USE OF ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DIAGNOSIS OF BRUCELLOSIS IN CATTLE IN YUCATAN, MEXICO

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Abstract

USE 0F ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA) FOR THE DIAGNOSIS OF BRUCELLOSIS IN CATTLE IN YUCATAN, MEXICO.

Sera (247) from non-vaccinated brucellosis negative herds, 328 negative sera from *Brucella abortus* strain 19 vaccinated herds (brucellosis free), and 95 sera positive to the Rose Bengal test (RBT) and Complement Fixation test (CFT) from *Brucella abortus*-infected herds, were used to determine the relative sensitivity and specificity of a FAO/IAEA I-ELISA kit and the Rivanol Agglutination Test (RAT), using the CFT as a "gold standard". A threshold value for the I-ELISA was determined to be 37 PP using the mean plus 3 standard deviations of the negative sera from vaccinated animals. The I-ELISA showed a high relative sensitivity (100%) and a good relative specificity (92.5%), using the threshold determined for local conditions. The RAT gave a lower sensitivity value than the CFT (97.8%) and good specificity (99.3%). The I-ELISA could be used as a screening test under Yucatán conditions or as a confirmatory test in places where vaccination is not carried out. The RAT lacks sensitivity and is therefore not recommended for use in final stages of eradication programs but could be used in control programmes or early stages of eradication campaigns as a confirmatory test.

1. INTRODUCTION

Yucatán is a brucellosis endemic area, and vaccination with the full dose of S-19 is a common practice. Vaccination practices are not uniform, and for the most cases there are no records, making considerations in diagnostic serology difficult. In Latin America, México has one of the highest incidences of brucellosis. In México the estimate of annual losses due to this infectious disease are thought to amount US$ 350,000,000 [1]. An important aspect of brucellosis is its strong zoonotic potential. In 1988, México reported 6303 cases of brucellosis in humans [2].

Although several seroprevalence studies have been done, different diagnostic techniques have been used and the results vary from zone to zone and for different laboratories within the country. Yucatán is not an exeption and none of the studies performed have been carried out following a reliable sampling design and using a reliable diagnostic protocol designed for conditions of the area. In previous studies, the Rose Bengal Plate Test (RBT), the Rivanol Agglutination test (RAT), the 2-mercaptoethanol agglutination test (2-ME), and an indirect ELISA (I-ELISA) were compared to Complement Fixation Test (CFT) in order to check their sensitivity and specificity. However, not enough samples were tested and the results were therefore of limited value [3].

Although the definitive diagnosis of infectious disease can be accomplish only through the direct demonstration and identification of the causative agent(s) by culture and isolation procedures, sometimes this may be difficult and beyond the expertise and capabilities of diagnostic laboratories, particularly those in developing countries. However, accurate presumptive diagnosis can be achieved from serological techniques used in combination with clinical observations and case histories [4].

Classical serological techniques (i.e. agglutination, precipitation, complement fixation and virus neutralization tests) have proved useful but they suffer from several drawbacks such as poor performance and lack of standardization. ELISA techniques have the potential to solve many of these problems [4]. ELISA has become widely used for both antigen and antibody detection in animal disease diagnosis. Unfortunately, little has been done concerning internationally

acceptable reference reagents or protocols, with a negative impact on international control of animal diseases and inter-country trade in livestock [5].

The objectives of the present study were to establish a serum bank and to use it to validate the FAO/IAEA I-ELISA kit, determining the threshold value for local conditions; to calculate the relative sensitivity and specificity of the FAO/IAEA I-ELISA test; to determine the relative specificity of other tests used, considering the CFT as the " gold standard"; to start a seroprevalence survey using the I-ELISA and to catalogue and analyze the data using computer facilities.

2. MATERIAL AND METHODS

For the comparison of the serological tests a serum bank was established using 247 sera from non-vaccinated negative brucellosis free herds, 328 negative sera from *Brucella abortus* strain 19 vaccinated herds (brucellosis free), and 95 sera positive to RBT and CFT from infected herds (at least 2% seropositivity in the herd using the above tests and showing clinical signs).

