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With 2 tables in the text

A Preliminary Study on Bovine Tuberculosis in Stallholder Farms and its Zoonotic Implication to Owners in Fitche Town, Central Ethiopia

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ملخص البحث

أعدت هذه الدراسة الأولية لتقصى وبائية مرض الدرن في الأبقار و مقارنة ذلك بمدى انتشاره في الإنسان في بلدة فيتشى الأثيوبية. شملت الدراسة 170 (مائة و سبعين) حيازة منزلية للأبقار تحتوى على عدد 735 رأساً بالإضافة إلى 170 أسرة مالكة لهذه الأبقار في هذه المنطقة. الوسائل التى استخدمت في الدراسة هي اختبار التيوبركلين، زراعة 15 عينة من ألبان الأبقار و10 مسحات أنفية واستبيان. أظهرت نتيجة اختبار التيوبركلين في الأبقار أن نسبة 15.3% (170/26) من القطعان و 4.2% (735/31) من الابقار المصابة بالدرن بينما لم تعط العينات التى زرعت نتائج إيجابية. حللت هذه النتائج إحصائيا وأظهرت وجود علاقة بين فصيلة الأبقار و الحالة الإنتاجية و الحالة الجسدية للأبقار أو معدل الإصابة و إدارة المزارع. اظهر الكشف على مالكي الأبقار بالمدينة أن 14 أسرة من جملة 170 (8.1%) مصابة بالدرن، كما تأكدت إصابة 17 شخصاً من تلك الأسر بظهور الأعراض ونتائج فحص اللعاب وصور الأشعة. تفيد تقارير مستشفى المدينة عن الفترة من سبتمبر 1991 إلى فبراير 2000 أن 1034 شخصاً قد زاروا المستشفى للعلاج من المرض. تظهر الدراسة أن هناك علاقة ما بين انتشار المرض في الأبقار وانتقاله للإنسان ولكن نسبة الحالات البشرية ليست خطيرة وبدرجة تسمح بامتلاك الأبقار بواسطة المواطنين وفي المقابل لابد من تفعيل دور الإرشاد الصحي منبهاً إلى أهمية قتل الميكروب بواسطة غلي الحليب كضرورة صحية.

Summary

A preliminary study was conducted in Fitche town from November 1999 to April 2000 to assess the epidemiology of bovine tuberculosis (BTB) on 170 herds containing 735 head of cattle and its zoonotic implications on 170 households owning these animals. The methods applied were comparative intradermal tuberculin test (CIDT), mycobacteriological examinations and questionnaire survey. Herd and individual animal prevalences of BTB were 15.3% (26/170) and 4.2% (31/735), respectively. Statistically, significant association was observed between breed(P=0.02), reproductive status (P=0.001), body condition (P=0.011) or management (P=0.010) and prevalence of BTB.

No growth of *Mycobaterium bovis* (*M. bovis*) was observed on Lewenstein-Jensen medium by culturing 15 milk samples and 10 nasal swabs from reactor cattle. Out of the 170 households, 14(8.23%) had human tuberculosis (TB) in their families; 17 confirmed cases (clinical, smear or chest X-ray examinations)

of human TB were recorded, of which 15 were pulmonary tuberculosis (PTB) and 2 were extrapulmonary (EPTB). Analysis of data registered in Fitche hospital from September 1991to February 2000 showed a total of 1034 TB cases, 209(20.2%) were EPTB. The association between reactor cattle and human TB in household was marginally significant (14/170 vs. 2/170, McNemars χ^2 =4, P=0.0455). In conclusion, the result of the present study revealed a low prevalence of BTB in stallholder dairy farms, thus encouraging their expansion in the future. On the other hand, although no *M. bovis* was isolated from the milk of reactor cows, public education on the boiling of milk before consumption is recommended.

