10.21608/avmj.2025.360233.1583 Assiut University web-site: www.aun.edu.eg

ROLE OF HARD TICKS IN TRANSMISSION OF *THEILERIA* IN IMPORTED CAMELS

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Received: 19 February 2024; Accepted**:** 16 April 2025

ABSTRACT

Theileriosis is one of the most prominent tick-borne haemoparasitic diseases affecting camels. Microscopic analysis of Giemsa-stained blood smears obtained from 100 tick-infested imported camels at Daraw quarantine, Aswan Gov., Egypt, showed 42% (42/100) were infected with *Theileria sp*. Moreover, infection rates significantly increased in autumn and spring (83% and 78%, respectively) compared to winter's 44%, while it notably declined during summer to 27%. Both erythrocytic and lymphocytic forms of *Theileria sp.* in the affected camels were described. The erythrocytic forms were identified as ring, rounded, and rod shapes, and macro and microschizonts were observed in lymphocytes. Four species of ticks were recognized throughout the monitoring period, all of which were molecularly confirmed to harbor *Theileria sp.* stages. *Hyalomma dromedarii* was the predominant tick species (90.1%), while other tick species present in small numbers were *H. excavatum* (4.9%), *H. rufipes* (2.5%) and *Amblyomma variegatum* (2.5%). Four distinct developmental stages were observed in the stained midgut smears of ticks: immature zygotes, early zygotes, developing kinete in zygote, and undifferentiated kinete. Additionally, the haemolymph revealed mature motile kinete, multinucleated rod-shaped vermicules and banana-shaped multinucleated vermicules. Multinucleated polymorphous sporoblasts were noted in the salivary gland smears. The accuracy of the microscopic detection of *Theileria sp.* in ticks was determined to be 90%, with specificity and sensitivity recorded at 87.5% and 100%, respectively. Overall, our results concluded that *Hyalomma sp.* and *Amblyomma variegatum* collected from imported camels were reservoirs of *Theileria sp*. Additionally, the present study confirmed the high prevalence of theileriosis in imported camels.

***Keywards*:** *Theileria sp*., camels, tick, *Hyalomma sp., Amblyomma variegatum*

INTRODUCTION

Camels play a significant role in Egypt's economy and cultural heritage by providing milk, meat, and wool (Salman *et al.,* 2022). They can effectively utilize limited food resources for their maintenance, growth and milk production, making them an integral part of nomadic lifestyles (Joshua *et al.,* 2008). According to the World Population Review (2022), there are approximately 164,000 camels in Egypt, while the global population stands

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at approximately 35 million (FAOSTAT, 2019). In Egypt, there has been a consistent increase in the slaughtered camel for meat (El-Naga and Barghash, 2016).

Camels are resilient animals that can endure the harsh conditions of desert environments due to their physical traits and unique physiological features, making them crucial for human survival (El-Naga and Barghash, 2016; Abass *et al.,* 2022). However, they are susceptible to various parasitic, bacterial, and viral infections (Selim *et al.,* 2023).

In both domestic and wild camel populations, infestations of hard ticks often result in serious illness and fatalities. Tick infestations can also cause skin irritation, affect appetite, impact body condition, damage the skin, and play a significant role in the transmission of various pathogens, including protozoa. *Theileria sp.* is considered one of the most important tick-borne blood parasites in ruminants (Abdally, 2008; El-Seify *et al.,* 2011).

Theileria sp. is an obligatory intracellular protozoan parasite transmitted by ticks. *Theileria* spp. are apicomplexan protozoa in the order Piroplasmida, family Theileridae, and genus *Theileria*, that infect lymphocytes and RBCs of a wide range of animal species, including camels found in tropical and subtropical areas (Mahran, 2004; El-Kelesh *et al.,* 2011; Youssef *et al.,* 2015).

During tick infestation, *Theileria* parasites enter the host as sporozoites, which quickly penetrate mononuclear leukocytes, develop into macroschizonts and stimulate the host cell's proliferation. Macroschizonts continue to mature into microschizonts and finally into merozoites, which are released from leukocytes. Merozoites then penetrate erythrocytes and transform into piroplasms (Youssef *et al.,* 2015).

Yakimoff *et al.* (1917) reported theileriosis in camels for the first time in Russia, naming it *Theileria camelensis*. The initial record of *Theileria* infection in camels in Egypt was documented by Mohamed (1935), who identified *T. camelensis* in camels in Aswan.

