

2022-03

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Academic Journals

<https://doi.org/10.5897/JVMAH2020.0895>

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Full Length Research Paper

Brucellosis infection dynamics in cattle and the impacts on production and reproduction in pastoral settings of Tanzania

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Received 12 November, 2020; Accepted 22 February, 2022

Brucellosis is endemic in pastoral settings of Tanzania with significant socio-economic implications. However, comprehensive studies to establish its impacts had not been elucidated. A longitudinal study was conducted in order to elucidate the dynamics and its impact on production and reproduction. Initially, 464 animals were enrolled with baseline seroprevalence in each herd. Animals were bled every three months to determine the incidence rate, impacts and trends in sero-status. In addition, individual animal reproductive information was collected. Milk yield was measured indirectly by estimating the calves' growth rate. Data were analysed using Epi Info 7.0 software where descriptive analyses were used to establish proportions, associations and relationships. Wilcoxon test was used to establish the growth rate differences. Forty-seven new c-ELISA seropositive animals were identified over the period of three months representing an incidence rate of 0.811 cases per animal-year at risk. Households with a high seroprevalence during baseline screening were observed to have high infection rate in the subsequent visit. There was no statistical association between new seropositive cases and seasons ($P>0.05$). Furthermore, positive to negative seroconversion was observed. Of the 94 females that were expected to parturate, 15% aborted with 29% of these being seropositive. Retained placenta was observed in 4.3% of the domestic ruminants. Of the 79 calves that were screened, 21.5% were seropositive with majority born from seropositive dams. Calves born from seropositive dams were 27 times more likely to be seropositive. Growth rate was not different ($p>0.05$) between calves suckling from seropositive and seronegative dams.

Key words: Brucellosis impacts, incidence rate, seropositivity.

INTRODUCTION

Brucellosis in animals has a potential to cause enormous economic loss through abortion, decreased milk yield, placental retention, impaired fertility and increased cost of treatment (Lokamar et al., 2020; Assenga et al., 2015).

Previous studies evaluating the impact of disease have been confined to dairy herds (Mdoe et al., 1991) and there are limited studies that have been extended to extensive farming systems such as pastoral herds

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(Shirima, 2005; Mokantla et al., 2004). It has been observed that in the pastoral farming system, the real inputs and economic outputs are often not well known by herd owners (Mokantla et al., 2004). This, together with lack of record keeping and significant livestock movements complicates any evaluation strategy on production and reproduction status at the herd level. Evaluation based on financial loss caused by brucellosis becomes even more difficult due to the differing nature of farming systems, varying herd sizes (Shirima, 2005; Assenga et al., 2015; Fernandez et al., 2018) and the purpose of livestock keeping such as prestige, social and cultural functions.

Also, in extensive farming systems, the causes of abortion and retained placenta are numerous, (Mokantla et al., 2004; Shirima, 2005) where other infectious diseases and management factors have been shown to play a major role (Mathew et al., 2017).

Livestock have a direct impact on the health and social well-being of pastoralists whose livelihoods are dependent upon livestock and livestock products. Low milk production may result in malnutrition in children who depend heavily on consumption of milk. High abortion rates result in small numbers of replacement stock which lead to decreased herd sizes and thus to poverty. Extra costs incurred for treating retained placenta and sometimes metritis increases the economic burden to livestock keepers.

The effect of brucellosis on milk yield has been quantified and found to significantly reduce yield to below average in dairy animals in Ethiopia though other studies found with no effect (Sintaro, 1994; Mellado et al., 2014). No similar study has been conducted in Tanzania in any farming system to quantify the impact of brucellosis on milk yield, retained placenta and abortion. Thus, quantifying abortion rates, milk production and the incidence of retained placenta attributed to brucellosis could generate useful information to inform policy for future formulation of appropriate control measures that ultimately may alleviate poverty in the sector. Therefore, this study aimed to determine the dynamics of brucellosis in cattle under pastoral farming system in Tanzania, evaluate the impact of brucellosis on abortion, retain placenta and milk yield for better management of the disease.

