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ScienceDirect
Preventive Veterinary Medicine 81 (2007) 323–326
PREVENTIVE VETERINARY MEDICINE
www.elsevier.com/locate/prevetmed

Short communication

Comparisons among two serological tests and microscopic examination for the detection of *Theileria annulata* in cattle in northern Sudan

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Received 27 January 2006; received in revised form 10 May 2007; accepted 15 May 2007

Abstract

We tested the agreement between microscopic examination (ME), a surface protein-detecting enzyme-linked immunosorbent assay (TaSP ELISA) and an indirect fluorescent assay (IFA) for detection of *Theileria annulata* in 2661 naturally infected cattle from northern Sudan (samples collected between June 2001 and July 2002). In the ME, we detected piroplasms in 364/2661 cattle (14%), and the kappas between the ME and the serological tests were poor (TaSP ELISA 10%; IFA 8%). The TaSP ELISA detected 885/2661 cattle as positive, and the Rogan-and-Gladen corrected true prevalence of this sample was estimated to be 30%. The relative sensitivity and specificity of the IFA (compared to the previously validated TaSP ELISA) were 70.7% and 81.8%, respectively.

Keywords: IFA; Microscopic examination; TaSP ELISA; *Theileria annulata*

1. Introduction

Ticks and tick-borne diseases are widespread in the Sudan (Anonymous, 1983). They represent a threat to exotic cattle and their crosses in the country and cause substantial losses of both animals and their products (Gamal and El Hussein, 2003). The most important tick-borne disease in the Sudan is tropical theileriosis (*Theileria annulata* infection of cattle) (El Hussein et al., 2004), which is endemic in the northern parts of the country (Anonymous, 1983). *Hyalomma anatolicum anatolicum* is the most efficient vector of *T. annulata* (Um El Hassan et al., 1983).

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0167-5877/$ – see front matter
doi:10.1016/j.prevetmed.2007.05.008

Laboratory diagnosis of tropical theileriosis is mainly by preparation of blood-smear and lymph-node biopsy for detection of piroplasms and schizonts, respectively (Norval et al., 1992). Serum antibodies appear 2–4 weeks after infection, and decline to variable levels after 24–26 weeks (Burridge and Kimber, 1973). The most commonly used serum-antibody assay for *T. annulata* has been the indirect fluorescent-antibody assay (IFA) (Burridge, 1971); however, it is tedious and subjective, and cross-reactions are considered a problem (Kiltz et al., 1986). ELISAs have the advantage over IFA of being less laborious and many samples can be tested quickly. Recently, *T. annulata* surface protein (TaSP) has been characterized (Schnittger et al., 2002) and its application in indirect ELISA has been documented (Bakheit et al., 2004) and validated (Salih et al., 2005). The sensitivity (Se) and specificity (Sp) of the TaSP ELISA both were estimated to be 93.5% by the latter authors.

The anticipated introduction of an attenuated cell-culture vaccine now under development in Sudan (Shariff et al., 2006) highlighted the need for application of more specific, sensitive, reproducible and easy to perform laboratory techniques. In the present study, we described the kappas between the ME and two serological tests (TaSP ELISA and IFA), and used the TaSP ELISA to calculate the relative Se and Sp of the IFA.

2. Materials and methods

2.1. Study design

The survey was conducted during the period from June 2001 to July 2002 in the Northern, Central, Western and Eastern Sudan and Blue and White Nile. Selection of these locations was based on them being the main potential areas for livestock rearing. In each location, samples were collected from at least four farms of cattle that were kept apart. Selection of farms was made randomly and the formal mechanism used was lottery. A total of 2661 blood-smears and serum were collected during the study period. Two thin blood-smears from an ear-vein puncture were prepared from each animal, air dried and fixed in absolute methanol for 3–5 min. The slides were labelled indicating the location, date and animal number. Blood was collected in a plain vacutainer tubes and sera were separated by centrifugation at 1500 rpm for 10 min. Each serum sample was collected using a sterile Pasteur pipette in an Eppendorf tube, labelled indicating location, date and animal number and then stored at $-20\ ^{\circ}C$ until used during the next 6 months.

2.2. Microscopic examination and serological tests

At least 30 microscopic fields were examined for *Theileria* spp. piroplasms under oil-immersion lens (100× magnification) after being stained with Giemsa. The presence of ≥ 1 piroplasm was considered positive. Microscopic examination was done in a manner that was blinded to the serological results.

Procedures followed in materials preparation and running the IFA were as described by Burridge (1971). *T. annulata* schizont-antigen slides were prepared from culture of low passage number (<20). Indirect ELISA was performed with *T. annulata* surface protein ‘‘TaSP’’ as described by Bakheit et al. (2004). The optical density (OD) values were expressed as percent positivity (PP), using the formula: (mean OD of the sample divided by mean OD value of positive control) multiplied by 100. The cut off was set at 31.5 PP with specificity and sensitivity of 93.5% using the TWO-ROC software (Salih et al., 2005). The Rogan-and-Gladen estimator (P_{RG}) (Rogan and Gladen 1978, cited in Greiner and Gardner, 2000) was used to correct the apparent