Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 8 August 2023

Research Article

Cross-sectional Study on Bovine Tuberculosis Based on Post Mortem and Bacteriological Examination in Four Abattoirs of Northern Algeria: Slaughterhouses as Source of Data for Disease Control

Hanane Damene[1], Razika Boukert[1], Naima Bouzid[2], Yassine Zerizer[2], Ali Berber[1], Naima Sahraoui[1] and Djamel Tahir[1,3]*

[1]*Institute of Veterinary Sciences, University Blida 1, Blida, Algeria*

[2]*Directory of Agricultural Services, Béjaia, Algeria*

[3]*Institute of Pasteur, Paris, France*

***Corresponding Author:** Djamel Tahir, Institute of Veterinary Sciences, University Blida 1, Blida, Algeria.

DOI: 10.31080/ASMI.2022.06.1284

Received: July 17, 2023

Published: July 25, 2023

Abstract

Bovine tuberculosis (BTB) remains a major zoonotic disease, causing economic losses as well as public health problems, particularly in developing countries. The aim of this study was to determine the prevalence of BTB and the associated risk factors in northern Algeria, based on the cattle population slaughtered in four abattoirs. Overall, 3,546 slaughtered cattle were inspected for detection of visible tuberculosis-like lesions between 2017 and 2019. Furthermore, tissue samples were collected from the affected carcasses (n = 232) and screened using the Ziehl–Neelsen (ZN) staining technique and mycobacterial culture. Visible tuberculosis-like lesions occurred in 6.5% (232/3,546) of the slaughtered animals. Whatever the technique used, the prevalence of BTB disease was found to be statistically associated with age, sex, and breed (p<0.0001). The thoracic lymph nodes including the tracheobronchial and mediastinal LN were mostly affected, at rates of 52.6% and 31.9%, respectively. The majority (69.4%; n = 161) of granulomas found in the thoracic lymph nodes were characterized by caseous necrosis (34%) or caseous-calcified lesions (35.3%). The present study demonstrated that despite the efforts of authorities to eradicate BTB, this disease is still enzootic in Algeria, since the prevalence remains important. Finally, our findings highlighted the importance of abattoirs as a source of information for recording the BTB epidaemiological situation in a given area, contributing to updating the design and implementation of control and prevention strategies.

Keywords: Bovine Tuberculosis; Cattle; Risk Factors; Post Mortem Inspection; Bacteriological Examination; Algeria

Introduction

Bovine tuberculosis (BTB) is a chronic, infectious, and contagious disease of cattle caused mostly by *Mycobacterium bovis* and occasionally by other members of the *M. tuberculosis* complex [1], such as *M. caprae, M. tuberculosis,* and *M. microti* [2-4]. *M. bovis* can also cause disease in humans and a wide range of domestic and wild animals [5]. Transmission of infection to cattle occurs from the environment (e.g., contact with contaminated feces), cattle, wildlife, and humans. The airborne route is the most important route of transmission in cattle [6], while zoonotic transmission to humans generally occurs after close contact with infected animals or consumption of unpasteurized contaminated dairy products [7].

BTB is characterized by progressive development of nodular granulomas, known as tubercles, which usually contain yellowish central caseous necroses, caseous-calcified or calcified, and are often encapsulated by connective tissue [8]. Lesions may remain localized or may generalize to other tissues and organs through hematogenous or lymphatic dissemination of the mycobacteria [9]. Generally, the disease occurs in organs rich in reticuloendothelial tissue as well as in the lymph nodes (particularly those of the head and thorax), lungs, spleen, liver and surfaces of cavities [10]. Based on macroscopic and microscopic criteria, tuberculous lesions in cattle lymph nodes can be classified into four development stages: stage I (initial), stage II (solid), stage III (minimal necrosis), and stage IV (necrosis and mineralization) [11-14]. The evolution of the infection is generally chronic and signs might be absent for months or years before the organ involvement is severe enough to cause functional impairment [9]. The switch from latent TB infection to active TB is due to loss of the delicate equilibrium between the host defense mechanisms and mycobacterial virulence factors owing to stresses such as immunosuppression or malnutrition [15].

BTB remains of great concern worldwide, particularly in developing countries where only 25% apply control and eradication program [16]. In Africa, the most available epidemiological data about BTB infection originate from post mortem carcass inspection and/or tuberculin skin testing. The infection rates reported in the literature range from 1% to 10% [17].