Numerous studies have documented infection rates of camel theileriosis in Egypt, ranging from 1.4% to 71.9% (El-Seify *et al.,* 2011; Hamed *et al.*, 2011; El-Naga and Barghash, 2016; Salman *et al.,* 2022).

Theileriosis leads to substantial economic losses in camel production and reproduction by reducing the quality of milk, meat, and other animal byproducts (Sazmand and Otranto, 2019; Selim *et al.,* 2023).

Clinically, the symptoms can range from moderate to severe; infected camels may exhibit weakness in the hind limbs, weight loss, swollen superficial lymph nodes, intermittent diarrhea, anemia shown as pale mucous membranes, excessive tear production, fever, loss of appetite, and leukopenia, which diminishes their resistance (El-Refaii *et al.,* 1998; El-Kelesh *et al.,* 2011; Hamed *et al*., 2011).

Acute cases of theileriosis are diagnosed based on clinical signs observed in infected animals, while confirmation relies on the microscopic examination of thin blood and lymph node smears stained with Giemsa, which remains the most common and reliable method of diagnosing the disease (Hamed *et al.,* 2011; Omer *et al*., 2021). Molecular techniques have increasingly become an essential tool for investigating the epidemiology and diagnosis of the disease (El-Naga and Barghash, 2016).

Therefore, the present study aimed to determine the prevalence of theileriosis in imported camels and identify the possible tick vectors of *Theileria sp.* through parasitological and molecular investigation.

MATERIALS AND METHODS

Ethical Approval:
The study adhered to the guidelines of the Ethical Committee of the Faculty of Veterinary Medicine, Assiut, Egypt, in accordance with the OIE standards for the use of animals in research (Approval no. 06/2024/0286).

Survey of Camels:
In this study, a total of 100 one-humped camels from Daraw quarantine in Aswan Governorate, Egypt, were examined during the period from October 2023 to September 2024.

Blood sample collection:
Following the protocol outlined by El-Naga and Barghash (2016), blood samples were collected under controlled conditions and promptly transported to the Animal Health Research Institute.

Tick collection and preservation:
Ticks were gathered from inspected camels by detaching different types of ticks from various areas of their bodies. All visible ticks were carefully removed by grasping them with curved forceps and rotating them anticlockwise (Youssef *et al.,* 2015). For smear preparation, some collected ticks were kept alive in separate plastic Falcon tubes with labels, while the rest were maintained in Falcon tubes with 70% alcohol for morphological and molecular identification (Youssef *et al.,* 2015; Hegab *et al.,* 2020). The collected ticks were transferred to the laboratory for further assessment.

Parasitological examination [blood smears]:
Thin blood smears were prepared from each blood sample and examined, according to El-Seify *et al.* (2011). The erythrocytic and lymphocytic forms of *Theileria* species were identified as previously described by Levine (1985) and El-Kelesh *et. al.* (2011).

Tick identification:
The collected ticks were permanently mounted following the methods of Pritchard and Kruse (1982) and Millar *et al.* (2000). Each tick was examined under a stereomicroscope for morphological characterization and identification. This process was conducted using the keys for hard ticks provided by Hoogstraal (1956) and Walker *et al.* (2003).

Dissection of the ticks:
A- Hemolymph collection for slide preparation:
Tick hemolymph smears were prepared according to the method described by Patton *et al.* (2012).

B- Salivary glands and midgut smears:
After immobilizing the tick, the salivary glands and midgut were removed following the methods described by Edwards *et al.* (2009) and Patton *et al.* (2012). Subsequently, they were crushed and spread on a glass slide for examination under a light microscope to detect *Theileria* stages (Youssef *et al.,* 2015).

Identification of *Theileria sp.* developmental stages inside ticks:
The morphological identification of *Theileria sp.* developmental stages in ticks was conducted in accordance with the protocols outlined by Young *et al.,* (1980) and El-Kelesh *et al.* (2011).

Molecular detection of *Theileria sp.* in ticks:
Ten tick samples, previously examined for *Theileria sp*. through parasitological methods, were further analyzed using PCR (Polymerase Chain Reaction) for confirmation. The samples were stored in labeled Eppendorf tubes containing 70% alcohol at -23°C.

