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The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs and in the absence of tsetse challenge

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Abstract

The decline of anti-trypanosomal antibody levels in cattle after treatment with trypanocidal drugs was investigated using an anti-trypanosomal antibody-detection enzyme-linked immunosorbent assay (ELISA). The decline of antibody levels differed between experimental animals but went through two phases. During the first 5 months after trypanocidal drug treatment, the decline is rapid with a monthly average decline of 10% of the average percentage positivity during the treatment. Between months 6 and 13 after treatment, the monthly average decline was only 3.6% of the average percentage positivity at the moment of treatment. It took a total of 13 months for all the experimental animals to become seronegative. The usefulness of the anti-trypanosomal antibody-detection enzyme-linked immunosorbent assay (ELISA) in the monitoring of tsetse control operations is discussed. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Trypanosoma; Antibody; ELISA; Diagnosis

1. Introduction

When determining the distribution, or studying the epidemiology, of bovine trypanosomosis use is usually made of parasitological diagnostic tests. These tests are simple but lack the diagnostic sensitivity required for an accurate assessment of the distribution of infected animals (Paris et al., 1982). Recently, an anti-trypanosomal antibody-detection enzyme-linked immunosorbent assay (ELISA) was adapted for use with dried blood spots on filter paper (Hopkins et al., 1998). The test has high diagnostic sensitivity and specificity. It has been used in large-scale bovine trypanosomosis surveys and for monitoring the effectiveness of tsetse control interventions in southern Africa (Van den Bossche et al., 1999, 2000). Although knowledge of the prevalence of anti-trypanosomal antibodies in cattle is very useful, interpretation of

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the results is often difficult. This is due to the persistence of anti-trypanosomal antibodies even after an animal has been cured (Bocquentin et al., 1990). To facilitate the interpretation of data on anti-trypanosomal antibody prevalence in cattle, a study was undertaken to determine the changes in the antibody levels after treatment with trypanocidal drugs.

2. Materials and methods

2.1. Experimental animals

The decline in anti-trypanosomal antibody levels was studied in adult Mashona breed cattle between May 1998 and August 1999. Thirty experimental animals were introduced in an area under natural tsetse challenge (*Glossina morsitans morsitans* and *G. pallidipes*) along the Zimbabwe/Mozambique border in Mudzi District (Mashonaland East Province), Zimbabwe (henceforth referred to as tsetse-infested area) in January 1996 (Warnes et al., 1999). Each month, blood taken from an ear vein of each experimental animal was examined for trypanosomes using the haematocrit centrifuge, phase contrast technique (Murray et al., 1977). Animals infected with trypanosomes received a curative treatment of diminazene aceturate (Berenil®, Hoechst), at a dosage of 7 mg/kg body weight for *T. brucei* or 3.5 mg/kg body weight for *Trypanosoma congolense* or *T. vivax*, by intramuscular injection. From May 1998 onwards, ear vein blood, contained in one heparinised microhaematocrit centrifuge capillary tube, was extruded onto a filter paper (Whatman no. 4, Whatman®). Eluted blood spots were screened for the presence of anti-trypanosomal antibodies using an indirect anti-trypanosomal antibody detection ELISA (Hopkins et al., 1998). Use was made of a *T. congolense* (IL 3000) antigen batch prepared from trypanosome bloodstream forms (Hopkins et al., 1998). The cut-off value was determined using blood spots collected from trypanosomosis-negative Mashona breed cattle kept in an adjacent tsetse-free area of Zimbabwe (Mutoko District, Mashonaland East Province, $n = 355$) and blood spots collected from trypanosome-infected animals in Zimbabwe (Mudzi District, Mashonaland East Province, $n = 150$) and Zambia (Petauke District, Eastern Province, $n = 42$). Sensitivity and specificity were calculated over a range of cut-off values. A cut-off of 28% positivity was used at which the test had a sensitivity of 88.5% and a specificity of 99% (Fig. 1). A rigorous system of quality assurance was adopted (Hopkins et al., 1998). The optical density (OD) of each ELISA-sample tested was expressed as a percentage (percentage positivity) of a strong positive reference standard (Wright et al., 1993; Hopkins et al., 1998). Criteria for data acceptance or rejection were identical to those described by Hopkins et al. (1998). Each sample was analyzed in triplicate, on three different microplates.

