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Trypanosomiasis Caused by *Trypanosoma evansi* in Indonesia

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ABSTRACT

It is possible that *Trypanosoma evansi* was introduced into Indonesia at the end of the 19th century since records of the occurrence of disease date from this time. Trypanosomiasis caused by *T. evansi* ("surra") is considered to be one of the most important parasitic diseases of domesticated livestock in Indonesia and occurs in most of the islands, including Irian Jaya. *Trypanosoma evansi* infections in buffalo and cattle are usually chronic or asymptomatic, and result in production losses, including reduced draught power. However, acute cases leading to sudden death have been reported, and occasional epidemics with a high death rate do occur. Infections in horses, which are the livestock species most susceptible to infection with *T. evansi*, are usually fatal, but surra in this species appears to be of importance only in Sulawesi. The abundance of biting flies throughout the Indonesian archipelago facilitates transmission of *T. evansi,* and the extensive movements of livestock over recent years within and between the islands have no doubt assisted in its dissemination. *Trypanosoma evansi* infects wild animals as well as domesticated species and it is possible, though not proven. that certain mammals, such as wild deer, act as reservoirs of infection.

Control of the disease requires identification of the infected animals, and in the field, parasitological diagnostic techniques involving microscopical examination of wet blood films, together with examination of Giemsa' s stained blood smears (thick or thin) are widely used. A number of serodiagnostic tests have been used for screening animals, including fluorescent antibody tests, card agglutination tests, and enzyme immunoassays to detect trypanosomal antibodies or antigen. These techniques have not been widely applied however, although they have been shown to have a higher sensitivity than parasitological techniques , and have been used in preliminary studies in controlling infection in animals at risk. Serological tests have given a more comprehensive indication of the distribution of surra, the prevalence in different areas, the risk from infection, and the economic impact of the disease. Control of surra has been directed at the treatment of clinical cases or prevention of disease outbreaks by prophylactic treatment of animals at risk. For many years, suramin has been the mainstay of treatment for surra in cattle, buffaloes and horses in Indonesia as it has both a curative and a prophylactic action. Not surprisingly, the long term use of a single drug has elicited the appearance of drug resistant stocks, but there is no indication of the extent of the problem. Diminazene aceturate has been shown effective in control of infections in buffalo in Central Java; isometamidium chloride appears to be less effective, although it has not been widely tested.

INTRODUCTION

Trypanosomiasis caused by *Trypanosoma evansi* ("surra") is considered to be one of the most important livestock diseases of horses, cattle and buffaloes in Indonesia. Infection is widespread throughout the archipelago and is on all the main islands, including Irian Jaya (Anon I 993). Occasional epidemic outbreaks of disease still occur, but in Indonesia *T.*

evansi usually causes production losses, including reduced draught power, due to chronic forms of the disease. In recent years there has been widespread movement of livestock throughout the islands and this must have facilitated the spread of infection, although to what extent is not really known.

Trypanosoma evansi was first reported in a horse in Semarang, Central Java (Penning, 1900) and cases of trypanosomiasis in cattle and buffaloes were reported soon after. Vigorous measures were taken to control the spread of infection including slaughter, isolation of infected animals and attempts to discourage flies using smudge fires. However, by 1917 it became apparent that infection was endemic in livestock throughout low lying regions of Java. Although the disease was widespread the prevalence infection varied from district to district. Some 25,000 cases were reported between 1920 and 1927, a quarter of them in horses and all of them fatal (Bakker 1930). Effective trypanocidal drugs became available by 1925 but the results of treatment were sometime disappointing. Horses are the most susceptible to infection with a high mortality rate, death often occurring within a few months of infection (Bakker 1930) if they remain untreated. Equine trypanosomiasis is now largely confined to the island of Sulawesi. Infections in buffaloes and cattle are usually mild or asymptotic but occasional epidemics with a high mortality do occur (Sukanto et al. 1989).