In Algeria where the prevalence of BTB varies between 3.6% and 6.5% [18,19], the control strategy adopted by the authorities to fight this disease is based on two components: test-and-slaughter and systematic post mortem examination of carcasses at slaughterhouses. The ante mortem screening consists of the Single Intradermal Cervical Tuberculin (SICT) test [20]. Therefore, in Algeria, all organized intensive cattle herds belonging to the state are tested once a year for BTB infection, but some are tested more frequently if they are considered at increased risk. Animals that have positive SICT results (reactors) are quickly isolated from the rest of the herd and eliminated by stamping out. In fact, this control strategy is faced with multiple constraints such as the non-screening of extensive cattle ranching. Consequently, these BTB infected cattle are not eliminated and continue to be a discreet source infecting other animals, risking zoonotic transmission as seem from evidence of the involvement of *M. bovis* in human TB infection in Algeria [19].

This study was designed to determine the risk factors associated with the prevalence of BTB in four slaughterhouses of northern Algeria using post mortem and bacteriological examination, with the aim of using this information as a starting point for early warning of BTB and anticipating its impact on public and animal health.

Materials and Methods

Ethical authorization

Research approval was obtained from the Institute of Veterinary Sciences of Blida before the commencement of the study and an authorization certificate was issued with reference number 391/PG-ISV/2017, while permission was obtained from the Inspection Vétérinaire de Wilaya, operating under the auspices of the Direction des Services Vétérinaires (DSV, Ministry of Agriculture) to access the abattoirs included in this study.

Study area

The present study was conducted over 2 years, from November 2017 to November 2019 at four municipal slaughterhouses in two neighboring departments of northern Algeria, Béjaia and Kherrata (from the Béjaia department) and Sétif and El-Eulma (from the Sétif department) (Figure 1). It is important to note that more than 80% of cattle population are located in Northern Algeria. The Béjaia department covers about 3,268 Km^2, with a Mediterranean climate (mean annual temperature and precipitation rate are 18 °C and 637 mm, respectively). The Sétif department covers about 6,500 Km^2, with a semi-arid climate (mean annual temperature and precipitation rate are 13.5 °C and 583 mm, respectively). The number of cattle and small ruminants has been estimated at 24,200 bovines, 71,447 sheep, and 36,659 goats in Béjaia and 143,710 bovines, 412,709 sheep, and 55,839 goats in Sétif.

Study design

Sanitary inspection and sample collection

The study population consisted of cattle slaughtered at four municipal abattoirs of northern Algeria during the study period.

