Research Article

A Survey Study on Sheep Theileriosis in Al-Fallujah City, Iraq

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Abstract | Theileriosis is a serious hemoprotozoal disease affecting both domestic and wild ruminants. The purpose of this study was to assess the prevalence of *Theileria* parasites in sheep within specific areas of the Al-Fallujah District, including Al-Fallujah Centre, Al-Shehabi, Al-Saqlawiyah, and Al-Karma. The study also aimed to analyze the impact of region, age group, month, and gender on infection rates during the period from November 2024 to March 2025. A total of ninety serum samples were collected from sheep blood and analyzed using a competitive enzyme-linked immunosorbent assay (ELISA). The overall prevalence of infection was 30%. Among the studied areas, the highest prevalence was recorded in Al-Saqlawiyah (50%), while the lowest was in Al-Fallujah Centre (16.6%), with a statistically significant difference ($P \leq 0.05$). Sheep older than 4 years exhibited the highest infection rate (35%), while the lowest rate was observed in the age group under 1 year (20%), also with a significant difference ($P \leq 0.05$). In terms of monthly variation, a significant difference ($P \leq 0.05$) was observed, with the highest infection rate in November (52%), followed by March (35.3%) and December (30%). February showed a 20% infection rate, while no cases were recorded in January. Regarding gender, females showed a higher infection rate (31.6%) compared to males (26.6%), with a statistically significant difference ($P \leq 0.05$). In conclusion, theileriosis was found to be relatively common in sheep, with significant variation based on location, age, month, and gender. This study is the first to report the prevalence of *Theileria* infection in sheep in the Al-Fallujah District using a competitive ELISA approach.

Keywords | ELISA, Sheep, Theileriosis, Al-Fallujah District, Iraq

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INTRODUCTION

Theileriosis is considered one of the most important protozoan diseases affecting animals in tropical and subtropical regions of the world (Irfan *et al*., 2023; Ali *et al*., 2024; Mustafa *et al*., 2024). In sheep, the disease is primarily caused by *Theileria ovis* and *Theileria herci*, which are transmitted by various tick species, including *Hyalomma* spp. and *Haemaphysalis* spp. (Mans *et al*., 2015). Infection with *Theileria* can lead to a range of clinical signs such as fever, coughing, lethargy, lymphadenopathy, emaciation, and in severe cases, death (Ali *et al*., 2020; El-Imam and Taha, 2015).

Theileria species can be identified through microscopic examination of blood and lymph node smears (Faraj and Assi, 2022). Several serological assays have been developed to detect recent infections, including the indirect fluorescent antibody test (IFAT), complement fixation test (CFT), indirect hemagglutination assay (IHA), and indirect enzyme-linked immunosorbent assay (iELISA), which has been widely used in seroepidemiological surveys of bovine theileriosis. These assays often target specific antigens such as the *Theileria luwenshuni* surface protein. Notably, the iELISA method demonstrated high diagnostic performance, with a reported specificity of 98.9% and sensitivity of 98.5% (Salman *et al*., 2014; Li *et al*., 2016).

In addition to serological methods, *Theileria* infection can also be detected using molecular techniques such as polymerase chain reaction (PCR) (Salih *et al*., 2024). However, traditional PCR methods may not effectively differentiate between clinically infected animals and carrier animals, as they do not quantify parasite load (Faraj and Assi, 2022). To address this limitation, quantitative PCR (qPCR) has been employed for more accurate diagnosis and quantification of *Theileria* species (Selim *et al*., 2021).

The present study aimed to detect *Theileria* infection in sheep using the ELISA method and to investigate the effects of location, month, age, and gender on infection rates.

MATERIALS AND METHODS

Collection of blood samples

Ninety blood samples were randomly taken from sheep aged less than one to four years, 30 males and 60 females, from different areas of Fallujah, Al-Shahabi, Al-Saqlawiyah, and Al-Karmah, for the period from November 2024 to March 2025 (Hussein *et al*., 2024).

For serological studies, 5 ml of jugular vein blood was collected after cleaning and disinfecting the area with (70%) ethyl alcohol. The blood was collected into sterile, anticoagulant-free tubes. Then allowed to clot, and the serum was isolated by centrifugation at 3,000 g/ 10 minutes. The separated serum was stored in 1.5 ml Eppendorf bottles at -20˚C until use.

Laboratory examination

An ELISA test kit for ovine *Theileria* (SunLong Biotechnology Co., Ltd., China) was used to detect antibodies in the serum samples. The procedure involved the standard ELISA steps: sample addition, incubation, washing, staining, and reaction termination. Serum samples were mixed with a diluent and sample buffer according to the manufacturer's instructions. After processing, the microplate was read using a microplate reader, and optical density (OD) values were recorded. The critical (cut-off) value was calculated based on the method described by Gao *et al*. (2002), following the protocols established for the assay.

Statistical analysis

A Chi-square test was used to analyze all data and compare the different factors affecting the infection rate. A *P* value of ≤ 0.05 was considered statistically significant (Kwak, 2023).

