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Status of bovine tuberculosis in Addis Ababa dairy farms

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Summary
The study was conducted to determine the status of bovine tuberculosis in Addis Ababa, Ethiopia, by a comparative intradermal tuberculin test of 1,869 animals in 106 farms. Epidemiological information was also collected, taking into account factors chosen for their epidemiological significance and local livestock husbandry characteristics. In addition, milk samples were collected from tuberculin reactors for mycobacterial isolation and characterisation. Chi-square statistic, simple regression and multiple stepwise logistic regression were used to analyse the data. Of the 106 farms examined, 46 (95% confidence interval [CI]: 33.8% to 53.4%) contained comparative skin test reactors. Of the 1,869 animals, 443 (95% CI: 21.8% to 25.7%) were comparative skin test reactors. Furthermore, about 8.5% of tuberculin sensitive cows (12 of a sample of 141) secreted acid-fast bacteria in their milk. The microbes are described in more detail in the paper. Factors identified as possibly increasing the risk of bovine tuberculosis in Addis Ababa were herd size (large herd), farming (housing) condition (poor), and age (older animals). Similarly, as body condition scores improved from poor to medium and then to good, the likelihood of positive results significantly decreased ($OR = 0.54$; $p < 0.01$). Other factors including breed, sex, and physiological status of animals did not seem to significantly contribute to tuberculin sensitivity. The finding that large-size and intensively (often poorly) managed herds were at greater risk of bovine tuberculosis suggests that the significance of bovine tuberculosis is increasing in Addis Ababa parallel to an increasing dairy operation. If measures are not taken promptly, the impact on the economy and public health could be enormous.

Keywords
Bovine tuberculosis – Dairy farm – Ethiopia – Exotic breed – Milk – Mycobacterium bovis – Tuberculin test.

Introduction

Ethiopia possesses the largest number of livestock in Africa, with an estimated 44 million cattle (7). However, due to various performance-limiting constraints, including low genetic potential of the animals, poor nutrition and prevailing diseases, the productivity of Ethiopian livestock has always been sub-optimal. This low level of livestock productivity is also reflected in a very low per capita consumption of animal protein, which is estimated at 20 kg/year (33).

In the tropics, introducing high-grade dairy cows is considered a quick and suitable option to meet the increasing demand for milk and milk products, compared to dairying based on indigenous cattle alone (16, 33). As a result, Channel Island breeds and Friesians were introduced into the country shortly after the Second World War. The increase in milk yield attained due to introduction of improved dairy breeds was often satisfactory; especially under improved feeding and intensive management conditions (1). On the other hand, several studies have indicated that bovine tuberculosis shows close links with intensive management systems, and can spread rapidly when there is a lack of adequate veterinary supervision, such as routine testing and certification practices (3, 10). It has also been ascertained that tuberculosis becomes a serious problem in cattle when European breeds are used to establish an intensive dairy industry (21, 29).

Bovine tuberculosis (BTB) is an endemic disease of cattle in Ethiopia. It has been reported from several regions of the country based on tuberculin tests (5, 20) and abattoir inspections (2, 6, 18, 34, 36). Although studies conducted so far have been unable to establish the nationwide prevalence, mainly due to lack of adequate disease surveillance and diagnostic facilities, the rate of carcass condemnation due to tuberculosis has been increasing over the past decade (6). Because of the gap in knowledge regarding tuberculosis status in milk-producing cows, this study was designed to look into the trend of BTB in Addis Ababa, where there has been unprecedented expansion in the dairy operation over the past few decades.

Materials and methods

Study area

The study was conducted from July 2005 to June 2006 in private dairy farms distributed in various suburbs of Addis Ababa. Geographically, Addis Ababa is located at an altitude of about 2,400 m above sea level, and receives a mean annual rainfall of 1,800 mm. The average minimum and maximum temperatures are around 10.7°C and 23.6°C, respectively.

