

MOLECULAR AND EPIDEMIOLOGICAL INVESTIGATION OF THEILERIA INFECTION IN CROSSBRED CATTLE IN ASSIUT PROVINCE, UPPER EGYPT

HESHAM A. SADEK ¹; MOHSEN I. ARAFA ¹; AHMED KAMAL DYAB ^{2,3,*}; AND
EHAB KOTB ELMAHALLAWY ^{4,5*}, AHMED GAREH ⁶

¹ Department of Parasitology, Animal Health Research Institute, Agriculture Research Center, Assiut Branch, Egypt.

² Department of Parasitology, School of Veterinary Medicine, Badr University in Assiut, (BUA) New Nasser City, Assiut, Egypt. ahmed.kdaib@bua.edu.eg

³ Department of Medical Parasitology, Faculty of Medicine, Assiut University, 71526 Assiut, Egypt.

⁴ Grupo de Investigación en Sanidad Animal y Zoonosis (GISAZ), Unidad de Investigación Zoonosis y Enfermedades Emergentes (ENZOEM), Departamento de Sanidad Animal, Universidad de Córdoba, Córdoba, Spain.

⁵ Department of Zoonoses, Faculty of Veterinary Medicine, Sohag University, Sohag, Egypt.

⁶ Department of Parasitology, Faculty of Veterinary Medicine, Aswan University, Egypt

Received: 4 July 2025; **Accepted:** 17 August 2025

ABSTRACT

Cattle in tropical and Mediterranean regions are susceptible to theileriosis, a serious tick-borne disease that impairs animal health and productivity and causes significant financial losses. This study aimed to determine the prevalence of *Theileria* spp. infection among crossbred cattle in Assiut Province, Upper Egypt, using microscopic examination and molecular PCR confirmation. A total of 200 blood samples were collected from crossbred cattle, including 84 apparently healthy animals and 116 animals presenting clinical signs suggestive of theileriosis. Microscopic examination of Giemsa-stained blood smears revealed an overall prevalence of *Theileria* spp. infection of 38.5% (77/200). Infected animals were more frequently observed among the clinically suspected group, with a significantly higher prevalence of 54.31%, compared to the apparently healthy group. Analysis of epidemiological factors revealed that age-related analysis showed that cattle older than one year had a higher infection rate (47.5%), and females exhibited greater prevalence than males. Seasonal variation was evident; infection rates peaked during the hot season at 44.23% and declined to 32.29% in the cold season, although these factors (age, sex and season) were not statistically significant ($P > 0.05$). Tick infestation was significantly associated with *Theileria* spp. infection ($P < 0.05$), indicating its crucial role in disease transmission. Molecular confirmation of selected heavily infected samples was performed using PCR targeting the *Tams1* gene, successfully amplifying a 721 bp fragment, confirming the presence of *T. annulata*. This study concludes a moderate prevalence of *Theileria* spp. infection in crossbred cattle in Assiut Province, highlighting the critical need for integrated tick management and diagnostic surveillance to mitigate health and economic impacts.

Key words: Cattle, Epidemiology, Molecular identification, PCR, *Theileria annulata*, Ticks, Assiut, Egypt.

Corresponding author: ³Ahmed Kamal Dyab and ⁵Ehab Kotb Elmahallawy

E-mail address: ahmed2015@aun.edu.eg; Email: eehaa@unileon.es

Present address: ³ Department of Medical Parasitology, Faculty of Medicine, Assiut University, 71526 Assiut, Egypt. ⁵ Grupo de investigación en Sanidad Animal y Zoonosis (GISAZ), Unidad de Investigación Zoonosis y Enfermedades emergentes (ENZOEM), Departamento de Sanidad Animal, Universidad de Córdoba, Córdoba, Spain.

INTRODUCTION

Cattle play a crucial role in global food security and rural economies by providing essential nutrients through their meat and dairy products. Beef is the third most widely consumed meat globally, serving as a rich source of protein, iron, phosphorus, zinc, selenium, and B-complex vitamins (Dold and Cocks, 2001). In Egypt, cattle productivity is increasingly threatened by tick-borne parasitic infections, particularly blood parasites from the families Theileriidae and Babesiidae (Rdwan *et al.*, 2010).

