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EPIDEMIOLOGICAL AND CLINICAL MANIFESTATIONS OF BLOOD PARASITIC INFECTIONS IN CATTLE IN ASSIUT GOVERNORATE EGYPT

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ABSTRACT

This research paper presents the epidemiological data and clinical manifestations of blood parasitic infections in cattle. A total of 130 blood samples were collected from animals exhibiting clinical signs suggestive of blood parasitic infection. Microscopic examination of blood smears revealed an overall infection rate of 46.2% among the examined cattle. *Theileria sp.* infections were found to be the most prevalent, with an overall prevalence of 40%. *Babesia sp.* infections had a lower overall prevalence of 6.2%, Microscopic examination also highlighted the intracellular localization of *Theileria* and *Babesia* parasites within red blood cells. The infected red blood cells exhibited morphological changes, appearing enlarged and distorted compared to unaffected cells. The study analyzed the risk factors for *Theileria sp.* and *Babesia sp.* infections in cattle, specifically focusing on age and gender. The prevalence of *Theileria sp.* infection was highest in cattle less than one year, with no significant difference observed among different age groups or genders. Similarly, there was no significant difference in *Babesia sp.* infection rates based on age or gender. These results provide valuable insights into the epidemiology and clinical manifestations of blood parasitic infections in cattle. The findings can contribute to the development of effective control and prevention strategies, highlighting the importance of regular surveillance and management practices in mitigating the impact of these infections on cattle health and productivity.

Keywords: Microscopic examination, Blood smears, *Theileria sp*., *Babesia sp*., Risk factors.

INTRODUCTION

Cattle play a significant role in Egypt's economy by enhancing the socioeconomic status of resource-poor farming communities and reducing poverty. They are the main livestock used in Egypt to provide milk and meat (Zaitoun *et al*., 2019).

Cattle blood parasites pose significant challenges to livestock health and productivity worldwide. Among the notable blood parasites affecting cattle are *Theileria* and *Babesia*, which are intracellular

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Apicomplexan protozoan parasites that infect erythrocytes, causing significant economic losses in the livestock industry (Almazán *et al.*, 2022). These parasites are transmitted by ticks, which act as vectors by injecting sporozoites into the bloodstream of susceptible hosts (Andersson *et al.*, 2017). Once inside the host, *Theileria* and *Babesia* undergo complex life cycles, involving replication and development within the host's erythrocytes. The parasites' ability to evade host immune responses and their impact on erythrocyte function makes them important pathogens to study (Lempereur *et al.*, 2017).

Egypt, with its diverse livestock population and favorable climatic conditions, provides an ideal environment for the transmission and spread of blood parasites among cattle. Several studies have documented the prevalence of *Theileria* and *Babesia* species in cattle (Rizk *et al.*, 2017). For instance, research conducted in different regions of Egypt reported the presence of *Theileria annulata*, *Theileria orientalis*, and *Babesia bigemina*, among other species (Fereig *et al.*, 2017; El-Dakhly Kh *et al.*, 2018; Selim *et al.*, 2022), Extensive research has been conducted on *Theileria* and *Babesia* parasites in Egypt due to their significant impact on the cattle industry (Anter, 2019). The movement of infected animals plays a crucial role in the spread of these infections. Introducing infected cattle into uninfected herds can create new areas of infection, while the movement of cattle between regions with varying parasite prevalence can introduce different species of *Theileria* and *Babesia* (Zhou *et al.*, 2019).

Infection with *Theileria* and *Babesia* parasites can lead to severe diseases collectively known as bovine theileriosis and babesiosis, respectively (Habibi *et al.*, 2020). These diseases are characterized by symptoms such as fever, anemia, lethargy, decreased milk production, weight loss, and sometimes death. *Theileria annulata*, in particular, causes tropical theileriosis, a disease of high economic importance in cattle (Yousef *et al.*, 2020). The impact of these diseases extends beyond direct losses due to morbidity and mortality, as infected animals may also suffer from reduced fertility and compromised meat and milk production (Agina *et al.*, 2020).

