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Epidemiological and diagnostic investigation on bovine theileriosis in Aswan Governorate, Egypt

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Abstract The present study was carried out to investigate the epidemiological and clinical status of bovine Theileriosis in Aswan governorate. During a 2-year study, 265 cattle were clinically suspected upon careful clinical examination as *Theileria annulata* (*T. annulata*) infected animals. Conventional diagnosis based on blood and lymph smears examinations showed that, the prevalence of Tropical Theileriosis in cattle in Aswan governorate was 56 (21.13%). Stained blood smears showed the presence of macro and/or micro-schizonts inside lymphocyte (Koch's blue bodies. Intraerythrocytic stages of *Theileria annulata* piroplasms inside RBCs. Polymerase chain reactions of *T. annulata* merozoite-piroplasms surface antigen Targeting gene: (Tams-1), revealed positive 29 (58%) animals confirmed by visualization of specific bands at 768 bp. Positive results could be detected in suspected cattle that showed positive or negative blood smear results that confirmed the high sensitivity of the PCR technique compared with the conventional method for diagnosis of bovine tropical Theileriosis. PCR proved to be a highly sensitive and accurate method for diagnosis of bovine tropical theileriosis especially in the detection of samples that was negative on blood and lymph smears.

Keywords Epidemiology · *Theileria annulata* · Bovine · Diagnosis

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Introduction

Among the highly important tick-borne diseases in Egypt is bovine Theileriosis. Theileriosis is a tick-borne protozoal disease of ruminants caused by hemoprotozoan parasites belonging to the genus Theileria (Demessie and Derso, 2015). It is considered as one of the most significant parasitic diseases (Jenkins 2018) due to the great economic impact on livestock of the world cattle population and economic losses because of high morbidity and mortality and significant effects on productivity and reproductivity of affected animals (Haghi et al. 2017; Kasozi et al. 2018). Theileria are obligate intracellular protozoan parasites that infect both wild and domestic bovidae throughout the world. *Theileria parva* and *Theileria annulata* are the most pathogenic species-affecting cattle (Kohli et al. 2014). They are transmitted by ixodid ticks and have complex life cycles in both vertebrate and invertebrate hosts (OIE 2014). The clinical signs in the infected animals include pyrexia, enlargement of superficial lymph nodes, nasal and ocular discharges, salivation, anemia, respiratory distress and eye lesions (Osman and Al-Gaabary 2007). Anemia develops due to oxidative damages to erythrocytes, increase in fragility and destruction in reticuloendothelial system (Hasanpour et al. 2008).

Infected animals remain carriers (latent Theileriosis), upon exposure of these animals to stress conditions; they become clinically diseased and show the characteristic signs of bovine Theileriosis, (Boussaadoun et al. 2015). These animals play a critical role in disease epidemiology (Gharbi et al. 2017) as they constitute a serious source of infection to susceptible cattle in non-endemic areas (Bilgic et al. 2013).

Direct microscopy of Giemsa-stained blood smears is the most commonly used tool for identifying blood parasites. However, in carrier animals or in animals with low parasitemia, microscopy may be unable to detect the causative

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protozoans due to a lack of sensitivity and specificity, El-Dakhly et al. (2020); Almeria et al. (2001); Jacobson (2006). Therefore, negative microscopic findings do not exclude the occurrence of such parasites, Weiland and Reiter (1988); Constable et al. (2017).

PCR assay helps in early detection of infection as well as detection of latent infected animals, Therefore, the application of PCR based techniques is highly essential for detection of piroplasmosis in latent carrier animals, Maharana et al. (2016). Little is known about the epidemiology of bovine piroplasmosis in Aswan Governorate due to the continuous importation of cattle from different countries where blood parasites are a common problem for different cattle breeds. Therefore, the aim of this study was directed to estimate the epidemiological situation of bovine Theileriosis among bovine population at Aswan governorate, Egypt and evaluate the efficacy of PCR technique in detection of *Theileria annulata* infection.

Material and methods

Ethics approval

All procedures were carried out according to the experimental standards approved by the Animal Research Ethics Committee at Faculty of Veterinary Medicine, Aswan University.

