THE PREVALENCE OF SERUM ANTIBODIES TO TICK-BORNE INFECTIONS IN MBALE DISTRICT, UGANDA: BY AGRO-ECOLOGICAL ZONE, GRAZING MANAGEMENT AND AGE OF CATTLE

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Abstract

Between August and October 2000, a cross-sectional study was conducted in smallholder dairy farms in Mbale district, Uganda to assess the prevalence of ticks and tick-borne diseases under different grazing systems and agro-ecological zones (AEZs) and understand the circumstances under which farmers operated. A questionnaire was administered to capture information on dairy farm circumstances and practices. A total of 102 farms were visited and sera and ticks were collected from 478 animals. Sero-prevalence of tick-borne diseases (TBDs) was determined using an ELISA. Acaricides were used indiscriminately but the intensity of their use varied with the grazing system and AEZ. Cattle from different farms mixed for various reasons. During the dry seasons farmers have to get additional fodder from outside their farms. The prevalence of ticks and serum antibodies to tick-borne infections differed across the grazing systems and AEZs. The highest serum antibody prevalence (>60%) was recorded in the lowland AEZ under the free range and tethering grazing systems. The lowest tick challenge and serum antibody levels (<50%) were recorded in the midland and upland AEZs under zero-

grazing system. These findings suggest that endemic stability to East Coast Fever, babesiosis and anaplasmosis is most likely to have existed in the lowland AEZ, particularly, under the tethering and free-range grazing systems. Also, endemic stability for babesiosis existed in the upland AEZs. Endemic instability for ECF existed in the midland and upland AEZs. These structured observational studies are instrumental in planning of control strategies for ticks and TBD's since production systems and the cattle population at high risk of the diseases in the district have been identified.

Introduction

Mbale District is located in the eastern region of Uganda bordering with the Republic of Kenya in the east (Figure 1). The district covers a total land area of 2,504 square kilometers and lies approximately between latitude 0° 45' N; and O ^{0} 35' N and longitudes 34^0 and 34^0, 35' E. The district is made of five counties of Bungokho, Bubulo, Manjiya, Budadiri and Bulamburi. The district has diverse climatic and environmental conditions due to variation in altitude, rainfall and agricultural activities. The topography of the district can be conveniently divided into three distinct types: (1) Lowland at an altitude of 1100-1350 m above sea level. This merges into extensive undulating plateau but is occasionally interrupted by a few upland and mountain ridge extensions. (2) The upland, which starts from the upper levels of Mbale plain, rises to form a hill and valley topography. The altitude is about 1800m above sea level. (3) The Elgon mountain with its highest point 4,322m above sea level. The orographic effect of Mountain Elgon massif and the district's proximity to Lake Victoria increase the amount of rainfall and tamper the severity of the dry periods. The distribution of this rainfall is irregular and depends on the location. Accordingly, three agro-ecological zones (AEZs) are identified. These are the Lowland AEZ, the Midland AEZ and the Upland AEZ; the three falling in the lowland and upland typographical types (Figure 1).

The district is densely populated and so there is a general shortage of land for agriculture. This land shortage is compounded by the hilly landscape making some parts inhabitable. The majority of the farmers, therefore, are smallholder farmers living in scattered

homesteads where they keep limited number of cattle under zero-grazing, tethering, fenced and free-range grazing systems depending on land availability and the AEZ. In the midland and upland AEZ's (Budadiri, Bulamburi and Manjiya counties) perennial crops (banana and coffee) are produced while in the lowland AEZ (Bubulo, Bungokho and lower parts of Bulamburi) annual crops (maize and beans) are produced.

The district enjoys a favourable climate for agriculture and plant growth. This climate, however, also provides ideal conditions for ticks, the vectors of tick-borne diseases (TBDs), namely *Rhipicephalus appendiculatus* for East Coast Fever (E.CF.), *Boophilus decoloratus* for babesiosis and anaplasmosis and *Amblyomma veriegatum* for heartwater (Branagan, 1973). So cattle in the district are under constant threat of severe tick infestations and TBDs unless tick control measures are undertaken (Maywald 1987, Perry 1994).

