Isolation nontuberculous mycobacteria and histopathological changes in lymph nodes collected at the abattoir in cattle reactive-positive to tuberculin dermal test

Angélica María Hernández Jarguín (amhjarguin@hotmail.com)
Universidad Autonoma de Tamaulipas Facultad de Medicina Veterinaria y Zootecnia
https://orcid.org/0000-0002-2198-3611
Julio Martínez-Burnes
Universidad Autonoma de Tamaulipas Facultad de Medicina Veterinaria y Zootecnia
Gloria M. Molina-Salinas
Instituto Mexicano del Seguro Social
Ned I. de la Cruz-Hernández
Universidad Autonoma de Tamaulipas Facultad de Medicina Veterinaria y Zootecnia
José L. Palomares-Rangel
Universidad Autonoma de Tamaulipas Facultad de Medicina Veterinaria y Zootecnia
Alfonso López Mayagoitia
University of Prince Edward Island
Hugo B. Barrios-García
Universidad Autonoma de Tamaulipas Facultad de Medicina Veterinaria y Zootecnia

Research article

Keywords: non-tuberculous mycobacteria, tuberculosis, granulomatous lesions, tuberculin test

Posted Date: March 3rd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-15889/v1

Isolation nontuberculous mycobacteria and histopathological changes in lymph nodes collected at the abattoir in cattle reactive-positive to tuberculin dermal test

Angélica M. Hernández-Jarguín,[1*] Julio Martínez-Burnes,[1] Gloria M. Molina-Salinas, [2] Ned I. de la Cruz-Hernández,[1] José L. Palomares-Rangel,[1] Alfonso López Mayagoitia[3] and Hugo B. Barrios-García[1]

[1]Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas. Cd. Victoria, Tamaulipas C.P. 87000, México.

[2] Unidad de Investigación Médica Yucatán, Unidad Médica de Alta Especialidad Hospital de Especialidades 1 Mérida, Yucatán, Instituto Mexicano del Seguro Social, CP 97150, México.

[3]Department of Pathology & Microbiology, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, PEI, C1A4P3. Canada

* Corresponding author:

Angélica M. Hernández-Jarguín

amhjarguin@hotmail.com

Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma de Tamaulipas. Cd. Victoria, Tamaulipas C.P. 87000, México.

Carretera Cd. Victoria- Mante Km 5, Cd. Victoria, Tamaulipas. México

C.P. 87000

Abstract

Background: The *Mycobacterium tuberculosis* complex causes a variety of diseases; in bovine, the common pathogen is *M. bovis* which is considered zoonotic. A separate group of mycobacteria, much less known, is "non-tuberculous mycobacteria (NTM) which are also infectious for animals and humans. The Mexican Official Norm (NOM-ZOO-031-1995) regulates *M. bovis* in cattle, but not NTM species, even though this last type of microorganisms has a confounding effect for the diagnosis of bovine tuberculosis. The objective of the study was to isolate and identify the NTM of bovine lymph nodes condemned in the slaughterhouse, to characterize the histological lesions in these tissues and to correlate bacteriological and postmortem findings with the antemortem skin test of the tuberculin.

Results: Mycobacteria were isolated from 54/528 (10.2%) of the lymph nodes; 29/54 (53.7%) of these isolates were identified as *M. bovis* and 25/54 (46.2%) as NTM; 3 bacteriological cultures were discarded due to contamination with fungi and in one case it was not possible to identify the species. Granulomatous and pyogranulomatous inflammation were observed in 6/21 (28.6%) and 7/21 (33.3%) of the NTM-positive lymph nodes, respectively. Necrosis and mineralization were only found in 6/21(28.6%) of the lymph nodes. The species of NTM associated with granulomatous lymphadenitis were *M. scrofulaceum, M. triviale, M. terrae* and *M. szulgai,* while those causing pyogranulomatous lesions were *M. szulgai, M. kansasii, M. phlei,* and *M. scrofulaceum.*

Conclusions: Considering the increase of mycobacterial infections in humans worldwide, the idetification of NTM that inducing Tuberculosis-like lesions in abattoir inspection is the first step to investigate the livestock-human-wildlife-environment interactions with especially focus on transmission dynamics.

