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Veterinary Parasitology 127 (2005) 189–198
veterinary parasitology
www.elsevier.com/locate/vetpar

Evaluation of the indirect fluorescent antibody test as a diagnostic tool for East Coast fever in eastern Zambia

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Received 18 June 2004; received in revised form 15 September 2004; accepted 15 September 2004

Abstract

Serological surveys using the schizont indirect fluorescence antibody test (IFAt) are routinely carried out to monitor the *Theileria parva* infection prevalence. The present study evaluates the diagnostic accuracy of the IFAt in eastern Zambia, where the transmission of *T. parva* is highly seasonal. The data set resulted from a sentinel herd ($n = 105$ animals) study carried out between 1995 and 2000 and was split into an epidemic period, during which the majority of the cattle became infected, and an endemic period with seasonal disease incidence in calves.

In the epidemic period the *T. parva* seroprevalence followed closely the build up of the herd immunity. In the endemic period the seroprevalence fluctuates considerably although most of the animals had been infected. Overall, the diagnostic sensitivity of the IFA test was 55% at cut-off titre 1:40 and 28% at cut-off 1:160. The specificity of the test was 86 and 95%, respectively. A logistic regression model demonstrates that the sensitivity is significantly lower when the *T. parva* transmission is low ($p < 0.01$). The analysis of receiver operator characteristic curves classifies the test as moderately accurate (area under the curve, AUC = 0.79) during the epidemic period and less accurate in the endemic period (AUC = 0.63).

Neonatal serology surveys yield a better estimate of the infection prevalence. The sensitivity of the neonatal test was 73% at cut-off titre 1:40 and 24% at cut-off 1:160.

Keywords: *Theileria parva*; East Coast fever epidemiology; Diagnosis; Fluorescent antibody test; Sensitivity; Specificity

1. Introduction

The cattle disease East Coast fever (ECF) is caused by infection with the parasite *Theileria parva* and is transmitted mainly by *Rhipicephalus appendiculatus*

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doi:10.1016/j.vetpar.2004.09.028

(the African brown ear tick). The clinical picture is dominated by high fever and generalized lymphadenopathy and the diagnosis is confirmed when schizonts are demonstrated in lymphnode biopsy Giemsa-stained smears. Mortality rates may reach up to 95% in susceptible cattle in epidemic situations (Medley et al., 1992). Mild and sub-clinical cases as well as those that are efficiently treated recover and remain carriers of the infection (Young et al., 1981). These animals are immune against homologous challenge and the immunity is assumed to be life-long when the animals are re-challenged (Norval et al., 1992).

Antibodies to *T. parva* antigen can be detected by means of the indirect fluorescence antibody test (IFAt). Because of the longer duration of the serological response Burridge and Kimber (1973) recommended the schizont antigen instead of the piroplasm antigen when using IFAt in epidemiological studies of ECF. The schizont IFAt has since then been used in several serological surveys in ECF infested Africa (Norval et al., 1992) and has also been the main tool in many assessments of endemic stability of ECF (Perry et al., 1989). In eastern and southern Zambia, IFAt seroprevalence studies are routinely carried out to identify regions where calf immunisations may be introduced as a method of ECF control (Belot et al., 2001). Billiouw et al. (2002) observed that in parts of Zambia where *T. parva* transmission is highly seasonal, the sero-conversion in re-challenged cattle is immediate but the duration of antibody detection very short. The same study also showed how the infection prevalence in the adult population might be estimated indirectly from maternal antibody serology surveys in newborn calves.

The purpose of the present study, carried out in eastern Zambia, was to determine the test characteristics, sensitivity and specificity, under field conditions. To the best of our knowledge this is the first time such an evaluation was done under field conditions. The methodology presented here may be used in other epidemiological situations.

2. Materials and methods

2.1. Animals and study site

We used data from a sentinel herd ($n = 105$ animals, Angoni breed) study carried out in the eastern province of Zambia in the period 1995–2000. The region is characterised by a unimodal rainfall pattern (October–April). The *T. parva* transmission occurs mainly in this rainy period (first wave of adult *R. appendiculatus*). A second period of *T. parva* challenge is observed in the months May–June coinciding with second wave of adult ticks and first wave of nymphs). A small peak of transmission occurs in September (second wave of nymphs).