Introduction

One of the regions of the world with rapidly growing human population is the sub-Saharan Africa.The current data shows that human population is 500 million in sub-Saharan region and it is expected to be 1300 million by the year 2025 (WHO, 1999; UNDP, 1999). By the year 2020, the Ethiopian population is estimated to be 139 million making the country the third most populous in Africa (WHO,1999).As a result, the demand for animal products is increasing both in terms of quantity and quality, requiring the intensification of animal production.This should be accompanied by the control of livestock diseases such as BTB.

Though BTB is endemic in Ethiopia, there is scarcity of information on its epidemiological pattern and zoonotic importance in the country (Abdo, 1993). The result of a few studies conducted in the country showed a prevalence ranging from 10.1% in stallholder farms to 50% in peri-urban intensive dairy farms (Ameni and Roger, 1998; Kiros, 1998; Ragassa, 1999; Bogale *et al*., 2001). Presently, the Ministry of Agriculture is promoting milk production by encouraging the expansion of stallholder dairy farms in different regions of the country. Such regions include Fitche town area, which is known for its dairy production and is the major source of milk for Addis Ababa. Though dairy production is practised in Fitche, the status of BTB in dairy farms is unknown. Furthermore, generation of information on its zoonotic implication on dairy farmers is vital so as to propose control measures. Therefore, this study was undertaken to assess the

epidemiology of BTB in stallholder dairy farms and its public health implications to owners of the small-scale diary farms in Fitche town.

Materials and Methods

Description of the study area:

This study was conducted in Fitche town, located 9^o 48N and 38^o 44E at about 115 km north of Addis Ababa, central Ethiopia. The altitude of Fitche is 2800m a.s.l, its minimum and maximum temperatures vary from 11.5-29^{o}C and 17.5-35^{o}C, respectively. It gets bimodal rainfall that ranges from 651-1115mm (National meteorological service) the predominant soil types of the area are black soil (38.2%) and bright red soil (38%). Mixed agricultural activities are practised in the area (North Shoa Agricultural Department).

Study subjects and sampling:

One hundred and seventy herds, each possessing at least either one crossbred or one exotic breed of cattle, were included in this study. Thus, 162 pure Holstein-Friesian and 573 cross breeds, all were above 6 months of age, were examined. Similarly, 170 households owning the study herds were considered in this study. Moreover, retrospective data from Fitche hospital were collected and analyzed. The body condition of each sampled animal was scored using the guidelines established by Nicholson and Butterworth (1986). Accordingly, the animals were categorized into lean (Score 1-3), medium (Score 4-6) and fat (Score 7-9) groups. The management of the farms was categorized into three categories (good, medium and poor) on the basis of housing condition (neatness, waste disposal, nature of the floor, presence of confinement), feeding (concentrate plus hay), possession of a recreation place, contact with other herds and provision with clean water.

Comparative Intradermal Tuberculin Test (CIDT):

Each study animal was inoculated with 0.1 ml of 20,000i.u/ml of bovine purified protein derivative PPD (AN5 strain, Bovituber, Rhone-Merieux) and 0.1 ml of 25,000 i.u/ml of avian PPD (D4ER strain, avituber, Rhone-Merceux) at two sites, 12 cm apart, in the middle of the neck. The injection sites were examined for swelling and thickness after 72 h of injection. The difference in skin thickness after and before injection at both sites was used for the interpretation of results. When the difference in skin thickness was greater at the site of injection of avian PPD than that at bovine PPD, then the animal

was considered as positive for *M. avium* or other atypical mycobacteria; but when the change in skin thickness was increased at both sites, difference in thickness of the two sites was considered. Thus, if the increase in skin thickness at the injection site for bovine PPD (B) was greater than that at the injection site for avian PPD (A) and the difference in thickness between B and A was less than 2mm, between 2 and 4 mm or above 4mm, the animal was considered to be negative, doubtful and positive, respectively.

