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Studies on the Transmission of *Theileria annulata* to Cattle by the Tick *Hyalomma lusitanicum*

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With 3 figures

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Summary

The role of the ixodid tick *Hyalomma lusitanicum* Koch 1844 as a vector of Mediterranean or tropical theileriosis (caused by the protozoan parasite *Theileria annulata* Dschunkowsky et Luhs 1904) in southern Spain was studied. *Hyalomma lusitanicum* was the most common tick, and the only species of the genus *Hyalomma* L., found on *T. annulata*-infected cattle from the theileriosis enzootic area studied (province of Cádiz, southern Spain). Likewise, we found that all sera of the cattle previously considered as suspected of theileriosis by clinical signs, tested for *T. annulata* antibodies, were positive and all blood samples of these suspected cattle examined had infected erythrocytes. Partially fed *H. lusitanicum* adults were collected in the field on *T. annulata*-infected cattle in this enzootic area and fed on an uninfected calf in an experimental farm free of theileriosis and ticks. At approximately 3 weeks post-tick feeding on the calf, this became positive for *T. annulata* antibodies and *T. annulata* merozoites were found in erythrocytes from blood smears. These results show the ability of *H. lusitanicum* to transmit the protozoan parasite *T. annulata* to susceptible cattle and indicate that *H. lusitanicum* is probably an important vector of *T. annulata* in the enzootic area surveyed.

Introduction

Mediterranean or tropical theileriosis is a tick-borne disease caused by the protozoan parasite *Theileria annulata* Dschunkowsky et Luhs 1904 which is transmitted to cattle by species of the genus *Hyalomma* L. (IRVIN, 1987). This disease affects cattle and other livestock throughout a wide area of north Africa, the Middle East, southern Europe, south of the former USSR, India and China (PURNELL, 1978). In most of the affected countries, ticks of the genus *Hyalomma* have been identified as vectors (IRVIN, 1987). Several species of *Hyalomma* have been reported in cattle from southern Spain, such as *H. lusitanicum* Koch 1844 and *H. marginatum* Koch 1844 (GARCÍA-FERNÁNDEZ and HUELI, 1984). Recently, *T. annulata* has been isolated in this region (VISERAS et al., 1997a) and a tissue culture vaccine against Mediterranean theileriosis has been developed and successfully tested in a field trial (VISERAS et al., 1997b, 1998). However, no studies have been reported to document the actual vector of the disease.

The aim of this study was to show whether *H. lusitanicum* can act as a vector of *T. annulata* in the surveyed enzootic area in southern Spain.

Materials and Methods

A study was carried out on 31 cases of suspected clinical Mediterranean theileriosis cattle throughout the last 2 years in an area of southern Spain (province of Cádiz, 36°00′ N to 37°00′ N and 5°15′ W to

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6°25′ W, approximately) in which the disease is enzootic (GARCÍA-FERNÁNDEZ et al., 1987; VISERAS, 1994), in order to identify the presence of the protozoan and the most common species of ticks in those cases. For this purpose, peripheral blood samples of the cattle were withdrawn from the jugular vein, and ticks were randomly collected on the cattle.

When possible, both clinical and laboratory diagnoses of the disease were carried out. For the former, the typical signs of Mediterranean theileriosis – enlargement of the lymph nodes (palpation), fever (rectal temperature), jaundice, anaemia (packed cell volume) – were considered; for the latter, samples of peripheral blood to prepare thin smears and sera were collected when clinical signs were observed. Blood smears were stained with 10 % Giemsa in phosphate-buffered saline pH 7.2 in order to identify the intraerythrocytic parasites. Specific antibodies against *T. annulata* in serum were determined using the immunofluorescent assay technique (IFAT) (BURRIDGE and KIMBER, 1972; GARCÍA-FERNÁNDEZ et al., 1996). The antigen was the schizonts of *T. annulata* from a culture of infected lymphoblastoid cells (VISERAS et al., 1997a). In two fatal cases, *post mortem* samples of blood, lymph nodes, liver, kidney, and spleen were taken, when considered necessary. The diagnosis of clinical cases of Mediterranean theileriosis was confirmed when the parasites were seen in the Giemsa-stained blood and tissue smears examined under the light microscope.

A healthy 3-month-old Friesian calf, *Bos taurus* L., was purchased from a theileriosis-free farm in an area where the disease is not enzootic. No parasites in blood smears nor any antibodies against *T. annulata* by IFAT were detected. The calf was splenectomized approximately 1 month before the start of the experimental transmission and then was housed in a tick-proof stall at an experimental farm in a theileriosis-free area. One day before the ticks were released on the calf, it was tested and was, once again, found to be *T. annulata*-free by Giemsa-stained blood smears and by IFAT.

