Outbreak investigation and control case report of brucellosis: Experience from livestock research centre, Mpwapwa, Tanzania

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Brucellosis screening was conducted between 2005 and 2010 at the National Livestock Research Institute headquarters, Mpwapwa, Tanzania, following an abortion storm in cattle. The initial screening targeted breeding herds; 483 cattle were screened using the Rose Bengal Plate Test (RBPT) followed by the Competitive Enzyme-linked Immunosorbent Assay (c-ELISA) as a confirmatory test. The seropositivity on c-ELISA was 28.95% in 2005; it subsequently declined to 6.72%, 1.17%, 0.16% and 0.00% in 2006, 2007, 2009 and 2010, respectively. *Brucella* seropositivity was not detected in goats. Seropositivity declined following institution of stringent control measures that included: gradual culling of seropositive animals through slaughter; isolation and confinement of pregnant cows close to calving; proper disposal of placentas and aborted foetuses; the use of the S19 vaccine; and restricted introduction of new animals. It was thought that the source of this outbreak was likely to have been from the introduction of infected animals from another farm. Furthermore, humans were found with brucellosis antibodies. Out of 120 people screened, 12 (10%) were confirmed seropositive to brucella antigen exposure by c-ELISA analysis. The majority of the seropositive individuals (80%) were milkers and animal handlers from the farm. Nine individuals had clinical signs suggestive of brucellosis. All cases received medical attention from the district hospital. This achievement in livestock and human health showed that it is possible to control brucellosis in dairy farms, compared to pastoral and agro-pastoral farms, thus providing evidence to adopt these strategies in dairy farms thought to be at risk.

Introduction

Brucellosis is an infectious and contagious bacterial disease. It primarily affects domestic and wild animals and has both economic and public health implications. It is of economic importance as it causes financial losses from abortions, sterility, decreased milk production, veterinary fees and costs of replacement animals (Radostits *et al.* 2000). In humans, it is characterised by headaches, joint pain, undulating fever and general body malaise (Bouley *et al.* 2012). Brucellosis has a worldwide distribution and is also an important disease in Tanzania.

The history of brucellosis in Tanzania (historically Tanganyika) dates back to 1928, when an outbreak of abortions was reported in exotic dairy animals introduced into the country. The affected animals were kept in Engare Nanyuki, Arusha and were confirmed brucellosis positive (Kitalyi 1984). From the time that the disease was introduced into the country, it has never been controlled. Brucellosis continued to spread in pastoral, agro-pastoral and dairy farming systems (Shirima *et al.* 2007). Mahlau and Hammond (1962) reported three outbreaks in indigenous cattle in the Maswa district, Lake zone, where in all cases the seropositivity was greater than 20% in the affected herds. This was followed by an abattoir survey in the same zone where seropositivity in cattle and goats was 15.0% and 1.3%, respectively (Mahlau & Hammond 1962). Nevertheless, extensive surveillance covering different farming systems was conducted by Jiwa *et al.* (1996); it revealed a prevalence of 10.8% in the same zone, with variable seropositivity noted in government ranches (15.8%), dairies (6.3%) and traditional herds (4.3%). Similar surveys conducted in other zones revealed brucellosis seropositivity at varying levels (Maiseli 1992; Shirima *et al.* 2007; Swai & Schoonman 2009; Weinhaupl *et al.* 2000). Lack of regular brucellosis screening in indigenous herds, ranches and dairy farms has resulted in the disease spreading countrywide (Jiwa *et al.* 1996; Minga & Balemba 1990; Shirima *et al.* 2007). Despite this spread, it is not certain which brucella species are in circulation; knowledge is necessary for implementing the appropriate control strategies. The present paper describes investigation of a disease outbreak following an abortion storm in 2005 in a cattle herd belonging to a research institution, and devises various integrated control strategies.

Materials and methods

Study area and animals

The present study was conducted at the National Livestock Research Institute (NLRI) (currently known as the Tanzania Livestock Research Institute [TALIRI]), Mpwapwa, which is located in the Dodoma region in the central zone of Tanzania. Since 1907, the institute has been mandated to conduct livestock production research. The TALIRI also has a farm that keeps cattle, goats, sheep and pigs for research purposes. The centre is known for developing a cattle breed known as the Mpwapwa breed (dual-purpose breed).

Outbreak investigation

Brucellosis outbreaks were first reported at the TALIRI in 1937 following the introduction of animals (Department of Veterinary Services 1937); however, the disease has been controlled until this latest event, when a similar disease presenting with an abortion storm was suspected. A team of five veterinarians, one from the Tanzania Veterinary Laboratory Agency (TVLA), Dar es Salaam, three from the Veterinary Investigation Centre (VIC), Mpwapwa, and one from TALIRI, Mpwapwa, were assigned to carry out the disease outbreak investigation. The epidemiologic investigation procedures employed to carry out the assignment included: case description, diagnosis verification, magnitude determination, and intensive follow-up (World Organisation for Animal Health [OIE] 2014). Case definition and other epidemiological information were obtained through person-to-person interviews.

