Identification of Babesia bovis and Cowdria ruminantium on the island of Unguja, Zanzibar

E. J. Flach, J. D. Woodford, S. P. Morzaria, T. T. Dolan, I. Shambwana

Babesia bovis and Cowdria ruminantium were identified for the first time in cattle on Unguja Island, Zanzibar. B bovis is common and widespread, although clinical disease had not been diagnosed previously. The vector of heartwater, Amblyomma variegatum, is found throughout Unguja but C ruminantium appears to be more localised in distribution than B bovis.

TICK-BORNE diseases are important constraints upon the improvement of livestock on the Unguja and Pemba Islands of Zanzibar. The common tick species found on the islands are Rhipicephalus appendiculatus, Boophilus microplus and Amblyomma variegatum. These ticks transmit Theileria parva, the cause of East Coast fever, Babesia bigemina and, or, Anaplasma marginale, and Theileria mutans, respectively, to cattle (Newson 1976, Tatchell 1976, Hofstedt 1977, Irvin 1984). Babesia bovis, transmitted by B microplus, has recently been identified on Pemba (Woodford and others 1989). Cowdria ruminantium, transmitted by Amblyomma species and the cause of heartwater, has not been identified on either island. This paper reports the presence of B bovis and C ruminantium on Unguja.

Materials and methods

Observations were made between July and December 1987 on three groups of cattle which were born and raised on Unguja.


SANGER, D. V., RowlANDS, D. J. & BROWN, F. (1977) Veterinary Record 100, 240

Group 1 consisted of 16 Bos taurus (Jersey, Friesian and Jersey x Friesian) bulls and steers aged 15 to 30 months, which had been used in a field trial of immunisation against East Coast fever at Kizimbani and Tunguu between October 1986 and March 1987 (Fig 1) (Pedersen 1987). These cattle were exposed for a further period of three weeks at Kipange in October and November 1987 during a second immunisation trial.

Group 2 consisted of 12 B taurus (Jersey and Jersey x Friesian) bull calves aged six to 12 months, which were obtained from a government dairy farm for a second East Coast fever trial. This group was immunised against East Coast fever by infection and treatment (Radley 1981) and exposed to challenge at Kipange together with group 1.

Group 3 consisted of 46 adult cattle; 44 Bos indicus (East African shorthorn zebu) and two B indicus x B taurus (zebu x Jersey and zebu x Friesian) aged between 15 months and 10 years. These animals were kept by smallholder farmers at Kipange, Kiwendi, Winzo and Jozani (Fig 1).

The animals in groups 1 and 2 were monitored regularly during natural exposure to an unlimited tick challenge in the second immunisation trial. Their rectal temperatures and clinical appearance were recorded daily and blood samples and lymph node biopsies were taken from any animal which showed fever (temperature over 39.4°C) or signs of tick-borne disease. The packed cell volume (PCV) was determined and blood smears and lymph node biopsy samples were stained with Giemsa's stain and examined for haemoparasites. All cattle which died during the trial were examined and smears were prepared and examined from blood, lymph node, visceral organs and brain.

Serum samples had been collected from all the animals entering the first immunisation trial in July 1986 but samples from only 11 of the 16 survivors (group 1) were available in 1987. The 28 cattle in groups 1 and 2 were bled for serum before field exposure at Kipange (either in August or October 1987). Serum was collected from the cattle in group 3 during a routine epidemiological survey. The three sets of sera were tested for antibodies to B bovis and B bigemina at the Centre for Tropical Veterinary Medicine, University of Edinburgh, using an enzyme-linked immunosorbent assay (ELISA) set up to investigate babesiosis on Pemba (Woodford and others, 1990).
animals had antibodies to both *Babesia* species in 1987. Six of the 12 calves in group 2 had antibodies to *B. bovis* and six to *B. bigemina*. Both animals which suffered clinical babesiosis had antibodies to *B. bovis* two to three weeks after recovery whereas the group 1 animal had been seronegative in July 1986. The 28 animals in groups 1 and 2 had been hand-sprayed with toxaphene (Coopertox; Coopers, Kenya) twice weekly for two months before these diagnoses but, nevertheless, en­gorging adult female *B. microplus* were seen on several oc­casions. Almost all local cattle (group 3) at Kipange, Kibweni and Winzo had antibodies to both *Babesia* species, whereas around Jozani (Pete, Kitogani and Muungoni) only 25 per cent were positive to *B. bovis* and 25 per cent to *B. bigemina* (Table 1).

