High Prevalence and Increased Severity of Pathology of Bovine Tuberculosis in Holsteins Compared to Zebu Breeds under Field Cattle Husbandry in Central Ethiopia

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Received 20 May 2007/Returned for modification 29 June 2007/Accepted 19 August 2007

Abstract

A comparative study on the prevalence and pathology of bovine tuberculosis (TB) was conducted on 5,424 cattle (2,578 zebus, 1,921 crosses, and 925 Holsteins), which were kept on pasture in the central highlands of Ethiopia, using a comparative intradermal tuberculin test, postmortem examination, and bacteriology. The overall prevalence of bovine TB was 13.5%; prevalence was higher in Holsteins than either zebus (22.2% versus 11.6%, \( \chi^2 = 61.8; P < 0.001 \)) or crosses (22.2% versus 11.9%, \( \chi^2 = 50.7; P < 0.001 \)). Moreover, the severity of pathology in Holsteins (mean ± standard error of the mean [SEM], 6.84 ± 0.79) was significantly higher (\( P = 0.018 \)) than the severity of pathology in zebus (5.21 ± 0.30). In addition, the risk of TB in Holsteins was more than twice (odds ratio [OR] = 2.32; 95% confidence interval [CI] = 1.89, 2.85) that in zebus. Animals between 5 and 9 years of age were at higher (OR = 2.37; 95% CI = 1.80, 3.12) risk of bovine TB than those 2 years of age or below. A significant difference (\( \chi^2 = 351; P < 0.001 \)) in the occurrence of TB lesions in lymph nodes was recorded; the mesenteric lymph node (mean pathology score ± SEM, 1.95 ± 0.08) was most severely affected, followed by the retropharyngeal (0.80 ± 0.05) and caudal mediastinal (0.8 ± 0.06) lymph nodes. Fifty-six percent (\( n = 145 \)) of the animals with gross TB lesions were culture positive; the lowest culture positivity was recorded in the skin lesions (27.3%) and the lesions of the mesenteric lymph node (31.5%). Both the skin test response and the postmortem findings suggested a higher susceptibility to bovine TB in Holsteins than zebus under identical field husbandry conditions (on pasture). In the light of increased numbers of Holstein cattle introduced into this area to raise milk production to satisfy the needs of Addis Ababa’s growing population, these findings highlight the need for a control program in these herds.

Bovine tuberculosis (TB) is caused by intracellular infection with the acid-fast bacterium Mycobacterium bovis. In cattle, exposure to this organism can result in a chronic disease that jeopardizes animal welfare and productivity and in some countries leads to significant economic losses (36). Moreover, human TB of animal origin caused by \( M. \) bovis is becoming increasingly important in developing countries. In sub-Saharan Africa, humans and animals share the same microenvironment and water holes, especially during droughts and the dry season, thereby potentially promoting the transmission of \( M. \) bovis from animals to humans. In industrialized countries, bovine TB is controlled by testing and slaughter of animals and pasteurization of milk, and therefore the risk of human infection is minimized. In Africa, however, bovine TB represents potential health hazards for both animals and humans. Nonetheless, in most African countries, \( M. \) bovis infection remains an uninvestigated problem (13). In general, the epidemiology and public health significance of bovine TB in Africa remain largely unknown. Many of the factors which account for this are politico-economic, including the high cost of testing, social unrest and ethnic war, displacement of large numbers of human and animal populations, and a lack of veterinary expertise and communication networks, as well as insufficient collaboration between neighboring countries (6). Moreover, scarce human and financial resources are absorbed by action against the incidence of other acute and fatal diseases, such as contagious bovine pleuropneumonia, foot and mouth disease, African and classical swine fever, and parasitic diseases (6).

In Ethiopia, although the endemic nature of bovine TB has been known since 1967, little information about its epidemiology and its public health significance is available. It is estimated that 82% of the milk is supplied unpasteurized by intra- and peri-urban producers to consumers, while only 18% is supplied by dairy enterprises in pasteurized form. The central highlands, mainly Selalle and Holeta (Fig. 1), are the major dairying areas, and as a result they are the main sources of milk for Addis Ababa, Ethiopia’s capital and main urban population center, where 8% of its inhabitants live. Furthermore, farmers in Selalle and Holeta are conscious of the milk market and produce milk for commercial sale, unlike the majority of Ethiopian farmers, who produce milk for home use. In Selalle and Holeta, farmers keep high-yield crossbred (zebu × Holstein) and Holstein dairy cattle mainly for milk production alongside native zebu breeds. It is of paramount importance to assess the...
difference in susceptibility to bovine TB among the native zebus and the exotic Holsteins or their crosses with zebus kept under identical husbandry conditions. Thus, this study was designed to compare the prevalence and severity of bovine TB among zebus, Holsteins, and zebu-Holstein crosses kept on pasture by traditional farmers in central Ethiopia.

