

Investigation of a syndrome characterised by passage of red urine in smallholder dairy cattle in East Usambara Mountains, Tanzania

E D Karimuribo^{a*}, E S Swai^b and P K Kyakaisho^c

ABSTRACT

A case-control study was carried out to investigate a syndrome in smallholder dairy cattle in East Usambara Mountains characterised by urination of clotted blood. Smallholder dairy farms with the problem (cases) were matched with nearest farms without the problem (controls). In total, 30 farmers from Mbomole (19), Shebomez (9) and Mlesa (2) villages in Amani division participated in the study. Using a structured questionnaire, information on risk factors associated with conditions characterised by passage of red urine in cattle was collected. In addition, serum samples from 80 smallholder dairy animals were collected and submitted for serodiagnosis of leptospirosis and babesiosis by microscopic agglutination test (MAT) and an indirect enzyme-linked immunosorbent assay (ELISA), respectively. Laboratory analysis showed that the seroprevalence of leptospirosis and babesiosis was 21.3% and 46.3%, respectively and there was no significant difference between 'case' and 'control' farms ($P > 0.05$), hence the occurrence of urination of clotted blood syndrome in Amani was not explained. However, bracken fern (*Pteridium aquilinum*) was found to be ubiquitous in the area, and also found to be widespread in all areas used as sources of animal fodder. Given the presence and distribution of bracken ferns and clinical signs and *post-mortem* lesions described by informants, chronic bracken-fern poisoning is more likely to be associated with the syndrome affecting animals in the study area. However, further investigation is required to confirm this observation so that appropriate control strategies can be devised.

Key words: clotted blood syndrome, East Usambara, smallholder dairy cattle, Tanzania, urine.

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INTRODUCTION

Smallholder dairy farming is a relatively recent undertaking in Tanzania. In Tanga region, most of the smallholder dairy animals were supplied by the Tanga Dairy Development Programme (TDDP), which was a bilateral organisation established in 1985 by the Tanzania government with assistance from the Dutch government. The main objectives of promoting smallholder dairy farming were to alleviate poverty and improve human health through availability of milk for human consumption and sale.

Smallholder dairy cattle were introduced in Amani, one of the 6 divisions of Muheza district in Tanga region located on slopes

of the East Usambara Mountains, during the mid-1980s by the TDDP¹⁰. Prior to this period, a few crossbred dairy cattle were owned by the National Malaria Research Institute. As of December 2004, the number of reporting farmers and dairy cattle in Amani were reported to be 553 and 1378, respectively¹¹. Smallholder dairy farming is perceived as one of the major sources of income to farmers in East and West Usambara Mountains⁸. During 2003, a syndrome characterised by passage of clotted blood in the urine was identified through participatory analysis of the situation by farmers under the Eastern Zone Client Oriented Research and Extension (EZCORE) in Amani to be an important animal health constraint affecting smallholder dairy animals in the area. A number of infectious and non-infectious conditions may present 'red urine', which may be associated with either haemoglobinuria or haematuria. Common conditions in cattle that are characterised by passage of red urine include babesiosis, bacillary

haemoglobinuria, bovine enzootic haematuria, contagious bovine pyelonephritis and leptospirosis¹⁴. The EZCORE programme funded this study which aimed at conducting an investigation in order to shed light on conditions likely to be associated with 'passage of clotted blood in the urine' syndrome so that farmers could be advised on proper control and preventive measures.

MATERIALS AND METHODS

Study area

This study was carried out during June and July 2005 in Amani division of Muheza district, located on the eastern slopes of the Usambara Mountains, in Tanga region. The study area is located c. 75 km northwest of Tanga city and 32 km north of Muheza town and lies between longitude 38.5–39.5°E and latitude 4.5–5.0°S. The average annual rainfall in Amani is 1945 mm (range 1377 to 3505 mm) and the annual mean temperature is 20.8 °C⁸. The division is located at an altitude ranging between 400 m and 1500 m above sea level and has high humidity. Most of Amani division is covered with tropical evergreen rainforest. The soils in the study area are of the type generally found in the rainforest areas, *i.e.* deeply weathered red loam soils derived from gneiss, granulite or pegmatite which is acidic in nature (pH 4.6 to 5.2).