2.2. Samplings and IFAt

All cattle of the herd, including the calves born in the herd during the study, were clinically examined on a weekly basis. Blood was sampled from all animals on a monthly basis (a total of 1192 samples were collected). In vitro cultured *T. parva* (Katete) infected lymphoblasts were used as antigen to detect antibodies in diluted plasma or serum (Burridge and Kimber, 1972). Anti-bovine fluorescein-labeled conjugate was applied after the antigen-antibody binding, producing fluorescent schizonts in positive samples.

2.3. Case-definition of T. parva (ECF) infection

Clinically suspect cases of ECF were confirmed parasitologically by demonstrating *T. parva* schizonts in lymphode smears. A positive diagnosis was equally upheld when after the clinical episode the animal converted serologically. Sero-conversion was the single criterion for the diagnosis of sub-clinical *T. parva* infections. Sero-conversion was defined as the first event of three consecutive positive samples at or above a cut-off titre of 1:40. The date of contact was set 1 week prior to the presence of schizonts and 1 month prior to sero-conversion.

2.4. ECF seroprevalence

A total of 56 cattle aged 2 years and above were enrolled in this part of the study. The seroprevalence in a particular month was calculated as the number of positive samples divided by the total number of samples collected that month. The actual infection status of the individual animal was updated monthly using the above listed diagnostic criteria. Once an animal became infected it was considered to remain infected for the rest of its life.

2.5. Sensitivity and specificity of the IFAt

We calculated the diagnostic sensitivity as the proportion of infected animals that are detected by the test and its specificity as the proportion of infection-free animals that yielded a negative result in the test. Interval estimation was done in Stata 8.0 (StataCorp., 2003). The survey methodology was used to correct for the effect of clustering when data resulted from repeated sampling of the same animals. Receiver operator characteristic (ROC) curves which plot sensitivity against (1: specificity) at the different dilutions used in the assay were obtained by means of post estimation after fitting a logistic regression model with infection status as response variable and the serological titre as explanatory variable. The area under a ROC curve (AUC) was used as a global summary statistic of diagnostic accuracy (Greiner et al., 2000). An arbitrary guideline suggested by Swets (1988) allowed us to distinguish between ''moderately accurate'' tests ($0.7 < \text{AUC} < 0.9$) and ''less accurate'' tests ($0.5 < \text{AUC} < 0.7$). To explore the temporal variation of the sensitivity, a logistic regression model was fitted to the data using the number of positive samples as binomial response variable with the total number of tests as denominator. A quadratic model used the continuous variable TIME (coded 1–72 for January 1995 to December 2000) and the square term of TIME to explain the between-years variation of the sensitivity. The seasonal variation observed each year was initially explored by introducing the variable MONTH (coded 1–12 for January to December) as a discrete variable (representing eleven indicator variables). A more parsimonious model replaced these eleven variables by the sine and cosine function of MONTH calculated, respectively as $\sin(\pi(2 \times \text{MONTH} - 1)/4)$ and $\cos(\pi(2 \times \text{MONTH} - 1)/4)$. The likelihood ratio test was used to determine the overall significance of a logistic model (G_0^2) and to test the contribution of a subset of parameters (improvement G_0^2).

2.6. The presence of maternal antibodies in newly born calves

Sixty-three calves were born from dams in the herd between 1995 and 2000. The presence of maternal antibodies was confirmed when one of three consecutive monthly serum samples, taken at month 0–2, tested positive on IFAt at cut-off titre 1:40. Antibody profiles were constructed for 16 calves for which complete information was available.

2.7. Sensitivity and specificity of IFAt detection of maternal antibodies

At any time during the study, the *T. parva* infection status of the dams, according to the above case definition, was known. This allowed us to calculate the sensitivity and specificity of the IFAt performed in newborn calves. The sensitivity of the test is the proportion of calves, born from immune dams, in which antibodies are detected. The specificity is defined as the proportion of calves, born from naive dams, in which antibodies could not be detected.