Although *T. evansi* is diagnosed by field veterinarians, and by Disease Investigation Centres, there have been no concerted efforts to determine the prevalence and incidence of *T. evansi* in Indonesia. Where studies have been undertaken infection rates have been found to vary considerably, depending on the location and the host species. On the basis of parasitological detection methods, 1.4%-6% of cattle, 5.8%-7% of buffaloes and 0% of horses were found infected in Sumatra, Java, South Kalimantan, Lombok, South Sulawesi and North Sulawesi (Partoutomo 1996). The lowest prevalence was found in Lombok which was associated with the dry climate of the island where vectors do not survive well. Partoutomo (1996) calculates the annual incidence per 1,000,000 animals from 1987-1991as 26.7 in Sumatra, 27.1 in Java, 185.8 in Kalimantan, 582.4 in Sulawesi, 226.3 in West Nusa Tenggara, 3.5 in East Nusa Tenggara and 71.5 in Irian Jaya. Buffaloes were considered to show a higher rate of infection and to be more affected by *T. evansi* than other ruminants (Partoutomo 1995). Imported buffaloes from Australia showed a higher mortality rate than local buffaloes in the same location (Payne et al. 1991) but nutritional stress and stress of working (Payne 1989; Partoutomo 1995) did not affect the level of parasitaemia. Much important work on the transmission of *T. evansi* was carried out in Indonesia by Niesehulz and his colleagues (Partoutomo 1996) in which the possible role of different fly species was determined, and information on the most likely vectors obtained; the most abundant species in Java were *Tabanus rubidus* and *T. striatus* (Partoutomo 1996).

The treatment of clinical cases of disease and prophylactic treatment of animals at risk have been used widely in Indonesia Suramin has been used for many years in horses, cattle and buffalo. The drug is effective at doses of 3-5 g/animal but is more likely to be active against early infection and acute disease rather than late, chronic infections (Douwes 1923; 1924). There have been reports of the appearance of drug resistant strains of trypanosomes (Wilson et al. 1985; Stevenson et al. 1985). Other trypanocidal drugs have been used; Diminazene was found to give variable effects in experimental studies in laboratory animals (Holz and Adiwinata 1956; Dieleman 1986; Prastyawati and Dieleman 1983; Stevenson et al. 1983). While isometamidium chloride only had only a temporary effect in cattle and buffaloes (Stevenson et al. 1985).

Several diagnostic techniques have been used for the diagnosis of surra in Indonesia, including wet blood film, blood smear, mouse inoculation (MI), microhaematocrit centrifugation technique (MHCT) and ELISA techniques. Wet blood films are used widely in the field because it is quick and simple although the sensitivity of the test is low. The MHCT was recommended as a definitive practical technique for diagnosis of surra in the field (Rukmana 1979).

TRYPANOSOMA EVANSI IN CENTRAL JAVA

During the past five years, a comprehensive survey on *T. evansi* has been carried out in Central Java by the Research Institute of Veterinary Science, Bogor, Indonesia and the Centre for Tropical Veterinary Medicine, Edinburgh (Davison 1997). Initially, the work was undertaken in five districts, namely Batang, Pemalang, Tegal. Brebes and Pekolongan, that have a buffalo population of about 90,000 animals. Cross sectional studies took place in 59 villages in the five districts and a total of 2,387 buffalo were sampled. In addition, a longitudinal study was undertaken in a group of 49 buffalo that showed no evidence of infection by parasitological or serological methods of diagnosis at the commencement of the study. The main objective was to estimate the prevalence and the incidence rate using two different antigen detection ELISA tests (Ag-ELISA) and to a more limited extent, antibody ELISA (Ab-ELISA) and the Card Agglutination Test for Trypanosomiasis (CATT). These data have not been acquired before: there is very little information on prevalence rates in different districts and villages, and no information on incidence rates of *T. evansi* infection. As part of the survey, the serological tests used were fully validated in term of diagnostic sensitivity and specificity, in order to acquire corrected estimates of the true prevalence. All animals were examined by the MHCT and 360 buffaloes were tested using MI techniques. Assays were carefully quality controlled to ensure consistency of results. The estimates of sensitivity for the Ab-ELISA, based on assays carried out with sera from known infected buffalo was 89%; this was higher than the results for Ag-ELISA or CATT tests. The specificity of the assays for antibody were higher than those for the Ag-ELISA, namely 92% for the Ab-ELISA and 100% for the CATT. The results of the assays are shown in Table 1.