Study design

Due to the sporadic nature and few cases of 'urination of clotted blood' syndrome in dairy animals in the study area, a case-control study design was considered suitable to investigate the syndrome. The sampling unit was a farm in which a 'case' farm was defined as one that had an animal passing clotted blood in the urine during the period of practising dairy farming in Amani division and a 'control' farm was a nearest smallholder farm neighbouring a 'case' farm in the study area but without animal(s) passing clotted blood in the urine. In total, 30 farms (15 farms in each category) were recruited to participate in the study and

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were from Mbomole (19), Shebomeza (9) and Mlesa (2) villages.

Of 80 animals that were sampled, 71 (88.8 %) were females and 9 (11.2 %) were males. The average age of sampled animals was 4.1 ± 2.6 years (range 1 month to 13.25 years). Most animals ($n = 69, 86.3 \%$) were home-bred and only 11 animals (13.8 %) originated from outside Amani division, mainly from other areas within Tanga region, although a few animals originated from distant areas such as Arusha (2) or Coast (1) regions. All animals sampled were under zero-grazing management systems where owners cut fodder to feed their animals indoors throughout the year.

Additional information was collected through in-depth interview of key informants, mainly animal health service providers (livestock field officers and community-based animal health workers) who attended and carried out *post mortem* examination of animals that died or were slaughtered because of suffering from 'urination of blood' syndrome. This included questions on how they manage affected animals and description of lesions seen during *post mortem* examination, which was supported by records available on each farm. In addition, on-farm inspection of cut fodder was carried out to identify common fodder species, including the presence of poisonous plants, in animal feeds on the day of the farm visit. This inspection was complemented by transect walks in areas used as sources of fodder (established pasture plots, valley bottoms and swampy areas, and forest reserve areas) in order to determine distribution of bracken fern in East Usambara Mountains.

A structured questionnaire was used to collect information on 'case' and 'control' farms with respect to risks and exposure to some factors that might predispose animals to conditions presenting with passage of red urine that were selected for investigation, *i.e.* babesiosis, leptospirosis and bracken-fern poisoning. The choice of conditions to investigate was based on the epidemiology, previous experience of animal health conditions in the area and limited resources available for the study. As an internal way of validating some information, questions to cross-check the validity of some responses were included in the questionnaire to control the quality of information collected. To minimise recall bias, information related to occurrence of events during 2004 was collected. In addition, blood samples were collected from 80 smallholder dairy animals by jugular venipuncture and serum separated by centrifugation at 3000 *g* for 20 minutes. Serum samples were then transported in

refrigerated cool boxes to a laboratory for serodiagnosis of babesiosis and leptospirosis.

Laboratory analysis

Laboratory analysis of serum samples was commissioned from specialised laboratories in Tanzania based at the Sokoine University of Agriculture Pest Management Centre (leptospirosis) and Veterinary Investigation Centre, Arusha (babesiosis). The microscopic agglutination test (MAT) using a panel of 5 serovars carefully selected based on previous experience in Tanga region¹⁹ and presence of carrier animals such as dogs and cats was used for serodiagnosis of leptospirosis. The antigens used included strains and locally prevalent serovars of the following serogroups (serovars in brackets): *Canicola* (*canicola*), *Grippytyphosa* (*grippytyphosa*), *Sejroe* (*hardjo*), *Icterohaemorrhagiae* (*icterohaemorrhagiae*) and *Pomona* (*pomona*). Briefly, the test was carried out in microtitre plates using PBS pH 7.2 as a diluent of test serum. *Leptospira* culture (5 days old) of the above serovars was used as antigen that was added to the microtitre plate wells before the mixture was incubated at 30°C for 2 hours. The results were read using a dark-field microscope and individual sera were considered positive if agglutination was present at dilutions of 1/160 or more (*i.e.* antibody titre of $\geq 1:160$), using agglutination of 50 % or more of the leptospire as the end point¹⁰.

