

Clinical and Some Risk Factors Associated with *Theileria* spp in Cows of Salah AL-Din Governorate, Iraq

Rafal Ali Hamad,
Dr. Omaila Ibrahim Mahmoud ,
Dr. Maher Saber
Department of Microbiology, Collage of Veterinary Medicine,
University of Tikrit, Iraq
rafal-ali@tu.iq

Abstract

The current study aimed to diagnosis *Theileria* spp in cattle by using microscopic examination of blood smears , to record the clinical signs appearing on animals infection and to investigate the relationship between the infection and some risk factors such as sex ,age and presence of ticks on the animal. Fifty blood samples were collected during the period from September 2021 to the end of December 2022 from different areas in Salah Al-Din government (Al-Hamra, Albu Ajil, Baiji, AL-Alam). The animals were suffering from one or more of clinical signs (high temperature, lack of appetite, lethargy, pallor mucous membranes, superficial lymphadenopathy and tick infection) . The results showed that the total rate of infection was 40% . No significant differences between age groups, with 30% in animals that are less than a year and 16% 1-2 years and 10% in 2-3 years . and 4% in age $3 <$ The study registered a significant difference according to gender, the rate of infection in male was 22 % and in females was 18% .

Keywords: *Theileria* parasite, Clinical signs, Risk factors, Epidemiology, cows.

العوامل السريرية وبعض عوامل الخطر المرتبطة بطفيلي الثيليريا في
ابقار محافظة صلاح الدين –العراق

الخلاصة

هدفت الدراسة الحالية لتشخيص الإصابة بطفيلي *Theileria.spp* في الابقار باستخدام التقنية الجزيئية تفاعل السلسلة المتبلر PCR والتتابع الجيني للنوع الى جانب الفحص السريري والتشخيص المجهرى . هدفت الدراسة لمعرفة العلاقة بين الإصابة وبعض العوامل كالجنس والعمر و وجود القراد على ابقار محافظة صلاح الدين . جمعت 50 عينة دم للفترة من منتصف ايلول عام 2021 الى نهاية كانون الاول من عام 2022 من مناطق من محافظه صلاح الدين (الحمرة ,البو عجيل ,بيجي ,العلم) من ابقار كانت تعاني من واحدة او اكثر من العلامات السريرية التي تمثلت بارتفاع درجة الحرارة وقلة الشهية والخمول وشحوب الاغشية المخاطية وتضخم العقد اللمفية السطحية والإصابة بالقراد . اظهرت نتائج الفحص المجهرى للمسحات الدموية المصبوغة بصبغة كمزا ان نسبة الإصابة الكلية كانت 40% كما بينت النتائج اختلاف معنويا في نسبة الإصابة بين مجاميع الفئة العمرية, اذ بلغت 30 % في الحيوانات التي تكون اعمارها اقل من سنة و 16 % في الحيوانات التي

تتراوح اعمارها بين 1-2 سنوات و 10 % في الحيوانات التي تتراوح اعمارها بين 2-3 سنوات و 4% في الحيوانات التي يتجاوز عمرها 3 سنوات . وجود فرق معنوي بين العمر ونسبة الاصابة . سجلت الدراسة فرقا معنوياً بالنسبة للجنس ومعدل الاصابة حيث بلغت نسبة الاصابة في الذكور 22% وفي الاناث 18%.

1-Introduction ;

The genus of *Theileria* includes many numbers of protozoan parasites that infect wild and field ruminants in the tropical and subtropical regions of the world [1]. The infection with blood parasites represents the most important major problems in livestock breeding due to economic losses that lead to a decrease in animal production and a high rate of mortality [2]. The geographical distribution of each species is determined by the distribution of vector ticks *Theileria* spp . undergoes sequential development in white blood cells and erythrocytes in two stages (Schizont, piroplasm it is in mammalian host), there is great variation in pathogenesis between species that infect ruminants, some of which are relatively benign in While others cause severe clinical disease [3] . The most important species that infect cows are *Theileria pava* and *Theileria annuleta* , Which cause a high level of mortality [2]. Bovin theileriosis is one of the most important parasitic diseases transmitted by ticks [4]. The disease was first discovered by researchers Theiler Arnold and Dschunkowsky , who described the disease for the first time in 1904 [2]. The parasite *Theileria parva*, which belongs to the Apicomplexa group , which is the causative agent of East Coast fever and kills more than one million head of livestock annually [5] . The sporozoite of this parasite are found in the salivary glands of infected ticks, where they are transmitted to host by feeding , at this stage, the sporozoites stage enters the lymphocytes and then develops into the schizont stage. this stage, induces a neoplastic transformation of the infected lymphocytes. Reproduction of infected cells and the occurrence of an immune response lead to the appearance of clinical signs. [6] . Which includes fever, anemia, abortion , lack of milk production, enlarged lymph nodes, shortness of breath and death [7].

