Prevalence of bovine tuberculosis in zebu cattle under traditional animal husbandry in Boji district of western Ethiopia

G. LAVAL* and G. AMENI

* Corresponding author : e-mail: geraudparis@hotmail.com
Villa Sarepian - 64310 F. - SARE

Summary

A study was conducted to determine the herd and individual animal prevalence of bovine tuberculosis (BTB) in zebu cattle in Boji district in western Ethiopia. A total of 62 herds consisting of 780 heads of cattle were included in this study. Of the 62 herds, 35 were tested with single intra dermal tuberculin test and another 27 herds with comparative intra dermal tuberculin test. The result of single intra dermal tuberculin test indicated a herd prevalence of 51% (18/35) and an individual animal prevalence of 4.1% (19/460). The herd prevalence was 19% (5/27) while the individual animal prevalence was 1.6% (5/320) with comparative intra dermal tuberculin test, 2.2% (7/320) of the animals being doubtful. The association between positive reactions and respiratory symptoms was statistically not significant (P>0.05). BTB was reported in zebu cattle kept under traditional farming system for the first time in Ethiopia. On the basis of the results of the present study, the potential risk of infection of man with M. bovis is discussed.

KeyWORDS : Mycobacterium bovis, intra dermal tuberculin test, zebu cattle, Boji district, Ethiopia, zoonosis.

Introduction

Bovine tuberculosis (BTB) caused by Mycobacterium bovis is a major cause of human gastrointestinal tuberculosis in developing countries [6]. Un-pasteurised contaminated milk and other secretions or tissues from animal hosts can serve as the source of infection for humans [14], milk through ingestion being regarded as the principal one. Primary invasion, through pharynx or intestine, results in lymphadenitis of adjacent lymph nodes [24]. Haematogenous dissemination to vertebrae can cause bone disorders that can result in a hunchback. Infection through inhalation of aerosol causes in humans the development of a primary lesion (tubercle) in the respiratory tract [24]. M. bovis is a member of the Mycobacterium tuberculosis complex, a group of mycobacterial species that includes M. tuberculosis, M. bovis, M. africanum and M. microti. Human infection with any of these species of mycobacteria can result in tuberculosis and, regarding the responsible species, the disease is similar clinically, radiologically, or pathologically [22]. Sub-populations at risk of M. bovis infection include any population consuming un-pasteurised contami-
number of notified cases of human tuberculosis is increasing from time to time in urban and rural areas of Ethiopia, with a country average case-notification rate of 197 per 100,000 [20], the role of *M. bovis* is not known because differential diagnosis between Mycobacterium species is not undertaken.

Though a few studies have reported high prevalence of BTB in commercial dairy farms in Ethiopia [3, 16] and Eritrea [21], the extent of the disease in traditional husbandry system is not known. More than 95% of the Ethiopian farmers are still keeping zebu cattle using the traditional animal husbandry system. This segment of the population consumes animal products raw and often shares the same sheltering with animals. This study was formulated to investigate BTB in zebu cattle in a traditional rural area of the district of Boji (West Wellega Zone), which was also the field site of a research project primarily designed to study the spread of contagious bovine pleuropneumonia (CBPP).

### 2. Description of the study area and the production system

The district of Boji (Fig. 1) is situated in the Administrative Zone of West Wellega, in the west part of Ethiopia at latitude of 9.36° N and a longitude of 35.59°E. Its surface area is 966.1 square km, which represents 4% of the West Wellega Zone. West Wellega has a population of 1.9 million, the district of Boji sharing 100,300 of it [10]. Boji has a population density of 103.8 inhabitants per square km and is located at an altitude varying from 1200 to 2100 m above sea level. The larger part of it is situated in the agro-ecological zone of Woina Dega [13], a zone of moderate altitude, between 1500 and 2300 m characterised by a relatively high rainfall level. A smaller part of the district in the Northeast is located in the lowland zone, named Kolla, with an altitude below 1500 m. The area has a monomodal rainfall from May to October attaining a peak in July. The other months are dry during the year except that sometimes the rain can start in March or April and continues until November. The rainfall fluctuates between 1300 and 2000 mm per year in West Wellega Zone from district to district and year to year (personal comm., West Wellega Department of Agriculture, 2002).