Keywords: non-tuberculous mycobacteria, tuberculosis, granulomatous lesions, tuberculin test

1. Background

Tuberculosis (TB) is one of the most devastating zoonotic diseases that, for centuries, has afflicted humans and animals worldwide (Bercovier and Vincent 2001; Fonseca et al. 2017). A report of the World Health Organization indicates that over 1.5 million people die each year because of TB, and as many as 2 billion people are actively or silently infected (WHO, 2017). The infection starts when humans or animals become exposed to genetically related species of mycobacteria, namely *Mycobacterium tuberculosis*, *M. bovis*, and the *M. tuberculosis* complex. These mycobacteria evade the immune and phagocytic systems and remain in infected organs for a long time causing chronic inflammation, debilitating disease and become a source of infection for other susceptible animals. For the last two decades, TB has been reviewed under the "One-Health" or "Global Health" perspective, where human and animal diseases are closely interconnected with the food and the environment. Strategies to monitor and eradicate TB are underway in practically all countries around the world (WHO, 2017).

In Mexico, the National Program for Control and Eradication of Bovine TB started 1990, and a few years later the guidelines for antemortem and postmortem diagnoses were streamlined under the Mexican Official Norm (NOM-031-ZOO-1995). The antemortem detection of bovine TB is done using a skin test that measures the immune response to mycobacterial antigens. For intradermal tuberculin test, the skin is first inoculated with a purified protein derivate (PPD) of *M. bovis* AN5 strain, followed 72 h later by a second inoculation of *M. avium* D4 strain. Animals infected with TB show a characteristic thickening of this skin at the site of the inoculation 72 h later.

The postmortem detection of bovine TB is accomplished by abattoir surveillance, where meat inspectors examine organs and lymph nodes for lesions indicative of TB. Affected tissues are condemned and submitted to the laboratory for histopathological and bacteriological analyses. Microscopically, tissues with TB show a characteristic granulomatous inflammatory response with the presence of intralesional acid-fast bacilli. The bacteriologic diagnosis of TB is based on the identification of *M. bovis* either by the bacteriological culture or more recently by PCR. Farm quarantine and meat condemnation at the slaughter plant causes significant economic losses to farmers and producers (Zaragoza, 2017).

One major problem for the antemortem and postmortem diagnosis of bovine TB is the confounding effect of the infection by non-tuberculous mycobacteria (NTM), organisms that are widely disseminated in the environment. Infection with NTM gives a false-positive

tuberculin test because these saprophytic bacteria cross-react with the immune response to the classical tuberculous mycobacteria (Waters et al. 2010, Waters et al. 2006; Jakko et al. 2009, Devulder et al. 2005). In addition to the cross immune response, the gross and microscopic lesions induced by NTM are sometimes indistinguishable from those produced by *M. tuberculosis, M. bovis* and *M. tuberculosis* complex (Waters et al. 2010, Waters et al. 2006). The NTM species most frequently isolated from TB-like lesions in cattle are *M. gastri, M. flavescens, M. phlei, M. triviale, M. terrae, M. nonchromogenicum, M. intracellulare, M. gordonae* (Latini et al. 1997, Proano et al. 2006, Rigouts et al. 1996). Immunosuppression is a risk factor for TB and NTM infections (Marras and Daley 2002, Falkinham J.O. III 2009). Although there is no evidence that NTM is transmitted from animals to humans, or between humans (Gadkowski and Stout 2008, Katoch 2004), the confounding effect of NTM in the antemortem and postmortem diagnosis of bovine TB is of significance in surveillance and meat inspection.

The study aimed to isolate and identify NTM from condemned bovine lymph nodes at the slaughterhouse, characterize the histological lesions in these tissues, and correlate bacteriological and postmortem findings with the antemortem tuberculin skin test.

3. Results

3.1. Collection of lymph nodes and gross changes

From 528 condemned lymph nodes at the abattoir, 235 (44.5%) showed gross lesions compatible with TB and 42/528 (8%) lymph nodes were from cattle that had tested positive for intradermal tuberculin (Table 1).

3.2. Mycobacterial isolation and biochemical identification

Mycobacteria were isolated from 54/528 (10.2%) of the lymph nodes; 29/54 (53.7%) of the mycobacterial isolates were identify as *M. bovis* and 25/54 (46.2%) as NTM; Biochemical typing for 21 NTM isolates yielded *M. szulgai* (n = 8), *M. scrofulaceum* (n = 4), *M. phlei* (n = 3), *M. kansasii* (n = 2), *M. chelonei* (n = 2), *M. triviale* (n = 1), *M. fortuitum* (n = 1), and *M. terrae* (n = 1); 3 NTM isolates were discarded because fungal contamination precluded biochemical typing and in one identifying the species was not possible.