In Iraq, theileriosis represents a major challenge and threat to the livestock industry. Therefore, many studies have been conducted in various governorates such as: Erbil, Sulaymaniyah, Baghdad, Hilla, Qadisiyah, and Basrah [8]. The current study aimed to investigate *Theileria* sp. In cattle in some areas of Salah Al-Din Governorate and its relationship with some risk factors.

Materials and Methods

Collecting samples

Fifty blood samples were collected from cattle of different ages and from some regions of Salah Al-Din Governorate, including Al-Hamra, Albu-Ajeel, and Baiji, which were suffering from some of clinical signs that were confirmed after clinical

examination which include ; lethargy, lack of appetite, high Fever, mucous membrane pallor, superficial lymphadenopathy in some cases, and tick infestation [9].

Blood samples and microscopic examination

Two ml of blood was drawn from the jugular vein using sterile plastic syringes, and blood smears were prepared by placing a drop of blood using a capillary tube on the surface of a clean glass slide and passing it through the slide diffuser at an angle of 45° and fixed with methyl alcohol 70% for 3-5 minutes, left to dry and then stained with Giemsa stain for 30 minutes and then examined under the microscope using the lens oil immersion lens X100 [9].

3- Results ;

General clinical examination

The most important clinical signs of *Theileria spp.* were recorded, which were observed in infected animals, which were included , enlargement of the lymph nodes in front of the shoulder blades, mucous membranes pallor, body temperature rising to 40°C, emaciation, lethargy, opacity of the cornea of the eye, and the presence of ticks on the udder, tail, or hind legs of the animal. as shown in Table (1)

Table 1: Clinical symptoms of animals infected with *Theileria sp.*

Clinical signs	The number of infected animals	Percentage%
High temperature	50	100%
Enlargement of lymph node	50	100%
Loose of appetite	45	90%
Hard breathing	22	44%
Corneal opacity	10	20%
Bloody diarrhea	3	6%
Paleness of the mucous membranes	25	50%
Present of ticks on animals	29.2	58.8%

Examination of blood smears

The results of the microscopic examination of the blood smears stained with the Giemsa stain showed the presence of the *Theileria* sp. parasite inside the red blood cells in its various forms, annular and comma-shaped, in addition to the change in the shapes of the blood cells as in the Figure (1). The infection rate was 40 %, as shown in Table 2)

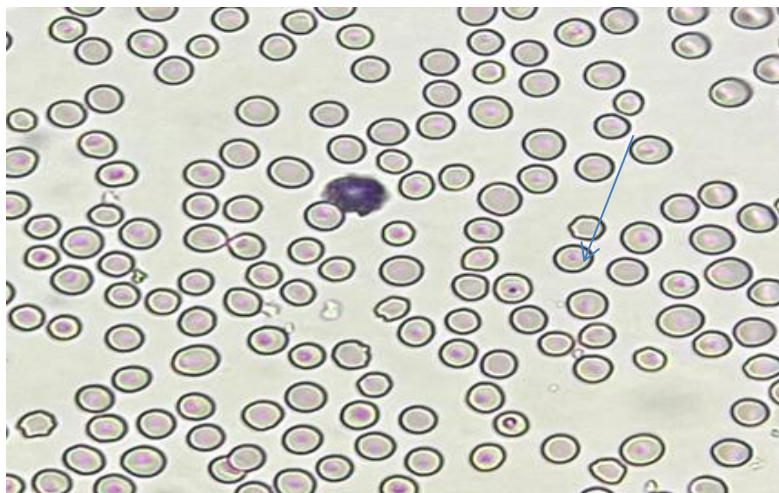


Figure 1; of a blood smear showing the presence of *Theileria* .sp parasite inside red blood cells Giemsa 100X

Table (2) Percentage of infection with *Theileria* parasite in cows using microscopic examination of blood smears

Examination method	Number of exanimated samples	Number of positive samples	percentage %
Microscopical examination of Blood smears	50	20	40%

