

Prevalence of Bovine Tuberculosis (BTB) by post-mortem inspection and bacteriological culture in the municipal slaughterhouse of Mejía canton (Pichincha-Ecuador)

Prevalencia de Tuberculosis Bovina (TBB) mediante inspección post-mortem y cultivo bacteriológico en el matadero municipal del cantón Mejía (Pichincha-Ecuador)

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Published

Edwards Deming Higher Technological
Institute. Quito - Ecuador

Periodicity

January-March
Vol. 2, Num. 1, 2023
<http://centrosuragraria.com/index.php/revista>
pp. 76-92

Dates of receipt

Received: May 21, 2022
Approved: October 31, 2022

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Abstract: This cross-sectional study was carried out at the Municipal Slaughterhouse of Canton Mejía between May 27 - July 17, 2013 in order to estimate the apparent prevalence of TBB tuberculosis in slaughtered cattle based on macroscopic post-mortem inspection and isolation of *M. bovis*. In total, 395 heads of cattle were examined during the study period, of which 4 animals were diagnosed with tuberculous lesions compatible with the disease at the lung level, giving an apparent prevalence of 1.01% (IC95%: 0.28% - 2.57 %); while, through bacteriological culture, isolates compatible with *M. bovis* were identified in 6 animals, giving an apparent prevalence of 1.52% (IC95% = 0.56% – 3.28%). Risk factors such as age, biotype, sex and origin of the animals were not significant in the apparent prevalence of the disease. In conclusion, a significant prevalence of macroscopic lesions and of *M. bovis* was obtained without obtaining significant association of the factors that predispose to a greater presentation of visible lesions and of the pathogen, therefore, control strategies could not be redirected in the different populations that could be at risk.

Keywords: Prevalence, Bovine tuberculosis, post-mortem, isolation, *M. bovis*, risk factors.

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Resumen: Este estudio transversal se realizó en el Matadero Municipal del Cantón Mejía entre el 27 de mayo y el 17 de julio de 2013 con el objetivo de estimar la prevalencia aparente de tuberculosis TBB en bovinos sacrificados a partir de la inspección macroscópica post-mortem y el aislamiento de *M. bovis*. En total, se examinaron 395 cabezas de ganado bovino durante el periodo de estudio, de las cuales 4 animales fueron diagnosticados con lesiones tuberculosas compatibles con la enfermedad a nivel pulmonar, dando una prevalencia aparente del 1,01% (IC95%: 0,28% - 2,57%); mientras que, mediante cultivo bacteriológico, se identificaron aislados compatibles con *M. bovis* en 6 animales, dando una prevalencia aparente del 1,52% (IC95% = 0,56% - 3,28%). Factores de riesgo como la edad, el biotipo, el sexo y el origen de los animales no fueron significativos en la prevalencia aparente de la enfermedad. En conclusión, se obtuvo una prevalencia significativa de lesiones macroscópicas y de *M. bovis* sin obtener asociación significativa de los factores que predisponen a una mayor presentación de lesiones visibles y del patógeno, por lo que no se pudieron reorientar las estrategias de control en las diferentes poblaciones que pudieran estar en riesgo.

Palabras clave: Prevalencia, Tuberculosis bovina, post-mortem, aislamiento, *M. bovis*, factores de riesgo.

Introduction

Bovine tuberculosis (BTB) is an infectious-contagious disease with a wide distribution in cattle worldwide (Michel et al., 2010) and a potential risk to human health (de Kantor et al., 2012). (de Kantor et al., 2012).. The disease is caused by some mycobacteria of the *Mycobacterium tuberculosis* complex that develop granulomatous lesions in different organs, especially in the lungs, lymph nodes (Acha & Szyfres 2001; OIE, 2021), liver and/or kidneys (Shitaye et al., 2006). In cattle, *Mycobacterium bovis* has been shown to be the main pathogen causing tuberculosis (Amanfu, 2006), which causes trade restrictions, morbidity and mortality of animals that consequently generate economic losses in livestock production (OIE, 2021).

