

Determination of the Causes of Death and Ill Health, with Particular Reference to Trypanosomiasis, Babesiosis and Anaplasmosis, in Cattle and Buffaloes Imported into Indonesia*)

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Abstrak.

Payne, R.C., I.P. Sukanto, A. Husein, D. Djauhari, Khadjatun & D. Waltner-Toewes. 1989. Penentuan penyebab kematian dan kesakitan, khususnya oleh tripanosomiasis, babesiosis dan anaplasmosis, pada sapi dan kerbau yang diimpor ke Indonesia. *Maj. Parasitol. Ind.* 3, 1990 (Edisi Khusus) : 99-107.

Penelitian yang dilakukan di daerah Aceh, Yogyakarta dan Garut bertujuan untuk menentukan kemungkinan penyebab morbiditas dan mortalitas sapi dan kerbau impor. Sampel darah sapi lokal dan impor dari Aceh diperiksa keadaan haematologinya dan serumnya diuji dengan menggunakan uji enzyme-linked immunosorbent assay (ELISA) untuk mendeteksi adanya antibodi terhadap *Trypanosoma evansi*, *Babesia bovis*, dan *Anaplasma marginale*. Hasil yang diperoleh menunjukkan bahwa Aceh merupakan daerah endemik terhadap ketiga parasit tersebut. Uji ELISA ini digunakan pula untuk mengukur jumlah antibodi *T. evansi* pada serum kerbau lokal dan impor di daerah Yogyakarta dan Garut. Hasil uji pada kerbau lokal menunjukkan bahwa kedua daerah tersebut adalah endemik terhadap *T. evansi*, sedangkan pada kerbau impor tidak diperoleh bukti bahwa hewan tersebut pernah terinfeksi sebelumnya. Naganol (Suramin, Bayer) digunakan untuk tindakan pencegahan terhadap *T. evansi* di daerah Yogyakarta, tempat ditemukan 12 ekor mati dari sejumlah 131 ekor kerbau yang diamati dan *T. evansi* merupakan penyebab kematian pada 3 ekor di antaranya. Kerbau impor di daerah Garut diberi Trypamidium (Isometamidium, Specia) untuk tindakan pencegahan, karena 33 ekor dari 45 ekor mati secara berturut-turut dan *T. evansi* ditemukan pada 23 ekor di antaranya. Di samping itu, *A. marginale* ditemukan pada 3 ekor hewan lain dengan parasitemia yang cukup tinggi. Hasil penelitian ini menunjukkan bahwa babesiosis, anaplasmosis dan tripanosomiasis patut dipertimbangkan sebagai penyebab yang memungkinkan terjadinya kematian sapi dan kerbau impor, yang sebelumnya tidak pernah mengalami infeksi oleh ketiga jenis parasit tersebut.

Abstract.

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Introduction.

Little is known about the epidemiology of tick-borne diseases in Indonesia. The current knowledge on *Anaplasma marginale* was reviewed by Wilson and Ronohardjo (1984) and the authors concluded that the parasites and its vector tick *Boophilus microplus* are endemic in livestock rearing areas. The haemoparasites *Babesia bigemina* and *B. bovis* are known to occur in cattle (Ronohardjo *et al.*, 1985), although little information is available on the prevalence of these parasites. The other main haemoparasitic disease of large ruminants in Indonesia is caused by *T. evansi*. Much research has been conducted on this organism since the early part of this century (Reviewed by Dielman, 1983) and trypanosomiasis is now thought to be endemic throughout most of the livestock rearing areas.

It is well known that the importation of native cattle into areas where *A. marginale*, *B. bovis* and *B. bigemina* are endemic results in very high mortalities unless the animals are vaccinated or chemotherapy is applied (Ristic and Carson, 1976; Callow 1977). In a study on buffaloes imported into Central Java from northern Australia, Waltner-Toewes *et*

al., (1986) concluded that imported native buffaloes were susceptible to infection with *T. evansi* and furthermore, that they were much more likely to die as a result of the infection than local buffaloes. Moreover, Doeve (1917) observed that buffaloes imported into areas of Indonesia where *T. evansi* was endemic suffered an acute form of the disease, but provided the animals were rested, well fed and protected from further infection they recovered completely. Buffaloes which were not adequately fed and were also made to work however, suffered loss of weight and condition and some eventually died.

