

EVALUATION OF AN ELISA KIT IN THE SEROLOGICAL DIAGNOSIS OF *BABESIA BOVIS* FOR EPIDEMIOLOGICAL STUDIES

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Abstract

EVALUATION OF AN ELISA KIT IN THE SEROLOGICAL DIAGNOSIS OF *BABESIA BOVIS* FOR EPIDEMIOLOGICAL STUDIES.

An enzyme linked immunosorbent assay (ELISA) kit for detect antibodies to *Babesia bovis*, an intraerythrocytic bovine parasite was evaluated using known negative and positive samples and the results were compared with an indirect immunofluorescent antibody test (IFAT). Results obtained with field samples were used to estimate seroprevalence of *B. bovis* in an endemic area to the cattle tick (*Boophilus microplus*) vector of bovine babesiosis. Percentage of positivity (PP) values (optical density of tested serum/mean optical density of positive control) on 274 negative samples, had major values ranged in the frequency of 4.0 to 7.0 PP. Comparison between ELISA and IFAT showed an agreement of 93.3% on field sera samples, collected in areas of low, good and high soil fertility in the region of Bagé (31° 20' 13''S, 54° 06' 21''W), RS, Brazil. From 5,082 tested sera, 3,751 (73%) were positive for *B. bovis* antibodies. No significant difference ($p > 0.05$) was observed between results from calves living in areas of low and good soil fertility (80 and 82% of seroprevalence, respectively). However, calves living in soil of high fertility showed a minor inoculation rate for *B. bovis*, (63% of seroprevalence) indicating needs of measures to prevent losses due to babesiosis.

1. INTRODUCTION

The use of the enzyme linked immunosorbent assay (ELISA) techniques since its first description in 1971 [1] is widely distributed for detect antigens and antibodies. Briefly, the assay involves: 1) the adsorption of antigen or antibody to a solid phase; 2) the addition of sample; 3) incubation and washing steps; 4) addition of enzyme labelled antigen or antibody, and 5) the addition of the enzyme substrate. The first ELISA technique for detecting antibodies against a bovine haemoparasite was described in 1976 [2] for *Babesia divergens*. Barry et al. [3] described the first ELISA for detecting antibodies to *B. bovis* showing agreement of more than 95% with the Indirect Fluorescent Antibody Test (IFAT) in a comparative study. Also the assay was able to detect *B. bovis* antibody 14 days after experimental infection showing more sensitivity than the IFAT. Waltisbuhl et al. [4] described an ELISA for *B. bovis* claiming more sensitivity than the IFAT and using horseradish peroxidase rather than alkaline phosphatase as labelled enzyme.

In order to introduce this technique and make it accessible for several laboratories in the world, the Joint FAO/IAEA Division, through its Animal Production and Health Section standardized an ELISA kit and distributed it to laboratories in Latin American countries.

This report refers to the results obtained with the ELISA kit for *B. bovis* under an FAO/IAEA Research Contract (No. 6522) tested at the "Instituto de Pesquisas Desidério Finamor".

2. MATERIALS AND METHODS

2.1. Reference sera samples

Aiming to establish a catalogue of serum bank, 274 samples from a herd of a tick-free area (Santa Vitória do Palmar, extreme south of the State of Rio Grande do Sul) were collected. Also blood

samples were obtained and stained by Giemsa for direct observation of haemoparasite. These sera being negative on IFAT and negative by light microscopic examination, were taken as negative reference samples. A total of 97 sera samples from *babesia*-vaccinated cattle with circulating *B. bovis* hemoparasites seen by light microscopic were collected and stored at -20°C prior testing. The specificity and sensitivity of the test was based in the results obtained with these sera samples.

2.2. Field samples

A survey for *B. bovis* antibodies was carried out in the region of Bagé, State of Rio Grande do Sul (31° 20' 13" S, 54° 06' 21" W, 216 m) where 5082 sera samples from calves age between 10 and 14 months-old were collected. The farms (68) were selected from three different zones according to type of soil (low, good and high fertility) and 20 calves selected at each farm.

2.3. Serological assay

The ELISA kit for *B. bovis* antibody was used as specified in the FAO/IAEA manual. In order to compare some serological results, an immunofluorescent antibody test (IFAT) for *B. bovis* locally produced and used routinely in the parasitology section at IPVDF was taken as reference. Herds with a prevalence rate between 15% and 80% were considered to be at risk from babesiosis outbreaks due to *B. bovis*.

