A COMPARATIVE STUDY OF AN ELISA TEST AND AN INDIRECT IMMUNOFLUORESCENCE TEST FOR SEROLOGICAL DIAGNOSIS OF BABESIA BOVIS INFECTION

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Abstract

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Detection of antibodies to *Babesia bovis* in cattle is essential for the understanding of the epidemiology of babesiosis and this study was concerned with comparing the indirect fluorescent antibody with the ELISA. Both assays gave rise to 100% sensitivity whilst the ELISA was shown to be marginally more specific at 98%. The ease of use and low cost of the ELISA would make it the more obvious choice in conducting future serological surveys for this parasite.

1. INTRODUCTION

Detection of antibodies to *Babesia bovis* in cattle is essential for the understanding of the epidemiology of babesiosis. Serological tests which have been commonly used include complement-fixation (CF) [1], indirect fluorescent antibody (IFA) [2] and inhibition of hemaglutination (IHA) [3]. More recently, enzyme linked immunoassays (ELISA) have been applied to the serological diagnosis of a number of haemoparasite infections of medical and veterinary importance throughout the world [4]. These tests have been shown to be more specific than those used previously [5]. Barry et al. [6] described the comparison of ELISA and IFA tests with known positive sera where they found more than 95% agreement between the two methods with the ELISA displaying a slightly better sensitivity.

Waltisbuhl et el. [7] reported an improved sensitivity in the ELISA by using horseradish peroxidase instead of alkaline phosphatase in the test. Johnston et al. [8] described an IFA technique which with some modifications, is being used routinely in our laboratory at the Instituto de Pesquisas Veterinárias Desidério Finamor, Porto Alegre, RS, Brazil.

The advantages of the ELISA over IFA are: a) it is more quantitative and not subjective, b) more tests can be performed in a given time, c) it is more sensitive. All these are important considerations when an epidemiological study of an infected region such as the state of Rio Grande do Sul is contemplated.

In this report we describe the comparison of an ELISA for detection of antibodies to *B. bovis* and an IFA test. The viability of the ELISA system for use as an epidemiological tool is also evaluated.

2. MATERIALS AND METHODS

2.1. Field serum samples

One thousand five hundred sixty bovine sera from two regions (Livramento and Bage) in the State of Rio Grande do Sul, southern Brazil, where babesiosis is considered to be enzootic, were collected and stored at -20°C prior to testing.

2.2. Reference sera

In order to check the specificity of the ELISA, serum samples were collected from 97 cattle, living in one area free of the cattle tick vector, *Boophilus microplus*, and found previously negative on IFA tests, considered at the time as standard. In addition light microscopic examinations of blood smears were also performed which demonstrated the absence of circulating parasites. Sera from 22 known
positive animals (as demonstrated by the presence of parasites in blood smears) were obtained from calves experimentally infected with a local strain of *B. bovis* (strain IPV1).

### 2.3. IFA test: (Preparation of the IFA antigen)

A splenectomized calf was inoculated with *B. bovis* parasites. At the peak of parasitemia (0.5% of parasitized erythrocytes at day 5), 100 ml of blood was withdrawn and immediately mixed with anticoagulant (4.5% sodium citrate). This was washed twice in PBS and centrifuged at 1,000 g with intervals of 10 minutes between washing. Sediment was passed through a fibrous cellulose powder (CF 11, Whatman) and inoculated intravenously into a second splenectomized calf. When the parasitaemia reached 5-7%, 500 ml of blood was collected and mixed with anticoagulant at a ratio of 7:1 (v/v). The blood so obtained was then washed three times in PBS with intermittent centrifuging spins at 1,000 g for 15 minutes. The washed erythrocytes were finally resuspended in PBS to give a packed cell volume of about 50%. Thin unfixed films were made from that suspension on cleaned microscope slides, dried at room temperature, wrapped in tissue paper, and covered with aluminium foil before being stored at -20°C.

