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Original Article

Blood protozoa infection in cattle in Minia province with reference to tick infestation

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Abstract: This study was conducted to investigate the prevalence of blood protozoa infecting cattle, beside an *in-vitro* study of the efficacy of some common used acaricides i.e (Chlorprifos, Deltamethrin and Ivermectin) against *Rhipicephalus annulatus* ticks. Blood and tick samples were collected from 240 cattle; in addition, to hemolymph smears from ticks were examined microscopically by using Giemsa's stained blood smear method. The study revealed that the overall prevalence of blood protozoa was 52.5%. *Theileria* infection was the highest with a rate of 44.58%, while, *Babesia* infection was 2.5%,and *Anaplasma* infection was 2.9%. and the mixed infection was 2.5% .Blood protozoa infection was lower in young cattle, with 41.67% in calves and 55.2% in adult cattle. Blood protozoa infection was higher in females (54.8%) than males (49.5%), however the mixed infection was found to be higher in males. The overall prevalence of tick infestation among examined cattle was 17.9%. The Autumn season had the highest tick infestation rate (21.6%), while the Spring season had the lowest prevalence rate (15%). The highest tick's infestation rate was recorded in December (30%), and the lowest was in March (5%). The tick microscopically identified indicates only the presence of *Rhipicephalus annulatus* ticks. The microscopic examination of tick hemolymph revealed the presence of piroplasmic developmental stages in 37.2% of examined tick samples. The *in-vitro* results showed that Chlorprifos had the highest efficacy of the tested acaricides, but Deltamethrin and Ivermectin demonstrated low efficacy. In conclusion, Blood protozoa are still a threat to cattle in the study area, with *Theileria* spp. infection being the greatest among tick-borne parasites, and high restrictions in the use of Deltamethrin and Ivermectin are recommended due to their low efficacy.

Key words: Prevalence, Babesia, Theileria, Anaplasma, ticks, cattle

Introduction

Millions of cattle die every year from infections caused by tick-borne pathogens (TBPs). This results in production losses and high costs for farmers all over the world for medical care and tick control. (**Bock, 2004)**. Some of the possible risks to global cattle production include *Babesia bigemina, Babesia bovis, Theileria annulata, and Anaplasma marginale* (**Jongejan et al., 1994)**. Bovine babesiosis (BB) is a prevalent and costly disease in Egypt's cattle business, mostly due to its ability to induce fatal hemolytic anemia and its severe effects on meat and milk output (**Fereig et al., 2017)**. Bovine babesiosis is a blood parasite disease caused by the reproduction of apicomplexan protozoa, specifically *Babesia bovis* and *B. bigemina*, within red blood cells. (**Niu et al., 2015)**. Cattle affected by babesiosis typically exhibit prominent clinical symptoms, such as a high body temperature, anemia, icteric, decreased appetite, muscular tremors, hemoglobinuria, and subsequently death (**Mark and Dohme., 2017)**.

Bovine theileriosis is a world tick-borne disease, and it has become one of the major restrictions affecting livestock production in Egypt. The disease is caused by hemoparasites., *Theileria annulata*, which is transmitted by the tick vector, *Hyalomma* species **(El-Dakhly et al., 2018)**. Theileriosis negative impact has caused a huge economic loss to cattle industry, particularly in beef production-oriented communities, such as high mortality, and cost of treatment and control measures **(Chen et al, 2022)**.

Bovine anaplasmosis is caused by many species of *Anaplasma*, including *Anaplasma marginale, A. centrale, A. ovis*, and the zoonotic *A. phagocytophilum* **(Dumler et al., 2001; Rar and Golovljova., 2011)**. *Anaplasma ovis* infection in cattle often does not show any symptoms, although it can lead to fever, loss of weight, depression, anemia, and in some cases mortality and abortion **(Noaman and Shayan., 2010)**. Acute anaplasmosis is caused by *Anaplasma marginale* causes progressive hemolytic anemia and can cause abortion, high temperatures, less milk production, weight loss, and even death **(Kocan et al., 2003)**. Conversely, *A. centrale*, an intraerythrocytic species closely related to *A. marginale* **(Dumler et al., 2001)**, is less harmful and only causes mild signs in cattle **(Rar and Golovljova., 2011)**. Ticks are of important consideration and have a harmful effect on livestock due to their ability to not only have direct impacts on infested cattle but also to transmit life-threatening infections (**Elsify et al., 2015)**. *Rhipicephalus annulatus* and *Hyalomma anatolicum* are the prevalent tick species that infest cattle in Egypt **(Alhossary et al., 2018).**

So, the aim of this study is to investigate the prevalence of blood protozoa infecting cattle in El-Minia governorate and tick infestations on cattle. Also, *in-vitro* studies were conducted to control ticks with common acaricides.

Material and Methods:

Study area

This study was conducted from November 2022 to December 2023. Tick and blood samples were collected from 240 cattle of native and cross breeds in Minia province **(coordinates: 28° 07′ 10″ N, 30°44′ 40″ E)**, Egypt.

Blood samples

Blood samples were randomly collected through ear vein from cattle, the skin over the ear vein was cleaned with 70% ethanol, and three blood smears were prepared for each cattle in the veterinary clinics and veterinary units. Smears were then fixed with methanol, stained with Giemsa's stain. Then,

were examined under a microscope (100x) with immersion oil for the identification of blood parasites as described by **(Soulsby.,1982)**. The blood smears were recorded as negative for piroplasms if no parasites were detected in 50 fields **(Moretti et al., 2010)**.

Tick samples

All animals were examined for tick infestation at the same time to study. The ticks were collected from cattle by using forceps to remove them with no damage to their mouthparts **(Soulsby, 1982)**. Collected ticks were examined in parasitology labs at Beni Suef University, Kafrelsheikh University, and Minia University for taxonomic identification using an Olympus stereo microscope, according to that given by **(Walker et al., 2003)**.

