

Development of IFA test to detect *Theileria annulata* and seroprevalence of the parasite in Tabriz area of Iran

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Abstract

Theileria annulata is one of the most famous species of theileria genus and transmit by *Hyalomma* spp. ticks. In Iran *Theileria annulata* infection is very distributed and it has been considered as an important disease that decreases efficient cattle production. The present work was undertaken to develop immunofluorescence assay test (IFAT) for detecting antibodies against *Theileria annulata* in cattle and to study the seroprevalence of the parasite in Tabriz area. From 100 tested sera 22 (22%) were found to be positive and 78 (78 %) were negative. The prevalence of infection showed a significant increase with respect to the age of animals. The rate of infection in sheep of 6-12 months old, 12-24 months old and older than two years was 12.1 %, 21.2% and 32.3%, respectively. Sixty-eight ticks (56 female, 12 male) were collected and ticks were identified to be *Hyalomma anatolicum anatolicum* based of their morphological characters.

Keywords: IFA test; *Theileria annulata*; *Hyalomma* spp; Tabriz.

Introduction

Theileriosis is a protozoan infection seen in cattle, sheep, buffalo, zebu and bison and causes severe infection especially in cattle (Dumanli *et al.*, 2005; Young, 1981). *Theileria annulata* is one of the most famous species of theileria genus and is transmitted by *Hyalomma* spp. ticks (Aktas *et al.*, 2004; Robinson, 1982; Samad *et al.*, 1983). In natural theileria infections, parasites invade to lymphoid cells and at a later stage of infection invade to the erythrocytes, the infection causes fever, anemia and icterus, and it can be caused to dead (Souslby, 1982). Current route to diagnosing the disease is based on clinical findings and microscopic examination of blood and lymph node smears stained with giemsa in acute cases (Dumanli *et al.*, 2005).

Frequently serological methods are employed in determining subclinical infections. Many different sero-diagnostic tests have been described among which the indirect immune-fluorescence assay (IFA) is the most widely used (Eren *et al.*, 1995; Sayin *et al.*, 2003).

In Iran *Theileria annulata* infection is very distributed and it has been considered as an important disease that decreases efficient cattle production. The present work was undertaken to develop an IFAT for detecting antibodies against *Theileria annulata* in cattle and to study the seroprevalence of the parasite in Tabriz area.

Materials and methods

Animals and Study Site: This survey was done between March and June 2009 in Tabriz and its surrounding villages. Tabriz climate is semi-arid with cold winters and mild summers. 100 blood samples were prepared from jugular Vein in sterile air-vacuumed tubes containing ethylenediamine tetraacetic acid (EDTA) and all of the cattle were in different age groups (one year old, two years old and older).

Antigen Preparation: An acute infection cow blood was used as IFA antigen. A part of infected blood collected and washed 3 times with PBS and then was resuspended in PBS at the original volume of blood. The washed blood diluted 1:4 in PBS and three spots of blood were placed on clean glass slides and air dried. The slides were wrapped in aluminum foil and stored at -70°C.

Sera: To develop the IFA test 5 positive control Sera, from natural infected cows and 5 negative control sera from newborn calf, before colostrum ingestion, were used. The seroprevalence survey was performed on a total of zoo sera, collected from Tabriz and suburb.

IFA Procedure: Microscope glass slides, spotted with infected blood, were fixed in a cold solution of 70% acetone and 30% methanol for 5 minute at room temperature. For preventing from false positive results, the slides were blocked negative reactions with 1% BSA (Bovine Serum Albumin) in PBS, overnight at room temperature. After blocking, the slides gently rinsed three times with PBS. Ten µl volumes of the test sera, diluted 1:40 in PBS, were added on the blood spots and the slides were incubated for 30 minutes at 37°C, in a humid chamber. The slides washed as before and probed with a commercial fluorescent conjugated rabbit anti-cattle IgG (Sigma chemicals USA), at the dilution of 1:200 in PBS. After incubation, time and washing, as above, the slides dried, mounted in a 10% buffered glycerin and examined by fluorescent microscope (Shiels *et al.*, 1986). The IFA test performed in pathobiology department in veterinary faculty of Islamic Azad University of Tabriz.

Results

The results of examining 100 serum samples obtained from different areas of Tabriz showed that out of 100 tested sera 22 (22%) were found to be positive and 78 (78%) were negative. The prevalence of infection showed a significant increase with respect to the age of animals (Fischers exact test, $P < 0.05$). The rate of infection in sheep of 6-12 months old, 12-24 months old and older than two years was 12.1%, 21.2% and 32.3%, respectively (Table 1). A total of 68 ticks (56 female, 12 male) were collected and ticks were identified to be *Hyalomma anatolicum anatolicum* based on their morphological characters (Fig. 1).

Discussion

To understanding the epidemiological status of parasites and for their control and monitoring, development and the use of serological tests are very important. Our results presented that *Theileria annulata* is widely distributed in Tabriz and suburb. Based on our results from 100 cattle sera samples 22 (22%) were to be found positive and 78 (78%) were negative. In Tabriz there are no previously seroepidemiological studies on *Theileria annulata* infection by IFA test. In the east of Turkey a serological survey by IFA test was carried out to detect the *Theileria annulata* infection prevalence, In this study, of the 1505 blood samples 526 (34.9%) were found to be positive (Dumanli *et al.*, 2005). This rate is higher than our results that could be related with their different climates and different population of ticks. In other studies in different part of Turkey, *Theileria annulata* infection rate have been reported to be present between 2.3 and 43.9% (Aktas *et al.*, 2004).

Table 1: The prevalence of *Theileria annulata* infection in sheep according to age groups.

Location	Sera 6-12 months	Positive (%)	Sera (12-24 months)	Positive (%)	Sera (>24 months)	Positive (%)
Tabriz city	33	4 (12.1)	33	7 (21.2)	34	11 (32.3)

Fig. 1. *Hyalomma anatolicum anatolicum*

In present study the prevalence of infection showed a significant (Chi-square test, $p < 0.05$) increase with respect to the age of the animals. The rate of infection in sheep of 6-12 months old, 12-24 months old and older than two years was 12.1 %, 27.2% and 55.8%, respectively (Table 1).

In a previous study carried out in Sudan showed that the prevalence of *Theileria annulata* is 9.3% (Hoogstraal, 2001). Another study carried out in Zambia demonstrated that from 37 calves born from immune dams, 27 were seropositive to *theileria annulata* (Billiouw *et al.*, 2005). Whereas in Sanliurfa it was demonstrated that from 191 serum samples, 19 were positive for *Theileria annulata* (Sevgili *et al.*, 2010). Comparison of these results has been revealed that the contamination rate in Iran is very high than other countries and must be take measured in this field.

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