

Evaluation of a commercially available ELISA kit for detection of antibodies to *Anaplasma marginale* and *Anaplasma centrale* in cattle in Australia and Zimbabwe

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ABSTRACT

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A newly available competitive inhibition ELISA kit for the serological diagnosis of anaplasmosis was evaluated in Australia and Zimbabwe. In Australia the performance of the test was compared with the card agglutination test (CAT). The assay was evaluated using negative sera collected from *Anaplasma*-free herds, positive sera from experimentally infected cattle and sera from *Anaplasma marginale*-endemic herds. The sensitivity and specificity of the ELISA in Australia were 100% and 83,3%, respectively, and the sensitivity and specificity of the CAT were both 100%. The agreement between the ELISA and CAT in the sera from endemic herds was 86,4% ($\kappa = 0,718$). The specificity of the ELISA in Zimbabwe was 100%. No meaningful estimate of sensitivity was possible in Zimbabwe because few known positive sera were available for testing, but all eight known positive sera that were available were clearly positive. We conclude that the ELISA is a useful alternative to the CAT for epidemiological studies. The ELISA kits have advantages over the CAT in that the ELISA is more robust and reagents are better standardized, but the kits are expensive.

Keywords: *Anaplasma*, card agglutination test, cattle, ELISA, serology

INTRODUCTION

Anaplasma marginale, an arthropod-borne intracellular rickettsia, is an important pathogen of cattle occurring predominantly in tropical and subtropical regions of the world. Anaplasmosis is associ-

ated with significant economic losses in relation to impaired production, mortalities and cost of control measures (De Vos 1991). Since 1974, a live vaccine containing the less virulent *Anaplasma centrale* has been commonly used in tick-infested areas of Australia (Callow & Dalgliesh 1980). The same vaccine has recently been used in Zimbabwe (Turton, Katsande, Matingo, Jorgensen, Ushewokunze-Obatolu & Dalgliesh 1998) as part of a project supported by the Australian Centre for International Agricultural Research (ACIAR). Reliable tests for detection of antibodies to *A. marginale* and *A. centrale* are required in both countries for generating epidemiological data necessary for assessing the need for, and effectiveness of, vaccination. In Australia a reliable test is also required for screening cattle destined for the live cattle export market.

The card agglutination test (CAT) (Amerault & Roby 1968) is currently used for the serological diagnosis

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of *A. marginale* and *A. centrale* in Australia. However, the test must be performed under strictly controlled laboratory conditions and it has proved unreliable in Zimbabwe. A competitive inhibition ELISA for detecting antibodies to *A. marginale* has been developed in the USA (Palmer, McElwain, McGuire, Kappmeyer, Davis, Stiller, Visser, Tebele, Ndung'u, Shkap, Pipano & Knowles 1993; Knowles, Torioni De Echaide, Palmer, McGuire, Stiller & McElwain 1996). We recently published a comparison of this test with the CAT for detection of antibodies to *A. marginale* and *A. centrale* in Australian cattle and concluded that the performance of the two tests was comparable (Molloy, Bowles, Knowles, McElwain, Bock, Kingston, Blight & Dalgliesh 1999). A slightly modified version of the ELISA is now available commercially in kit form. Here we report on a small-scale evaluation of this kit in Australia and Zimbabwe.

The ELISA kits (VMRD Inc., Pullman WA 99163 USA) were used in strict accordance with the manufacturer's instructions. All sera were tested in duplicate and the positive and negative control sera provided in the kit were included on each plate. Percent

inhibition (PI) was calculated as a percentage of the negative control. The CAT was performed at the Tick Fever Research Centre (TFRC) as previously described (Wright & Leatch 1996). Positive results were classified as 1+, 2+ or 3+ depending on the degree of agglutination.

Estimates of sensitivity, specificity, overall agreement and the chance-corrected coefficient of agreement (kappa) were calculated according to Fleiss (1981). Approximate 95% confidence limits for sensitivity, specificity and kappa were also calculated as described by Fleiss (1981). Analysis of variance was used to compare mean PI values for African negative sera and Australian negative sera.