MATERIALS AND METHODS

Study area and animals. The study was conducted in two areas (Fig. 1), namely, West Shewa (Holeta area) and North West Shewa (commonly called Selalle), which are located in the central highlands of Ethiopia. The climate of the two areas is predominantly temperate and thus conducive to dairy production. Smallholder farmers rear dairy cattle for production of milk that is sold to consumers. These areas were selected because relatively high concentrations of Holsteins and/or crosses are found alongside native zebus (mainly of the Arsi breed). In addition, the two study areas are among the few areas of Ethiopia where the two types of cattle and their cross-breeds are kept under similar conditions by traditional farmers, making a comparative study possible. The study districts and villages were further selected on the basis of the concentration of Holsteins and their interaction with zebus. The total number of Holsteins and crosses in the study area is estimated to be 40,000, of which about 30,000 are managed under a traditional farming system (grazing in the field) by local farmers, while the other 10,000 are managed under intensive or semi-intensive farming systems. These 123 cattle were obtained from among the 5,424 cattle tested for bovine TB prevalence and were selected on the basis of the level of skin induration and willingness of the owners to sell them for this research purpose. The spectrum of PPD-B and PPD-A responses was similar between the two breeds, and the response to PPD-B and PPD-A was greater than 4 mm postmortem examination and pathology scoring. A total of 153 animals (80 Holsteins and 73 zebus) with positive skin test reactions were slaughtered for postmortem examination and pathology scoring. All 73 zebus and 50 of the 80 Holsteins were obtained from grazing herds that were kept under identical husbandry conditions. Thus, this study was designed to compare the prevalence and severity of bovine TB among zebus, Holsteins, and zebu-Holstein crosses kept on pasture by traditional farmers in central Ethiopia.

Comparative intradermal tuberculin test. Purified protein derivatives (PPDs), which are crude proteins extracted from mycobacteria, were used for this study. Avian PPD (PPD-A) is extracted from Mycobacterium avium, while bovine PPD (PPD-B) is extracted from M. bovis. The two antigens were used to increase the specificity of the skin test. For each of the 5,424 cattle, two sites on the right side of the mid-neck, 12 cm apart, were shaved, and the skin thicknesses were measured with calipers. One site was injected with an aliquot of 0.1 ml containing 2,500 IU/ml PPD-B (Veterinary Laboratories Agency, Addelstone, Surrey, United Kingdom). Similarly, 0.1 ml of 2,500 IU/ml PPD-A (Veterinary Laboratories Agency) was injected into the second site. After 72 h, the skin thickness at the injection sites was measured, and the difference between the reaction sizes at the two injection sites was determined. An animal was classified as tuberculin positive if the increase in the skin thickness at the injection site for PPD-B was at least 4 mm greater than the increase in skin thickness at the injection site for PPD-A. In addition, to assess skin test responses to PPD-A and PPD-B individually, skin indurations greater than 4 mm after each PPD injection were considered positive.

Body condition scoring. The body condition of each of the study animal was scored using the guidelines established by Nicholson and Butterworth (32). Accordingly, on the basis of observation of anatomical parts such as vertebral column, ribs, spines, tail of, etc., the study animals were classified as lean (score, 1 to 3), medium (4 to 6), or fat (7 to 9). The body condition was scored at the beginning and at the end of the study.

Postmortem examination and pathology scoring. The body condition was scored at the beginning and at the end of the study. The body condition was scored at the beginning and at the end of the study. The body condition was scored at the beginning and at the end of the study.

Isolation of mycobacteria. Suspicious tissues from 145 cattle were further processed for isolation of mycobacteria in accordance with Office International des Epizooties (4) protocols. Briefly, tissue specimens for culture were collected in sterile universal bottles in 5 ml of 0.9% saline solution and then transported to the laboratory by maintaining a cold chain. In the laboratory, the specimens were sectioned using sterile blades and then homogenized with a mortar and pestle. The homogenate was decontaminated by adding an equal volume of 4% NaOH by centrifugation at 3,000 rpm for 15 min. The supernatant was discarded, and the sediment was neutralized by 1% (0.1 N) HCl using phenol red as an indicator. Neutralization was achieved when the color of the solution changed from purple to yellow (5). Next, 0.1 ml of suspension from each sample was spread onto a slant of Lowenstein-Jensen medium. Duplicate slants were used, one enriched with sodium pyruvate and one enriched with glycerol. Cultures were incubated aerobically at 37°C for about 5 to 8 weeks with weekly observation for growth of colonies.