Tick-borne infections are known to exert the greatest limitations for improved cattle production by causing serious debility, morbidity, mortality and production losses in susceptible exotic cattle, their crosses as well as in indigenous breeds of cattle raised in tick-borne disease free areas (Bram 1982, Yeoman 1966, Norval *et al*. 1992, Okello-Onen *et al*. 1994, Perry and Randolph 1999). Generally the occurrence and importance of TBDs is a reflection of complex interactions involving the causative organisms, the tick vectors, the vertebrate hosts and the environment (Norval *et al.* 1992). These interactions are driven and modified by a wide variety of factors ranging from climate, soil and vegetation to human activities including crop/livestock production systems and measures taken to control ticks and TBDs.

Control of TBD's in East Africa has proved difficult largely because of lack of epidemiological information (Norval et al. 1992) and because control strategies commonly applied are not integrated in the production system (Perry 1994). In most parts of Africa, earlier investigations were not location/production system-specific and did not target biological, management and social economic parameters of the production system to establish the presence and the magnitude of the TBD's problems (Pegram and Chizyka

1987, Amir and Knipscheer 1989). So the control efforts, in most cases have not been commensurate to the magnitude of the disease problem. In Uganda the influence of the different management systems; the disease control practices and the changing patterns of land use on the epidemiological status of the TBDs are not well understood. In the Kenya highlands (Deem *et al.* 1993, Gitau *et al..* 1994, Gitau *et al.* 1997, O'Callaghan 1998 and Gitau et al. 2000) have demonstrated that the prevalence of *Theileria parva* infections and the reported ECF morbidity, mortality and case-fatality can vary significantly by AEZs and grazing system and that these differences have important implications for both the impact and control of ECF.

The impetus to carry out this study in Mbale District came out of a diagnostic survey using participatory rural appraisal methodologies (LSRP Mbale District diagnostic and planning reports, 1999) which ranked TBDs and helminthosis as the most important constraints to dairy production. Formulation of appropriate control strategies was deemed the solution.

This study was, therefore, production system-specific with the objective of understanding to assess the prevalence of ticks and serum antibodies to tick-borne infections and the farmer circumstances and practices in Mbale District by AEZ, grazing system and age of the animals. The purpose was to characterize the potential risks and associated epidemiological states. This would help identify potential endemically stable and unstable areas. Existence of endemic stability in the district would imply that control could be selective, strategic and focused only on susceptible target cattle populations. This would reduce costs and environmental pollution associated with intensive acaricide use.

Materials and methods

Study design:

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The farms were selected by a stratified random sampling method. The strata were AEZs, grazing system and age of the animals. A sampling frame of the parishes (the smallest administrative units) and villages in each subcounty was obtained from the District Veterinary Office, Mbale. The parishes were classified according to the three AEZs and 6 parishes in each AEZ were selected using random numbers. The antibody prevalence was not known *a priori* and so a 50% prevalence and a 20% tolerable error were assumed when determining the number of farms to include in the study. The sample size was determined according to the method described by Martin et al. (1987). The number of required farms per AEZ was calculated to be 25. Since farm-to-farm variation was expected in some cases (although average serum antibody prevalence by parish was expected to be constant within an AEZ), there was need to inflate the sample size by 40%. This gave 35 farms in the 6 parishes of each AEZ. For each AEZ, it was determined *a priori* to select 8 farms from the fenced grazing system and 9 farms from each of the remaining grazing systems (zero-grazing, tethering and free range). The veterinarians in charge of the selected parishes were then asked to compile a sampling frame of the farms in each of the 4 grazing systems in the selected parishes. The required number of farms in each grazing system was selected using tables of random numbers. However, of the 105 farms selected for study, three farmers declined to participate. Except in a few cases (where there were many animals on a farm), all animals in the age groups 0-3, 4-12, 13-18 and >18 months, were sampled. Where the number of animals in an age group exceeded 10, systematic random sampling was applied to select at least 5 animals. A total of 478 animals were examined and sampled.

