



A comparison between indirect ELISA and tuberculin skin test in the diagnosis of bovine tuberculosis in Kenya

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Abstract

Bovine tuberculosis (BTB) is an important zoonotic disease which has remained persistent in many areas of the World. Control and eradication has proved problematic due to the challenges in effective screening and diagnosis. Limited information on the status of BTB in Kenya presents a need to investigate its occurrence in cattle population. The study was carried out using an indirect antibody Enzyme Linked Immunosorbent Assay (ELISA-IDEXX-USA) to assess the agreement with Comparative Intradermal Tuberculin Test (CITT) test results between August and December 2013 in Laikipia County. The study also determined the apparent prevalence of cattle BTB antibodies in Laikipia County. A total of 276 bovine serum samples were tested using MPB70 and MPB83 recombinant proteins as capture antigens in the ELISA kit. Data were recorded in Microsoft Excel and exported to SPSS 16.0 for analysis. Apparent prevalence was calculated as a proportion at 95% CI, Kappa statistics computed, and test of significance assessed by Chi-square and Fisher exact test. The results showed an individual animal apparent prevalence of 3.9% (11/276) and a herd prevalence of 58.3% (7/12). There was no significant association between BTB infection and both animal level factors, breed and sex. Kappa agreement test between ELISA test and TST showed a good agreement at K= 0.65. This study reported the prevalence of BTB in cattle for the first time in Laikipia County Kenya using the IDEXX ELISA. The ELISA test was comparable to the tuberculin test which is used as the gold standard for screening TB on live animals. The two tests can be used alongside for series or parallel interpretation to achieve desirable diagnostic sensitivity and specificity in disease control programs.

Keywords: Bovine; Tuberculosis; Kenya; Indirect Antibody ELISA; TST

Introduction

Bovine tuberculosis (BTB) is a zoonotic and a chronic infectious disease of cattle caused primarily by *Mycobacterium bovis* (M. bovis) and characterized by development of avascular granulomatous nodules. Most body tissues and organs can be affected, but lesions are most frequently seen in the lymph nodes of the head and thorax, in the intestines, lungs, liver, spleen

and peritoneum (Clarke, 1998). *Mycobacterium bovis* primarily infects cattle but it can infect other animals and humans (Kaneene and Pfeiffer 2006, Thoen *et al.*, 2006). *Mycobacterium caprae* has been identified as a cause of BTB in some parts of central Europe (Naranjo *et al.*, 2008).

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The genus *Mycobacterium* consists of more than 100 species and most of these are found in the environment and are not normally pathogenic to humans or animals however a small number of them are pathogenic to various species of animals (Sam *et al.*, 2008). BTB is endemic in many areas of the World and is associated with significant economic losses due to reduced cattle productivity and cost of control and eradication programs in addition to being a zoonotic pathogen that can infect humans (Steele, 1995). Bovine tuberculosis has a worldwide distribution with some countries of Europe having made a lot of success in control programs, it is however still endemic in Africa (Ayele *et al.*, 2004, Alvarez *et al.*, 2012). A study in Kenya and Tanzania using Single Comparative Intradermal Tuberculin Test (SCITT) showed prevalence of 4% to 6% and 1.28% to 2.37 % respectively in both countries (Mugabi *et al.*, 2016). In Kenya a study reported prevalence of *M. bovis* at 2.05 % among slaughter cattle around Nairobi in 2009 (Gathogo *et al.*, 2012). A recent study employing examination of direct stained smears and recovered media isolates on slaughter lesions in Laikipia Kenya found 0.3% of the carcasses having zoonotic mycobacteria (Kuria *et al.*, 2018). There is little transmission awareness knowledge about BTB by communities in both cattle keeping and non-cattle keeping farming families in Kenya (Kangethe *et al.*, 2007).

Eradication of BTB has been difficult to achieve in various countries due to the presence of wildlife reservoirs, increased animal movement and/or pastoral practices and limitation of available diagnostic and screening methods (Schiller *et al.*, 2010 and Farias *et al.*, 2012). Diagnosis of BTB remains extremely challenging and currently there is no one single test that can fulfill all the criteria necessary to identify infected animals (Schiller *et al.*, 2010). A number of new specific antigens have been tested on immunoassays for the diagnosis of BTB. The capture antigens MPB70 and MPB83 used in this IDEXX ELISA kit have shown good diagnostic reliability (Amadori *et al.*, 2002 and Farias *et al.*, 2012).

In Kenya, most studies done have concentrated on post-mortem diagnosis in slaughter lesions. Diagnosing on live animals through CITT and serological methods can be of great importance in

understanding risk factors and contributing to control programs information in different production systems. This study aimed to compare the antibody ELISA and CITT diagnostic methods and report the agreement between the two tests in the diagnosis of BTB.