Theileriosis is caused by intracellular protozoan parasites of the genus *Theileria*, which infect erythrocytes and leukocytes in cattle. These parasites, primarily transmitted by ixodid ticks, impose significant health and economic burdens on livestock populations (Kohli *et al.*, 2014; Silva *et al.*, 2010). Taxonomically, *Theileria* spp. belong to the family Theileriidae, suborder Piroplasmida, within the phylum Apicomplexa (Rdwan *et al.*, 2010). Among these species, *T. annulata* and *T. parva* are the most pathogenic and widely recognized as the main causative agents of tropical theileriosis and East Coast fever disease, respectively (Gebrekidan *et al.*, 2016).

Clinically, theileriosis manifests as fever, anaemia, jaundice, anorexia, weight loss, infertility, and immunosuppression. Advanced infection can lead to leukocyte infiltration in ocular tissues, causing corneal opacity and potential vision impairment (Durrani *et al.*, 2010; Mohammed and Elshahawy, 2018). Tropical theileriosis caused by *T. annulata* leads to high morbidity and mortality, reduced milk production, stunted growth, and substantial economic losses. This substantial infection burden greatly reduces animal productivity and represents a major threat to the livestock industry globally, including Egypt. Theileriosis is one of the most significant parasitic diseases in Egypt,

affecting cattle health and productivity (El-Alfy *et al.*, 2022). Crossbred cattle are not just more productive, but also display better fecundity, health, growth, and heat flexibility, making them compatible for Egypt's climate and production systems. However, there is still a lack of comprehensive epidemiological data regarding theileriosis in Upper Egypt, especially in crossbred cattle. So, this study aims to assess the prevalence of tropical theileriosis in Assiut Province, examine key risk factors, and utilize molecular techniques to accurately identify *Theileria* species, ultimately supporting enhanced disease control strategies in the region, and that will be translated into healthier cattle, higher productivity, and improved livelihoods for farmers.

MATERIALS AND METHODS

1. Ethical Considerations

This study was approved by the Research Ethics Committee of the Faculty of Veterinary Medicine, Assiut University, Egypt (Approval No. 06- 2025- 0318).

2. Study Location

Assiut Province, located at a latitude of 27°10'51.46" N and a longitude of 31°11'1.25" E, has a desert climate with an average annual temperature of 23°C, ranging from 1°C to 45°C and minimal precipitation. In Upper Egypt, the cold season (December- February) is defined by temperatures between 15 and 27°C, and the hot season (March- November) is defined by temperatures over 28°C (Abdel-Rady *et al.*, 2023).

3. Animals

A total of 200 blood samples were collected from small stakeholder farms, including 116 clinically affected animals and 84 apparently healthy cattle, categorized into three age groups: 80 calves (< 1 year old), 56 young cattle (1 - 3 years old), and 64 adults (>3 years old). The sample set comprised 84 males and 116 females.

4. Sample Collection

Approximately 5 mL of blood was drawn from the jugular vein of each animal into EDTA tubes, following standard protocols (Gupta and Singla, 2012). Samples were transported in an icebox to the Assiut Regional Laboratory at the Animal Health Institute within a few hours of collection for parasitological analysis, or stored at 4°C until further processing.

5. Blood Microscopic Analysis

Thin blood smears were prepared, fixed in absolute methanol, stained with 10% Giemsa solution, and examined under an Olympus microscope at 100× magnification (Garcia, 2007). Identification of erythrocytic and lymphocytic forms of *Theileria* species followed the criteria outlined elsewhere (Levine, 1985).

6. Molecular Analysis

A representative set of 20 blood samples with high parasitic load under microscopic examination were selected for PCR confirmation. The samples were stored in labeled Eppendorf tubes at -23°C until further processing.

6.1. DNA Extraction

Genomic DNA was extracted using the QIAamp DNA Mini Kit according to the manufacturer's protocol (Qiagen, 2016).

6.2. PCR Amplification

Polymerase chain reactions were conducted in a total volume of 25 µL. Each PCR reaction included 12.5 µL of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µL of forward and reverse primers, 5.5 µL of nuclease-free water, and 5 µL of genomic DNA template. Amplification was performed using an Applied Biosystems 2720 thermal cycler, following the cycling parameters outlined in Table (1).

6.3. PCR Product Analysis

Amplified PCR products were analyzed using 1.5% agarose gel electrophoresis, and sizes were compared with a GelPilot 100 bp Plus DNA ladder. Visualization was carried

out using an Alpha Innotech gel documentation system (Biometra), and images were processed and analyzed using the accompanying imaging software.

