

ISOLATION OF THEILERIA AND BABESIA FROM GUT AND OVARY OF HARD TICKS: *Hyalomma a. anatolicum* IN BAGHDAD.

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ABSTRACT

The application of thick blood smear technique based on Giemza- stain confirmed the presence of high rate of ticks infection 58.3% is revealed to endemic area of Theileriosis and Babesiosis in Iraq. Abdomen area (gut and ovary) of hard ticks: *Hyalomma a. anatolicum* revealed high rate of infection with *Theileria* sp.43% and *Babesia* sp. 15.2% . *Babesia* was recorded for the first time in Iraq from ticks.

Females appeared positive smears more than males that total rate of infection (39) 54.1%. Distribution of infection rate in ticks was discussed with two periods of collection from May to October 2009. We concluded from this study that *Hyalomma a. anatolicum* tick at least one of *Theileria* & *Babesia* sp. Infective for cattle in Iraq; and this technique to be useful in identifying the species of protozoa in potential tick vectors.

Keywords: *Babesia*, *Theileria*, hard ticks, *Hyalomma anatolicum* , salivary gland.

INTRODUCTION

Parasitic infections may be carried from one host to another by means of arthropod vectors. Many *Theileria* species cause diseases in cattle, of which one of the most economically important is East Coast fever (ECF), caused by *T. parva*. A more comprehensive review of the subject has been published by Irvin (1987). The distribution of the two most important cattle species, *T. parva* and *T. annulata*, correlates well with the distribution of their vectors. *Theileria parva* is transmitted predominantly by *Rhipicephalus appendiculatus*, which is restricted to eastern, central and southern Africa; *T. annulata* is transmitted by several *Hyalomma* species, which are distributed widely over North Africa, southern Europe, the Middle East, India, southern Russia and China.

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There is virtually no overlap of these vectors and therefore the possibility of confusing these two parasites is low. (Conrad *et al.*,1987).

Babesiosis, caused by infection with intraerythrocytic parasites of the genus *Babesia*, is one of the most common infections of free-living animals worldwide and is gaining increasing interest as an emerging zoonosis in humans. (Mary *et al.*,2000) . All babesial parasites described to date are transmitted by ixodid ticks to their vertebrate hosts. Recognized *Babesia* species of domestic animals by Kuttler(2004). The parasites replicate in the vertebrate hosts' red blood cells and are called piroplasms due to their pear-shaped appearance when within the infected host cells.(Kakoma and Mehlhorn,1993; Telford *et al.*,1993)

In Iraq, the vectors of Theileriosis in cattle were: *Hyalomma a. anatolicum* 94.4%, *Boophilus annulatus* 4.4% ,*Rhipicephalus sanguineus* 1.4%.(Tarish ,1982).

This study investigated the presence of some species of protozoa in the abdomen (gut and ovary) of hard ticks: *Hyalomma a. anatolicum* as the widest distribution of cattles in Iraq.

MATERIALS AND METHODS

A total of 72hard ticks (9♂, 63♀) were collected from udder and perioneal region of cattles, their age between 1 – 3 years in Rashidia – Baghdad, for two periods: first period during May & July, second period during September & October 2009.

All the ticks are diagnosed as *Hyalomma a. analolicum* by Iraqi Natural History Research Center and museum. Thick blood smear applied with simple modified as follow:

- 1- Inject tick with drop of Dextrose slain.
- 2- Cut anterior section of ticks, apply of blood drop on clean slide and leave it to dry.
- 3-Cut posterior section, apply of blood drop on clean slide and leave it to dry.
- 4-Fix the slides with absolute methanol for 5 min.
- 5-Dry the slides.
- 6-Put the slides in Giemsa- stain (10%) for 1 hour.
- 7-Wash the slides with tap water.
- 8-Examined under light microscope with high power100X.

RESULTS AND DISSCUSION

Microscopic of Giemsa-stained blood films revealed the presence of numerous *Theileria* and *Babesia* piroplasms for both sexes. Table 1.

Table 1: Number of positive Ticks and percentage of infection ticks with *Theileria* and *Babesia* species.

protozoa	No. of positive ticks	% of infected	No. of ♂ infected	% of infected	No. of ♀ infected	% of infected
<i>Theileria</i> sp.	31	43%	3	33.3%	28	44.4%
<i>Babesia</i> sp.	11	15.2%	—	—	11	17.4%
total	42	58.3%	3	4.1%	39	54.1%

*Significant difference $p < 0.05$ between two sex.

The current study, described the application of thick blood smear technique based on Giemsa- stain to detect the possible presence of protozoa species in ticks. Giemsa-stained blood films confirmed the presence of numerous *Theileria* parasites. (Chae *et al.*,1998)

The total rate of ticks infection 58.3% is high rate revealed to endemic area of Theileriosis and Babesiosis with or without appeared of clinical signs as in Iraq. Females appeared positive smears more than male as result for large quantities of blood that sucking from animals; that similar with Tarish(1982) who recorded *Theileria annulata* 65.1% in female and 34.9% in male of *Hyalomma a. anatolicum* from cattle.