Another aliquot of the serum samples was subjected to an indirect enzyme-linked immunosorbent assay (ELISA) in order to evaluate the level of antibodies to *Babesia bigemina* and *B. bovis* using standard procedures⁹. The results were expressed as percentage positivity (PP) values of optical densities relative to that of a strongly positive control serum¹⁸. The

threshold levels of PP for positivity of *B. bigemina* and *B. bovis* were 15 % and 25 %, respectively.

Data storage and analysis

A database was developed to store quantitative data using Epi Info software³. The same programme was used to compute descriptive statistics of variables collected during the study. Comparison of proportions of independent variables was done using chi-square (χ^2) tests while analysis of variance (ANOVA) was used to test for significant differences between means of the continuous variables. Bartlett's test for inequality of population variance was used to assess homogeneity of variances and a small *P*-value ($P < 0.05$) was used as basis for adopting the Kruskal-Wallis test of statistical significance of the 2 groups that were compared instead of ANOVA. A critical probability of 0.05 was adopted throughout as a cut-off point for statistical significance between groups compared.

RESULTS

Cattle ownership and animal health conditions perceived by farmers to be important.

All 30 selected smallholder dairy farms were visited and farmers interviewed in the 3 villages of Amani division (a 100 % voluntary response rate). The average number of dairy cattle owned per household was 5.4 ± 4.1 (mean \pm standard deviation), which ranged between 2 and 20 animals per household. The majority of farms (>53 %) had 3 or 4 dairy animals; however, on only 2 farms were there more than 15 animals. Most farmers had recently entered smallholder dairy farming (Fig. 1).

A number of disease conditions were reported to be prevalent in Amani area.

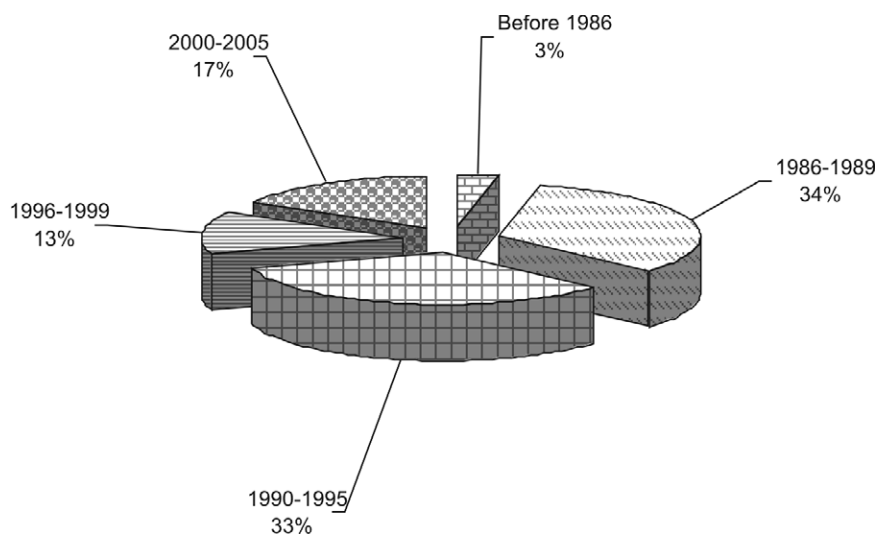


Fig. 1: Distribution of smallholder dairy farmers who participated in the study by year in which they received their first dairy animal.

Overall, the most common diseases mentioned were anaplasmosis (85.7 %), mastitis (64.3 %) and urination of clotted blood (50.0 %). Other conditions reported to occur less commonly were bloat (3.6 %), pneumonia especially in calves (3.6 %), hoof overgrowth (3.6 %) and trypanosomiasis (3.6 %). The respondents were also asked to rank diseases they had mentioned in the order of importance. The 3 priority diseases to farmers were mastitis (1st), urination of clotted blood (2nd) and anaplasmosis (3rd).