7. Tick Collection and Identification

Engorged ticks were gently removed from various anatomical regions of the examined cattle using curved forceps. The removal was done gently, with a counterclockwise rotation to minimize damage to the specimens (Youssef *et al.*, 2015). Animals were classified into four categories according to their infection and tick infestation status: (1) infected and infested, (2) non-infected but infested, (3) infected but not infested, and (4) non-infected and non-infested. Ticks were preserved in 70% ethanol in labeled Falcon tubes for subsequent morphological identification. Adult tick species were identified under both dissecting and compound microscopes based on established taxonomic keys (Walker *et al.*, 2003).

8. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software version 9.5.1, with associations evaluated using the Chi-square (χ^2) test. A p-value < 0.05 indicated a significant difference.

RESULTS

1. Detection of *Theileria* spp. using the ordinary microscope

1.1. Prevalence of *Theileria* spp. in crossbred cattle in Assiut province.

In this study, Giemsa-stained blood smears were examined under a light microscope to detect the presence of *Theileria* spp. The overall positivity rate was 38.5% (77 out of 200) among the tested crossbred cattle in Assiut Province. Cattle exhibiting clinical symptoms had a significantly higher positivity rate (54.31%) compared to clinically healthy animals (16.67%). The most common clinical signs observed in positive animals included fever, reduced

appetite, eye discharge, swollen lymph nodes, and cloudy appearance of the cornea.

When stratified by age, calves younger than one year had the highest positivity rate (47.5%), while older cattle (over three years) showed the lowest rate. Female animals demonstrated a higher positivity rate (41.38%) compared to males (34.52%).

Seasonal variation was evident, with the highest positivity recorded during the hot season (44.23%). Moreover, cattle with hard tick infestations had the highest rates of positivity for *Theileria* spp. (Table 2).

1.2. Morphological characterization of isolated *Theileria* spp. using a light microscope

Microscopic examination of Giemsa-stained smears revealed intraerythrocytic *Theileria* spp. piroplasm, which appeared

as rounded, oval, or comma-shaped bodies within RBCs. Frequently, multiple piroplasm forms were observed per erythrocyte, staining intensely purple to blue with Giemsa (Fig. 1A–C). Examination of lymphocytes also identified macroschizonts, known as Koch's Blue Bodies, which appeared as large chromatin-rich structures with deep blue staining (Fig. 1D–F)

2. Molecular findings

Molecular identification was performed to confirm the microscopic findings, focusing on blood samples with high parasite load. These samples tested positive for *Theileria* spp., with a specific amplification product of 721 bp detected by gel electrophoresis, confirming the presence of the *Tams1* gene of *Theileria annulata*