In Ecuador, cattle production has intensified (Proaño-Pérez et al., 2011), favoring the maintenance and spread of *M. bovis* infection due to direct contact (Palmer et al., 2006). In developed countries, control and eradication programs have reduced and/or eliminated the disease in cattle populations; however, BTB is still prevalent in developing countries (FAO, 2012), possibly due to a limited use of laboratory tests, low efficiency in post-mortem inspection due to lack of veterinary

inspectors and highly qualified personnel (de Kantor & Ritacco 2006); Proaño-Pérez et al., 2011).

BTB has been reported in several Latin American countries (de Kantor et al., 2012), where in the northern highlands of Ecuador, it is endemic in cattle with different prevalences, using different diagnostic methods (Echeverría et al., 2014); which may also be related to the geographical area, breeds, ages and breeding methods (Regassa et al., 2010). In Ecuador, a policy of skin testing and slaughter of positive animals is being applied on a voluntary basis; likewise, complementary diagnostic tests such as veterinary inspection and bacteriological culture can be used as an option for disease surveillance.

The present study was carried out to determine the prevalence of TBB by post-mortem inspection and bacteriological culture in cattle slaughtered at the Municipal Slaughterhouse of Cantón Mejía in the Province of Pichincha-Ecuador.

Materials and methods

This study was conducted between May 27 - July 17, 2013 at the Municipal Slaughterhouse of Cantón Mejía, which is located in the southeast of the Province of Pichincha, at the coordinates 11° 50' 22" South; 39° 36' 44" West longitude and at an altitude of 2000 meters above sea level. The slaughtering center mainly receives cattle for slaughter from the cattle ranches of Cantón Mejía, although there was an important influx of zebu cattle from the extensive systems of the Ecuadorian coast.

Study population: During the study period, 395 samples were obtained from animals slaughtered in the slaughterhouse. Sample selection was systematically randomized, with an average of 49 samples collected per day. The animals that were inspected and sampled included 71 (18%) mestizos, 103 (26%) zebras and 222 (56%) Europeans. Zebuino type animals are under an extensive breeding system; purebreds and mestizos are under an intensive breeding system because they are primarily engaged in milk production. The main sources of cattle that supplied the slaughterhouse in this study were from the cantons of Mejía with 49% (194/395), Santo Domingo de los Tsáchilas with 34% (133/395), Rumiñahui with 8% (31/395), Saquisilí with 5% (20/395) and Quito with 4% (17/395). Slaughterhouse hygiene was similar to

others in Pichincha province, including poor drainage and poor lighting. The average number of animals slaughtered in the slaughterhouse was 1200 cattle/month.

An observational, descriptive, cross-sectional, descriptive epidemiological study was carried out, which involved macroscopic inspection of the animals that arrived at the slaughterhouse and the collection of 3 tissue samples from selected cattle for the isolation of *M. bovis*. In addition, the age, biotype, sex and origin of each animal were recorded in order to determine the association between the study variables in relation to the presence or absence of macroscopic lesions and *M. bovis*.

Age was categorized according to age group: under 2 years, young; 2 to 6 years, adult; and over 6 years, old as determined by dentition characteristics (Pace & Wakeman, 2003). The European, zebu and mestizo biotype was according to Pourrain (2007) and, the origin was established according to the animal mobilization guides.

Post-mortem examination: Inspection was performed according to the procedures used by Proaño-Pérez et al. (2011), consisting of visual examination, palpation and incision (using knife disinfected with 10% chlorine bleach) at approximately 2 mm and 2 cm intervals in the lymph nodes (left tracheobronchial and caudal mediastinal) and lungs, respectively, to detect the presence of lesions compatible with BTB.

Sample collection: Based on macroscopic inspection, 1185 lung/ganglionic tissue samples (two lymph node and one lung sample) were obtained from the 395 animals. Precautionary measures were taken during sample collection to avoid cross-contamination of samples or possible biological risk. Each sample was collected individually in sterile airtight bags, previously labeled, to be stored under isothermal conditions in a cooler and transported, the same day, to the laboratories of the Centro Internacional de Zoonosis, in Quito, where they were stored frozen at -20°C until the time of processing in the mycobacteria laboratory.