Case definition

History provided by the farm veterinarian indicated that pregnant animals expected to calve within 2–3 months were aborting. Clinically aborted animals had no significant clinical signs. Based on herd records, aborted cows were in the third trimester. For the purpose of the present investigation, the definition for a positive brucellosis abortion case was based on three categories:

- Suspected cases were those animals characterised by abortion at the third trimester with or without retained placenta and no fever noted.
- Probable cases fulfilled the suspect criteria and epidemiological information, and also tested positive on Rose Bengal Plate Test (RBPT).
- Confirmed cases fulfilled probable case criteria and also tested positive on Competitive Enzyme-linked Immunosorbent Assay (c-ELISA).

Serological screening of livestock

As part of intensive follow-up, blood samples were collected for screening. Plain vacutainers were used to collect whole blood from each animal and labelled using individual animal ear-tag numbers. Blood samples were left at the ambient temperature for at least 30 min after collection, to avoid problems of albumin coagulation that prevents sera formation during centrifugation. Samples were centrifuged at 3022 g for 5 min, using a Mobile spin centrifuge (Vulcon technologies, USA) at VIC-Mpwapwa. Tubes were removed and sera were decanted into Eppendorf tubes (Eppendorf-Netheler-Hinz GmbH, Hamburg, Germany) in duplicate. All sera were labelled and kept in the freezer at approximately -20 °C. The RBPT and c-ELISA kits, with control sera, were used for screening and confirmation, respectively. The antigen used for screening was *Brucella abortus* antigen, which was kindly donated by the Veterinary Laboratory Agency (VLA) Weybridge, UK (batch numbers SG269 and SG276). The RBPT and c-ELISA tests were performed as described by VLA protocol (Perret *et al.* 2001). Interpretations of results were based on agglutination for RBPT and visual observation for any colour development and optic density readings for c-ELISA using the ELISA reader at 450 nm. The samples were considered to be positive when the binding ratio was greater than 10 and at a cut-off point of 0.83 (Optic Density).

The first screening was performed in June 2005, when 483 out of 490 pregnant and lactating cows were sampled. Seven animals were not in the herd during bleeding and were thus not screened. The follow-up activities were conducted between May and June on an annual basis until 2010 (Table 1). Screening was skipped in 2008 due to logistic reasons; in 2006, the number was almost doubled due to inclusion of experimental animals. The farm had four groups of animals that were kept separate (production herd, experimental herd, yearlings with both males and females, and goat flock). The experimental herd, yearlings and goat flock were tested once and not followed because they were all negative.

Serological screening of humans

People working in the institute were approached after receiving permission from the institute Director and in consultation with the District Medical Officer. Individual consent was also sought before participation in the present study. The people enrolled were TALIRI residents who either drank milk from the farm or worked with TALIRI animals. The TALIRI residents who neither drank milk from the farm nor worked with animals were excluded.

TABLE 1: Number of cattle screened annually at Tanzania Livestock Research Institute, Mpwapwa.

Year	Number of cattle screened	Proportion positive on RBPT (%)	Proportion positive on c-ELISA (%)	Proportion positive on both RBPT and c-ELISA (%)
2005	487	33.5	29.0	28.0
2006	714	11.0	9.2	5.6
2007	342	1.7	1.2	0.9
2009	632	0.8	0.2	0.2
2010	131	3.0	0.0	0.0

c-ELISA, Competitive Enzyme-linked Immunosorbent Assay; RBPT, Rose Bengal Plate Test.

Based on their willingness to participate, in 2006 120 residents were screened using RBPT and confirmed by c-ELISA. Clinical signs suggestive of brucellosis were collected from each individual after sampling.

Data storage and analysis

Data generated were entered in a Microsoft Excel 2007 spread sheet. Some of the variables collected from interviews were summarised using narrative text, whereas serological data were analysed using descriptive statistics.

Results

Epidemiologic investigation

Magnitude of the problem and herd history

Based on the previous records, the incidences of abortion storms were not reported at TALIRI for decades. During the first visit, 30 out of the 126 heavily pregnant cows were found to have aborted within a period of 2 weeks. The herd structure consisted of 40 non-pregnant heifers, 180 yearlings, 80 breeding bulls, and 490 pregnant and lactating cows. There were also 200 goats present on the farm, but they were kept separate from the cattle. The geographical boundaries protected the farm animals from co-mingling with other neighbouring herds. Two years before the incidences of abortion storm in cattle, 100 cattle were introduced into the herd from a different farm belonging to another institution. These animals were screened for brucellosis on the farm of origin and found to be seronegative by the RBPT before being introduced into the TALIRI farm.