For the experimental transmission, 80 (72 males and eight females) adult *H. lusitanicum* ticks were used (Fig. 1a, b). These ticks were collected in the field on a cow of breed 'Retinta' with a 0.1 % *T. annulata* parasitaemia. This animal was in an area in which Mediterranean theileriosis is enzootic [San José del Valle (36°37′ N 5°51′ W), province of Cádiz, southern Spain] (GARCÍA-FERNÁNDEZ et al., 1987). After collecting, the ticks were cleaned with nistatin antifungal agent (Mycostatin® suspension to 10 % v/v) to avoid saprophyte fungi on the tick body surface. Subsequently, they were placed in plastic tubes with cotton and gauze stoppers. The tubes were then kept in the dark, at 28°C, 75 % relative humidity, according to HUELI (1979, 1984) and FUJISAKI et al. (1988). The ixodid ticks were maintained under these conditions for 24 h and then put into linen bags for attaching and feeding on the calf's ears according to BAILEY (1960).

During the infection process, rectal temperature and adenopathies were monitored every 2 days. When the lymph nodes became enlarged, lymph node biopsy smears were prepared (PIPANO et al., 1989) in order to detect *T. annulata* schizonts. Packed cell volume was checked and Giemsa-stained smears were

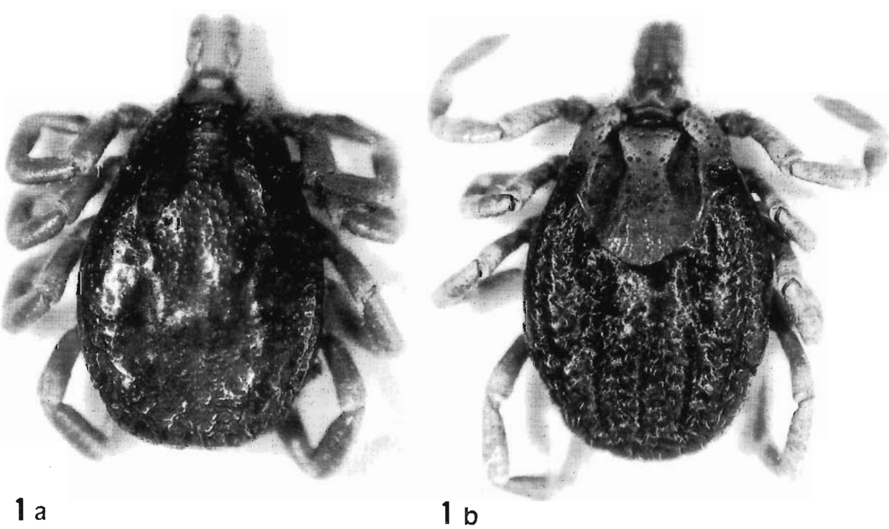


Fig. 1. Male (a) and female (b) *Hyalomma lusitanicum*, dorsal view (× 12).

observed twice a week to determine the parasitaemia. The antibody titres against *T. annulata*, by IFAT, were also monitored every 6–7 days.

Results

The 31 samples of possible clinical theileriosis cases were analysed in laboratory tests and the results specifically confirmed the *T. annulata* infection in all cases. *Hyalomma lusitanicum* (Fig. 1a, b) was shown to be the most frequently parasitizing ixodid tick on cattle (82.9 % of 469 ticks collected). Other ticks collected were *Rhipicephalus bursa* (Canestrini et Fanzago 1877) 15.1 %, *Boophilus annulatus* (Say 1821) 1.7 %, and *Dermacentor marginatus* (Sulzer 1776) 0.2 %. Thirty cattle (96.8 %) were parasitized by *H. lusitanicum*, five (16.1 %) by *R. bursa*, two (6.5 %) by *B. annulatus*, and one cattle (3.2 %) by *D. marginatus*. One cattle was not parasitized by ticks when sampled due to previous acaricide treatment by the owner.

In the experimental transmission, the adults of *H. lusitanicum* remained in the ear bags feeding on the calf for 5 days. From the initial 80 ticks, 19 (23.75 %) fed successfully – two females and 17 males – while the rest failed to attach to the calf's skin.

A slow-developing parasitaemia occurred in the calf. The infection was detected in blood smears at 23 days post-tick feeding and increased over time until it reached 12 % of erythrocytes and even more than one parasite per erythrocyte was sometimes observed (Fig. 2a, b). However, no schizonts were detected on smears of the lymph nodes nor in the blood.