A- DNA extraction.
According to QIAGEN, (2024), the QIAamp DNA Mini kit was utilized for DNA extraction from the samples. In brief, 25 mg of the sample was homogenized using TissueLyser with proteinase K (20

µl) and ATL buffer (180 µl), followed by incubation for three hours at 56 °C. The lysate was then mixed with AL buffer (200 µl), and incubated at 72 °C for 10 minutes, before adding 100% ethanol (200 µl). The lysate was centrifuged, washed, and eluted with 100 µl of the elution buffer provided in the kit.

B- Oligonucleotide Primers

The primers used were supplied by Metabion (Germany) and are listed in Table 1.

C- PCR amplification and Analysis of the PCR Products

The amplification and analysis of the PCR products were carried out as previously described by Nayel *et al.* (2012).

Table 1: Primer sequences, amplicon sizes, cycling conditions, and target genes.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
Theileria 18S rRNA	GTC TTG TAA TTG GAA TGA TGG CCA AAG ACT TTG ATT TCT CTC	370 bp	94˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 40 sec.	72˚C 10 min.	(Nayel *et al.,* 2012)

Statistical analysis:

The data was analyzed using the "GraphPad Prism, version 9.5.1 for Windows" software package (GraphPad Software, LLC, USA). The Chi-square test was utilized to compare categorical variables between the groups. Results were considered highly significant when the P-value was less than 0.001. Sensitivity, specificity, and accuracy were calculated using (MedCalc for Windows, version 22.0.18, (MedCalc Software, Mariakerke, Belgium, https://www.medcalc.org) (Altman, 1991).

RESULTS

Microscopic analysis of Giemsa-stained blood smears obtained from 100 tick-infested imported camels in Daraw quarantine during the study period showed that 42% (42/100) were infected with *Theileria sp*. (Table 2). Additionally, the infection rates significantly increased in autumn and spring (83% and 78%, respectively) ($\chi 2=12.35$, $p< 0.01$), compared to winter's 44%, while it notably declined during summer to 27% (Table 2).

Parasitological analysis of the camels' blood smears (Figure 1) identified both erythrocytic and lymphocytic (schizont) forms of *Theileria sp.* within the RBCs and lymphocytes of the affected camels. The erythrocytic forms were identified as ring shapes (1.2-1.4 µm), rounded forms (1.3-1.5 µm), and rod shapes (1.4-1.6 µm x 0.6 µm). Furthermore, macro and micro-schizonts were observed in lymphocytes, measuring 6-8.5 µm and 3.8-4 µm, respectively. Each had chromatin granules of sizes 1.0-1.25 µm and 0.45-0.6 µm in diameter, respectively.

Morphologically, the taxonomic identification of tick specimens taken from the inspected camels showed that four species of ticks were recognized throughout the monitoring period: *Hyalomma dromedarii, H. excavatum, H. rufipes and Amblyomma variegatum* (Figure 2, 3 and 4). *Hyalomma dromedarii* was the predominant tick species (90.1%), while the other tick species were present in small numbers: *H. excavatum* (4.9%), *H. rufipes* (2.5%), and *A. variegatum* (2.5%).

Molecular analysis confirmed the presence of *Theileria sp.* in all tick species identified in the camels we studied (Figure 6).

Four Theileria developmental stages were observed by microscopic examination of Giemsa-stained ticks' midgut smears (Figure 5 A, B, C, and D). These stages include immature zygotes with a uniform, purple-stained cytoplasm and distinctly condensed nuclear material at one pole (7 µm); early zygotes showing chromatin material at cell boundaries (5.25 µm); developing kinete within the zygote (6.65 µm), and undifferentiated kinete, characterized by a homogeneous cytoplasm and a round nucleus that occupies the anterior end (10.3 µm long x 7 µm wide).

Examination of the haemolymph (Figures 5 E, F, and G) revealed mature motile kinetes, which appeared as club-shaped structures with a dark polar cap at their anterior end (15.5 µm long x 3.7 µm at the broad end); multinucleated rod-shaped vermicules (ranging from 4 to 7 µm long x 1 µm wide) and banana-shaped multinucleated vermicules (5 x 1 µm). Additionally, a multinucleated polymorphous sporoblast containing sporozoites at the periphery was observed in salivary gland smears measuring 25 µm x 22.5 µm (Figure 5 H).

PCR testing served as the gold standard for evaluating the specificity and sensitivity of the microscopic method for detecting *Theileria sp*. stages in ticks (Figure 6). The infection rates determined by microscopic examination and PCR testing were 70% and 80%, respectively. The accuracy of microscopic examination was 90%, with specificity and sensitivity recorded as 87.5% and 100%, respectively.