'Urination of clotted blood' syndrome

Of the 15 'case' farms visited, only 4 (26.7 %) had 1 or more animals urinating blood at time of the field visit. According to the farmers, cases of urinating clotted blood were observed since 1986. The trend of occurrence of cases of animals urinating clotted blood as reported on 11 farms between 1986 and 2004 is summarised in Fig. 2. From these results, the trend of occurrence of cases of animals urinating clotted blood is increasing.

According to both farmers and animal health service providers, urination of clotted blood was reported to occur in phases. It was reported that during early stages of the disease, affected animals show mild signs characterised by change of colour of urine, which turns brownish and was described by informants as 'black tea-like urine'. This phase may continue for about 1 month when the next phase ensues. The second phase is characterised by passage of frank bloody urine, which is watery in nature. During this phase, affected animals are alert and their appetite remains good. The third phase is characterised by passage of clotted blood in urine. Farmers usually notice 'clots of blood' on the floor of the animal house in the morning. At this stage, the perineal region of affected animals is usually soiled with clotted blood and this is the stage when owners of affected animals become concerned and start seeking alternative interventions. Most affected animals during this stage are emaciated. For lactating cows, there is significant drop in milk yield, which was estimated to decrease by almost half (50 %) of the original production. A number of management strategies were reported to have been attempted, which included antibiotic injection (usually penicillin-dihydrostreptomycin preparations or oxytetracyclines), antiprotozoals (usually diminazene diaceturate such as Berenil[®]) and even some vitamins, mainly Vitamin K. Livestock field officers also reported encouraging good management of affected animals, including provision of a good plane of nutrition, especially feeds rich in proteins (such as cotton seed cake)

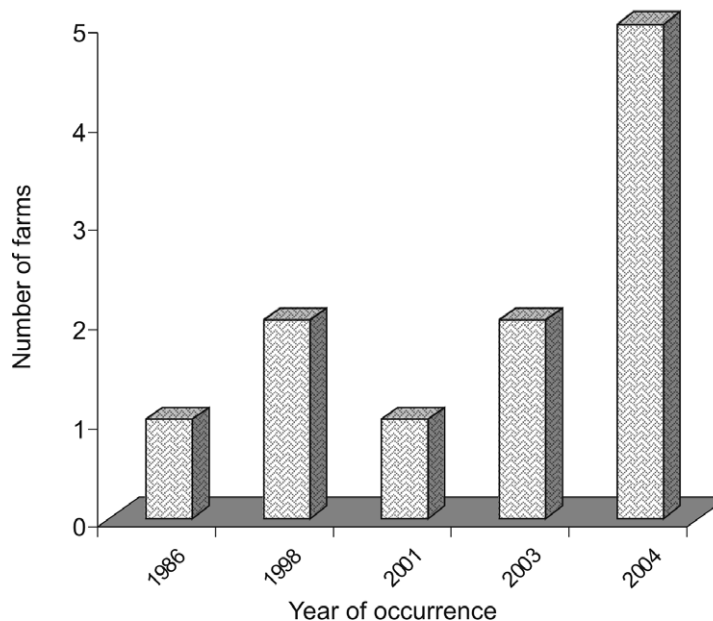


Fig. 2: Trend of occurrence of cases of urination of clotted blood in Amani division between 1986 and 2004.

and minerals. Although the interventions above were considered to help in reducing the severity of the condition, both farmers and animal health service providers considered that affected animals could not recover from this syndrome and the fate would always be death. Due to past experience and beliefs, almost all 'case' animals are slaughtered during the third phase of clinical manifestations that is associated with urination of clotted blood. Lesions reported by livestock field officers who had conducted *post-mortem* inspection were confined to the urinary tract system, mainly in the urinary bladder. Some of the lesions were described as failure of bladder to collapse even if it does not contain urine. When opened, the urinary bladder of affected animals was reported to have a smaller lumen containing thick, bloody contents. The wall of the bladder was thickened and 3-layered, with the innermost layer being raw while peripheral ones were composed of dead tissue.