3.3. Histopathology

Histopathologically, the 21 lymph nodes positive for NTM revealed two distinct morphologic types of inflammation. (1).- Granulomatous, characterized by an external rim of fibroblasts

that contain macrophages, giant cells, central caseous necrosis, mineralization and scant neutrophils. (2).- Pyogranulomatous inflammation, which besides macrophages, giant cells, necrosis and mineralization, also showed abudndant neutrophils. Granulomatous and pyogranulomatous inflammation were observed in 6/21 (28.6%) and 7/21 (33.3%) of the NTM-positive lymph nodes, respectively. Necrosis and mineralization were only observed in 6/21(28.6%) of the lymph nodes. Two lymph nodes (2/7; 28.6%) with pyogranulomatous inflammation also revealed intralesional fungi. AFB were microscopically observed in 5/6 (83.3%) of the lymph nodes showing granulomatous inflammation and in 4/7 (57.1%) with pyogranulomatous inflammation. Eight (8/21; 38.1%) lymph nodes yielded NTM but did not have any microscopic lesions or changes associated with TB (Table 2).

The species of NTM associated with granulomatous lymphadenitis were *M. scrofulaceum, M. triviale, M. terrae* and *M. szulgai,* while those related to pyogranulomatous lesions were *M. szulgai, M. kansasii, M. phlei,* and *M. scrofulaceum.* However, the NTM species, except *for M. fortuitum* and *M. chelonei,* were also isolated from lymph nodes with no microscopic lesions (Table 2).

Five animals were identified as positive at antemortem intradermal tuberculin test, and another animal from a herd with positive reactors to tuberculin was considered as "exposed." One tuberculin-positive animal had granulomatous lesions that were associated with *M. terrae*. The animal considered as "exposed" that had pyogranulomatous changes yielded *M. scrofulaceum*. Two tuberculin-positive animals showing no lesions were associated with *M. phlei* and *M. chelonei*. On the other hand, *M. kansasii* and *M. szulgai* were isolated from the two tuberculin-positive animals that lacked tissues for histopathology.

4. Discussion

4.1 *Species of NTM isolated from lymph nodes:*

The species of NTM isolated from the lymph nodes condemned by the meat inspectors at the slaughter plant in Tamaulipas were *M. kansasii*, *M. szulgai*, *M. scrofulaceum*, *M. terrae*, *M. phlei* and *M. chelonae*. Our findings do not match the results of a similar study conducted in the southern part of the state of Mexico (Zaragoza et al., 2017) where *M. neoaurum, M. parafortuitum and M. confluentis* were the main isolates. The species of NTM reported here agree with those previously reported in Mexico in human lungs (Pérez-Martínez et al., 2008; Cortés-Torres et al., 2013; Escobar-Escamilla et al., 2014; Orduña et al., 2015), water (Castillo

Rodal et al., 2012; Pérez-Martinez et al., 2013) and salad samples (Cerna-Cortés et al., 2015). The number of NTM species reported in the literature exceeds 130, and according to some reports the most common NTM isolates from cattle are *M. gastri, M. flavescens, M. phlei, M. triviale, M. terrae, M. nonchromogenicum, M. intracellulare, M. gordonae M. thermorresistible, M. xenopi, M. fortuitum, M. chelonae, M. ulcerans* and *M. kansasii, M. avium, M. neoaurum, M. confluentis* and *M. vaccae* (Bercovier and Vincent, 2001; Proano et al., 2006; Gortazar et al., 2011; Biet and Boschiroli, 2014; Katale et al., 2014; Zaragoza et al., 2017; Cheng et al., 2017; Ghielmetti et al., 2018; Bolaños et al., 2018). It could be surmised from these findings that the environment in Northern Mexico and other regions in America, Europe, Asia and Africa share the same species of saprophytic mycobacteria.

Tuberculous bacteria disseminate in tissues by lymhatic drainage, making the lymph nodes the prime anatomical site for meat inspection. The majority of the lymph nodes condemned and evaluated in our study were mesenteric and mediastinic indicating that the main port of entry for NTM is through the lungs and digestive systens (Belachew, 2017). It should be noted that 38.1% of healthy lymph nodes tested positive for NTM suggesting that lymphoid tissues harbour mycobacteria without eliciting inflammation, or that no sufficient time elapsed to trigger a microscopically visible inflammatory response.