3. Results

A total of 71 *T. parva* infections were diagnosed in the study period. Fifty-three were clinical cases of which 24 died. Eighteen sub-clinical cases were diagnosed. Thirty-four animals remained naive. Most of these animals were calves born towards in the last 6 months of the study.

3.1. Seroprevalence

Fig. 1A and B show the seroprevalence at titres 1:40 and 1:160 together with the ''true'' infection prevalence in the animals older than 2 years. The monthly sample sizes ranged from 1 to 30 with median 21. Based on this curve the study period was divided into two periods of 3 years. The first is the epidemic period in which the herd becomes infected and the seroprevalence appears to follow quite closely the build up of the immunity from 0 to 100%. In the second, endemic period the true prevalence oscillates between 80 and 100% while the seroprevalence fluctuates hectically and drops to minimum levels in the last 6 months of the study.

3.2. Sensitivity and specificity of the IFAt

Overall, the diagnostic sensitivity of the IFAt was 55% (95% CI: 50–58%) at cut-off titre 1:40 and 28%

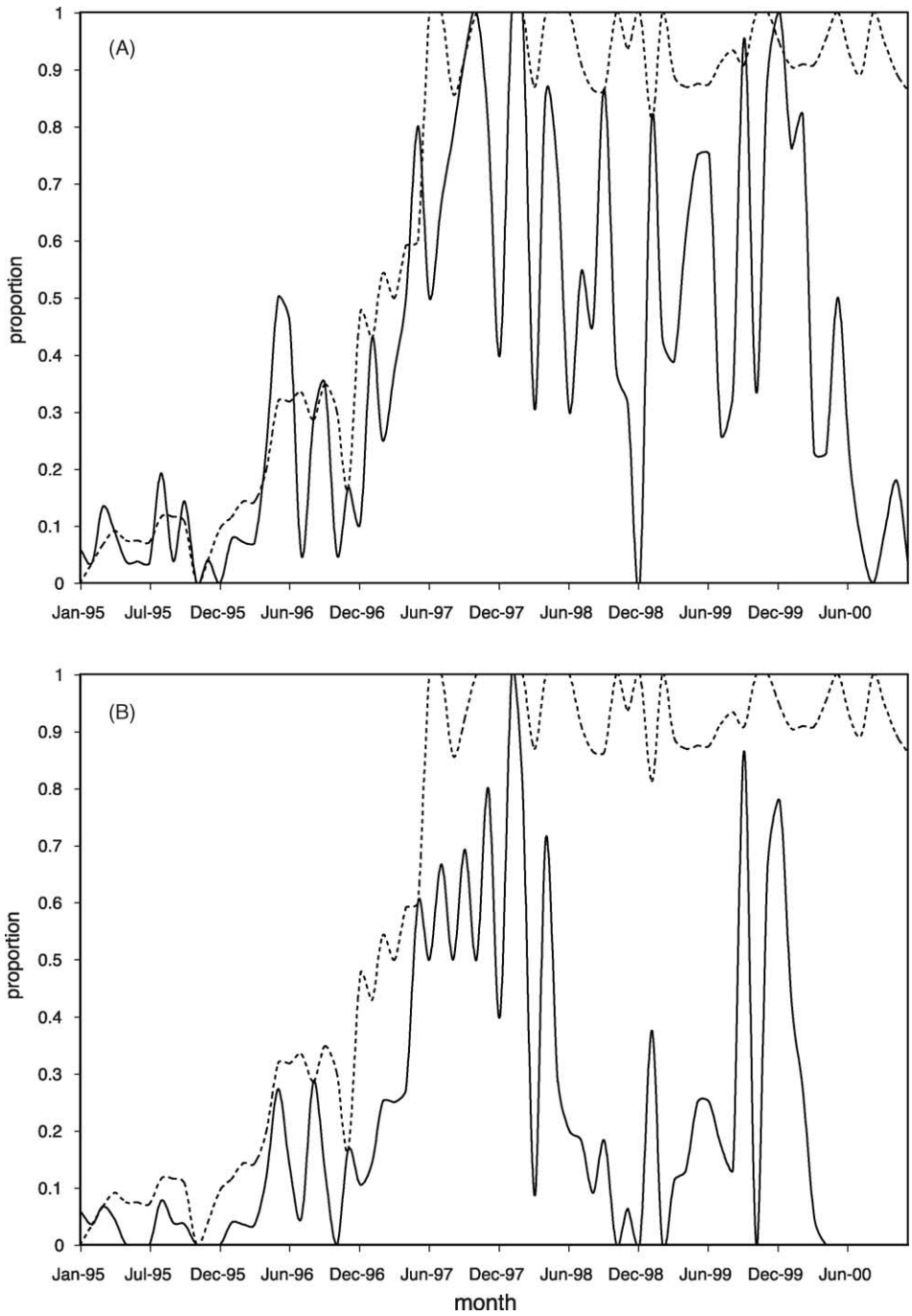

Fig. 1. Seroprevalence and "true prevalence" in animals older than 2 years. (A) Cut-off titre 1:40; (B) cut-off titre 1:160; (- - -) proportion animals infected; (—) proportion positive test results.