The method described by Alton et al. [6] and Morgan et al. [7] was used for the RBT. The antigen was supplied by Productora Nacional de Biológicos Veterinarios (PRONABIVE). For the RAT, the method described by Morilla and Bautista was used [8]). The antigen was provided by PRONABIVE. Serum dilutions used were 1:25, 1:50, 1:100 and 1:200. The CFT was the microtiter method as used at the Central Veterinary Laboratory (CVL), Weybridge, England [9], except that samples were run at doubling dilutions up to 1:64, due to strong prozone effect found previously at lower dilutions. The concentration of International Units (I.U.) of antibody were derived from standard tables. The positive and negative controls and antigen were supplied by the CVL, Weybridge, UK.

An ELISA kit developed by the Joint FAO/IAEA Division was used, following the procedure exactly as indicated in the protocol provided. The antigen was a hot water/hot phenol extract from *Brucella abortus* and the conjugate was horseradish peroxidase-labeled mouse monoclonal anti-bovine IgG1. The substrate was H_2O_2 and the chromogen was ABTS. The optical density (OD) of each well was measured using a Immunoskan Plus automatic reader (BDSL) linked to a computer using the FAO/IAEA BREIA 1.01 program to interpret results. The reader, the program and the computer were provided under an FAO/IAEA Research Contract.

The basis of interpretation of each test is shown in Table I. For the RBT and RAT, this is provided in the papers, which describe the techniques. For the CFT, the antibody levels used were calculated using a table supplied by the Center for Tropical Veterinary Medicine, Edinburgh, U.K.

The I-ELISA threshold was determined using the sera negative to all tests from vaccinated animals, then calculating the mean (x) of the percentage positivity (PP) of the animals and adding three standard deviations (SD).

TABLE I. ANTIBODY LEVELS FOR INTERPRETATION OF TESTS

	Vaccinated	Non-vaccinated
RBT	reaction	reaction
RAT	1:50	1:25
CFT	50 i.u.	20 i.u.
I-ELISA*	37%	28%

* These values are the cut-off point calculated using the kit for each of the groups

The relative sensitivity and specificity of each test compared to the others and the Predictive Value for the I-ELISA were calculated using the methods described by Thrusfield [10]. The Epi-Info version 5.01b software [11] was used for sorting and analyzing the data.

3. RESULTS

The cut-off point for the I-ELISA was found to be 37 PP of the strong positive control. Tables II-V show the results for the RAT and I-ELISA tests compared to those for the CFT using sera from vaccinated, non-vaccinated cattle and overall. Sensitivity and specificity of all the tests compared to CFT are also shown.

TABLE II. SENSITIVITY AND SPECIFICITY OF RIVANOL TEST COMPARED TO CFT (n=688)

	CFT positive	CFT negative	Sensitivity (%)	Specificity (%)
Non-vaccinated Riv. +ve	88	4	97.8	98.5
Non-vaccinated Riv. -ve	2	271	-	-
Vaccinated Riv. +ve	-	-	-	-
Vaccinated Riv. -ve	-	323	-	-
Overall Riv. +ve	88	4	97.8	99.3
Overall Riv. -ve	2	594	-	-

Riv. = Rivanol Agglutination test

TABLE III. SENSITIVITY AND SPECIFICITY OF I-ELISA TEST COMPARED TO CFT (n=681).

	CFT positive	CFT negative	Sensitivity (%)	Specificity (%)
Non-vaccinated E. +ve	83	14	100	94.9
Non-vaccinated E. -ve	0	261	-	-
Vaccinated E. +ve	-	31	-	90.4
Vaccinated E. -ve	-	292	-	-
Overall E. +ve	83	45	100	92.5
Overall E. -ve	0	553	-	-

TABLE IV. SENSITIVITY AND SPECIFICITY OF I-ELISA TEST COMPARED TO RIVANOL TEST (n=691).