Abdomen area (gut and ovary) of ticks revealed high rate of infection with *Theileria* 43% and *Babesia* 15.2%, that mean reproduction process may be happened in gut of ticks in infected with *Theileria* and migrated to ovary of ticks in infected with *Babesia* (Siegmund *et al.*,2010).

Giemsa –stained blood film from *Hyalomma a. anatolicum* showing polymorphic *Theileria* parasite forms: Ring form (Fig.1), and multiple parasites are seen within hemolytic erythrocytes (Fig.2). *Babesia* forms appear as annular(Fig.3), pear- shaped(Fig.4) and bicornate form (Fig.5);The appearance of all these forms in ticks indicate that Iraq still now a day endemic area of Theileriosis & Babesiosis.

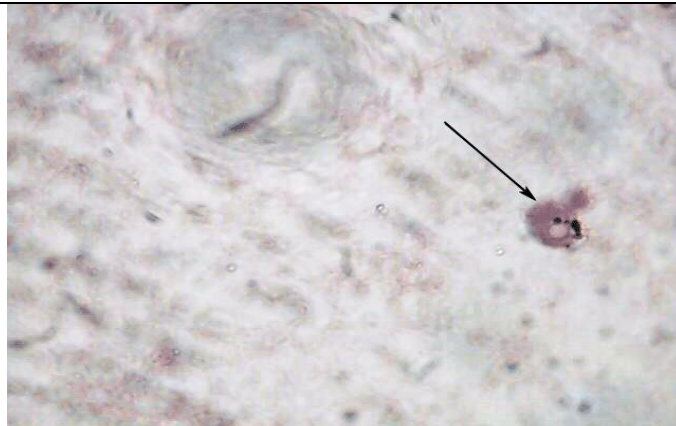


Fig.1: Ring form of *Theileria sp.* in ticks. 100X.

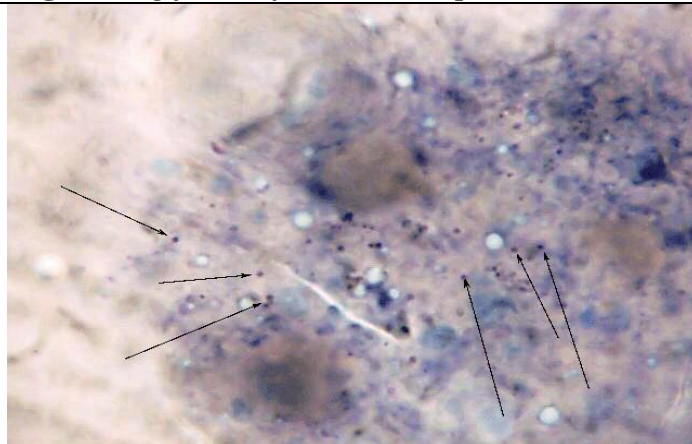


Fig.2: Multiple *Theileria sp.* are seen within hemolytic erythrocytes in ticks. 100X.



Fig.3: Annular form of *Babesia sp.* in tick. 100X.

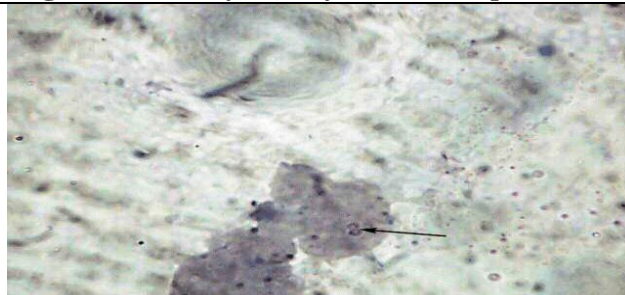


Fig.4: Pear – shaped of *Babesia sp.* in tick. 100X.

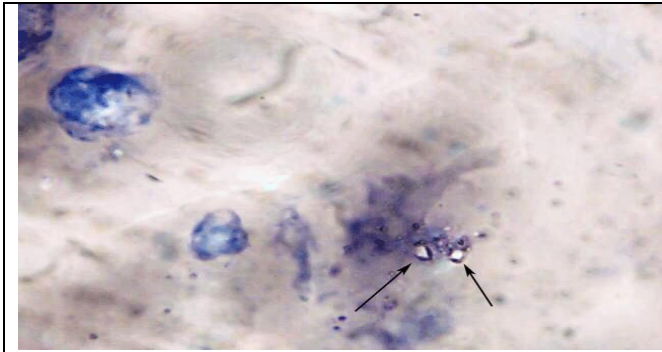


Fig.5: *Bicornate form of Babesia sp. in ticks.100X*

The finding of *Babesia* sp. from abdomen of ticks is significant, because the definitive host is the tick ;Once ingested by an appropriate tick, gametes unite and undergo a sporogonic cycle resulting in sporozoites. Transovarial transmission has been documented for " large" *Babesia spp.* but not for the "small" *Babesia*, such as *B. microti*.(Zintl *et al.*, 2003) .