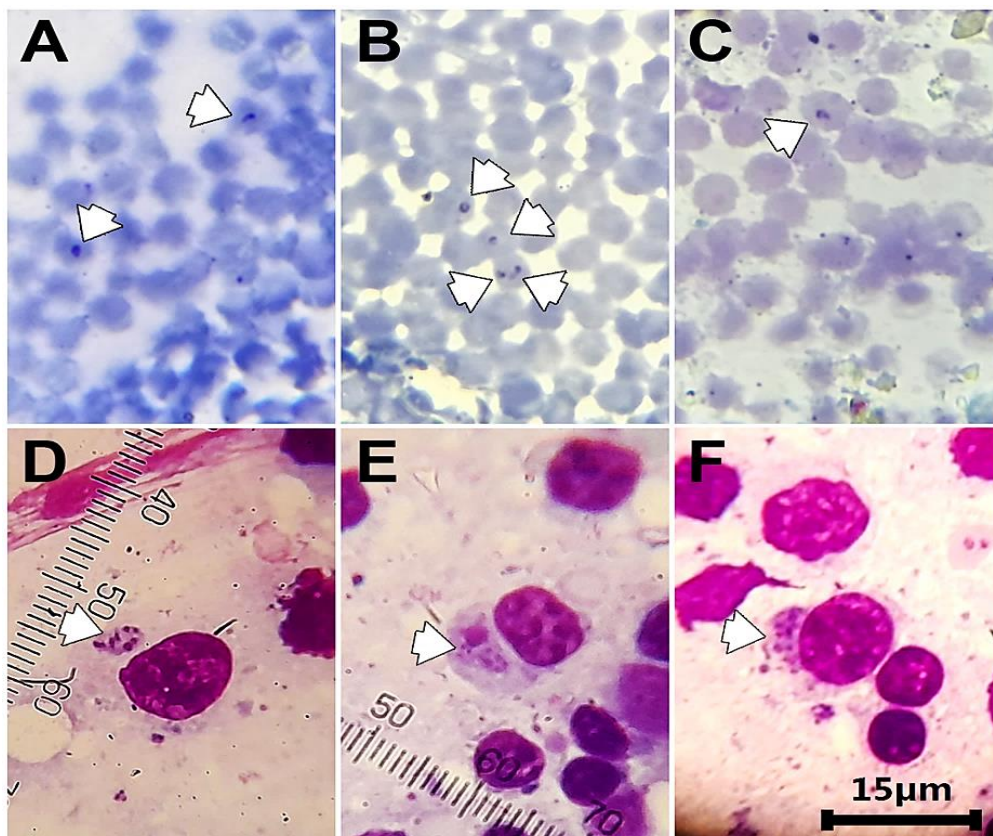


Figure 1. Giemsa-stained blood smears from cattle infected with *Theileria* spp. (A–C) Intraerythrocytic piroplasm stages displaying variable shapes (round, oval, and comma-shaped) within red blood cells (white arrowheads). (D–F) Macroschizonts (Koch's Blue Bodies) identified within lymphocytes (white arrowheads). All images were captured under oil immersion at 100× magnification.

Table 1: Primer sequence, amplicon size, cycling conditions and target gene.

Target gene	Primers sequences	Amplified segment (bp)	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
<i>Theileria annulata</i> (<i>Tams1</i>)	GTAACCTTT AAAAACGT GTTACGAA CATGG GTTT	721bp	94°C 5min.	94°C 30sec.	55°C 40sec.	72°C 40sec.	72°C 10min.	(28)

Table 2: Prevalence of *Theileria* Infection in Relation to Clinical Signs, Age, Sex, Season, and Tick Infestation.

Variable	No. exam.	No. of infected (%)	X ²	P. value
Total	200	77 (38.5%)		
Clinical condition				
Asymptomatic	84	14 (16.67%)		
symptomatic	116	63 (54.3%)	29.16	0.0001*
Age				
< 1 year	80	38 (47.5%)		
1-3 year	56	21 (37.5%)	5.670, 2	0.0587
>3 year	64	18 (28.13%)		
Gender				
Male	64	29 (34.5%)		
Female	116	48 (41.38%)	0.9671, 2	0.6166
Season				
Hot	104	46 (44.23%)		
Cold	96	31 (32.29%)	3.005	0.083
Tick infestation				
Infected-infested	200	48 (24%)		
Non-infected- infested	200	49 (24.5%)		
Infected-non infested	200	29 (14.5%)	9.6	0.01*
Non-infected-non infest.	200	74 (37%)		

* Statistically significant differences (P<0.05).

DISCUSSION

Theileriosis, caused by *Theileria* species and transmitted by ixodid ticks, remains a major protozoan disease affecting cattle in Egypt, leading to significant economic losses through morbidity, mortality, reduced productivity, and increased veterinary costs. Among the species, *Theileria annulata*, which causes tropical theileriosis, poses the greatest threat to cattle health in endemic areas, especially under traditional farming systems (Al-Hosary *et al.*, 2018).

In this study, 38.5% of examined cattle tested positive for *Theileria* species based on microscopic analysis of blood smears. This rate aligns with earlier findings from Assiut (Abdel-Rady *et al.*, 2008; Abou-El-Naga *et al.*, 2005) and the New Valley Governorate (Sayed *et al.*, 2020). While this rate is lower than those reported in India (86%) and Saudi Arabia (76.5%), it is higher than those from El-Wadi El-Gadid (9.31%) and Qalubia Governorate (14.61%) (Al-Emarah *et al.*, 2012; El-Dakhly *et al.*, 2018; EL-Metenawy, 2000; El-Moghazy *et al.*, 2014). These differences likely result from variations in geography, vector

presence, animal management, and host immunity (Gul *et al.*, 2015). Among clinically suspected animals, 54.31% tested positive for theileriosis, which is consistent with the results reported in literature (El-Deeb *et al.*, 2009; Kalyan *et al.*, 2016). The 16.67% positivity rate among apparently healthy cattle highlights the role of subclinical carriers in maintaining the transmission cycle between hosts and ticks (Kundave *et al.*, 2015), (El Damaty *et al.*, 2022), (El Dakhly *et al.*, 2020), who found that the cattle tested had a *T. annulata* infection without any clinical symptoms. These animals are important to the study of disease epidemiology as they are a major source of infection for vulnerable cattle in non-endemic areas (Madkour *et al.*, 2023).