The production system observed in the district of Boji combines cereal-based agriculture and livestock farming. The farms are of small size and are characterised by a subsistence economy. The cultivation practices in Boji are similar to other equivalent agro-ecological zones in the Ethiopian highlands. Cattle farming play a major role in the production system. Cattle population in the district of Boji during the study was 55,700 (Personal Comm. Boji district Agricultural Office, 2002) and the average herd size was 10.5 heads of cattle [17]. The cattle in Boji district are of the Horro breed, an intermediate Sanga-zebu type and also called « Zenga » [1]. The main objective of livestock farming is the use of oxen for farm works, especially ploughing activities. The other animal production purposes in Boji district are manure, milk and live animals. Manure is used as an organic fertiliser but never used as fuel. Milk is collected twice a day from lactating cows, on average 1.2 litres per day, and processed at farm into butter and cottage cheese. Butter can be marketed in the local market and cottage cheese, which is un-cooked, is always consumed at home. Live animals can be slaughte-
red at the farm for meat consumption and hides sold at the local market. One special feature of the livestock practices in Boji is the widespread use of animal exchanges between farmers, a possible risk factor in the spread of contagious diseases in this region [17]. The rearing of small ruminants is not well developed.

3. Materials and methods

3.1. STUDY ANIMALS AND SAMPLING.

Seventy herds were monitored during one year, between December 2000 and November 2001 in the district of Boji, primarily for the need of a study on CBPP. These herds were selected according to two criteria: (i) they comprised at least five owned animals at the beginning of the monitoring period to ensure survey continuity at the herd level; (ii) some herds (the objective being 50% of them) were selected because they were suspected to be newly contaminated by CBPP; they should have animals that recently presented respiratory symptoms, which was investigated using farmers’ interviews. Each animal was identified by a numbered ear-tag. The monitoring had demographic and health components. The selected herds were only representatives of one part of the district, namely the highlands, and located within a radius of 15 km around Bila (district town). The mean herd size during the monitoring was 16.2 heads of cattle. According to the initial serological results, 25 to 30% of the monitored herds had at least one infected animal with CBPP during the study period. Among the 70 herds, intra dermal tuberculin test was conducted on 780 heads of cattle above 6 months from 62 herds. The other 8 herds were not included in the study because of small size (less than 4 animals available for testing) or inconvenience to owners.

3.2. SINGLE INTRA DERMAL TUBERCULIN TESTING

Using the method described by OIE [23], single intra dermal tuberculin (SID) test was conducted on 460 heads of cattle in 35 herds. The middle neck of each of the study animal was the injection site for tuberculin. After the thickness of the site was measured with callipers, 0.1 ml of bovine tuberculin (Bovituber PPD, Merial, France) containing 20,000 CTU/ml of purified protein derivative (PPD) was injected into the dermis of each animal. Three days (72 hours) after injection, the site was measured again for swelling. When the swelling was equal to or less than 2 mm, between 2 mm and 4 mm, and equal to or greater than 4 mm the individual results were interpreted as negative, doubtful and positive, respectively. At herd level and because of the small size of the herds, the result was interpreted as positive if at least one animal was diagnosed as positive.

3.3. COMPARATIVE INTRA DERMAL TUBERCULIN TESTING

Because of the finding of several doubtful reactors by the single intra dermal tuberculin test, it was decided to conduct comparative intra dermal tuberculin (CID) test on the rest of the study animals. This test was conducted on 320 animals in 27 herds. The skin thickness at two sites in the middle neck of each study animal were measured and 0.1 ml (20,000 CTU/ml) of bovituber PPD at one site and 0.1 ml (25,000 IU/ml) of avian tuberculin PPD (Avituber PPD, Merial, France) at the other site were injected into the dermis. Three days (72 hours) after injection, the sites were measured again for swelling. The test was interpreted as indicated by OIE [23]. A reaction was considered to be positive if the bovine reaction was non negative (B<2 mm) and more than 4 mm greater than the avian reaction. The reaction was considered to be doubtful if the bovine reaction was positive and from 1 to 4 mm greater than the avian reaction. The reaction was considered to be negative if the bovine reaction was negative (B<2 mm) or if the bovine reaction was positive, but equal to or less than a positive avian reaction.