Rate of infection according to the age and sex

The current study recorded the highest infection rate in young animals whose age exceeds one year, 30% (15/20) and 16% (8/15) in animals whose ages ranged between 1-2 years and 10% (5/10) in animals ranging from Their ages range between 2-3 years, as shown in Table (3)and figure 2 . The infection rate in males was 8% (4/ 15)and in females 52 %(26 /35)as shown in Table (4) and Figure(3)

Table(3)Rate of infection according to the age

Age/Year	The number	Positive cases	percentage %
<1	20	15	30 %
1-2	15	8	16%
2-3	10	5	10 %
3<	5	2	4 %
The Total	50	30	60%
χ^2	3.403		
P value	P >0.33		

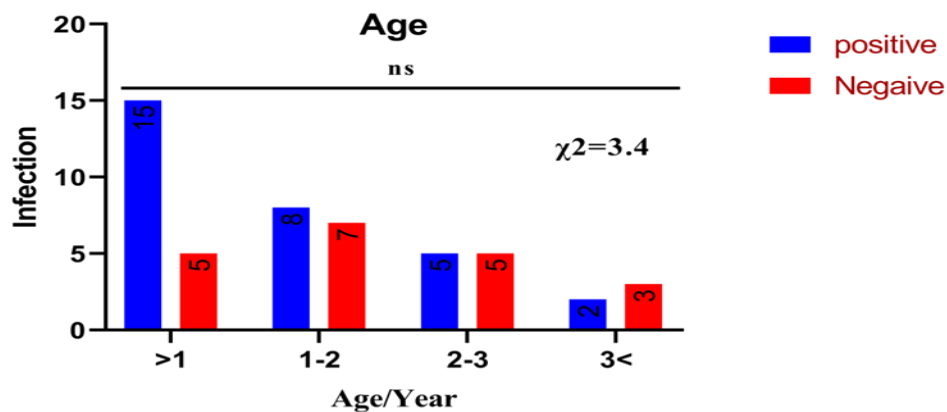


Figure (2) Number of cattle infected with the parasite according to the age of the animal

Ns: There is a significant difference between age and infection with Theileria parasite

Table (4) The rate of infection according to the sex

Animal sex	The number	The positive number	Percentage of positive cases/rows	Percentage of negative cases/rows
Female	35	26	52%	18%
Male	15	4	8%	22%
The total	50	30	60%	40%
χ^2		9.921		
P value		0.0016		

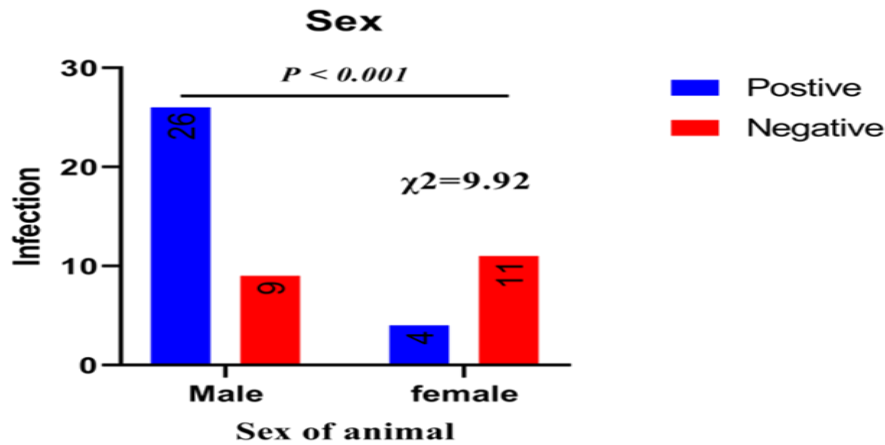


Figure (3) Number of cattle infected with the parasite according to the sex of the animal. There is a significant difference ($P < 0.001$) between the sex and the infection with Theileria parasite

Table(5) The rate of infection according to the area

Area of infection	Number of sample	percentage%
ALbu Ajil	20	40%
Baiji	5	10%
AL-Alam	10	20%
AL-Hamra	15	30%
The total	50	100%

Table(6) The rate of infection according to the months

Months	Number of sample	percentage%
May	10	20%
June	15	30%
July	20	40%
August	5	10%
The totle	50	100%