Isolation of mycobacteria: A standard procedure for bacterial isolation (OIE, 2009) was used, using aseptic techniques in the level III microbiology laboratory, in a type 2B biological safety cabinet (II BSC), to avoid cross-contamination between samples and their possible biological risk. A portion equal to that used for maceration was stored at -80°C, in case a repeat test is necessary. Fat and connective tissue

were removed from each sample and approximately 1 cm³ was taken. The 3 samples, obtained from one individual, were pooled for homogenization for 10 minutes and decontamination was with 4% NaOH in a 1:1 or 1:2 volume (modified Petroff's method). Subsequently, it was incubated at 37°C for 20 minutes with intermediate shaking every 5 minutes. Then, it was centrifuged at 3000 rpm for 20 minutes, the supernatant was decanted in 5% phenol solution and the sediment was re-suspended in 500 µl H₂O to neutralize with 600 µl H₂SO₄ (0.1N), using phenol red as pH indicator. Four drops of the obtained solution were inoculated in Löwenstein-Jensen (L-J) culture media with pyruvate (0.4%) and in Stonebrink medium, in both media in duplicate. The tubes were incubated at 37°C for a maximum period of 8 weeks, with weekly observations. Any colony growth that presented phenotypes suggestive of mycobacteria was observed by the Ziehl-Neelsen technique, for confirmation of acid fast bacilli alcohol resistant (BAAR).

Identification of *M. bovis*: A bacteriological culture positive for *M. bovis* was taken into account for its growth time (Gathogo et al., 2012). In Stonebrink medium the average growth time of 5 weeks was observed (Corner et al., 2012) and in Löwenstein-Jensen a longer time (8 weeks) was considered (OIE, 2009) and with little colony growth (Toledo et al., 1999). Also taken into account were characteristics of appearance, color and shape that are clues to suspect *M. bovis* (OIE, 2021); however, they are not always constant in all strains of a species (Lévy-Frébault & Portaels, 1992).

In a Microsoft Excel spreadsheet version 2010, a database was created containing information on age, biotype, sex, origin of each animal, positivity for visible lesions (VL) and *M. bovis*. Apparent prevalence was determined as a proportion of carcasses with visible lesions and/or *M. bovis* isolates, out of the total carcasses examined and pool of samples processed, respectively (Thrusfield, 2008).. Confidence intervals for apparent prevalences were calculated with 95% confidence using the PropCIs package of the statistical program "R" (version 3.0.2.). Additionally, the Rcomander package of R was used to perform Chi-square tests, Fisher's exact test and a univariate and multivariate binomial analysis using a generalized linear logistic regression model (GLM) to identify whether possible risk factors such as age, biotype, sex and origin are associated with the occurrence or positivity of visible

lesions or *M. bovis*. For all the analysis performed, a p-value of less than <0.05 was taken as statistically significant.

3. Result

The apparent prevalence of lesions compatible with BTB by macroscopic post-mortem inspection in cattle slaughtered at the Municipal Slaughterhouse of Cantón Mejía during the study period was 1.01% (4/395) [CI95% = 0.28% - 2.57%] (Table 1). Of the 4 cases of visible lesions at the lung level, *M. bovis* colonies were isolated in only one sample. Macroscopically, small tubercles surrounded by a fibrous capsule not easily detached from the lung tissue were observed. When obtaining apparent prevalence of macroscopic lesions in slaughter cattle, by individual variables (Table 1), the results show that only adult animals (1.19%) tend to develop macroscopic lesions compared to young and old animals. Similarly, mestizo cattle (1.45%) are more affected than European (1.25%) and Zebu cattle. Also, only females (1.35%) presented visible lesions. Finally, the presence of lesions was only found in cattle from Cantón Mejía (2.06%).

Table 1.- *Apparent prevalence of visible lesions total and with respect to age, biotype, sex and canton*.*

Factor	Visible lesions	
	(POS)/T	BP % (95% CI)
Apparent prevalence	4/395	1.01% (0.28 - 2.57)
Age (years)		
< 2	0/34	(0 - 10.28)
2 a 6	3/336	1.19(0.33 - 3.02)
> 6	0/25	(0 - 13.72)
Biotype		
Cebuino	0/104	(0 - 3.48)
European	3/222	1.25(0.28 - 3.90)
Mongrel	1/69	1.45(0.04 - 7.81)
Sex		
H	4/296	1.35(0.37 - 3.42)
M	0/99	(0 - 3.66)
Canton		
Saquisilí	0/20	(0 - 16.84)
Mejía	4/194	2.06(0.56 - 5.19)
Quito	0/17	(0 - 19.51)
Rumiñahui	0/31	(0 - 11.22)

S. Domingo 0/133 (0 - 2.74)

* POS = positive for macroscopic lesions compatible with BTB; T = total carcasses examined, PA = apparent prevalence, CI = confidence interval.