Data and sample collection

Ten milliliters of blood was collected from each animal in vacutainer tubes by jugular venipuncture. Two to three milliliters of this blood was transferred to small vials coated with EDTA from which thin and thick blood smears were made and stained with Giemsa to determine if it is infected with haemoparasites. Part of this blood was collected in capillary tubes to determine the Packed Cell Volume (PCV), using Haematocrit Centrifugation Technique (HCT). The rest of the blood in the vacutainer tubes was

stored in iceboxes while in the field and later refrigerated overnight in the laboratory. Serum samples were separated the following day and were stored at (-20°C). Antibodies against tick-borne parasites were detected in sera using an ELISA technique (Katende *et al.* 1998). The results were expressed as percent positivity (PP) according to Wright et al. (1993), simply expressed as (optic density of test serum/optic density of strong positive) x100. For *Theileria parva* and *T. mutans*, a sample was considered positive if the PP value was 20 or above; for *Babesia bigemina* and *Anaplasma marginale*, the cut-off PP value was 15, while for *B. bovis* the cut-off point was 30.

Whenever swollen parotid and/or prescapular lymph nodes were palpated, lymph node biopsy smears were made and stained with Giemsa to detect *Theileria* schizonts under microscopy. This was to provide information on the morbidity of East Coast Fever (ECF).

Ticks were counted from one side of the animal body. The tick species; their sex and the state of feeding of female ticks were also determined (Kaiser *et al.* 1982). At each farm, a structured questionnaire with close-ended questions was administered to the farmer or farm manager to capture information on farm circumstances (management practices; farmer's needs/problems or challenges; coping strategies and opportunities; gender roles and criteria for choosing disease control technologies).

Data storage and analysis

Data derived from questionnaires and laboratory examinations were stored in Microsoft Excel. In order to eliminate the interference of maternal antibodies in sera of calves (0 – 3 months old), sera from older calves aged 4 – 12, was used to detect sero-conversion and to assess the epidemiological states of TBDs. Animals were classified as positive or negative depending on whether they were above or below the PP cut-off point. Univariate associations between explanatory variables (e.g., AEZ and grazing system) and the outcome (PP group) were initially tested using the Chi square test. Logistic regression analyses were then performed using the GENMOD procedure in SAS (Statistical

Analysis Systems Institute, Cary, NC). Logistic regressions were performed on antibody prevalence for *T.parva, B. bigemina* and *A. marginale* using PP values as outcome variables against the potential risk factors. The associations were presented as odds ratios and their 95% confidence limits. Binomial proportions and their 95% confidence limits for each stratum of AEZ and grazing system were generated for all antibody prevalence data.

Results

Farm characteristics

Most of the dairy farm-related activities were carried out by the household head (in some cases these were widows), assisted by other members of the family including children. Hired labour was also used particularly in the rural areas. Housewives were increasingly involved in farm–related activities in areas bordering Mbale town. The source of water varied by grazing system and AEZ; the free ranging farmers mostly watered their animals directly in the rivers/swamps. The major dry season feeding strategy was to feed animals on fodder obtained outside the farm. Cattle from different farms mixed for various reasons: at watering points (for free range and tethering), during communal grazing (free range and tethering grazing systems) and when seeking a bull (under all grazing systems - including zero-grazers).

Distribution of the sampled animals

Of the animals sampled in the different grazing systems 14.3% and 34.5% were from fenced and free ranging grazing systems, respectively, while 25.5% were from each of the two grazing systems of zero-grazing and tethering. This reflected the predominance of the free-ranging grazing system in the district. In addition, of the sampled animals, 43.9%, 22.7% and 33.4% were from lowland, midland and upland AEZ, respectively. Further, 14.5%, 27.5%, 5.3% and 52.7% of animals sampled were 0-3, 4-12, 13-18 and

>18 months old, respectively. About 70% of the animals had a good body score (>3.0) although 5I.5% had a PCV below 28%, a 'PCV profile shift' indicative of anaemia in cattle (Woo, 1970).

Tick challenge

The commonest tick species recorded in decreasing order of abundance were *Rhipicephalus appendiculatus, Amblyomma variegatum, Boophilus decoloratus and R. evertsi evertsi*. Of these ticks, *R. appendiculatus* showed the highest challenge in the free range grazing system (Figure 2a). The challenge was extremely low in the zero-grazing system. The other tick species occurred in very low numbers. Considering all the tick species, the highest challenge was recorded in the lowland AEZ, followed by the midland AEZ (Figure 2b). The highest number of standard ticks (ticks that had fed and would engorge and detach in the following 24 hours) was recorded in the upland AEZ, particularly, in the free range and tethering grazing systems (Figure 2c).

