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Bovine tuberculosis on small-scale dairy farms in Adama Town, central Ethiopia, and farmer awareness of the disease

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Summary

A cross-sectional study was conducted on 378 households, owning 524 head of cattle, to determine the prevalence of bovine tuberculosis (TB) and assess its public health implications. Stratified systematic sampling and a questionnaire were used, in addition to the comparative intradermal tuberculin test, mycobacterial culture and biochemical and drug susceptibility tests.

Using a confidence interval (CI) of 95%, the herd and animal prevalences were 15% (11.4%, 18.6%) and 11% (8.32%, 13.68%), respectively. A significant variation in prevalence (P) was observed in regard to herd size ($\chi^2 = 32.3$, $P < 0.0001$); age group ($\chi^2 = 11.47$, $P < 0.05$); and reproductive status ($\chi^2 = 10.9$, $P < 0.05$). The herd prevalence was significantly associated with feeding practices ($\chi^2 = 6.6$, $P < 0.05$) and respiratory signs ($\chi^2 = 8.7$, $P < 0.01$). A significant ($\chi^2 = 8.4$, $P < 0.01$) association was recorded between the presence of reactor cattle and human TB cases in a household. Thirty-five percent (132/378) of the respondents knew about bovine TB while only 32% (121/378) were aware it could be transmitted from cattle to humans. *Mycobacterium bovis* and *M. tuberculosis* were isolated from 18.2% and 11.4% (n = 44) of the milk of reactor cows, respectively.

In conclusion, the herd and animal prevalences of bovine TB were moderate. The fact that human TB patients and reactor cattle were present in the same household suggests the transmission of mycobacterial species between cattle and their owners, while the awareness of these farmers about bovine TB and its transmission was generally poor.

Keywords

Bovine tuberculosis – Ethiopia – Mycobacterium bovis – Prevalence – Public health – Risk factor – Tuberculosis – Zoonosis.

Introduction

There is a growing demand throughout the world for livestock products, such as milk and meat. Consequently, livestock production is changing from a subsistence activity to a global food activity (11). The total world production of milk has increased during the past 25 years but not at the same rate as the human population (49%, as opposed to 53%, respectively). More than 90% of the world milk supply is attributed to dairy cows (25). Smallholder milk production is increasingly dominating the dairy industry in eastern and southern Africa (9). Since indigenous zebu cattle alone do not have the capacity to meet the increasing demand for milk and milk products in the tropics, cross-breeding to incorporate the productive capacity of *Bos taurus* with the hardiness of zebu breeds is the favoured alternative (17). Genetic improvement to intensify dairy production should be linked to the control of diseases associated with such intensification; for example, bovine tuberculosis (TB) (27).

Bovine TB is of significant economic importance in two different production systems where it appears in two separate forms:

– pulmonary TB in highly productive cattle in intensive dairy production systems

– gastro-intestinal TB in extensive as well as nomadic animal production systems (24).

Bovine TB remains one of the most devastating diseases of cattle in developing countries throughout the world (5). The disease is important not only because of its detrimental effect on animal production, but also for public health reasons (10, 21). Infection in humans occurs largely through children consuming infected milk but TB can also be spread through inhalation (21). Nowadays, human TB due to *Mycobacterium tuberculosis* in association with the human immunodeficiency virus (HIV) is increasing, and this may suggest a similar increase in human TB cases due to *M. bovis* in association with HIV (13). This could be extremely important in Africa, where people live in close proximity with cattle and the standard of hygiene when handling animal products is usually very low (10).

In Adama Town, small-scale dairying using Holstein cattle, or Holsteins cross-bred with zebu, is becoming common, accompanying the expansion of the town and its increasing demand for dairy products. As smallholder dairying expands, it is pertinent to ask whether there is also a rise in the incidence of bovine TB. The aim of this study was to determine the prevalence of bovine TB on small-scale farms and farmer awareness about the disease.

Materials and methods

Study subjects and sampling

The study was conducted in Adama Town, central Ethiopia, which is located 95 km south-east of Addis Ababa (39.17° N and 8.33° E), at an altitude of 1,622 meters above sea level in the Rift Valley. A total of 378 households owning 524 cattle (76 zebu, 136 cross-bred and 312 Holsteins) were included in the study. Adama Town is divided into 14 *kebeles* (a *kebele* is the smallest administrative district in Ethiopia, similar to a neighbourhood or ward). A list of the households owning dairy cattle in each of the 14 *kebeles* was obtained with the help of that *kebele's* administration. A household was used as the sampling frame. To calculate the sample size, the expected herd prevalence was estimated to be 42.6%, on the basis of earlier studies (2).