The apparent prevalence of *M. bovis* by bacteriological culture, from 395 animals slaughtered at the Municipal Slaughterhouse of Cantón Mejía, was 1.52% (6/395) [IC95% = 0.56 - 3.28]. Of the 6 positive isolates, 5 were obtained from samples without visible lesions. Of the 6 isolates considered to be *M. bovis*, 3 were obtained on L-J culture media with pyruvate and the other 3 on Stonebrink culture media. The colonies were small, raised, friable, unpigmented after approximately 6 to 14 weeks, rounded, smooth and beige in color. When obtaining the apparent prevalence of *M. bovis* in slaughter cattle by individual variables (Table 2), the results show that old animals (4%) tend to have a relatively higher frequency of *M. bovis* compared to adult (1.49%) and young animals. Likewise, European cattle (2.25%) presented a higher presence of the microorganism than mestizo (1.45%) and zebu cattle. Also, the female sex (2.03%) was the only one that tested positive for the pathogen. Finally, the highest prevalence of *M. bovis* was found in animals from Canton Rumiñahui (6.45%) in relation to Canton Mejía (2.06%) and the other cattle from the different origins did not present isolations of the microorganism.

Table 2.- Apparent prevalence of *M. bovis* total and with respect to age, biotype, sex and canton*.

Categories	M. bovis	
	(POS)/T	BP % (95% CI)
Apparent prevalence	6/395	1.52% (0.56 - 3.28)
Age (years)		
< 2	0/34	(0 - 10.28)
2 a 6	5/336	1.49 (0.48 - 3.44)
> 6	1/25	4 (0.10 - 20.35)
Biotype		
Cebuino	0/104	(0 - 3.48)
European	5/222	2.25 (0.74 - 5.18)
Mongrel	1/69	1.45 (0.04 - 7.81)
Sex		
H	6/296	2.03 (0.75 - 4.36)

M	0/99	(0 - 3.66)
Canton		
Saquisilí	0/20	(0 - 16.84)
Mejía	4/194	2.06 (0.56 - 5.19)
Quito	0/17	(0 - 19.51)
Rumiñahui	11355	6.45 (0.79 - 21.42)
S. Domingo	0/133	(0 - 2.74)

* M. bovis = Mycobacterium bovis, POS = positive for M. bovis, T = total tissue pool seeded, PA = apparent prevalence, CI = confidence interval.

In this study, when analyzing the age, biotype, sex and origin of the animals in relation to the positivity or presence of macroscopic lesions compatible with the disease and M. bovis, no significant association was found. Therefore, it could not be inferred that the factors analyzed influenced the apparent prevalence of macroscopic lesions and M. bovis, which also means that there is the same probability for any category analyzed of presenting visible lesions or M. bovis in cattle slaughtered in the Municipal Slaughterhouse of Cantón Mejía.

Different countries or regions can fit into one of three categories, depending on the prevalence of the disease: less than 1%, low; between 0.1% and 1%, intermediate; greater than 1%, high (de Kantor & Ritacco, 2006); according to the category, different control strategies should be followed in order to reduce the prevalence of the disease, in addition to making use of diagnostic tests with greater sensitivity (de Kantor & Ritacco, 2012). The apparent prevalence of lesions compatible with BTB of 1.01% in the Municipal Slaughterhouse of Cantón Mejía by post-mortem macroscopic inspection in the present study represents a high prevalence, and among the factors that can influence the prevalence of the disease are the production systems to which the cattle are subjected (intensive dairy cattle breeding), the geographic area and the size of the farm (small, medium or large) (Proaño-Pérez et al., 2011); however, a high prevalence is possible in housed cattle as infection through the respiratory tract is more likely to subsequently produce lesions at the level of the lungs (de Kantor & Ritacco, 2012) .