Cultural methods:

All activities related to cultivation of mycobacteria were manipulated in a biological safety cabinet. Milk samples from 15 reactors were processed for culturing. Accordingly, 50 ml of milk was drawn from the four quarters of each cow towards the end of milking and centrifuged at 3500 rpm for 15 minutes at room temperature. The cream was removed with a sterile swab, the supernatant taken off and the sediment decontaminated with 4% NaOH and centrifuged again in the same condition and neutralized with 1% Hcl. Thereafter each sediment was inoculated on to Lowenstein-Jensen medium with pyruvate. The culture was incubated at 37^{o}C for about 8-12 weeks. Sub-culturing and Ziehl Neelsen staining of the suspected colonies were made. Nasal discharges were collected from 10 reactors using sterile swabs which were cultured using a similar procedure and incubated under the same conditions indicated for milk culture.

Questionnaire survey:

The farmers (head of each household) were interviewed on the awareness of the transmission of TB from cattle to man and vice versa, habit of milk and meat consumption, recent history of TB in his/her family members, if so, the type of TB (PTB or EPTB) and response of the cases to treatment.

Data management and analysis:

The data collected from the field was entered into computer using Microsoft Excel and transferred to STATA version 6 for statistical analysis. All categorical variables were expressed in percentages.

The association between each risk factor and the outcome variable was assessed using Chi-square. When assumptions of Chi-square failed Fisher's exact test was used. For the paired type of data McNemars Chi-square was used.

Results

Prevalence:

Of the 170 herds tested, 26 were positive, thus giving a herd prevalence of 15.3%. An individual animal prevalence of 4.2% (31/735) was recorded. Nevertheless, no growth of *M. Bovis* was obtained from the milk and nasal swab samples collected from reactors.

Association of Risk Factors with the Prevalence:

Table 1 shows the association between the different risk factors and the prevalence of BTB. Breed (P=0.02), reproductive stage (P=0.001), body condition (P=0.011) and management (P=0.010) were significantly associated with the prevalence of BTB. On the other hand, no significant association was statistically obtained between prevalence and either age group (P=0.121) or sex group (P=0.06).

Zoonotic Implication

Out of the total 170 households interviewed during the study period, 14 (8.23%) had TB case(s) in their families. In these 14 households, a total of 17 cases of TB [15(88.2%) with PTB and 2 (11.8%) with EPTB] were recorded. Besides, 78.9% of the households had the habit of consuming raw milk.

Only 10(5.9%) of the households shared the same shelter with their cattle. Nevertheless, the association between reactor cattle and human TB cases in the households (Table 2) was marginally significant (14/170 vs 26/170, McNemars $\chi^2 = 4$, P=0.0455).

The consciousness of the households about the transmission of BTB from cattle to man improved as the educational background of the household increased. Analysis of secondary data from Fitche hospital indicated that from 1034-recorded TB cases from September 1991 to February 2000, 825 (79.8%) were PTB while the rest 209 (20.2%) were EPTB.

Table 1: Comparison of the different risk factors affecting reactivity of cattle to tuberculin test in Fitche, central Ethiopia.

Characteristic	No. examined	Number(%) positive	P-value
Age			0.121
<1	9	0(0)	
(1, 3]	264	13(4.9)	
(3,6]	183	9(4.9)	
>6	197	9(4.6)	
Sex			0.066
Male	141	2(1.4)	
Female	594	29(4.9)	
Breed			0.022
Cross	573	19(3.4)	
Exotic	162	12(7.4)	
Reproductive cycle			0.0001
Heifer	143	2(1.4)	
Lactating	206	14(6.8)	
Early pregnant	76	9(11.8)	
Mid-pregnant	47	2(4.3)	
Late pregnant	46	2(7.7)	
Dry	40	0(0)	
Body condition			0.011
Poor	126	0(0)	
Medium	449	24(5.4)	
Good	160	7(4.4)	
Management			0.010
Poor	133	9(6.8)	
Medium	337	18(5.3)	
Good	265	4(1.5)	

Table 2: The association between reactor cattle and human TB cases in the households in Fitche, Central Ethiopia.