FIG. 1. Map of the study area. Selalle and Holeta are known for their dairy production. Farmers in these areas keep three major breeds of cattle (Holstein, zebu, and Holstein × zebu) under similar conditions. Unlike the majority of Ethiopian farmers, who use milk for home consumption, farmers in Holeta and Selalle sell milk, and the two areas are the major sources of milk for Addis Ababa.
TABLE 1. Association of host-related risk factors with the prevalence of bovine TB in the central highlands of Ethiopia, determined using multivariable analysis

| Factor                      | No. (%) of animals Examed | Positive for TB | OR (95% CI) Crude | OR (95% CI) Adjusted |
|-----------------------------|---------------------------|----------------|-------------------|---------------------|
| Sex                         |                           |                |                   |                     |
| Male                        | 2,289                     | 316 (13.8)     | 1                 | 1                   |
| Female                      | 3,135                     | 416 (13.3)     | 0.96 (0.82, 1.12) | 0.94 (0.79, 1.11)   |
| Breed                       |                           |                |                   |                     |
| Zebu                        | 2,578                     | 298 (11.6)     | 1                 | 1                   |
| Cross                       | 1,921                     | 229 (11.9)     | 1.04 (0.86, 1.26) | 1.14 (0.94, 1.38)   |
| Holstein                    | 925                       | 205 (22.2)     | 2.18 (1.88, 2.65) | 2.32 (1.89, 2.85)   |
| Age (yr)                    |                           |                |                   |                     |
| <2                          | 892                       | 73 (8.2)       | 1                 | 1                   |
| 2–5                         | 1,868                     | 237 (12.7)     | 1.63 (1.24, 2.15) | 1.63 (1.23, 2.16)   |
| 5–9                         | 1,792                     | 317 (17.7)     | 2.41 (1.84, 3.15) | 2.37 (1.80, 3.12)   |
| >9                          | 872                       | 105 (12.0)     | 1.54 (1.12, 2.10) | 1.51 (1.09, 2.09)   |
| Body condition              |                           |                |                   |                     |
| Lean (thin)                 | 1,373                     | 152 (17.1)     | 1                 | 1                   |
| Medium (meat)               | 3,802                     | 511 (13.4)     | 1.25 (1.03, 1.51) | 1.13 (0.93, 1.39)   |
| Fat                         | 249                       | 69 (27.7)      | 3.08 (2.23, 4.26) | 2.53 (1.80, 3.37)   |

Data analysis. Individual animal prevalence was defined as the number of positive reactors per 100 animals tested. Logistic regression analysis was used to assess the association between prevalence and animal risk factors using STATA statistical software (STATA Corporation, College Station, TX). The differences in prevalence between the different breeds and proportions were compared using the Pearson and Chi-square test statistic. The odds ratio (OR) was calculated to assess the strength of association between different factors with the prevalence of bovine TB. The Mann-Whitney test was used to compare pathology scores between cattle types.

RESULTS

Prevalence of bovine TB assessed by tuberculin skin testing. The overall prevalence of bovine TB, as judged by the number of skin test-positive cows, was 13.5% (n = 5,424) in dairy cattle reared in the central highlands of Ethiopia. The prevalence of bovine TB was significantly higher in Holsteins than in either zebus (predominantly of the Arsi breed) (22.2% versus 11.6%, χ² = 61.8; P < 0.001) or zebu-Holstein crosses (22.2% versus 11.9%, χ² = 50.7; P < 0.001). In contrast, the skin test preva

Relationship between skin reactions to PPD-B and PPD-A. To assess individual skin test responses to PPD-A and PPD-B, skin indurations greater than 4 mm after PPD injection were considered positive. A strong association (Pearson χ² = 1,300; P < 0.001) between skin reactions to PPD-A and PPD-B was noted. As indicated in Table 2, 4.8% of the study animals responded positively to both PPD-A and PPD-B.

hand, 8.7% of them reacted only to PPD-B, while 1.3% reacted only to PPD-A.

Necropsy findings. Lesion distribution and disease severity were established in 153 skin test-positive animals that had gross pathological lesions typical of bovine TB when examined postmortem. Gross lesions typical of bovine TB were detected in 95% (145/153) of the reactor animals studied. The occurrence of TB lesions in the lymph nodes and lungs is presented in Table 3 as percentage of total animals assessed. A significant difference (χ² = 351; P < 0.001) in the tropism of TB lesions in lymph nodes was found: the percentage of mesenteric lymph nodes containing lesions was highest (94.5%), followed by retropharyngeal (74.5%) and caudal mediastinal lymph nodes (64.4%). In contrast, no tropism of TB lesions for particular parts of the lung was observed (χ² = 4.43; P = 0.49).