The sample size was calculated according to Putt *et al.* (20), using a confidence interval of 95%. Thus, 378 households were included. Proportional allocation was used to distribute these households evenly among the 14 *kebeles*. Although systematic sampling was used within the *kebeles*, random sampling was assumed, since systematic sampling is equivalent to random sampling.

Comparative intradermal tuberculin test

The animal's skin was shaved at two sites (12 cm apart) on the right-hand side of the mid-neck area. The skin thickness was measured with calipers before the tuberculin was injected. Aliquots of 0.1 ml of 2,500 international units (IU) per millilitre (ml) of bovine purified protein derivative (PPD) (Veterinary Laboratories Agency, Addlestone, United Kingdom), and 0.1 ml of 2,500 IU/ml of avian PPD (Veterinary Laboratories Agency, Addlestone) were injected into the dermis at these sites. After 72 hours, the thickness of the skin at the injection sites was measured, using calipers. The results were interpreted in accordance with the recommendations of the World Organisation for Animal Health (OIE) (29). Briefly, when the change in skin thickness was greater at the avian PPD injection site, the animal was considered positive for mycobacterial species other than the mammalian type (*M. tuberculosis* and *M. bovis*). However, when an increase in thickness was observed at both sites, the difference in thickness was considered. Thus, if the increase in thickness at the injection site for bovine PPD (B) was greater than that at the avian PPD site (A), and if B minus A was less than 2 mm, the animal was classified as negative for bovine TB. If B minus A was between 2 mm and 4 mm, or above 4 mm, the animal was classified as either suspect, or positive, respectively.

Milk culture

Towards the end of milking, the dairy farmers collected approximately 30 ml of the last few streams into sterile universal bottles from each quarter of tuberculin-positive dairy animals. The procedure indicated by Kazwala *et al.* (14) was followed for culturing milk. Milk samples were centrifuged at 3,000 revolutions per minute (rpm) for 15 min and the supernatant was discarded. The sediments were suspended in 2 ml of sterile physiological saline solution and decontaminated with an equal volume of sterilised 4% sodium hydroxide solution for 10 min. One to two drops of 0.05% phenol red indicator was added and then the suspension was neutralised using 2N sodium hydrochloride. (Neutralisation is confirmed when the colour of the solution changes from purple to yellow.) The suspension was centrifuged at 3,000 rpm at 4°C for 15 min, and the sediment was inoculated onto two slants of Lowenstein-Jensen (LJ) media (i.e. on pyruvate-supplemented LJ and glycerol-supplemented LJ). The media were checked for mycobacterial growth each week.

Positive cultures were subcultured onto another set of media and incubated for another three to four weeks, for further identification.

Differential tests

The mycobacterial isolates were identified using:

– colony morphology

– the nitrate reduction test

– the pyrazinamidase test

– the thiophene-2-carboxylic acid hydrazide (TCH) susceptibility test.

These tests were undertaken according to World Health Organization guidelines (28).

Questionnaire

The roles of various risk factors in the occurrence and spread of bovine TB among cattle, and between cattle and people, were assessed by a questionnaire. The farmers were asked questions about, among other things:

– their awareness of the transmission of bovine TB from cattle to humans and vice versa

– their consumption of meat and milk

– any recent history of TB cases in the family.

Data analysis

The animal prevalence level was defined as the number of positive reactors per 100 animals tested. The herd level prevalence was calculated as the number of herds with at least one reactor animal per 100 herds tested. Chi-square (χ^2) and multivariable logistic regression analyses (STAT software) were used to examine the effect of risk factors on animal prevalence (P). A P-value < 0.05 was considered to be statistically significant. However, the authors acknowledge that, since this analysis does not take into account the clustering of animals by household, the significance of the risk factors could have been overestimated and the level of animal prevalence may not be entirely precise.

Results

Herd prevalence

A herd prevalence of 15% (95% CI: 11.4%, 18.6%) was recorded in Adama Town, using the comparative intradermal tuberculin (CIDT) test. The differences in prevalence among the different sized herds were statistically significant (χ^2 = 32.3, P < 0.0001). Cattle in herds numbering six animals or above were ten times more likely to become infected (odds ratio [OR] = 10.8; 95% CI = 4.12, 28.42) with bovine TB than those in herds of two animals or fewer (Table I). A statistically significant association was found between the feeding practices of the herds and their reactivity to the tuberculin test (χ^2 = 12.63, P < 0.05). The association between respiratory problems and reactivity to the CIDT test was also statistically significant (χ^2 = 8.66, P < 0.01).