CIDT herds results	TB Status of house holds for		
	Total	**Positive**	**Negative**
Positive	26	2	24
Negative	144	12	132
Total	170	14	156

(14/170 household vs. 26/170* herds, McNemars $\chi^2 = 4$, P = 0.0455).*
**=no. positive / no examined.*

Discussion

The prevalence recorded in the present study is low compared to the previously reported prevalence in the country (Ameni, 1996; Kiros, 1998; Ragassa, 1999; Bogale *et al*., 2001; Ameni *et al*., 2002). This may be due to the fact that the present study was conducted on herds of small sizes in contrast to previous studies, which were conducted on relatively large herd-sized farms. O'reilly and Daborn (1995) have indicated that the transmission of BTB from cattle to cattle is largely influenced by herd size, i.e. the larger the herd size the greater the chance of transmission. Moreover, when larger proportion of the study animals was grazing in the field, the level of confinement is reduced to a certain degree, which in turn minimizes the rate of infection in the herd (OIE, 1996).

Similar to the result of this study, higher prevalence has also been recorded in pure Holestein as compared to the crossbreeds (O'Reilly and Daborn, 1995; Barwinek and Taylor, 1996; Kiros, 1998; Ragassa, 1999). The probable reason could be the fact that genetically improved cattle suffer more severely from poor housing, under-and malnutrition and subsequently become more susceptible to infection (Barwinek and Taylor, 1996). Contrary to the results reported by other investigators (0'Reilly and Daborn, 1995; Kiros, 1998; Gragassa, 1999), relatively high prevalence was recorded in good managed animals.This could be due to the fact that most of the herds kept under good management were kept in houses while those categorized under poor management were dependent on grazing in the field. It has been indicated that grazing in the field minimizes the level of confinement thereby reducing the chance of infection (Cook, 1997). Radostits *et al*. (1994) also believed that housing predisposes to TB, hence the disease is more common and serious in animals that are kept in house.

The prevalence increased with age reaching the maximum around the age of 6 years and decreased afterwards. O'Reilly and Daborn (1995) indicated that the chance of infection with *M. bovis* increases as the age increases. Large number of reactors was observed in animals with better body condition; similar results were also reported by Cook (1997), Ameni (1996) and Kiros (1998). This finding could be due to the fact that animals with good body condition have a better immune status and thus respond to any foreign protein better than those with poor body condition, which may be immuno-compromised as the result of malnutrition and/or other stress factors (Cook, 1997). The prevalence was influenced by the reproductive stage of female animals. This is because some of the reproductive stages (Late pregnancy and lactation) are stressful and thus reduce the immune reaction of the female animal to tuberculin test.

No growth of *M. bovis* was obtained from milk and nasal swabs of reactor animals in the present study. Previously, different authors (Ameni, 1996; Kiros, 1998; Ameni *et al*., 2002) have isolated *M. bovis* from milk of reactor cows in Ethiopia. It is established that all reactor cows do not excrete TB bacilli in their milk. In this connection, it was indicated that even if animals are infected with *M. bovis*, the chance that they excrete the bacilli in their milk is only 1% (Grange and Yates, 1994).

The existence of human TB patients and reactor cattle in a household suggests that either the former or the latter could be a source of infection for the other. The transmission could be cyclical, i.e. cow—man—cow (Cosivi *et al.*, 1998). Similar finding was reported in Wolaita Soddo, southern Ethiopia by Ameni *et al*. (2001). Different authors (Cosivi *et al.*, 1998; Ashford *et al*., 2001) have indicated that PTB as well as EPTB of animal origin would continue to be a public health problem especially in areas where prevalence of infection in cattle is high and where raw milk and its products are commonly consumed.

The presently recorded prevalence was lower than the previously reported and thus, encouraging the expansion of stallholder dairy farms. On the other hand, although no *M. bovis* was isolated from the milk of reactor cows, public education on the boiling of milk before consumption is recommended.

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