Types of inflammation:

Enlargement of a lymph node, clinically referred to as lymphoadenomegaly, is the “red flag” that prompts meat inspectors to carefully examine these tissues and look for inflammatory reactions such as granulomas or abscesses. Histopathological and microbiological studies are indispensable to properly characterize the lesions and identify the NTB organisms. The host response elicited by mycobacteria is characteristically granulomatous, regardless of the bacterial species or the affected organ (Ackerman 2017). This stereotyped inflammatory response relates mainly to the lipids of the bacterial cell wall and the ability of the mycobacteria to overcome phagocytosis (Ackerman, 2017).

It remains unclear, however, why *M. scrofulaceum, M. triviale, M. terrae,* and *M. szulgai* were isolated mostly from granulomatous lymphadenitis while *M. scrofulaceum, M. kansasii, M. szulgai* and *M. phlei* from pyogranulomatous lymphadenitis. According to the literature, granulomatous and pyogranulomatous inflammation could be merely different stages of the inflammatory process, being a pyogranuloma less chronic and active, while a granuloma is more chronic and better organized (Ackerman 2017). In similar studies based on the examination of lymph nodes, liver and lungs in cattle, have been reported the *M. fortuitum*, *M.*

avium, *M. kansasii*, *M .lentiflavum* and *M. intracellulare* as the principally NTM species associated to chronic inflammatory response and form typical tuberculous granuloma (Katale et al., 2014; Cheng et al., 2017; Gcebeet and Hlokwe, 2017; Ghielmetti et al., 2018). Interestingly, none of the NTM species isolated in our study involved co-infection with *organisms of the M. tuberculosis* complex. The histopathological findings of infections caused by nontuberculous mycobacteria are indistinguishable from those caused by *M.* tuberculosis complex (Claudio Piersimoni et al., 2008).

The role of neutrophils in the pathogenesis mycobacterial lesions is only partially understood. It has been observed in experimental mycobacterial infections that neutrophils favour the recruitment of other cells involved in the formation of granulomas (Seok et al., 2010). The permanence of neutrophils at the site of injury depends on the production of chemical mediators such as TNF-α (Kasahara et al., 1998). Neutrophils arrive in two "waves," the first relates to the innate response against the pathogen and the second to the adaptive response (Remote et al. 2019). As a result, neutrophils can be present in the initial host response and remain in the chronic stages of inflammation.

Pyogranulomatous lymphadenitis has been previously reported in cattle and black deer infected with *M. kansasii* (Waters et al. 2010; Hall et al. 2005). This NTM species has also been isolated from calcified microabscesses in the lymph nodes, lungs, and liver of pigs, feral pigs, camels, goats, and cows (Jarnagin et al. 1983).

Granulomatous lymphadenitis is not unique to mycobacterial infections and occurs also with other bacterial and fungal infections. *Coccidioides immitis* and the algae Chlorella sp are two systemic mycoses endemic in northern Mexico which are important impersonators of TB. Both of thses infections cause granulomatous and pyogranulomatous lymphadenitis in ruminants grossly indistinguishable from mycobacterial diseases (Del Rocío et al. 2016; Ramirez et al. 2010). Lymph node enlargement is not unique to infectious diseases, it often occurs in neoplastic diseases, particularly lymphosarcoma, which is prevalent in cattle worldwide (Marshak 1962). None of the condemned specimens which were submitted to the laboratory slaughterhouse in Tamaulipas had microscopic evidence of bovine lymphosarcoma or any other neoplastic disease.

Although a study reported that NTM sampled from field conditions are more likely to interfere with the current intradermal diagnostic test for bovine TB detection (Thacker et al., 2013). Our findings and those reported by Bolaños, 2018 and Ghielmetti , 2018 suggest that *M. terrae*, *M. kansasii*, *M, szulgai, M. scrofulaceum, M. phlei, M. chelonae, M. engbaekii, M. arupense, M.*

nonchromogenicum, *M. heraklionense* and *M. persicum* isolated from slaughtered cattle and milk samples from dairy cows, cause interference in the routine antemortem intradermal tuberculin test for bovine TB leading to false-positive results and considerable economic losses (Water et al., 2006). The cross-reactivity in cattle infected with *M. bovis* and NTM has become a serious problem for TB surveillance, which explains why the lymph nodes of some of the tuberculin-positive animals in Tamaulipas yielded NTM and not *M. bovis*. This cross-antigenic reaction results from the close phylogenetic relationship shared by NTM and *M. tuberculosis* (Jakko et al. 2009, Devulder et al. 2005). Despite this close phylogenetic relationship, there is no reliable evidence that NTM are transmitted from animals to humans, or between humans (Gadkowski and Stout 2008, Katoch 2004. Although it has high phylogenetic homology because it is of the same genus, it can be different by rRNA amplification (Zaragoza, 2017). However, it can induce several types of lesion.