Prevalence of diseases characterised by passage of red urine

Leptospirosis

A total of 80 serum samples from dairy cattle in Mbomole (49), Shebomezha (25)

and Mlesa (6) villages were subjected to MAT. Of the 80 samples analysed, 17 (21.3 %) had antibody against at least 1 of the 5 serovars used at a titre equal or above the cut-off point of 1:160. The mean age of seropositive animals (5.3 ± 3.4 years, range 0.25–13.25 years) was significantly higher than that of seronegative animals (3.7 ± 2.3 , range 0.08–10.5 years) ($P = 0.027$). The overall serovar-specific seroprevalence in the screened animals at the cut-off titre of 1:160 is presented in Fig. 3. The highest prevalence was that of *L. hardjo* (12.5 %) followed by *L. icterohaemorrhagiae* (3.8 %), *L. grippotyphosa* (2.5 %) and *L. pomona* (2.5 %). *Leptospira canicola* was not found in the area. Table 1 shows distribution of positive sera by antibody levels. The highest titre was 1:640 detected for *L. hardjo* (1), *L. icterohaemorrhagiae* (1) and *L. pomona* (2) serovars.

Distribution of seropositive animals by study village is summarised in Table 2. At the cut-off titre of $\geq 1:160$, *L. hardjo* was prevalent in animals sampled from Shebomezha (8.0 %), Mbomole (14.3 %) and Mlesa (16.7 %) villages. For *L. icterohaemorrhagiae*, seropositive animals were from Shebomezha (4.0 %) and Mbomole (4.1 %) villages only. For indication of

Table 1: The distribution of positive sera by *Leptospira* antibody levels.

Serovar	Antibody titre			Total (%)
	1:160	1:320	1:640	
<i>L. canicola</i>	–	–	–	0 (0.0)
<i>L. grippotyphosa</i>	2	–	–	2 (11.8)
<i>L. hardjo</i>	4	5	1	10 (58.8)
<i>L. icterohaemorrhagiae</i>	2	–	1	3 (17.6)
<i>L. pomona</i>	–	–	2	2 (11.8)

exposure to the 5 *Leptospira* serovars screened, the highest seroprevalence was that of *L. icterohaemorrhagiae* recorded in Shebomeza (68.0 %) and the least prevalence was for *L. canicola* recorded in Shebomeza (16.0 %) and Mbomole (8.2 %) villages only. The seroprevalence for the 5 serovars was also grouped by 'case' and 'control' animals as sampled during the study (Table 3). Overall, of 39 samples from 'case' animals analysed, 10 animals (25.6 %) had antibody levels detected at $\geq 1:160$ level while in the 'control' group, 7 (17.1 %) out of 41 animals screened had antibody levels detected at the same level. The differences were not statistically significant ($P > 0.05$), indicating that leptospirosis was not responsible for the occurrence of 'clotted-blood syndrome' in the study area.

Babesiosis

Of the 80 samples analysed for babesiosis, 37 (46.3 %) were positive to either *B. bigemina* or *B. bovis* antibodies (Table 4). For *B. bigemina* and *B. bovis*, the seroprevalence was 38.8 % and 31.3 %, respectively. There was no significant difference between the seroprevalence in animals sampled on 'case' farms (41.0 %) and 'control' farms (51.2 %) ($P > 0.05$). Similar observation was noted with respect to the seroprevalence of *B. bigemina* in animals from 'case' (33.3 %) and 'control' (43.9 %) farms ($P > 0.05$). The same trend was also observed for *B. bovis*, where the seroprevalence on 'case' and 'control' farms was 33.3 % and 29.3 %, respectively ($P > 0.05$). The seroprevalence of *B. bigemina* and *B. bovis* did not differ significantly from the source (homebred *vs* brought-in) ($P > 0.05$), age ($P > 0.05$) or sex ($P > 0.05$) of animals either.