The high apparent prevalence (greater than 1%) found in the current study by post-mortem inspection is comparable with the results found at the slaughterhouse level by Proaño-Pérez et al. (2011) [2.33%

(16/687) and 2.42% (17/703) in 2007 and 2008, respectively] and Espinoza (2011) [Pelileo 7.92% (55/307) and Ambato 4.30% (32/744)], in the same way similar findings have been reported by Torres (2009) in Argentina [1.2% (140,840/12,018,251), Biffa et al. (2009) in Ethiopia [10.2% (337/3,322)], Aliyu et al. (2009) in Nigeria [4.05% (12,259/302,700)], Gathogo et al. (2012) in Kenya [18.95% (176/926)] and Koro et al. (2013) in Cameroon [1.03% (168/16,316)]. On the other hand, the prevalence found in this study presents a difference with the average prevalence (0.1% - 1%), found in the Ecuadorian coast such as: Guayas (0.47% 16/3,425), Santo Domingo de los Tsáchilas (0.21% 6/2,778) (Proaño-Pérez et al., 2011); and in other countries such as: Argentina [0.9% (116,798/13,483,515) in 2009) (Torres, 2009), California, Mexico (0.12 and 0.46% in 1995 and 1996, respectively) (Hernández et al., 1997) and Minas-Gerais, Brazil [0.71% (681/954,640)] (Baptista et al., 2004) and similarly shows a difference with the low prevalence (less than 0.1%) in Ethiopia [0.050% (699/1,336,266)] (Shitaye et al., 2006).

A higher prevalence of BTB compared to other regions of medium and low prevalence could be related to the lack of an adequate testing and slaughter policy, intensive management practices, where there is greater contact between animals and the lack of preventive measures that make the disease endemic; however, it should be considered that macroscopic lesions could not be detected in 83% (5/6) of the animals infected with *M. bovis* (confirmed by isolation) (de Kantor & Ritacco, 2012). These results may be due to the fact that not all organs and tissues present visible lesions, because the animal is in an early stage of the disease, and it may be the case that not all organs and tissues present visible lesions, because the animal is in an early stage of the disease. The three lesions from which it was not possible to isolate *M. bovis* may be due to other granulomatous diseases that are not correctly examined (OIE, 2019); on the other hand, the three lesions from which it was not possible to isolate *M. bovis* may be due to other granulomatous diseases (Mellau et al., 2012). (Mellau et al., 2010) such as Actinomycosis, Acnino bacillosis and Leukosis, which have been diagnosed in the Machachi animal farm (OIE, 2021), or other opportunistic pathogenic microorganisms that cause this type of lesions (Oloya et al., 2010). (Oloya et al., 2007)..

In addition, there is no adequate control of cattle movement in the country, and there are deficiencies in post-mortem inspection protocols;

therefore, these procedures should be improved and adjusted to the conditions of each area in Ecuador, or propose a protocol that can be followed to obtain favorable results, which have been shown to increase their level of efficiency fourfold, and thus can be used in the surveillance of the disease. Finally, the microorganisms that can cause lesions compatible with the disease and that generate confusion in the macroscopic diagnosis should be studied (de Kantor & Ritacco, 2012).

There have been no previous data on apparent prevalence of *M. bovis* by bacteriological culture from tissues of slaughtered cattle; therefore, the present result is not comparable with other studies. The apparent prevalence of *M. bovis* was 1.52% (6/395) in the Municipal Slaughterhouse of Cantón Mejía by bacteriological culture. The isolation of *M. bovis* was performed from 5 animals that did not present visible lesions, results that are related to the reports of Echeverría et al. (2014), who demonstrated by nested PCR (for *M. bovis*) a prevalence of 4.33% (25/578), in two areas of the northern highlands of Ecuador.

In 75% (3/4) of the animals that presented encapsulated granulomatous lesions, isolation of the microorganism was not possible because it has been shown to be negatively correlated with the growth of *M. bovis* in culture media; however, the highest isolation can be achieved from caseous/purulent lesions (Gathogo et al., 2012). However, isolation of the organism from tissues with or without lesions may be affected by a) the effect of the decontaminant because mycobacteria are sensitive to sodium hydroxide (Miller et al., 2002), b) choice of culture medium used (Corner et al., 2012) and c) loss of viability during sampling (Latini et al., 1997) or during the period of time between sample collection and processing (Araújo et al., 2005), since concentrations higher than 10 bacilli/ml are required to obtain a positive culture (Nava & Prieto, 2001).