Because of continuing programme of animal importations there is an urgent need to obtain information about the epidemiology of blood parasites of large ruminants in Indonesia. Better advice can then be given to government agencies on control procedures for buffalo and cattle imported areas which are free from these diseases. In view of this requirement a limited serological survey was undertaken to determine the prevalence of antibodies to *T. evansi*, *A. marginale* and *B. bovis* in local and imported cattle in Aceh Province and of *T. evansi* in local and imported buffaloes in Central Java.

Imported Cattle in Aceh Province

A group of 3,125 Sahiwal cross (*Bos taurus* cross *Bos indicus*) heifers and bulls were imported in mid 1985 New Zealand, where *A. marginale*, *B. bovis*, *B. bigemina* and *T. evansi* do not occur. Subsequent to distribution to smallholder farmers many cattle became sick and died, *T. evansi* and *B. bigemina* were reported to be associated with outbreaks of disease, and heavy tick infestations were observed (Ward, 1985).

Imported Buffaloes in Central Java

The importation of 657 feral buffaloes in February 1986 from northern Australia provided an opportunity to study the response to infection with *T. evansi* in previously unexposed buffaloes. *A. marginale*, *B. bovis* and *B. bigemina* are endemic in much of northern Australia (Callow, 1977), but the area is free from *T. evansi* (Bainbridge, 1977).

Materials and Methods

The present investigation was divided into two parts, the first of which comprised a seroepidemiological survey on the prevalence of antibodies to *T. evansi*, *A. marginale* and *B. bovis* in indigenous Acehenese cattle, undertaken in order to assess the risk of transmitting parasites to susceptible, imported cattle.

Serum samples were also collected from imported Sahiwal cross cattle to evaluate the blood parasite infection rate.

In the second part of the study, the prevalence of *T. evansi* in indigenous buffaloes in Central Java and Garut was determined and the reaction to infection of imported buffaloes was monitored over several months.

Sampling Procedure

Aceh : Cattle

Sampling locations were selected in areas where imported and local livestock were concentrated and outbreaks of disease had been reported by the Dinas Peternakan (DP). Clusters of cattle were identified for sampling, these included as many animals as possible from several locations. These clusters were stratified into area, breed and age group. Blood was collected for haematological and serological examinations on one occasion only, from 102 local cattle of various ages, 157 imported Sahiwal cross cattle and 48 Sahiwal cross calves born locally. Sixty one blood smears were prepared from 42 anaemic cattle and 19 calves.

Central Java : Buffaloes

Prior to dispersal the imported buffaloes were held in quarantine for two weeks; during this time DP personnel administered vaccinations against foot and mouth disease and haemorrhagic septicaemia and intramuscular injections of Trypanidium (Isometadum chloride, Specia) were given as a prophylactic against *T. evansi*. The buffaloes were checked for body condition and whole blood and serum samples were collected from 288 randomly selected animals for haematological and serological examinations.

The buffaloes were distributed to farms within the area covered by the Yogyakarta Disease Investigation Centre (DIC) in late February. Three villages in the Kabupatens of Bantul, Sleman and Kulon Progo were selected where samples could be collected regularly from groups of 47, 43 and 41 imported buffaloes. Visits were made in March, June, August and November of 1986 and June 1987.

In response to a request from the regional DP the investigation was extended to include Garut, where 45 buffaloes from a subsequent importation in May 1986 were located. This area was visited in August and October 1986 and January 1987.

Laboratory Examinations

During each visit whole blood and serum samples were collected from imported and local animals. The packed cell volume (PCV) was determined by the microhaematocrit method and the plasma-buffy coat interphase in the capillary tubes was examined for trypanosomes (MHT method, Woo, 1970).