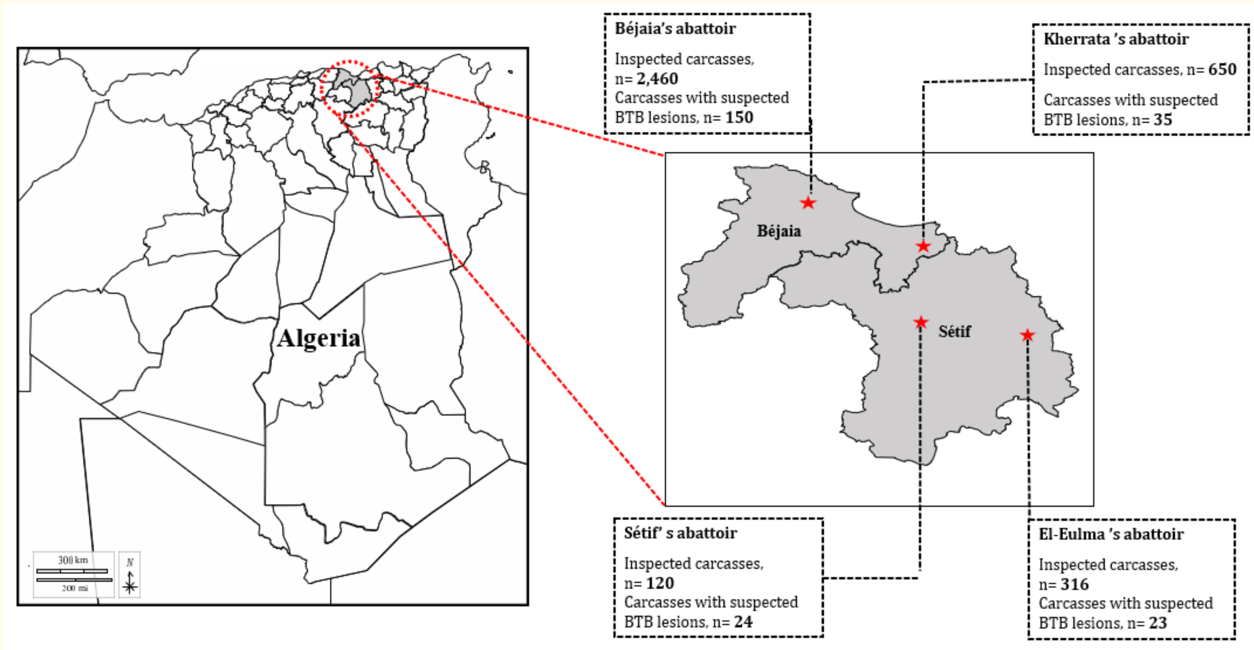

Figure 1: Maps of Algeria showing the geographical distribution of the included slaughterhouses in Béjaia and Sétif departments.

All cattle included in this study were examined using ante- and post mortem inspection. During the ante mortem inspection, individual animal data such as age, sex and breed were collected. During post mortem inspection (PMI) performed by visual examination, palpation, and incision for detection of suspected BTB lesions, the organs (lung, pleura, heart, liver, intestine, kidneys, and spleen) and lymph nodes (tracheobronchial, mediastinal, apical, hepatic, retropharyngeal, mandibular, parotid, mesenteric, prescapular, precrural) of each carcass were examined. When visible or suspected BTB lesions were noted, the tissue specimen was sampled and transferred into sterile containers and stored at -20 °C until bacteriological analysis. In parallel, information that related to the morphological appearances of lesions such as the type of TB (localized or generalized), anatomical infection site, and nature of lesions (caseous with or without mineralization or suppurative) was recorded in the clinical file.

Smear microscopy and mycobacterial culture

All laboratory analyses were performed at the Service de Contrôle de la Tuberculose et les Maladies Respiratoires of Béjaia province.

Each tissue specimen was cut in tiny pieces and macerated by sterile pestle and mortar in 10 ml of sterile distilled water. The homogenate was harvested in a sterile centrifuge tube and decontaminated using an equal volume of 4% NaOH. Thereafter, the suspension was mixed and concentrated by centrifugation at 3000 rpm for 15 min. The sediment obtained was neutralized with 20 ml of sterile distilled water and re-centrifuged at 3000 rpm for 15 min. The supernatant was discarded while the sediment was used for ZN staining and culture.

For microscopy, the heat-fixed smears were completely covered with carbol-fuchsin stain and were heated until vapors started rising. After 10 min, the slides were rinsed with tap water and decolorized with 25% sulfuric acid for 3 min. After decolorization, the rinsed slides were counterstained with methylene blue solution for 1 min. Finally, the stained smears were observed under an optical microscope (×100) to detect the acid-fast bacilli [21]. Regarding the culture, 0.1 ml of each sediment was inoculated onto both Lowenstein Jensen (supplemented with glycerol) and Coletsos media. The cultures were incubated at 37°C for 8-12 weeks, during which they were examined once a week to monitor the growth of bacterial colonies. All the growth cultures were stained by ZN to confirm the presence of acid-fast-bacilli (AFB).

Statistical analysis

Data recorded from the surveys were entered into a Microsoft Excel® (MS Excel 2016) spreadsheet. To obtain information on potential associations between the prevalence of BTB and sex, age, or breed of the slaughtered cattle, Pearson's chi-squared test was applied. The differences were considered statistically significant when the *p*-value was < 0.05.

Results

Structure of slaughtered cattle populations

During the study period, a total of 3,546 cattle were inspected for detection of suspected BTB lesions at Béjaia (n = 2,460), Kherrata (n = 650), Sétif (n = 120), and El-Eulma (n = 316) slaughterhouses. Among the 3,546 slaughtered cattle, 77.1% were younger than 2 years, 21.7% were 2-5 years, and 1.2% were more than 5 years of age. The majority of slaughtered cattle were male (95.3%). The cross-breeds presented a higher proportion (68%) compared to other cattle breeds (Table 1). A total of 232 carcasses with lesions suggestive of TB were sampled.