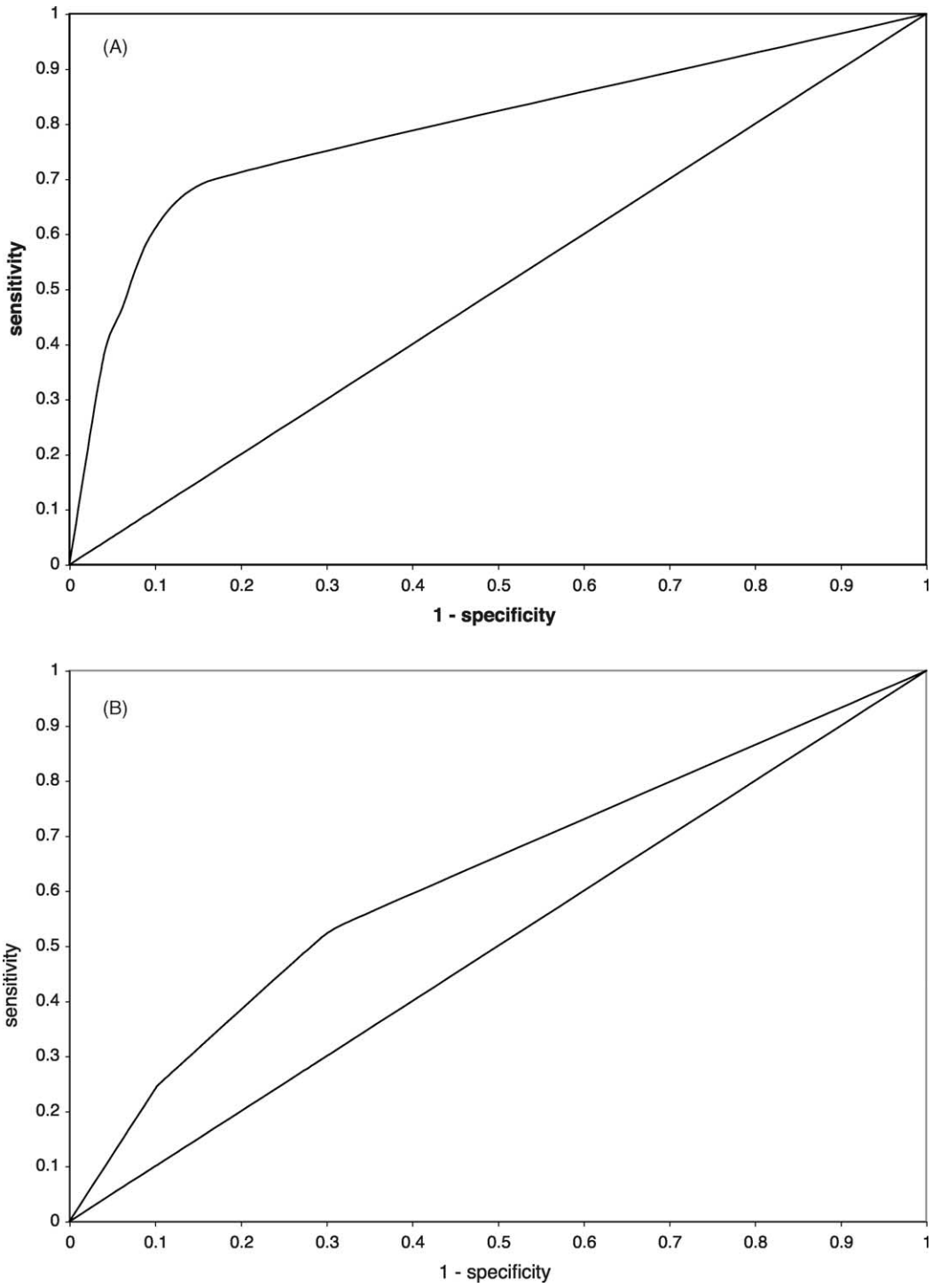

Fig. 2. Receiver operating characteristics (ROC) curves. (A) Epidemic period; (B) endemic period.