Discussion

The current study recorded clinical signs associated with *Theileria* infection observed in infected animals, which was represented by enlargement lymph nodes, high temperature to 40 °C, loss of appetite, hard breathing, corneal opacity, bloody diarrhea, general weakness, presence of ticks on the udder and tail and the hind legs. These signs are close to what was recorded by Nuri, [10] and compatible with Muhanguzi *et al.* [7] in their study. The rise in temperature occurs as a result of high blood parasitic level that leads to free generators of fever as a result of analysis of lymphocyte cells [11] where height of temperature leads to anorexia [12], as well as hypertrophy of the superficial lymph nodes as a result of the proliferation of parasite within the lymphocytes, as infected cells stimulate massive irregular proliferation of T-lymphocytes reactive follicular hyperplasia and reticular endothelial hyperplasia in the affected node [13]. Mucous membranes appear pale as a result of reduced red blood cells and hemoglobin concentration [14]. Hard breath occurs as a result of the accumulation of protein fluid in the alveolar spaces and enlargement of the pulmonary veins and infiltration of inflammatory cells within the interstitial tissue of the lung [15]. show some neurological signs that occur as a result of clutching capillaries in the central nervous system by infected cells [16]. The severity of the infection It depends on its virulence parasitic, host susceptibility level, and parasite strain [17] AL-Khalid [18] that the sensitivity of the host depends on the age, strain and immune situation of the host. The imported strain show high sensitivity to the disease while in local animals shows high resistance against disease

Microscopic Diagnosis

The current study showed that the rate of infection in cattle was 40% by using microscopic examination of blood samples stained with Giemsa, where this result is agreed with Ban Abdul-Hussain [19]. The prevalence was 51.5 % in Baghdad, while this result was higher than reported by Haider and Qassim [20]. which was 41.42 % in a study conducted in Dhi Qar Approach to Kawan 2019 [21], which was 35% in the Baghdad governorate, The result of the current study less than it [22] Which was 69% in Basra. In neighboring and Arabic countries, the rate of infection was included: Amman 72% [23] Sudan results were 70%, [24] and 69% in Iran [25] which were higher than in Libya 22.7 % by [26] and in Tripoli and In foam, Angella 35.4 % and in Egypt 38% by [27] Higher than [28] in Saudi 15%. In Pakistan 12.8% 29] in Algeria 11.69% [30] and in India was 1.6% [31] Environmental conditions are a major factor epidemiology of infection. where climate plays an important factor in the spread of the disease because it controls the activity and reproduction of the vector ticks [32]. where the peak of the parasite spread in Summer and Autumn [33] Previous studies showed that temperature and relative humidity are among the most important environmental factors affect the lifecycle cycle of the parasite [34]. Also the spread of

the disease depends on the geographical area and other factors such as climatic conditions, tick reproduction, age, sex, management, management and immunization and affected the spread is also affected by the breed of the livestock in tick resistance and susceptibility to infection [35]. as well as, this difference is due to several reasons for this for low parasitism in the blood and misguided of the parasite and carriers of the disease [13].

The study showed that all ages have the susceptibility to the infection with *Theileria* where the study . the highest infection rate in Young animals . Also ,in another study whose is less than a year 30% while recorded 16% in the ages of 1-2 and 10% in the animals between the age 2-3 years and 4 % in age 3< ,these results are identical to was mentioned AL-Hasnawi 2006 [36] that the proportion of infection is higher than in Yong animals. Also ,in another study [20] indicated that the disease affects calves. Which range from 2-3 weeks as mentioned [37] The highest infection rate in animals under six months was 69% compared to 22.27% in the animals above six months while he pointed out [36] indicated The animals aged between 2 to 3 years are higher rate compared to the youngest , that which agreed with Farooq [38] as it indicated that the highest infection rate was in the age of more than two years , followed by age groups from 6 months to two years , where he disagreed with AL - Khalidi [17] , which confirmed that large animals were carrier of infection most often and showed resistance against the disease, the presence of antibodies in young animals may be a cause of low infection in small ages [39] as for the relationship of sex and infection the study showed the infection it can affect both sexes but in varying degrees, the incidence in male rate was 52% in females 8% in male close this result was close with other studies registered in Male 52% and in females 97.94% [20] in females and in males 2.6% [40] and that can be explained through the fact that females It have higher a hormonal pressure , they are less immune and more susceptible [41] as the infection of hard ticks in large quantities leads to great stress and anemia, which inhibit the immune response to the host and reduces productivity, leading to losses in meat and milk production and increase the infection rate of diseases [42].

Conclusion ;

Based on the results of the research , the *Theileria* spp is wide spread in Iraq especially in tikrit Governorate

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