Preparation of hemolymph smears from ticks

The surface of a tick was disinfected twice using 75% ethanol and once using phosphate buffered saline (PBS) prior to the preparation of hemolymph smears. The ticks were seen and dissected using a stereomicroscope, as described by (**Patton et al., 2012)**. The hemolymph was obtained from the incision following the removal of the lower part of a leg. The sample was collected on a slide, then, dryness with heat and fixation with methanol for 1:2 minute. It was then stained with Giemsa's stain and seen under a microscope at a magnification of 100x, using immersion oil **(Burgdorfer, 1970)**.

Midgut and salivary gland smears

Following the immobilization of the tick, it was washed with a small amount of PBS in order to prevent the tissues from drying out. The scutum was removed using a micro scissor. At this point, the presence of connective tissue and tracheae can be seen and must be eliminated in order to examine the underlying structures. The anterior salivary glands were observed as grape-like structures located at the proximal end of the tick. In addition, there are other clusters of salivary glands situated in close proximity to the midgut. The gut was observed as a dark red, spider-like structure. Tick gut or salivary glands were used to smear preparations. This was done by placing the specimen on a glass slide, crushing it with another slide, and spreading it out in a single movement. The smears were subsequently dried using air, treated with methanol for 5 minutes to fix them, and then stained with a 10% solution of Giemsa stain for 30 minutes. The stained smears were then seen under an oil immersion lens with a magnification of 1000X using a light microscope manufactured by Olympus (**Qayyum et al., 2010**).

Susceptibility of collected ticks to common acaricides in vitro acaricidal activity

Deltamethrin (98%), Chlorprifos (99%), and Ivermectin powder (97%) were obtained from a local Egyptian company specializing in pesticides and chemicals (Laboratory Chemicals Trading Company). The three acaricides, Deltamethrin (D), Chlorpyrifos (Ch), and Ivermectin (IV), were dissolved in pure ethyl alcohol at different concentrations. Deltamethrin was dissolved at a rate of 5%, Chlorpyrifos at a rate of 25%, and Ivermectin at a rate of 1%. Five dilutions were prepared by diluting D and Ch in 70% ethyl alcohol. A 1% stock solution of Ivermectin was made using 100% ethanol, as described by (**Rodriguez-Vivas et al., 2014)**. Distilled water was used to dilute a 1% ethanol solution containing 2% Triton X-100 (TX 1%). After then, the TX solutions were used to prepare different Ivermectin dilutions (5, 2.5, 1.25, 0.75, and 0.38 mg/mL).

Adult immersion test (AIT) for Adult ticks

The adult engorged females of *R. annulatus* were used for this technique. This test was carried out in accordance with **(Drummond et al.,1973)**. Ten engorged female ticks were submerged for two minutes for D and Ch, while in IV, they were submerged for 10 minutes in tubes containing

10 mL of various treatments before being dried and incubated in Petri dishes at 26 to 28 °C and 80% relative humidity. In the same experiment, 5 replicates of 10 ticks were used for each treatment. The control ticks were immersed in 70% ethyl alcohol for two minutes. Dead adult ticks that were black in color, immobile, and had no egg deposits were counted. For live ticks, the egg production index (EPI) was determined using the following formula:

$$\textbf{(EPI} = \frac{\text{Weight of egg mass}}{\text{Initial weight of engorged female}} \times 100\textbf{)}$$

(**Klafke et al., 2020).**

Statistical analysis

Data of the different treatments was statistically analyzed using IBM SPSS for Windows, v.22 (IBM, Armonk, NY, USA). ANOVA was used to compare the different treatments and subsequent Duncan's estimated the differences between means ($\alpha = 0.05$). Lethal concentrations, causing 50% mortality were calculated with SPSS v.22.

Results

.1. Prevalence of blood protozoa among cattle at Minia province

The overall prevalence of blood protozoa infection in the microscopically examined blood samples of cattle was 52.50%(126/240) (table,1). The highest infection rate (61.66%) was recorded in Spring. While Autumn recorded the lowest infection 35.00 % (table. 2). The monthly infection rate revealed that March and February had the highest rates (75 % and 70%) respectively. While, April and July recorded the same infection rate 60%, also, June, August and September recorded the same rates of infection 55%. The lowest infection rate was recorded in October 20%. (table. 3).

Theileria infection recorded 44.58% from total examined cattle (107/240) and 84.9% of the total infected cattle, while, *Babesia i*nfection recorded 2.5% from total examined cattle (6/240)and 4.7% of the total infected cattle. *Anaplasma* infection recoded 2.9% from total examined cattle (7/240) (5.5% of the total infected cattle). While, mixed infection recorded 2.5% from total examined cattle (6/240) (4,76% of total infected cattle)). (1 cattle found to had mixed infection with the three blood protozoa, 2 cattle had mixed infection with both *Theileria* and *Anaplasma*, 2 cattle had mixed infection with both *Theileria and Babesia*, and 1 cattle had mixed infection with both *Anaplasma a*nd *Babesia),* but negative for other blood protozoal infection (table. 1) & (figure. 1,2,3,4,5).

In studying the age and sex influences on the infection rates, results showed that young cattle had a lower blood parasite infection (41.67%) compared to adult cattle which recorded 55.2% infection rate. Also females found to have high infected rate (54.8%) than males (49.5%)(table. 4)

3.2 Tick infestation

3.2. 1. Prevalence of tick infestation among cattle at Minia province

The overall prevalence of tick infestation among examined cattle was 17.9%. Regarding to seasonal prevalence, the highest infestation rate was recorded in Autumn (21.6%), and the lowest was in Spring (15%) (table 5). Meanwhile, the highest infestation rate was recorded in December (30%), and the lowest was in March (5%) (table. 6). 51% of tick-infested cattle were infected with blood parasites. The microscopic examination of tick hemolymph revealed the presence of piroplasmic developmental stages in 37.2% (16/43) (table. 6). The tick microscopically identified indicates only the presence of *Rhipicephalus annulatus* ticks.