Study animals

According to the data compiled by the Livestock Marketing Authority in Ethiopia (23), there are about 97,000 cattle in the study area, of which 12,200 are exotic breeds (and their crosses) kept for dairying purposes. Study animals were selected from this population by 1-stage cluster sampling, based on the sample frame provided by Shola Zonal Veterinary Laboratory, Addis Ababa. Accordingly, 70 specialised dairy farms were randomly selected, and all animals (n = 1,572) from the selected farms were included in the study. Additionally, 36 farms keeping only local zebu breeds (n = 297) were randomly selected from the same area for a comparative study. In all the farms, young stock less than three months old were excluded from the analysis (32). Similarly, cows in late gestation and those who had recently calved were not included in the study for fear of immune suppression that usually occurs in dairy cows starting from three weeks pre-calving to three weeks post-calving (31).

Comparative intradermal tuberculin test

Animals were restrained and two sites, 12 cm to 15 cm apart, on the lateral side of the mid neck (22) were shaved to measure a skin-fold thickness using a caliper (Harpenden skin calipers, John Bull, United Kingdom). Then, aliquots of 0.2 ml (14,000 TU/ml) of bovine purified protein derivative (PPD) (Bovitubal, strain AN5, Bioveta, Czech Republic) and 0.1 ml (28,000 TU/ml) of avian PPD (Avitubal, strain D_4ER, Bioveta, Czech Republic) were injected obliquely into the deeper layer of the skin using separate preset syringes with short sterile needles. The injection sites were examined and skin fold thickness measured, once again, at 72 h post injection. The result was interpreted according to the manufacturer's instructions and the World Organisation for Animal Health (OIE) *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals* (35), i.e. an animal is considered positive if the increase in skin fold thickness at the bovine PPD injection site minus the increase at the avian PPD injection site exceeds 4 mm.

Bacteriology

Milk samples (n = 141) of approximately 100 ml each were collected into sterile universal bottles from previously washed udders of tuberculin sensitive cows (5, 19). The samples were kept in a cool box and transported to the tuberculosis laboratory of the Ethiopian Health and Nutrition Research Institute (EHNRI), Addis Ababa, and processed for cultivation in a Microflow Biological Safety Cabinet (Woerden, the Netherlands).

Individual milk samples were transferred into a sterile tube and centrifuged at 3,000 *g* for 15 min at 4°C. After discarding the supernatant, the deposit was re-suspended in 2 ml of sterile water, decontaminated with 2 ml NaOH (2%) for 15 min, and then neutralised with concentrated HCl using phenol red as indicator (28). After centrifugation (as above), about 2 ml of the sediment was heavily seeded onto the surfaces of standard Löwenstein-Jensen medium (standard-LJ) and on LJ medium supplemented with 0.4% pyruvate (LJ-pyruvate) for primary isolation of mycobacteria. Samples were incubated

at 37°C, at an angle for the first week and in the upright position for up to 11 weeks, with weekly observation for visible growth (12).

Whenever visible colonies were seen, smears were taken, air-dried and heat-fixed by passing several times through a Bunsen flame and stained by the Ziehl-Neelsen method to confirm the presence of acid-fast bacilli (9). Briefly, the heat-fixed smears were stained with carbol-fuchsin, heated gently to steaming and allowed to stand for about 10 min. The stain was poured-off and washed with tap water, then the slides were kept in 25% sulfuric acid and 96% ethyl alcohol solutions for one minute each (they were washed with tap water after each step). The smears were then counterstained with methylene blue for three minutes, washed with tap water, air-dried and examined by light microscope (Olivetti) with a 100× oil-immersion objective. Positive cultures were sub-cultured onto another set of LJ media and incubated for another three to four weeks for further identification, using catalase (12), nitrate reduction (28) and niacin production (9) tests.

Data collection and analysis

The size of the farm, farm condition and location were recorded. In each of the farms, animals were identified by their tag or their name, along with breed, sex, physiological status, age and/or parity. Body condition scoring was made noting anatomical structures such as the tail head, brisket, transverse processes of the lumbar vertebrae, ribs and hips (31). For data analyses, a two-stage statistical process was applied. In the first stage, the variables were screened, as appropriate, using comparison of proportions, the χ^2 (Chi square) statistic, and simple regression. In the second stage, factors that screened through ($p < 0.10$) were evaluated using unconditional multiple logistic regression (SPSS 11.5 for Windows), applying a backward stepwise model, with removal based on the likelihood-ratio statistic until all p-values were <0.05.