During January 2020–December 2021, a total number of 265 male adult cattle of 2–3 years and different breeds (Native, Frisian and Crossbreed) belonging to different localities in Aswan Governorate were employed in this study. All animals were clinically examined for evidences of Tropical Theileriosis.

- Blood samples were collected directly from the ear vein of 265 animals and used for preparation of blood smears (Coles 1986).
- Whole blood sample on E.D.T.A as anticoagulant (1 mg/1 ml) were collected from 50 suspected animals by jugular vein puncture and then stored at (− 20 °C) till use in DNA—extraction.
- Lymph node aspiration from enlarged lymph nodes for preparation of lymph smears immediately after collection was carried out (Charles 2002).
- Thin films were prepared from blood and lymph samples, according to (Coles 1986; Minnat et al. 2016).
- DNA extraction: Blood samples of 50 cattle were collected into EDTA containing tubes and stored at − 20 °C. Genomic DNA extraction was done in the parasitology department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt, using (Geneaid, New Taipei, Taiwan) DNA extraction kit. DNA extracts were stored at − 20 °C pending genetic analysis.

DNA amplification: Polymerase chain reactions of *T. annulata* merozoite- piroplasm surface antigen Targeting gene, (Tams-1). (F 5′-GTT AAT GCT GCA AAT GAG GAT G3′, and R5′-GGACTGATGAGAAGACGATGAG-3) were performed according to Kirvar et al. (2000).

- PCR reaction: Briefy, each 25 µL reaction consisted of 25 µL of 12.5 µL 2X master mix, 1 µl of the F primer (10 pmol/µl), 1 µl of the R primer (10 pmol/µl), 3 µl DNA, and 7.5 µl nuclease free water. Cycling conditions were initial denaturation for 5 min at 95 ºC, 37 cycles of denaturation for 30 s at 95 ºC, annealing for 60 s at 54 ºC and elongation for 1 min at 72 ºC. Then the final extension at 72 ºC for 7 min was allowed. Amplified products were visualized on a 1.5% agarose gel under UV transillumination after staining with ethidium bromide.

Statistical analysis

- Statistical analysis of epidemiological data on the effect of the age, sex, breed, locality, and season on the occurrence of the disease was conducted using chi-square (significance level at $P < 0.05$) (https://www.socscistatistics.com/tests/chisquare2) and Odds ratio analysis with MedCalc Statistical Software version19.1.2.

Results

During a 2-year study, 265 cattle were clinically suspected upon careful clinical examination as *Theileria annulta* infected animals.

Clinical examination

Most of these animals suffered from one or more clinical signs suggestive of bovine theileriosis. These include fever, emaciation, corneal opacity, enlargement of superficial lymph nodes, respiratory distress, diarrhea with blackish feces, drop in milk yield, heavy tick infestations and some animals showed paleness of the visible mucous membranes, Fig. 1a–f. Clinical examination revealed that 248 (93.75%) were infested with ticks of genus *Hyalomma* (*Hyalomma anatolicum anatolicum)*, 215 (81.25%) showed fever, 232 (87.5%) showed marked enlargement of superficial lymph nodes, 67 (25%) showed corneal opacity, 67 (25%) showed respiratory distress and 16 (6.25%) showed diarrhea Table 1.

Conventional testing

The prevalence of conventionally confirmed Theileriosis among 265 clinically suspected cattle using Giemsa-stained thin blood and lymph smears examination was 56 (21.13%). Giemsa stained blood smears showed presence