3.2.2. In-vitro acaricidal activity of chlorprifos, deltamethrin and ivermectin against adult ticks of *R. annulatus*

Chlorprifos showed the highest significant efficacy of the acaricide on *R. annulatus* ticks and the lowest resistance, but deltamethrin had the highest resistance with low efficacy *on R. annulatus* ticks.

The high concentrations (2.5, 5, 10& 20%) of Chlorprifos achieved 100% mortality. The adulticidal effect of Chlorprifos against *R. annulatus* adult ticks showed that the LC_{50} was 0.876 % and LC_{90} was 1.729 %. On other side, deltamethrin and ivermectin showed the lowest adulticidal effect, which recorded (1.80 % & 2.65 %) for LC_{50} respectively and (4.04 % &15.91%) for LC_{90} respectively. (Table 7)

4. Discussion

Three prevalent tick-borne diseases (TBDs) that cause significant economic losses to cattle in Egypt are theileriosis, babesiosis, and anaplasmosis **(Elsify et al., 2015)**.

The current study, conducted from November 2022 to December 2023, found that out of 240 blood samples examined, 126 (52.5%) were infected with blood parasites, of which 107 were infected by *Theileria* species. This resulted in a prevalence rate of 44.58% for the total examined cattle and 84.9 percent for the total infected cattle. Several studies in Egypt investigated the prevalence of *Theileria* parasites in cattle and obtained similar results. Among those, (**Abdel-Rady et al., 2008)** detected that out of 120 cattle, 31 (25.8%) animals in Assuit, Sohag, and El-Wadi El-Gadid provinces harbored *T. annulata* parasites in Giemsa-stained thin blood smears. Furthermore, **(Abdel-Rady et al., 2010)** reported an infection rate of 65.6% using PCR. Furthermore, (**Al-Hosary et al., 2015)** found that Giemsa-stained thin blood smears revealed 30.98% of cattle (145/468) positive for *theileriosis* in El-Wadi El-Gadid. Meanwhile, (**Elsify et al., 2015)** recorded an infection rate of 9.56% with *Theileria annulata* in cattle in Menoufia, Behera, Giza, and Sohage provinces, Egypt, using light microscopy of Giemsa-stained thin blood smears. Meanwhile (**El-Dakhly et al., 2020)** recorded that *theileria annulata* 22.0% (33/150) in cattle Beni-Suef, El-Fayoum, and El-Wadi El-Gadid using PCR.

In a similar African country, Algeria According to **(Ayadi et al., 2016)**, 10.4% and 16.4%, respectively, of Giemsa-stained thin blood smears were positive for *Theileria* before and after the tick season. Furthermore **(Koonyosying et al., 2022)** recorded that the infection rate of *Theileria orientalis was* 36.50% by microscopic examination of a blood sample from cattle from central and northern Thailand**.** Meanwhile **(Khatoon et al., 2021)** recorded that the prevalence of theileriosis was 67.22% in cattle in Udaipur, Rajasthan, by blood smear examination.

The higher infection rate could be attributed to several cattle carriers of theileriosis without clinical signs and cattle with chronic theileriosis, as mentioned by (**Cacci et al., 2000) and (Kirvar et al., 2000**).

Also, with (**Noaman., 2013)**, recorded that the recovered cows from acute or primary *theileriosis* and *babesiosis* remained infected for a long period and even for the rest of their lives, acting as reservoirs of infection for ticks and causing natural transmission of the disease. Recovered cattle are the primary agents of infection spread, and due to their proximity to industrial dairy cattle farms, there is a possibility of disease transmission to other farms by vectors. Moreover, **(Altay et al., 2008)** detected that cattle with subclinical theileriosis and babesiosis become chronic carriers of the piroplasm and, hence, sources of infection for tick vectors. Therefore, latent infections are important in the epidemiology of diseases.

Theileria is quite prevalent in El Minia, despite the rarity of *Hyalomma*. This might be because of the mechanical transmission of the disease by insect bite, colostrum, or iatrogenic (medical procedures or treatments such as blood transfusion, reuse of needles, or IV set). According to **(Hammer et al., 2016)**, it has been observed that *T. orientalis* may be mechanically transmitted intravenously with tiny amounts of blood and can be detected up to 5 months after infection. Animals infected

using this method can have a substantial impact on the spread of the infection by serving as carriers without showing any symptoms. Additional methods of blood transmission involve the activity of arthropods that bite. Colostral transfer can potentially serve as a means of transmission. **(Spickler et al., 2010)** also mentioned that iatrogenic transmission can occur via blood (e.g., on re-used needles).

In the current study, the theileria infection rate is the highest in Spring (53.3%) from total examined cattle, and the lowest in Autumn (28.3%). In the current study, *theileria* infection rates in males were slightly higher (44.8%) of total examined males than females (44.3%) of total examined females. On the contrary, those reported by (**Goyal., 2018)** recorded that the prevalence was higher in females (44.83) as compared to male cattle (35.71%) by nested polymerase chain reaction (nPCR). Also, with (**Khatoon et al., 2021)**.

