Short communication

Survey of *Theileria lestoquardi* antibodies among Sudanese sheep

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Abstract

The prevalence of *Theileria lestoquardi* antibodies in Sudanese sheep from nine geographical areas in Sudan was determined using indirect fluorescent antibody “IFA” test. Out of 315 samples examined, 51 (16.2\%) were found positive and ranged between 23.4\% in River Nile State and 10\% in Kasala and Darfour Provinces with an overall prevalence of 16.2\% indicating widespread distribution of the infection. We also report on presence of antibodies reactive to *Theileria annulata* in sheep sera.

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1. Introduction

*Theileria lestoquardi* (Morel and Uilenberg, 1981), previously known as *Theileria hirci* is a tick-borne protozoan parasite of sheep, which occurs in south-eastern Europe, northern Africa, western and central Asia (Uilenberg, 1981b) and in India (Sisodia, 1981). Of the three *Theileria* species occurring in sheep, *T. lestoquardi* is considered the only one that is highly pathogenic (Hooshmand-Rad and Hawa, 1973; Uilenberg, 1981b). The parasite causes malignant ovine theileriosis, which assumes acute, subacute or chronic forms causing high mortality rates among sheep. *Theileria ovis* and *Theileria seperata*, on the other hand, are less pathogenic and have lower importance than *T. lestoquardi* (Soulsby, 1982; Arnold and Dias, 1983; Uilenberg, 1983).

Malignant ovine theileriosis was first described in Sudan by Mason (1915), then reported in Khartoum State (Nagwa, 1986; Tageldin et al., 1992; Latif et al., 1994). In northern Sudan
it was reported by ElHussein et al. (1993), ElGhali and ElHussein (1995) and Ahmed (1999) who showed that the disease caused substantial losses among sheep.

Many serological tests have been used for detection of antibodies produced by *Theileria* species. These include complement fixation, capillary agglutination and indirect haemagglutination tests, but their reliability is not always proven (Uilenberg, 1981a). Indirect fluorescent antibody (IFA) test has for long time been used and currently represents the reference test used in investigation of tropical theileriosis (Darghouth et al., 1996). The test has also been used for detection of antibodies produced by *T. lestoquardi* (Hawa et al., 1976). More recently, Leemans et al. (1997) used schizont-based IFA test to detect antibodies against *T. lestoquardi* in sheep sera collected from the field.

In the present study, we report the prevalence of *T. lestoquardi* antibodies in sheep in Sudan.

2. Materials and methods

2.1. Samples collection

Serum samples were collected from apparently healthy adult sheep, in nine geographical areas in Sudan, viz. River Nile, Khartoum, White Nile, Blue Nile, Western Kordofan, South Kordofan, Kasala, Red Sea and Darfour States (Fig. 1). The samples were labeled and stored at −20°C until used.

2.2. Antigen preparation

The schizont antigen was prepared from a local *T. lestoquardi* and *Theileria annulata* cell line at low passage (<20 passage) according to the method described by FAO (1984) in 12-well Teflon-coated multispot slides (Highveld Biological, USA). Antigen-coated slides were individually wrapped in tissue paper and packed in aluminum foil with five slides in each packet. The slide packets were labeled and stored in airtight, waterproof plastic containers at −20°C until used.

2.3. Conjugate

Rabbit anti-sheep immune gammaglobulin (IgG) conjugated to fluorescein isothiocyanate (FITC) was obtained from Nordic Immunological Laboratory, The Netherlands.

The conjugate was used at dilution in phosphate buffer saline (PBS) that gives no loss of titre of positive control serum in the IFA test, but which at the same time gives a reaction not greater than 1/10 with the negative control. This was achieved by dilution 1/80. Evans blue at a concentration of 0.01% was added to the conjugate as a counterstain.

2.4. Control sera

Positive control (C +ve) sera was obtained from Razzi institute, Iran. Negative control (C −ve) sera was obtained from BDSL, UK. Control sera (C −ve and C +ve) were diluted directly to 1/80.
2.5. Test sera

Test sera were diluted in PBS 1/80 for screening the antibodies to *T. lestoquardi* and *T. annulata* according to FAO (1984).
2.6. Indirect fluorescent antibody “IFA” test

The procedures followed in materials preparation and running the test were essentially as described by Burridge et al. (1974) and FAO (1984). Examination was carried out using Olympus Vanox incident-light excitation fluorescent microscope (Japan). The slides were examined under 40× objective using a mountant composed of nine parts glycerol and one part PBS.

3. Results

The results of examining 315 serum samples obtained from nine geographical areas are shown in Table 1. The prevalence rate of *T. lestoquardi* antibodies ranged between 23.4% in River Nile State and 10% in Kasala and Darfour Provinces with an overall prevalence of 16.2%.