The study of cattle blood parasites, specifically *Theileria* and *Babesia*, holds immense importance for the cattle industry in Egypt. This research paper seeks to delve into the prevalence and associated risk factors of these parasites within cattle populations across various regions of Assiut Governorate. By conducting this study, we aim to enhance our comprehension of the prevalence rates and risk factors associated with *Theileria* and *Babesia* infections. This research endeavor strives to contribute to the development of targeted control strategies that can effectively mitigate the impact of these parasites on livestock health. Ultimately, the findings of this study have the potential to improve overall livestock health outcomes, leading to enhanced productivity and sustainability within the cattle industry in Egypt.

MATERIALS AND METHODS:

i. Study Area:

The study was conducted in Sidfa, EL-Badary, EL-Fath and Drunka, Assiut Governorate, Egypt, which is located at approximately 27.1828° N latitude and 31.1837° E longitude (Ahmed *et al.*, 2020). The region's environmental factors, including humidity, create suitable conditions for the thriving of blood parasites. Moreover, the presence of vectors such as ticks, poor cattle management practices, and the trade and movement of cattle all contribute to the risk of blood parasite spread in Assiut Governorate (Abdelbaset *et al.*, 2022).

ii. Sample Collection:

ii.a. Animals:

A total of 130 cattle from Assiut Governorate, Egypt, were included in this cross-sectional study to determine the prevalence of bovine blood parasites. The animals were selected based on exhibiting signs of fever, along with

infestation by hard ticks species. Additionally, a group of apparently healthy cattle belonging to the same owners, referred to as the contact group, were also sampled. The study animals in all selected areas were categorized into three age groups: that is, calves (less than 1 year), young (1–3 years) and adults (above 3 years). The age of the animals was estimated by looking at the dentition pattern of the animals (Frandson and Spurgeon, 1992)**,** and both sexes were included in the study.

ii.b. Samples:
Blood samples were collected from the jugular vein of each selected animal using sterile vacutainer tubes (EDTA tubes). Care was taken to avoid contamination during the collection process, and samples were labeled with unique identifiers for proper identification and tracking. After collection, samples were transported to the Veterinary Laboratory of the College of Veterinary Medicine, Assiut University, and were processed on the day of collection or stored at +4 °C to be processed the next day (Gupta and Singla, 2012).

iii. Laboratory Examination:
In the laboratory, the collected blood samples were processed for the detection and identification of Blood parasites. The following techniques were employed:

iii.a. Direct Microscopic Examination:
A drop of blood was placed on a slide with a coverslip and examined under the microscope to see the living or motile parasite (Soulsby, 1982).

iii.b. Thin blood film Examination:
Thin blood films were prepared on clean glass slides, air-dried, and fixed with methanol 99%. The slides were stained with 10% Giemsa stain and examined under a light microscope at high magnification (Oil immersion lens) Olympus Microscope. The presence of intraerythrocytic parasitic stages were recorded. Morphological features, such as the shape and size of infected RBCs, were observed to aid in species identification (Soulsby, 1982).

iv. Data Analysis:
The statistical analysis was performed using the SPSS software, version 20. The chi-square tests in SPSS were used to determine the significance of associations between variables. The prevalence of single infection, and total infection with *Theileria sp.* and *Babesia sp.* was assessed using the chi-square test. Additionally, chi-square tests were conducted to examine the relationship between age (categorized as >1 year, 1-3 years, and <3 years) and gender (male and female) with the presence of these parasites. This allowed for the evaluation of associated risk factors. The resulting p-values from the chi-square tests were used to determine the level of significance for each association. A p-value below 0.05 was considered statistically significant, indicating a significant association between the variables.

Ethical Considerations:
Ethical approval for the study was obtained from the Faculty of Veterinary Medicine, Assiut University, Egypt. The study was conducted in compliance with all relevant Egyptian laws pertaining to research and publication. The ethical approval number for this research project is [06/2023/0118].

RESULTS

i. Epidemiological data and Clinical manifestation of cattle revealing blood piroplasms infection:
In this study, a total of 130 blood samples were collected from animals. Upon examination, it was determined that 60 of these samples, representing an infection rate of 46.2%, were found to be infected with blood piroplasms. Conversely, 70 animals (53.8%) tested negative for piroplasms and exhibited no clinical signs linked to these infections (Table 1).

Table 1: Prevalence of Piroplasm Infections in Cattle.

