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Comparison of radioimmunoassay with the complement fixation test and the indirect haemolysis test in the field diagnosis of bovine brucellosis

By R. J. CHAPPEL and J. HAYES

Department of Agriculture, Attwood Veterinary Research Laboratory, Mickelham Road, Westmeadows 3047, *Victoria, Australia*

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SUMMARY

Sera were collected from female cattle in 118 commercial herds being subjected to a programme to eradicate brucellosis by test and slaughter, in an area in which vaccination of heifer calves with *Brucella abortus* strain 19 was compulsory. Of 4583 sera positive by the Rose Bengal plate test, the brucellosis radioimmunoassay was positive for 1524, the complement fixation test for 1363 and the indirect haemolysis test for 1141. These figures, and supporting evidence from the eradication programme, suggest that the radioimmunoassay may be a useful supplementary test in problem herds.

INTRODUCTION

The efficiency of serological diagnosis affects progress towards the eradication of bovine brucellosis by test and slaughter. False negative serological reactions contribute to the existence of problem herds which resist eradication (Cordes & Carter, 1979). The brucellosis radioimmunoassay (RIA) (Chappel *et al.* 1982*a*) is a sensitive test designed to reduce the number of false negative serological diagnoses.

In this study, sera collected from commercial herds undergoing brucellosis eradication were used to compare the results of the RIA with those of the complement fixation test (CFT) and the indirect haemolysis test (IHLT).

MATERIALS AND METHODS

Sera were collected from female cattle in 118 commercial beef and dairy herds in the State of Victoria. These herds were being subjected to a programme to eradicate brucellosis by test and slaughter, and formed part of a field trial in which the IHLT and the CFT were compared as definitive eradication tests. Vaccination of heifer calves by eight months of age with *Brucella abortus* strain 19 was compulsory in Victoria, and all sera were collected at least 12 months after vaccination. Sera were collected on 532 occasions, a mean of 4·5 times per herd.

All sera were submitted to the Rose Bengal plate test (RBPT) (Anon. 1980), and RBPT-positive sera were tested by RIA (Chappel *et al.* 1982*a*), the CFT using

Table 1. *Reactions of RBPT-positive sera in the RIA, CFT and IHLT*

RIA	CFT	IHLT	No. of sera
+	+	+	1098
+	+	−	214
+	−	+	27
+	−	−	185
−	+	+	2
−	+	−	49
−	−	+	14
−	−	−	2994
Total			4583

Table 2. *Serological history of a herd undergoing eradication of brucellosis by test and slaughter*

(The animals in the first two categories were culled and the others left in the herd.)

	Serology of RBPT-positive animals				
RIA	+	+	+	+	−
CFT	+	+	−	−	−
IHLT	+	−	+	−	−
Week 0	2	6	.	1	6
Week 6	.	.	.	2	4
Week 33	1	.	.	1	3
Week 48	1	.	1	1	6
Week 56	1	1	.	.	2

warm fixation (Anon. 1977) and the IHLT (Plackett, Cottew & Best, 1976). Sera and the results of the RBPT and CFT were kindly provided by Mr B. A. Rogerson and Ms L. J. Shenfield of this laboratory, and IHLT results by Dr G. G. Alton and Dr P. Plackett of CSIRO.

The minimum diagnostic value for the RIA was taken as 5 units. For the CFT a 50% reaction at a dilution of 1 in 4 was taken as diagnostic, and for the IHLT a 50% reaction at a dilution of 1 in 8.

RESULTS

Of 4583 sera positive by the RBPT, 1524 were positive by the RIA, 1363 by the CFT and 1141 by the IHLT (Table 1). The RIA was positive in 12% more sera than the CFT and in 34% more than the IHLT. It was positive in 96% of CFT-positive sera, 99% of IHLT-positive sera, and 99·8% of sera positive by both the CFT and the IHLT.

Cattle that were positive by the RIA but negative by both the CFT and the IHLT sometimes retained this serological pattern for several weeks or months. It was possible to follow 25 such animals over a series of serum collections until the pattern changed. In 13 cases the RIA eventually became negative, whilst in the other 12 the CFT and/or the IHLT eventually became positive.

In a number of the herds studied, all RBPT-positive cattle were negative by both the CFT and the IHLT at one serum collection, but the herd subsequently broke down to give reactors in both of these tests. In some such herds, there were one or more RIA-positive cattle when the other two tests gave no indication of infection. The serological history of what was subsequently considered to be a problem herd is given in Table 2. It was not possible to trace the serological history of all individual cattle. However the animal positive by all tests in week 48 had been positive by the RIA alone in week 0, and that positive on the same occasion by RIA and IHLT was one of those RIA-positive in week 6.

DISCUSSION

The RIA was positive for many sera negative by the CFT, and for an even greater number negative by the IHLT. Depending on which sera were from infected animals, these reactions represent some combination of false positive reactions in the RIA and false negative reactions in the other two tests.

There is reason to believe from previous work that the RIA is, in many of these cases, detecting infected animals. In vaccinated cattle that were experimentally infected, the RIA gave fewer false negative reactions in early infection than the CFT or IHLT, and it gave fewer false negatives throughout the experimental period in culture-positive animals whose serological response was only transient (Hayes & Chappel, 1982). The RIA preferentially measures high avidity antibody, whose concentration can be expected to increase as antigenic exposure is prolonged (Carpenter, 1965). It is therefore likely that in chronically infected cattle the sensitivity of the RIA relative to the other tests is even greater than in early infection. Furthermore, cattle whose production of serum antibody is lowest, making infection hardest to detect, tend to produce antibody of highest avidity (Williams & Halliday, 1980). Chronic infection may thus account for some of the additional cattle detected by the RIA.

Two pieces of evidence from the present study give practical support to this contention. First, of animals positive by the RIA alone that could be followed until their serological pattern changed, about half subsequently became positive to other tests. In these cattle the RIA presumably indicated infection. The others, in which the RIA later became negative, may or may not have been infected when the RIA was positive. Secondly, some herds experienced breakdowns after being negative by the CFT and the IHLT, indicating that there had been infection undetected by these tests. In some cases the RIA gave one or more positive reactions during the otherwise clear period.

High levels of serum IgG_2 antibody can cause false negative reactions in the CFT (Plackett & Alton, 1975). A previous survey of sera from Victorian cattle indicated that this occurred frequently, affected sera being positive by the RIA and often also by the less sensitive IHLT (Chappel *et al.* 1978). However, that study was made early in the eradication programme, and may have included many older infected cattle whose IgG_2 levels are higher (Williams & Millar, 1978). In the present investigation only 27 sera were CFT-negative but positive by both RIA and IHLT, suggesting that IgG_2 interference was not a main cause of CFT false negative reactions.

Some RIA-positive sera, particularly those that are also CFT-positive, are attributable to persistent reactions after strain 19 vaccination. However, the RIA was found to give only about as many persistent vaccination reactions as the CFT, somewhat more than the IHLT but far fewer than the RBPT (Chappel *et al.* 1982*b*). Furthermore, the IHLT, although it gave fewer vaccination reactions than the CFT or RIA, was the least sensitive of the three tests to experimental infection (Hayes & Chappel, 1982). The IHLT is valuable where the major concern is to eliminate false positive reactions, whereas the RIA may be useful where it is important to minimize the number of false negatives.

Using the RIA to detect a larger proportion of infected animals is likely to give no advantage in cases where eradication using other tests proceeds smoothly. Reducing the number of false negative reactions can be expected to be of most value in problem herds. Field experience with some such herds in Victoria indicates that the use of the RIA has had value in helping to eliminate infection.

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