The results of the questionnaire showed that 39% (147/378) of the households had at least one human TB patient in their family. Of these households, 21.8% (33/147) owned reactor cattle. The association between the presence of a human TB patient and reactor cattle in a household was statistically significant (χ^2= 8.41, P < 0.01).

Characteristics at the animal level

Disease prevalence at the animal level, by the CIDT test, was 11% (95% CI: 8.32, 13.68). The prevalence of bovine TB varied significantly among different age groups (χ^2 = 11.0, P < 0.05), and according to the breed (χ^2 = 8.6, P < 0.05) and physical condition of the animal (χ^2 = 11.5, P < 0.01). The difference in reactivity to the CIDT test among females at different reproductive stages was statistically significant (χ^2 = 10.78, P < 0.05). The association between animal-related risk factors and animal prevalence is presented in Table II.

Table I
The effect of herd size on the prevalence of bovine tuberculosis in Adama Town, central Ethiopia

Herd size	Number of herds tested	Number (%) of herds testing positive	χ^2	P-value	Odds ratio (95% confidence interval)
≤ 2 animals	267	27 (10.2%)	32.28	< 0.0001	1
3 to 5 animals	91	19 (20.9%)			2.34 (1.23, 4.45)
≥ 6 animals	20	11 (52.4%)			10.82 (4.12, 28.42)
Total	**378**	**57 (15.1%)**			

Table II
The effect of risk factors on the animal prevalence of bovine tuberculosis in Adama Town, central Ethiopia

Variable	Category	Number of animals	Prevalence (%)	Adjusted odds ratio (95% confidence interval)
Age (years)	≤1	24	4%	1
	(1-3)	157	10%	2.4 (0.31, 19.3)
	(3-6)	230	16%	4.4 (0.58, 33.45)
	> 6	113	5%	1.3 (0.15, 11.13)
Sex	Male	15	7%	1
	Female	509	11%	0.6 (0.23, 14.15)
Breed	Zebu	76	4%	1
	Cross-bred	136	8%	2.1 (0.58, 7.92)
	Holstein	312	14%	4.1 (1.24, 13.46)
Body condition	Poor	190	5%	1
	Medium	297	14%	3.0 (1.45, 6.06)
	Good	37	19%	4.2 (1.48, 11.88)
Lactation	Lactating	408	11%	1
	Not lactating	47	13%	1.2 (0.48, 3.01)
Reproductive status	3rd trimester	114	7%	1
	2nd trimester	130	8%	1.1 (0.42, 2.89)
	1st trimester	156	13%	1.9 (0.83, 4.6)
	Not pregnant	55	22%	3.7 (1.41, 9.68)
	Heifer	54	15%	2.3 (0.81, 6.51)

Principal risk factor for transmitting tuberculosis from cattle to humans

About 46% (174/378) of the respondents regularly drank raw milk. Fewer than half, i.e. 33.1% (125/378), boiled fresh milk before consumption, although these people also consumed soured milk products without heat treatment. Of the respondents, only 37.3% (140/378) fed their babies on boiled milk, whereas 62.6% (238/378) did not regularly boil milk for their babies.

Farmer awareness of bovine tuberculosis

The results of questioning these small-scale dairy farmers on their awareness of bovine TB are summarised in Table III.

Isolating *Mycobacteria* from milk

Primary growth of bacilli was observed in milk from 36.4% (16/44) of the reactor herds. Of the observed cultures, 68.8% (11/16) were found to be acid-fast bacilli. These were further subcultured, then subjected to the pyrazinamidase test and TCH susceptibility test. Six isolates were confirmed to be those of *M. bovis*; three were *M. tuberculosis*, while two were found to be other mycobacterial species.