Materials and Methods

Study Area and Design

The study was purposively carried out in Laikipia County, Kenya due to availability of sera samples and existing records for animals that had earlier been used for Comparative Intradermal Tuberculin Test (CITT). Blood for sera preparation from different farms in Laikipia County was collected from between 2011 and 2013 with the animals undergoing CITT. A cross-sectional study employing indirect antibody ELISA test (IDEXX-USA) was carried out between August 2013 and December 2013 to screen for Bovine Tuberculosis (BTB). The CITT had been done in 2012 and the sera stored at the Central Veterinary Investigation Laboratories (CVIL), Kabete Nairobi. Data available for the sera included the farm, sub-county, breed and sex of the animals. The samples were from all the three sub counties of Laikipia County.

Sample size determination

A total of 276 samples were tested due to the availability of test kits against a minimum proportion sample calculation of 87 assuming a prevalence of 6% (Dohoo *et al.*, 2012). The a priori estimate of 6% was informed by one of the studies showing the highest prevalence of BTB in Kenya (Lekoolool 2011).

Laboratory analysis

The antibody ELISA test was carried out in accordance with the manufacturer's instructions. Antigen coated plates sufficient for planned samples were tested at a time and withdrawn from the desiccated bag. A total of 100µl (microliters) of the diluted negative control, positive control and serum samples were dispensed in the wells. The wells were covered and incubated at 18-26°C for 60 minutes then washed 3 to 5 times with 300µl of wash solution avoiding drying in between washes. Thereafter, 100µl of the conjugate was then dispensed into the wells, incubated for 30 minutes and then washed 3 to 5 times with 300µl. The substrate was

then dispensed into the wells at 100µl, covered and incubated for 15 minutes. The stop solution was then dispensed last into the wells and absorbance optical density (OD) read at 450 nm. The presence or absence of antibody to *M. bovis* is determined by calculating the sample to positive (S/P) ratio for each sample. The S/P ratio is derived by subtracting the mean kit negative-control optical density (OD) from each sample and dividing this value by the corrected positive-control value (mean positive-control OD minus mean negative-control OD). An S/P greater or equal to 0.30 is considered positive for *M. bovis* antibodies.

Statistical Analysis

Data from the antibody ELISA results was stored in Excel 2007 (Microsoft Corporation, USA) and exported to SPSS 16.0 (SPSS Inc., 233 South Wacker Drive, Chicago IL) for analysis. Apparent prevalence was determined as a proportion of the positive cases out of total positives at 95% confidence interval (CI). The kappa statistics was used to estimate the degree of agreement between the two diagnostic tests. Chi-square and Fisher exact test techniques were applied to test significance of association between BTB infection status and animal- and farm-level predictors.

Results

The distribution of the samples collected from the 12 farms in three sub counties of Laikipia County and their test status are shown in Table 1. Laikipia East had the highest number of samples 171/276 (70%) with 9/171 (5.3%) being positive to *M. bovis* antibodies. Laikipia North and West districts had only one positive sample each with the latter contributing the least number of samples. The overall apparent prevalence was 3.99% (11/276).

The farm with the highest number of samples was 33.7% (93/276) with the least being 0.4% (1/276) (Table 2). The farm with highest number of positive samples was 4.5% (3/67) and seven farms (7/12) had at least one positive sample while the other five ranches/farms did not have any positive animal. Out of 12 farms sampled, the prevalence was 58.63% (7/12). Out of the 276 samples tested, 94.6% (261/276) were from female animals.

Table 1

Number of Bovine tuberculosis positive samples to ELISA from Laikipia County by Sub County

<u>Sub county</u>	<u>Number positive</u>	<u>Total sample</u>
Laikipia West	1 (1.09%)	92
Laikipia East	9 (5.26%)	171
Laikipia North	1 (7.67%)	13
Total	11 (3.99%)	276

About 6.7% (1/15) of serum samples from male animals tested positive for antibodies against *M. bovis* while 3.8% (10/261) of the serum samples from females tested positive. There was no significant association between sex of the animal and occurrence of BTB (Fisher Exact test, p= 0.9310).

Table 2

Samples tested from Laikipia County by farms, indicating numbers of positive animals to Bovine tuberculosis ELISA

<u>Farm Number</u>	<u>Number positive</u>	<u>Total</u>	<u>% Positive</u>
1	3	67	4.48
2	0	17	0
3	0	1	0
4	0	6	0
5	2	30	6.67
6	0	4	0
7	1	5	20
8	1	93	4.35
9	2	35	5.71
10	1	6	16.67
11	1	5	20
12	0	7	0
Total	11	276	3.99

The Boran breed had the highest number of samples that were tested at 51.8% (143/276) with Ankole having the least number at only one sample (Table 3). About 9.4% (26/ 276) did not have the breed recorded during sampling. The

highest breed BTB prevalence was in Ayrshire at 7.1% (2/28) followed by Friesian with 6.85% (3/44), Boran with 3.4% (5/143) and Zebu breed with 3.1% (1/32). Ankole and Brown Swiss breeds, and the 26 samples without breed were all negative. When the animals were classified by breed into two groups, exotic (*Bos taurus*) and indigenous (*Bos indicus*), there was no statistically significant association (P=.4)