Results

Table I depicts the study farms and the results of a summarised tuberculin test using univariate analysis; animal level analysis using descriptive statistics is similarly reported in Table II. The results of the multiple logistic regression, as obtained from the model in which all the variables were entered simultaneously, are presented in Table III. Factors significantly associated with an increased risk of tuberculin reaction are herd size (OR = 1.5; $p = 0.003$), housing condition (OR = 0.57; $p < 0.001$), age (OR = 1.19; $p < 0.001$), and body condition score (OR = 0.54; $p < 0.001$).

Table I
Association between herd tuberculin test results and recorded variables

Variable	Tuberculin test			χ^2 (p-value) **
	Total	Positive	% positive (95% CI)*	
Herd size				
< 10	45	9	20.0 (9.6 to 34.6)	29.3 (< 0.001)
10 to 19	34	14	41.2 (24.6 to 59.3)	
≥ 20	27	23	85.2 (66.3 to 95.8)	
Management condition				
Poor	30	20	66.7 (47.2 to 82.7)	8.0 (0.0048)
Good	76	26	34.2 (23.7 to 46.0)	with Yates' correction

CI: confidence interval
* exact binomial 95% CI
** (on the assumption that all other factors are balanced)

Furthermore, out of the 141 milk samples collected from tuberculin reactor cows (Table IV), 12 (8.5%) were positive for acid-fast bacilli (AFB): five of the isolates yielded negative results for catalase, niacin and nitrate tests, indicating that *Mycobacterium bovis* was the most probable aetiology; one isolate gave negative results for catalase but positive results for both niacin and nitrate tests, indicating that *M. tuberculosis* was the most probable aetiology; three other cultures gave negative reactions for catalase and niacin tests but positive reactions for nitrate tests. Three of the acid-fast bacilli positive cultures failed to grow to a workable colony-size during the subculture and hence were discarded (Table IV).

Discussion

Congruent with an existing report (5), this study has documented an exceptionally high prevalence of BTB in Addis Ababa dairy farms (Table I). The overall prevalence of BTB (Table II) is also higher than has been reported previously (4). Since BTB persists in a farm unless mitigating measures are put in place (3, 24), cattle movements without preliminary test and certification could have spread the infection from infected focal farms to newly established ones.

Several studies have indicated that as herd size increases, the risk of cattle within the herd showing a positive reaction also increases (5, 10). In the present study, the herd tuberculin test result showed a statistically significant association (OR = 1.5) with herd size; also, the proportion of reactors increased parallel to an increasing herd size (Fig. 1). This finding is consistent with previous reports (5, 10, 14), and may arise from the fact that increased contact in larger herds favours lateral spread of infection within a herd, making the prevalence of infection greater than in small herds. Similarly, herd tuberculin results (Table III)

Table II
Distribution of tuberculin test results (odds ratio and 95% confidence interval) for 1,869 cattle in Addis Ababa according to breed, sex, physiological state, parity/age, and body condition scores

Variable	Test result				χ^2 (p-value)*
	Positive	Negative	% positive	Odds ratio (95% CI)	
Breed					
Exotic	440	1132	28.0	38.1 (12.2-119.0)	99.1 (< 0.001)
Local	3	294	1.0	1	Using Yates' correction
Sex					
Female	424	1218	25.8	0.3 (0.2-0.4)	32.6 (< 0.001)
Male	19	208	18.4	1	Using Yates' correction
Body condition score					
Poor	66	117	36.1	3.1 (2.2-4.4)	
Medium	232	518	30.9	2.4 (1.9-3.1)	72.1(< 0.001)
Good	145	791	15.5	1	
Age category (years)					
> 6	62	107	36.7	9.2 (3.8-22.2)	
> 3-6	373	489	43.3	12.1 (5.2-27.9)	97.9 (< 0.001)
1-3	50	225	18.2	3.5 (1-5-8.5)	
< 1	6	95	5.9	1	
Parity classes					
Parity ≥ 6	8	25	24.2	1.2 (0.5-2.7)	
Parity 3-5	155	248	38.5	2.3 (1.7-3.2)	33.7 (< 0.001)
Parity 1 and 2	144	432	25.0	1.2 (0.9-1.7)	
Heifers	82	305	21.2	1	
Physiological states					
Lactating	220	473	31.8	3.4 (1.4-8.1)	
Lactating and pregnant	56	101	35.7	4.1 (1.6-10.1)	15.0 (0.002)
Pregnant	26	94	21.7	2.0 (0.8-5.3)	
Dry cows	6	44	12.0	1	