Parasite	Apicomplexa species	Number of bovines	Percentage
Infection	*Theileria sp. showing clinical signs*	44	33.8%
	Theileria sp. without clinical signs	8	6.2%
	Babesia sp.	8	6.2%
Positive	_	60	46.2%
Negative	_	70	53.8%
Total Examined	_	130	100%

Among the 130 animals tested, it was discovered that 52 animals (44%) tested positive for *Theileria sp.* infection. Among these animals 44(33.8%) displayed various clinical signs commonly associated with *Theileria* infection, including fever, enlarged lymph nodes, Opacity in the eye, and respiratory distress with nasal discharge. Intriguingly, another 8 animals (6.2%) tested positive for *Theileria sp*., but did not exhibit any discernible clinical signs (Figure1).

Figure 1: Illustrates various clinical manifestations observed in the cattle infected with *Theileria sp*. infection x1. a) Ticks attached to the cattle's neck and wither region. b) Shows nasal discharge. c) Opacity in the eye. d) Depicts a cattle with ticks attached to the skin above the udder region.

On the contrary, 8 animals (6.2%) showed clinical signs specifically attributed to *Babesia sp.* infection, such as fever and hemoglobinuria, and were consequently diagnosed as positive for *Babesia sp.* through the examination of blood films (Figure2).

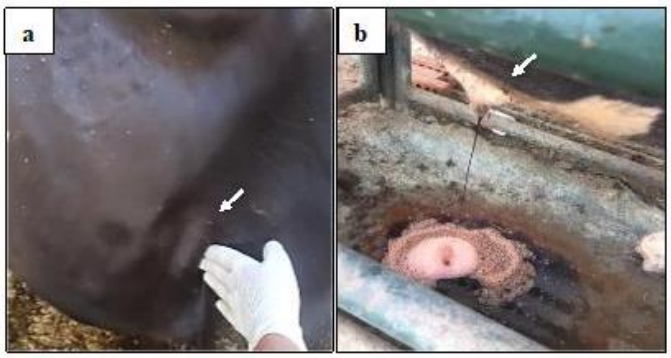

Figure 2: Demonstrates clinical manifestations observed in the cattle infected with *Babesia sp*. infection x1. a) Shows an enlarged Prefemoral lymph node in the cow. b) Hemoglobinuria.

ii. Characteristics of the Detected Blood Parasites under Microscopic Examination:
Microscopic examination provides visual evidence of the intracellular localization of *Theileria* parasites within the RBCs. The presence of *Theileria sp.* within RBCs can cause changes in RBC Morphology as the infected RBCs appear enlarged and distorted compared to unaffected RBCs. highlighting their presence within the host's blood stream (Figure 3).

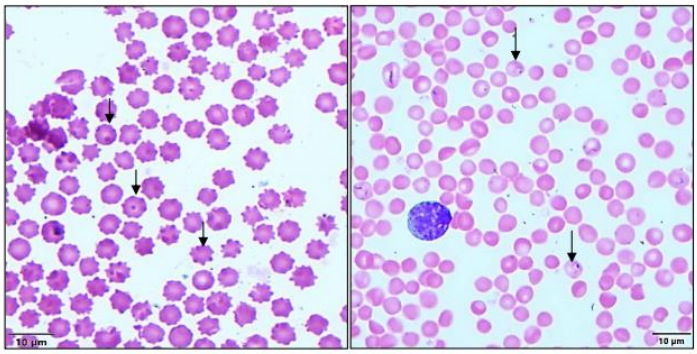

Figure 3: Reveal the presence of *Theileria* parasites within red blood cells (RBCs). These parasites are observed as small, round to dot-shaped structures x100.