Variable		Slaughterhouses				
		Béjaia	Kherrata	Sétif	El-Eulma	Total
		No.	No.	No.	No.	No. (%)
Age	< 2 years	1925	497	76	235	2733 (77.1)
	2-5 years	535	153	23	58	769 (21.7)
	> 5 years	0	0	21	23	44 (1.2)
Sex	Male	2454	650	60	216	3380 (95.3)
	Female	6	0	60	100	166 (4.7)
Breed	Cross-breed	1813	417	28	154	2412 (68)
	Local	380	223	26	66	695 (19.6)
	Imported	267	10	66	96	439 (12.4)

Table 1: Structure of cattle populations slaughtered in the four abattoirs of this study (2017-2019).

Prevalence of suspected BTB lesions and risk factors

The prevalence of suspected BTB lesions according to age, sex, and breed is presented in Table 2. A significant association was observed in the prevalence of suspected BTB lesions according to age (p = 0.00001), sex (p = 0.00001), and breeds (p = 0.017). The highest infection rates occurred in cattle aged older than 5 years (27.3%), followed by cattle aged 2-5 years (9.4%), and those younger than 2 years (5.4%). Our results revealed that females were more affected by BTB (19.3%) than male cattle (6%). Imported breeds were affected to a higher degree (8.1%) compared to crossed (7.1%) or local breeds (3.6%).

Distribution of BTB like-lesions in lymph nodes

BTB like-lesions were mostly observed on the tracheobronchial lymph nodes (52.6%; 122/232) and mediastinal lymph nodes (31.9%; 74/232), followed by the hepatic (15.9%; 37/232), apical (10%; 23/232), retropharyngeal (8.6%; 20/232), and other visceral and carcass lymph nodes.

Variable		No. of inspected carcasses	Number of positive cases (%)					
			carcasses with suspected BTB lesions	*p*-value	smear ZN	*p*-value	culture	*p*-value
Age	< 2 years	2733	148 (5.4)	0.00001	52 (1.9)	0.0036	110 (4)	0.00001
	2-5 years	769	72 (9.4)		17 (2.2)		60 (7.8)	
	> 5 years	44	12 (27.3)		4 (9.1)		11 (25)	
Sex	Male	3380	200 (6)	0.00001	62 (1.8)	0.099	158 (4.7)	0.00001
	Female	166	32 (19.3)		11 (6.6)		23 (13.8)	
Breed	Cross-breed	2412	172 (7.1)	0.017	53 (2.2)	0.00002	134 (5.5)	0.006
	Local	712	26 (3.6)		8 (1.1)		20 (2.8)	
	Imported	422	34 (8.1)		12 (2.8)		27 (6.4)	
Total		3546	232 (6.5)		73 (2.0)		181 (5.1)	

Table 2: Prevalence of BTB infection and the associated risk factors reported for slaughtered cattle during the study period (2017-2019).

Based on the macroscopic characteristics, numerous lesions of the thoracic lymph nodes showed caseous necrotic areas with or without mineralization (35.3% and 34%, respectively) (Table 3/Figure 2A and 2B), followed by hemorrhagic lesions with caseous (1.7%) (Table 3/Figure 2C) and suppurative lesions (1.3%) (Table 3/Figure 2D).

Nature of lesions	No. of lesions	Count of lesions (%)
Hemorrhagic lesion with necrosis	4	1.7
Caseous	79	34
Caseous-calcified	82	35.3
Purulent	3	1.3
Not recorded	64	27.6

Table 3: Distribution of suspected BTB lesions observed in thoracic lymph nodes based on macroscopic characteristics.

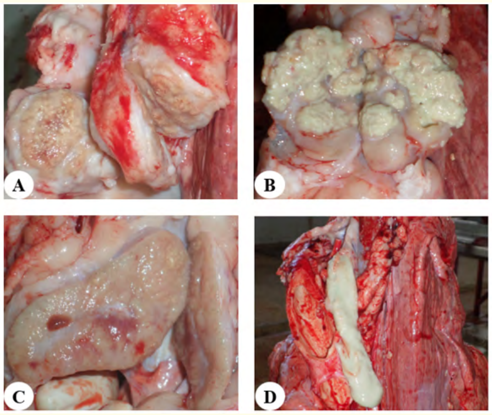

Figure 2: Macroscopic appearances of tuberculous lesions in thoracic lymph nodes. (A) Poorly defined caseous necrotic foci with extensive mineralization areas, surrounded by a thick fibrous capsule in the mediastinal lymph node. (B) Multiple irregularly confluent tubercle foci consisting of yellow caseous material in the cranial mediastinal lymph node. (C) Hemorrhagic lesion with minimal necrotic area in the left tracheobronchial lymph node. (D) Left tracheobronchial lymph node occupied by purulent material resulting from liquefaction of caseous lesion.