In the present investigation, 6 cattle (2.5% of the total examined cattle) were *Babesia*-infected. These findings coincided with those reported by **(El-Bahy et al., 2018)**, who found that the infection with *Babesia* species was 9.42% in Behaira using Giemsa-stained blood smears, and these results were confirmed by PCR as well. Moreover, (**Nayel et al., 2012)** found that the prevalence of *Babesia* spp. was 8.15% by direct microscopy using Giemsa-stained thin blood smears in Menofia. Moreover, (**Ibrahim et al., 2012)** recorded the prevalence of *B. bigemina* and *B. bovis* in cattle by nestedPCR, which were found at 5.30 and 3.97%, respectively, in Behaira and Fayoum provinces. Moreover, (**El-Dakhly et al., 2020)** recorded that *Babesia bigemina* 19.33% (29/150) in cattle Beni-Suef, El-Fayoum, and El-Wadi El-Gadid using PCR**.** Moreover, **(Koonyosying et al., 2022)** recorded that the infection rate of *Babesia* spp. is 1.22 percent by microscopic examination of blood samples from cattle from central and northern Thailand**.**

In the present investigation, 7 cattle (2.9% of the total examined cattle) were infected with *Anaplasma*. These findings coincided with those reported by (**Mahmoud et al., 2022)**, who revealed that 7.5% had an *anaplasma* infection with the microscopic examination of blood smears moreover (**El-Dakhly et al., 2020)** recorded that *A. marginale was* 10.6% (16/150) in cattle from Beni-Suef, El-Fayoum, and El-Wadi El-Gadid using PCR. **(Koonyosying et al., 2022)** recorded that the infection rate of *A. marginale* is 34.15% by microscopic examination of a blood sample from cattle from central and northern Thailand.

In current study, the rate of infection with *anaplasma* was higher in females (4.5%) than males (0% but there as mixed). This result was in agreement with those reported by (**Mahmoud et al., 2022)**, who recorded that the infection rate concerning gender indicated that it was higher in females (7.57%) than males (6.67%).

In the current study, there was no significant seasonal influence on the prevalence of *Babesia* and *Anaplasma*, which was in agreement with what was reported by (**Velusamy et al., 2014)**.

In the present investigation, the mixed infection with blood parasites was 2.5% from total examined (4.76% from total infected cattle), and the mixed infection was found to be higher in males (7.5% from total infected male cattle) than females (2.7% from total infected female cattle). (**Khatoon et al., 2021)** recorded that the prevalence of mixed hemoprotozoan infection was 18.54% in cattle in Udaipur, Rajasthan, by blood smear examination.

In the current study, the prevalence of the tick's infestation was found to be 17.9% (43 infested cattle/240 examined cattle). These findings coincided with those reported by **(Ramadan et al.,2016)** revealed that 6.17% of cattle were infested by ticks in Qalyobia governorate moreover (**Sayed et al.,2020)**

recorded that 116 out of 414 examined samples (28%) were positive for tick infestation in cattle in the New Valley Governorate. Moreover (**Ica et al., 2007)** examined a total of 300 cattle for tick infestations found Ticks infested 117 (39%) of the 300 cattle.

The highest infestation rate was recorded in December (30%), and the lowest one was in March (5%). The highest infestation rate was recorded in winter **(18%),** and the lowest prevalence was in spring (15%). The study found that 51% of tick-infested cattle were infected with blood parasites. Contrary to expectations, it seems that ticks that infest cattle are more abundant in the winter season compared to the summer. This observation can be explained by the infestation of ticks throughout the summer season, particularly in regions where animals have light coats. This makes the ticks more visible to farmers, who then take appropriate measures to eliminate them by administering tick-repellent drugs. In contrast, in winter, ticks multiply in areas of the body with dense fur, posing a challenge for farmers to identify them. As a result, farmers may not take action to apply tick-repellent compounds to the animals. This promotes the growth and spread of ticks among a large number of cattle.

Through the examination of hemolymph for the developmental stages, it was found that 16 (37.2%) of the 43 examined samples contained the developmental stages of *Babesia*, *Theileria*, and *Anaplasma*. This result was in agreement with that reported by (**Ramadan et al., 2016),** who recorded that the microscopic examination of tick hemolymph revealed the presence of piroplasmic developmental stages in 39.23% and 49.4% of hemolymph samples obtained from *Rhipicephalus turanicus* and *Rhipicephalus praetextatus*, respectively.

Chemotherapeutic control remains the foundation of tick control programs for the eradication of livestock infestations in the developing world. (**Bianchi et al., 2003).** The current study tested Deltamethrin, Ivermectin, and Chlorprifos *in vitro* against adult ticks and found that Chlorprifos, at a concentration of 2.5 mg/L, exhibited a greater efficacy on tick mortality than Deltamethrin and Ivermectin**.** Chlorpyrifos is a toxic, crystalline organophosphate acaricide that inhibits acetylcholinesterase and is used to control insect pests. There is a progressive decrease in the efficiency of acaricidal drugs through the development of resistance **(Aboelhadid et al., 2018).** Epidemiological investigations have suggested that a reduction in acaricide-treatment frequency that permits high tick attachment rates allows for the development of endemic stability (**You., 2014)**. To this end, a regular screening of compounds is required to determine their efficacy.

Conclusions

Theileria spp. was the most prevalent tick-borne blood protozoa in the examined cattle. The mixed infection was found to be higher in males than females. The absence of ticks in cattle did not affect the rate of infection with blood parasites. Chloripyrifos has higher efficacy as an acaricide compared to Deltamethrin and Ivermectin.

Ethics

This study was approved by the Ethics Committee of Faculty of Veterinary Medicine Science, Suez Canal University, Egypt with approval number (2018123).

Conflict of interest

The authors declare that they have no conflict of interest.