In relation to age, younger cattle (under one year old) exhibited the highest positivity rate (47.5%), followed by animals aged 1 to 3 years (37.5%), and the lowest in cattle over three years (28.13%). Although the difference was not statistically significant ($P = 0.0587$), it may be due to the small sample size, consistent with previous studies that identify younger animals as more susceptible (EL-Masry *et al.*, 2006; Al-Hosary *et al.*, 2018; Ahmed *et al.*, 2019). Conversely, another study (Selim *et al.*, 2022) reported higher positivity in older cattle, which might be attributed to differences in exposure and acquired immunity.

In relation to sex as a potential risk factor, although 41.38% of females tested positive compared to 34.52% of males, the difference was not statistically significant ($P = 0.6166$), agreeing with studies showing minimal sex-related variation (Ayadi *et al.*, 2017; Madkour *et al.*, 2023; Selim *et al.*, 2022). While some studies found higher rates in males (Abdel-Rady *et al.*, 2010; Boussaadoun *et al.*, 2015; Al-Hosary *et al.*, 2018), others linked increased female positivity to physiological stressors such as pregnancy and lactation in the absence of effective tick control. The higher positivity during the hot season (44.23%) supports the

understanding that tick activity peaks in warm conditions. These findings agree with previous research (Abdel-Rady *et al.*, 2005, Al-Hosary *et al.*, 2018).

Regarding the correlation between tick infestation and *Theileria* sp. infection rate, tick infestation was examined in 200 cattle, with 97 infested. Positivity rates varied among groups: 24% in tick-infested positives, 24.5% in tick-infested negatives, 14.5% in non-infested positives, and 37% in non-infested negatives. A statistically significant association ($P < 0.01$) was found between tick infestation and *Theileria* positivity, indicating the pivotal role of ticks in disease transmission. Positivity in non-infested cattle may reflect the biology of certain tick species that spend much of their life off-host and transmit pathogens through transstadial mechanisms (Varma, 1993).

Monitoring the spread of *Theileria annulata* in endemic areas requires precise and reliable diagnostic methods. Among molecular techniques, PCR targeting the *Tams1* gene has proven to be highly sensitive and specific, enhancing diagnostic accuracy and aiding in tracking transmission patterns and guiding targeted control measures. In the present study, molecular detection via PCR amplification of a specific 721 base pair fragment of the *Tams1* gene confirmed the presence of *T. annulata* in the samples analyzed. These findings confirm *T. annulata*'s endemicity within the study area, aligning with previous reports from similar regions (Ahmed *et al.*, 2019, Al-Hosary *et al.*, 2020, Madkour *et al.*, 2023, Sayed *et al.*, 2020, Yousef *et al.*, 2020).

CONCLUSIONS

This study confirms that *Theileria annulata* remains a widespread and damaging blood parasite affecting cattle health in Egypt. Our study confirmed bovine theileriosis using PCR combined with microscopic examination, revealing that a significant

proportion of cattle in Assiut Governorate in Upper Egypt are infected. Urgent implementation of targeted treatment for infected animals and comprehensive tick eradication programs in both livestock and their environments using chemical and biological acaricides are critical to prevent the spread of the disease. Furthermore, expanded research is essential to understand how parasite genetic diversity influences virulence, providing a foundation for tailored control strategies. Collaboration among veterinarians, farmers, and researchers is vital to developing effective interventions that protect cattle health and ensure food security in the region.

Author's contributions:

H.A.S., M.I.A., A.K.D., and A.G involved in the conception of the research idea and methodology design, supervision, and performed data analysis and interpretation. H.A.S., A.K.D., A.G, and E.K.E involved in methodology, drafted and prepared the manuscript for publication and revision. All authors read and approved the final manuscript.

Institutional Review Board Statement:

This study was conducted with the approval of the approval of the Faculty of Veterinary Medicine, Assiut University, Egypt, under ethical approval number 06- 2025- 0318.

Conflicts of interest:

The authors declare no conflict of interest.

Data Availability Statement:

The data that support the findings of this study are contained in the manuscript.

Informed Consent Statement:

Not applicable.

