed to determine the prevalence of *Theileria annulata* infection in cattle in Babylon province, Iraq, and to evaluate the effects of sex, age, and geographical distribution on the infection rate using microscopic examination. A total of 120 blood samples were randomly collected from cattle (43 males and 77 females) from different regions of southern Babylon province, including Al-Qasim, Al-Hamaza Al-Gharbi, Al-Talia, and Al-Shomali, during the period from September 2025 to January 2026. Thin and thick blood smears were prepared, stained with Giemsa stain, and examined microscopically for the detection of *Theileria annulata*. Statistical analysis was performed using SPSS version 27 and Microsoft Excel, and the chi-square test was applied to assess the significance of differences among variables. The overall prevalence of *Theileria annulata* infection was 27.5% (33/120). A significantly higher infection rate was observed in females (37.6%) compared to males (9.3%) ($p < 0.05$). Age-related analysis revealed that cattle less than one year old had the highest infection rate (53.1%), followed by animals aged 1-3 years (19.1%), while the lowest rate was recorded in cattle older than three years (17%) ($p < 0.05$). Moreover, a statistically significant variation in infection rates was detected among the studied regions ($p < 0.01$), with the highest prevalence reported in Al-Shomali (61.5%).

Keywords: *Theileria annulata*, bovine, giemsa stained, blood smear, Babylon, Iraq

Introduction

Theileriosis is a haemoprotozoan disease transmitted via biting of ticks and this disease leads to major economic losses in livestock including cattle (Kundave *et al.*, 2015) [9]. Ticks (ixodid ticks) act as vectors in spreading of the disease in hot or moderate climates that inject *Theileria* spp. into the bovine blood. Eight different *Theileria* species cause bovine theileriosis including *Theileria annulata*, *Theileria parva*, *Theileria orientalis*, *Theileria velifera*, *Theileria lowrenci*, *Theileria taritrgi*, and *Theileria sergenli* that produce moderate or asymptomatic sickness in cattle (Khudor *et al.*, 2022) [8]. The most typical clinical signs initially are anorexia, elevated body temperature, enlargement of the superficial lymph nodes, pale mucous membranes, then iteration dehydration, saliva in the mouth, lacrimation, nose discharge, nervous signs, and breathing problems at the later stages (Faraj and Assi, 2022) [5]. The prevalence and active regions of vector ticks are critical components of bovine *Theileria* species epidemiology. The disease, which is difficult to completely eliminate, is associated with obvious regional and seasonal epidemics. Severe infections of cattle result in death, which causes considerable economic losses and potential threats to the cattle industry.

Materials and Methods

Sample Collection

120 Blood samples of cattle (43 male and 77 female) were collected during the period from (September 2025 to January 2026) randomly from the south of Babylon (Al- Qassim, Al-hamaza al-gharbi, AL-Talia and Al-shomali). Blood is usually collected from jugular vein of each cattle using vacuum tube then, 5 ml of blood was drawn by a sterile needle with a vacutainer EDTA- sterile tubes and immediately transported in the ice box for laboratory diagnosis (microscopic examination).

Microscopic Examination

Blood Smear

Blood samples were used to make thin and thick films, which were then allowed to air dry, fixed with 100% methyl alcohol for 15 minutes, and stained with 10% Giemsa stain for 30 minutes before being examined under an x40 light microscope, according to Rashmi (2013) ^[13].

Staining with Giemsa stain

Giemsa stain is one of the synthetic dyes used to differentiate parasites. Prepare blood film, air dry and place in a staining rack (put drop from blood and make Thin blood smear) the blood smear is fixed in absolute methanol for 3-5 minutes. - Staining is performed at room temperature for 15-30 -Wash thoroughly with running water. The smear surface is washed directly.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2010 and SPSS for Windows version 27. Using the chi-squared test, differences between groups were examined. Zhao *et al.* (2022) ^[17] used P-values less than 0.05 as the significance criterion for all statistical studies.

Results

The results of microscopic examination in the present study found that the total infection rate of *Theileria annulata* in cattle was 27.5% (33/120), as shown in Table (1).

Table 1: Total rate of *Theileria annulata* infection in cattle by microscopic examination in Babylon province

Number of examined animals	Number of infected animals	% of infected
120	33	27.5%

According to sex, the females had a greater overall infection rate (37.6%) with *Theileria annulata* than males (9.3%) (Table 2).

Table 2: infection rate of *Theileria annulata* according to sex:-

Sex	No. of examined animals	Positive samples	Percentage (%)
Female	77	29	(37.6%)
Male	43	4	(9.3%)
Total	120	33	(27.5%)
χ^2	11.13		
p-value	0.0008		

Statistical Significance ($p < 0.05$)

According to sex, the infection rate was higher in females (37.6%; 29/77) compared to males (9.3%; 4/43).

Table 3: Infection rate of *Theileria annulata* according to age by microscopic examination

Age group	No. of examined animals	Positive samples	Percentage (%)
Less 1 year	32	17	(53.1%)
1-3 years	47	9	(19.1%)
> 3 years	41	7	(17%)
Total	120	33	(27.5%)
χ^2	14.42		
p-value	0.00074		

($p < 0.05$) significantly differences among the infected and age

Highest infection rate of *Theileria annulata* in cattle was (53.1) % (17/32) at age group (less than 1 year), while moderate 19.1% (9/47) in age between (1-3 years) and the lowest infection rate was 17% (7/41) at age (more than 3years). Table (3).