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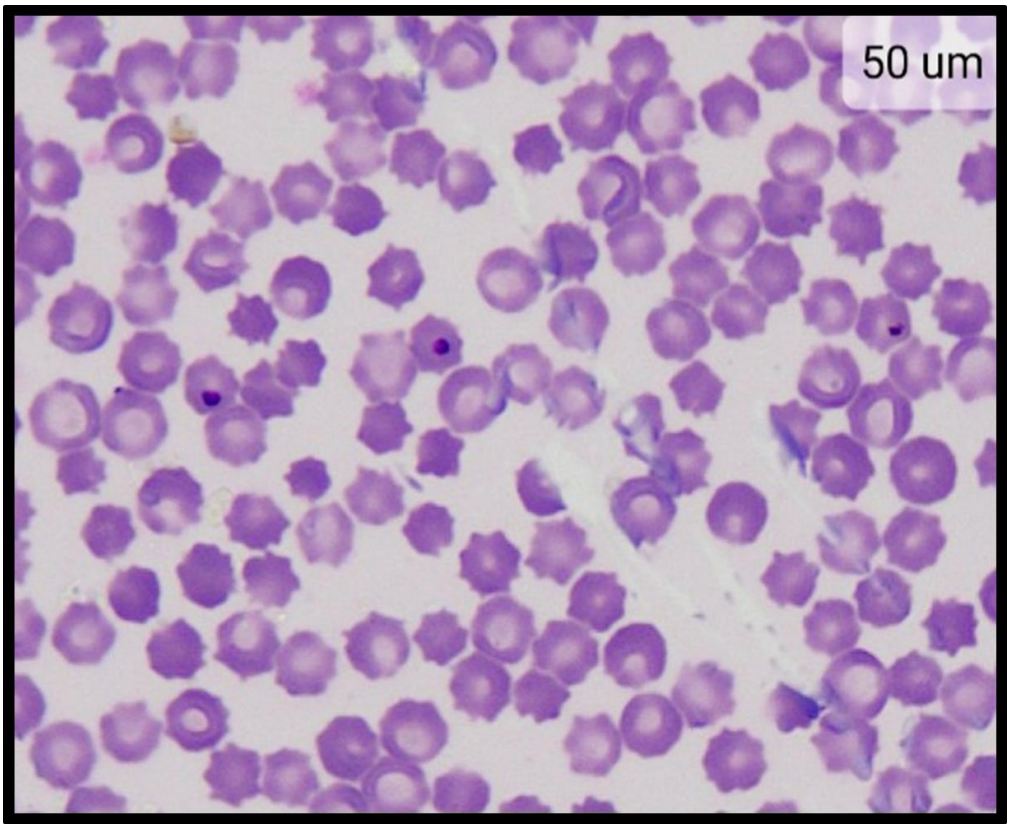

Figure 1.showed *Theileria annulata* merozoites in RBCs.

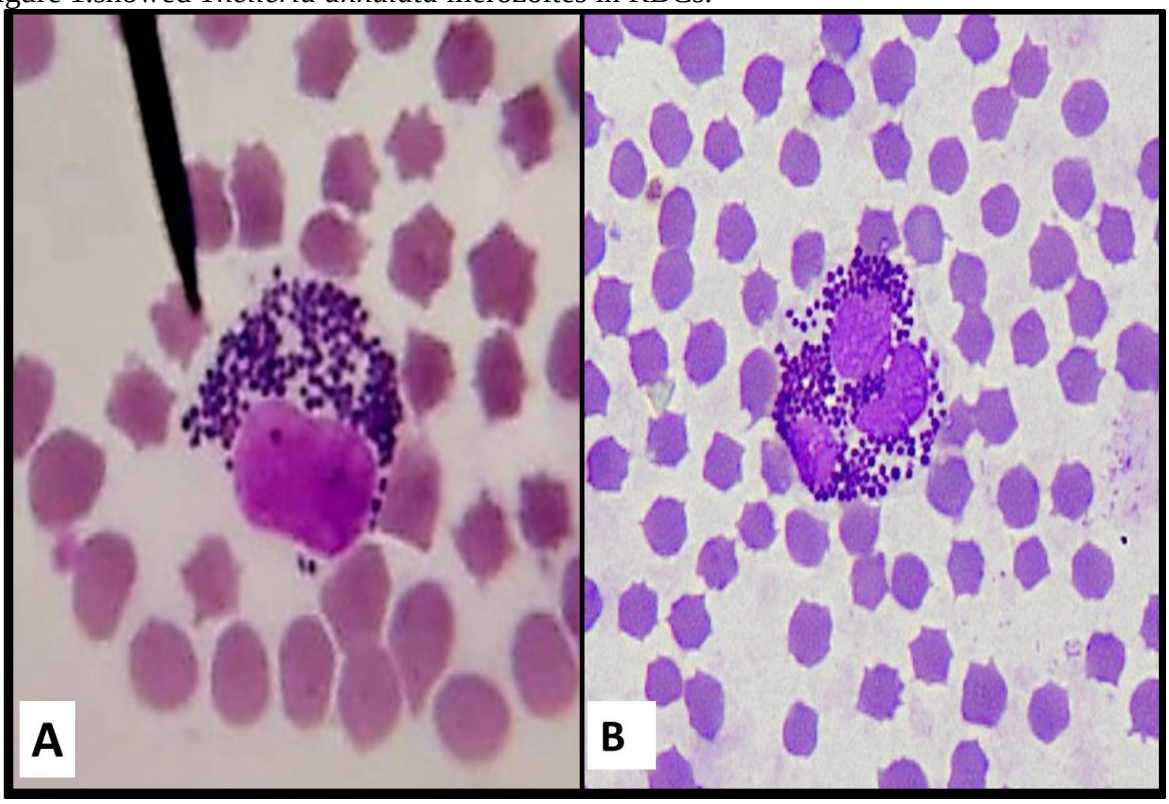

Figure 2**. A &B** showed *Theileria annulata* Koch blue body.