REFERENCES

- Abdel-Rady, A.; Ahmed, LS.; Mohamed, A. and AL-Hosary, A. (2010):* Using polymerase chain reaction (PCR) for diagnosis of bovine theileriosis in Upper Egypt. IJAVMS, 4, 67–74.
- Abdel-Rady, A.; Kotb, S. and AbdEllah MR. (2008):* Clinical, Diagnostic and therapeutic studies on theileriosis (*Theileria annulata*) in cattle in Upper Egypt. SCVMJ, 13, 387-395.
- Abdel-Rady, A.; Karmi, M.; Youssef, M.; Abdel-Raouf, AM. and Madkour, B. (2023):* Some epidemiological studies on *Theileria annulata* infection in Egypt. Res J. Vet. Pract. 11(1): 7-12.
- Abou-El-Naga, TR.; Abdou, TA. and Mahmoud, MA. (2005):* Clinicopathological Studies on *Theileria annulata* Infection in Siwa Oasis, Egypt. J. Vet. Med. Res., 15, 40–46.
- Ahmed, S.; Abd El-Hakim, O.; Maher, A. and Elnaker, Y. (2019):* Tropical theileriosis, epidemiology and Molecular diagnosis in cattle in New Valley Province, Egypt. AJVS, 63, 31–41.
- Al-Emarah, GYA.; Khudor, MH. and Daham, H. (2012):* Clinical, haematological and biochemical study to cattle naturally infected with *Theileria annulata* in north of Basrah province. AL-Qadisiyah J. Vet. Med. Sci., 11, 54–62.
- Al-Hosary, A.; Ahmed, L.; Ahmed, J.; Nijhof, A. and Clausen, PH. (2018):* Epidemiological study on tropical theileriosis (*Theileria annulata* infection) in the Egyptian Oases with special reference to the molecular characterization of *Theileria* spp. Ticks and tick-borne diseases, 9, 1489–1493.
- Al-Hosary, A.; Răileanu, C.; Tauchmann, O. and et al. (2020):* Epidemiology and genotyping of *Anaplasma marginale* and co-infection with *piroplasms* and other Anaplasma-taceae in cattle and buffaloes from Egypt. Parasites & vectors, 13, 495.
- Ayadi, O.; Rjeibi, MR.; Benchikh Elfegoun, MC. and Gharbi, M. (2017):* Prevalence and risk factors of tropical theileriosis, and sequencing of *Theileria annulata*, the causative

- pathogen, in Setif region (Algeria) before and after tick season. *Revue d'élevage Et De médecine vétérinaire Des Pays Tropicaux*, 69, 161–166.
- Boussaadoun, MA.; Gharbi, M.; Sayeh, L.; Soudani, MC. and Darghouth, MA. (2015):* Epidemiological situation of bovine tropical theileriosis (*Theileria annulata* infection) in the Northwest Tunisia. *J. Adv. Parasitol.*, 2, 69-74.
- Dold, A.P. and Cocks, M.L. (2001):* Traditional veterinary medicine in the Alice district of the Eastern Cape Province, South Africa: Research in action. *South Afric. J. Sci.*, 97, 375-379.
- Durrani, AZ.; Mehmood, N. and Shakoori, AR. (2010):* Comparison of three diagnostic methods for *Theileria annulata* in Sahiwal and Friesian cattle in Pakistan. *Pak. J. Zool.*, 42, 467-72.
- El-Alfy, ES.; Abbas, I.; Baghdadi, HB. and et al. (2022):* Molecular Epidemiology and Species Diversity of Tick-Borne Pathogens of Animals in Egypt: A Systematic Review and Meta-Analysis. *Pathogens (Basel, Switzerland)*, 11, 912.
- El-Dakhly, KM.; Arafa, W.; Ghanem, SS. and et al. (2018):* Microscopic and molecular detection of *Theileria annulata* infection of cattle in Egypt. *J. Adv. Parasitol.*, 5, 29-34.
- El-Dakhly, KM.; Arafa, WM. and Soliman S. (2020):* Molecular detection, phylogenetic analysis, and genetic diversity of *Theileria annulata*, *Babesia bigemina*, and *Anaplasma marginale* in cattle in three districts of Egypt. *Acta. Parasitol* 65:620–627.
- El Damaty, HM.; Yousef, SG.; El-Balkemy, FA, et al., (2022):* Seroprevalence and risk factors of tropical theileriosis in smallholder asymptomatic large ruminants in Egypt. *Front Vet Sci* 9. DOI 10.3389/fvets.1004378
- El-Deeb, WM. and Younis, EE. (2009):* Clinical and biochemical studies on *Theileria annulata* in Egyptian buffaloes (*Bubalus bubalis*) with particular orientation to oxidative stress and ketosis relationship. *Vet. Parasitol.*, 164, 301–305.
- EL-Masry, NM.; EL-Dessouky, SA. and Abo-Elkheir, SA. (2006):* Parasitological and biochemical studies on cattle theileriosis at Dakahlia Province with special references to its control. *Assiut Vet. Med.*, 52, 165–178.
- EL-Metenawy, TM. (2000):* Prevalence of Blood Parasites among Cattle at the Central Area of Saudi Arabia. *The J. Protoz. Res.*, 10, 6–13.
- El-Moghazy, HM.; El Ebied, MH.; Mohamed, AG. and EL-Sayed, AA. (2014):* Epidemiological studies on bovine babesiosis and theileriosis in Qalubia Governorate. *Benha vet. Med. J.*, 27, 36–48.
- Garcia, Ls. (2007):* Diagnostic Medical Parasitology, 5th Edition, ASM Press, 1752 N St. NW, Washington, 2007, DC20036-2904, USA. p. 881-894.
- Gebrekidan, H.; Gasser, RB. and Baneth, G. (2016):* Molecular characterization of *Theileria orientalis* from cattle in Ethiopia. *Ticks and Tick-borne Diseases*, 7, 742-747.
- Gul, N.; Ayaz, S.; Gul I.; Adnan, M.; Shams, S. and Akbar, N. (2015):* Tropical theileriosis and east coast fever in cattle: present, past and future perspective. *Int. J. Curr. Microbiol. Appl. Sci*, 4, 1000-1018.
- Gupta, SK. and Singla, LD. (2012):* Diagnostic trends in parasitic diseases of animals. *Vet. Diag.: current trends*, 2012, 81-112.
- Kalyan, S.; Prasad, H.; Gunjan, DP.; Behera, SK.; Behera, JB. and et al. (2016):* Theileriosis in crossbred cows and its therapeutic management: first report from Lushaihill district of Mizoram. *J. Parasit. Dis.*, 40, 605–610.
- Kohli, S.; Atheya, U.K. and Thapliyal, A. (2014):* Prevalence of theileriosis in cross-bred cattle: its detection through blood smear examination and polymerase chain reaction in