Figure 2 shows the severity of pathology of bovine TB determined using a semiquantitative scoring system (1, 43). Extending the findings presented in Table 3, the mesenteric lymph nodes constituted the most severely affected lymph nodes (mean pathology score ± standard error of the mean [SEM], 1.95 ± 0.08), followed by retropharyngeal (0.80 ± 0.05) and caudal mediastinal (0.8 ± 0.06) lymph nodes. The severity of pathology in Holstein and zebus was also assessed, and as shown in Fig. 3, the severity of pathology in Holsteins (mean pathology score ± SEM, 6.84 ± 0.79; median score [range], 6.0

TABLE 2. Responses to PPD-A and PPD-B

| PPD-A result | No. (%) of animals with PPD-B result | Total no. (%) |
|--------------|-------------------------------------|---------------|
| Positive     | 258 (4.8)                           | 68 (1.3)      | 326 (6.1)      |
| Negative     | 474 (8.7)                           | 4,624 (85.3)  | 5,098 (93.9)   |
| Total        | 732 (13.5)                          | 4,692 (86.5)  | 5,424 (100)    |

* Positive and negative reactions were defined as skin indurations of >4 mm and ≤4 mm, respectively. Pearson χ² = 1,300; P < 0.001.

TABLE 3. Distribution of tuberculous lesions in the lymph nodes and lobes of lungs of 145 skin test positive cattle with lesions in at least one tissue or organ

| Tissue                  | No. (%) of animals | χ²; P    |
|-------------------------|--------------------|---------|
| Lymph nodes and skin    |                    |         |
| Mandibular              | 145                | 19 (13.1)| 351; 0.0000  |
| Retropharyngeal         | 145                | 108 (74.5)|           |
| Cranial mediastinal     | 145                | 54 (37.2)|           |
| Caudal mediastinal      | 145                | 92 (63.4)|           |
| Left bronchial          | 145                | 59 (40.7)|           |
| Right bronchial         | 145                | 49 (33.8)|           |
| Mesenteric              | 145                | 137 (94.5)|           |
| Skin                    | 145                | 11 (7.6)|           |

Lobes of lungs

| Tissue        | No. (%) of animals | χ²; P    |
|---------------|--------------------|---------|
| Left apical   | 145                | 6 (4.1)| 4.43; 0.49  |
| Left cardiac  | 145                | 10 (6.9)|           |
| Left diaphragmatic | 145 | 10 (6.9)|           |
| Right apical  | 145                | 8 (5.5)|           |
| Right cardiac | 145                | 6 (4.1)|           |
| Right diaphragmatic | 145 | 13 (9.0)|           |
| Right accessory| 145               | 11 (7.6)|           |
[2 to 42]) was significantly higher \((P = 0.018,\) Mann-Whitney test) than the severity of pathology in zebus \((5.21 \pm 0.30; 5.0 [1 to 17])\).

**Bacteriology.** Fifty-six percent \((81/145)\) of the animals with gross TB lesions yielded a positive culture from at least one lymph node or other tissue sample. As indicated in Fig. 4, the highest proportion of culture positivity \((71.43\%)\) was observed in right bronchial lymph nodes, while the lowest percentage was observed in the skin \((27.3\%)\) and in the mesenteric lymph nodes \((31.5\%)\). However, culture positivity of suspicious lesions did not differ \((\chi^2 = 0.13, P = 0.72)\); comparison of proportions by use of the dose format of Epitable [Epinfo, version 6] between Holstein \((54\%, n = 50)\) and zebu \((51\%, n = 73)\) breeds kept under identical field husbandry conditions.