Bovine enzootic haematuria

Chronic bracken-fern poisoning is

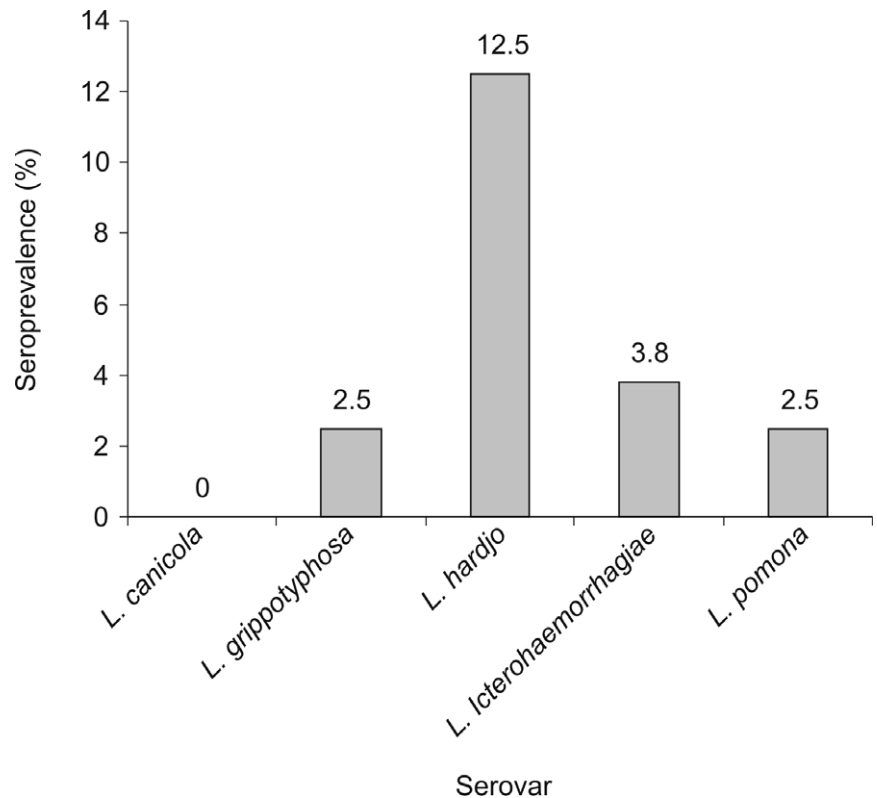


Fig. 3: Seroprevalence of *Leptospira* serovars in smallholder dairy cattle sampled in Amani ($n = 80$).

known to be responsible for bovine enzootic haematuria in many countries. The risk of animal exposure to this plant was assessed by on-farm inspection for this plant in animal feeds on farm visit day. We also assessed the ability of both farmers and animal attendants to identify bracken fern and also which intervention strategies were used to avoid feeding this poisonous plant to dairy cattle. During farm visits, it was observed that bracken fern was widespread in Amani area. The plant was observed to be inter-cropped with pasture species in most of the fodder plots visited. The bracken fern, according to farmers and attendants, was reported

to be mainly found in valley bottoms (47.0 %) and swampy areas (18.2 %), which are locally known as 'vitivo', and forest reserve (12.2 %). All areas mentioned to have bracken ferns are main sources of fodder cut for feeding smallholder dairy cattle in Amani division. At least 70 % of the fodder inspected on the day of farm visit had bracken fern and there was no significant difference between the 'case' and 'control' farms ($P > 0.05$) (Table 5). Other plant species found in the fodder were Guatemala grass (*Tripsacum laxum*) (100.0 %), a wild plant locally known as 'tikini' (*Asystasia gangetica*)²⁰ (96.7 %) and leguminous plants (93.3 %). Less common

Table 2: The serovar-specific seroprevalence of leptospirosis in 3 villages that participated in the study.

Village (n)	Seroprevalence, % (n)				
	<i>L. canicola</i>	<i>L. grippityphosa</i>	<i>L. hardjo</i>	<i>L. icterohaemorrhagiae</i>	<i>L. pomona</i>
Mbomole (49)	0.0 (0)	4.1 (2)	14.3 (7)	4.1 (2)	4.1 (2)
Mlesa (6)	0.0 (0)	0.0 (0)	16.7 (1)	0.0 (0)	0.0 (0)
Shebomeza (25)	0.0 (0)	0.0 (0)	8.0 (2)	4.0 (1)	0.0 (0)
Overall (80)	0.0 (0)	2.5 (2)	12.5 (10)	3.8 (3)	2.5 (2)

Table 3: The serovar-specific seroprevalence of leptospirosis in the 'case' and 'control' groups screened in the study.