While, *Babesia* species, parasites can be visualized as pear-shaped or oval structures within the red blood cells. They typically appear as paired pyriform organisms, commonly referred to as "pear-shaped" bodies. Babesia parasites vary in size, ranging from approximately 1 to 5 micrometers in length (Figure 4)

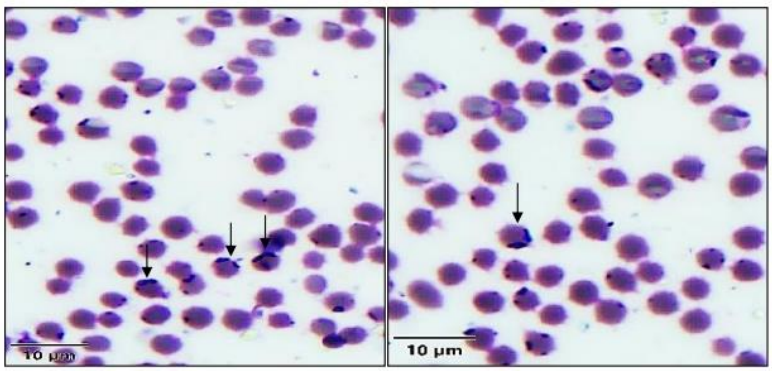

Figure 4: Illustrating the presence of paired pyriform organisms of Babesia species within the red blood cells (RBCs) x100.

iii. The risk factors for *Theileria sp.* and *Babesia sp.* infections in cattle, focusing on age and gender.

The data in Table (2) the risk factors for *Theileria sp.* and *Babesia sp.* infections in cattle, specifically focusing on age and gender. For *Theileria sp.*, the prevalence of infection was highest in cattle less than 1 year (49.1%), followed by cattle aged 1-3 years (36.8%) and cattle older than 3 years (28.6%). However, the p-value (0.1) indicates that the difference in infection rates among the age groups is not statistically significant. In terms of gender, the prevalence of *Theileria sp.* infection was similar in males (34.6%) and females (43.6%), with no significant difference observed (p-value = 0.3).

Regarding *Babesia sp.*, the prevalence of infection was relatively low in all age groups, with the highest prevalence in cattle older than 1 year (8.8%), followed by cattle aged 1-3 years (5.3%) and cattle older than 3 years (2.9%). However, the p-value (0.5) suggests that the difference in infection rates among the age groups is not statistically significant. Similarly, there was no significant difference in *Babesia sp.* infection rates between males (9.6%) and females (3.8%) (p-value = 0.3).

Table 2: Present the risk factors for *Theileria sp.* and *Babesia sp.* infections in cattle, focusing on age and gender.

Risk Factor		*Theileria sp.* (%)	*Babesia sp.* (%)	Total (%)
Age	> 1 year (n=57)	28 (49.1%)	5 (8.8%)	33 (57.9%)
	1-3 years (n=38)	14 (36.8%)	2 (5.3%)	16 (42.1%)
	< 3 years (n=35)	10 (28.6%)	1 (2.9%)	11 (31.4%)
	P.value	0.1	0.5	
Gender	Male (n=52)	18 (34.6%)	5 (9.6%)	23 (44.2%)
	Female (n=78)	34 (43.6%)	3 (3.8%)	37(47.4%)
	P.value	0.3	0.2	

P-value > 0.05 no significance

DISCUSSION

In the recent study, a total of 130 blood samples taken from animals exhibiting clinical signs suggestive of a blood parasitic infection, such as fever, pale visible mucus membrane, weakness, enlarged lymph nodes, respiratory distress, and emaciation (Almazán *et al.*, 2022). Microscopic examination of the blood smears revealed that 46.2% of the animals were infected with blood piroplasms. Consistently, this study (Zhou *et al.*, 2019) reported a total infection rate with piroplasms 43.48% in China.

Additionally, our investigation revealed that *Theileria* sp. infections were more prevalent (40%), similar to Sayed *et al*., (2020), who reported that *Theileria* infection was 38.65% in the New- Valley governorate. While higher than Hosny *et al*., (2010), who found that *Theileria* infection was 31.58% in Fayoum governorate. Also, Zaitoun *et al*. (2019) revealed that *Thileria* infection was 26.67% in Assiut Governorate. The difference may be attributed to geographical and, climatic conditions, as well as management practices (Gul *et al.*, 2015). *Babesia sp.* infections were less prevalent 6.2% which is similar to these authors (Prado *et al.*, 2022; Hossain *et al.*, 2023) who reported a higher infection with *Theileria* than *Babesia*. The higher prevalence of *Theileria* species compared to *Babesia* species is likely attributed to several factors, including the availability of suitable tick vectors, reservoirs, and amplification hosts. These factors contribute to the increased transmission and maintenance of *Theileria* in a given ecosystem (Remesar *et al.*, 2019).