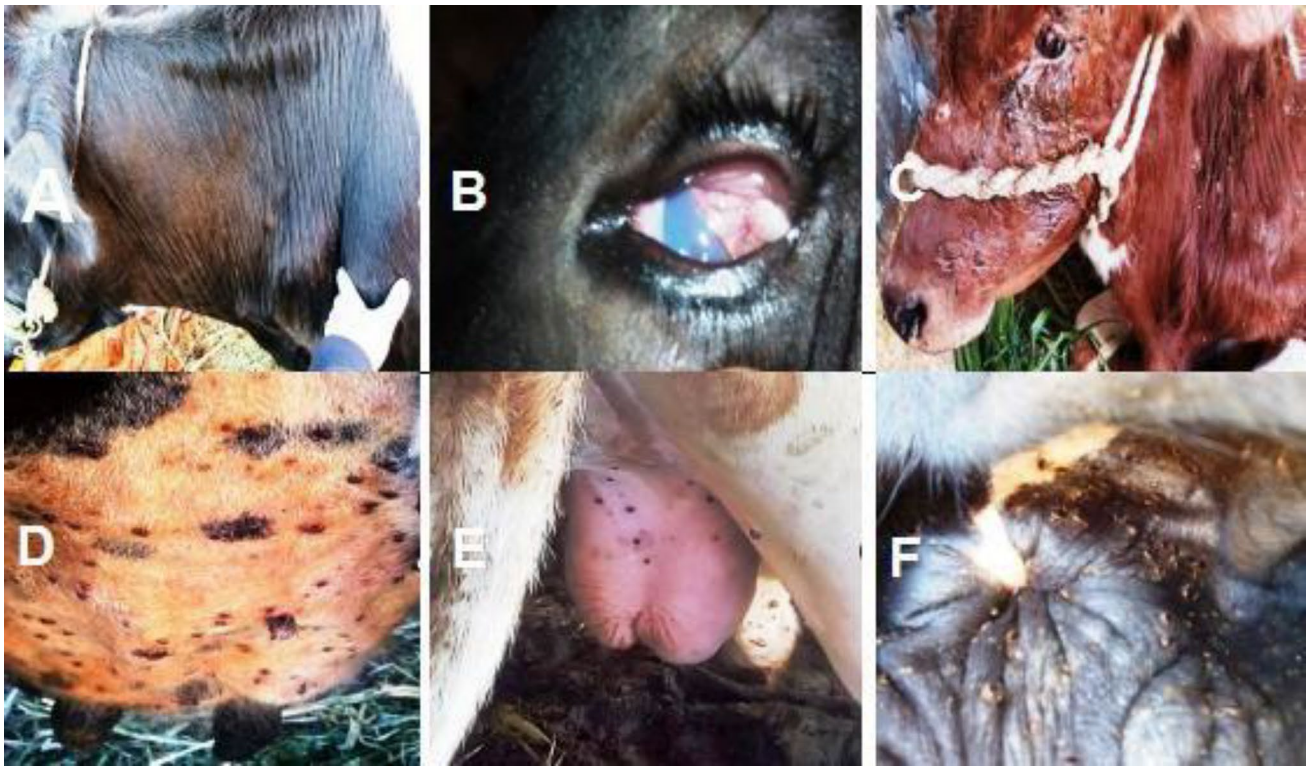

Fig. 1 **a** Enlargement of prescapular L.N. in *Theileria annulata* infected cattle. **b** Corneal opacity in *Theileria annulata* infected cattle. **c** Severe eye and skin affections around the eye. **d** Heavy infestation of udder with ticks. **e** Ticks infestation on scrotum and perineal region. **f** Heavy infestation with ticks around the anus

Table 1 Frequency of *T. annulata* infection in cattle according to breed, sex, and season risk factors

Variable	Positive	Prevalence %	95% CI	*P*-value	
Breed	Native ($n=36$)	9	25	0.11–0.47	0.4 ^{NS}
	Frisian ($n=79$)	17	21.5	0.13–0.34	
	Imported ($n=150$)	30	20	0.13–0.29	
Sex	Male ($n=261$)	55	21.1	0.15–0.27	0.8^{NS}
	Female ($n=4$)	1	25	0.01—1.4	
Season	Hot months ($n=159$)	31	19.5	0.13–0.28	0.5^{NS}
	Cold months ($n=106$)	25	23.5	0.15–0.35	
Total ($n=265$)	56	21.13	0.15–0.27		

The result is significant at $P<0.5$. The result is not significant at $P>0.5$

of a macro-schizonts inside lymphocyte (Koch's blue bodies), micro-schizonts inside lymphocyte, raptured schizonts, *Theileria annulata* piroplasms inside RBCs, Fig. 2a–d. Giemsa stained lymph smears showed shizonts of *Theileria annulata* inside lymphocytes (koch's blue bodies), Fig. 2e and f.