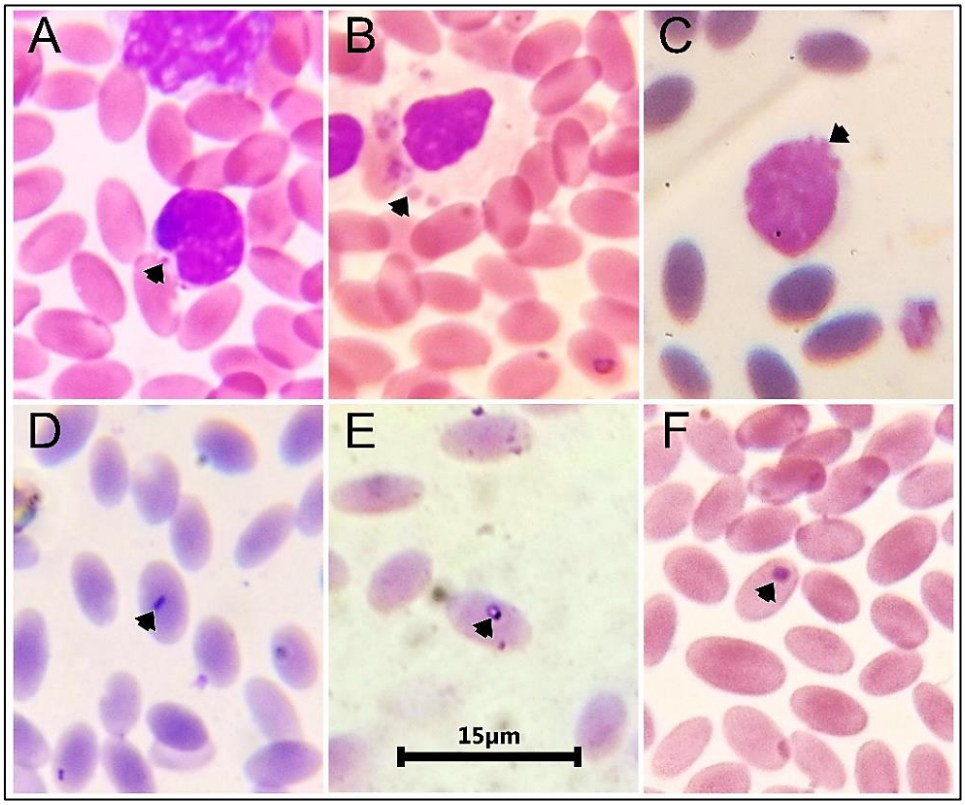

Figure 1: Giemsa-stained camel' blood smear showing, [A]: microschizonts of *Theileria sp.* inside lymphocytes (black arrow head); [B – C]: macroschizonts inside lymphocytes (black arrow head); [D]: rod shaped erythrocytic stage of *Theileria sp.* (black arrow head); [E]: ring form of *Theileria sp*. inside RBCs (black arrow head); and [F]: the rounded form of *Theileria sp.* inside RBCs (black arrow head), (magnification X 1000).

Table 2: Seasonal infection rates among the examined imported camels.

Season **	examined	infected	%
Winter	41	18	44%
Summer	44	12	27%
Autumn	6	5	83%
spring	9	7	78%
Total	100	42	42%

** High statistical difference among different seasons (P< 0.01, Chi-square $\chi^2 = 12.92$).
** High statistical difference between different species of ticks (P< 0.0001, $\chi 2 = 244.5$).

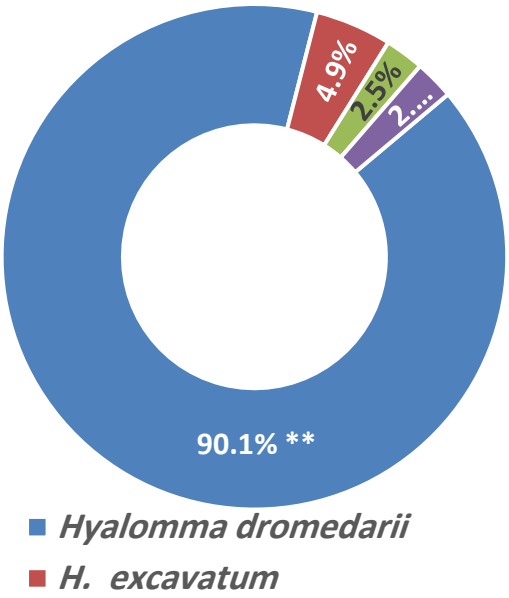

Figure 2: Showing the distribution of different identified species of ticks.