- Dehradun district, Uttarakh and, India, *Vet. World*, 7, 168-171.
- Kundave, VR.; Patel, AK.; Patel, PV.; Hasnani, JJ. and Joshi, CG. (2015):* Detection of theileriosis in cattle and buffaloes by polymerase chain reaction. *J. Parasit. Dis.*, 39, 508–513.
- Levine, ND. (1985):* Veterinary Protozoology, 1st Edition, Iowa State Univ. Press Ames, 1985, p. 313- 328.
- Madkour, BS.; Karmi, M.; Youssef, MA.; Abdelraouf, A. and Abdel-Rady, A. (2023):* Epidemiological and diagnostic investigation on bovine theileriosis in Aswan Governorate, Egypt. *J. Parasit. Dis.*, 47, 124–130.
- Mohammed, ES. and Elshahawy I. (2018):* The current prevalence of bovine babesiosis and theileriosis infection in Egypt. *J. Bacteriol. Mycol.*, 6, 306–311.
- Nourollahi-Fard, S.R.; Khalili, M. and Ghalekhani, N. (2013):* Detection of *Theileria annulata* in blood samples of native cattle by PCR and smear method in Southeast of Iran. *J Parasit Dis*, 2013, 39(2), 249–252. <https://doi.org/10.1007/s12639-013-0333-2>
- QIAGEN GmbH (2016):* QIAamp DNA Miniand Blood Mini Handbook. 5th ed. Hilden (Germany). Available at <https://www.qiagen.com/us/resources/resourcedetail?id=62a200d6-faf4-469b-b50f-2b59cf738962&lang=en>
- Rdwan, MEI.; Talkhan, F. and Ali, MA. (2010):* Cattle Babesiosis and Associated Biochemical Alteration in Kalubya Governorate. *Nature and Science*, 8, 29–36.
- Sayed, S.; Sotohy, S.; Saleh, M.; Abdelazeem, A. and Dyab, A. (2020):* 'prevalence of Thielieriasis and its vector in cattle in New-valley governorate, Egypt', *JESP*, 50, 93-97.
- Selim, A.; Weir, W. and Khater, H. (2022):* Prevalence and risk factors associated with tropical theileriosis in Egyptian dairy cattle. *Vet. World*, 15, 919.
- Silva, MG.; Marques PX. and Oliva A. (2010):* Detection of *Babesia* and *Theileria* species infection in cattle from Portugal using a reverse line blotting method. *Vet. Parasitol.*, 174, 199–205.
- Varma, MRG. (1993):* Ticks and mites (Acari). In: Lane, R.P., Crosskey, R.W. *Medical Insects and Arachnids*. Springer, Dordrecht, 1993.
- Walker, AR.; Bouatour, A.; Camicas, JL. And et al. (2003):* Ticks of domestic animals in Africa: a guide to identification of the species. Bioscience report, 42 Comiston Drive, Edinburgh EH10 5 QR Scotland, UK.
- Youssef S.Y., Yasien S., Mousa W.M.A. et al. (2015):* Vector identification and clinical, hematological, biochemical, and parasitological characteristics of camel (*Camelus dromedarius*) theileriosis in Egypt. *Trop Anim Health Prod.*, 47, 649–656.
- Yousef, SG.; El Balkemy, FA.; El-Shazly, YA. and El Damaty, HM. (2020):* Clinical picture and haemogram profile associated with *Theileria annulata* infection in cattle before and after therapeutic intervention. *Advan. Anim. and Vet. Sci.*, 8, 290–296.