On the other hand, the results of the reaction with *T. annulata* antigen are shown in Table 2. The prevalence rate ranged between 10.6% in River Nile State and 5% in Kordofan State with an overall prevalence of 9.3%.

Table 1

The prevalence rate of *T. lestoquardi* antibodies using the IFA test based on schizont antigen among sheep in Sudan

<table>
<thead>
<tr>
<th>Location</th>
<th>Number examined</th>
<th>Number positive</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>River Nile</td>
<td>47</td>
<td>11</td>
<td>23.4</td>
</tr>
<tr>
<td>White Nile</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>Blue Nile</td>
<td>50</td>
<td>7</td>
<td>14</td>
</tr>
<tr>
<td>Western Kordofan</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td>South Kordofan</td>
<td>48</td>
<td>9</td>
<td>18.8</td>
</tr>
<tr>
<td>Khartoum</td>
<td>30</td>
<td>5</td>
<td>16.7</td>
</tr>
<tr>
<td>Kasala</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Darfour</td>
<td>30</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>Red Sea</td>
<td>30</td>
<td>6</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>315</td>
<td>51</td>
<td>16.2</td>
</tr>
</tbody>
</table>

Table 2

The prevalence rate of *T. annulata* antibodies using the IFA test based on schizont antigen among sheep in Sudan

<table>
<thead>
<tr>
<th>Location</th>
<th>Number examined</th>
<th><em>T. lestoquardi</em></th>
<th><em>T. annulata</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>River Nile</td>
<td>47</td>
<td>11 (23.4)</td>
<td>5 (10.6)</td>
</tr>
<tr>
<td>Kasala</td>
<td>20</td>
<td>2 (10)</td>
<td>1 (5)</td>
</tr>
<tr>
<td>Western Kordofan</td>
<td>30</td>
<td>4 (13.3)</td>
<td>3 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td>17 (17.5)</td>
<td>9 (9.3)</td>
</tr>
</tbody>
</table>

The values in parentheses are percentages.
4. Discussion

This preliminary survey was carried out in nine geographical areas in Sudan (Fig. 1), known to be natural grazing areas for sheep breeding.

Results presented here show that *T. lestoquardi* is widely distributed in Sudan. Findings reported here in regard to antibodies prevalence rate, extend results obtained by different authors (Nagwa, 1986; ElHussein et al., 1993; Latif et al., 1994; ElGhali and ElHussein, 1995; Ahmed, 1999; Taha, 2000).

Among the nine investigated areas in Sudan, River Nile State showed the highest prevalence rate (23.4%). This finding could be attributed to the fact that, sheep in River Nile State are mainly raised along the River Nile and Atbara River banks, where favorable microhabitat for survival and reproduction of the dominant tick vector *Hyalomma annatolicum annatolicum* exists (Hoogstraal, 1956; Ahmed, 1999). These ticks are usually found in animal sheds which are largely made of mud bricks and tree branches. This finding corresponds largely with the results obtained by Ahmed (1999) who reported that 22% out of 800 apparently healthy sheep showed patent theilerial piroplasmosis in River Nile State, northern Sudan.

The prevalence rate 16.7% reported here from Khartoum State was much lower than that reported by Nagwa (1986). This may reflect sampling factors as animals sampled in the latter study were obtained from resident animals, while our samples were obtained from markets, where mostly non-resident animals are brought for sale.

Although Blue Nile area is reported to be free of *H. annatolicum annatolicum* ticks (Jongejan et al., 1987), the present study showed 14% prevalence rate among sheep in the area. This may indicate either recent introduction of the known vector, *H. annatolicum annatolicum*, into the area or alternative vectors (e.g. *Rhipicephalus sanguineus* group, *Rhipicephalus evertsi evertsi*, *Hyalomma impleatum* and *Hyalomma rufipes*, Jongejan et al., 1987) may be involved in the transmission cycle. Further investigation regarding this subject in the Blue Nile State is needed.

In spite of the fact that sheep is not considered to be a natural host of *T. annulata* (Uilenberg, 1981a,b; Robinson, 1982), it can become infected with this parasite. However, the developmental cycle of *T. annulata* in the sheep seemed to be incomplete since no piroplasms were detected (Leemans et al., 1999). Hence, the presence of antibodies to *T. annulata* may not be surprising since the known vector for *T. annulata* and *T. lestoquardi* in Sudan is *H. annatolicum annatolicum* and in the areas investigated, both cattle and sheep are raised together.

These findings imply that, under field condition in Sudan, sheep are at risk of theileriosis where tick management is not strictly practiced. As mortality due to malignant ovine theileriosis is high and may reach 100% (Tageldin et al., 1992), the economic impact and control measures of the disease should be further investigated.

In conclusion, the present study showed that infection with *T. lestoquardi* is widely distributed in Sudan.

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References