* on the assumption that all other factors are balanced
CI: confidence interval

Table III
Results of unconditional multiple logistic regression analysis (presented as adjusted odds ratio and 95% confidence interval)

Variable	Coefficient	SE	p-value	Odds ratio	95% CI
Parity	0.003	0.02	0.882	1.003	0.96-1.04
Physiological status	0.02	0.06	0.809	1.02	0.90-1.15
Breed	–5.59	5.91	0.344	0.004	0.00-402.08
Sex	12.48	12.76	0.328	Very large	0-very large
Herd size	0.40	0.11	0.003	1.50	1.20-1.86
Housing condition	–0.68	0.13	0.000	0.57	0.39-0.66
Age	0.17	0.03	0.000	1.19	1.12-1.25
Condition score	–0.62	0.10	0.000	0.54	0.47-0.65

SE: standard error
CI: confidence interval

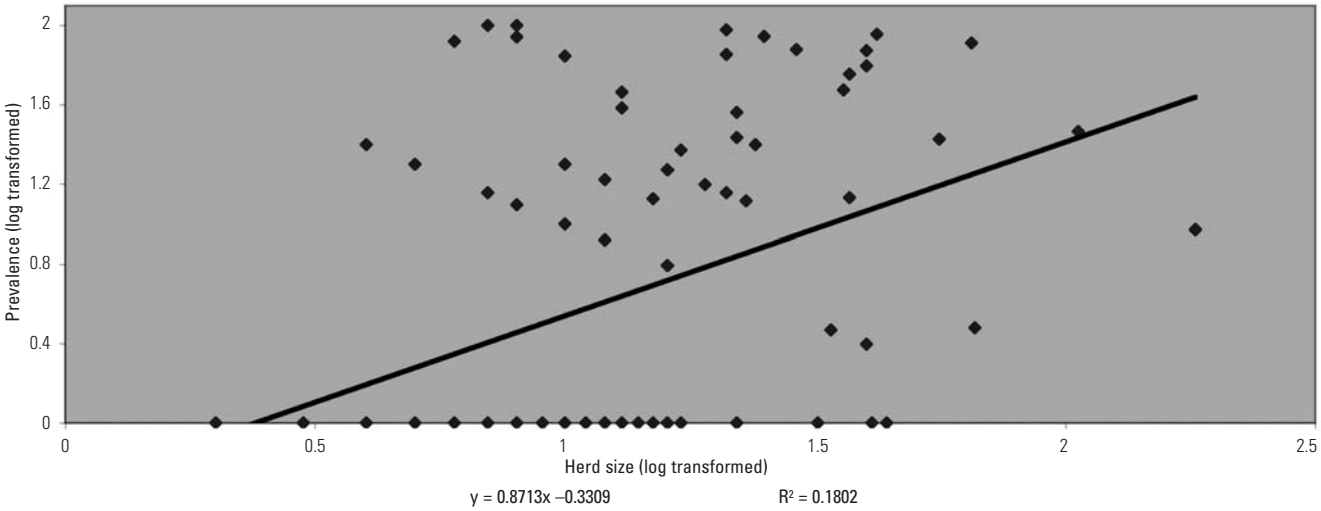

Fig. 1
Relationship between herd size and proportion of tuberculin reactors in a herd

showed a statistically significant association with herd management/housing conditions, signifying that poor housing and other poor managerial inputs increase the risk of tuberculosis (17). It can therefore be generalised that the status of BTB could be improved by adopting sanitary measures that improve hygiene conditions on farms. Previous workers (4, 10, 24) similarly documented higher infection rates in farms under poor management conditions.

The finding that older animals are more likely to be reactors compared to young animals (Table II) is in agreement with previous reports (5, 14, 20), and might indicate the relatively longer duration of exposure than an inherent age predisposition (29). However, Orme (27) related an increased incidence of tuberculosis in older people to a waning of protective capability, a situation confirmed in murine systems where an age-related effect on resistance was detected. Older ages are not often seen in cattle, and latency in cattle is still not well proven. On the other hand, in contrast to an earlier report from the laboratory of the authors (5), body condition score showed a statistically significant association with tuberculin sensitivity (Table III). This conforms well to the established fact that an animal's resistance to tuberculosis is reduced by a shortage of feed and/or unbalanced diet, attributable to a deficiency of proteins, minerals and vitamins in the diet (17). Previous workers (6, 13) similarly reported higher BTB prevalence in animals with poor body condition compared to those with good body condition scores.