The experimental animals with anti-trypanosomal antibody levels equal to or larger than 50% positivity were treated with diminazene aceturate (Berenil®, Hoechst) at 7.0 mg/kg body weight and transferred immediately to a tsetse-free zone (Mutoko District, Zimbabwe). After transfer, blood spots continued to be collected at monthly intervals and the percentage positivity of each blood spot was determined.

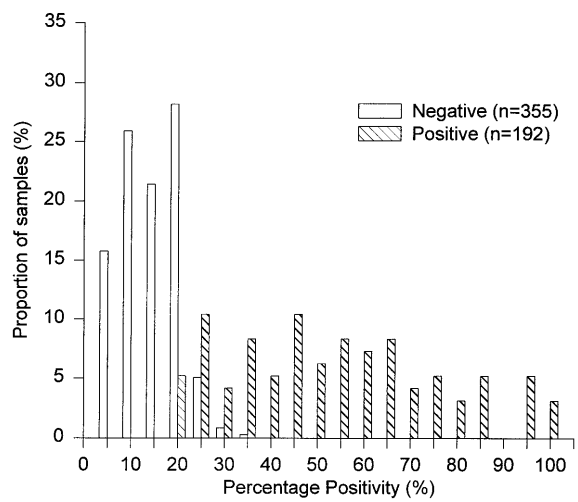
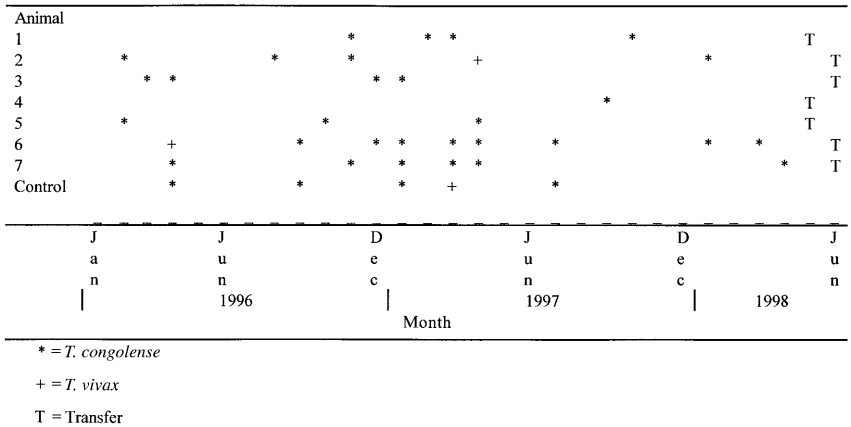


Fig. 1. Distribution of percentage positivity values for blood spots collected from trypanosome-infected ($n = 192$) and uninfected ($n = 355$) cattle.

Table 1
Monthly incidence of trypanosomosis in the experimental animals (1–7) before transfer to the tsetse-free area and in the control animal kept in the tsetse-infested zone[a]



Two animals, one in the tsetse-infested and one in the tsetse-free area, were not transferred and served as controls. Blood spots were collected at monthly intervals and analyzed as described above.

2.2. Data analysis

The monthly average percentage positivity and the standard deviation of the replicate measurements were calculated for each animal. The decline in anti-trypanosomal antibody levels (expressed as percentage positivity) over time in each experimental animal was examined by piecewise linear regression analysis using the monthly averages. The significance of the slope of the regression lines was tested by an analysis of variance (ANOVA) (Sokal and Rohlf, 1998). All analyses were performed using the statistical package SPSS (SPSS Inc.).

3. Results

In all the experimental animals (animals one to seven), trypanosomosis had been diagnosed at least once during the period preceding their transfer (Table 1). The monthly average incidence of trypanosomosis was $15.9 \pm 3.2\%$. The majority of infections (93.7%) were due to *T. congolense*. Between January 1996 and June 1998, the monthly average incidence of trypanosomal infections in the control animal kept in the tsetse-infested area was 16.7% (Table 1). One trypanosomal infection (*T. congolense*) was diagnosed in month 9 (March 1999).

3.1. Decline in percentage positivity in individual animals

1. Control animals: During the 12 months observation period, the average percentage positivity of blood spots collected from the uninfected control animal in the tsetse-free zone varied between months but never exceeded the cut-off value (Fig. 2). The control animal, kept in the tsetse-infested zone, developed a week positive antibody response in month 2 (August 1998). A significant anti-trypanosomal antibody response was only developed from month 6 onwards (Fig. 2).