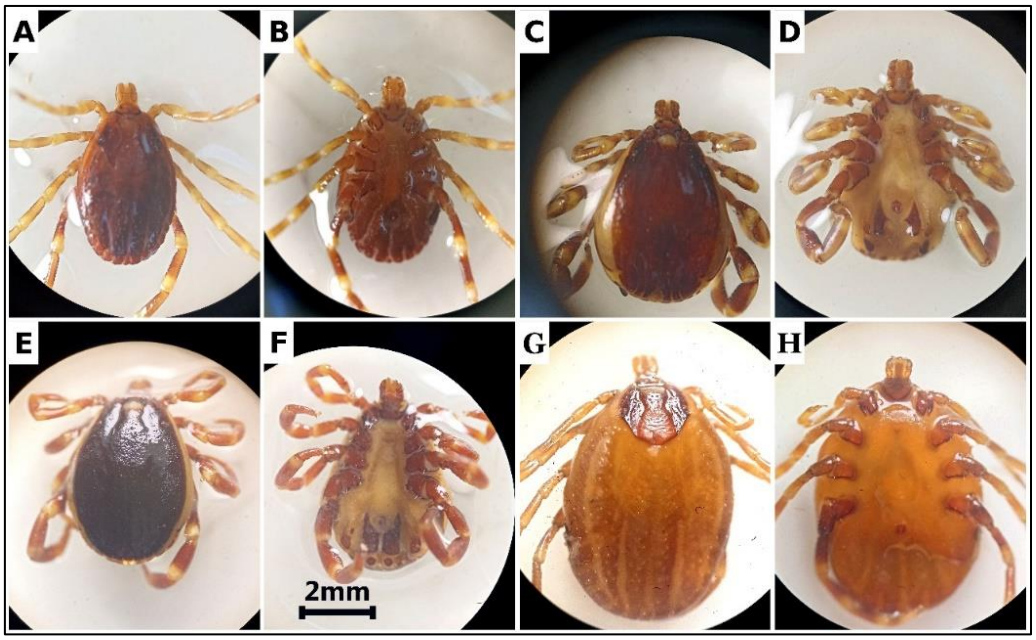

Figure 3: Showing wet mounts of the dorsal and ventral aspects of *Hyalomma dromedarii* female tick [A, B]. Dorsal and ventral aspects of an *H. dromedarii* male tick [C, D]. Dorsal and ventral surfaces of an *H. rufipes* male tick [E, F]. Dorsal and ventral surfaces of an *H. excavatum* female tick [G, H].

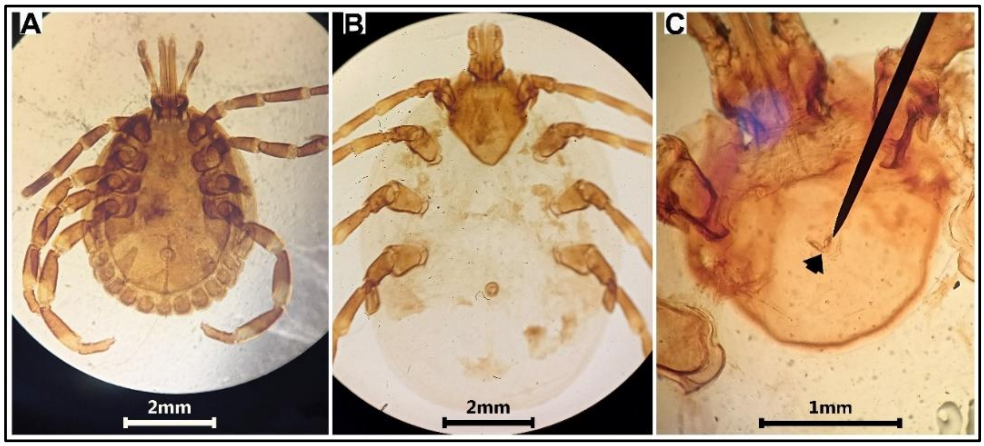

Figure 4: Showing permanent mounts of [A]:dorsal aspect of *Amblyomma variegatum* male; [B]:dorsal aspect of *Hyalomma dromedarii* female; [C]: ventral aspect of *Hyalomma dromedarii* female (notice the genital aperture posterior lips had a narrow V-shaped black arrow head).