3. RESULTS

Figure 1 shows the distribution of percentage of positive values (PP) obtained with the negative reference samples for *B. bovis*. Major of these values ranged in the frequency of 4.0 to 7.0 PP being that an average of 4.96 PP was observed on 274 examined sera from negative cattle.

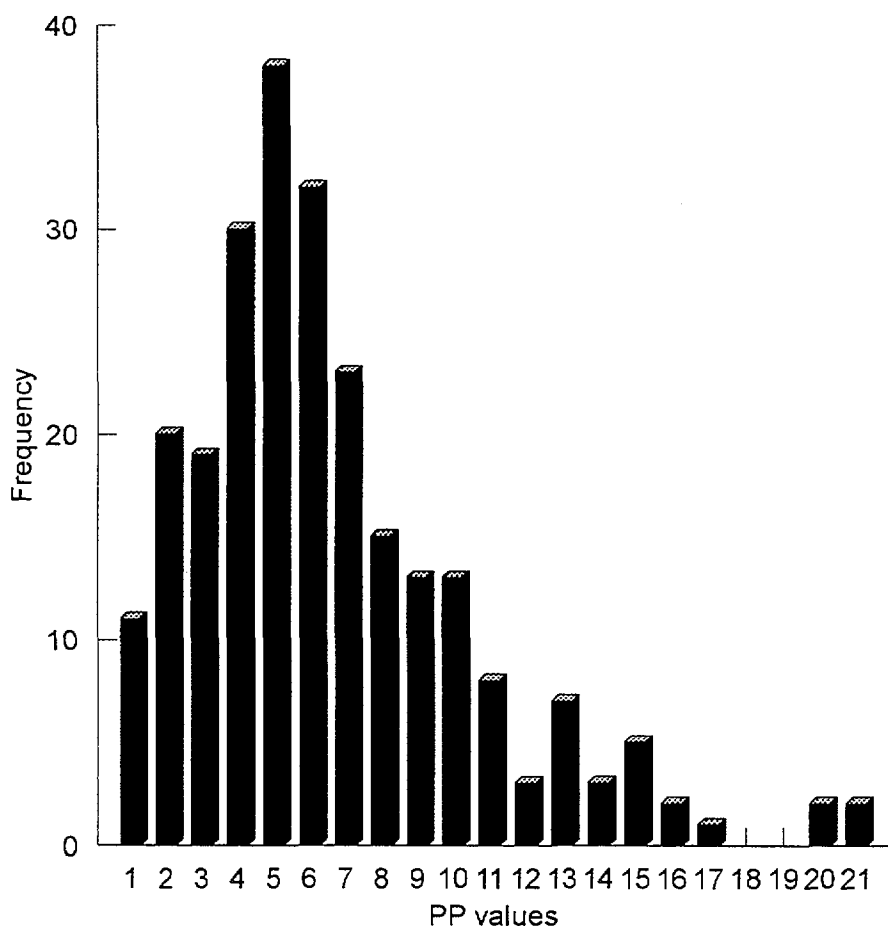


FIG. 1. Distribution of PP values for an ELISA kit of *B. bovis* (values for known negative samples).

A comparative picture (Figure 2) between IFAT and the ELISA kit on field samples showed an agreement of results in 93.3% of the tested samples. Nevertheless 3.9% were positive by ELISA and negative by IFAT and 2.7% were positive by IFAT and negative by ELISA.