Distribution of infection with *Babesia* appeared highly significant differences between two periods. Table 2. Fig.6

Table 2: *Distribution of infected ticks during two periods of 2009.*

Protozoa	First period (May & July) 34 ticks No. positive	%	Second period (Sept. & Oct.) 38 ticks No. positive	%
<i>Theileria</i> sp.	14	41.1%	17	44.7%
<i>Babesia</i> sp.	1	2.9%	10	26.3%
Total	15	44.1%	27	71%

**Significant difference $p < 0.01$ between two periods.*

The second period (September & October) of testing ticks revealed high rate of infection 71% with *Theileria* & *Babesia* sp. that may be because decreasing in the immune of cattle due to the peak of the infection with Theileriosis & Babesiosis in these months.

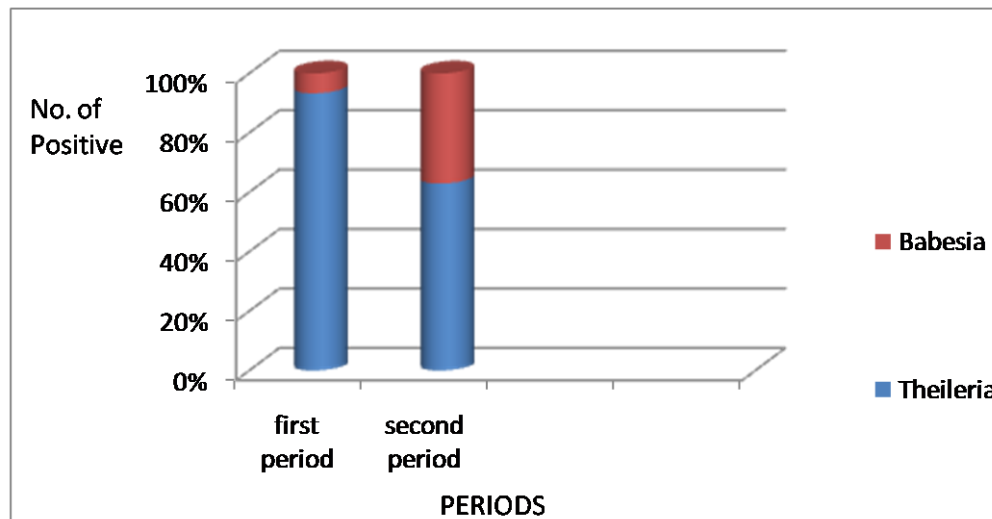


Fig.6: Distribution of infection of ticks during two periods 2009.

There is no significant difference between two periods of infected with *Theileria* sp; that signal for endemic disease in cattle in Iraq. The mortality rate for tropical theileriosis can also vary from 3% to nearly 90%, depending on the strain of parasite and the susceptibility of the animals. (Pipano and Shkap,2000) There is highly significant difference between two periods of infected with *Babesiosis* sp; that mean the infection may be begun in first period (May & July) and reach the peak at second period (September &October). (Fig.1)We concluded from our results that *Hyalomma a. anatolicum* tick at least one *Theileria* &*Babesia* sp. Infective for cattle in Iraq; and thick blood smear with Giemsa technique to be useful in identifying the species of protozoa in potential tick vectors.

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عزل التاييليريا والبابيزيا من القناة الهضمية والمبيض للقراد الصلب نوع هايلوما اناتولكم اناتولكم في بغداد.

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المستخلص

أظهر فحص المسحات السميكة لدم القراد المصبوغ بالكمزا انتشار التاييليريا والبابيزيا بنسبة كلية ٥٨.٣% تشير الى توطن المرضين في العراق. وجود طفيلي التاييليريا بنسبة ٤٣% وطفيلي البابيزيا بنسبة ١٥.٢% في منطقة البطن (القناة الهضمية والمبيض) للقراد الصلب نوع هايلوما اناتولكم اناتولكم، علما إن تسجيل البابيزيا من القراد لأول مرة في العراق. أظهرت مسحات دم إناث القراد نسبة إصابة أعلى من الذكور بلغت (٣٩) ٥٤.١%. ناقشت الدراسة توزيع الإصابة خلال الأشهر من أيار إلى تشرين الأول من عام ٢٠٠٩. نستنتج من الدراسة ان القراد الصلب نوع هايلوما اناتولكم اناتولكم ناقل حيوي لمرضي التاييليريا والبابيزيا في الابقار في العراق، والذي ظهر من فحص الدم المصبوغ بطريقة الكمزا .

الكلمات المفتاحية: البابيزيا، التاييليريا، القراد الصلب، هايلوما اناتولكم، الغدد اللعابية.