It has been ascertained that a high prevalence of BTB is

Table IV
Biochemical test of the 12 acid-fast bacilli positive cultures compared with the laboratory maintained strains

Sample ID	Primary growth on ith week	Catalase test	Niacin test	Nitrate test	Specific findings
C	9	N	N	N	*Mycobacterium bovis*
F	9	N	N	N	*Mycobacterium bovis*
G	6	N	N	N	*Mycobacterium bovis*
J	11	N	N	N	*Mycobacterium bovis*
K	7	N	N	N	*Mycobacterium bovis*
I	4	N	P	P	*Mycobacterium tuberculosis*
A	7	N	N	P	+
B	11	N	N	P	+
H	5	N	N	P	+
D	8	NT	NT	NT	§
L	9	NT	NT	NT	§
M	11	NT	NT	NT	§
*M. bovis**	NA	N	N	N	*Mycobacterium bovis*
M. TB H37RV*	NA	N	P	P	*Mycobacterium tuberculosis* (4+)

*: reference strains NA: not applicable P: positive §: workable colony could not be obtained in the subculture
N: negative NT: not tested +: intermediate between *M. bovis* and *M. tuberculosis*

often recorded in intensive dairy farms where European breeds of cattle have been used to establish a dairy industry (21). Many researchers (15, 21, 29) have therefore concluded that imported breeds of cattle may be less resistant to BTB compared with the autochthonous cattle breeds in the tropics. In our study (Table III), breed-based analysis showed no statistically significant breed predisposition (OR = 0.004; CI = 0.00-402.08), although infection rate was comparatively higher in exotic compared to indigenous cattle breeds (Table II). It can therefore be concluded that the difference observed is a reflection of the variation in the management systems. Dairy cows (mainly of exotic breeds) are kept under intensive farming systems that promote close contact between animals, thereby favouring the spread of *M. bovis*. This would make extensive farming systems safer than zero grazing and more effective in preventing transmission of BTB (8, 25, 30).

In this study, 8.5% of 141 milk samples collected from tuberculin sensitive cows contained AFB; five (3.5%) of the samples proved to contain *M. bovis* (Table IV). A similar finding (33) has been documented previously although with a relatively higher *M. bovis* isolation rate (5). The isolation of *M. tuberculosis* (a specialised human pathogen) from milk indicates a possible reciprocal transmission between man and cattle. Several other workers (11) have similarly isolated *M. tuberculosis* from milk. The other three isolates (Table IV), which exhibited biochemical reactions intermediate between *M. tuberculosis* and *M. bovis* when subjected to niacin production and nitrate reduction tests (26) probably belong to *M. africanum*. This agent has public health importance (9) but facilities were not available to conduct further characterisation tests.

This study has shown that the prevalence of BTB is increasing in Addis Ababa parallel to an increase in dairy operations. Furthermore, a considerable number of infected cows secrete *M. bovis* (and related species) in milk. The problem is further compounded by the practice of pooling milk, either in the farms or at milk collection centres, signifying a considerable health hazard to milk consumers. This surely indicates that if measures are not taken promptly, the impact on the economy and public health could be enormous.

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La situation de la tuberculose bovine dans les fermes laitières à Addis-Abeba

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Résumé

Afin de déterminer la situation de la tuberculose bovine à Addis-Abeba, Éthiopie, 1 869 animaux répartis dans 106 exploitations ont été soumis au test comparatif intradermique à la tuberculine. Des informations épidémiologiques ont également été recueillies, centrées sur les facteurs jouant un rôle épidémiologique ainsi que sur les caractéristiques locales des pratiques d'élevage. En outre, des échantillons de lait ont été prélevés sur des vaches ayant réagi au test de la tuberculine, afin d'isoler et de caractériser les mycobactéries. Les données ont été analysées au moyen du test statistique du χ^2, du modèle de régression simple et du modèle de régression logistique pas à pas. Au niveau des exploitations, sur les 106 fermes examinées, 46 (avec un intervalle de confiance [IC] de 95 % compris entre 33,8 % et 53,4 %) possédaient des vaches ayant réagi au test comparatif intradermique. Au niveau des animaux, sur les 1 869 animaux testés, 443 (avec un IC de 95 % compris entre 21,8 % et 25,7 %) ont réagi au test comparatif intradermique. En outre, près de