Giemsa stained blood smears from sick or anaemic cattle (PCV < 24%) were examined microscopically for blood parasites using 1,000 magnification. The ELISA (enzyme-linked immunosorbent assay) described by Luckins (1977) was employed to detect serum antibodies to *T. evansi*. The assay developed by Barry *et al.*, and (1986) was employed to select serum antibodies to *A. marginale* *B. bovis*. Antibodies were detected using the ELISA developed by Barry *et al.*, (1982) with modifications (Sukanto, 1988).

Results

Aceh : Cattle

Local cattle

The number of serologically positive reactors in indigenous cattle in Aceh Province is shown in Table 1. The majority of samples collected from adult cattle were positive in the ELISA for *B. bigemina* (88%), *B. bovis* (100%) and *T. evansi* (71%). High prevalence rates of antibodies to *B. bigemina* (60%) and *B. bovis* 100%) were detected in samples from indigenous calves whereas only 20% were positive for *T. evansi*.

Imported cattle

Prevalence rates of antibodies to the three parasites in imported cattle were *B. bigemina* 57%, *B. bovis* 96% and *T. evansi* 40%. Seroprevalence rates in the Sahiwal cross calves born in Aceh were *B. bigemina* 29%, *B. bovis* 92% and *T. evansi* 8% (Table 1).

Microscopic examinations of blood smears revealed *B. bigemina* in 3 local cattle and 2 of the imported Sahiwal cross animals.

Parasitaemias were low and in each case only occasional parasites were detected.

Central Java : Buffaloes

Local buffaloes

T. evansi was detected by MHT examinations in 2 of 72 local buffaloes samples in Bantul, Sleman and Kulon Progo, 55 of 72 were positive for *T. evansi* in the ELISA.

MHT examinations revealed *T. evansi* in 4 of 31 samples collected from local buffaloes in Garut, 21 of 31 were positive in the ELISA.

Samples collected in the quarantine station

T. evansi was not detected in blood samples from imported buffaloes, furthermore the serum samples were all negative in the ELISA.

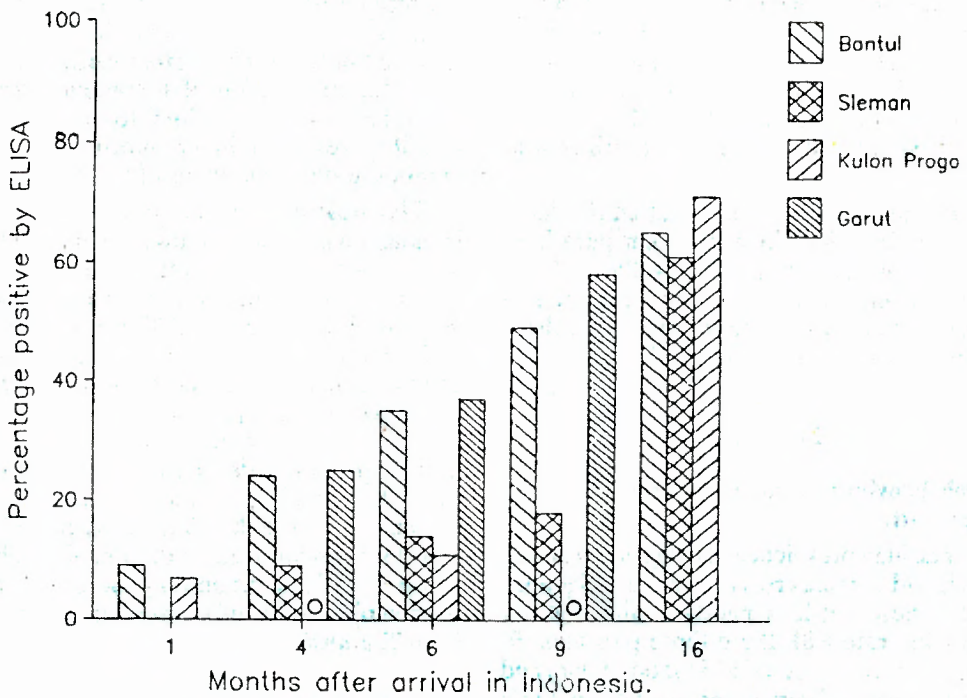
Observations in imported buffaloes following distribution