Intensive follow-up

Serology and control measures

The first screening targeted mature female cows in the production herd (pregnant and lactating cows) where 483 out of 490 cows were tested using the RBPT, followed by the c-ELISA as a confirmatory test. In 2005, seropositivity was 28.95%, which subsequently declined to 6.72%, 1.17%, 0.16%, and 0.00% in 2006, 2007, 2009 and 2010, respectively (Table 1). The experimental herd and goat flock was tested once, and found to be seronegative.

Out of the 120 human sera that were tested, seropositivity was 10% (Table 2). Seropositivity was higher in men (12%) compared to females (7%), with no statistically significant difference (p = 0.728); however, out of the 10 infected men, 8 (80%) were engaged in milking, herding the cattle and assisting during calving. Nine seropositive individuals had joint pains, body weakness and irregular fevers, whereas three showed no symptoms.

Control and eradication of brucellosis through test and slaughter, coupled with vaccination and restriction of animal movements, was adopted with some modification, based on the resources and transmission pathways to control the disease. Gradual culling of marked seropositive cattle, isolation of pregnant cows into designated pens 2 weeks before calving, proper disposal of placentas and aborted foetuses, vaccination of eligible calves (6–8 months) using the S19 vaccine, and restricted introduction of new animals were integrated during the entire period; however, vaccinated animals were not screened for brucellosis to avoid cross-reaction with the test resulting in false positives. Marked seropositive cattle were isolated and slaughtered under veterinarian supervision within the institute at certain intervals (gradual culling) for salvage purposes. Residents were advised to boil milk destined for human consumption.

Discussion

In the present study, the confirmatory diagnosis of bovine brucellosis was attained by using serological tests. The c-ELISA seropositivity in cattle was associated with a history of abortion storm and was in agreement with several other studies (Schelling *et al.* 2003; Shirima *et al.* 2007; Swai 1997). The results of the present study indicate that brucellosis infection could be present on dairy farms that have had no history of the disease, in the absence of routine surveillance. Introduction of animals originating from other herds has probably been the source of infection on the farm. Although these animals were tested before purchasing, false negative cases could have been the source of infection. False negative cases resulting from screening with serological tests such as RBPT were probably due to the failure to detect chronic infections, recently aborted cases, or an inherent weakness of the assay whose sensitivity ranges from 63% – 99% (Bishop, Bosman & Herr 1994; Jeff 2013).

In several countries, control and eradication of brucellosis has been achieved through test and slaughter, vaccination, and restriction of animal movements (McDermott, Grace & Zinsstag 2013). In other countries, similar methods have been adopted with some modification, based on the available resources and transmission pathways. In the present study, gradual culling of marked seropositive cattle, isolation of pregnant cows into designated pens 2 weeks before calving, proper disposal of placentas and aborted foetuses, vaccination of eligible calves (6–8 months) using the S19 vaccine, and restricting introduction of new animals were integrated during the entire period. Although the risk factors for human transmission were beyond the scope of the present investigation, the majority of seropositive humans were animal handlers and/or milk drinkers. The human results indicated that > 80% of seropositive cases were mainly due to occupation; this was consistent with other observations (Niwael 2001; Minja 2002; Schelling *et al.* 2003; Shirima *et al.* 2007, 2010). The remaining proportion

TABLE 2: Human serum samples tested using Competitive Enzyme Immunosorbent Assay at Tanzania Livestock Research Institute, Mpwapwa.

Gender	Seropositive	Seronegative	Total
Male	10	81	91
Female	2	27	29
Total	**12**	**108**	**120**

could probably be due to drinking unboiled milk originating from infected cows. These findings may necessitate public education to promote boiling of milk before drinking, and to properly handle animals during calving and aborted foetuses by using protective materials such as latex gloves. Although seropositive animals that were destined for gradual culling were isolated, extra care was required by attendants to reduce the risk of exposure to humans.

The integration of different approaches employed for brucellosis control at the TALIRI-Mpwapwa farm has shown significant success in reducing disease incidence. It can therefore be concluded that brucellosis can be controlled and prevented with the appropriate human resources, good collaboration with the existing diagnostic institutions, and awareness of brucellosis-control strategies. Owner awareness enhances the compliance to biosecurity measures, as well as the agreement to test and slaughter seropositive animals. Control strategies used at the TALIRI-Mpwapwa farm are strongly recommended for other organised farms, with or without modifications, depending on farm husbandry and management.

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Competing interests

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' contribution

G.M.S. (Tanzania Veterinary Laboratory Agency) was the team leader and ran the c-ELISA and interpreted the data. S.N.M. (National Livestock Research Institute) and O.N.M. (Veterinary Investigation Centre) were responsible for designing, serum separation and implementing control measures. B.A.S. (University of Wyoming) performed the critical revision of the manuscript.

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