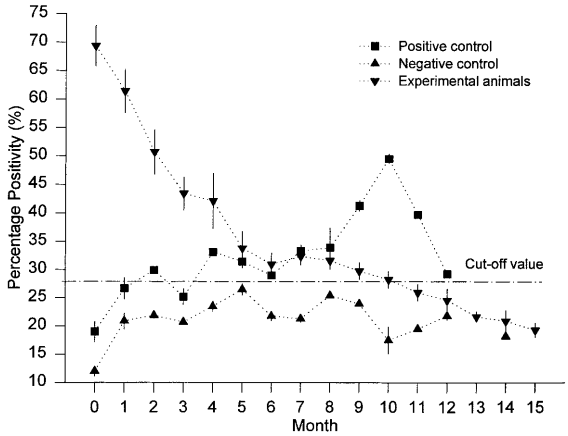


Fig. 2. Monthly average percentage positivity (%) (± 1 S.D.) of the positive and negative control animal and monthly average percentage positivity (%) (± 1 S.E.M.) of all experimental animals in consecutive months after transfer to the tsetse-free zone (month 0 being the month of treatment and transfer).

2. Animals transferred to tsetse-free zone: a total of seven animals (henceforth referred to as animals 1–7) were treated and transferred from the tsetse-infested to the tsetse-free zone in May or June 1998 (Table 1). In all the animals, the percentage positivity decreased over time (Fig. 3). The average percentage positivity of blood spots at the moment of transfer was $70.6 \pm 1.9\%$ (Fig. 2). It declined rapidly in the absence of challenge and reached $37.1 \pm 2.3\%$ 5 months after transfer (Fig. 2). From month 6 onwards, the average percentage positivity continued to decline but at a lower rate. It reached a level lower than the cut-off value (28%), 13 months after treatment (Fig. 2). The decline of anti-trypanosomal antibodies was almost linear with a change in slope 6 months after treatment (Figs. 2 and 3). Therefore, the decline in average percentage positivity was analyzed over two periods, i.e. months 0–5 and 6–14.

Table 2
Linear regression of anti-trypanosomal antibody level on months after treatment (months 0–5) for each of the experimental animals

Animal	a[a]	b[b]	R^2[c]	P-value[d]
1	78.9	−9.1	97.8	<0.001
2	76.8	−7.4	94.5	<0.001
3	71.9	−5.2	39.3	<0.004
4	70.8	−6.7	98.9	<0.001
5	62.7	−8.1	90.8	<0.001
6	60.4	−7.5	98.0	<0.001
7	51.0	−4.7	92.8	<0.001

[a] a, Intercept.
[b] b, Regression coefficient.
[c] R^2, Adjusted R^2.
[d] P-value, significance of slope.

3.2. Decline in percentage positivity during the first 5 months after transfer

The decline in the anti-trypanosomal antibody (Figs. 2 and 3) levels during the first 5 months after treatment was almost linear (Table 2). With the exception of animal three, which had a temporary increase in anti-trypanosomal antibody level 4 months after treatment (Fig. 3), the 'time after treatment' explained between 90.8 and 98.9% of the variation in the anti-trypanosomal antibody level (Table 2).

The decline in anti-trypanosomal antibody levels after treatment (quantified by the slope of the regression lines (b)) was significant in all the animals (Table 2). However, the slope differed significantly between animals ($P < 0.05$) but was not affected by the antibody level at the moment of treatment ($r = -0.51$, $P > 0.05$). The monthly decline in percentage positivity was, on average, 10% of the average percentage positivity at the moment of treatment.

3.3. Decline in percentage positivity between months 6 and 14 after transfer

With the exception of animal seven, the anti-trypanosomal antibody level (Figs. 2 and 3) continued to decline significantly between months 6 and 14 after treatment (Table 3 and Figs. 2 and 3). However, the rate of decline was substantially lower compared with the one observed during the first 5 months. Between month 6 and 14, the monthly average decline in percentage positivity was 3.6% of the average percentage positivity in month 6 after treatment.