Table 1 : Diagnostic sensitivity and specificity of Ag-ELISA, Ab-ELISA and CATT for the diagnosis of *Trypanosoma evansi* in buffaloes in Indonesia

Test	Sensitivity* number of animals - 139	Specificity** number of animals - 263
AB-ELISA	89 (84, 94)	92 (86, 96)
AG-ELISA	71 63. 79	78 73. 83
CATT	78 72. 85	100 97, 100

Figures in brackets denote 95% confidence interval

* Estimates based on sera collected from buffalo that showed parasitological evidence of infection by MHCT and MI.

* * Estimates based on sera collected from buffalo from Australia.

Comparison of the assays by calculation of post-test probabilities of infection, and

Table 2: Uncorrected district-specific prevalence values (P^T), with associated 95% confidence intervals (CI)(in brackets) and standard errors (SE), obtained using different diagnostic tests for *Trypanosoma evansi*

District	MHCT			MI			Ag-ELISA		
	P^T (o/o)	CI	SE	P^T (solo)	CI	SE	P^T (olo)	CI	SE
Batang	6	(2, O)	0.02	16	(6,27)	0.06	58	(42, 75)	0.09
Pekalongan	6	(3. O)	0.02	7	(4. 1 1)	0.02	39	(.29. 50)	0.05
Pemalang	3	(O, 5)	0.0 1	8	(-5. 1)	0.07	47	(38. 56)	0.05
Tegal	1	(O. 3)	0.01	9	(-2, 9)	0.05	58	(43. 74)	0.08
Brebes	4	(1, 6)	0.01				62	(53, 72)	0.05

The corrected prevalence rates for a number of villages in two districts that showed significant differences in their overall prevalence values are shown in Figures 3 and 4. It can be seen that even within a district there is often considerable variation between the infection rates in buffalo in different villages.

Table 3 : Brebes District: Corrected village-specific prevalence values (P) of *Trypanosoma evansi* infections in buffaloes, with 95% confidence intervals (CI) (in brackets), obtained using Antigen-ELISA

Visit	Village	Total number of buffaloes	Sample size	Ag-ELISA	
				P(%)	CI
1	Kutamendala	267	99	63	(52, 72)
2	Indrajaya	280	173	74	(68, 81)
3	Tanggeran	120	97	61	(50, 71)
4	Pulosari	76	19	26	(9, 51)
4	Tengki	70	9	41	(14, 79)
4	Pagejugan	30	12	17	(2, 48)
4	Pasar Batang	80	8	54	(16, 84)
5	Tembonag Ray	280	103	57	(48, 67)

likelihood ratios suggested that the Ab-ELISA would be more likely to correctly classify uninfected buffaloes and that the CATT would be more likely to correctly classify truly infected buffaloes (Davison, unpublished observations). Thus, antibody assays would be useful screening tools, whereas the CATT would be a useful penside diagnostic test to identify infected buffaloes for trypanocidal drug treatment. The district prevalence values for the Ag-ELISA and parasitological tests are shown in Table 2. Prevalence values differed between districts and in the case of Brebes the prevalence estimated by Ag-ELISA was significantly higher than in Pekalongan or Pemalang (Table 2).