RESULTS AND DISCUSSIONS

According to Table 1, the overall infection rate with theileriosis in this study was 30%. This is comparable to infection rates reported in other studies, such as 33.8% by Aktas *et al*. (2005) and 22.8% by Al-Amri and Hasso (2002). The current prevalence is higher than the 7.5% reported in Mosul Governorate (Al-Fatly, 2012), but lower than the 52.4% recorded by Hamid and Al-Obaidi (2023). Similar variations have been observed in neighboring countries; for instance, Iran and the Sultanate of Oman reported infection rates of 11.9% and 17%, respectively (Dhim and Aaiz, 2014; Razmi *et al*., 2006). In Sudan, a slightly higher infection rate of 22.1% was documented (Muslih *et al*., 1988), whereas Egypt reported a much higher prevalence of 77.93% (Ahmed *et al*., 2003). These differences may be attributed to factors such as variations in sheep breeds, environmental conditions, the immune status of the animals, sample size, and vector density.

Table 1: Incidence of *Theileria* infection in sheep as detected by ELISA.

Number of samples	Positive	Percentage (%)
90	27	30

Our study revealed significant heterogeneity (P ≤ 0.05) in infection rates across different study areas. The highest prevalence was observed in Al-Saqlawiyah (50%), while the lowest was in Al-Fallujah Centre (16.6%) as shown in Table 2. These significant differences (P ≤ 0.05) could be related to variations in the distribution of vector ticks (hard ticks) across these regions, differences in immunity acquired from previous infections, or disparities in the regular use of treatments between areas. Tageldin *et al*. (2005) similarly reported that infection rates varied across different regions of Pakistan, largely due to differences in climate.

The highest prevalence of *Theileria* infection was observed in sheep older than 4 years (35%), followed by those aged 1–4 years (30%), while the lowest prevalence was recorded

in the age group under 1 year (20%), with a significant difference (P ≤ 0.05) (Table 3). This finding contrasts with that of Fadel *et al.* (2010), who reported a higher prevalence in lambs (78–85%) and a lower prevalence (9%) in adult animals. However, it aligns with Mohsin *et al.* (2022), who documented a low infection rate in lambs under six months of age. Similarly, Luo and Yin (1997) observed a greater incidence of infection in sheep older than three years. The lower infection rate in lambs may be attributed to the passive transfer of immunity from their dams (Naz *et al.*, 2012) or the protective effects of treatments administered to young animals (Khalaf, 2009).

Table 2: Prevalence of *Theileria* infection in sheep according to study areas.

Areas	Number of samples	Positive	Percentage (%)
Al-Karma	22	4	18
Al-Fallujah center	24	4	16.6
Al-Shehabi	20	7	35*
Al-Saqlawiyah	24	12	50*
Total	90	27	30

*significantly higher than other areas (P<0.05); X^2 value=16.0

Table 3: Age-related frequency of *Theileria* infection in sheep.

Age (year)	Number of samples	Positive	Percentage (%)
<1	20	4	20
1-4	30	9	30*
>4	40	14	35
Total	90	27	30

*significantly higher than other age groups (P<0.05); X^2 value=86.0

Regarding monthly prevalence, the highest infection rate was recorded in November (52%), followed by March (35.3%) and December (30%). February showed a lower infection rate of 20%, while no infections were detected in January. These differences were statistically significant (P ≤ 0.05) (Table 4). This finding contradicts the observations of Mohsin *et al.* (2022) and Ahmed *et al.* (2008), who reported no significant variation in *Theileria* infection rates across different months. The discrepancy in monthly infection rates in this study may be attributed to seasonal variations in temperature and humidity, which influence tick infestation levels (Salih, *et al.*, 2024).

The current investigation revealed a significant difference (P ≤ 0.05) in *Theileria* infection prevalence between females and males. Females showed a higher prevalence (31.6%) compared to males (26.6%), as presented in Table 5. This statistically significant association (P ≤ 0.05) between gender and infection may be attributed to stress factors related to milk production and gestation in females (Zahid *et al.*, 2005). Additionally, females may have a longer lifespan in the population since males are often sent to slaughter earlier. In contrast, Mohsin *et al.* (2022) reported no significant effect of gender on infection rates, which they attributed to equal exposure of both sexes to the same environmental conditions and vectors.

Table 4: Monthly distribution of *Theileria* infection in sheep.

Months	Number of samples	Positive	Percentage (%)*
November	23	12	52
December	20	6	30
January	15	0	0
February	15	3	20
March	17	6	35.3
Total	90	27	30
Total	90	27	30

*significantly varied between all months (P<0.05); X^2 value=15.6

Table 5: Gender-wise prevalence of *Theileria* infection in sheep.

Gender	Number of samples	Positive	Percentage (%)*
Male	30	8	26.6
Female	60	19	31.6
Total	90	27	30
Total	90	27	30

*significantly varied between both genders (P<0.05); X^2 value=6.1

CONCLUSIONS

The prevalence of theileriosis in sheep across different areas of the region was 30%, with significant variations observed among study locations, months, age groups, and sexes. This study provides the first scientific documentation of a high prevalence of theileriosis in sheep within these specific areas. Additionally, the findings highlight competitive ELISA as a reliable diagnostic tool for detecting infection in the vector host, offering valuable potential for improving epidemiological surveillance and informing control strategies in the future.

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NOVELTY STATEMENT

This study is the first to decomented a survey of the parasite Theileria spp. In sheep from Fallujah city, Iraq through

ELIZA techneq. The results highlight disease conditions and the influence of age, gender, and months, in addition to the different rural areas where animals are found.

AUTHOR'S CONTRIBUTION

All authors contributed to the manuscript equally in collecting blood samples, analyzing them using ELISA, organizing data, and academic writing.

Ethical approval

The study was approved by the Council of the College of Veterinary Medicine, University of Fallujah, under approval number [8], dated 25 May 2025.

Conflict of interest

The authors have declared no conflict of interest

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