### TABLE 1: Cattle with antibodies to *Babesia bovis* and *B. bigemina*

<table>
<thead>
<tr>
<th>Area</th>
<th>Mean age (years)</th>
<th>Range</th>
<th>Number of animals with antibodies in ELISA tests for <em>B. bovis</em></th>
<th><em>B. bigemina</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Kipange</td>
<td>5-6</td>
<td>3-10</td>
<td>12/12</td>
<td>12/12</td>
</tr>
<tr>
<td>Kibweni</td>
<td>5-1</td>
<td>1-3-10</td>
<td>10/11</td>
<td>11/11</td>
</tr>
<tr>
<td>Winzo</td>
<td>4-8</td>
<td>2-8</td>
<td>14/15</td>
<td>14/15</td>
</tr>
<tr>
<td>Jozani</td>
<td>5-2</td>
<td>1-5-9</td>
<td>2/8</td>
<td>2/8</td>
</tr>
</tbody>
</table>

### Identification of *C. ruminantium*

During the period of field exposure at Kipange five animals in group 1 and one animal in group 2 died of heartwater, which was confirmed by the identification of *C. ruminantium* in brain crush smears (Purchase 1945). In five of the cases the disease ran an acute or peracute course with one to three days of pyrexia and depression followed by death. In the sixth an­imal the disease occurred shortly after a moderate East Coast fever reaction. *T. parva* schizonts were seen in smears from tissues of this and two other animals after death. Nervous signs, mainly hyperaesthesia and incoordination, were shown by two animals and three had diarrhoea. On post mortem ex­amination there was a generalised congestion of mucous membranes, hydrothorax and hydropericardium in all cases. Gastrointestinal haemorrhages were found in two of the ani­mals which had diarrhoea. Heartwater has been diagnosed in one field case since this second East Coast fever trial. *C. ruminantium* was detected in brain crush smears from a four­month-old zebu × Friesian calf kept at Mgeni-haji (Fig 1) which had shown nervous signs before death.

### Discussion

These results suggest that *B. bovis* is common and wides­pread in indigenous cattle on Unguja. Clinical disease has not previously been diagnosed so it is probably endemic in the cattle population, as on Pemba (Woodford and others 1990). The lower prevalence of antibodies in cattle around Jozani, an area of marginal grazing and low cattle density, is probably a reflection of a reduced reinfection rate.

The vector of heartwater, *A. variegatum*, is found through­out Unguja but *C. ruminantium* appears to be more localised in distribution, because five of the animals which died of heartwater in trial 2 had survived field exposure without tick control for 50 days at Kizimbani and 13 days at Tunguu (Fig 1). Heartwater was an important cause of death in East Coast fever immunisation trials in mainland Tanzania (Uilenberg and others 1977) and both *B. bovis* and heartwater were among the non-theilerian causes of mortality in trials in Malawi (Radley 1985). The presence of these two parasites further complicates the efforts to increase the crossbred cattle population on Unguja and makes it unlikely that immunisa­tion against East Coast fever alone would reduce mortality significantly, unless other tick-borne diseases were con­trolled.
Acknowledgements.—We should like to thank the director of the Department of Livestock Development, Ministry of Agriculture, Livestock Development and Natural Resources, Zanzibar for permission to publish these results. This work would not have been possible without the able assistance of Azani Ussi, Ramadhah Ali, Said Khalafan and Zakia Omar. Dr F. Musisi’s help in the first diagnosis of heartwater is warmly appreciated. The staff of the protozoology section, Centre for Tropical Veterinary Medicine, Edinburgh, collaborated with J. Woodford in establishing a B. bovis ELISA with which to investigate babesiosis in Zanzibar. Mr J. Katende, ILRAD, kindly supplied B. bigemina antigen. This work was carried out while E. F. was receiving a postgraduate training award from the Overseas Development Administration, United Kingdom and J. D. W. was employed by the Development Corporation Division, Department of Foreign Affairs, Eire. This is ILRAD publication number 772.

References

Efficacy of an inactivated vaccine against hydropericardium syndrome in broilers

M. Afzal, I. Ahmad

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Field trials were used to assess the efficacy of an inactivated vaccine against hydropericardium syndrome in broiler chickens. A single vaccination at 10 to 12 days old was effective for the control of the syndrome; mortality in the vaccinated birds was 0-52 per cent compared with 5-34 per cent in unvaccinated birds kept at the same premises. Vaccination was also effective when carried out in the face of an outbreak; mortality in the vaccinated infected birds was 2-33 per cent compared with 10-27 per cent in unvaccinated infected birds. The data indicate that a formalinised vaccine prepared from the liver of experimentally infected birds could be used for the control of hydropericardium syndrome.

HYDROPERICARDIUM syndrome, or Angara disease, an apparently new disease, has resulted in large economic losses to the broiler industry in Pakistan. The disease first appeared in a broiler growing area near Angara Goth in Karachi in August and September 1987 (Anonymous 1988). The disease spread to all the broiler growing areas in the country within a year. The disease has typically been observed in three- to six-week-old growing broiler chickens and results in mortality up to 30 to 60 per cent. The syndrome has been characterised by the accumulation of a clear, straw-coloured fluid in the pericardium, a swollen, discoloured and friable liver and pale enlarged kidneys with distended tubules (Anonymous 1988, Anjum and others 1989, Cheema and others 1989, Jaffery 1989). Histologically, basophilic intranuclear inclusion bodies have been observed in the hepatocytes (Ahmad and others 1989, Anjum and others 1989, Cheema and others 1989). Although the exact aetiology of the syndrome remains unknown, an adenovirus has been isolated from the liver of infected chickens (Cheema and others 1989).

The efficacy of an inactivated vaccine against the hydropericardium syndrome has been reported in a preliminary experimental challenge-protection study (Chishti and others 1989). This report describes field trials with the same inactivated vaccine.