METHODOLOGY

Study site and design

Cross sectional study was conducted in Ngorongoro district to establish herd prevalence. The study was conducted in five pastoral herds (labelled A-E for confidentiality purposes) from Ngorongoro district, Arusha region for 12 months.

Herds enrolment for longitudinal study

Herds were selected conveniently for longitudinal study one month

after cross sectional brucellosis screening. Herds inclusion criteria were:

- (i) Households with ≥ 70 cattle and field Rose Bengal Plate Test seropositivity $\geq 10\%$ at herd level. (Screened one month prior the initiation of longitudinal follow up). Herds with a high seroprevalence were chosen so as to give the greatest chance of detecting the impact of brucellosis within these herds.
- (ii) Consented with herd owners.

Blood sampling and analysis

Animals were bled every three months for a duration of twelve months. Blood samples were collected from the jugular vein using plain vacutainer tubes. Blood samples were processed on the same day of collection. Blood samples were left at ambient temperature for at least 30 min after collection to allow clotting and serum separation. In the field, these samples were centrifuged at 3022 g for five minutes using a mobile spin centrifuge (Vulcan Technologies, USA). Tubes were removed and serum decanted into Eppendorf tubes (Eppendorf-Netheler-Hinz GmbH, Hamburg Germany) in duplicate. All sera were kept in the cool box and transported for storage at an approximately -20°C in the laboratory. Information of the individual animal and herd level was obtained from the owner. Individual animal information included abortion, retained placenta following normal delivery or abortion, deliveries, cost of treating retained placenta and pregnancy status were collected every three months. Replacement of animals which dropped out of the study was done by restraining any animal from the herd. Newly recruited animals were tagged for identification. However, in some herds the owners were reluctant to tag the new recruits and bled during dry season with the fear of having less blood.

Serological analysis

The procedure employed for c-ELISA testing for brucellosis was according to VLA protocol (Perret et al., 2001). By using the ELISA reader Multiscan RC Version 6.0 (Laboratory systems, Helsinki Finland) at 450 nm, the plate results were obtained and interpreted. The cut-off value for c-ELISA positivity was based on the conjugate control where the cut-off taken a 60% of the mean of the optic density (OD) of the 4 conjugate control wells. Any test sample giving an OD equal to or below this value, was considered positive. All results were expressed as a percentage of the conjugate control and referred to as percentage positive values (pp-values).

Monitoring calf growth rate as a proxy to milk production

Calf growth rate was estimated by measuring their heart girth using a weighing measuring tape. Calves stood squarely on four legs while restrained; the measuring tape was placed around the animal just behind the hump and forelegs, and heart girth measurements taken. Heart girth measurements were also carried out at three months intervals. An increase in girth measurement (cm) was considered as an increase in growth. Seventy nine calves were enrolled in the study. Each calf serostatus was matched with the respective dam serostatus.

Data storage and analysis

Data were entered in the Microsoft Excel 97 spread sheet. The association between seasonality (wet vs dry) and seropositivity was determined using the Chi-square statistic and descriptive analysis

Table 1. The number of cattle screened in each visit.

Household ID	Visit-1(baseline)	Visit-2	Visit-3	Visit-4	Visit-5
A	103	85	72	68	62
B	70	33	26	19	22
C	94	6	20	NA	NA
D	93	58	54	61	NA
E	104	90	4	NA	NA
Total	464	328	223	194	126

NA =Household not visited as a result of difficulties in locating herds due to seasonal movements.

was also used to calculate percentage proportions to parturition, retained placentae and abortion. Figures were produced with Microsoft Excel. The increase in heart girth measurements for calves suckling from *Brucella* seronegative and seropositive dams were compared for any difference using the Wilcoxon test. The incidences and survival probabilities were calculated as described by Thrusfield (1995) and Woodward (2005). Incidence Rate (IR)= Number of new cases in the three months period/ [(Number of cattle at risk at start of the time period + Number of cattle at risk at the end of that period/2)]. "A new case "in this study refers to any animal that seroconverted from being c-ELISA seronegative to c-ELISA seropositive.