Prevalence of BTB infection according to the risk factors based on bacteriological examination

The prevalence of BTB infection was statistically associated with age either by ZN staining (p = 0.0036) or in bacterial culture (p = 0.00001). Cattle > 5 years had a higher prevalence of BTB compared to other age categories when using ZN staining (9.1%) and bacterial culture (25%). As for sex, the culture revealed a significant difference (p = 0.00001) between females (13.8%) and males (4.7%). However, no significant association was noted (p = 0.099) for sex using the ZN staining technique. Concerning cattle breeds, we noted a significant difference in the prevalence using both ZN staining (p = 0.00002) and bacterial culture positivity (p = 0.006). The highest prevalence of BTB was recorded for imported bovines using ZN staining (2.8%) and culture (6.4%) compared to other breed categories (Table 2).

Discussion

The detection of BTB cases through PMI combined with bacteriological examination of suspected lesions is a crucial step for the confirmation of BTB infection [22]. However, in endemic countries, slaughter inspection is often used, without resorting to laboratory examinations, as the main method for BTB control and epidemiological surveillance [21,23-25]. The present study estimated the prevalence of BTB infection, using both PMI and bacteriological tests. Based on animal age, results showed a positive correlation with the prevalence of BTB infection. The higher prevalence (27.3%) of BTB among old cattle might be explained by the chronic nature of the disease, whereby the animals might have acquired the infection at a young age but only developed the lesions and the disease in old age [26], as well as the probability that exposure to the pathogen and reactivation of latent infections increases with longevity [27]. As for sex, cows were found more affected than males, and this may be explained by the fact that females remain longer in the herds for milk production while males are slaughtered at an early age [28]. Furthermore, the stress of lactation and gestation make females more vulnerable to infection [29]. Our results demonstrated that imported cattle breeds were more affected by the disease compared to the crossed and local breeds. Anterior studies done in Africa suggested that European breeds may be less resistant to BTB infection than the indigenous breeds [30,31].

Our results showed a high tropism of tuberculous lesions in the thoracic lymph nodes, particularly the tracheobronchial and mediastinal lymph nodes. This result was consistent with previous studies that reported the predominance of an alteration of thoracic lymph nodes in naturally infected cattle [6,32-34]. In fact, the transmission route determines the location of BTB lesions. In cattle, the infection mostly occurs via airborne pathway [6].

In this study the PMI allowed us to determine the development stages of tuberculous lesions, based on morphological characteristics. The highest frequency of suspected BTB lesions were characterized by caseous necrosis, with or without mineralization, and the lowest were suppurative (liquefied) lesions. Generally, the lesions in ruminants are described as caseous and, to a lesser degree, liquefactive [35]. Numerous studies have reported the predominance of caseous necrosis and calcification lesions encountered in natural infections by *M. bovis* [23,33,36,37], suggesting the chronic process of the disease [14].

The prevalence of suspected BTB lesions reported here (6.5%) remains significant and alarming. Some infected animals escape stamping out because of the moderate sensitivity of SICT (approximately 84%) [38], as well as the lack of traceability (correct identification) for many individuals in the herd. To eradicate BTB in Algeria, the campaign adopted by the authorities should combine three essential components: test and slaughter, along with surveillance for the disease in abattoirs and trace-back to the property of origin.

Conclusion

The present study confirmed that BTB remains prevalent in Northern Algeria. This suggests a lack of effectiveness to BTB control and eradication programs. Owing to the resource limitations, it is not feasible to apply the laboratory tests (*i.e.*, bacteriology, histopathology and molecular tests) in routine diagnosis to confirm the BTB infection in samples with visible lesions. Therefore, meat inspection remains a cost-efficient method for disease surveillance. However, the lack of a cattle identification system among herds represents an obstacle to applying trace-back to identify the origin of tuberculous foci. Our study demonstrated the need to re-evaluate national BTB control and eradication programs through the implementation of an efficient identification system for all the national livestock and application of large-scale BTB eradication programs, ensuring adequate compensation for farmers for the slaughter of their infected cattle.

