Research Article

Some Epidemiological Studies on *Theileria annulata* Infection in 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 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%). Giemsa stained blood smears showed presence of macro-schizont inside lymphocyte (Koch's blue bodies), micro-schizonts inside lymphocyte, raptured schizont and intraerythrocytic stages of *Theileria annulata* piroplasms inside RBCs..Polymerase chain reactions of *T. annulata* merozoite-piroplasm surface antigen Targeting gene: (Tams1) 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 proved the high sensitivity of PCR test compared with the conventional method for diagnosis of bovine tropical Theileriosis. PCR proved a highly sensitive and accurate method for diagnosis of bovine tropical Theileriosis especially in detection of blood and lymph smears negative cases.

Keywords | Epidemiology, *Theileria annulata*, Bovine, Diagnosis, PCR

Received | October 12, 2022; **Accepted** | December 25, 2022; **Published** | February 25, 2023
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Citation | Abdel-Rady A, Karmi M, Youssef M, Abdel-Raouf AM, Madkour B (2023). Some epidemiological studies on *Theileria annulata* infection in egypt. Res J. Vet. Pract. 11(1): 7-12.
DOI | http://dx.doi.org/10.17582/journal.rjvp/2023/11.1.7.12
ISSN | 2308-2798

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 (Mousa 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, respiratorydistress and eye lesions (Osman and Al- Gaabary, 2007). Anemia develops due to oxidative damages to erythrocytes, increase in fragility and destruction in retic-

uloendothelial 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 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 usedtool for identifying blood parasites. However, in carrier animals or in animals with low parasitemia, such method may be unable to detect the causative protozoans due to lake of sensitivity and specificity, El-Dakhly et al. (2020); Almeria et al. (2001); Jacobson LS (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, Biswa 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 common problems and breeding of different breeds of cattle. 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 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 cattleof 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 (1mg/1ml) 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).

DNA extraction

Blood samples of 50 cattle were collected into EDTA containing tubes and stored at −20 °C. Genomic DNA extraction was done in 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, (Tams1). (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.

RESULTS

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

Clinical examination

Most of these animals suffered from one or more of bovine Theileriosis suggestive clinical signs. 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, Figure 1 (A, B, C, D, E and F).

Clinical examination revealed that 248 (93.75%) were infested with tick, 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.

Table 1: Clinical examination of Theileriosis suspected animals.

Clinical signs	Positive
Infestation with ticks	248 (93.6%)
Fever	215 (81.1%)
Enlargement of superficial lymph nodes	232 (87.5%)
Corneal opacity	67 (25%)
Occurrence of respiratory distress	67 (25%)
Diarrhea	16 (6.25%)

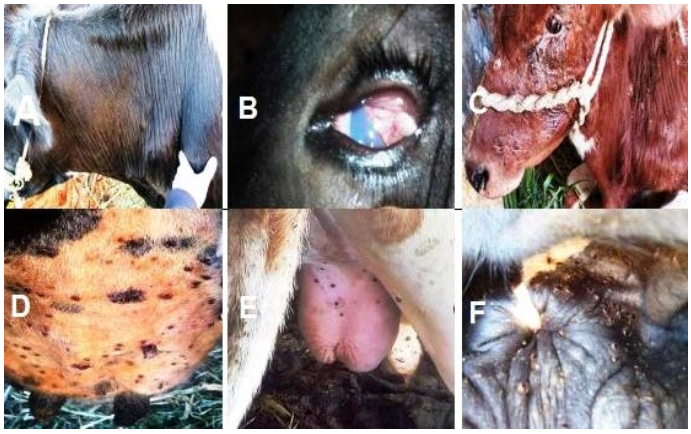

Figure 1: A) Enlargement of prescapular L.N. in *Theileria annulata* infected cattle. B) Corneal opacity in Theileria annulata infected cattle. C) Severe eye and skin affections around the eye. D) Heavy infestation of udder with ticks. E) Ticks infestation on scrotum and perineal region. F) Heavy infestation with ticks around the anus.

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 of macro-schizont inside lymphocyte (Koch's blue bodies), micro-schizont inside lymphocyte, raptured schizont, *Theileria annulata* piroplasm inside RBCs, Figure 2 (A, B, C, D). Giemsa stained lymph smears showed shizont of Theileria annulata inside lymphocytes (koch's blue bodies), Figure 2 (E and F).

PCR assay

PCR applied on 50 selected samples of suspected Theileria infected cattle including positives and negative lymph smears examination confirmed the infection of cattle with *Theileria annulata* in 29 (58%) among selected clinically suspected cattle (Figure 3).

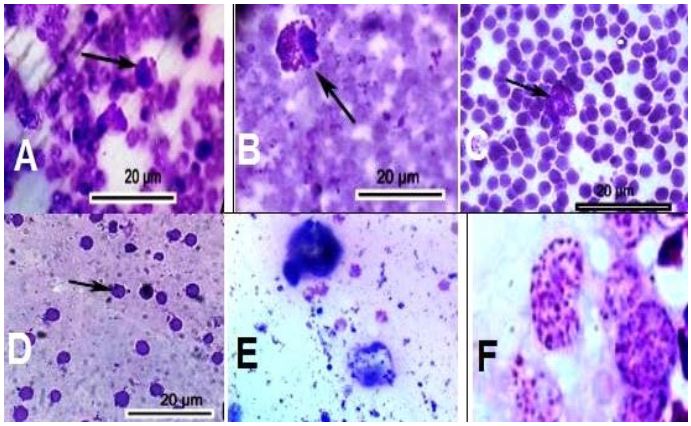

Figure 2: Blood and lymph smears of *Theileria annulata* infected cattle. A) arrow refers to macro-schizont inside lymphocyte (Koch's blue bodies).B) arrow shows micro-schizont inside lymphocyte. C) arrow shows raptured schizont. D) arrow shows *Theileria annulata* piroplasm inside RBCs. E and F) schizont of *Theileria annulata* inside lymphocytes (koch's blue bodies) in lymph smears.