A total of 60 sera was collected from cattle in regions of Queensland known to be free of *A. marginale* and its tick vector. Serum samples were also obtained from a further 60 cattle experimentally infected at TFRC with either *A. marginale* (33 cattle) or the vaccine strain of *A. centrale* (27 cattle). In all cases infections were confirmed by microscopic detection of the rickettsia in thin blood films. A further 59 sera

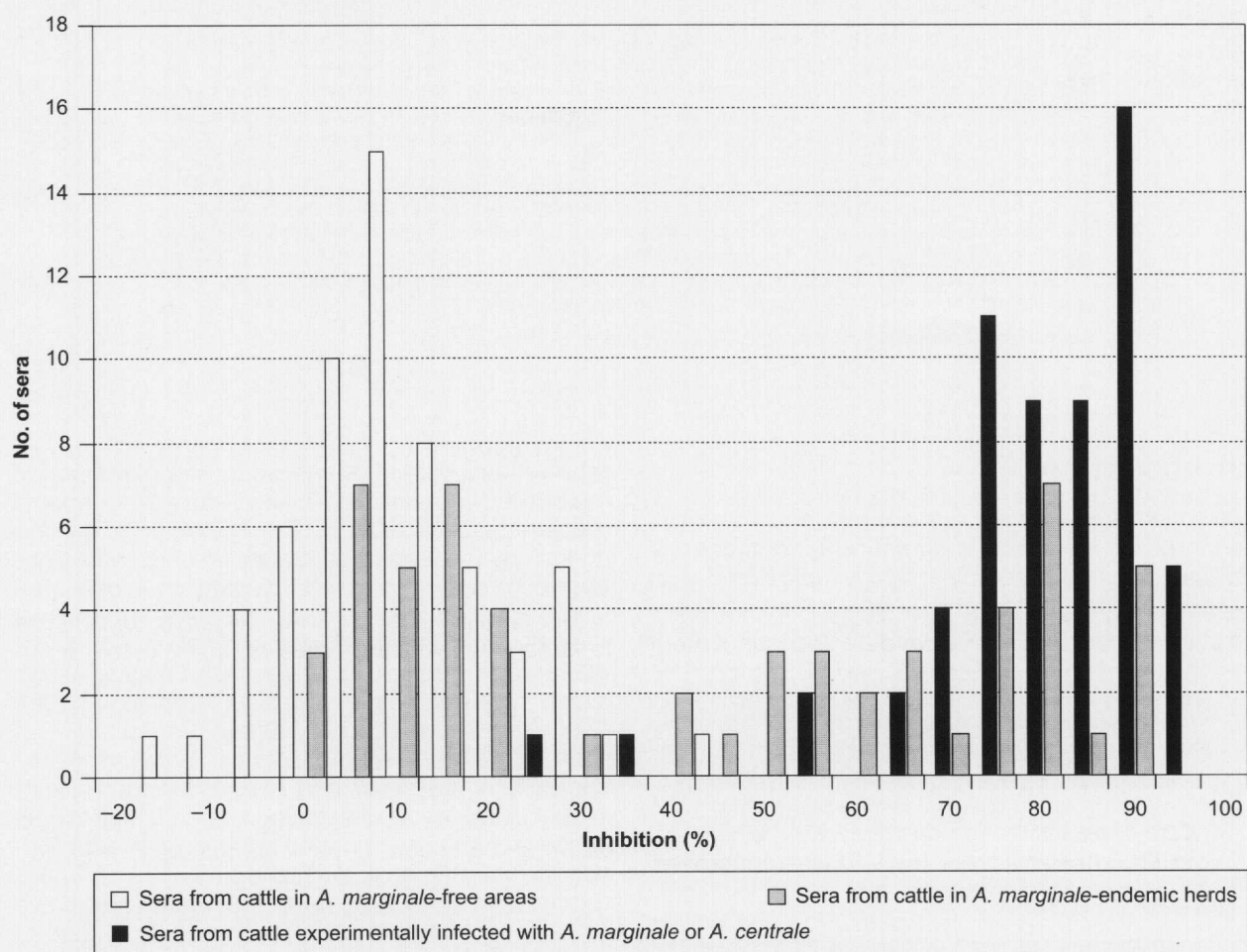


FIG. 1 Frequency distributions of ELISA PI values for sera collected in Queensland from 60 cattle in *A. marginale*-free areas, 60 cattle experimentally infected with *A. marginale* (33 sera) or *A. centrale* (27 sera) and 59 cattle in *A. marginale*-endemic herds

were obtained in Queensland from cattle of unknown infection status in five different *A. marginale*-endemic herds that had not been vaccinated with *A. centrale*. In Africa, sera were collected from 122 cattle maintained under *Anaplasma*-free conditions at the Central Veterinary Laboratory in Harare or at the Onderstepoort Veterinary Institute in South Africa and from eight cattle experimentally infected with an *A. marginale* strain isolated in Zimbabwe.