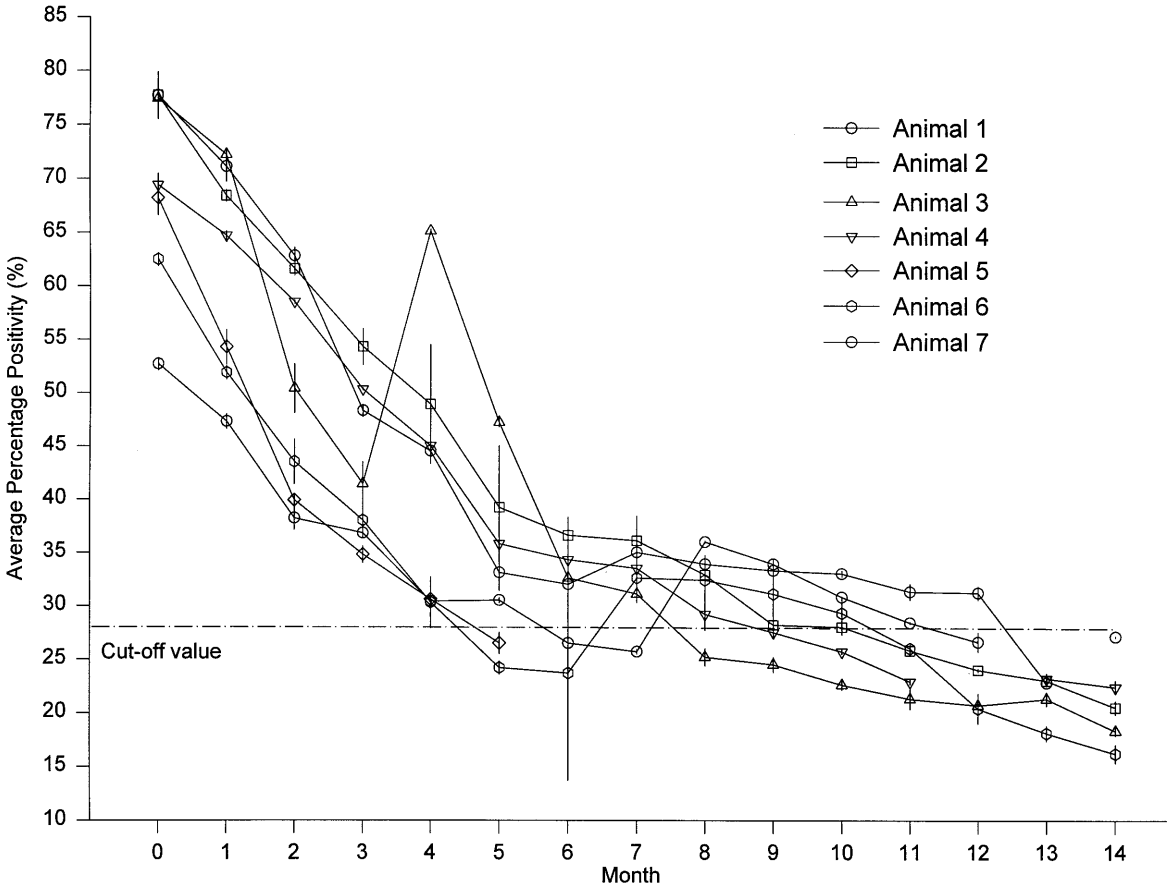


Fig. 3. Average percentage positivity (%) (± 1 S.D.) of animals 1–7 in consecutive months after treatment.

The slope of the regression lines differed significantly between animals ($P < 0.05$) but again was not affected by the antibody level at the moment of treatment ($r = -0.76$, $P > 0.05$). In three animals, the 'time after treatment' explained most of the variation in the anti-trypanosomal antibody level (Table 3).

Approximately 50% of the experimental animals became seronegative 10 months after treatment. All the animals had become seronegative 13 months after treatment (Fig. 3).

4. Discussion

The level of anti-trypanosomal antibodies, collected from the experimental cattle, declined rapidly after treatment with trypanocides. The rate of decline differed significantly between animals. Other workers have observed a similar rapid decline in the level of anti-trypanosomal antibodies after treatment (Wilson and Cunningham, 1971; Luckins, 1977; Dwinger et al., 1988; Bocquentin et al., 1990). Luckins (1977), using a microplate ELISA with various antigens, found that antibodies persisted up to 83 days after treatment with diminazene aceturate. Bocquentin et al. (1990), using an ELISA with *T. congolense* antigen, detected anti-trypanosomal antibodies up to 116 days after treatment but persistence differed significantly between experimental animals. All these results concur with those obtained using the indirect fluorescent antibody test (IFAT) as anti-trypanosomal antibody detection method (Wilson

and Cunningham, 1971; Zwart et al., 1973; Bocquentin et al., 1990). However, contrary to the above-mentioned work, the majority of our experimental animals still had anti-trypanosomal antibodies after this initial phase of rapid decline. It took another 8 months before the anti-trypanosomal antibodies had disappeared in all experimental animals. During this period (months 6–13), the antibody level continued to decline significantly, though at a much lower rate.