The odds of the animals being infested with *R. appendiculatus* were approximately 1.3 times (lowland fenced), 183 times (lowland free range) and 365 times (lowland tethering) greater than in animals kept in the upland AEZ under zero-grazing system (Table 1). The animals within the age groups 0 – 3 and 4 – 12 months were at less risk of being infested with *R. appendiculatus* than the older groups (> 18 months).

As for *B. decoloratus,* the animals kept in the lowland (free range grazing system) and midland (tethered and zero-grazing systems) were at less risk of being infested than the animals in the upland AEZ under zero-grazing system (Table 2). As expected, calves (0–3 months old) were at a less risk of being infested with *B. decoloratus* than the older groups (>18 months).

Tick control

The farmers who practiced fenced and zero-grazing systems had better knowledge of the problems caused by ticks and TBDs than those who practiced tethering and free range grazing systems. Ticks and tick-borne diseases were controlled using acaricides belonging to the groups of organophosphates (OPs), amidines and synthetic pyrethroids.

These acaricides were used indiscriminately in all the grazing systems. However, they were used at different intensities, depending on AEZ and grazing system. Synthetic pyrethroids were popular in the fenced and zero-grazing systems, while amidines were equally used in the free-range, zero- grazing and tethering systems (Figure 3). OPs were popular in the free range and zero grazing systems. The commonest methods of acaricide application were hand-dressing, hand-spraying and pour-on; and the extent of their use varied in the different grazing systems. Some farmers in the free-range, zero-grazing and tethering grazing systems did not practice tick control (Figure 3).

Prevalence of serum antibodies to tick-borne infections

The prevalence of antibody to tick-borne infections differed across the grazing systems, age of animals and AEZs. In animals 4-12 months old, the serum antibody prevalence for *T. parva* was >60% in the free-range cattle in the lowland AEZ (Figure 4). Except for tethered cattle under the midland AEZ, the prevalence of antibodies to *T. parva* was below 50% in the midland and upland AEZs.

The prevalence of antibodies to *B. bigemina and A. marginale* in the 4-12 month age group varied greatly across the AEZs and grazing systems. *B. bigemina,* antibody levels above 60% occurred only in the lowland (free range grazing system) and upland (fenced and tethering grazing system). Animals older than 12 months under free range (lowland AEZ) and under fenced and tethering grazing systems (upland AEZ) also had a high prevalence of antibodies against *B. bigemina*. In cattle 4-12 months old, *A. marginale*, antibody levels above 60% were recorded only in the lowland AEZ under free range grazing system. The sero-prevalence of antibodies to *T. parva* was associated ($p < 0.05$) with AEZ, age and grazing system. The odds of being exposed to *T. parva* infection were 7.8 times greater (Lowland free-range grazing system) and 4.0 times greater (lowland tethering grazing system) than in animals kept in the upland AEZ under zero grazing (Table 3).

The situation in the case of *A. marginale* and *B. bigemina* was different from that of *T. parva* (Tables 4 and 5). In the case of *A. marginale*, there was a significant association ($p < 0.05$) between sero-prevalence and both age and AEZ. The prevalence decreased with increasing elevation in the case of the fenced and free-range systems (Figure 4).

As for antibodies to *B. bigemina*, there was an association ($p < 0.05$) between sero-prevalence and AEZ. Animals (4-12 months old) in the upland AEZ had higher sero-prevalence (Figure 4). The prevalence of antibodies to *B. bigemina* was, also, associated with age and grazing system ($p < 0.05$).

Discussion

The present study provided an opportunity to understand the farm circumstances and farm practices that have a bearing on the occurrence of ticks and TBDs in smallholder dairy farms in Mbale district. This logical approach has been adopted earlier by Gitau *et al*. (1997, 2000).

The highest tick challenge was recorded in the lowland AEZ, followed by midland and upland AEZs. This concurs with the results from Kenya highlands reported by Gitau *et al*. (1997). These results can be explained by the distribution of the ticks and farming practices in these strata. Climatically, the lowlands are more suitable for *R. appendiculatus* than the midlands and uplands (Mathysse and Colbo, 1987). Also, in the lowlands most of the animals were the local indigenous breeds kept under free-range grazing system; a system known to increase the spread of ticks among cattle herds. Many farmers in this AEZ reported not to have practiced tick control.

