



Comparison of indirect fluorescent antibody test and enzyme linked immunosorbent assay in the detection of exposure of cattle to *Theileria parva* in Kenya

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ABSTRACT

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Appraisal of the indirect fluorescent antibody test (IFAT) and antigen enzyme linked immunosorbent assay (ELISA) serological tests as carried out to detect cattle exposed to *Theileria parva* at the National Veterinary Research Centre, Muguga (NVRC), Kenya is reported. Using sera from *T. parva* naive cattle and cattle experimentally exposed to *T. parva*, the two tests were appraised in terms of their sensitivity and specificity. IFAT and ELISA had the same sensitivity of 90 % while ELISA had a higher specificity (90 %) than IFAT (80 %). A comparison was also made of the capability of the two tests to detect exposure of dairy cattle to *T. parva* prior to immunization against East Coast fever (ECF). The positive outcome from the IFAT was significantly higher ($\chi^2 = 30,36$; $P < 0,001$) than that from the ELISA. The agreement between the two tests was low (Kappa = 0,21). The two tests indicated a higher risk of ECF in the study area than was expected. Indications are that the ELISA has been effectively adopted at NVRC.

Keywords: East Coast fever, ELISA, IFAT, *Theileria parva*

INTRODUCTION

The National Veterinary Research Centre (NVRC) at Muguga has a national mandate to carry out research on the epidemiology and control of tick-borne diseases in Kenya. East Coast fever (ECF) (*Theileria parva* infection in cattle) is the most economically important tick-borne disease in Kenya (Kariuki 1990). Serological tests have been developed or adapted and applied in the screening and surveillance of ECF. The indirect fluorescent antibody test (IFAT) against the *T. parva* schizont antigen (Burrige & Kimber 1972) has been used routinely at NVRC since the development of techniques for growing *T. parva* macroshizonts in cell culture systems (Malmquist, Nyindo & Brown 1970). However, there have been frequent

changes in the trained staff and those mandated to carry out the test. Changes of the equipment used in carrying out the test were also frequent. An appraisal of the current performance and reliance of this test as it is currently performed is therefore imperative.

More recently, an antigen enzyme linked immunosorbent assay (Ag ELISA) was developed and applied at the International Livestock Research Institute (ILRI) to test exposure of animals to *T. parva*. (Katende, Goddeeris, Morzaria, Nkonge & Musoke 1990). This test was intended to be adopted by national laboratories in the region, including NVRC as a routine serological test. To ensure effective adoption of the test by the target laboratories, critical evaluation and capacity building were essential. Currently, these requirements are limited. The regional national laboratories are faced with the crucial decision on which test

to apply routinely in the detection and quantification of exposure of cattle to *T. parva*.

In Kenya, immunization against ECF using the infection and treatment method (Radley, Brown, BurrIDGE, Cunningham, KIRIMI, Purnnell & Young 1975) is being evaluated for widespread field application. There is therefore a need to assess the risk of ECF in the target immunization regions. Such evaluations are best based on data from longitudinal epidemiological studies but important inferences could be derived from serological analyses. Presently, there is shortage of appropriate epidemiological data for many regions of Kenya. This complicates the choice of regions where immunization against ECF could be most economically viable.

The objective of this study was to appraise the performance of the IFAT and ELISA as carried out at NVRC. The two tests were also compared in their capacities to detect cattle exposed to *T. parva* prior to an ECF immunization trial carried out on smallholder dairy farms.

MATERIALS AND METHODS

Appraisal of IFA and ELISA tests

The IFAT (BurrIDGE & Kimber 1972) and ELISA (Katende *et al.* 1990) serological tests as applied to detect exposure to *T. parva* were appraised in terms of their sensitivity and specificity at NVRC. In this case, the sensitivity of the test was defined as its capability to identify individual cattle as being positive when they were truly exposed to *T. parva* antigen. Similarly, specificity was defined as the test's capability to identify individual animals as being negative when they were truly not exposed to *T. parva* antigen.