### 2.4. IFA test procedures

The IFA tests were performed as previously described [8]. Previous to testing, all sera were diluted 1:40 in PBS. The conjugate was anti-bovine IgG fluorescein isothiocyanate (produced in rabbit, by Sera-lab, UK) diluted 1:60 in PBS.

### 2.5. ELISA test

The ELISA reagents were those supplied by the Joint FAO/IAEA Division of the International Atomic Energy Agency (IAEA), Vienna, Austria. They were used as specified in the ELISA kit for detection of *B. bovis* and all the tests procedures were according to the recommendations by the Joint FAO/IAEA Division, included in the reference kit. Basically in this test, the antigen dilution used was 1:600 in carbonate bicarbonate buffer, pH 9.6, and the sera to be tested was diluted 1:200 in PBS with 0.05% tween-20 (PBS-T) with 5% of powder milk. Rabbit anti-bovine IgG conjugated with horse radish peroxidase (provided by the IAEA) was used diluted in PBS-T plus 5% of powder milk. The substrate used was orthophenyldiamine (OPD) and as stopper, a solution of 1NH₂SO₄ was used. Flat-bottomed microplates (Linbro, Flow laboratories, USA) were used throughout and read at optical density of 492 nm in a microplate reader (Titertek Multiskan, Flow laboratories). Sensitivity and specificity was calculated using the formula presented in Table II.

### 3. RESULTS

#### 3.1. ELISA/IFA comparison

The results obtained with the ELISA and IFA techniques on the 1560 field samples examined are shown in Table I and Figure 1. There was a 90.1% (1406/1560) agreement between positive and negative results in both tests. The remainder 9.9% were represented by samples which were 4.2% (66) positive for IFA and negative for ELISA and samples which were 5.6% (88) negative for IFA and positive for ELISA.

<table>
<thead>
<tr>
<th>IFA</th>
<th>ELISA</th>
<th>IFA/ELISA</th>
<th>IFA+</th>
<th>IFA-</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
<td>66</td>
<td>88</td>
<td>1560</td>
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<td>1116</td>
<td>1028</td>
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</tbody>
</table>

### TABLE I. COMPARATIVE RESULTS BETWEEN IFA AND ELISA ON FIELD SAMPLES TESTED FOR DETECTION OF ANTIBODIES AGAINST BABESIA BOVIS
FIG. 1. Comparative results between IFA and ELISA for Babesia bovis of field samples.

3.2. Specificity and sensitivity

Of 97 examined known negative sera, specificity was shown to be 97.9% for IFA and 98.9% for ELISA. The 22 sera known to contain antibodies to *B. bovis* were all positive in both ELISA and IFA.

### TABLE II. CALCULATION OF SPECIFICITY AND SENSITIVITY

<table>
<thead>
<tr>
<th>Test status</th>
<th>True status</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diseased</td>
<td>Not diseased</td>
</tr>
<tr>
<td>Diseased</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>Not diseased</td>
<td>c</td>
<td>d</td>
</tr>
<tr>
<td>Totals</td>
<td>a + c</td>
<td>b + d</td>
</tr>
</tbody>
</table>

Sensitivity = a / (a + c) Specificity = d / (b + d)
4. DISCUSSION

The introduction of washing erythrocytes before a second inoculation into a splenectomized calf, seems to abbreviate timing of *B. bovis* parasitemia. The inoculation of a number of leucocytes from the first calve into a second one is markedly reduced, decreasing possibilities for immediate antibody production which probably interferes in the quality of the produced antigen.

An explanation for the 9.1% of different results between IFA and ELISA might be the possibility of cross-reaction with other hemoprotozoa (*B. bigemina* or *Anaplasma marginale*) previously reported by Waltisbuhl et al. [7]. In our study concurrent infection may have accounted to such discrepancies. Also false positives reactions to *B. bovis* were observed in sera from cattle infected with *A. marginale*. ELISA has been reported as more sensitive than IFA [6] and this can explain the few more positives samples detected by this technique (88 positive by ELISA and negative by IFA). The use of a more sensitive technique is usually desirable. This seems to be the case of the ELISA kit provided by Joint FAO/IAEA Division.

REFERENCES