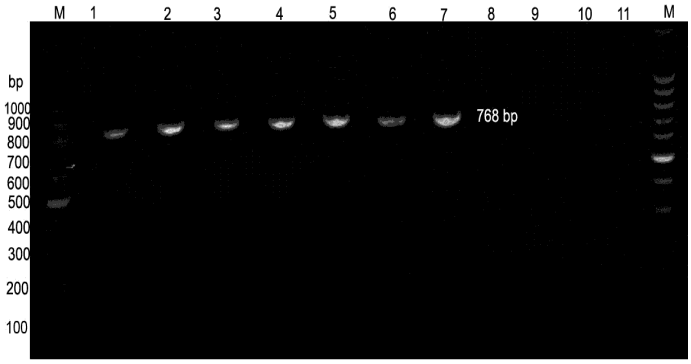

Figure 3: PCR findings of blood of *Theileria annulata* suspected infected cattle. M: Ladder of 100 base pair. Lane 1: control +ve *T. annulata* (Dept. of parasitology, Beni-Suef University) showed the specificamplicon size of 768 bP. Lanes 2, 3, 4, 5, 6 and 7: *T. annulata* +ve samples showed the specific 768 bp amplicon size. Lanes 8, 9, and 10: -ve samples.Lane 11: a control -ve.

Seasonal distribution

The study revealed that the conventionally confirmed *Theileria annulata* infection in clinically suspected cases was higher during hot months (March- November) 11.69%, as compared to 9.4% during cold months (December- February), Table 2.

Table 2: Seasonal occurrence of bovine theileriosis

Season	No, of animals	Positive
Hot months	159	31 (11.7%)
Cold months	106	25 (9.4%)
Total	265	56 (21.13%)

Prevalence in different breeds

The prevalence of bovine Theileriosis were 25%, 21.5%, and

20% in native, Frisian and Crossbreed cattle respectively.

DISCUSSION

Bovine Theileriosis is an important disease with a world-wide 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 (Hazem et al., 2014). However, little is known about theepidemiology 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 – 41 ºC, enlargement of lymph node, 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, Figure 1 (A, B, C, D, E and F). El-Dakhly et al. (2018), AL-Hosary (2018) and Reham 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, and 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). Irvin and Mwamachi, (1983), explained the corneal opacity because 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-schizont inside lymphocytes (Koch's blue bodies), micro-schizont inside lymphocytes, rapturedschizont and *intraerythrocytic* stages *of Theileria annulata* piroplasm inside RBCs, Figure 2 (A, B, C, D, E). These agreed with the results obtained by Gomes et al. (2017). Giemsa stained lymph smears showed shizont of Theileria annulata inside lymphocytes (koch's blue bodies) Plate2 (E and F). This also agrees with the findings of Biswa 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 (Biswa et al., 2016).

In the present study, Polymerase chain reactions of *T. annulata* merozoite- piroplasm surface antigen Targeting gene: (Tams1) was used for molecular confirmation of Theileriosis. Positive results were confirmed by visualization of specific bands at 768 bP., Figure 3. Selected 50 blood samples from theileriosis suspected cattle that showed positive and negative blood smear results were subjected for examination by PCR. The results revealed positive 29 (58%) animals. 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 comes in agreement with Abdel Rady et al. (2010). Variation of prevalence of tropical Theileriosis in different seasons may beattributed to the effect of climatic condition on the tick's activity, which increased in summer season. In this study the conventionally confirmed *Theileria annulata* infection of clinically suspected cases was higher during hot months (March- November) 11.69%, as compared to 9.4% during cold months, December- February, Table 2. Based on the meteorological data of Aswan Governorate, currently the hot months presented for 9 consecutive months starting from March to November, and the hottest month in is July. The cold months are January, February and December and the coldest month is January. These results agreed with that obtained by, Mousa et al. (2017). These results come in agreement with Sotohy et al. (2019) who foundthat the highest prevalence of infection by blood film examination was (39.50 %) in Summer, followed by (37.15 %) in Spring, then (34.70 %) in Autumn and the lowest rate was (13.95 %) in Winter. In Upper Egypt, higher infection prevalence was reported during hot months when compared with non-hot months, 33.98 and 13.73%, respectively as reported by AL-Hosary, (2013).

Concerning the prevalence of theileriosis in different cattle breeds investigated inthis study, the prevalences were 25%, 21.5%, and 20% in native Frisian, and Cross breed cattle respectively, Table 3. This comes in agreement with Abou-El-Naga et al. (2005) who found that that infection rate of tropical theileriosis in crossbreed cattle was higher than that of native cattle 40.3% and 29.4% respectively.

Table 3: Prevalence of Theileriosis in different cattle breeds

Native		Frisian		Imported	
No.	**Positive**	**No.**	**positive**	**No.**	**positive**
36	9 (25%)	79	17 (21.5%)	150	30 (20%)

CONCLUSION

Seasonal dynamic showed that, the highest prevalence rate was detected in hot months (March- November). The breed susceptibility of bovine Theileriasis showed a higher prevalence in imported cattle than native one. PCR proved a highly sensitive and accurate method for diagnosis of bovine tropical Theileriosis especially in detection of blood and lymph smears negative cases. The current study revealed that imported cattle breeds are generally most susceptible than native one.

ACKNOWLEDGEMENTS

The authors thank the veterinarians and farm owners for their support and help in providing data and samples collection throughout the study.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHORS CONTRIBUTION

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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