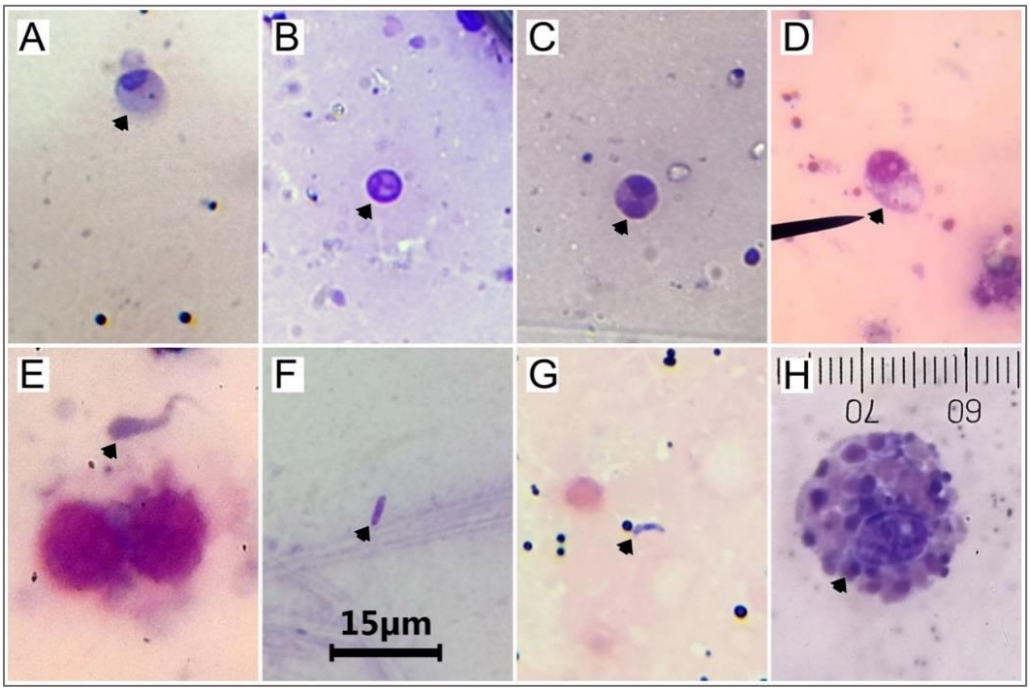

Figure 5: Giemsa-stained developmental stages of *Theileria sp*. in midgut, hemolymph and salivary gland smears of feeding ticks [A-H] (X 1000).

(A) Immature zygotes with a homogeneous, purple stained cytoplasm and distinct nuclear material that was condensed at one pole in the midgut.
(B) Early zygote with chromatin material at cell margins in the midgut.
(C) Developing kinete in zygote in the midgut.
(D) Undifferentiated kinete in the midgut, characterized by a homogeneous cytoplasm and a round nucleus situated at the anterior end.
(E) Club-shaped mature motile kinete in hemolymph appeared as a club-shaped structure with dark polar cap at the anterior end.
(F) Rod-shaped multinucleated vermicules in hemolymph.
(G) Banana-shaped multinucleated vermicule in hemolymph.
(H) Polymorphous multinucleated sporoblast with sporozoite at the periphery of salivary gland smears.