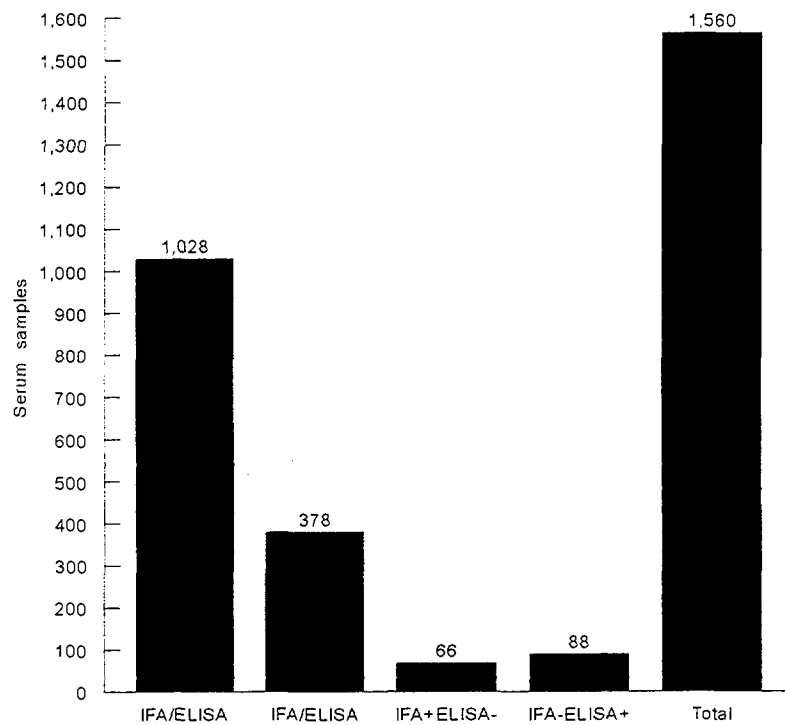


FIG. 2. Comparative results between IFA and ELISA for *Babesia bovis* on field samples.

The results with 5082 field samples (Figure 3) showed that 3,751 (73.8%) were positive for antibody against *B. bovis*. From the three zones, percentages of 80%, 82% and 63% of prevalence were observed respectively for soils with low, good and high fertility.

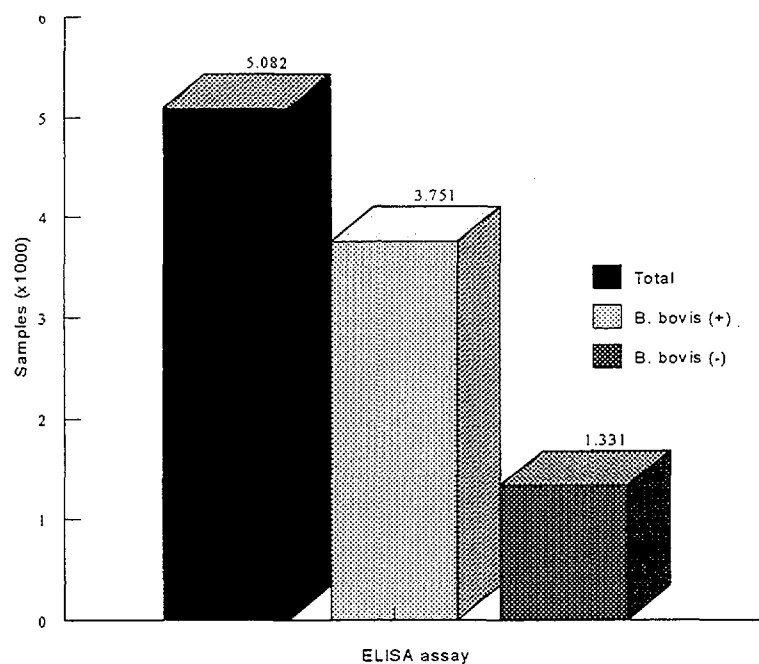


FIG. 3. Epidemiology of TBD in RGS state, Brazil, Survey in Bagé, RGS.

No significant difference ($p>0.05$) was observed between results from calves living in areas of low and good soil fertility. Calves in both these areas were in an area of enzootic stability for babesiosis, since the cattle tick vector (*Boophilus microplus*) is endemic. However, when improving soil fertility and the changes in the pastures management, a lower number of cases was seen in calves indicating a difference in the inoculation rate for *B. bovis*.

4. DISCUSSION

The comparison between ELISA and IFAT demonstrated an agreement of 93.3% and revealed the value of the ELISA system which is especially suitable for screening large numbers of samples. Results are expressed in optical density (OD). Thus defined positive and negative values can be expressed and used for further comparisons. For epidemiological surveys this method is very useful and highly applicable. The known negatives and positives samples tested showed the high sensitivity and specificity of the technique, although cross reactions with *B. bigemina* and *Anaplasma marginale* were not tested. Sera from animals after 14 days after inoculation with *B. bovis* attenuated strain gave positive results for antibody. The antigen dilution recommended (1/200) and the sera dilution (1/200) worked well in our conditions.

Nevertheless a few differences were observed with regard to conjugate titration (variations from 1/9000 to 1/13000 were detected). As described by Mahoney et al. [5] it is possible to predict areas where low and good soil fertility are found, and where a situation of enzootic stability for babesiosis occurs. However, in the area where the soil is very fertile, an enzootic instability was found, indicating that measures to prevent losses due to *B. bovis* should be adopted.

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