The relationship between baseline seroprevalence and incidence rate was assessed by using Pearson correlation coefficient as described by Woodward (2005). Data were analysed using Epi Info 7.0 software (CDC) where descriptive analyses was used to calculate proportions, Chi-square to establish associations and Pearson correlation to determine the relationship between incidence and baseline seroprevalence. However, Wilcoxon test was used to establish the growth rate differences.

RESULTS

Infection dynamics

At the beginning of the study, five livestock households were enrolled with 332 seronegative and 132 seropositive cattle, respectively. During the period of twelve months loss to the study of animals occurred in both seronegative and seropositive domestic ruminants. Lost to follow up occurred due to several factors including movements to sites where visiting and sampling was not possible, sales or gifting, slaughter, deaths, or attacked by wild animals (Table 1). Furthermore, failure to replace animals lost to the study was due to owners not agreeing to recruit new animals especially during the dry season when they consider animals to have less blood due to shortage of feeds and water, and some were not willing to tag their animals.

The incidences of brucellosis c-ELISA seropositivity between visits 1-2 and 2-3 were calculated on the basis of five households. Two households (C and E) were excluded in the calculations for incidences between visits 3-4 as animals were moved to new sites looking for water and pastures. The incidence rate of brucellosis c-ELISA seropositivity between the first and second visits was 0.181(181/1000) cases per animal-3 months at risk

equivalent to 0.728 (728/1,000) cases per animal-year at risk. However, herd E had more cases than any other herds and excluding it, and the incidence of brucellosis c-ELISA seropositivity declined to 0.079 (79/1000) cases per animal-three months at risk, equivalent to 0.316 (316/1,000) cases per animal-year at risk. The incidence rate varied depending on the number of cattle at risk and new cases at different visits (Table 2). The relationship between household seroprevalence and incidence of brucellosis c-ELISA seropositivity in the subsequent visits, showed a strong positive linear relationship as measured by Pearson correlation coefficient ($r=0.93$). Households that had higher seroprevalence at baseline were observed to have a high incidence rate on the subsequent visit (Table 3).

Relationship between incidence rate of brucellosis c-ELISA seropositivity and seasonality

Households were visited during both the wet and dry seasons. The wet season started in November and ended in June, whereas the dry season started in July and ended in October. New cases were categorised by season of the year with 36 (61%) new cases diagnosed during the wet season and 23 (39%) during the dry season. Although brucellosis seropositive cases were encountered more during the wet season the difference was not statistically significant ($p=0.05$, $\chi^2=0.08$, 95%CI= 0.0093-0.0733) when compared with dry season.

Impact of brucellosis infection on production

A follow-up was undertaken on 210 mature female domestic ruminants over a period of twelve months in all five households. Among these, 26% had a history of previous abortion. Of the 94 cows that were expected to parturate, 80 gave birth and 14 aborted (Table 4). Twenty five percent of cows that gave birth were c-ELISA seropositive whereas, 29% of the aborted cows were c-ELISA seropositive. Retained placenta was observed in 4.3% of the cows (normal birth and aborted). Among cattle that had retained placenta, 67% were c-ELISA

Table 2. The incidence of brucellosis c-ELISA seropositivity at three months interval for twelve months period.

Visits	Number of cattle at risk at the start	Number of cattle at risk at the end	New cases at that period	Incidence
2 (after 3 months from baseline screening)	332	186	47	0.181
3 (after 6 months from baseline screening)	186	156	7	0.041
4 (after 9 months from baseline screening)	156	157	3	0.091
5 (after 12 months from baseline screening)	157	102	2	0.015

Table 3. The relationship between seroprevalence at the initial sampling point (baseline) and incidence rate after three months in individual households.

Household	Initial seroprevalence in cattle (%)	Incidence rate in cattle
A	12.2	0.027
B	34.4	0.083
C	25.4	0.090
D	12.5	0.067
E	24.1	0.065

Table 4. Proportion of cows with normal births and abortions for 12 months of study.