PCR analysis of 50 suspected *Theileria annulata* infected cattle that were either positive or negative on lymph smear examination indicated 29 (58%) samples as PCR positive (Fig. 3).

Analysis of risk factors associated with *T. annulata* infection

The analysis of microscopic data (Table 1) showed that the prevalence rate of *T. annulata* was non-significantly ($P = 0.4$) increased with animal breed. The highest prevalence was recorded in native breed (25%, 95 % CI: 0.11–0.47) compared to Frisian (21.5%, 95%CI: 0.13–0.34) and imported breed (20%, 95% CI: 0.13–0.29). The prevalence

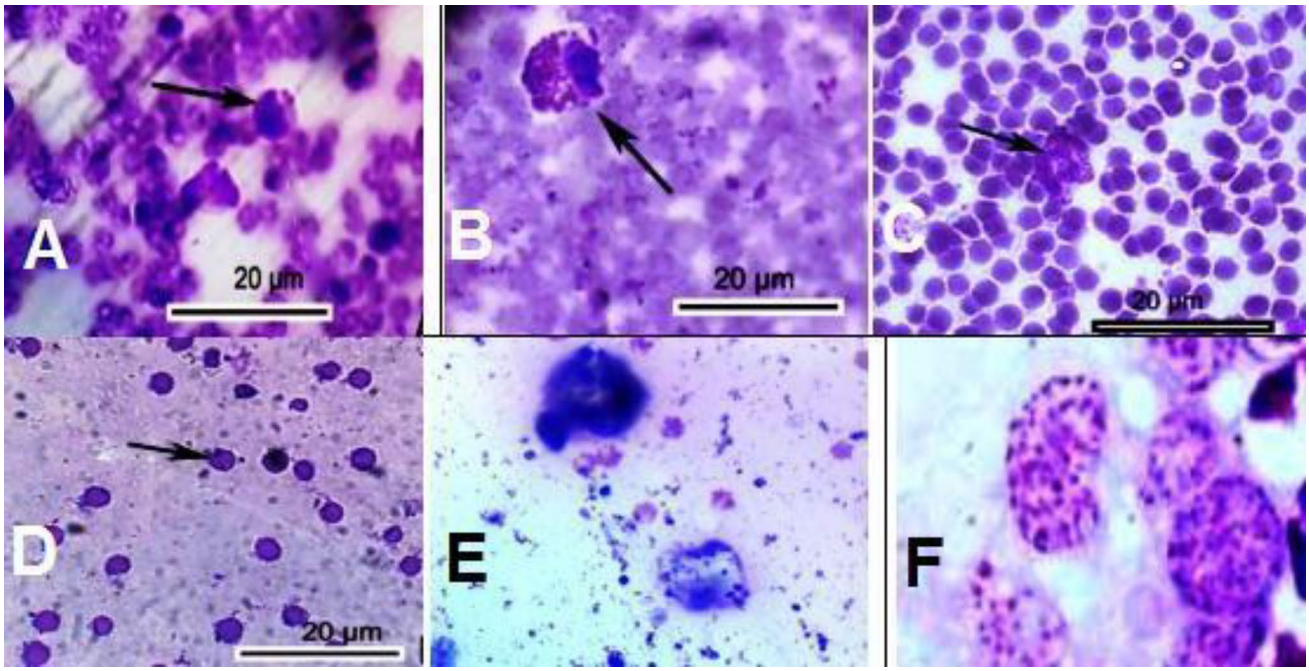

Fig. 2 Blood and lymph smears of *Theileria annulata* infected cattle. **a** The Arrow indicate a macro-schizont inside lymphocyte (Koch's blue bodies). **b** The Arrow indicate a micro-schizont inside lymphocyte. **c** The Arrow Indicate a raptured schizont. **d** The Arrow indicate *Theileria annulata* piroplasms inside RBCs. **e** and **f**. Schizonts of *Theileria annulata* inside lymphocytes (koch's blue bodies) in lymph smears