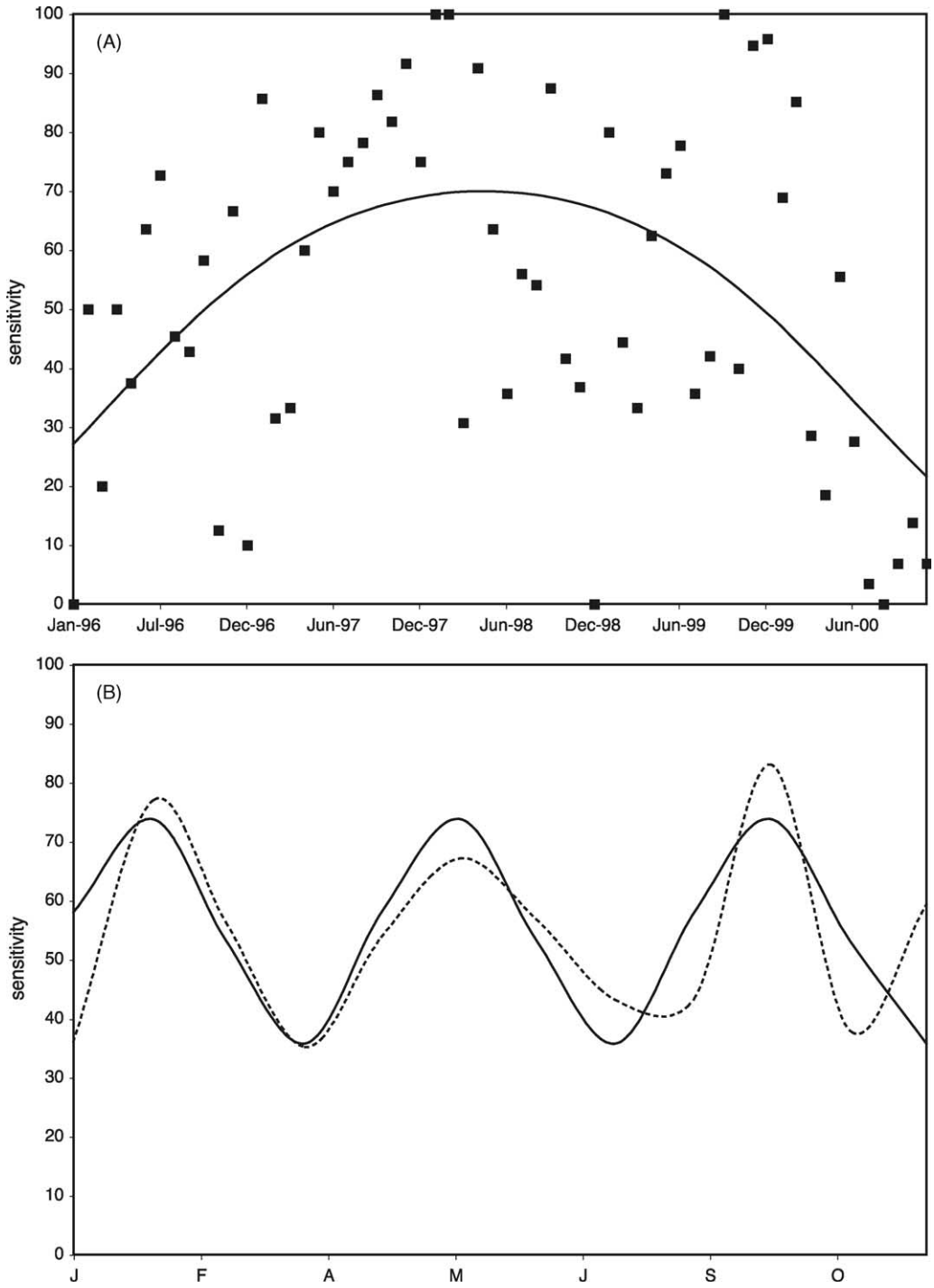

Fig. 3. Monthly variation in IFAt sensitivity. (A) Observed and fitted quadratic logistic regression model in function of