One significant aspect of this research is the exploration of risk factors associated with *Theileria sp.* and *Babesia sp.* infections in cattle, particularly age and gender. The prevalence rate of *Thieleria sp.* was found to be higher in young animals, particularly those under one year old (49.1%). However, there was no significant difference in parasitic distribution with age. These findings are consistent with previous studies that have also reported higher infection rates in younger animals (Al-Hosary *et al.*, 2018). Also, the prevalence of *Babesia* infection was more common in animals under one year old 8.8% compared to older animals. These results highlight the vulnerability of young animals to these parasitic infections (Nyabongo *et al.*, 2021).

In term of sex, our study found that there was no significant difference in the distribution of *Theileria* spp. infection based on sex in cattle. This is consistent with some previous studies that also reported non-significant differences in the prevalence between females and males (Selim *et al.*, 2022). However, there are conflicting findings in the literature, with some studies reporting a higher prevalence in males and others reporting higher prevalence in females (Zhou *et al.*, 2019). It is possible that these variations could be influenced by factors such as geographical location, breed, and management practices (Nyabongo *et al.*, 2021). Additionally, it is worth noting that the prevalence of *Babesia* infection was found to be higher in males compared to females in our study, which is consistent with similar findings reported in other research studies (Idris *et al.*, 2018; Fesseha *et al.*, 2022).

CONCLUSION

In conclusion, this research provides important epidemiological data on blood parasitic infections caused by *Theileria* and *Babesia* species in cattle in Assiut governorate, Egypt. *Theileria* infections were more prevalent, with various clinical manifestations observed in the infected animals. Age and gender did not appear to be significant risk factors for either *Theileria* or *Babesia* infections. These findings contribute to our understanding of blood protozoa infections in cattle and can inform future control and prevention strategies in the studied area. Further studies involving molecular characterization of the identified parasites would be valuable for a more comprehensive analysis of blood parasite infections and their impact on cattle health.

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المظاهر الوبائية والسريرية لعدوى طفيليات الدم في الأبقار بمحافظة أسيوط مصر

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يعرض هذا البحث البيانات الوبائية والمظاهر السريرية لعدوى طفيليات الدم في الأبقار.حيث انه تم جمع مجموعه ١٣٠ عينة دم من الحيوانات التي تظهر عليها علامات سريرية توحي بالعدوى الطفيلية في الدم. أظهر الفحص المجهري لمسحات الدم وجد أن معدل الإصابة الإجمالي بلغ 46.2% بين الأبقار التي تم فحصها. وقد وجد أن العدوى بالثايلريا هي الأكثر انتشارًا، حيث يبلغ معدل انتشارها الإجمالي ٤٠٪,بينما كان معدل انتشار عدوى البابيزيا الإجمالي أقل بنسبة 6.2%، كما سلط الفحص المجهري الضوء على وجود طفيليات الثايلريا والبابيزيا داخل الخلايا داخل خلايا الدم الحمراء. أظهرت خلايا الدم الحمراء المصابة تغيرات شكلية، حيث ظهرت متضخمة ومشوهة مقارنة بالخلايا غير المصابة. حللت الدراسة عوامل الخطر للثايلريا و البابيزيا في الماشية، مع التركيز بشكل خاص على العمر والجنس بالنسبه للثايلريا وكانت الإصابة أعلى في الماشية التي يقل عمرها عن سنة واحدة، مع عدم وجود اختلاف كبير بين مختلف الفئات العمرية أو الجنسين. وبالمثل، لم يكن هناك اختلاف كبير في البابيزيا بالنسبه لمعدلات الإصابة على أساس العمر أو الجنس. توفر هذه النتائج رؤى قيمة في علم الأوبئة والمظاهر السريرية للعدوى الطفيلية في الدم في الماشية. يمكن أن تساهم النتائج في تطوير استراتيجيات فعالة للمكافحة والوقاية، مع تسليط الضوء على أهمية ممارسات المراقبة والإدارة المنتظمة في التخفيف من تأثير هذه العدوى على صحة الماشية وإنتاجيتها.