The colonies of acid fast bacilli that represented 40% (4/10) could be atypical mycobacteria that have been isolated from Löwenstein-Jensen medium or Stonebrink medium; among which the following have been found: *Mycobacterium terrae*, *Mycobacterium avium*, *Mycobacterium chelonae*, *Mycobacterium gordona*, *Mycobacterium fortuitum*, *Mycobacterium flavescens*, *Mycobacterium smegmatis*, *Mycobacterium smegmatis*, *Mycobacterium flavescens* and *Mycobacterium smegmatis* (Cleaveland et al., 2007). (Cleaveland et al., 2007), *Mycobacterium smegmatis* (Cleaveland et al., 2007) *Mycobacterium avium* intracellulare-scrofulaceum, *Mycobacterium gordonae* and *Mycobacterium szulgai* (Proaño-Pérez et

al., 2006). These mycobacteria are sometimes found causing infection in cattle and possibly affect production performance (Biet et al., 2005). (Biet et al., 2005). Similarly, it could be *Mycobacterium caprae* or *Mycobacterium tuberculosis* that has been diagnosed in cattle (Ameni et al., 2011).

The prevalence may be influenced by the season, since old or poor condition animals are usually sold at certain times of the year (Boukary et al., 2012), usually at Livestock Fairs and with respect to bacteriological culture results, they indicate the presence of *M. bovis* in the farms of origin (Milian-Suazo et al., 2000), could help to re-direct surveillance strategies with better diagnostic tests.

The higher prevalence of visible lesions and *M. bovis* in older cattle could be explained by the longer time spent in endemic environments, long term productive activity (Acha & Szyfres, 2001), chronicity of the disease and immunosuppression (Humblet et al., 2009). (Humblet et al., 2009).. The greater presence of lesions found according to biotype could be attributed to the fact that the few mestizo cattle remain together with European cattle, which are more susceptible than zebu and crossbred cattle (Humblet et al., 2009). (Omer et al., 2001; Cadmus et al., 2010).

Despite an apparent resistance of TBB in zebu cattle (Inangolet et al. (Inangolet et al., 2008)(Inangolet et al., 2008), the disease has been diagnosed in this type of cattle, due to the increased contact between high-producing animals and the overcrowding of dairy production systems (Proaño-Pérez et al., 2011). (Proaño-Pérez et al., 2011). In this study, the presence of lesions and *M. bovis* was only found in females, but males used as steers could also present the disease (Kazwala et al., 2011). (Kazwala et al., 2001).. Finally, the lesions found in animals from Cantón Mejía could be due to the fact that the disease is endemic in this area and adequate control measures have not been carried out, especially in this area with a high density of dairy cattle ((Proaño-Pérez et al., 2011).

M. bovis infection was higher in Cantón Rumiñahui because it is related to an endemic area, a possible sporadic outbreak and/or inadequate control of cattle movement. A null or decreased prevalence in other areas is more likely to be related to the type of production (extensive systems), since transmission through the respiratory route is minimal.

4. Conclusions

In conclusion, this study, which was carried out in an area with high cattle movement, showed a high prevalence of lesions compatible with bovine tuberculosis and *M. bovis* in cattle slaughtered in the Municipal Slaughterhouse of Cantón Mejía. Tuberculosis control programs must be carried out in the different cattle populations in order to have adequate epidemiological data collection and, in the future, to have access to export markets for cattle and livestock products. Due to the scarcity of information on tuberculosis in cattle at the slaughterhouse and laboratory levels, studies such as this one, but with significant values, could help redirect control strategies in the different populations predisposed to contracting BTB, so that the economic and public health impacts of the disease can be reduced.

Acknowledgments

We are grateful for the scientific and financial support of the Centro Internacional de Zoonosis; to Dr. Marco Coral for his help with part of the statistics. I also thank Bioq. Paulina Fernández for her collaboration in the laboratory and finally the staff of the Cantón Mejía Slaughterhouse, Dr. Antonio Viteri, Dr. Pablo Tuyapanda and Dr. Carlos Luna for their collaboration during the field work.

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