In Australia, the commercial ELISA kit was compared with the CAT. Frequency distributions of ELISA PI values for the sera from *Anaplasma*-free areas, the sera from experimentally infected cattle and the sera from *A. marginale*-endemic herds are shown in Fig. 1. Based on the results of testing the sera from *Anaplasma*-free areas and the sera from experimentally infected cattle, and using the prescribed positive PI threshold of $\geq 25\%$, the sensitivity and specificity of the ELISA (with lower and upper 95% confidence limits) were 100% (92.5, 100) and 83.3% (71.0, 91.3), respectively. For the same sets of sera, the sensitivity and specificity of the CAT (with lower and upper 95% confidence limits) were each 100% (92.5, 100). The frequency distribution of PI values for the 59 sera from *A. marginale*-endemic herds was bimodal suggesting that the ELISA was effectively differentiating between infected and uninfected populations. ELISA and CAT results for these sera were compared (Table 1).

TABLE 1 Cross-tabulation of ELISA and CAT results for 59 sera collected from cattle in *A. marginale*-endemic herds in Australia

ELISA	CAT				n
	-	1+	2+	3+	
-	19	4	2	1	26
+	1	1	6	25	33
n	20	5	8	26	59

Most discrepancies were in sera that gave a weak positive (1+) result in the CAT. There were five sera in this category and the ELISA detected four of them as negative. In these 59 sera the percent agreement between the two tests was 86.4% and the corresponding kappa statistic (with approximate lower and upper 95% confidence limits) was 0.718 (0.538, 0.898).

The ELISA was not evaluated in comparison with the CAT in Zimbabwe because the CAT has not proved reliable in that laboratory and is not routinely used. Instead, the ELISA was evaluated independently using the 122 known negative sera and eight known positive sera that were available. Frequency distributions of PI values are shown in Fig. 2. Again us-

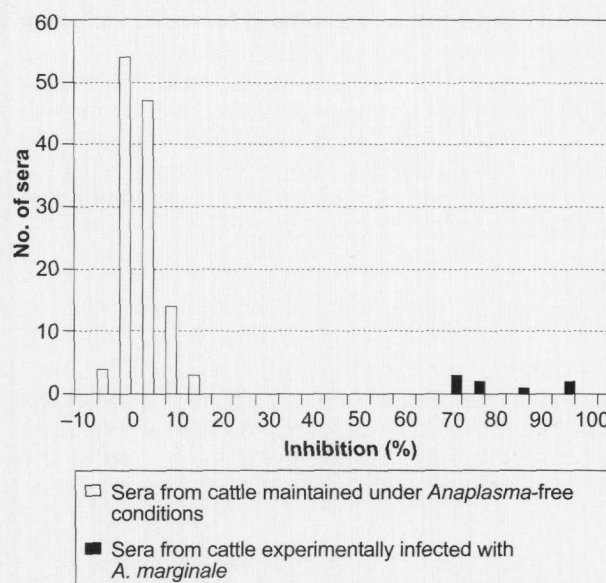


FIG. 2 Frequency distributions of ELISA PI values for 122 sera collected from cattle maintained under *Anaplasma*-free conditions in Zimbabwe or South Africa and eight sera collected from cattle experimentally infected with a Zimbabwean isolate of *A. marginale*

ing the prescribed positive PI threshold of $\geq 25\%$, the specificity of the ELISA (with lower and upper 95% confidence limits) was 100% (96.2, 100). The number of positive sera available in Zimbabwe was insufficient to give a meaningful estimate of sensitivity but all eight sera produced PI values $> 67\%$.

In this comparison, the specificity of the commercial ELISA kit in Australia (83.3%) was lower than that of the CAT (100%) and lower than the specificity estimated in our previous evaluation of this assay in Australia (99.5%) (Molloy *et al.* 1999). However, agreement between the ELISA and the CAT in sera from *A. marginale*-endemic herds was similar in both studies (86.4% compared with 87.1% in the earlier study). The mean PI for African negative sera (0.9) was significantly lower (LSD = 2.3, $P = 0.05$) than the mean PI for Australian negative sera (13.7) and the specificity of the ELISA in Zimbabwe (100%) was higher than in Australia. This difference could relate to factors such as the breed of cattle tested or the presence of cross-reacting antibodies to some other disease agent. The specificity of the ELISA in Australia could be improved by using a higher positive threshold (a positive threshold of $\geq 35\%$, for example, would increase the specificity to 96.7% and maintain sensitivity at 98.3%). As always, the choice of threshold should depend on the specificity and sensitivity requirements of a particular application.

Despite the specificity of the ELISA being lower in Australia than in Zimbabwe, the test performed well enough in both countries to be useful for epidemiological work. Largely as a result of the relatively high

cost of the ELISA kits, the numbers of sera tested here were limited and the data should be considered in conjunction with the evaluation reported by Molloy *et al.* (1999). The kits have obvious advantages over the CAT in terms of standardisation of reagents and technique and overall quality control but cost is the factor that is likely to determine how widely the test is adopted.

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