Materials and methods
Preparation of vaccine

The vaccine was prepared from livers collected from naturally or experimentally infected birds. The method for the experimental reproduction of the disease has been described previously (Cheema and others 1989). Briefly, three-week-old chicks were inoculated subcutaneously with a 30 per cent suspension of liver tissue collected from birds infected in a natural outbreak. The liver tissues were stored at −30°C and used within one month. The vaccine for the field trials was prepared as described previously (Chishti and others 1989) with minor modifications. The liver tissue was homogenised in a blender (Matsushita Electric Industrial) to make a 10 per cent suspension in 0·85 per cent saline. The suspension was sonicated in 400 ml batches at 50 watts for three minutes using a 19 mm probe (Labsonic 2000; B. Braun Melsungen). Formaldehyde (0·1 per cent) was added with continuous stirring. Antibiotic (streptomycin 1 mg/ml and penicillin 1000 iu/ml) were added and the preparation was left overnight at 4°C. Next day, the vaccine was distributed into 200 ml vials and stored at 4°C until use. Each batch was used within one month of its preparation. The sterility of each batch of vaccine was checked by injecting 1·0 ml of vaccine into 10-day-old broiler chicks.
TABLE 1: Efficacy of an inactivated vaccine against hydropericardium syndrome in broilers

<table>
<thead>
<tr>
<th>Occurrence of disease</th>
<th>Number of farms</th>
<th>Number of birds Vaccinated</th>
<th>Unvaccinated</th>
<th>Vaccinated Mortality</th>
<th>Unvaccinated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease present</td>
<td>15</td>
<td>38,800</td>
<td>13,100</td>
<td>418 (10-8%)</td>
<td>1399</td>
</tr>
<tr>
<td>Disease absent</td>
<td>15</td>
<td>40,900</td>
<td>13,100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>30</td>
<td>79,700</td>
<td>26,200</td>
<td>418 (0-52%)</td>
<td>1399 (5-34%)</td>
</tr>
</tbody>
</table>

Field trial

The vaccine was tested at poultry farms registered through the Directorate of the Poultry Development Centre, Rawalpindi. Ten- to 12-day-old broiler chicks were injected subcutaneously with 0-25 ml of the vaccine, either by Poultry Development Centre vaccinators or by the poultry farmers. Control birds were left unvaccinated on each farm, in each shed, and separated by a wire mesh from the vaccinated birds. Farmers were asked to report all dead birds to the Poultry Development Centre; each farm was visited weekly and the data recorded.

Results

Thirty farms, with a total population of 105,900 broiler chicks, were included in the study; all of them had previously suffered from the hydropericardium syndrome. During the trials, the mortality due to the syndrome was 0-52 per cent among the vaccinated birds and 5-34 per cent among the unvaccinated birds (Table 1); the difference was statistically significant (P < 0-01). The disease did not occur on 15 farms. On four of the 15 farms where disease did appear, there was no mortality among the vaccinated birds. In the other flocks, the mortality was significantly lower in the vaccinated birds (1-08 per cent) than in the unvaccinated control birds (10-7 per cent). Mortality among the vaccinated birds occurred mainly during the fourth and fifth weeks after vaccination.

It was also observed that on the farms where the disease did not appear the weight gains of the vaccinated and unvaccinated birds were similar, whereas in the infected flocks the vaccinated birds gained more weight than the unvaccinated birds.

Two flocks were vaccinated in the face of outbreaks of the disease. Both of these flocks were three weeks old and mortality of 0-39 per cent had occurred during the three days before they were vaccinated. Vaccination significantly (P < 0-01) reduced the mortality in the infected flocks (Fig 1). Mortality due to hydropericardium syndrome continued among the vaccinated birds for five days after vaccination and only occasional deaths occurred subsequently; among the unvaccinated control birds mortality continued for 16 days, and the total losses in this group were 10-3 per cent.

Discussion

The data presented in this report indicated that an inactivated vaccine prepared from the livers of infected birds provided good protection against hydropericardium syndrome in the field. However, the protection provided by this inactivated product did not last long and some cases of disease occurred in the vaccinated birds during the fourth and fifth weeks after vaccination. Since broilers are normally marketed at six weeks old in Pakistan, vaccination at 15 to 18 days old, or two vaccinations at 10 and 21 days old might be expected to give better protection.

The data presented in Fig 1 indicate that vaccination with this inactivated product was useful even in the face of an outbreak of the disease. It seems that good immunity is developed within five days after vaccination. Whether the protection is cell-mediated or humoral is unknown.

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References


Analysis of the movements of cutting horses

A CUTTING horse sorts individual calves from a herd of cattle and keeps them separate; it must be able to react quickly to the movements of a calf and accelerate rapidly in either direction. In competitions a good horse can win large prizes. The abilities of 12 cutting horses, which were ridden by the same trainer, were analysed by recording their movements by high-speed cinematography in a standard test in which they had to follow a flag which was moved mechanically.

The horses which had won more prize money reacted more quickly to the start and end of the movements of the flag, and kept closer to the flag throughout the test.