Acknowledgments

We thank all the STMR staff in Béjaia as well as the veterinarians who participated in this study.

Declaration of Competing Interest

The authors declare that they have no competing interests.

Bibliography

1. Humblet MF., *et al*. "Classification of worldwide bovine tuberculosis risk factors in cattle: a stratifiedapproach". *Veterinary Research* 40.5 (2009): 1-24.
2. Cvetnic Z., *et al*. "*Mycobacterium caprae* in cattle and humans in Croatia". *The International Journal of Tuberculosis and Lung Disease* 11.6 (2007): 652-658.
3. Sulieman MS., et al. "Identification of acid fast bacteria from caseous lesions in cattle in Sudan". *Journal of Veterinary Medicine*, *Series B* 49.9 (2002): 415-418.
4. Michelet L., *et al*. "*Mycobacterium microti* interferes with bovine tuberculosis surveillance". *Microorganisms* 8.12 (2020): 1850.
5. Thoen CO., *et al*. "*Mycobacterium bovis* infection in animals and humans". *John Wiley and Sons* 2 (2008): 1-329.
6. O'Reilly LM., *et al*. "The epidemiology of *Mycobacterium bovis* infections in animals and man: a review". *Tubercle and Lung Disease* 76 (1995): 1-46.
7. Grange JM. "*Mycobacterium bovis* infection in human beings". *Tuberculosis* 81.1-2 (2001): 71-77.
8. De Kantor IN., *et al*. "An update on bovine tuberculosis programmes in Latin American and Caribbean countries". *Veterinary Microbiol*ogy 112.2-4 (2006): 111-118.
9. Domingo M., *et al*. "Pathology of bovine tuberculosis". *Research in Veterinary Science* 97 (2014): S20-S29.
10. Corner LA., *et al*. "Efficiency of inspection procedures for the detection of tuberculous lesions in cattle". *Australian Veterinary Journal* 67.11 (1990): 389-392.
11. Wangoo A., *et al*. "Advanced granulomatous lesions in *Mycobacterium bovis*-infected cattle are associated with increased expression of type I procollagen, γδ (WC1+) T cells and CD 68+ cells". *Journal of Comparative Pathology* 133.4 (2005): 223-234.
12. Vordermeier HM., *et al*. "Correlation of ESAT-6-specific gamma interferon production with pathology in cattle following *Mycobacterium bovis* BCG vaccination against experimental bovine tuberculosis". *Infection and Immunity* 70.6 (2002): 3026-3032.
13. Palmer MV., *et al*. "Lesion development and immunohistochemical changes in granulomas from cattle experimentally infected with Mycobacterium bovis". *Veterinary Pathology* 44.6 (2007): 863-874.
14. Carrisoza-Urbina J., *et al*. "Atypical granuloma formation in *Mycobacterium bovis*-infected calves". *PLoS One* 14.7 (2019): e0218547.
15. Acha PN., *et al*. "Zoonoses and Communicable Diseases Common to Man and Animals. Bacteriose and mycoses". *Scientific and Technical Publication (DC: Pan American Health Organization, Washington)* 580.3 (2001): 283-299.
16. Word Organisation for Animal Health (OIE). "Contronlling of bovine tuberculosis: a one health chalenge". *Bulletin* (2019): 2-82.
17. Demelash B., *et al*. "Prevalence of bovine tuberculosis in Ethiopian slaughter cattle based on post-mortem examination". *Tropical Animal Health and Production* 41.5 (2009): 755-765.
18. Sahraoui N., *et al*. "Molecular characterization of *Mycobacterium bovis* strains isolated from cattle slaughtered at two abattoirs in Algeria". *BMC Veterinary Research* 5. 1 (2009): 4.
19. Damene H., *et al*. "Broad diversity of *Mycobacterium tuberculosis* complex strains isolated from humans and cattle in Northern Algeria suggests a zoonotic transmission cycle". *PLoS Neglected Tropical Diseases* 14.11 (2020): e0008894.
20. Word Organisation for Animal Health (OIE). "Bovine tuberculosis:The tuberculin test Manual of diagnostic tests and vaccines for terrestrial animals". *OIE Terrestrial Manual* 6 (2009): 6-7.
21. World Health Organization (WHO). "Laboratory services in tuberculosis control. Geneva Switzerland: Part II: Microscopy". *World Health Organization* (1998): 1-63.