Low tick challenge was demonstrated in all zero grazing strata including lowland AEZ because in zero grazing systems tick challenge is almost exclusively through the importation of ticks on fodder from neighbouring farms, roadsides and/ or swamps and fodder banks. Since there is very little suitable habitat to support tick populations on zero-grazing farms, the tick challenge had to be very low. Therefore, there was a low

probability that ticks on these farms would transmit their infections to other cattle and so the low prevalence of serum antibodies to TBDs pathogens in all AEZs that practiced zerograzing.

The odds ratios for *R. appendiculatus* infestation in respect of lowland free range (183) and lowland tethered (365) appear very big and probably unrealistic, so are the respective confidence intervals (53-907 and 46-8434). This is explained by the low numbers of animals with *R. appendiculatus on them in* the upland zero grazing system, which we used as the reference group. On the contrary most of the animals in the lowland grazing system had *R. appendiculatus,* hence the observed high odds ratios for these grazing systems.

The highest number of standard ticks was recorded in the upland AEZ and these ticks were mainly *Boophilus* species because at this elevation (600- 2,300 metres above sea level), this tick is the most abundant (Matthysse and Colbo, 1987). Another reason is that farmers in the Upland AEZ tended not to worry about their animals being infested with ticks since ECF, a well known tick-borne killer disease, was not a threat here. The tick vector*, R. appendiculatus* is very rare at these elevations (Matthysse and Colbo, 1987). The farmers therefore laxed in their tick control regimes or did nothing to control the ticks: ticks were allowed to feed to engorgement and hence the common finding of standard ticks in the upland AEZ. Standard ticks are ticks that have fed successfully and would engorge and detach in the following 24 hours (Kaiser et al. 1982). This was possible because either the animals were naïve to tick infestation and so highly susceptible or because the ticks were not removed by any means by the farmer.

The detection of antibodies to all the TBDs pathogens in calves as young as 0-3 months was expected since, at this age, passively transferred colostral antibodies are still in high titre in the serum of the animals (Burrdge and Kimber 1973). So, in this study, a special effort was made to scrutinize the sero-prevalence of antibodies to TBDs pathogens in calves (4-12 months old). This was done because it is known that by the age of 2-4 months, the passively transferred colostral antibodies have waned to undetectable levels (Burridge and Kimber, 1973). So it was assumed that the antibodies detected in this age

group (4-12 months) were due to infection from infected ticks and so indicated exposure to tick infection.

There was variability in serum antibody prevalence to the three TBDs and degree of tick infestation across the AEZs, grazing systems and age of animals. So did tick control standards vary with AEZs and grazing systems. Consideration of a combination of these facts suggests existence of different epidemiological states for the three diseases in the district. These findings suggest that endemic stability to the three TBDs (East Coast Fever, babesiosis and anaplasmosis) was most likely to have existed in the lowland AEZ, particularly, under the tethering and free-range grazing systems where sero-prevalence to TBDs pathogens and tick challenge was highest. An endemically stable state is characterized, among other things, by high antibody prevalence (>70%) and a constant tick challenge; while endemic instability is characterized by low antibody prevalence of <30% (Norval *et al*.1992, Deen *et al.* 1993, Perry and Young 1993). Animals in the midland and upland AEZs were in endemically unstable state (for ECF) because *R. appendiculatus* challenge here was low because of the sub-optimal climatic conditions for this tick at these elevations. Seroprevalence of antibodies *to B. bigemina* (>60%) and a high *B. decoloratus* tick challenge was found in animals (4-12 months old) in the upland AEZs, suggesting that also endemic stability to babesiosis is most likely to have existed in the upland AEZs. This explains the laxity in tick control measures instituted at the high elevations: a sizable number of farmers were not perturbed by *B. decoloratus* infestation of their animals. This difference in epidemiological states of East Coast Fever across AEZs and grazing system has been reported previously (Gitau *et al.* 1997) in the Kenya highlands.

Our findings underscore the importance of structured observational studies in guiding the planning of ticks and TBDs control strategies since control strategies should be tailored to different production systems and cattle population at high risk of the diseases. This is better done after the factors associated with acquisition of infection; infection patterns and the production losses associated with these infections have been established through a longitudinal study. This is our next study in the same district.

Acknowledgments

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