Table III
Farmer awareness of bovine tuberculosis and its mode of transmission

Farmer knowledge examined in questionnarie	Number (%) of respondents (out of 378) who were aware
Aware of bovine tuberculosis (TB)	132/378 (35%)
Know that cattle transmit bovine TB to humans	121/378 (32%)
Know that humans transmit TB to cattle	11/378 (3%)
Know that milk is a source of infection	129/378 (34%)
Know that meat is a source of infection	49/378 (13%)
Know that cough spray is a source of infection	23/378 (6%)

Discussion

The herd prevalence recorded by this study was lower than the previously reported herd prevalence in Wuchale-Jida district, central Ethiopia (2). One reason could be because the majority of the herds in this study were smaller in size and did not mix. However, the herd prevalence was higher than that (5.5%) reported by Redi (22) in Asella Town, south-eastern Ethiopia. On the other hand, the individual animal prevalence (11%) was similar to that reported previously by Ameni *et al.* (3) and Omer *et al.* (18), who recorded 14.2% and 14.5% in southern Ethiopia and Eritrea, respectively. Asseged *et al.* (4) reported a similar

animal prevalence in and around Addis Ababa, the capital of Ethiopia.

In accordance with findings from other studies (2, 4, 6), this analysis showed that, as herd size increased, there was a corresponding increase in the prevalence of bovine TB. This is because, as stated by Radostits *et al.* (21), bovine TB is a disease of overcrowding. Thus, when the number of animals in a herd increases, the transmission of the bacillus is promoted. Animals with no grazing are at a higher risk of infection than those kept on free grazing and mixed grazing.

This result agrees with the established observation that bovine TB is more common and more serious in housed animals than those at pasture (21). The closer the animals are packed together, the greater the chance of transmitting the disease (24). This study also demonstrates that, consistent with previous reports (1, 3, 15, 16, 30), the prevalence of bovine TB is higher in Holstein cattle than zebu. Fewer reactor animals were recorded in the younger age groups and reactivity to the CIDT test increased with age, up to six years of age, after which it declined. This accords with findings by other researchers (2, 3, 4, 6). It is possible that the infection may not become established in young animals but, as they get older, their chance of acquiring infection also increases, due to the increased time of exposure.

Francis *et al.* (12) and Wood *et al.* (26) have indicated that pregnant animals show lower reactivity as a result of stress-induced immuno-suppression, which accords with the findings of this study. This could be because animals lose sensitivity to tuberculin shortly before and after calving (21). There was a significant association between respiratory pathology and reactivity to CIDT, which could indicate that inhalation is the most common route of infection into housed cattle (21). Moreover, Daborn *et al.* (8) reported that 38.8% of TB lesions were found in the pulmonary system and 3.7% in the intestinal tract. Similarly, O'Reilly and Daborn (19) have reported that 68.5% (116/168) of the tuberculous cattle had TB lesions in the lungs and associated lymph nodes, suggesting that TB in cattle primarily involves the pulmonary system.

As suggested earlier (6), the presence of both a human TB patient and reactor cattle in a household could indicate that either the human TB patient was a source of infection for the cattle or *vice versa*. The disease transmission may also be cyclical: bovine to human to bovine (7). Close physical contact between the owner and his or her cattle, especially at night, and the consumption of raw milk or milk products facilitate the transmission of bovine TB (7, 27). This was also observed in the study area. Cases of human TB of animal origin will continue to pose a serious public health problem, especially in areas where raw milk or its products are commonly consumed (7). The isolation of *M. bovis* from humans and *M. tuberculosis* from milk suggests that both animals and humans may continue to infect each other.

The significance of this study lies in the fact that *M. tuberculosis* was isolated from milk. It is well established that cattle infected with *M. bovis* can excrete the bacillus in their milk. However, it is not likely that cattle infected with *M. tuberculosis* would excrete the bacillus in their milk since they rarely develop TB due to *M. tuberculosis*. Nevertheless, in addition to the results of the present study, *M. tuberculosis* has also been isolated from cows' milk in Ethiopia by Kiros (16) and Regassa *et al.* (23). The other possible source of *M. tuberculosis* in milk is contamination by the cough spray from infected farmers during milking. Whatever the case, the presence of *M. tuberculosis* in milk is of grave public concern, as it is primarily raw milk that is consumed in Ethiopia.

La tuberculose bovine chez les vaches laitières des petits élevages familiaux à Adama, Éthiopie centrale, et le niveau de connaissance des éleveurs

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Résumé

Une étude transversale a été réalisée dans 378 élevages familiaux comportant en tout 524 bovins, afin de déterminer la prévalence de la tuberculose bovine et d'en évaluer les conséquences sur la santé publique. La méthode d'échantillonnage systématique stratifié ainsi qu'un questionnaire ont été utilisés pour l'étude, qui a également recouru au test comparatif intradermique à la tuberculine, à la culture des mycobactéries et à des épreuves de sensibilité aux médicaments.