According to table (3) statistically significant difference in *Theileria annulata* infection was observed among different age groups ($p < 0.05$), with the highest prevalence recorded in animals less than one year old.

Table 4: infection rate of *Theileria annulata* according to the regions of study different regions of Babylon

Regions	Number of examined animals	Number of positive animals	Infestation rate%
Al - shomali	26	16	61.5%
Al-hamaza al-gharbi	24	8	33.3%
Al-talia	31	4	12.9%
Al-Qasim	39	5	12.8%
Total	120	33	27.5%
χ^2	23.05		
p-value	0.00004		

Significant difference ($p < 0.01$)

These findings suggest that geographical factors play an important role in the distribution of *Theileria annulata* infection. The higher prevalence observed in certain regions may be related to differences in tick density, environmental and climatic conditions, animal management practices, and the effectiveness of control measure

Chi-square analysis revealed a statistically significant difference in *Theileria annulata* infection among the examined regions ($p < 0.05$).

Discussion

The present study revealed a relatively high prevalence of *Theileria annulata* infection among cattle in southern Babylon province, with an overall infection rate of 27.5%. This finding indicates that tropical theileriosis continues to represent a major parasitic challenge for cattle production systems in endemic regions. Similar prevalence levels have been reported in areas characterized by favorable environmental conditions for tick survival and transmission (Uilenberg, 1995; Jongejan and Uilenberg, 2004) ^[15, 7]. The higher infection rate observed in female cattle compared to males may be attributed to prolonged exposure to tick infestation due to longer retention of females within herds for breeding and milk production purposes. In addition, physiological stress associated with pregnancy and lactation has been shown to reduce immune competence, increasing susceptibility to tick-borne infections (Radostits *et al.*, 2007). Comparable trends were reported by Brown (1997) ^[3], who indicated that management-related stressors significantly influence the epidemiology of tick-borne diseases in cattle. Age-related analysis demonstrated that calves less than one year old exhibited the highest prevalence of *T. annulata* infection. This increased susceptibility in young animals can be explained by the immaturity of their immune system and the absence of acquired immunity against the parasite. Norval *et al.* (1992) ^[12] reported that repeated exposure to *Theileria* spp. leads to partial immunity in older cattle, resulting in reduced clinical disease and lower detectable parasitemia. Similarly, Dolan (1989) ^[4] highlighted that infections in adult cattle are often subclinical due to the development of immune tolerance. Marked regional variation in infection rates was observed in the current study, with Al-Shomali region

showing the highest prevalence. This variation strongly suggests the influence of environmental and ecological factors such as humidity, vegetation density, tick abundance, and grazing systems. Bishop *et al.* (2004) [2] emphasized that ixodid tick distribution is closely linked to climatic conditions, which directly affects the transmission dynamics of *Theileria annulata*. Furthermore, inadequate implementation of tick control measures may contribute to the persistence of infection in highly affected areas (Gharbi *et al.*, 2011) [6]. The significant association between infection prevalence and geographical location highlights the importance of targeted control strategies based on regional risk assessment. Integrated tick control programs, improved herd management, and early diagnosis are essential to reduce the economic impact of tropical theileriosis (OIE, 2018). Overall, the findings of this study confirm that *Theileria annulata* infection in cattle is multifactorial, influenced by host-related and environmental factors, and remains a major constraint to cattle productivity in endemic areas. The microscopic study by analysis of Giemsa-stained for blood samples, the confirms that *Theileria annulata* infection rate in cattle is widespread in Babylon province, recorded overall prevalence was 27.5%. And the data, rate of infection were significantly with the factors such as age, gender, and geographic. The Female showed a higher prevalence rate while the males were lowest. The young cattle in age groups one year old were the most affected highest prevalence rate in Al-Shomali, recorded the higher infection rate the role of environmental conditions and tick density in disease transmission. Further research the molecular diagnostic approaches are advised to improve detection accuracy and to better understand the epidemiology of *Theileria annulata* in Iraq.

Conclusion

The present study concluded that *Theileria annulata* infection is prevalent among cattle in Babylon province, with an overall infection rate of 27.5%, as confirmed by microscopic examination of blood smears. This finding indicates that bovine theileriosis remains an important parasitic disease affecting cattle health and productivity in the study area. The results demonstrated a significant effect of sex on infection rate, where females showed a markedly higher prevalence (37.6%) compared to males (9.3%) ($p < 0.05$). This difference may be attributed to physiological stress, pregnancy, lactation, and management practices that increase females' susceptibility to tick infestation and infection. Age was also identified as a significant risk factor for *T. annulata* infection ($p < 0.05$). Cattle less than one year old exhibited the highest infection rate (53.1%), suggesting that younger animals are more vulnerable due to their immature immune systems and limited prior exposure to the parasite. Furthermore, a statistically significant variation in infection rates was observed among different regions of Babylon province ($p < 0.01$). The highest prevalence was recorded in Al-Shomali, indicating that geographical location, tick density, climatic conditions, and animal husbandry practices play crucial roles in the epidemiology of bovine theileriosis.

Conflict of Interest

Not available

Financial Support

Not available

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