Group (n)	Seroprevalence, % (n)				
	<i>L. canicola</i>	<i>L. grippityphosa</i>	<i>L. hardjo</i>	<i>L. icterohaemorrhagiae</i>	<i>L. pomona</i>
Case (39)	0.0 (0)	0.0 (0)	15.4 (6)	5.1 (2)	5.1 (2)
Control (41)	0.0 (0)	4.9 (2)	9.8 (4)	2.4 (1)	0.0 (0)
Overall (80)	0.0 (0)	2.5 (2)	12.5 (10)	3.8 (3)	2.5 (2)

Table 4: Seroprevalence of babesiosis in serum samples from Amani division.

Variable	No. samples	No. positive	Percent age	95 % CI
Overall <i>Babesia</i> [†]	80	37	46.3	35.0–57.8
<i>B. bigemina</i>	80	31	38.8	28.1–50.3
<i>B. bovis</i>	80	25	31.3	21.3–42.6
Overall <i>Babesia</i> [†] 'case'	39	16	41.0	25.6–57.9
Overall <i>Babesia</i> [†] 'control'	41	21	51.2	35.1–67.1
<i>B. bigemina</i> 'case'	39	13	33.3	19.1–50.2
<i>B. bigemina</i> 'control'	41	18	43.9	28.5–60.3
<i>B. bovis</i> 'case'	39	13	33.3	19.1–50.2
<i>B. bovis</i> 'control'	41	12	29.3	16.1–45.5

[†]Overall *Babesia* represents overall results for either *B. bigemina* or *B. bovis* combined as 1 variable.

Table 5: Type of plants found in the fodder inspected on the day of farm visit.

Plant species	Case (n = 15) Frequency, % (n)	Control (n = 15) Frequency, % (n)	Overall (n = 30) Frequency, % (n)
Bracken fern	60.0 (9)	80.0 (12)	70.0 (21)
Leguminous plants	93.3 (14)	93.3 (14)	93.3 (28)
<i>Asystasia gangetica</i> (' <i>tikin</i> ')*	93.3 (14)	100.0 (15)	96.7 (29)
<i>Caliandra</i> spp	60.0 (9)	53.3 (8)	56.7 (17)
Guatemala grass	50.0 (15)	50.0 (15)	100.0 (30)
Napier grass	60.0 (9)	46.7 (7)	53.3 (16)

*Local name for a leguminous wild plant commonly used as fodder in the area.

plants in fodder were *Caliandra* spp. (56.7 %) and napier grass (*Pennisetum purpureum*) (53.3 %). The majority of leguminous plants observed in fodder were tick clover (*Desmodium* spp.) (75.0 %) and *Glycine wightii* (25.0 %).

DISCUSSION

Farmers who took part in this study are typical smallholders, like in other areas in Tanzania, who own a small number of animals per household¹². The majority of farmers (63 %) had few (<10) years exposure to dairy farming, indicating that they had little experience in this undertaking. The animal health conditions perceived to be important in East Usambara area are different from those considered to be of utmost importance in other smallholder dairy-farming areas in Tanzania, which are mainly tick-borne diseases, especially East Coast fever (ECF), babesiosis, anaplasmosis and heartwater^{7,14,16}. In extensive management systems, major animal health constraints in Tanzanian cattle include contagious bovine pleuropneumonia, tick-borne diseases, foot-and-mouth disease, trypanosomosis and lumpy skin disease⁷. The unique situation of cattle diseases in East Usambara may be partly explained by geo-climatic factors and isolation of the Amani area, which has mountainous terrain and is only accessed by 1 road. This limits the number of new animals likely to introduce diseases and vectors of diseases in the area⁸. Of particular concern was the syndrome

characterised by 'urination of clotted blood', which is not common in other smallholder dairy-farming areas in Tanzania.