entire time period; (B) observed and fitted sine-cosine model; (C) observed and fitted combined model.

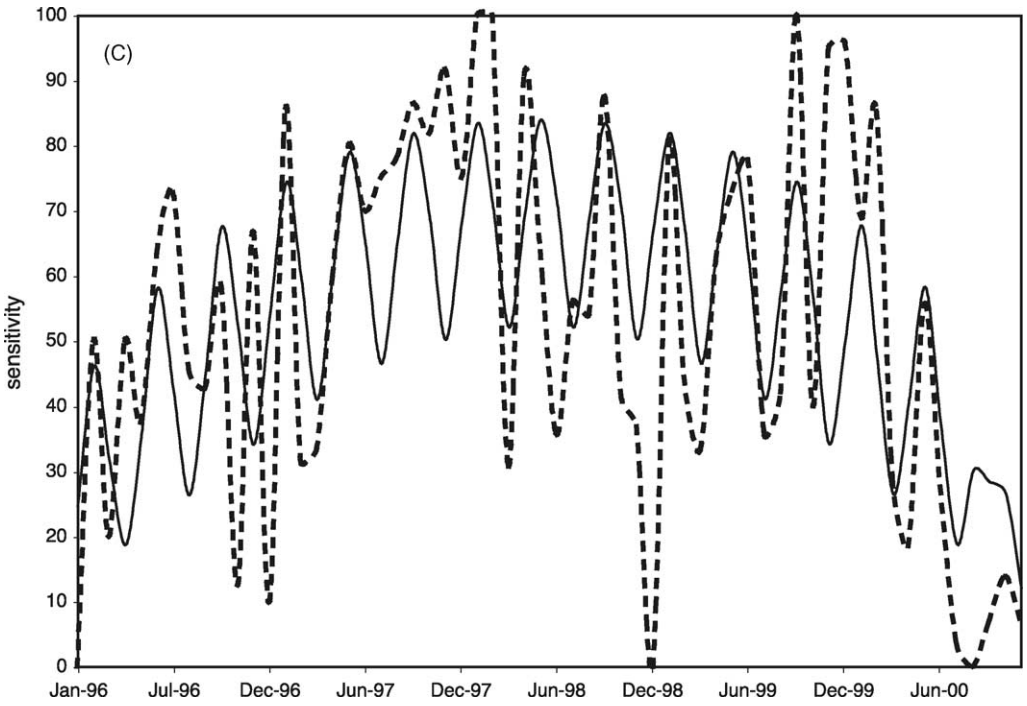

Fig. 3. (*Continued*).