8,5 % des vaches sensibles à la tuberculine (soit 12 sur un échantillon de 141) excrétaient des bactéries acido-alcoolo-résistantes par le lait. Les auteurs décrivent ces micro-organismes en détail. Les facteurs qui ont probablement augmenté le risque de tuberculose bovine à Addis-Abeba sont la taille importante des troupeaux, les pratiques d'élevage en stabulation, les conditions sanitaires médiocres et le fait que les animaux étaient assez vieux. De même, suivant que la condition physique des animaux était notée mauvaise, médiocre ou bonne, la probabilité qu'ils donnent des résultats positifs décroissait (rapport de cotes [*odds ratio*] = 0,54 ; p < 0,01). Il n'a pas été trouvé de corrélation significative entre d'autres facteurs (tels que la race, le sexe ou le statut physiologique des animaux) et la sensibilité à la tuberculine. L'observation selon laquelle les troupeaux de grande taille et souvent mal tenus sont davantage exposés à la tuberculose bovine laisse supposer que l'incidence de cette maladie à Addis-Abeba augmente en même temps que s'intensifie l'exploitation des races laitières. Si des mesures ne sont pas prises rapidement, les conséquences risquent d'être très graves pour l'économie et la santé publique.

Mots-clés
Éthiopie – Ferme laitière – Lait – Mycobacterium bovis – Race exotique – Test à la tuberculine – Tuberculose bovine.

Situación de la tuberculosis bovina en explotaciones lecheras de Addis Abeba

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Resumen
Los autores describen un estudio encaminado a determinar la situación de la tuberculosis bovina en Addis Abeba, Etiopia, para lo cual se practicó la prueba de la tuberculina intradérmica comparativa a 1.869 animales de 106 explotaciones. También se reunió información epidemiológica, teniendo en cuenta una serie de factores elegidos en función de su importancia epidemiológica y de las características de la producción ganadera local. Además, se extrajeron muestras de leche de reactores de tuberculina para el cultivo, eventual aislamiento y caracterización de micobacterias. Para analizar los datos se emplearon el estadístico ji cuadrado, la regresión simple y la regresión logística múltiple escalonada. De las 106 explotaciones estudiadas, en 46 (intervalo de confianza [IC] del 95%: 33,8% a 53,4%) se detectaron casos positivos a la prueba intradérmica comparativa. De los 1.869 animales analizados, 443 (IC 95%: 21,8% a 25,7%) resultaron positivos a esa prueba. Por otra parte, alrededor de un 8,5% de las vacas sensibles a la tuberculina (12 de una muestra de 141) secretaban bacterias acidorresistentes en la leche. En el cuerpo del artículo se describen con más detalle los microorganismos. Los factores que presumiblemente elevan el riesgo de tuberculosis bovina en Addis Abeba son: el tamaño del rebaño (grande), el tipo de cría (estabulación) y la condición (deficiente) y edad (avanzada) de los animales. Análogamente, a medida que la condición del animal pasa de deficiente a media y de media a buena, baja significativamente la probabilidad de resultado positivo (OR = 0,54; p < 0,01). Otros factores como la

raza, el sexo o el estado fisiológico del animal no parecían contribuir significativamente a la sensibilidad a la tuberculina. La conclusión de que los rebaños numerosos y mantenidos en régimen de producción intensiva (a menudo en malas condiciones) presentaban mayor riesgo de tuberculosis bovina lleva a pensar que la importancia de esa enfermedad en Addis Abeba está aumentando paralelamente a la intensificación de la producción lechera. Si no se adoptan medidas rápidamente, ello tendrá enormes repercusiones en la economía y la salud pública.

Palabras clave
Etiopia – Explotación lechera – Leche – Mycobacterium bovis – Prueba de la tuberculina – Raza exótica – Tuberculosis bovina.

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