The seroprevalence of leptospirosis recorded in the current study (21.3 %) is higher than that reported earlier in Tanga (10.8 %) and in Muheza district (10.3 %)¹⁹. With the exception of *L. tarassovi*, however, findings of the current study tally with findings by Swai *et al.*¹⁹ who also reported predominance of *L. hardjo* followed by *L. pomona* and *L. grippotyphosa* serovars in Tanga region. Findings of this study which indicated that older cattle were more likely to be seropositive to leptospira than young ones have also been reported in other countries¹⁰. Lack of a statistical difference in the seroprevalence of leptospirosis on 'case' and 'control' farms indicates that leptospirosis was not primarily responsible for the 'urination of clotted blood' syndrome in the study area. Similarly, lack of significant difference between the seroprevalence of *B. bigemina* and *B. bovis* between animals in the 'case' and 'control' groups implies that babesiosis was not the main factor responsible for cases of urination of clotted blood amongst dairy animals in Amani. However, the seroprevalence of *B. bigemina* and *B. bovis* reported in this investigation (38.8 % and 31.3 %, respectively) was higher than that reported earlier (<20 % for *B. bigemina* and <10 % for *B. bovis*) in the same area^{17,18}. Given the findings of the overall *B. bigemina* and

B. bovis seroprevalence of >50 %, occurrence of *Babesia* infection in Amani is considered to be in an 'endemic unstable' state¹⁵. It is possible that following phasing out of the then TDDP in the study area in early 2000s and subsequent supervision of all Heifer-in-Trust (HIT) scheme activities by farmers' groups and networks might have resulted in poor control of vectors (ticks) and consequently higher prevalence of tick-borne diseases. Recent studies on tick control in smallholder farms in Tanga region including Amani have identified a gross misuse (under and over dilution) of on-farm diluted acaricide on these farms¹⁶. The relationship between sero-conversion of most tick-borne pathogens (including *B. bigemina* and *B. bovis*) and tick-control methods (spray or hand-dressing) was found to be poor, suggesting that the current control regimens are ineffective in preventing animals from being exposed to ticks. This was also a concern of key informants responsible for animal health delivery in Amani division during field visits.

Although there was no significant difference between the proportion of fodder containing bracken fern on 'case' and 'control' farms, the ubiquitous nature, clinical signs and *post mortem* lesions reported by informants in this study strongly suggest that the 'urination of clotted blood' syndrome was due to bracken-fern poisoning. Although the aetiology of bovine enzootic haematuria is still not well known, previous studies in other countries have associated it with chronic exposure of animals to bracken fern^{6,13}. The toxic principles of this plant include thiaminase 1, aplastic anaemia factor and a carcinogenic factor known as ptaquiloside²⁵. The ptaquiloside principle is mainly responsible for lesions of bovine enzootic haematuria, although other factors such as bovine papillomavirus type 2 have also been reported to play part in this condition²¹. It was found that smallholder dairy animals in East Usambara are constantly exposed to low amounts of bracken fern, which supports involvement of bovine enzootic haematuria in the 'urination of clotted blood' syndrome reported in the area. Usually this condition has a long incubation period before lesions develop in the urinary bladder, which leads to clinical signs in affected animals¹³. This supports findings in the current study of a gradual increase in the number of cases reported over time and it is most likely that the number of cases will continue to increase unless immediate intervention is made to prevent exposure of dairy cattle to bracken fern.

It can be concluded that through the current study, it is evident that small-

holder dairy cattle were exposed to *Babesia* and *Leptospira* infections. Seropositivity to *Leptospira* could have been due to natural infection, as there has been no vaccination against this condition in the study area and Tanga region¹⁹. The seropositivity to *Babesia* is most likely explained by exposure of animals to ticks and this could have been due to the ineffective tick-control methods used. Finally, the syndrome characterised by intermitted passage of clotted blood is most likely to be bovine enzootic haematuria caused by chronic ingestion of small quantities of bracken fern, a poisonous plant found to be ubiquitous in Amani division. However, confirmatory study is needed to support this proposition.

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