(95% CI: 24–32%) at cut-off 1:160. The diagnostic specificity was 86% (95% CI: 82–89%) and 95% (95% CI: 94–97%), respectively. The ROC curves, plotted in Fig. 2A and B, indicate that the IFA test is moderately accurate in the epidemic period (AUC = 0.79; S.E. = 0.03) and less accurate in the endemic period (AUC = 0.63; S.E. = 0.02). The monthly variation of the sensitivity of the IFAt at cut-off 1:40 (Fig. 3A)

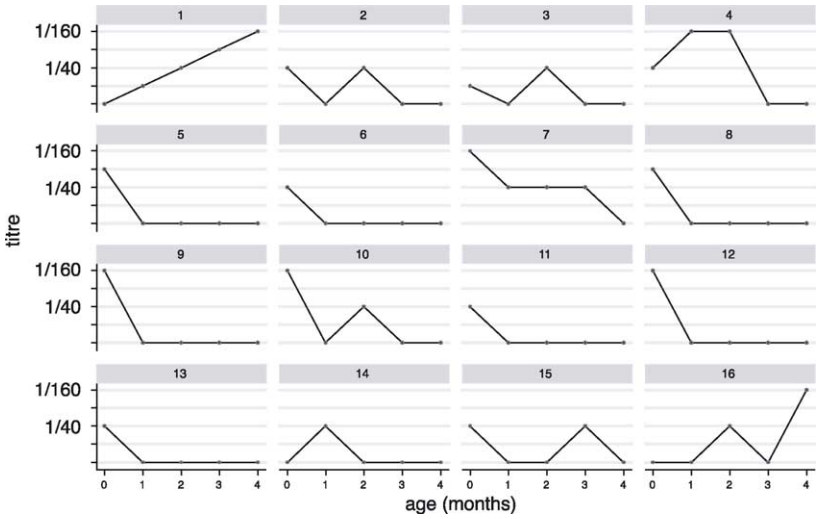

Fig. 4. Maternal antibody profiles observed in 16 calves aged 0–4 months.

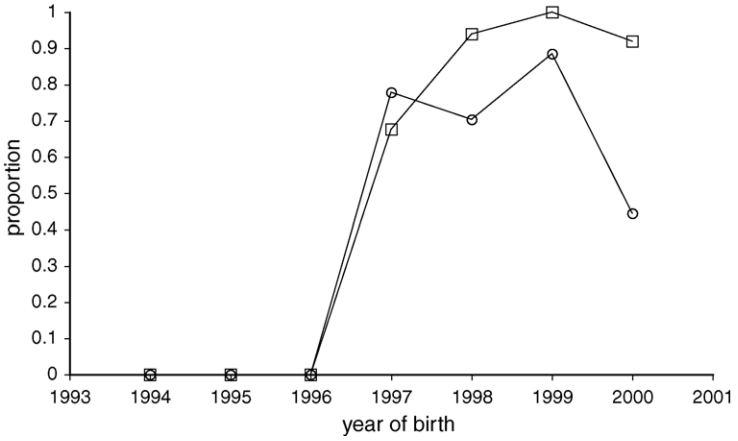

Fig. 5. Maternal antibody prevalence in calves and infection prevalence in dams.

suggests an increasing trend in the epidemic and decreasing trend in the endemic period. A quadratic logistic regression model captures a significant amount of this variation ($G_0^2 = 59.7$, d.f. = 2, $p < 0.01$). Part of the remaining scatter is explained by a seasonal pattern (improvement $G_0^2 = 67.2$, d.f. = 2, $p < 0.01$). Fig. 3B represents the average sensitivity for each month over all years along with the fitted value of a simple sine cosine seasonality model. The final model combines both sub-models ($G_0^2 = 120.9$, d.f. = 4, $p < 0.01$) (Fig. 3C).

3.3. IFAT study in newborn calves

Of 37 calves born from immune dams 27 were seropositive at 1:40 (sensitivity 73%; 95% CI: 56–86%). The sensitivity at cut-off 1:160 was only nine animals in 37 detected (24%; 95% CI: 12–41%). The specificity of the test was 24 negative results in 26 calves born from naive dams (92%; 95% CI; 73–99%) at both cut-offs. Fig. 4 shows the profiles of maternal antibody titres for sixteen calves. Thirteen of the 16 calves (81%) tested positive in the first month and seven of those tested negative thereafter. The proportion of calves with positive IFAt at 1:40 per year of birth is shown in Fig. 5 along with the expected proportion, i.e. the proportion of dams that were immune at the time of parturition. The curve shows that the neonatal sero-survey yields a good, indirect, estimate of the infection prevalence in the dam population except for the final year of the study.