Avec un intervalle de confiance (IC) de 95 %, la prévalence à l'échelle des troupeaux et la prévalence individuelle étaient respectivement de 15 % (11,4-18,6) et de 11 % (8,32-13,68). Une variation significative de la prévalence (P) a été constatée suivant la taille du troupeau (χ^2 = 32,3 ; P < 0,0001), la classe d'âge (χ^2 = 11,47 ; P < 0,05) et le statut reproducteur (χ^2 = 10,9 ; P < 0,05). Une corrélation significative a été constatée entre la prévalence au niveau du troupeau, les pratiques d'alimentation (χ^2 = 6,6 ; P < 0,05) et les troubles respiratoires (χ^2 = 8,7 ; P < 0,01). Une corrélation positive (χ^2 = 8,4 ; P < 0,01) a été constatée dans les élevages familiaux entre la présence de bovins réagissant à la tuberculine et les cas de tuberculose humaine. Parmi les éleveurs qui ont répondu au questionnaire, 35 % (132/378) connaissaient la tuberculose bovine, mais seulement 32 % savaient qu'elle peut être transmise des bovins à l'homme. *Mycobacterium bovis* et *M. tuberculosis* ont été isolés, respectivement, de 18,2 % et de 11,4 % des vaches laitières ayant réagi à la tuberculine (n = 44).

Les auteurs en concluent que la prévalence de la tuberculose bovine, tant au niveau des troupeaux que des animaux individuels, est relativement faible. La présence simultanée, dans certains élevages familiaux, de cas de tuberculose humaine et de bovins réagissant positivement à la tuberculine témoigne de la transmission des mycobactéries des bovins à leurs propriétaires et confirme que ces derniers sont généralement mal informés du risque de transmission de la tuberculose bovine à l'homme.

Mots-clés

Éthiopie – Facteur de risque – Mycobacterium bovis – Prévalence – Santé publique – Tuberculose – Tuberculose bovine – Zoonose.

Tuberculosis bovina en pequeñas explotaciones lecheras de Adama (Etiopía central) y conocimientos de los ganaderos sobre la enfermedad

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Resumen

Los autores describen un estudio transversal realizado en 378 granjas que contaban con 524 cabezas de ganado para determinar la prevalencia de la tuberculosis bovina y evaluar sus repercusiones en la salud pública. Con ese fin, además de las pruebas tuberculínicas comparativas administradas por vía intradérmica, los cultivos micobacterianos y las pruebas bioquímicas y de farmacosensibilidad, se utilizaron un muestreo estratificado sistemático y un cuestionario.

Al aplicarse un intervalo de confianza del 95% (IC 95%), la prevalencia en los rebaños y animales fue del 15% (11,4-18,6) y el 11% (8,32-13,68) respectivamente. Se observó una importante variación de la prevalencia (P) en función del número de cabezas de los rebaños (χ^2 = 32,3; P < 0,0001), la cohorte de edad (χ^2 = 11,47; P < 0,05) y el estado reproductivo (χ^2 = 10,9; P < 0,05). La prevalencia en los rebaños estaba estrechamente relacionada con la alimentación (χ^2 = 6,6; P < 0,05) y los síntomas respiratorios (χ^2 = 8,7; P < 0,01). Se registró una importante relación (χ^2 = 8,4; P < 0,01) entre la presencia de animales reaccionantes y de casos de tuberculosis humana en una granja. El 35% (132/378) de los encuestados conocía la tuberculosis bovina, pero sólo un 32% (121/378) sabía que podía transmitirse del ganado a los seres humanos. Se aislaron *Mycobacterium bovis* y *M. tuberculosis* en un 18,2% y un 11,4% (n = 44) de las vacas lecheras reaccionantes respectivamente.

En conclusión, se mostró que la prevalencia de la tuberculosis bovina en los rebaños y animales era moderada. La presencia simultánea de enfermos de tuberculosis humana y de animales reaccionantes en una misma granja podría indicar la transmisión de especies micobacterianas entre el ganado y sus propietarios. Por lo general, los conocimientos de los granjeros sobre la tuberculosis bovina y su transmisión eran insuficientes.

Palabras clave

Etiopía – Factor de riesgo – Mycobacterium bovis – Prevalencia – Salud pública – Tuberculosis – Tuberculosis bovina – Zoonosis.

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