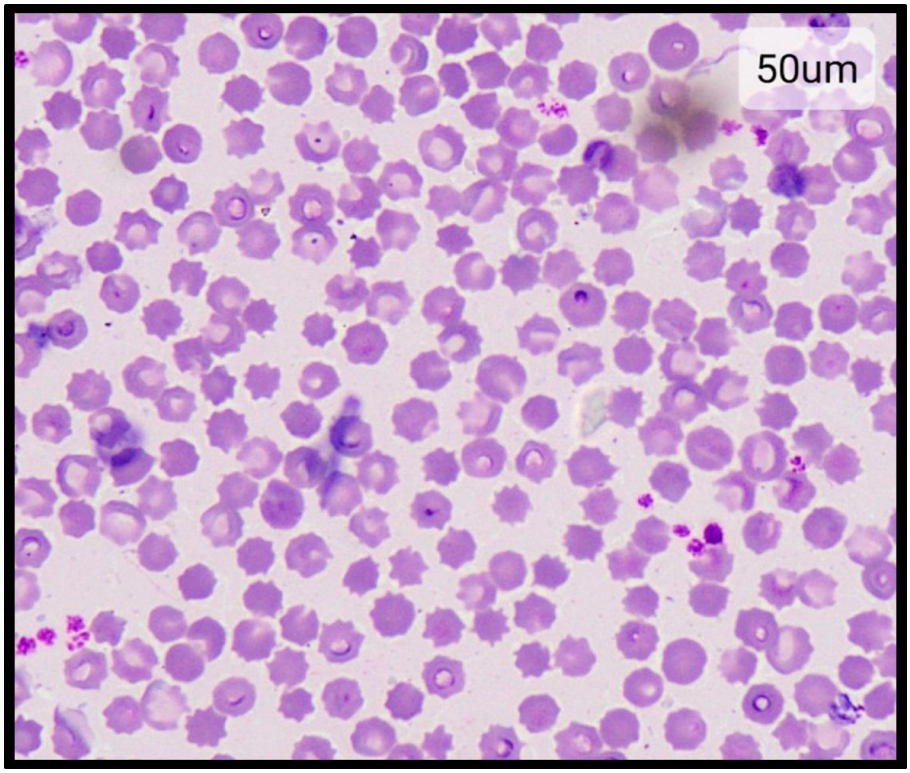

Figure 3.showed *Babesia bigemina* merozoites in RBCs infected cattle**.**

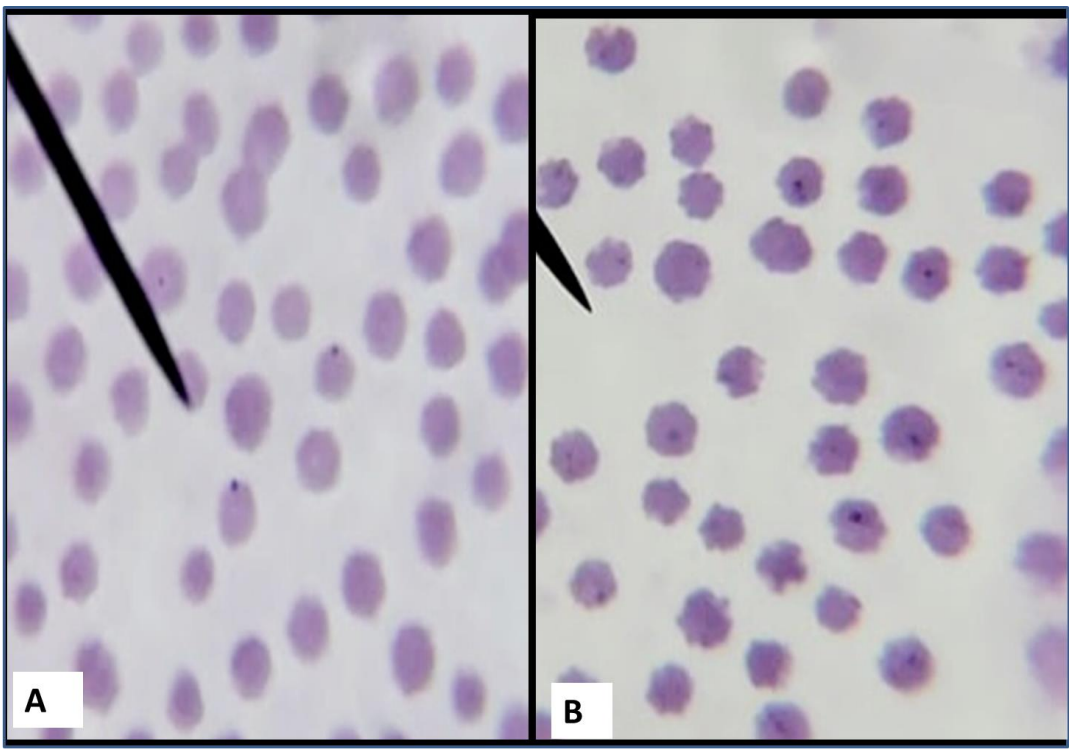

Figure 4. **A**.showed *Anaplasma marginale* (dots at periphery of RBCs)**.**
B. showed Anaplasma centrale (dots at the center of RBCs).

