

Targeting *tams-1* gene results in underestimation of *Theileria annulata* infection in diseased cattle in Egypt

Ahmed M. Ghoneim* and Asmaa O. El-Fayomy

Department of Zoology, Faculty of Science, Damietta University, New Damietta, Damietta, Egypt

Abstract

Tropical theileriosis is considered one of the most economically important tick borne diseases that cause significant mortality and morbidity to livestock. In the context of epidemiological studies on livestock in Egypt, this report investigated the spread of *Theileria annulata* among diseased farm cows (*Bos indicus*) over one year. Blood samples collected from 130 cows were investigated by routine staining and 64 samples were investigated by PCR assay using two different probes targeting *tams-1* gene. Microscopy revealed the infection of 33.8% of animals with *Theileria* while PCR detected infection in 51% of animals with one primer pair and the other primer pair detected the infection in 31% of animals. Combined PCR results indicated the infection of 68.8% of animals with *T. annulata*. Seasonal fluctuation of parasite infection was evident with the highest infection percentage and prevalence recorded during summer based on both microscopy and PCR data. For the first time, the current study reports the presence of two *T. annulata* isolates based on *tams-1* gene partial sequence in Egypt. Targeting polymorphic genes for parasite detection may result in underestimation of infection and target gene diversity has to be considered. The high infection with these pathogens in the clinically ill cows necessitates implementing serious programs to minimize their economic burden on the Egyptian farming industry.

Keywords

Tams-1 gene, PCR, epidemiology, *Theileria*, seasonal, *Bos indicus*, livestock

Introduction

Tick-borne diseases cause major problems to health and management of domestic cattle in tropical and subtropical regions of the world (Jongejan and Uilenberg 1994). Tropical theileriosis is one of the most economically important tick borne diseases (Callow 1984). It is caused by the Apicomplexan protozoan parasite *Theileria* transmitted by Ixodidae ticks (Caeiro 1999, Preston 2001, Silva *et al.* 2010) and affects millions of cattle in different regions of Africa. *T. annulata* is considered as the cause of tropical theileriosis (Spooner *et al.* 1989).

Infective sporozoites, injected during tick feeding, rapidly enter target cells, escape from the surrounding host-cell membrane and differentiate to schizonts that interact with different host-cell components (Dobbelaere and Rottenberg 2003). This interaction includes host cell signaling pathways that regulate proliferation and cell survival (Chaussepied and Langsley 2011) and thus cause blastogenesis and clonal expansion of predominantly T and B cells (Fawcett *et al.* 1982, Baldwin *et al.* 1988, Spooner *et al.* 1989). Merozoites released

from these schizonts subsequently infect red blood cells and become trophozoites. Lymphocytic stage of *Theileria* (schizont) is the cause of many of the severe disease manifestations like lymphadenopathy, pyrexia, thrombocytopenia, and panleukopenia (Homer *et al.* 2000).

Although microscopy is considered as the “gold standard” for detecting acute infections with piroplasms (Nayel *et al.* 2012), it is not suitable for detecting low parasitemia (Friedhoff and Bose 1994, Bose *et al.* 1995). Serological and indirect fluorescent antibody tests can result in false data due to cross-reactions or improper specific immune response (Billiow *et al.* 2005). Recently, molecular tools have been developed for detection and quantification of many infectious pathogens and they proved to be highly accurate and sensitive. Reverse line blotting assay is currently considered the most sensitive test for detecting *T. annulata* (Gubbels *et al.* 1999, Georges *et al.* 2001, Bilgic *et al.* 2010), however, this technique is a relatively cumbersome assay and is not entirely suitable for the routine diagnosis of *Theileria* infections (Santos *et al.* 2013). The alternative and most common PCR assays used for detecting *T. annulata*

*Corresponding author: am_ghoneim@du.edu.eg