At herd level, the result was interpreted as positive if at least one animal was diagnosed as positive. No graphic interpretation was carried out at the herd level because of the small size of the herds and the small number of reactions observed.

3.4. CLINICAL EXAMINATION

Out of the 780 tested heads of cattle, 508 were continuously monitored during one year before tuberculin testing. Enumerators were visiting the study herds every two weeks and recording clinical signs of sick animals with particular emphasis on respiratory tract. Respiratory symptoms such as cough, nasal discharge and dyspnoea were recorded and respiratory frequency was measured. Symptoms of digestive systems were also recorded.

3.5. DATA ANALYSIS

Herd prevalence was defined as the number of reactor herds out of 100 herds tested while individual animal prevalence was defined as the number of reactor animals out of 100 tested animals. Both herd and individual animal prevalences were expressed in percentage; they were estimated from the results of diagnostic tests (visible prevalences) and may differ from the real prevalences. The chi-square test was used to analyse association between respiratory symptoms and positive or doubtful reactions to the bovine tuberculosis only (for both SID and CID tested groups). Association was considered significant at the 0.05 level.

4. Results

4.1. SINGLE INTRA DERMAL TUBERCULIN TEST

If considering overall animals from both SID and CID tested groups and when only interpreting the bovine tuberculosis reaction (with the SID test criteria), the number of positive, doubtful and negative animals was 32, 20 and 728 respectively (i.e. 4.1%, 2.6% and 93.3%) and 29 herds were found positive with 1 or 2 positive animals in each.

Out of 35 SID tested herds, 18 showed positive reactors at...
4 mm threshold of sensitivity, giving a herd prevalence of 51%. If the threshold had been fixed at 2 mm, then 24 (69%) herds would have been positive. Within the positive herds (at 4 mm threshold), the average number of positive, doubtful and negative animals was 1.06, 0.44, and 13.0 respectively. On average 11.6 animals were tested (negative) within the 17 negative herds. The result of SID test for individual heads of cattle is indicated in Table I for different age-sex categories of animals (SID tested group only).

When only interpreting the reaction to bovine tuberculin (with the SID test criteria) in the CID tested group, the number of positive, doubtful and negative reactions was 13, 5 and 302 respectively (i.e. 4.0%, 1.6% and 94.4%); 11 herds were found positive out of 27.

4.2. COMPARATIVE INTRA DERMAL TUBERCULIN TEST

Using CID test criteria, only 5 out of 27 herds tested positive (19%); they showed one single positive, zero doubtful and an average 11.8 negative animals. Seven other animals tested doubtful within 5 other herds. Additionally, among the negative animals, 13 showed a reaction to avian tuberculin with a skin swelling greater than 2 mm and were considered as non-specific reactors. The result of CID test for individual heads of cattle is indicated in Table II for different age-sex categories of animals.

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Number of animals tested</th>
<th>P</th>
<th>D</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4 years</td>
<td>F</td>
<td>88</td>
<td>2</td>
<td>2</td>
<td>84</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>82</td>
<td>3</td>
<td>3</td>
<td>76</td>
</tr>
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<td>10</td>
<td>5</td>
<td>167</td>
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<tr>
<td></td>
<td>M</td>
<td>108</td>
<td>4</td>
<td>5</td>
<td>99</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>460</td>
<td>19</td>
<td>15</td>
<td>426</td>
</tr>
<tr>
<td>Average per herd</td>
<td></td>
<td>13.14</td>
<td>0.54</td>
<td>0.43</td>
<td>12.17</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td></td>
<td>100</td>
<td>4.1</td>
<td>3.3</td>
<td>92.6</td>
</tr>
</tbody>
</table>

P = Positive (> 4 mm) ; D = Doubtful [2-4mm] ; N = Negative (< 2mm)

Table I — Prevalence of bovine tuberculosis per age-sex categories in 35 cattle herds in the Boji district (West Wellega Zone) of western Ethiopia using single intra dermal tuberculin (SID) test

4.3. OBSERVATION OF SYMPTOMS

Among 508 animals monitored, 12 showed a chronic syndrome characterised by diarrhoea and/or emaciation. All tested negative, 10 with SID test and 2 with CID test, including one showing a non-specific reaction (B= 5mm ; A = 4.5mm). Association between chronic syndromes and non-specific reactions could not be shown statistically.