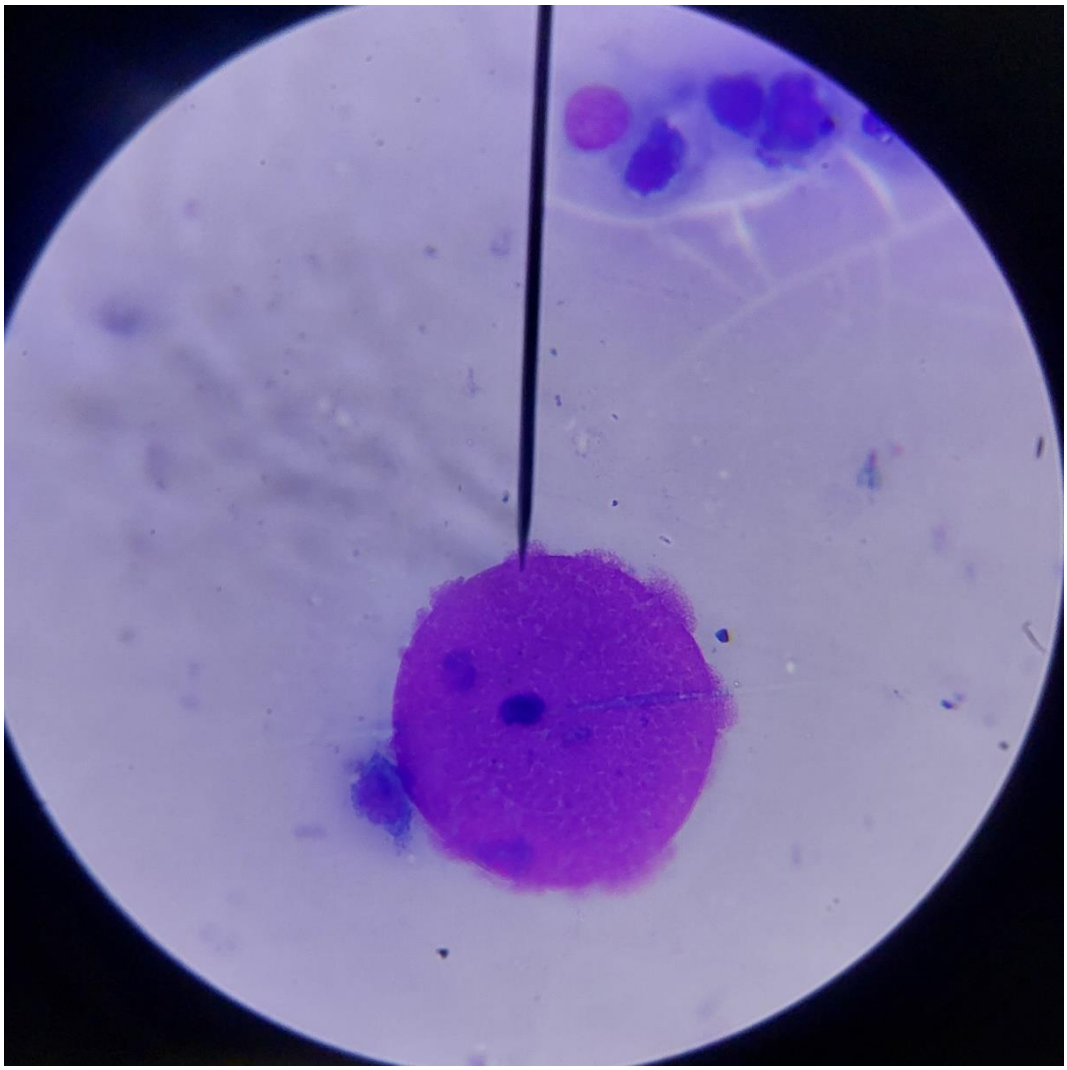

Figure 5.Mature oocyte filled with ripe sporocysts of Babesia species (x1000).

Table 1. Prevalence of blood protozoa infection in cattle examined in the current study at Minia province, Egypt

Item	Number	Percentage
Total Blood samples examined	240	
infected samples	126	52.5%
Theileria infection	107	44.58%
Babesia infection	6	2.5%
Anaplasma infection	7	2.9%
Mixed infected samples	6	2.5%

Table 2. Seasonal prevalence for blood protozoa infection in cattle examined by blood film smears at Minia province , Egypt

Seasons	Samples examined	Samples infected		*Theileria*		*Babesia*		*Anaplasma*		Mixed	
		No.	%	No.	%	No.	%	No.	%	No.	%
Winter	60	34	56.6	29	48.3	1	1.6	1	1.6	3 (1Th+B+A) (1Th+A) (1Th+B)	5
Spring	60	37	61.66	32	53.3	1	1.6	3	5	1 (Th+A)	1.6
Summer	60	34	56.6	29	48.3	3	5	2	3.3	0	0
Autumn	60	21	35	17	28.3	1	1.6	1	1.6	2 (1Th+B) (1B+A)	3.3
Total	240	126	52.5	107	44.6	6	2.5	7	2.9	6	2.5

Table 3. Monthly Prevalence of blood protozoa infection in cattle examined by blood film smears:

Month	**Samples examined**	**Samples infected**		***Theileria***		***Babesia***		***Anaplasma***		**Mixed**	
		No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
January	20	10	50	9	45	1	5	0	0	0	0
February	20	14	70	12	60	0	0	1	5	1 (Th+B)	5
March	20	15	75	12	60	0	0	3	15	0	0
April	20	12	60	10	50	1	5	0	0	1 (Th+A)	5
May	20	10	50	10	50	0	0	0	0	0	0
June	20	11	55	9	45	1	5	1	5	0	0
July	20	12	60	10	50	2	10	0	0	0	0
August	20	11	55	10	50	0	0	1	5	0	0
September	20	11	55	8	40	1	5	0	0	2 (1A+B) (1Th+B)	10
October	20	4	20	3	15	0	0	1	5	0	0
November	20	6	30	6	30	0	0	0	0	0	0
December	20	10	50	8	40	0	0	0		2 (1Th+B+A) (1Th+A)	10
Total	240	126	52.5	107	44.6	6	2.5	7	2.9	6	2.5

Table 4. Age and sex influences for blood protozoa infection in cattle examined by film smears at Minia province ,Egypt

Age &Gander	Blood samples examined	Blood samples infected		*Theileria*		*Babesia*		*Anaplasma*		Mixed	
		No.	%	No.	%	No.	%	No.	%	No.	%
Adult	192	106	55.2	92	86.7	9	8.5	8	7.5	3(2Th+B)(1B+A)	2.8
Calf	48	20	41.67	17	100	0	0	0	0	3 (2Th+A) (1Th+A+B)	15
Males	107	53	49.5	48	44.8	1	0.9	0	0	4 (1Th+A+B) (2Th+A) (1Th+B)	3.7
Females	133	73	54.8	59	44.3	5	3.7	7	4.5	2 (1 Th+B) (1B+|A)	1.5

Table 5. Seasonal prevalence of ticks infestation on cattle at Minia province ,Egypt