4. Discussion

4.1. Validity of IFAt as an estimator of the T. parva infection prevalence

In any attempt to classify the epidemiological states of ECF, the infection/antibody prevalence has consistently been included as one of the defining characteristics. Indeed, when moving from the very unstable state of ECF towards endemic stability, the prevalence is generally accepted to show a consistent increase. It is thus not surprising that for the past decades the IFAt seroprevalence survey has been an essential tool in the studies of the ECF epidemiology and that it has contributed considerably to the understanding of the epidemiological situation throughout *T. parva* infested Africa (Norval et al., 1992). Minor drawbacks have been reported, e.g. subjectivity in observing the degree of fluorescence and possible cross-reactions with *Theileria taurotragi*. However, the present study demonstrates that, when estimating *T. parva* infection prevalence in eastern Zambia, the IFAt does not always yield consistent results. The test particularly lacks sensitivity to detect infection in periods of low *T. parva* transmission. In the endemic regions of East Africa the *T. parva* challenge is quasi-continuous throughout the year so that antibody levels may remain detectable at all times. This condition ensures a maximum efficacy of an IFAt survey. In eastern Zambia, where the vector abundance and consequently the *T. parva* challenge

are highly seasonal, the sensitivity of the IFAt varies with the presence and the intensity of the *T. parva* transmission. Our analysis concludes that the IFAt performs increasingly well in the epidemic period when the intensity of the *T. parva* transmission builds up. Thereafter, the test performs quite poorly with an increasing lack of sensitivity, which is only interrupted by the seasonal waves of transmission. The specificity of the test was never affected to such an extent.

4.2. Neonatal sero-surveys

The presence of maternal antibodies reflects the infection history of the dam. Calf-bearing cows are fairly representative for the general adult cattle population. Calves are seronegative at birth. One day after the intake of colostrum, maternal antibodies are detected at very high titres when the dam is immune (Marcotty et al., 2002). Furthermore, hyperimmunised dams become highly seropositive shortly before parturition irrespective of day of boosting (Marcotty, unpublished data). The neonatal IFAt appears thus to present a potential alternative to estimate the infection prevalence. In this study the neonatal serology survey performs better than the classic one although also here the sensitivity dropped in the last year of the study when transmission dropped to minimum levels. Most of the calves enrolled that year were born in the low transmission months October and November. Neonatal serology surveys are not used in veterinary medicine but are performed routinely for monitoring the seroprevalence of HIV among pregnant women as a proxy to the heterosexual active population (Shapiro et al., 1989; Siedler et al., 1998).

4.3. Bias

The evaluation of the diagnostic sensitivity and specificity requires an independent criterion, which defines an animal's true disease status. The reference test in this field study was the detection of *T. parva* schizonts detected in an animal presenting with the clinical signs and symptoms typical for ECF. However, sub-clinical cases, which in our study could only be diagnosed by means of IFAt sero-conversion itself, were also included in our evaluation of the IFAt performance. This theoretically introduced the risk of selection bias. It was not clear whether this influenced our conclusions. It was in any case important to include the sub-clinical cases since these surviving animals have an important influence on the epidemiology of ECF, especially on the evolution towards endemicity. These carriers create a new permanent reservoir of the infection, which gives rise to less lethal infections since lesser doses are being transmitted. The criterion of three consecutive positive tests, using cut-off 1:40, definitely avoided misclassification of naive animals. The conservative criterion also made it unlikely for cross-reacting haemoparasites to interfere except, perhaps, for *T. taurotragi* (Norval et al., 1992). The criterion was also not too conservative in this study, as seroconversion in the great majority of calves was conclusive and isolated positive test results before the seroconversion, which made it difficult to pinpoint the exact date of *T. parva* contact, were the exception.

In the neonatal survey, antibodies due to natural infection could have been mistaken for maternal antibodies. The majority of the calves, however, showed maternal antibodies before the age of 1 month. For the other calves, the probability to become infected before the age of 3 months without showing clinical signs is very small and, if a calf became infected at such a young age, then the dam was most likely infected as well.

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