Conclusions

NTM were isolated from condemned bovine lymph nodes at the slaughterhouse, and the histological lesions revealed granulomatous and pyogranulomatous lymphadenitis. The NTM associated with granulomatous lymphadenitis were *M. scrofulaceum, M. triviale, M. terrae,* and *M. szulgai*, while those related to pyogranulomatous lesions were *M. szulgai, M. kansasii, M. phlei* and *M. scrofulaceum.* Interestingly, *M. terrae* was associated with granulomas from a bovine positive at intradermal tuberculin test, while *M. scrofulaceum* was associated with pyogranulomas from a bovine "exposed".

Considering the increase of mycobacterial infections in humans worldwide, along with the results of the present study in cattle, there is a need to investigate the livestock-human-wildlife-environment interactions with especially focus on transmission dynamics. There is a need also for molecular studies of *M. terrae* and NTM inducing TB-like lesions in abattoir inspection. Finally, it is imperative to recognise the relevance of NTM infection on the interpretation and differential diagnosis of TB cattle.

2. Methods

2.1 Collection of lymph nodes

Five hundred and twenty-eight bovine lymph nodes (n=528) were collected between 2007 and 2011 at an abattoir in Tamaulipas, Mexico as part of a surveillance program done by certified veterinary inspectors for the National Program for Control and Eradication of Bovine TB in Tamaulipas. Antemortem diagnosis of TB in animals was based on immunological response to an intradermal PPD and D4 inoculation. Lymph nodes condemned at the slaughter house were cut serially and examined for gross lesions compatible with TB. One-half of the lymphoid tissue was preserved in 6% sodium borate for bacteriological analyses while the other half was immersed in 10% buffered formalin for histological examination. The specimens were sent to the Diagnostic Laboratory of the University of Tamaulipas and processed according to the Mexican Official Norm (NOM-031-ZOO-1995).

2.2 Mycobacterial isolation and biochemical identification

All samples were handled and processed in laminar-flow hood type II following established guidelines. After removing the sodium borate, the lymph nodes were placed in a sterile mortar where the fat was removed, the tissue cut into small fragments and manually macerated with sterilized sand. Twenty mL of distilled sterile water was added to the specimen for a second maceration. The macerate was then diluted with a 4% solution of sodium hydroxide and phenol red at a 1:1 ratio and incubated at 37ºC/20 shaking for 20 min. The suspension was centrifuged at 1157.13 *g* for 20 min, and after removing the supernatant, the sediment was neutralized with a 1N hydrochloric acid solution until the pink color of the indicator turned yellow as detailed in the Petroff protocol (Buijtels PC, 2005).

Two Stonebrink media tubes (Pronavibe, México) and one Lowenstein-Jensen culture media tube (Pronavibe, México) were inoculated with the neutralized residue and incubated at 37°C in 5% CO_2 for nine weeks, or until typical colonies were observed. Mycobacteria was first confirmed by the microscopic observation of acid-fast bacilli (AFB) using the Ziehl- Neelsen stain. Mycobacterial cultures were evaluated for metabolic activity, niacin production, and nitrate reduction, catalase production at 22 ºC and 68 ºC, urease production, and hydrolysis of Tween 80. Bacterial growth and tolerance were also assessed in MacConkey agar without violet crystal and with 5% sodium chloride.

2.3. Histopathology

Formaline-ixed lymph nodes were processed, embedded in paraffin and cut at 4-5 µm using standard methods. Tissue sections stained with hematoxylin-eosin were microscopically evaluated for histologic lesions, and Ziehl-Neelsen stained tissues were assessed for acid-fast bacilli.

Declarations

Abbreviations

NTM: non-tuberculous mycobacteria
TB: Tuberculosis
PPD: purified protein derivate
AFB: acid-fast bacilli

Ethics approval and consent to participate

Not applicable

Consent for publications

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding

We thank the project "Molecular epidemiology of *Mycobacterium tuberculosis* complex in strains isolated from bovines in the state of Tamaulipas" TAMPS-2008-C17-107247, CONACYT.

Authors' contributions

Design of the work, analysis and interpretation of data, and wrote of the manuscript: AH, JM, GM, HB; Mycobacterial isolation and biochemical identification: AH; Histopathologic processing and evaluation: AH, JM, NC, JP, AL; have substantively revised: AH, JM, GM, HB, AL. All the authors revised and approved the final version of the manuscript.