Season	Number of cattle examined	Number of infested cattle with tick	Percentage of infestation
Winter	60	11	18%
Spring	60	9	15%
Summer	60	10	16.60%
Autumn	60	13	21.60%
Total	240	43	17.90%

Table 6. Monthly prevalence of ticks infestation on cattle at Minia province

Month	Number of cattle examined	Number of infested cattle with tick	Percentage of infestation	No of examined hemolymph	No of infected Hemolymph
January	20	3	15%	3	1
February	20	2	10%	2	1
March	20	1	5%	1	1
April	20	4	20%	4	1
May	20	4	20%	4	Zero
June	20	3	15%	3	2
July	20	4	20%	4	2
August	20	3	15%	3	1
September	20	4	20%	4	3
October	20	5	25%	5	2
November	20	4	20%	4	Zero
December	20	6	30%	6	2
Total	240	43	17.90%	43	16 (37.2%)

Table 7. Acaricide activity of deltamethrin, chlorprifos and ivermectin against *R. annulatus*:

Conc. (mg/L)	**Deltamethrin 5%**					**Chlorprifos 25%**					**Ivermectin**				
	0.005	0.05	0.5	1	2	1.25	2.5	5	10	20	0.38	0.75	1.25	2.5	5
Mortality rate	0.00 ±0.00 c	0.00 ±0.00 c	0.00 ±0.00 c	23.33 ±3.33 b	53.33 ±5.67 a	73.33 ±3.33 b	100.0 ±0.00 a	100.0 ±0.00 a	100.0 ±0.00 a	100.0 ±0.00 a	0.00 ±0.00 d	23.33 ±3.33 c	40.00 ±5.77 b	60.00 ±5.77 a	70.00 ±5.77[a]
LC50 (95%CL)	1.80 (1.59-2.11)					0.876 (0.576-1.054)					2.65 (1.29-47.73)				
LC90 (95%CL)	4.04 (3.17-5.94)					1.729 (1.519-2.155)					15.91 (5.01-1686583.69)				
X^2 (df)	4.90 (3)					2.651 (3)					18.57 (3)				
Slope ±SE	3.65±0.46					4.337±0.970					1.64±0.174				
P	0.179					0.449					0.00				

Mean in the row followed different letter are significantly different(Duncan's multiple test.
P<0.05. CL confidential limits; X^2 Chi-square; LC lethal ; df degree of freedom.
P*>0.05 is non-significant.

ملخص عربي

الدراسة الحالية هي دراسة استقصائية لطفيليات الدم التي تصيب الماشية في ظل غياب او وجود القراد في محافظة المنيا. تم جمع عينات دم من 240 رأس من الماشية من السلالات المحلية والهجينة. تم فحص العينات مجهريا باستخدام طريقة الصبغ بصبغة جيمزا للدم. وكشفت الدراسة أنه من بين 240 عينة دم تم فحصها، تبين إصابة 126 عينة بطفيليات الدم، بنسبة إصابة بلغت52.5%. حيث أصيبت 107 أبقار (44.58% من إجمالي الأبقار المفحوصة (84.9% من إجمالي الأبقار المصابة)) بعدوى الثيليريا. و 6 أبقار (2.5% من إجمالي الأبقار المفحوصة (4.7% من إجمالي الأبقار المصابة)) مصابة بعدوى البابيزيا، 7 أبقار (2.9% من إجمالي الأبقار المفحوصة (5.5% من إجمالي الأبقار المصابة)) مصابة بالأنابلازما، و 6 أبقار مختلطة (2.5% من إجمالي الأبقار المفحوصة (4,76% من إجمالي الأبقار المصابة). معدل انتشار طفيل الثيليريا هو الأعظم بين الأمراض الأخرى التي ينقلها القراد، ولكن البابيزيا كانت الأقل في معدل الإصابة. وتبين أن معدلات الإصابة بطفيليات الدم أقل في الماشية الصغيرة (41.67% في العجول التي يقل عمرها عن 11 شهرًا) و55.2% في الماشية البالغة؛ وكانت الإصابة المختلطة أعلى في العجول (15%) مقارنة بالبالغين (2.8%). ظهرت ذروة الإصابة بطفيليات الدم في الربيع (61.6%)، يليه الصيف والشتاء متساويا (56.6%). تم تسجيل انخفاض معدل الانتشار خلال فصل الخريف (35٪). ولكن شهرياً أعلى معدل إصابة بطفيليات الدم كان في شهر مارس (75%)، يليه شهر فبراير (70%)، بينما أقل شهر كان شهر أكتوبر (20%). وكان معدل انتشار طفيليات الدم أعلى عند الإناث (54.8%) مقارنة بالذكور (49.5%)؛ إلا أن العدوى المختلطة كانت أكثر شيوعاً عند الذكور (3.7%) مقارنة بالإناث (1.5%). بلغ معدل انتشار الإصابة بالقراد بين الماشية التي تم فحصها 17.9٪. أعلى نسبة إصابة بالقراد سجلت في شهر ديسمبر (30%)، وأقلها في شهر مارس (5%). وسجلت أعلى نسبة إصابة في الخريف (21.6%)، وأقلها في الربيع (15%). ووجدت الدراسة أن 51% من الماشية المصابة بالقراد كانت مصابة بطفيليات الدم. كشف الفحص المجهري للهيموليمف القراد عن وجود مراحل نمو بيروبلازما في 37.2% من الحالات. اختبرت الدراسة الحالية المبيدات القرادية الشائعة دلتامثرين، وإيفرمكتين، وكلوربريفوس في المختبر ضد القراد البالغ، ووجدت أن الكلوربريفوس، بتركيز 2.5 ملغم/لتر، أظهر فعالية أكبر في قتل القراد مقارنة بالدلتامثرين والإيفرمكتين، اللذين أظهرا مقاومة عالية وانخفاضًا في معدل وفيات القراد. فعالية ضد القراد ريبيسيفاليس انيولاتس.