**DISCUSSION**

The prevalence of bovine TB recorded by the present study \((13.5\%)\) is moderate. Previously, a similar prevalence \((14.2\%, n = 416)\) \((3)\) was reported for Wolaita Soddo, in southern Ethiopia, where cattle farming is similar to that in the central highlands. In both locations, farmers keep cross-bred cattle and Holsteins in addition to zebus for milk production. Large numbers of cattle were enrolled in the present study, and the area coverage was also larger than in previous studies. Thus, the results of this study represent an accurate picture of the disease in parts of the country where farmers keep three different breeds \((zebu, zebu \times Holstein, and Holstein)\) under field \((semintensive)\) management conditions. However, a lower prevalence \((4.1\%; n = 460)\) was reported for zebu cattle under traditional management in the Boji district of western Ethiopia \((22)\). In contrast, a significantly higher prevalence of positive skin test results \((46.8\%; n = 1,171)\) was reported for 12 intensive dairy farms which keep crossbreed and Holstein cattle \((2)\). Thus, as also indicated by previous studies \((1)\), the prevalence of bovine TB is predominantly affected by cattle management and, to a lower degree, by cattle breed. The result of the present study showed that the prevalence of bovine TB was significantly higher in Holsteins than in crosses or in zebus kept under identical husbandry conditions. Furthermore, although the distribution of the pathology in reaction-positive animals of the two breeds was comparable, the severity of pathology was significantly higher in Holsteins than in Arsi zebus. This study therefore extends an earlier pilot study that also showed a trend of lower pathology scores in zebus, although because fewer animals were assessed in the earlier study, the difference in disease severity was not statistically significant \((1)\). Historical reports also indicated that *Bos taurus taurus* animals \((the group to which Holsteins belong)\) are more susceptible to bovine TB than *Bos taurus indicus*, i.e., zebus \((9, 37)\), although few contemporary data, particularly on African cattle and on Holstein cattle versus native breeds, are available. Our study therefore substantiates and extends these earlier findings.

Experimental studies also support the notion of susceptibility differences between *B. t. taurus* and *B. t. indicus* breeds: experimental *M. bovis* infection of calves of taurine cattle and zebus with 50 mg of a bovine strain of the tubercle bacillus with standard virulence indicated marked resistance in zebu calves, while calves of Ankole \((taurine cattle of African origin)\) and European breeds were susceptible \((9)\). Thus, in general, increased resistance to bovine TB has been attributed to zebu breeds, and the significance of different breed susceptibilities in cattle in developed countries, which are almost exclusively of *B. t. taurus* background, has been discounted \((34)\). The gene *Nramp1* has been shown to correlate with resistance to *Brucella*...
abortus and M. bovis BCG in cattle (36). In mice, the Nramp1 protein plays an important role in resistance (17). This protein is a divalent cation transporter and has affinity for both iron and manganese (19, 20). However,Nramp1 polymorphisms associated with resistance in mice failed to protect cattle against TB (7). In humans, some evidence predicts a role for Nramp in resistance (21). However, to reach a conclusion, more extensive studies are needed to define such resistance genes, which could help to support targeted breeding strategies for developing more resistant cattle breeds (25, 27, 42). Other factors, such as nutrition, also influence the susceptibility of cattle to bovine TB. In a case control study, Griffin et al. (18) found an association between recurrent herd outbreaks of bovine TB and the presence of rough grazing, which suggested that nutritional deficiencies lead to reduced resistance to bovine TB. Doherty et al. (14) demonstrated significantly lower numbers of lymphocyte subpopulations in nutritionally deficient cattle. Susceptibility to M. bovis infection may also be enhanced in cattle persistently infected with immunosuppressive viruses, such as bovine viral diarrhea virus or bovine immunodeficiency virus (28). Similarly, responses to the tuberculin skin test depend on the capabilities of the cellular immune response, which in turn is affected by the level of nutrition in terms of protein energy and micronutrients (35). Thus, animals in good physical condition respond to tuberculin skin tests better than those in poor physical condition, as was also observed in this study.

The proportion of reaction-positive animals increased with age, reaching a maximum in animals between 5 and 9 years of age and then declining. Similarly, studies in Canada and Northern Ireland indicated an increased incidence of bovine TB with increased age (30). The reason could be, as suggested earlier by Mackay and Hein (24), the possible influence of increased age (30). The reason could be, as suggested earlier by Mackay and Hein (24), the possible influence of increased age (30).

In conclusion, this study determined the prevalence, based on skin test reactivity, of bovine TB in cattle reared under low-intensity farming conditions in the central highland of Ethiopia. The prevalence was significant yet considerably lower than in cattle reared under more intensive farming conditions. We also found significantly lower prevalence in native zebu breeds than exotic Holstein cattle; this difference in breed susceptibility was also highlighted by the demonstration of increased disease severity in Holsteins. In the light of the introduction of increased numbers of Holstein cattle into this area to raise milk production to satisfy the needs of Addis Ababa’s growing population, these findings highlight the need for a control program in these herds.

ACKNOWLEDGMENTS

The Wellcome Trust is acknowledged for its financial support.

The technical support of Surane Gemeda and the other technical staff of the Animal Health and Zoonoses Unit of the Aklilu Lemma Institute of Pathobiology is also acknowledged.

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