During the 12 month follow-up period, specific antibody levels against *T. annulata* were clearly detected 19 days after feeding ticks (Fig. 3), showing variable concentrations throughout the post-tick feeding time, and reaching maximum titres (of 5120) 4 months later. Rectal temperature and packed cell volume showed no significant changes.

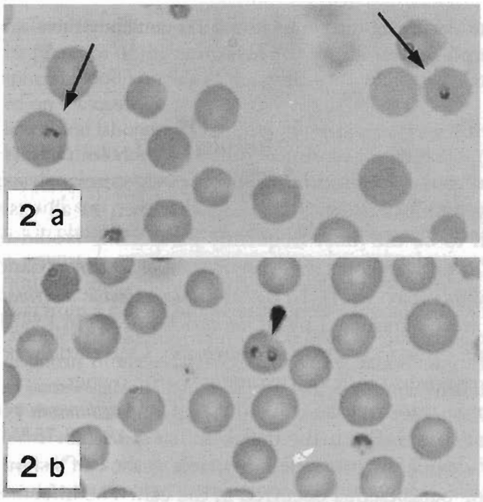
Discussion

The great majority (82.9 %) of ticks collected from infected animals in the studied enzootic area were *H. lusitanicum* (Fig. 1a, b), and all sampled cattle, except the acaricide-treated one, were parasitized by *H. lusitanicum* with the absence of any other *Hyalomma* species. In addition, all sampled cattle were *T. annulata*-infected and some fatal cases due to Mediterranean theileriosis occurred. These data lead the authors to suspect that this tick is a possible Mediterranean theileriosis vector in southern Spain. Moreover, whenever *T. annulata* is found worldwide, a vector of the genus *Hyalomma* is usually considered responsible (IRVIN, 1987).

On the other hand, JARRETT et al. (1966) pointed out that the incubation period of theileriosis-infected animals largely depends on the infection dose. It seems logical therefore that the prepatent period also depends on the infection dose. In this sense, it should be taken into account that parasitaemia in the cow where the ticks were collected from, and used in the experimental transmission, was extremely low at the moment of sampling. In addition, SANGWAN et al. (1989), in an epidemiological survey on *Theileria* transmission by *H. anatolicum* adults, concluded that male ticks are less able to transmit theileriosis than females, which could have a more important role than males in transmitting this parasite. We must bear in mind that from the 19 ticks that were attached and fed successfully on the calf in our experiment, only two were females.

For these reasons, it can be explained that an infection has been achieved (Figs 2 and 3) which has not led to any serious clinical symptoms but has led to the transmission of *T. annulata* by *H. lusitanicum* adults. They suggest the ability of this species to be a vector of the disease at least under experimental conditions.

Finally, the results seem to show the experimental transmission of *T. annulata* by *H. lusitanicum*. Also, bearing in mind the following facts: (a) 30 cattle from 31 confirmed cases of Mediterranean theileriosis from an enzootic area in southern Spain were parasitized by this species of tick; (b) a great majority of ticks were *H. lusitanicum* (82.9 %); and (c) no other *Hyalomma* species have been found on the cattle examined; it can be assumed that *H. lusitanicum* is able to transmit Mediterranean theileriosis to cattle under field conditions and, probably, it



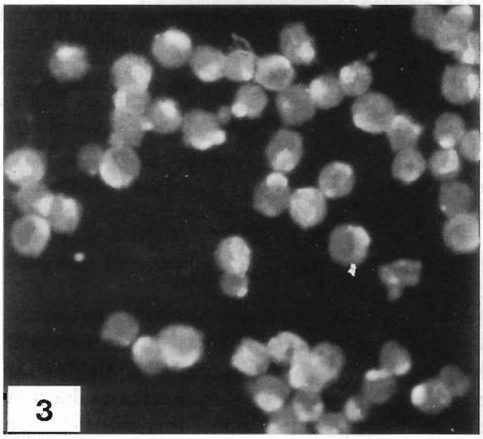


Fig. 2. *Theileria annulata* infection in the blood of the experimentally infected calf (Giemsa stain × 1400). Note erythrocytes with one (a) or two (b) piroplasms.
Fig. 3. Positive immunofluorescent assay technique (IFAT) using schizonts of *Theileria annulata* from established cultures as antigen (× 540).

acts as a vector of the disease in this enzootic area in southern Spain and, possibly, in other enzootic areas for Mediterranean theileriosis where this ixodid tick occurs.

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