T. evansi was detected by the MHT in one imported and two local buffaloes during the first visit to the Yogyakarta region, in March 1986. Subsequently DP personnel treated all the imported buffaloes with Naganol (Suramin, Bayer) to provide prophylactic cover. In addition local buffaloes on farms adjacent to those where the imported animals were kept, were given curative doses of Naganol in an attempt to prevent transmission of parasites to susceptible animals. *T. evansi* was not detected again until the last visit in June 1987 when two imported buffaloes were MHT positive. During the course of the investigation 12 imported buffaloes died, the cause of death in 3 of these animals was diagnosed at the DIC as *T. evansi*, while the remainder died of other causes.

Results of the ELISA demonstrate an increase in the number of imported buffaloes positive in the ELISA (Figure 1) and at the time of the last visit in June 1987 the percentage positive by ELISA in the three

Table 1. Percentage of cattle in Aceh Province positive by ELISA for *A. marginale*, *B. bovis* and *T. evansi*

Animals	<i>A. marginale</i>	<i>B. bovis</i>	<i>T. evansi</i>
Local adults	88%	100%	71%
Local calves	60%	100%	20%
Sahiwal cross	57%	96%	40%
Sahiwal cross calves	29%	92%	8%



O—No sample

Figure 1. Seroconversion in imported buffaloes

areas were Bantul 65%, Sleman 61% and Kulon Progo 71%.

Garut : Buffaloes

In Garut, *T. evansi* was detected by the MHT in 14 of 44 buffaloes sampled during the first visit in August 1986, subsequently the imported buffaloes were treated with Trypanidum by DP staff. By January 1987 however, thirty two buffaloes had died (Table 2). *T. evansi* was detected by the MHT in 11 buffaloes prior to death and examination of post-mortem blood smears revealed the organism in 12 others. Antibodies to *T. evansi* were detected by the ELISA in 2 of the dead buffaloes although parasites were not detected by MHT. On the final visit in January 1987 *T. evansi* was detected in 9 of the surviving 12 buffaloes, all the survivors were treated with Suramin but one more buffalo died a month later. The majority of buffalo deaths occurred in the early part of the rainy season between September and December.

Anaplasmosis may have caused the death of three buffaloes in which high parasitaemias of *Anaplasma marginale* (805, 30% and 25% infected red cells) were detected in post-mortem blood smears, two of the three were concurrently infected with *T. evansi*.

Discussion

Aceh Province : *Anaplasma* and *Babesia* in local cattle

The high prevalence of *B. bigemina* and *B. bovis* and *T. evansi* reactors in the indigenous Acehenese cattle suggests a high natural infection rate with these three parasites. *B. bigemina* was detected in 5 blood smears and the following observations indicate that this parasite is endemic in the area. *B. microplus* tick were seen on the majority of animals sampled. *B. bigemina* was detected and although babesiosis is reported to occur in northern Sumatra, the frequency of outbreaks is sporadic (Anon., 1985). If the

conditions of enzootic stability, outlined by Mahoney and Ross (1982) were not satisfied severe outbreaks of babesiosis could be expected. The majority of indigenous cattle are probably infected with *A. marginale*, *B. bovis* and *B. bigemina* at an early age when maternal antibodies and natural resistance help to reduce the severity of reactions. Cases of anaplasmosis and babesiosis in adult indigenous cattle are not a frequent occurrence in Aceh Province and it may be concluded that the diseases are enzootically stable in the area.

Reactions in imported cattle in Aceh

The high prevalence of antibodies to *T. marginale* and *B. bovis* confirm that the imported cattle were exposed to infection in Aceh. Although a high mortality rate was reported in the Sahiwal cross cattle (Ward, 1985) the application of therapeutic drugs (Imizol and Oxytetracycline) to sick animals, possibly resulted in chemoprophylactic immunization in a number of cases.