Source of test sera

Forty male calves of Friesian breed and aged 4–6 months, were purchased for various other experiments from a farm free from tick-borne diseases. This farm had had no history of tick-borne diseases for the previous 5 years as a result of an intensive and effective tick control programme through application of acaricides. After an acclimatization period of 35 d on intensive tick control in tick proof barns at NVRC, the calves were bled and sera separated within 48 h. These calves formed the group termed as "*T. parva* non-exposed". Another 50 calves of the same age category and breed from the same farm were immunized against ECF using the infection and treatment method (Radley *et al.* 1975). *T. parva* Marikebuni stabilate (Irvin, Dobbelaere, Mwamachi, Minami, Spooner & Ochama 1983) was used in the immunization and was blocked with Tetroxy LA (long-acting oxytetracycline formulation) (Bimeda Veteri-

nary Pharmaceuticals Ltd., Ireland). These calves were monitored daily in terms of rectal temperature, pre-scapular lymph node biopsy smears and ear vein blood smears from day 14–28 post immunization. During this period, all the blood and lymph node smears were dried, fixed in methanol, stained with Giemsa stain and examined under a light microscope for haemoparasites. From this group, 41 calves developed detectable schizont parasitosis at various times during the monitoring period. All 41 calves detected to have schizont parasitoses were bled on day 35 post immunization for the purpose of this study. These calves formed the group designated "*T. parva* exposed".

From each of the 81 blood samples sera was separated and two aliquots made within 48 h of collection. The two sets of serum samples were then code-labelled and submitted to an independent serology section to perform the tests. The results were then used to calculate the sensitivity and specificity of each of the two tests. For the IFAT, an animal was considered exposed if its serum exhibited a positive reaction at a dilution of 1:40 or higher. A percent positivity (PP) value of 15 was used as the cut-off point for the ELISA. The cut-off PP value for ELISA was computed from the optical density readings from a reference highly positive control sera (Katende *et al.* 1990).

Estimating risk of ECF using IFAT and ELISA

The study area

The immunization trial was conducted in Githunguri division in Kiambu district in the central highlands of Kenya. This is a high potential agricultural area with an annual rainfall of 704–1 474 mm (Jaetzold & Schmidt 1983). The proximity of this area to Nairobi city has led to an intensive mixed farming system. Smallholder dairy cattle production is an important sector of agriculture. ECF is one of the constraints to livestock production in this area (Gitau 1992). The ECF immunization trial has been described in detail by Muraguri, Mbogo, McHardy & Kariuki (1998).

Estimating apparent prevalence of T. parva

Prior to an ECF immunization trial in Githunguri division, blood samples were collected from 258 cattle on 64 smallholder dairy farms. These cattle were mainly crosses of exotic (Friesian, Guernsey or Ayrshire) and local breeds. The mean age of the cattle in the trial was 1.5 years. Two serum aliquots were made from each blood sample. The IFAT and ELISA were performed on the two code-labelled aliquots of sera by an independent serology team. The apparent prevalence of *T. parva* antibodies was estimated for each test as the proportion of the number of test sera samples showing a positive result compared with the total number of cattle sampled.

The derived apparent prevalence was used to estimate the true prevalence of *T. parva* antibodies in the division using the conversion formula described by Martin (1984). Annual incidence of ECF was estimated for the study region as described by Lilienfeld & Lilienfeld (1980).

Statistical analysis

The data was compiled into a dataset and stored in Microsoft Excel (computer program by Microsoft Corporation, USA 1992). The statistical significance of the difference between the proportions of positive results was analyzed by the McNemar's Chi square test (McNemar 1969). Assessment of the agreement between the two tests was analyzed through calculation of the Kappa value (Fleiss 1981).

RESULTS

The performance of the IFA test on sera from the experimentally *T. parva* "exposed" and "non-exposed" calves is summarized in Table 1. Table 2 shows the performance of the ELISA on sera from the same calves. The sensitivity and specificity of IFAT as performed at NVRC was 0,9 (90%) and 0,8 (80%) respectively. The false positive rate of this test was 20,0% while the false negative rate was 9,8%. The ELISA had a sensitivity and specificity of 0,9 (90%). The false positive and false negative rates for the ELISA were 10,0% and 9,8% respectively.