Eighty-six (86) animals showed respiratory symptoms, with acute or chronic expression. The relationship between

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Number of animals tested</th>
<th>B ≤ 2mm</th>
<th>B &gt; 2mm</th>
<th>&lt; 1mm</th>
<th>[1 – 4mm]</th>
<th>&gt; 4mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 4 years</td>
<td>F</td>
<td>66</td>
<td>65</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>47</td>
<td>44</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>≥ 5 years</td>
<td>F</td>
<td>156</td>
<td>128</td>
<td>8</td>
<td>3</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>71</td>
<td>65</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>320</td>
<td>302</td>
<td>18</td>
<td>6</td>
<td>7</td>
<td>5</td>
</tr>
<tr>
<td>Average per herd</td>
<td></td>
<td>11.85</td>
<td>11.19</td>
<td>0.67</td>
<td>0.22</td>
<td>0.26</td>
<td>0.19</td>
</tr>
<tr>
<td>Prevalence (%)</td>
<td></td>
<td>100</td>
<td>94.4</td>
<td>5.6</td>
<td>1.9</td>
<td>2.2</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table II — Prevalence of bovine tuberculosis per age-sex categories in 27 cattle herds in the Boji district (West Wellega Zone) of western Ethiopia using comparative intra dermal tuberculin (CID) test.
bovine tuberculosis (M. bovis) is a zoonotic disease that can affect both humans and animals. The prevalence of bovine tuberculosis is influenced by various factors such as age, breed, and management practices. In Ethiopia, the prevalence of tuberculosis in cattle is said to be low; however, it varies significantly among different regions. The prevalence in dairy herds, which are kept under intensive or semi-intensive farm management, was lower compared to beef and dual-purpose herds. The low prevalence of bovine tuberculosis in the Boji district (West Wellega Zone) of western Ethiopia is attributed to the traditional animal husbandry system found in the area. In the study, animals were kept in open-air even in the night, which is expected to reduce the rate of transmission of M. bovis. Individual reactors occurrence observed in Boji is the circulation of the organism and investigate into its biochemical and molecular characteristics. Both culture and identification of the mycobacterial species are complicated, potentially dangerous and require the expertise, which is rarely available in developing world.

### 5. Discussion

The prevalence of tuberculosis in cattle is said to be low when it is 5% and at this point, the test and slaughter control method might be considered an economical option for control [6]. The results in Boji district showed prevalence near to 5%. Nevertheless in the Ethiopian context, the test and slaughter control method cannot be implemented because of obvious financial and logistical constraints. This prevalence was lower than results of previous studies conducted with intra dermal tuberculin tests (single or comparative) in intensive dairy farms showing individual prevalence of 50% [2] and 38% [16] in Ethiopia and 14.5 % in Eritrea [21]. It was also lower than 14.2% [3] found in small-holder dairy farms in Ethiopia. In fact, the previous studies were conducted on either exotic or crossbred animals that were kept under intensive or semi-intensive farm management. However, the present study was purely conducted on zebu breed under traditional farming system. In herds considered as positive in our study, the number of positive reactors was low with only 1 or 2 animals (out of an average 12 to 14 animals tested). The prevalence of TB was influenced by breed of cattle and type of farming (intensive, semi-intensive, extensive), housing and gathering of animals at grazing and watering areas. The fact that zebu cattle are relatively resistant to BTB [26] substantiates the lower prevalence recorded by the present study. Furthermore, in the case of the traditional animal husbandry system found in Boji, animals are kept in open-air even in the night, which is expected to minimise the rate of transmission of M. bovis. Individual variation of allergic reactivity to tuberculin is a major limitation of intra dermal tuberculin tests. Intensity of reaction may vary between individuals and absence of reaction (anergy) is common especially in case of intercurrent disease occurrence (parasites, CBPP), length of infection with M. bovis (terminal anergy) or during transitory periods for various reasons (use of treatments, vaccination, pregnancy...); furthermore old animals above 5 years commonly show lower reactions to tuberculin tests. In the study area, high prevalence of parasitic diseases (internal and external, trypanosomiasis) and CBPP, as well as the structure of the herd (more animals above 5 years than young animals) may be additional factors that contribute to explain the apparently low prevalence of bovine tuberculosis.