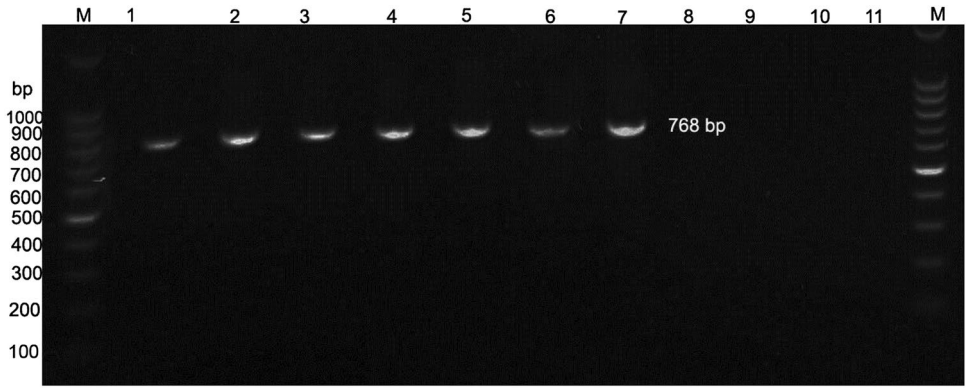

Fig. 3 PCR findings of blood of *Theileria annulata* suspected infected cattle. 1 M: Ladder of 100 base pair. 2 Lane 1: *Theileria annulata* positive control (Department of Parasitology, Beni-Suef University) indicate a 768 bp band. 3 Lanes 2–7: A representative set of *Theileria annulata* positive samples that show the 768 bp band. 4 Lanes 8, 9, and 10: *Theileria annulata* negative samples also negative blood smears. 5 Lane 11: Negative control

was non-significantly ($P = 0.8$) higher among females (25%, 95% CI: 0.01–1.4) than males (21.1%, 95%CI: 0.15–0.27). The infection rate was non-significantly ($P = 0.5$) higher in cold months (23.5%, 95%CI: 0.15–0.35) than hot months (23.5%, 95% CI: 0.13–0.28). The breed, sex and season factors showed no association with the *T. annulata* infection prevalence.

Discussion

Bovine Theileriosis is an important disease with a worldwide distribution affecting many animal species with a major impact on cattle. In Egypt, it constitutes a serious problem since it causes severe impacts on the livestock productivity and reproductivity (EL Moghazy et al. 2014).

However, little is known about the epidemiology of piroplasmosis in Aswan governorate.

A total of 265 Theileriosis suspected male cattle in different locations in Aswan governorate were examined clinically, microscopically and molecularly using PCR technique. Prevalence of *Theileria annulata* infection among cattle was determined firstly on clinical basis and examination of blood and lymph node smears of infected animals using Giemsa stain.

Clinical examination of cattle in this study showed rise of temperature that ranged between 40 and 41 ºC, enlargement of superficial lymph nodes, anorexia, cessation of rumination, ocular discharge and general weakness. Constipation was also observed in some cases followed by diarrhea and blackish feces. Frothy nasal, cough and respiratory distress were observed. Corneal opacity and lacrimation were observed in some cases, Fig. 1a–f. El-Dakhly et al. (2018), AL-Hosary (2018) and Antera et al. (2019) previously reported similar clinical picture in several studies. In addition, Yousef et al. (2020) reported similar clinical signs, which included fever, anorexia, enlargement of superficial lymph nodes, lacrimation, and corneal opacity. Enlargement of superficial lymph nodes could be explained by lymphoid hyperplasia in the early stage of the disease, (Mahmmod et al., 2011). The corneal opacity was explained by Irvin and Mwamachi, (1983) as a result of white blood cells infiltration and migration of infected lymphocytes. Blackish feces observed in some acutely infected cattle can be explained on the basis of hemorrhage as a result of the massive destruction of lymphoid tissues and ulceration in abomasum and intestines induced by Theileria spp. as discussed by Abdou et al. (2005), Abdel-Rady et al. (2008), Hoda and Osman (2009) and Hosein (2022).