	Riv. +ve	Riv. -ve	Sensitivity (%)	Specificity (%)
Non-vaccinated E. +ve	89	6	98.9	97.8
Non-vaccinated E. -ve	1	271	-	-
Vaccinated E. +ve	-	31	-	90.4
Vaccinated E. -ve	-	293	-	-
Overall E. +ve	89	37	98.9	93.8
Overall E. -ve	1	564	-	-

Riv. = Rivanol Agglutination test

TABLE V. SENSITIVITY AND SPECIFICITY OF RIVANOL TEST COMPARED TO I-ELISA TEST (n=691).

	E +	E -	Sensitivity (%)	Specificity (%)
Non-vaccinated Riv. +ve	89	1	86.4	99.6
Non-vaccinated Riv. -ve	14	263	-	-
Vaccinated Riv. +ve	39	0	100.	100
Vaccinated Riv. -ve	0	285	-	-
Overall Riv. +ve	128	1	90.1	99.8
Overall Riv. -ve	14	548	-	-

Riv. = Rivanol Agglutination test

The predictive value of the I-ELISA, was 34% and 100% for the positive and negative results respectively, using a calculated prevalence of 4% for the Yucatán state, a sensitivity and specificity of 100% and 92% respectively.

4. DISCUSSION

The cut-off point (37 PP) was determined using the negative vaccinated population because it improved the specificity of the test by 0.7% (from 91.6% to 92.3%) over the cut-off determined using the negative non-vaccinated population (28 PP). This is comparable to the 35 PP recommended in the kit protocol, without loss of sensitivity. The reason for considering the vaccinated negative population instead of the negative non-vaccinated population was because vaccination is a common practice in Yucatán and a large number of negative vaccinated animals would have been misclassified as positive, thereby decreasing assay specificity. Sensitivity of the I-ELISA was 100% relative to the CFT (gold standard). Specificity was lower in the overall population due to the inability of the test to differentiate vaccinated from infected animals, however, the specificity for the different groups (94.9% for non-vaccinated and 90.4% for the vaccinated animals) indicates the possibility of using the I- ELISA test as a confirmatory test perhaps as a replacement for the more complicated CFT or the rivanol agglutination test, especially in areas where vaccination with *Brucella abortus* S-19 is not carried out. The lower specificity of the I-ELISA compared to CFT, although the former uses a Mab specific to detect IgG1 could be explained by the fact that only capture enzyme immuno assays permit precise isotype analysis of antibodies of a distinct isotype (12).

The RAT showed a relative sensitivity of 97.8% compared to CFT. This makes the RAT less desirable to use as a confirmatory test in the final stages of eradication programmes, because of the danger of leaving false negative animals in the herd. This finding agrees with previous studies performed in Yucatan [3], however, the relative specificity of the RAT compared to CFT was good (99.3%) giving comparable results to other studies which indicated that the RAT could be used to differentiate between vaccination and infection titers [7,13] therefore it could be used as a confirmatory test during control of brucellosis and early stages of eradication campaigns.

The relative sensitivity and specificity of the I-ELISA compared to RAT test were also calculated. The results show that the I-ELISA has a comparable performance to the RAT in both aspects. However, when the comparison of the relative sensitivity and specificity of the RAT to I-ELISA was done, it showed that the relative sensitivity of the RAT was low, (90.1%), although the specificity was high (99.8%). This indicates that the I-ELISA is more sensitive than the RAT as performed in Yucatán. However, both tests demonstrated specificities which were comparable.

The I-ELISA Predictive Value for the positive (34 %) and negative (100 %) results, confirmed that this is a highly sensitive and but less specific test for our conditions, so it can be used as a screening test, but the positive results would have to be confirmed with a more specific laboratory test. A more realistic picture of the utility of the I-ELISA in comparison with other serological tests, will be obtained when it is field tested, and the prevalence survey in Yucatán is completed.

From the results presented here the following conclusion may be drawn. It is recommended that under the Yucatan conditions when possible the CFT should be used for identification of reactors after initial screening with a highly sensitive test (i.e. RBT or I-ELISA) which is more simple and quicker than CFT. The RAT seems to have a high specificity but a lower sensitivity compared to the CFT and might therefore not identify some reactors.

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