Visit	Normal births	Abortions
2 (after 3 months from baseline screening)	26	9
3 (after months from baseline screening)	25	2
4 (after 9 months from baseline screening)	20	2
5 (after 12 months from baseline screening)	9	1
Total	80	14

seropositive and majority occurred in aborted cases. These cases were handled by farmers themselves using antibiotics or local herbs. The cost of attending and using antibiotics in treating retained placenta ranged from 3 to 10 US\$ with an average of 5 US\$ per case.

Seroprevalence of brucellosis in calves

A total of 79 calves were screened and 21.5% were found to be c-ELISA seropositive. Forty seven percent of female calves were c-ELISA seropositive compared to 53% male calves. Of the c-ELISA seropositive calves, 82% were born from c-ELISA seropositive dams. One calf became c-ELISA seropositive three months after its dam had seroconverted. Twelve percent of the c-ELISA seropositive calves were born from c-ELISA seronegative dams. Of 62 c-ELISA seronegative calves, 21% were born from c-ELISA seropositive dams. A significant statistical association was observed between serostatus in calves and dams (OR 27, 95% CI = 5.46, 133.49),

indicating that calves born from c-ELISA seropositive dams were 27 times more likely to be c-ELISA seropositive compared to calves from seronegative dams.

The current study showed that six calves shown positive to negative seroconversion at different visits. For example, some calves had positive-negative-positive or positive-positive-negative-positive c-ELISA serostatus during the period of one year.

The influence of dam's sero status on calf growth rate

The median heart girth of calves suckled from seropositive dams was 94.5 cm whereas, those suckled from seronegative dams was 93 cm. Using the Wilcoxon test, the lower sum of the heart girth ranks (97.4) lie between the critical value from the Wilcoxon table (77-155) based on the two groups ($n_1 = 8$, $n_2 = 20$) and thus the difference was not statistically significant ($p > 0.05$).

DISCUSSION

From the current study, the overall incidence of brucellosis in the five households during the first three months interval was 0.181 cases per animal-three months at risk. However, subsequent visits revealed variations in the incidence of brucellosis c-ELISA seropositivity suggesting changes on the risk factors and herd control measures undertaken. Also, drop out of some animals may contribute to declined incidences in the subsequent visits. This reflects how difficult to conduct follow up studies in such dynamic settings.

Over the period of twelve months, 59 new brucellosis c-ELISA seropositive cases were encountered. Of the new cases identified, 93% were females. A high proportion of females being c-ELISA seropositive could be due to the fact that females are more prone to *Brucella* infection compared to males based on their behaviour of licking each other after parturition. Furthermore, *Brucella* organisms have a special affinity for a sugar alcohol called erythritol present in the placenta. This sugar is elevated during pregnancy and stimulates growth of *Brucella* organisms following (Bishop et al., 1994). Therefore, the effect of erythritol in female animals could possibly be the cause of the difference in *Brucella* seropositivity or this could be due to other physiological mechanisms. Similar findings were observed during the initial screening of the herds.

It was observed from the current study that a linear relationship existed between household baseline seroprevalence and the incidence of brucellosis c-ELISA seropositivity on the subsequent visits. This could be explained by the fact that the higher the number of infected animals in the herd, the higher the risk within the herd. Similar studies were conducted by Lithg-Pereira et al. (2004) and Fernandez et al. (2018) where flocks which delayed culling *Brucella* seropositive animals had more new brucellosis seropositive cases in the subsequent screening. The risk may be even higher if a large proportion of infected animals are reproductively mature females, as following parturition, they may spread the infection to susceptible animals. This emphasizes that immediate/gradual culling of female positive reactors may be an important control measure once coupled with other strategies such as vaccination and environmental hygiene to prevent further spread of Infection between animals and subsequently to humans (Zhang et al., 2014; Nyerere et al., 2019).