22. Corner LA. “Post mortem diagnosis of *Mycobacterium bovis* infection in cattle”. *Veterinary Microbiol*ogy 40.1-2 (1994): 53-63.

23. Shitaye JE., *et al*. “A prevalence study of bovine tuberculosis by using abattoir meat inspection and tuberculin skin testing data, histopathological and IS6110 PCR examination of tissues with tuberculous lesions in cattle in Ethiopia”. *Veterinarni Medicina* 51.11 (2006): 512.

24. Olea-Popelka FJ., *et al*. “Risk factors for disclosure of additional tuberculous cattle in attested-clear herds that had one animal with a confirmed lesion of tuberculosis at slaughter during 2003 in Ireland”. *Preventive Veterinary Medecine* 85 1-2 (2008): 81-91.

25. Shittu A., *et al*. “Factors associated with bovine tuberculosis confirmation rates in suspect lesions found in cattle at routine slaughter in Great Britain, 2003-2008”. *Preventive Veterinary Medecine* 110.3-4 (2013): 395-404.

26. Kasaye S., *et al*. “Prevalence of camel tuberculosis at Akaki abattoir in Addis Ababa, Ethiopia”. *African Journal of Microbiology Research* 7.20 (2013): 2184-9.

27. Pollock JM., *et al*. “*Mycobacterium bovis* infection and tuberculosis in cattle”. *The Veterinary Journal* 163.2 (2002): 115-127.

28. Boukary AR., *et al*. “Risk factors associated with bovine tuberculosis and molecular characterization of *Mycobacterium bovis* strains in urban settings in Niger”. *Transboundary and Emerging Diseases* 59.6 (2012): 490-502.

29. Habarugira G., *et al*. “Bovine tuberculosis in Rwanda: Prevalence and economic impact evaluation by meat inspection at Société des Abattoirs de Nyabugogo-Nyabugogo Abattoir, Kigali”. *Journal of the South African Veterinary Association* 85.1 (2014): 1-5.

30. Elias K., *et al*. “Status of bovine tuberculosis in Addis Ababa dairy farms”. *Revue scientifique et technique* 27.3 (2008): 915.

31. Skuce RA., *et al*. “Bovine tuberculosis (TB): a review of cattle-to-cattle transmission, risk factors and susceptibility”. *Bacteriology Branch Veterinary Sciences Division Agrifood and Biosciences Institute* (2011).

32. Teklu A., *et al*. “Tuberculous lesions not detected by routine abattoir inspection: the experience of the Hossana municipal abattoir, southern Ethiopia”. *Revue Scientifique et Technique-Office International Des Epizooties* 23.3 (2004): 957-964.

33. Liebana E., *et al*. “Pathology of naturally occurring bovine tuberculosis in England and Wales”. *Veterinary Journal* 176.3 (2008): 354-360.

34. Proaño-Pérez F., *et al*. “Post-mortem examination and laboratory-based analysis for the diagnosis of bovine tuberculosis among dairy cattle in Ecuador”. *Preventive Veterinary Medecine* 101.1-2 (2011): 65-72.

35. Converse PJ., *et al*. “Cavitary tuberculosis produced in rabbits by aerosolized virulent tubercle bacilli”. *Infection and Immunity* 64.11 (1996): 4776-4787.

36. Menin Á., *et al*. “Asymptomatic cattle naturally infected with *Mycobacterium bovis* present exacerbated tissue pathology and bacterial dissemination”. *PloS One* 8.1 (2013): e53884.

37. AVSEVER ML., *et al*. “New spoligotyping pattern of *Mycobacterium bovis* isolates from farm animals in Turkey”. *Ankara Üniversitesi Veteriner Fakültesi Dergisi* 64.1 (2017): 37-43.

38. De la Rua-Domenech R., *et al*. “Ante mortem diagnosis of tuberculosis in cattle: a review of the tuberculin tests, γ-interferon assay and other ancillary diagnostic techniques”. *Research Veterinary Science* 81.2 (2006): 190-210.