Acknowledgements

We thank Diagnostic Laboratory of the Faculty of Veterinary and Zootechnical Medicine of the Autonomous University of Tamaulipas for the infrastructure and equipment for samples procesing. We also acknowledge an scholarship-CONACYT-México for master's studies, grant 306393 and registration number 248068.

Table 1. Total of samples, tuberculin skin tests, gross lesions and number of mycobacteria isolated

Year	Total of samples	Tuberculin reactors	Gross lesions compatible with TB	*M. bovis*	NTM Isolated
2007	109	11	43	8	4
2008	169	13	69	12	6
2009	19	8	8	2	2
2010	139	2	67	3	5
2011	92	8	48	4	4
Total	528	42	235	29	21

Table 2. Association between microscopic findings and species of mycobacteria isolated

Number of samples	%	Microscopic Changes	NTM isolated
6	28.6	Granulomas	*M. scrofulaceum, M. triviale, M. terrae, M. szulgai*
7	33.3	Pyogranulomas	*M. szulgai, M. kansasii, M. phlei, M. scrofulaceum*
8	38.1	Without changes	*M. szulgai, M. phlei, M. chelonei, M. fortuitum* y *M. scrofulaceum*
21	100%		

References

1. Ackerman MR. (2017). Inflammation and healing. In: Pathologic Basis of Veterinary Disease, Zachary JF editor. *Elsevier*; 73-131.

2. Bercobier H & Vicent V. (2001). Mycobacterial infections in domestic and wild animals due to *Mycobacterium marinum, Mycobacterium fortuitum, Mycobacterium chelonae, Mycobacterium porcinum, Mycobacterium farcinogenes, Mycobacterium smegmatis, Mycobacterium scrofulaceum, Mycobacterium xenopi, Mycobacterium kansasii, Mycobacterium simiae* and *Mycobacterium genavense*. *Rev. Sci. Technol.* 20:265–290.

3. Del Rocío Reyes-Montes M, Pérez-Huitrón MA, Ocaña-Monroy JL, Frías-De-León MG, Martínez-Herrera E, Arenas R, & Duarte-Escalante E. (2016). The habitat of *Coccidioides* spp. and the role of animals as reservoirs and disseminators in nature. *BMC infectious diseases*, *16*(1), 550.

4. Devulver G, De Montclos MP & Flandrois JP. (2005). A multigene approach to phylogenetic analysis using the genus *Mycobacterium* as a model. *Int. J. Syst. Evol. Microbiol.* 55:293–302.

5. Falkinham JO 3rd. (2009). Surrounded by mycobacteria: nontuberculous mycobacteria in the human environment. *J. Appl. Microbiol.* 107:356–67.

6. Fonseca KL, Rodrigues PN, Olsson IAS & Saraiva M. (2017). Experimental study of tuberculosis: From animal models to complex cell systems and organoids. *PLoS pathogens,* 13(8), e1006421.

7. Gadkowski LB & Stout JE. (2008). Cavitary pulmonary disease. *Clin. Microbiol. Rev. 21*(2), 305–333.

8. Hall PB, Bender L & Garner MM. (2005). Mycobacteriosis in a black-tailed deer (*Odocoileus hemionus colombianus*) caused by *Mycobacerium kansasii.* J. Zoo Wildl. Med. 36:115–117.

9. Hoffner SE. (1994). Pulmonary infections caused by less frequently encountered slow-growing environmental mycobacteria. *Eur. J. Clin. Microbiol. Infect. Dis. 13:937–941.*

10. Holland SM. (2001). Nontuberculous mycobacteria. *Am. J. Med. Sci.* 321, 49-55.

11. van Ingen J, de Zwaan R, Dekhuijzen R, Boeree M & van Soolingen D. (2009). Region of difference 1 in nontuberculous *Mycobacterium* species adds a phylogenetic and taxonomical character. *J. Bacteriol.* 191(18), 5865-5867.

12. Jarnagin JL, Himes EM, Richards DW, & Harrington JR. (1983). Isolation of *Mycobacterium kansasii* from lymph nodes of cattle in the United States. *Am. J. Vet. Res.* 44(10):1853–1855.

13. Katoch VM. (2004). Infections due to non-tuberculous mycobacteria (NTM). *Indian J. Med. Res.* 120:290–304.

14. Latini O, Canal AM, Ferrara ME, Sequeira MD, Sequeira G, Bagnaroli R, & Torres, P. (1997). Confiabilidad en la determinación de prevalencia de infección por *Mycobacterium bovis* en ganado bovino por decomisos en frigoríficos. *Arch Med Vet.* 29(2):197-204.