Initially, in order to identify suitable animals, blood samples were collected from 434 buffalo and each animal was tattooed on the ear. The buffalo were sited at seven locations within the village complexes. All sera were assayed for the presence of trypanosomal antibody and antigen and parasitological detection was based on examination of blood by

the MHCT technique and MI. From the buffalo sampled, 258 were selected on the basis of their availability and degree of co-operation given by the farmer. These animals were sub-divided into four groups based on their serological/parasitological status. Group A consisted of 48 buffalo that were positive by both serological assays and/or positive parasitologically; Group B, consisted of 63 buffalo that were positive only by Ag-ELISA, Group C, 73 animals that were positive only by Ab-ELISA and Group D, a control group

Table 4: Pemalang district: Corrected village-specific prevalence values (P) of *Trypanosoma evansi* infections in buffaloes, with associated 95% confidence intervals (CI) (in brackets), obtained using Antigen-ELISA

Visit	Village	Total number of buffaloes	Sample size	Ag-ELISA	
				P(%)	CI
1	Kabunan	325	36	41	(26, 59)
1	Saradan	46	25	85	(64, 96)
1	Penggarit	172	50	67	(53, 82)
2	Wonogiri	70	52	26	(16, 41)
2	Tegal Sari	450	78	63	(51, 74)
3	Karangbrai	120	70	41	(30, 54)
3	Sarwodadi	36	23	50	(31, 73)
3	Wonokromo	38	28	24	(11, 45)
3	Mojo	78	4	54	(7, 93)
4	Pedurungan	150	109	52	(43, 62)
4	Surajaya	400	96	33	(24, 44)
5	Pegongsoran	200	40	39	(25, 57)

of 74 buffalo that tested negative by both assays and from which trypanosomes could not be isolated.

Treatment Regimen. Group A animals were targeted for trypanocidal drug treatment with diminazene aceturate (Berenil) and treatment was commenced in November. All buffalo were treated at a dose rate of 7 mg/kg body weight. Follow-up visits were made in December, March, June, October and December. At each visit, sera were collected and parasitological examination carried out. Serological assays were carried out serially, with the more specific Ab-ELISA used for screening and then any samples that tested positive were tested by Ag-ELISA to confirm if the animal was positive or not. Any animals in Group A that showed evidence of re-infection, or persistent infection, was treated again with Berenil at the same dose rate. By using these criteria for designating an animal as positive, the positive predictive value of the tests is increased. The point prevalence rates in the different groups are shown in Figures 1-4.

There is a decrease in the prevalence in buffalo in Group A compared with the other groups. In these animals there was a progressive increase in prevalence during the study period. In Groups B and C, the prevalence increased markedly from the second and third visits. In Group D this rise was slower; this was probably due to the fact that these animals consisted of animals that did not harbour infection, whereas in Groups B and C, where buffalo showed the presence of antigen or antibody, a proportion of them were already infected. There was a decrease in both serological and parasitological prevalence in these

three groups in December, possibly occasioned by the dry season, when transmission would have been low. Although there was a difference in prevalence rates between the treated and the untreated groups, there was no evidence that the incidence of infection was reduced by

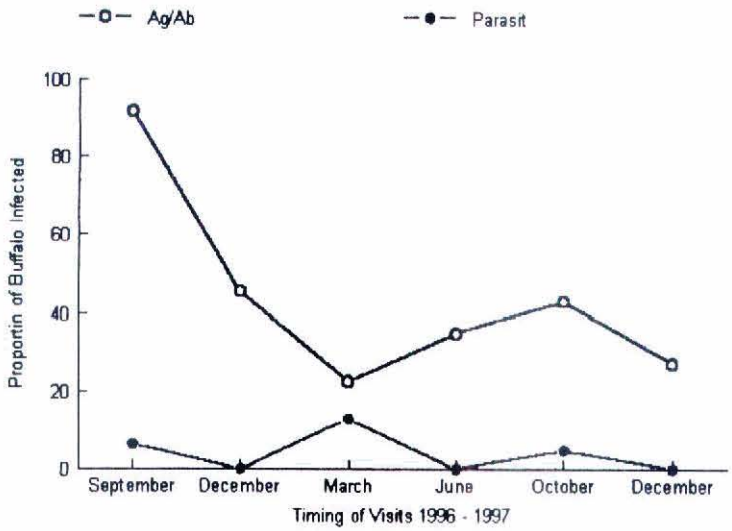

Figure 1: Point prevalence values estimated by serology and parasitology in buffaloes from Group A treated with the trypanocidal drug diminazene aceturate.