Table 3

Prevalence of *Mycobacterium bovis* by Breeds in Laikipia County

Breed	Number positive	Total	% Positive
Ayrshire	2	28	7.1
Friesian	3	44	6.8
Boran	5	143	3.5
Zebu	1	32	3.1
Ankole	0	1	0
Brown swiss	0	2	0
Non-specified	0	26	0
Total	11	276	3.99

Comparison of antibody ELISA and CITT

Out of the 276 samples tested from Laikipia County, 254 had tuberculin skin test results that were interpreted and recorded. Eight (8) samples tested positive on both CITT and ELISA with 239 tested negative for both with an observed proportional agreement of 0.97 (97%). Four (4) samples tested positive for CITT but tested negative for ELISA while three (3) testing positive for ELISA tested negative for CITT while 239 tested negative for both. Kappa showed strong agreement between the two *M. bovis* tests at K=0.65.

Discussion

This study was performed on stored bovine serum samples to estimate the status of BTB infection in Laikipia County using an antibody ELISA and compare the test with CITT results. The use and performance of the IDEXX *M. bovis* antibody ELISA kit has been evaluated in various studies (Eyob *et al.*, 2014 and Waters *et al.*, 2011). Capture antigens, especially recombinant MPB70 and MPB83 have been used in antibody ELISA as

alternative and/or complementary diagnostic tools to improve detection of cattle that do not react to tuberculin skin test (Smith *et al.*, 2006). The MPB83 and MPB70 are major antigens highly expressed by *M. bovis* and considerably less abundantly expressed by *M. tuberculosis* (Juarez *et al.*, 2001). The MPB83 antibodies have been detected in cattle infected with *Mycobacterium kansasii*, making it the only known *Mycobacterium* species cross reacting with the antigen (Green *et al.*, 2009, Waters *et al.*, 2000).

The overall prevalence of BTB reported in seven Counties of Kenya by Muasya, 2015 was 3.57% with a range of 4.74% in the County with highest prevalence and 0% the least. Like other countries in the region, Kenya lacks adequate data and reference records on BTB status. A past study in Kenya reported a 2.0% prevalence of *M. bovis* among slaughter cattle in two abattoirs around Nairobi (Gathogo *et al.*, 2012). The origin of cattle in that study was 18 counties of Kenya covering approximately 77% of the landmass. This is lower than what this study found in Laikipia County. Another study using TST reported way higher prevalence of 10% in dairy and non-dairy herds around Nairobi area (Kang'ethe *et al.*, 2007). A study in Kenya investigating the epidemiology of BTB at the wildlife-livestock interface in Maasai Mara and Amboseli ecosystems in the southern part of Kenya reported a prevalence of 14.8% in wildlife compared to an overall prevalence of 2.3% in livestock (Lekolool 2011). The findings of this study are similar to the overall findings of Lekolool however he reported a bigger variation between wildlife and livestock. Laikipia County being a wildlife area, a similar pattern could be expected.

Elsewhere, an estimated prevalence of less than 1% was reported in a study conducted around Dar-es-Salaam area using single Intradermal Tuberculin Test (SITT) with 6.8% being doubtful (Weinhaupl *et al.*, 2000). An animal-level prevalence of 2% and a herd-level prevalence of 51% were reported in cattle among the pastoral herds of Uganda's Karamoja region (Oloya *et al.*, 2006 and Oloya *et al.*, 2007). The herd-level prevalence is similar to what we found in this study however the animal-level prevalence reported a notable difference.

Unlike in our study a cross-sectional risk factors study conducted in Tanzania from 1994 to 1997,

revealed that male cattle were significantly more infected by BTB than female animals (Kazwala *et al.*, 2001). Another across-sectional study in Uganda from 2006 to 2007 showed significantly more females positive to the skin test than males (Inangolet *et al.*, 2008). However, in the Ugandan study, there was no significant association between age of the animal and BTB occurrence and this may have meant that the animals were not living long enough to acquire the infection given that BTB is generally a chronic disease. In epidemiological studies carried out in Zambia and Tanzania it was observed that the duration of exposure increases with age (Cook *et al.*, 1996 and Cleaveland *et al.*, 2007). In Laikipia County, the results showed a good Kappa between the two *M. bovis* tests at $K=0.65$ according to the classification by Altman (1991). A similar Kappa test comparison between TST and ELISA test utilizing MPB70/MPB83 capture antigens in Brazil demonstrated adequate agreement beyond that due to chance with a Kappa of 0.688 (Souza *et al.*, 2008).

Conclusion

This study showed that sex and breed were not significantly associated with BTB infection. The performance of the ELISA test showed strong agreement beyond that due to chance when compared to the tuberculin skin test. Therefore, the use of the antibody ELISA is recommended since it is feasible, reliable, cheaper and less time consuming.

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