15. Marras TK, Daley CL. (2002). Epidemiology of human pulmonary infection with nontuberculous mycobacteria. *Clin. Chest.* 23:553–567.

16. Monaghan ML, Doherty ML, Collins JD, Kazda JF, & Quinn PJ. (1994). The tuberculin test. *Vet. Microbiol.* 40(1-2):111-124.

17. Proano-Perez F, Rigouts L, Brandt J, Dorny P, Ron J, Chavez MA, Rodriguez R, Fissette K, Van Aerde A, Portaels F, Benitez-Ortiz W. (2006). Preliminary observations on *Mycobacterium* spp. in dairy cattle in Ecuador. *Am. J. Trop. Med. Hyg.* 75:318–323.

18. Ramírez-Romero R, Rodríguez-Tovar LE, Nevárez-Garza AM, & López A. (2010). Chlorella infections in a sheep in Mexico and minireview of published reports from humans and domestic animals. *Mycopathologia,* 169(6):461-466.

19. Rigouts L, Maregeya B, Traore H, Collart JP, Fissette K, & Portaels F. (1996). Use of DNA restriction fragment typing in the differentiation of *Mycobacterium tuberculosis* complex isolates from animals and humans in Burundi. *Tuber. Lung Dis.* 77(3):264-268.

20. Arend SM, de Haas P, Leyten E, Rosenkrands I, Rigouts L, Andersen P, & Van Soolingen D. (2005). ESAT-6 and CFP-10 in clinical versus environmental isolates of *Mycobacterium kansasii. J. Infect. Dis.* 191(8):1301-1310.

21. Waters WR, Palmer MV, Thacker TC, Payeur JB, Harris NB, Minion FC, Greenwald R, Esfandiari J, Andersen P, McNair J, Pollock JM, Lyashchenko KP. (2006). Immune responses to defined antigens of *Mycobacterium bovis* in cattle experimentally infected with *Mycobacterium kansasii*. Clin. Vaccine Immunol. 13(6):611-9.

22.Waters WR, Whelan AO, Lyashchenko KP, Greenwald R, Palmer MV, Harris BN, Hewinson RG, Vordermeier HM. (2010). Immune responses in cattle inoculated with *Mycobacterium bovis*, *Mycobacterium tuberculosis*, or *Mycobacterium kansasii*. Clin. Vaccine Immunol. 17(2):247-52.

23. World Health Organization (Ed.). (2013). *Global Tuberculosis report 2013.* World Health Organization.

24. Buijtles PC, & Petit PL. (2005). Comparison of NaOH-N-acetyl cysteine and sulfuric acid decontamination methods for recovery of mycobacteria from clinical specimens. *J. Microbiol Methods.* 62(1):83-88.

25. Marshak RR, Corriell LL, Lawrence WC, Croshaw JE, Schryver HF, Altera KP, & Nichols W W. (1962). Studies on bovine lymphosarcoma: I. Clinical aspects, pathological alterations, and herd studies. *Cancer Res.* 22(2): 202-217.

26. Zaragoza Bastida A, Rivero Pérez N, Valladares Carranza B, Isaac-Olivé K, Moreno Pérez P, Sandoval Trujillo H, Ramírez Durán N. (2017). Molecular Identification of *Mycobacterium* Species of Public Health and Veterinary Importance from Cattle in the South State of México. *Can. J. Infect. Dis. Med. Microbiol.* 2017:6094587.

27. Pérez-Martínez I1, Ponce-De-León A, Bobadilla M, Villegas-Sepúlveda N, Pérez-García M, Sifuentes-Osornio J, González-y-Merchand JA, Estrada-García T. (2008). A novel identification scheme for genus *Mycobacterium*, *M. tuberculosis* complex, and seven mycobacteria species of human clinical impact. *Eur. J. Clin. Microbiol. Infect. Dis.* 27:451.

28. Perez-Martinez I, Aguilar-Ayala DA, Fernandez-Rendon E, Carrillo-Sanchez AK, Helguera-Repetto AC, Rivera-Gutierrez S, Estrada-Garcia T, Cerna-Cortes JF, Gonzalez-Y-Merchand JA. (2013). Occurrence of potentially pathogenic nontuberculous mycobacteria in Mexican household potable water: a pilot study. *BMC Res. Notes* 11; 6:531.