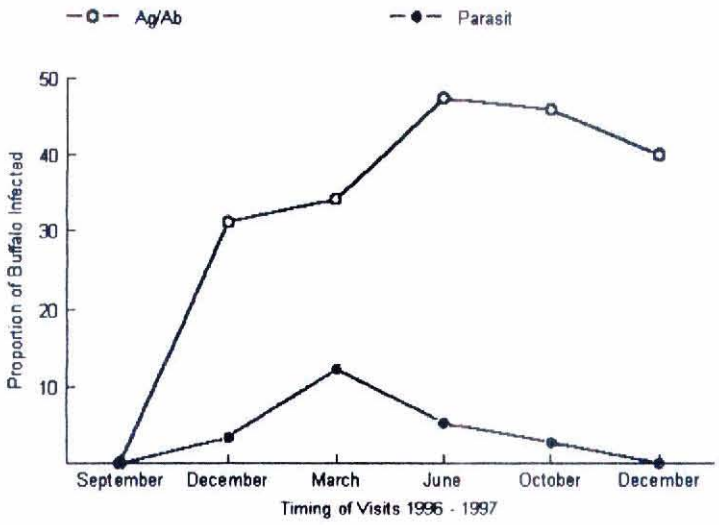

Figure 2: Point prevalence values estimated by serology and parasitology in buffaloes from Group B initially positive by Ag-ELISA only.

treatment. In Group D, the initially uninfected group, the cumulative incidence (CI) was 0.77. In Group B it was 0.75 and Croup C, 0.68. However. in Group A, in the treated animals the CI was still 0.77. There was no statistically significant difference between these values for the various groups. This finding gives some support to the hypothesis that in order to control infection it is necessary to treat all infected animals within the population. The treatment protocol targeted only a small proportion of the total buffalo population (800), and since prevalence rates were as high as 40%, many animals that were harbouring infection went untreated and served as reservoirs of infection.

Economic Impact of T. evansi in buffalo reared under village conditions in Indonesia. One hundred and twenty seven adult female buffalo kept by smallholder farmers in villages in Pemalang, Central Java were monitored for nine months to measure the impact of

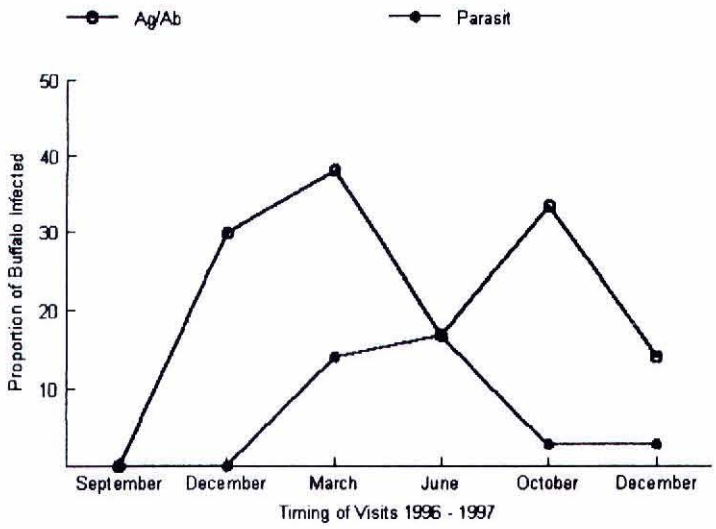

Figure 3: Point prevalence values estimated by serology and parasitology in buffaloes from Group C initially positive by Ab-ELISA only.

