A comparative study on the epidemiology and immuno-pathology of bovine tuberculosis in *Bos indicus* and *Bos taurus* cattle in Ethiopia

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Abstract

Bovine tuberculosis is a disease of dual effect, having public health and economic implications. The present study was conducted on its epidemiology and immuno-pathology in Holstein and Zebu breeds of cattle. Skin test, post mortem examination and pathology scoring, bacteriology, whole blood gamma interferon assay, ELISPOT assay, and lateral flow assay were used. An overall prevalence of 13.5% (n=5,424) was recorded; both prevalence (χ^2 =61.8; P<0.001) and severity of pathology (mean pathology scores ± SEM: 6.84±0.79 vs. 5.21±0.30; P=0.018, Mann-Whitney test) were significantly higher in Holstein than in Zebu. Similarly, IFN-γ responses to avian PPD (0.49±0.10 vs. 0.39±0.07), bovine PPD (0.63±0.11 vs. 0.43±0.07), or the ESAT6-CFP10 protein cocktail (0.43±0.01 vs. 0.30±0.05) were significantly higher (for all antigens: p<0.02) in Holstein than in Zebu cattle. However, both Holstein and Zebu exhibited similar T cell and antibody responses to different mycobacterial antigens i.e. no repertoire difference was observed between the two breeds. Thus, the present study showed increased susceptibility of Holsteins to bovine TB as compared to Zebu, similarity between Holsteins and Zebus in their antigen responses, and a positive correlation between IFN-γ responses and severity of pathology of bovine TB. [*Ethiop.J.Health Dev.* 2008;22(Special Issue):132-134]

Introduction

Despite the ability of several countries to eradicate bovine tuberculosis successfully, many countries continue to encounter *M. bovis* infection in their cattle population (1,2). Reasons for the failure of these countries to control/eradicate the disease include the presence of a feral reservoir of *M. bovis* in developed world, and the inability of developing countries to apply the test and slaughter control method. Other alternative control methods such as the use of vaccination, improvement of cattle husbandry and/ or breeding of relatively resistant cattle breeds may be particularly applicable in developing countries. It was stated that housing predisposes cattle to tuberculosis so that the disease is more common and serious in these forms of husbandry (3). Zebu type cattle are thought to be more resistant to tuberculosis than European cattle and the effects of the disease on these cattle are much less severe as stated earlier (4). One of the difficulties in generating more data on such observations was the problem of getting the two breeds under identical cattle husbandry. Zebu is limited to Africa and Asia while European breeds are mainly found in Europe and other developed countries. Therefore, the objectives of this study were assess immune responses to mycobacterial antigens and investigate the epidemiology and immuno-pathology of bovine TB in *Bos indicus* and *Bos taurus* cattle in central Ethiopia.

Materials and methods

Study animals and sampling: The study was conducted in dairy rearing areas of *Holeta* and *Selalle*, central Ethiopia. The sites were selected because of the concentration of Holstein or/and crosses alongside native Zebus (mainly of the Arsi breed). The target population is smallholders that keep at least one Holstein/cross. There are 30,000 Holsteins/crosses kept by smallholders in the study area. Similarly, about equal number of zebus are kept in the target herds. For epidemiological studies, 10% (n=2846) of the Holsteins/crosses were sampled and about 9% of the Zebus (n=2578) were sampled. Thus, a total of 5424 cattle were sampled for the epidemiological study. For the investigation of immune response and pathology, 123 (50 Holsteins and 73 Zebus) skin test reactors were recruited from the above sampled population on the basis of the level of skin indurations and willingness of the farmers to sell their animals. Furthermore, 30 reactor Holsteins were recruited from one intensive farm.

Mycobacterial antigens: ESAT-6 family, heat-shocked proteins of mycobacteria, and secreted antigens/lipoproteins (5).

Comparative intradermal tuberculin test: Two sites on the right side of the mid-neck, 12 cm apart, were shaved and the skin thicknesses were measured. One site was injected with an aliquot of 0.1ml containing 2500 IU/ml bovine purified protein derivative (PPD) (Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, U.K.). Similarly 0.1ml of 2500 IU/ml avian PPD (Veterinary Laboratories Agency, Addlestone, Surrey KT15 3NB, U.K.) were used according to the Office International des Epizooties (6).

Whole blood gamma interferon assay: Whole blood cultures stimulated with mycobacterial antigens were incubated at 37°C in a humid 5% CO_2 atmosphere, for 48 h and supernatants harvested (7) and frozen. Levels of IFN-γ in the supernatants were measured with ELISA using the bovine IFN-γ (Bovigam) test kit

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(Commonwealth Serum Laboratories, Victoria, Australia) as per the manufacturer's instructions.

Enzyme-linked immunospot assay: Peripheral blood mononuclear cell (PBMC) was isolated from 16 Zebus and 14 Holsteins and the ELISPOT was done according to (5).

Multiple antigen print immunoassay: MAPIA was performed as described previously (8,9) on sera of 16 cattle (eight Holsteins and eight Zebus).

Post mortem examination and pathology scoring: The severity of the gross lesions was scored by applying the semi-quantitative procedure developed by Vordermeier *et al*. (10), with minor modifications to facilitate performance under field conditions (11).

Bacteriology: Suspicious tissues were further processed for isolation of mycobacteria as described by the OIE (6).