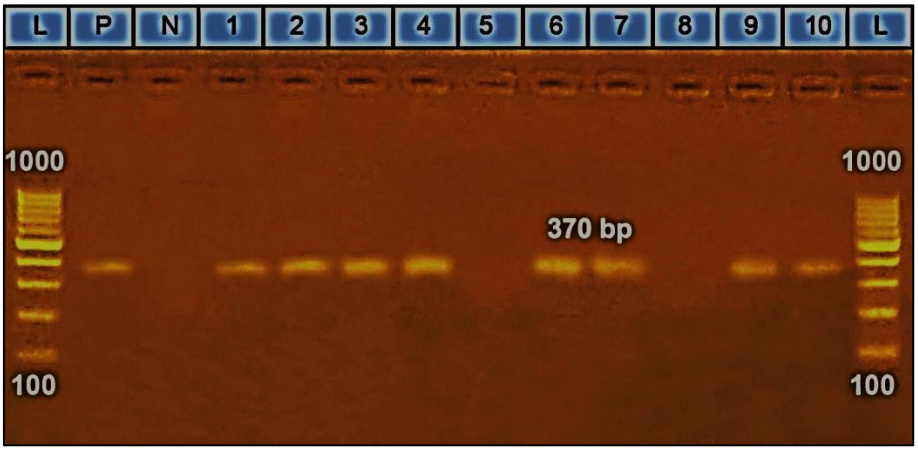

Figure 6: Agarose gel electrophoresis of PCR for *Theileria* 18S rRNA (370 bp) specific for characterizing *Theileria sp.* Lane L: 100 bp DNA ladder. Lane P: Positive control. Lane N: Negative control. Lanes 1, 2, 3, 4, 6, 7, 9, and 10: Positive for *Theileria sp.* Lanes 5 and 8: Negative for *Theileria sp.*

DISCUSSION

Theileriosis is one of the most prominent tick-borne haemoparasitic diseases affecting camels, characterized by significant economic repercussions due to its serious clinical symptoms (Abouzaid *et al.,* 2022). In the current study, the overall occurrence of *Theileria sp.* in camels was found to be 42% through microscopic examination (Table 2). These findings are consistent with earlier research conducted by El-Fayoumy *et al.* (2005) (44.8%), El-Seify *et al.* (2011) (48.58%), and El-Naga and Barghash (2016) (50.8%).

Various researchers in Egypt have reported both higher and lower rates for *Theileria sp.*, such as El-Refaii *et al.* (1998) at 62.1% (46/74), Hamed *et al.* (2011) at 6.75% (15/224) and Abouzaid *et al.* (2022) at 15% (15/100). These discrepancies may be attributed to factors, such as the characteristics of the study area, differences in camel breeds (considering that Egypt imports camels from various countries), the immune status of the animals, camel population density, hygiene practices, diagnostic approaches, tick infestation prevalence and climatic conditions, which significantly influence vector transmission (El-Kelesh *et al.,* 2011; Hamed *et al.,* 2011; El-Naga and Barghash, 2016).

In terms of the seasonal abundance of *Theileria*, it showed significantly higher infection rates ($\chi 2=12.35$, $p< 0.01$) during autumn and spring (83% and 78%, respectively) compared to winter (44%) and summer (27%) (Table 2). These results are consistent with the findings of Abouzaid *et al.* (2022), who reported the highest infection rates in autumn (8%), and Alimam *et al.* (2022), who observed an increase in *Theileria sp.* during spring and autumn compared to summer and winter. The seasonal variation in blood parasite infections in animals may be influenced by how climatic conditions impact tick activity (Fadly, 2012; Abouzaid *et al.,* 2022; Alimam *et al.,* 2022).

This study identified both the erythrocytic forms (rod, rounded and ring) and lymphocytic forms (macro and micro-schizont) of *Theileria* in circulating red blood cells and lymphocytes, respectively (Figure 1). This finding is consistent with previous descriptions by Levine (1985), Nassar (1992), El-Refaii *et al.* (1998), Mahran (2004), and Hamed *et al.* (2011).

In the current study, four tick species were identified (Figures 2, 3, and 4). *Hyalomma dromedarii* was the most prevalent species at 90.1%, followed by *H. excavatum* at 4.9%, *H. rufipes* at 2.5%, and *Amblyomma*

variegatum at 2.5%. These findings are consistent with those reported by van Straten and Jongejan (1993), Abdally (2008), Youssef *et al.* (2015), Hassan *et al.* (2017), and Salman *et al.* (2022). This indicates that *H. dromedarii* serves as the primary vector for the transmission of *Theileria* species to camels. Hoogstraal (1956), El-Refaii *et al.* (1998), El-Kelesh *et al.* (2011), and Youssef *et al.* (2015) support this conclusion**.**

Regarding the developmental stages of *Theileria sp.* in ticks (Figures 5 A, B, C, and D), four distinct developmental stages were observed in the stained midgut smears of ticks (immature zygotes, early zygotes with chromatin material at the cell boundaries, developing kinete in zygote, and undifferentiated kinete). Additionally, the examination of the hemolymph revealed mature motile kinete, multinucleated rod-shaped vermicules, and banana-shaped multinucleated vermicules (Figures 5 E, F, and G). Furthermore, a multinucleated polymorphous sporoblast was noted in the salivary gland smears (Figure 5 H). Similar findings were documented by Zapf and Schein (1994), El-Kelesh *et al.* (2011), Hegab *et al.* (2020), and Hegab *et al.* (2023) in haemolymph and mid-gut, as well as by El-Kelesh *et al.* (2011) and Youssef *et al.* (2015) in salivary glands.