The prevalence of antibodies to *T. evansi* in indigenous cattle was lower than that of *A. marginale* and *B. bovis*, which argues a lower infection rate. This was reflected by the generally lower number of *T. evansi* reactors in the imported Sahiwal cross cattle and their calves. The low number of Sahiwal cross calves which had positive reactions to *T. evansi* (4/48) may have been a result of keeping the animals under cover. Tabanid flies, the principle vectors of *T. evansi* in Indonesia (Nieschultz, 1930), feed in sunlight and do not readily enter dark stalls. Calves which were kept inside may therefore have been subjected to only a minimal challenge from *T. evansi*.

Imported Buffaloes in Java

The gradual increase in seropositive reactors detected in Yogyakarta, suggests that as the effect of Naganol declined a protective immunity was generated in a number of animals as a result of natural infection with *T. evansi*. In Garut, studies on

Table 2. The number of imported buffaloes in Garut in which evidence of infection with *T. evansi* or *A. marginale* was detected

Dead buffaloes (33)	
<i>T. evansi</i> detected by the MHT only	11
<i>T. evansi</i> detected in post-mortem blood smears only	11
<i>T. evansi</i> detected in both MHT and in PM blood smears	1
<i>T. evansi</i> not detected ELISA positive	2
Total	25
<i>A. marginale</i> detected in post mortem blood smears	6
Parasites detected in blood smears before and after death	2
Total	8
Number concurrently infected with <i>T. evansi</i> and <i>A. marginale</i>	6
Surviving buffaloes (12)	
<i>T. evansi</i> detected by MHT	9
<i>T. evansi</i> not detected, ELISA positive	1
<i>A. marginale</i> detected in blood smears	5
Number concurrently with <i>T. evansi</i> and <i>A. marginale</i>	5

Trypanidium was employed as prophylactic in Garut, studies on stocks of *T. evansi* isolated in different areas of Indonesia demonstrated widespread resistance to this drug (Partoutomo *et al.*, 1985) and it is unlikely to have had any beneficial effect in this instance. Naganol was given to several of the sick buffaloes in Garut but in three animals which subsequently died, treatment may have been applied too late to have any effect. In a review of *T. evansi* in Indonesia Dielman (1983) concluded that Naganol was only effective therapeutically if administered early in an infection. Most of the deaths occurred in Garut in the early part of the rainy season, at a time when fields are being prepared for planting and forage is scarce. Reactions to infection with *T. evansi* may therefore have been aggravated by nutritional and work stress. The only other blood parasite detected in imported buffaloes was *A. marginale*, the high parasitaemias observed in several animals suggests that a proportion of the imports were susceptible to the organism and in 3 cases in Garut the infections apparently resulted in death.

As a conclusion it could be said, that this study demonstrates that *A. marginale*, *B. bovis* and *T. evansi* and possibly *B. bigemina* are endemic in Aceh Province and that cattle imported from areas free from these diseases rapidly become infected. In order to prevent losses in imported cattle a result of haemo-parasitic diseases, preventive measures must be taken. These measures could include one of the following alternatives, vaccination of imported cattle against *B. bigemina*, *B. bovis* and *A. marginale* (ideally in the country of origin) or importation of cattle from areas where these diseases are endemic. There is an urgent need for epidemiological studies on which to base recommendations for minimizing the effects of *T. evansi* in imported cattle. Mean while protection against this parasite should be given for 6-12 months by treating all cattle with Naganol.

Data collected during this investigation supports the hypothesis that *T. evansi* is a

cause of mortalities in imported buffaloes. The most effective method of protecting buffaloes, previously unexposed to *T. evansi*, appears to chemoprophylactic intervention with Naganol. It cannot be said with certainty what killed the buffaloes in Garut. *T. evansi* and *A. marginale* are suspected as being causative agents but the outcome of single infection with each of the parasites or mixed infections in previously unexposed buffaloes needs to be examined.

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