Results of cross-tabulation of the performance of the two tests as carried out on 258 samples of sera from Githunguri division are shown in Table 3. The derived agreement of the tests was 83% (83 + 68/258). On statistical analysis using McNemar's Chi square test, IFAT had a significantly higher positive result than ELISA ($\chi^2 = 30,36$; $P < 0,001$). The Kappa estimate for agreement between the two tests was 0,21.

The apparent prevalence, true prevalence and annual incidence of *T. parva* infection in Githunguri division as derived using results of the IFAT was 0,64, 0,63 and 0,49 respectively. Using ELISA test results, the corresponding estimates for apparent prevalence, true prevalence and annual incidence of *T. parva* infection were 0,42, 0,40 and 0,30 respectively.

DISCUSSION

IFAT as performed at NVRC to detect exposure of cattle to *Theileria parva* had a high sensitivity (90%) but a relatively low speci-

ficity (80%). The sensitivity derived in this study was lower than that reported earlier by Burrige & Kimber (1972) who reported a sensitivity of 98%. Norval, Perry, & Young (1992) also stated that IFAT as applied to detect exposure to *T. parva* had low specificity as a result of cross-reaction with *T. annulata* and *T. taurotragi*. The distribution of *T. taurotragi* and that of *T. parva* overlaps in much of eastern, central and southern Africa (Norval *et al.* 1992). In this case, the high McNemar's chi square value ($\chi^2 = 30,36$; $P < 0,001$) in favour of IFAT was suspected to be as a result of the cross reactivity of *T. parva* with *T. taurotragi*. A similar result where IFAT had a consistently higher positive result was reported by Kwena, Muraguri & Mwangi (1996), using a different set of sera from the same region. It was indicated that further studies would therefore be required to assess the epidemiology of *T. taurotragi* that was suspected to be prevalent in the study region.

While the specificity in this study was relatively low, there is a need to ascertain that the source of these experimental animals was actually free from *T. parva* exposure. This farm has been a traditional source of experimental animals for NVRC and thus frequent

TABLE 1 Performance of the IFA test on sera from *T. parva* exposed and non-exposed calves

True animal status	No. of calves IFAT (+)	No. of calves IFAT (-)	Total tested
<i>T. parva</i> exposed	37	4	41
<i>T. parva</i> non-exposed	8	32	40
Total tested	45	36	81

TABLE 2 Performance of the ELISA on sera from *T. parva* exposed and non-exposed calves

True animal status	No. of calves ELISA (+)	No. of calves ELISA (-)	Total tested
<i>T. parva</i> exposed	37	4	41
<i>T. parva</i> non-exposed	4	36	40
Total tested	41	40	81

TABLE 3 Comparison of the performance of IFAT and ELISA on sera from smallholder dairy cattle in Githunguri division, Kiambu district, Kenya

Test result	IFAT (+)	IFAT (-)	Total tested
ELISA (+)	83	25	108
ELISA (-)	82	68	150
Total tested	165	93	258

monitoring of the effectiveness of its tick control programmes and continuous sero-monitoring are essential.

The ELISA had similar sensitivity to the IFAT (90%) but a higher specificity (90%). These high estimates were an indicator of effective adoption of the test at NVRC. The ELISA also allows processing of a larger number of samples than IFAT and has minimum inter-operator variations (Norval *et al.* 1992). However, analysis of the agreement between the two tests resulted in a Kappa value of 0.21 (21%) which indicates low level of agreement beyond chance. A kappa value of 40% and below indicates poor agreement. The high level of derived agreement between the tests (83%) could therefore be attributed to chance. Further assessment of the tests, especially on individual animal results was therefore indicated. Critical evaluation and validation of the cut-off point for the ELISA was necessary. It was therefore imperative to maintain the two tests until adequate standardization was achieved. However, efficacy of the tests requires to be analyzed against their financial implications. The choice of a serological test also depends on the precision required in the investigations being carried out.

The estimated incidence of ECF in this study for Githunguri division was higher than that reported elsewhere by Gitau (1992). The sera used in this study were collected from cattle in farms where at least one case of ECF had been diagnosed in the previous year. The selection criteria were therefore biased towards farms with a high risk of ECF, which may not reflect the general ECF situation in the region.

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