To improve the precise detection and identification of *Theileria sp.* in ticks (Figure 6), the PCR test was used as the gold standard to evaluate the specificity and sensitivity of microscopic examinations. *Theileria sp.* was detected by PCR in all identified tick species from the examined camels in our study. These results are consistent with the findings of Walker *et al.* (2003), Hassan *et al.* (2017), and Salman *et al.* (2022). The accuracy of the microscopic method was determined to be 90%, with specificity and sensitivity recorded at 87.5% and 100%, respectively. The infection rates identified through microscopic examination and PCR were 70% and 80%, respectively. This finding is supported the study of Ramadan *et al.* (2016), where 12.5% out of 40 ticks with microscopically negative hemolymph tested positive for *Theileria sp*. using molecular methods. Similarly, Hegab *et al*. (2023) reported an infection rate of 0.73% in tick hemolymph examined microscopically, contrasting with 10.42% detected by PCR.

CONCLUSION

This research revealed an overall infection rate of 42% for *Theileria sp.* among imported camels, which significantly increased during spring and autumn. Four tick species were identified: *H. dromedarii, H. excavatum, H. rufipes,* and *A. variegatum*, all implicated in the transmission of *Theileria* through molecular analysis. *H. dromedarii* was identified as the primary vector responsible for the transmission of *Theileria* species to camels.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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دور القراد الصلب في نقل الثيليريا في الإبل المستوردة

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يعد مرض الثيليريا من أبرز الأمراض الطفيلية التي تنتقل عن طريق القراد والتي تصيب الإبل. وقد أظهر الفحص المجهري لشرائح الدم المصبوغة بالجيمسا والتي تم الحصول عليها من 100 إبل مستورد مصاب بالقراد في محجر دراو بمحافظة أسوان أن معدل الاصابة بالثيليريا هو42٪. كما لوحظ ارتفاع معنوي في معدلات الإصابة في فصلي الخريف والربيع (83٪ و78٪ على التوالي) مقارنةً بالشتاء (44٪)، بينما انخفضت بشكل معنوي خلال فصل الصيف إلى 27٪. توصيف كل من مراحل وأشكال طفيل الثيليريا في الكريات الدم الحمراء والخلايا الليمفاوية للإبل المصابة حيث وجدت اشكال حلقية ومستديرة وعصوية داخل كريات الدم الحمراء بينما لوحظ تواجد المُتَقَسِّمات الكبرى (macroschizont) والمُتَقَسِّمات الصغرى (microschizont) داخل الخلايا الليمفاوية. بالفحص المورفولوجي للقراد على تلك الابل، تم التعرف على أربعة أنواع من القراد حيث كان *الهيالوما دورومیداري* هو النوع السائد بنسبة 90.1٪، بينما كانت أنواع القراد الأخرى موجودة بأعداد صغيرة وهي *الهيالوما اكسكافيتم* (4.9٪) ، *هيالوما روفيوبس* (2.5٪) *واميبليوما فاريجيتم* (2.5٪). أكد الفحص المورفولوجي والجزيئي أن جميع هذه الانواع تحوي مراحل لطفيل الثيليريا حيث لوحظ وجود أربع مراحل نمو مميزة في مسحات المعي الوسطى للقراد وهي الزيجوتات غير الناضجة، والزيجوتات المبكرة، و الكينيت النامي داخل الزيجوت والكينيت غير المتمايز. بالإضافة كشف فحص الهيموليمف عن وجود الكينيت الناضج والفيرمكيول متعددة النوى بينما وجد الاسبوروبلاست متعددة النوى في مسحات الغدد اللعابية. كانت دقة التشخيص المجهري للثيليريا في القراد 90٪، بينما بلغت حساسية ونوعية التشخيص 87.5٪ و100٪ على التوالي. خلصت النتائج إلى أن أنواع القراد السابق ذكرها تعمل كمستودع لطفيل الثيليريا في الإبل المستوردة. بالإضافة إلى ذلك، أكدت الدراسة الحالية ارتفاع معدل انتشار مرض الثيليريا في تلك الإبل.