In addition to tuberculin tests, the identification of M. bovis from reactor animals would be confirmatory diagnosis. Nevertheless, identification depends on the ability to culture the organism and investigate into its biochemical and molecular characteristics [8]. Both culture and identification of the mycobacterial species are complicated, potentially dangerous and require the expertise, which is rarely available in developing world.

No association was observed between positive reactors and respiratory symptoms. The high occurrence of respiratory symptoms observed in the study animals can be explained by CBPP, a major respiratory disease of cattle that was prevalent in the study area. Many animals showed doubtful or non-specific reactions. Non-specific reactors are mainly caused by infection either with M. avium or M. paratuberculosis. As a consequence to close antigenic relationship between M. avium and M. paratuberculosis, cattle with clinical Johne’s disease (caused by M. paratuberculosis) were found to react to avian tuberculin by Quinn et al. [25]. Our results may suggest the occurrence of Johne’s disease in the study area. Nevertheless, clinical signs characteristic of Johne’s disease, such as chronic diarrhoea and emaciation, could not be statistically associated to non-specific reactors among study animals. Another assumption to explain the non-specific reactors occurrence observed in Boji is the circulation of M. avium hold by poultry and possibly transmitted to cattle. Poultry keeping is commonly practised in Boji district; chicken were observed scavenging with cattle around houses. Clinical observations and post-mortem examinations of poultry were not carried out during the study albeit they could help to better understand the occurrence of avian tuberculosis at the farm level; such searching should be implemented if further investigation are conducted on this topic in Ethiopian highlands.
The prevalence of bovine tuberculosis in Zebu cattle under traditional animal husbandry is a significant concern. In Europe and North America, 0.5% to 1.0% of human tuberculosis cases are estimated to be caused by Mycobacterium bovis infection [15]. This is after the intensification of BTB control programs. However, in the earlier years before the launching of control programs, the proportion of human tuberculosis cases due to M. bovis was between 5% and 20% [14]. In developing countries like Ethiopia, where BTB is still common and pasteurisation of milk is not practised, an estimated 10% to 15% of human cases tuberculosis is caused by M. bovis [4]. In the 1990s, an estimated 9 million cases of tuberculosis occurred each year worldwide, approximately 10% of which among individuals infected with HIV [19]. From the country and regional data suggesting that 1% to 15% of those cases may be caused M. bovis, an annual incidence worldwide of between 90,000 and 1,350,000 cases of M. bovis associated tuberculosis can be estimated [4]. In Ethiopia, the study conducted by Gellete et al. [12] indicated an overall HIV sero-prevalence of 44.4% among tuberculosis patients. The same authors indicated a significantly higher HIV sero-positivity for extra pulmonary than for pulmonary cases of human tuberculosis. The higher probability that these extra pulmonary cases are caused by M. bovis may thereby indicate the role of M. bovis in HIV patients. The incidence of HIV infection in Ethiopia is kept on increasing not only in the towns but also among rural community, mainly composed of farmers who are closely associated with animals.

The results of the present study showed the occurrence of BTB in zebu cattle, as diagnosed by tuberculin test although no association was observed between reactors and respiratory symptoms. Complementary post-mortem investigations of lesions in slaughterhouses would give further information concerning the prevalence of BTB. Similar studies should be conducted in different parts of the country to establish the magnitude of the disease in both animal and man. Additionally, public education on the cooking of milk before consumption is indicated.

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