Giemsa stained blood smears showed presence of macro-schizonts inside lymphocytes (Koch's blue bodies), micro-schizonts inside lymphocytes, raptured schizonts and intraerythrocytic stages of Theileria annulata piroplasm inside RBCs, Fig. 2a–e. These agreed with the results obtained by Minnat et al. (2016) and Gomes et al. (2017). Giemsa stained lymph smears showed shizonts of Theileria annulata inside lymphocytes (koch's blue bodies) Fig. 2e and f. This also agrees with the findings of Maharana et al. (2016). Microscopic examination of Giemsa stained blood smear is routinely used for diagnosis of piroplasmosis, because it is simple to perform, quick and cost effective technique and remains the most rapid confirmatory method for detecting such infections in acute phase of the disease. However, lack of sensitivity makes it difficult to detect carrier cases or chronic phases of piroplasmosis (Maharana et al. 2016).

In the present study, polymerase chain reactions of *T. annulata* merozoite- piroplasm surface antigen Targeting gene: (Tams-1) was used for molecular confirmation of Theileriosis. Positive results were confirmed by visualization of specific bands at 768 bp, Fig. 3. Blood samples selected from50 cattle with suspected theileriosis that were positive or negative on blood smear analysis were subjected for examination by PCR. The results revealed 29 positive animals (58%). Such results proved the high sensitivity of PCR test compared with the conventional method for diagnosis of bovine tropical Theileriosis. Polymerase chain reaction (PCR) provides a highly sensitive and specific diagnosis tool in both clinically infected and carrier animals this is in agreement with Abdel-Rady et al. (2010).

In this study, Table 1. Based on the meteorological data of Aswan Governorate, cattle were 1.2 times more likely to be infected in the cold season than in the hot season and there is no statistical significance between infection rate and season. This finding agrees with a study from Pakistan that found the infection in winter was 12.9%, followed by summer (11.4%) (Asif et al. 2020). Previous study at the Egyptian oases reported a non-significant variation in the prevalence between the hot months (67%) and the non-hot months (57.6%) (AL-Hosary 2018). This may be due to the change in the environmental and climatic conditions suitable for the spread of ticks and making cattle more susceptible to a Theileria infection (Dharanesha et al., 2017). However, in Egypt, a previous study from Port Said and Dakahlia governorates reported higher infection in hot months than in cold months (Ghoneim and El-Fayomy, 2014), which is likely due to difference in the environment, location, sample size and animal susceptibility.

Concerning the prevalence of theileriosis in different cattle breeds investigated in this study, the prevalence's were 25%, 21.5%, and 20% in native Frisian, and Cross breed cattle respectively, Table 1. This comes in agreement with Abou-El-Naga et al. (2005) who found that that infection rate of tropical theileriosis in cross-breed cattle was higher than that of native cattle 40.3% and 29.4% respectively.

Conclusion

Although this is the first study to address the prevalence of bovine theileriosis in the Aswan governorate of Upper Egypt, it has primarily focused on the animal level and supports growing concerns about bovine theileriosis and climate change indicated elsewhere. The study revealed an increased prevalence in Aswan and coincided with a 21.13% positivity by stained blood smear. It should be borne in mind that, despite the high positivity of infection, the observed risk factors (age, sex, season and breed) were not associated with the occurrence of the disease. The study provides epidemiological data on *T. annulate* in cattle from three localities in Aswan and will be the basis for future studies on unexplored regions and different animal species for well-structured

prevention and control measures. The PCR is considered as more sensitive and accurate technique in the studies for diagnosis of both carrier and infected animals.

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Author Contributions All authors contributed to the study conception, design, material preparation, data collection and analysis. All authors read and approved the final manuscript.

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Data Availability The data used in this manuscript is publicly available.

Code Availability The code will be made available under request to the corresponding author.

Declaration

Conflict of interest The authors have declared no conflict of interest.

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