Hence, our results suggest that the persistence of anti-trypanosomal antibodies after treatment with trypanocidal drugs is much longer than has been suggested previously. This is in accordance with observations made by Authié et al. (1993) and Hopkins (1997). In both cases, anti-trypanosomal antibodies were present up to 10 months after treatment of a *T. congolense* infection with diminazene aceturate. The decline in the levels of anti-trypanosomal antibodies observed by Authié et al. (1993) was similar to the one in our experiments. The antibody levels decreased progressively until day 50 and remained higher than the pre-challenge levels for several months (Authié et al., 1993). The question remains why the persistence of anti-trypanosomal antibodies is so much longer in our study compared with those of many others. The most likely explanation is the difference in the analytical sensitivity of the various anti-trypanosomal antibody detection tests. The high analytical sensitivity of our test, compared with those used by other workers, can be explained as follows. First, the ELISA used in our experiment made use of a *T. congolense* antigen for coating (Hopkins et al., 1998). *T. congolense* is the dominant trypanosome species in the area. Second, the cut-off value used in our test was determined by making use of highly representative reference samples (Greiner et al., 1997). Indeed, the cut-off value was determined using blood spots collected from Mashona breed cattle kept in an adjacent tsetse-free area of Zimbabwe. The positive blood spots were collected from parasitological positive animals in the trial area and eastern Zambia. The accuracy of the cut-off can still be questioned. However, notwithstanding the fluctuations in the average percentage positivity of the negative control animal, the average percentage positivity never exceeded the cut-off value (28%). Moreover, during a 5 months serosurveillance exercise conducted in the tsetse-free area only five (2.2%) of a total of 222 animals had trypanosomal antibodies value higher than the cut-off value (Van den Bossche, unpublished results). Both the observations suggest that an appropriate cut-off value was used. Furthermore, if the experimental animals had been seronegative 5 months after treatment, the average percentage positivity would not be expected to decline significantly over time but fluctuate around the same value as was the case in the negative control animal.

Table 3
Linear regression of anti-trypanosomal antibody level on months after treatment (months 6–14) for each of the experimental animals

Animal	a[a]	b[b]	R^2[c]	P-value[d]
1	40.9	−1.0	44.8	<0.01
2	48.9	−2.1	93.9	<0.001
3	41.1	−1.7	86.4	<0.001
4	43.1	−1.6	86.8	<0.001
6	41.8	−1.6	45.9	<0.001
7	34.8	−0.6	15.6	>0.05

[a] a, Intercept.
[b] b, Regression coefficient.
[c] adjusted R^2, Correlation coefficient.
[d] P-value, significance of slope.

The antibody-ELISA has been extremely useful in determining the distribution and epidemiology of bovine trypanosomosis (Van den Bossche et al., 1999, 2000). The dynamics of the anti-trypanosomal antibody levels in cattle after treatment with trypanocides has another practical implications. When monitoring the effectiveness of tsetse control operations, sentinel herds of cattle are often used. These herds are examined at regular intervals and the parasitological incidence of trypanosomosis is determined. The value of this type of monitoring depends on the sensitivity of the diagnostic tests and since the diagnostic sensitivity of tests for the parasitological diagnosis of trypanosomosis is low, results from such surveillance exercises should be interpreted with caution. The apparent absence of an infection does not necessarily mean the complete absence of challenge.

More sensitive diagnostic methods are required to establish unequivocally the absence of challenge. The anti-trypanosomal antibody detection ELISA may offer this possibility. The above given results suggest that, in the absence of challenge, the level of anti-trypanosomal antibodies declines steeply in animals treated with trypanocidal drugs. Hence, establishing sentinel herds consisting of seropositive cattle that have been treated with trypanocides and determining the decline in the anti-trypanosomal antibody level over time may be a useful adjunct to evaluating the effectiveness of a tsetse control intervention.

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