Statistical analysis: Logistic regression analysis was used to assess the association between prevalence and animal risk factors using STATA statistical software, (STATA Corporation, 4905 Lakeway Drive, College Station, Texas 77845 USA). The Mann-Whitney test was used to compare pathology scores and IFN-γ responses between cattle types.

Results

Prevalence: The overall prevalence of bovine tuberculosis was 13.5% (n=5,424) in central highlands of Ethiopia. The prevalence was significantly higher in Holstein than either in zebus (22.2% vs. 11.6%, χ^2 =61.8; P<0.001) or in zebu-Holstein crosses (22.2% vs. 11.9%, χ^2 =50.7; P <0.001).

Effect of risk factors on prevalence: Holsteins were more than twice as likely to present as tuberculin positive than Zebu cattle (OR=2.32; CI=1.89, 2.85). Similarly, animals aged between 5 and 9 years were at higher risk of infection (OR=2.37; CI=1.80, 3.12) with TB compared to those aged 2 years or below.

IFN-γ responses to mycobacterial antigens: IFN-γ responses to avian PPD (0.49±0.10 vs. 0.39±0.07), bovine PPD (0.63±0.11 vs. 0.43±0.07), or the ESAT6-CFP10 protein cocktail (0.43±0.01 vs. 0.30±0.05) were significantly higher (for all antigens: p<0.02) in Holstein than in Zebu (Arsi) cattle, whilst responses to the positive control PHA, or to saline (i.e. no antigen) control wells, were not significantly different between the two breeds. Holstein cattle that were kept in-house produced significantly higher IFN-γ in response to avian PPD (0.63±0.10 vs. 0.49±0.10), bovine PPD (0.85±0.14 vs. 0.63±0.11), and the ESAT6-CFP10 protein cocktail.

T cell response to mycobacterial antigens in zebu and Holsteins: Higher T cell count (response) was observed against heat-shock protein 65 (Hsp65) both in Holsteins and Zebus. Nevertheless, no significant difference (P<0.05) was observed in T cell count (response) between Holsteins and Zebu in their recognition of mycobacterial antigens.

Antibody response to mycobacterial antigens in Holsteins and zebu: Both Holstein and Zebu cattle exhibited similar antibody responses to different mycobacterial antigens. Although generally weak responses were observed in both breeds, stronger antibody responses were recorded to *M. bovis* culture filtrate (MBCF) and 16kDa alphacrystallin/MPB83 fusion proteins (16/83).

Breed and pathology: The severity of pathology in Holsteins [mean pathology scores $\pm$ SEM: 6.84±0.79; median scores (range): 6.0 (2-42)] was significantly higher (P=0.018, Mann-Whitney test) than the severity of pathology in Zebu cattle [mean scores $\pm$ SEM 5.21±0.30; median (range): 5.0 (1-17)]. The mesenteric lymph nodes were the most severely affected (mean pathology scores±SEM, 1.95±0.08) followed by the retropharyngeal (0.80±0.05) and the caudal mediastinal (0.8±0.06) lymph nodes.

Bacteriology: Fifty-six percent (81/145) of the animals with gross TB lesions were culture positive. Culture positivity of suspicious lesions did not differ (χ^2 =0.13, P=0.72; EPI6) between Holstein (54%, n=50) and Zebu (51%, n=73) breeds under identical field husbandry.

Discussion

Moderate prevalence was recorded by this study. Previously, a similar prevalence (14.2%, n=416) (12) was reported in southern Ethiopia, where cattle farming is similar to Holeta and Selalle. However, a lower prevalence (4.1%; n=460) was reported in Zebu cattle under traditional management in the Boji district of western Ethiopia (13) while a significantly higher prevalence (46.8%; n=1,171) was reported in 12 intensive dairy farms which keep crossbreed and Holstein cattle (14). The prevalence was significantly higher in Holsteins than in either cross-breeds or in Zebus. Thus, the prevalence of bovine TB is affected by cattle husbandry and cattle breed (11). Furthermore, the severity of pathology was significantly higher in Holstein than in Arsi Zebus. Historical reports also indicated that *Bos taurus* (the group to which Holsteins belong) are more susceptible to bovine TB as compared to Zebu cattle (3,4).

The level of IFN-γ responses to the tested mycobacterial antigens was significantly lower in Zebu compared to Holstein cattle, which would support the correlation of IFN-γ release with the severity of pathology of bovine TB in Holstein (10). It is also noteworthy that the IFN-γ responses observed in Holstein cows in Ethiopia were considerably lower than those reported for Holsteins in the

United Kingdom, Ireland, or New Zealand (15). A likely explanation could be that a higher proportion of Holstein cattle in Ethiopia suffer from advanced disease as the test and slaughter is not applied in Ethiopia. In addition, multiple parasitic infections, which prevail in the study population (personal observation), could also modulate the IFN-γ responses to mycobacterial antigens. A previous study showed that infection with either *Fasciola spp*. or *Strongylus spp.* significantly reduced skin indurations to bovine PPD in *M. bovis* infected heifers compared to de-wormed *M. bovis* infected heifers (16).

This study has shown a comparable T cell and antibody responses to mycobacterial antigens in both Holsteins and Zebus. Strong T cell response to Hsp65 was found in both Holstein and Zebu breeds. Similarly, strong antibody response was observed to *M. bovis* culture filtrate (MBCF) and 16/83 in both breeds

The present study showed susceptibility of Holsteins to bovine TB as compared to Zebus, similarity between Holsteins and Zebus their antigen recognition repertoires and a positive correlation between IFN-γ responses and severity of pathology of bovine TB.

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