29. Cortés-Torres N, González-y-Merchand JA, González-Bonilla C, García-Elorriaga G. (2013). Molecular analysis of mycobacteria isolated in Mexican patients with different immunodeficiencies in a tertiary care hospital. *Arch Med Res*. 44:562–9.

30. Escobar-Escamilla N, Ramírez-González JE, González-Villa M, Torres-Mazadiego P, Mandujano-Martínez A, Barrón-Rivera C, Bäcker CE, Fragoso-Fonseca DE, Olivera-Díaz H, Alcántara-Pérez P, Hernández-Solís A, Cícero-Sabido R, Cortés-Ortíz IA. (2014). Hsp65 phylogenetic assay for molecular diagnosis of nontuberculous mycobacteria isolated in Mexico. *Arch Med Res.* 45(1):90–97.

31. Orduña P, Castillo-Rodal AI, Mercado ME, Ponce de León S, López-Vidal Y. (2015). Specific Proteins in Nontuberculous Mycobacteria: New Potential Tools. *Biomed. Res. Int.* 2015:964178.

32. Castillo-Rodal, A.I., Mazari-Hiriart, M., Lloret-Sánchez, L.T., Sachman-Ruiz, B., Vinuesa, P., López-Vidal, Y. (2012). Potentially pathogenic nontuberculous mycobacteria found in aquatic systems. Analysis from a reclaimed water and water distribution system in Mexico City. *Eur. J. Clin. Microbiol. Infect. Dis.* 31(5):683-94

33. Cerna-Cortes JF, Leon-Montes N, Cortes-Cueto AL, Salas-Rangel LP, Helguera-Repetto AC, Lopez-Hernandez D, Rivera-Gutierrez S, Fernandez-Rendon E, Gonzalez-y-Merchand JA. (2015). Microbiological Quality of Ready-to-Eat Vegetables Collected in Mexico City: Occurrence of Aerobic-Mesophilic Bacteria, Fecal Coliforms, and Potentially Pathogenic Nontuberculous Mycobacteria. *Biomed Res Int.* 2015: 789508.

34. Gortazar C, Torres MJ, Acevedo P, Aznar J, Negro JJ, de la Fuente J, Vicente J. (2011). Fine-tuning the space, time, and host distribution of mycobacteria in wildlife. *BMC Microbiol.* 11(1): 27.

35. Biet F, & Boschiroli ML. (2014). Non- tuberculous mycobacterial infections of veterinary relevance. *Res. Vet. Sci*, 97(Suppl.), S69–S77.

36. Cheng G, Xu D, Wang J, Liu C, Zhou Y, Cui Y, Liu H, Wan K, Zhou X. (2017). Isolation and identification of multiple drug resistant nontuberculous mycobacteria from organs of cattle produced typical granuloma lesions. *Microb. Pathog*. 107:313–316.

37. Katale BZ, Mbugi EV, Botha L, Keyyu JD, Kendall S, Dockrell HM, Michel AL, Kazwala RR, Rweyemamu MM, van Helden P, Matee MI. (2014). Species diversity of non-tuberculous

mycobacteria isolated from humans, livestock and wildlife in the Serengeti ecosystem, Tanzania. *BMC Infect. Dis.* 14:616.

38. Ghielmetti G, Friedel U, Scherrer S, Sarno E, Landolt P, Dietz O, Hilbe M, Zweifel C, Stephan R. (2018). Non-tuberculous Mycobacteria isolated from lymph nodes and faecal samples of healthy slaughtered cattle and the abattoir environment. *Transbound Emerg. Dis*. 65:711-718.

39. Bolaños CAD, Franco MMJ, Souza Filho AF, Ikuta CY, Burbano-Rosero EM, Ferreira Neto JS, Heinemann MB, Motta RG, Paula CL, Morais ABC, Guerra ST, Alves AC, Listoni FJP, Ribeiro MG. (2018). Nontuberculous mycobacteria in milk from positive cows in the intradermal comparative cervical tuberculin test: implications for human tuberculosis infections. *Rev. Inst. Med. Trop. Sao Paulo.* 60:e6.

40. Gcebe N, Hlokwe TM. Non-tuberculous Mycobacteria in South African Wildlife: Neglected Pathogens and Potential Impediments for Bovine Tuberculosis Diagnosis. Front. Cell. Infect. Microbiol. 7:15.

41. Thacker TC, Robbe-Austerman S, Harris B, Van Palmer M, Waters WR. (2013). Isolation of mycobacteria from clinical samples collected in the United States from 2004 to 2011. *BMC Vet.* Res. 9:100.