دراسات جزيئية ووبائية حول عدوى التيليريا في الأبقار الهجين في محافظة أسيوط ، صعيد مصر

احمد كمال دياب ، هشام صادق ، محسن عرفه ، ايهاب قطب المحلاوي ، احمد جراح

Email: ahmed2015@aun.edu.eg; eehaa@unileon.es

Assiut University website: www.aun.edu.eg

داء التيليريا هو مرض رئيسي ينتقل عن طريق القراد يصيب الماشية في المناطق الاستوائية والمتوسطية، ويسبب خسائر اقتصادية كبيرة ويؤثر سلبيًا على صحة الحيوان وإنتاجيته. هدفت هذه الدراسة إلى تحديد معدل انتشار عدوى التيليريا بين الماشية المهجنة في محافظة أسيوط بصعيد مصر، باستخدام الفحص المجهرى. بعد ذلك، تم اختيار مجموعة من العينات التي أظهرت إيجابية قوية عن طريق الفحص المجهرى للتأكد من خلال تقنيات التعريف الجزيئي. تم جمع ٢٠٠ عينة دم من الماشية المهجنة، بما في ذلك ٨٤ حيوانًا سليمًا ظاهريًا و١١٦ حيوانًا تظهر عليها علامات مرضيه توحى بالتيليريا. كشف الفحص المجهرى لعينات الدم المصبوغة بالجيوسا عن معدل انتشار عام لعدوى تيليريا بنسبة ٣٨,٥% (٢٠٠/٧٧). لوحظت الحيوانات المصابة بشكل متكرر بين المجموعة التي أظهرت أعراض المرض، مع معدل انتشار أعلى بكثير بلغ ٥٤,٣١%، مقارنة بالمجموعة السليمة ظاهريًا. كشف تحليل العوامل الوبائية أن الماشية التي يزيد عمرها عن عام واحد أظهرت معدل إصابة أعلى (٤٧,٥%) مقارنة بالحيوانات الأصغر سنًا. أظهرت الماشية الإناث انتشارًا أكبر من الذكور. كان التباين الموسمي واضحًا، حيث بلغت معدلات الإصابة ذروتها خلال الموسم الحار (٤٤,٢٣%) وانخفضت خلال الموسم البارد (٣٢,٢٩%). ومع ذلك، أشار التحليل الإحصائي إلى أن العمر والجنس والموسم لم يرتبطوا بشكل كبير بحالة الإصابة ($P > 0.05$). في المقابل، ارتبطت الإصابة بالقراد بشكل كبير بعدوى *Theileria spp.* ($P < 0.05$)، مما يسلط الضوء على دورها المحوري في انتقال المرض. تم إجراء التأكيد الجزيئي لعينات تمثيلية مختارة أظهرت درجة أعلى من الإيجابية وحمل طفيلي مرتفع عن طريق الفحص المجهرى باستخدام تفاعل البوليميراز المتسلسل (PCR) الذي يستهدف جين *Tams 1*، الذي يشفر مستضد السطح لـ *Theileria annulata*. أكد التضاعف الناجح لجزء بطول ٧٢١ زوجًا قاعديًا في هذه العينات الإيجابية مجهرًا وجود *T. annulata*. خلصت هذه الدراسة إلى انتشار التيليريا بين الأبقار المهجنة في محافظة أسيوط، مع ارتفاع ملحوظ في معدلات الإصابة لدى الحيوانات المصابة. وبينما لم يُظهر العمر والجنس والموسم تأثيرًا ذا دلالة إحصائية، ارتبطت الإصابة بالقراد ارتباطًا وثيقًا بخطر الإصابة. وتؤكد هذه النتائج على ضرورة وضع برامج متكاملة لإدارة القراد، والمراقبة التشخيصية المنتظمة، للسيطرة على داء التيليريا والحد من آثاره الاقتصادية والصحية على الأبقار في المنطقة.