infection with *T. evansi* on productivity. The buffalo were divided into three groups according to the presence or absence of infection with *T. evansi*, and whether or not they were treated for the disease. Group 1 was comprised of 45 buffaloes that tested positive for *T. evansi* at the onset of the monitoring period and were treated with diminazene aceturate initially, and, if required, during the monitoring period. Group 2 consisted of 37 buffalo that were positive for *T. evansi* throughout the monitoring period. These animals were not treated. Croup 3 was comprised of 45 animals that remained negative for *T. evansi* throughout the survey. Animals infected with *T. evansi* were more likely to die or be sold through ill health compared to uninfected animals. The combined mortality/emergency sale rate for infected and uninfected animals were 22%and 0% respectively. Animals sold through ill health fetched a lower price than health animals. Calving rates were also marginally lower among infected animals. These lower calving rates, higher mortality and emergency sales rates resulted in lower herd growth rates: infected herds produced some S27 less weight per animal per year compared to *T. evansi* free animals. Most of this reduction was attributed to mortality losses and emergency sales rather than a reduction in growth rates. The productivity of animals that were treated with Berenil approximately matched that of the *T. evansi*-free animals, yielding weight gains valued at $25 per animal. Mortality and emergency sales were greatly reduced from 22% for the infected group to 8% for the treated group, but still exceeded the loss rates for the uninfected group. However, treated animals that were not lost or sold due to disease appeared to gain weight quicker than the uninfected group .

Net returns to an annual course of treatment thus amounted to $14 per infected animal per annum. This is equivalent to a benefit to cost ratio of 2.4, or a return of 135% on treatment costs. However, farmers are currently unlikely to invest in treatment of the disease,

as farmers reportedly are unable to distinguish between infected and non-infected animals. Comprehensive treatment of all animals at risk would only be economically justifiable in this

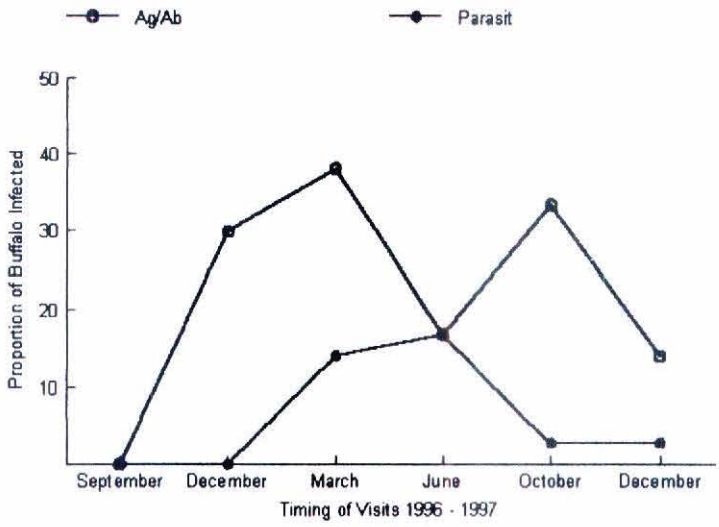

Figure 4: point prevalence values estimated by serology and parasitology in buffaloes from Group D. initially negative by all diagnostic techniques.

village context if *T. evansi* prevalence rates exceeded 40%. This in fact did occur at some time during the course of the investigation, but for most of the period point prevalence rates were <35%. At current levels of prevalence, the average cost of the disease per animal is $3.4, or 36% of the cost of treatment. At these levels of disease prevalence, farmers can only reap the benefits of treatment if they are able to target infected animals. This is only likely to be possible if infected animals can be diagnosed for the farmer at a reasonable cost (i.e. less than $14) and with relative ease.

The studies have given the first estimate of the economic impact of *T. evansi* and an indication of the positive benefits of treatment in village situations in areas where the disease is endemic, and rarely presenting as an infection with high mortality. These sort of data are essential in evaluating the likely effects of trypanosomiasis on livestock and the resources required to control the disease. They have also indicated the need for more effective diagnostic strategies coupled with rational treatment regimens that will enable effective control of trypanosomiasis.

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