The current findings revealed that the incidence rate of infection was higher during the wet season (although not statistically significant) compared to the dry season. High numbers of new brucellosis c-ELISA seropositive cases during the wet season coincided with the high parturition rate. This could explain the high numbers of new brucellosis c-ELISA seropositive cases during this period as environmental contamination could be expected to be high through exposure to foetal fluids and placentae

(Nyerere et al., 2019). Environmental contamination during the wet season may have a significant effect as it creates a favourable climate for *Brucella* organisms to survive longer thus providing more exposure time to animals at risk (Zhang et al., 2014; Bishop et al., 1994; Nyerere, et al., 2019). Furthermore, congregation of animals especially females in the kraal facilitate licking each other after calving or abortion thus spreading the infection to animals at risk.

Results of the current study showed that 15% of animals had abortion and cost the livestock owner an average of \$5 USD to attend each case of retained placenta. These observations were an indication that brucellosis attributed to abortions and retained placenta. This could be supported by the cross-sectional findings where 12% (Attributable risk) of abortions were attributed to brucellosis and an association between c-ELISA seropositivity and retained placenta was reported (Shirima, 2005). In addition, to calf loss and cost of treating retained placenta, brucellosis may interfere with calving pattern and results in long calving intervals and impairs milk production. Although the impact of brucellosis on abortion may be confounded by other causes, any intervention will result in benefits such as increased number of replacement animals, reduced costs of treating retained placenta and ultimately preventing human infection. This is especially important in pastoral poor communities where livestock and livestock products are crucial for their livelihood and welfare.

There was a significant statistical association between c-ELISA seropositive calves and c-ELISA seropositive dams with calves born from seropositive dams, being 27 times more likely to be infected compared to calves born from seronegative dams.

A higher proportion of seropositive calves from seropositive dams as observed in this study may indicate that the source of infection could be either through uterine transmission or ingestion of contaminated colostrum or milk. Similar suggestions were put forward by others (Fernandez et al. 2018; Bishop, et al., 1994) where transovarial transmission and ingestion of milk from infected dams were considered as the major sources of infection in calves. Calves that were c-ELISA seropositive while their dams were seronegative could be due to the fact that in the pastoral herds calves can suckle from different dams provided they are docile. Such practice of leaving calves suckling to other dams could be a means of transmitting brucellosis to calves within a herd. Other sources of infection could be through ingestion of contaminated pastures as some of calves graze on pastures nearby.

Furthermore, 21% of seronegative calves were born from seropositive dams. This could be explained by the fact that calves born from seropositive dams their antibodies may fall to undetectable level probably due to failure of infection establishment (Nicoletti, 1990; Fernandez et al., 2018) or due to elimination of infection

and return to seronegative status (Bishop, et al., 1994). Although these calves were serologically negative, other studies have shown that they harbour the organisms as positive cultures following cultivation of tissues from seronegative calves were observed (Crawford et al., 1990). Another interesting finding from this study was the tendency of some calves to exhibit variation in serostatus at three months intervals. Such an observation made it difficult to ascertain the serostatus at the calf-hood stage. Therefore, based on these observation calves born from positive and negative dams of the same herd may be treated as suspicious regardless of their serostatus and should be excluded from breeding programmes as suggested by others (Cattlin and Sheehan, 1986; Fernandez et al., 2018).

There was no statistically significant difference in growth rate between calves suckling from seropositive and seronegative dams. Lack of significant differences could be because brucellosis has not caused significant effect on the milk yield or may be confounded by other factors that were not controlled based on the nature of the study. Also, intervention by herd owners allowing calves to suckle from other dams when their dams have little milk affects this observation (Personal observation, 2015). In addition, the small size of the longitudinal study, especially in light of significant loss to follow-up meant that it was not possible to stratify the analyses to account for some possible confounders for the outcome variables investigated. These include variations in the ways calves were managed, breed variations and possible suckling of animals by children.

Conclusion

Therefore, it could be concluded from this study that the incidence rate in an individual household/ herd was mainly determined by the number of animals infected in the household. Also, nevertheless seasonal pattern was not observed to influence brucellosis spread further studies with enough sample size and duration could be useful in developing strategic control interventions. Furthermore, the inconsistencies in serostatus observed in calves suggested future problems at herd level with replacements and in controlling the disease. This may call for further investigation to explore epidemiological variations necessary during mitigations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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