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Pathology

Bovine Tuberculosis with Special Reference to its Spread in Egypt: A Review Article

Hamdy Abd EL-aziz Salem; Anhar Ali; Enas Abd EL-aziz Tahoun; Anis Anis.

Department of pathology, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Menofia, Egypt.

*Corresponding author: aniszaid@vet.usc.edu.eg

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ABSTRACT

Bovine tuberculosis (BTB) is one of the most recognized endemic zoonotic diseases in the world, including Egypt. *Mycobacterium bovis* belongs to the strain of Mycobacterium TB complex, which is the main cause of bovine tuberculosis, which spreads via inhalation of infected droplets or consumption of infected food and water. Bovine tuberculosis causes severe economic loss, which has major implications on animal health, ranging from decreased milk output, weight losses, infertility, meat condemnation or even mortalities. Antemortem and postmortem examinations are used for the diagnosis of bovine tuberculosis. Antemortem procedures rely on either cellular or humoral immunological responses, whereas postmortem diagnosis is based on proper visual inspection and palpation. The tubercles are mainly found in lymph nodes, notably those in the bronchial, retropharyngeal, and mediastinal regions, with some exhibiting cheesy material when cut during meat inspection processes on animal carcasses at slaughterhouses. However, the bacteria can also spread throughout the blood, cause a systemic infection, and damage other organs. The histopathological examination is distinguished by the granuloma formation, including central necrosis, chronic inflammatory cell aggregation, mineralization, and peripheral fibrosis. Other diagnostic tests, such as bacterial isolation and PCR, are necessary to establish a final diagnosis. Vaccination, hygienic measurements and proper diagnosis are critical for preventing and controlling TB in humans and animals.

Key wards: Bovine tuberculosis; *Mycobacterium bovis*, Zoonosis, Diagnosis, Pathological examination.

INTRODUCTION

History of bovine tuberculosis

Tuberculosis has been recognized from thousands of years and affects many mammals. It is considered the greatest cause of deaths in the last 200 years when compared to other dangerous such as AIDS, cholera, influenza, malaria, plague, and smallpox (Donoghue *et al*., 2017; Elsayed and Amer, 2019).

Bovine tuberculosis is a zoonotic chronic bacterial infectious disease that negatively affects both human and animal health (Jolma *et al*., 2021) due to lower milk and meat productivity, costs associated with testing and slaughter, the culling of ill animals, carcass condemnation and international trade restrictions (Algammal *et al*., 2019). It is caused primarily by *M. Bovis* and infrequently by other pathogenic mycobacteria (Borham *et al*., 2022). The bacillus that causes tuberculosis in humans, (*Mycobacterium tuberculosis*), was found by Koch (1882), whereas *Mycobacterium bovis* was discovered by Smith (1898).

Classification of mycobacteria

Mycobacterium is a member of the order Actinomycetales and the family Mycobacteraceae (Pfyffer, 2015). A multitude of characteristics, including growth rate and pathogenicity, are used to classify organisms within the genus. It is categorized into three types based on its pathogenicity: (1) Mycobacterium tuberculosis Complex (MTBC) (Obligatory pathogens); (2) Mycobacterium other than tuberculosis (MOTTs), a large group of potentially pathogenic mycobacteria, such as the Mycobacterium avium complex, which can cause a variety of clinical diseases in both humans and animals; and (3) *Mycobacterium leprae* (saprophytic bacteria) (Stanford, 2012).

Obligatory pathogens are members of the Mycobacterium tuberculosis complex (MTBC) group, which also includes *Mycobacterium tuberculosis, Mycobacterium bovis, Mycobacterium caprae, Mycobacterium africanum, Mycobacterium canetti, Mycobacterium microtti and Mycobacterium pinnipedii* (Santos *et al*., 2020).

The potentially pathogenic mycobacteria which includes mycobacterium avium subspecies avium and mycobacterium avium subspecies Para tuberculosis, are members of the mycobacterium other than tuberculosis (MOTTs) group (Choi *et al*., 2018). A great majority of mycobacteria discovered in the nature are saprophytic like *Mycobacterium leprae, Mycobacterium gordonae, Mycomacterium fortuitum, Mycobacterium ulcerans and M. septicum* have all been linked to human and animal disorders (Ashbolt *et al.,* 2015).

Cellular morphology of mycobacteria

Mycobacteria are slender, straight or slightly curved, non-motile, non-capsulated, slow growing, obligate aerobic rods that measure 0.2-0.6 µm by 1.0-10 µm. The waxy shell (capsular structure) of mycobacteria makes it challenging for the host's defense mechanisms to get rid of them, resulting in a persistent, progressive illness, As well as act as a protective effect against antibiotic. (Batt, *et al.,* 2020).

The pathogen has several distinct properties because of its complex cell wall, including: Acid fastness due to increase amount of lipid and mycolic acid, Glycolipid complexes play an essential role in granuloma development, The lipid layer's hydrophobic characteristics make it resistant to chemical agents and difficult to stain with standard procedures (Kuria *et al*., 2019).

Susceptible Hosts and Reservoirs

Mycobacterium bovis is the main pathogen among mycobacteria, infecting many species including humans, cattle, buffalo, camel, sheep, goats, and pigs, with horses having a high natural resistance (Refaya *et al.,* 2020). *Mycobacterium bovis* has also been found in elephants, rhinoceroses, foxes, monkeys, opossums, bears, warthogs, and large cats (Alemu *et al*., 2016).

Mycobacterium bovis and *Mycobacterium caprae* are the two most frequent tuberculosis pathogens in goats and sheep with just a few cases caused by *M. tuberculosis* (Didkowska *et al*., 2021). When the granulomas develop in goats, they resemble human tuberculosis in terms of their cavernous nature and liquefactive necrosis (Guta *et al*., 2014). Although Mycobacterium tuberculosis is the most prevalent cause of tuberculosis in humans, *M. bovis* can also infect people (Zoonotic tuberculosis) (Oleo-Popelka *et al*., 2017).

Source of infection, Mode of transmission and risk factors

The environment has a large impact on mycobacterium tuberculosis complex transmission such as close contact between animals happens during the milking station, as well as in drinking and eating troughs (Ghodbane *et al*., 2014). The infected dust particles or aerosols from coughing or sneezing animals with open tuberculosis are the main sources of infection in cattle (Bhembe *et al.*, 2017).

Additionally, it's important to note that oral transmission of bovine tuberculosis to calves occurs when they nurse from sick cows that excrete mycobacterium in their milk (Serrano *et al*., 2018). Dogs and cats are more likely to become infected through oral transmission due to their habits (drinking infected milk, feeding on infected carcasses, or coming into contact with infected pus secreted through open lesions) (Fitzgerald *et al*., 2016).

Consumption of polluted feed and water is considered to be another mode of transmission and also results in non-pulmonary form of the disease (Sa'idu *et al*., 2015). Cutaneous infection, vaginal infection during coitus, placental or umbilical infection, and transmission via udder diseases are some more atypical routes of infection (Constable *et al*., 2017). As well as Mukherjee *et al*. (2018) confirmed that humans act as a source of infection for cattle when discovered identical Mycobacterium tuberculosis spoligotypes in peoples and neighboring animals.

Many risk factors were responsible for occurrence of disease in animals including cow breed and age, Furthermore, host-independent characteristics such as herd size, management practices, and cattle purchasing (Aliraqi *et al*., 2020).

Pathogenesis of TB

Inhalation of infected droplets expelled from the lungs is the main route of TB infection; however, the bacteria can also be ingested via contaminated milk, water, or food. When infection occurs via aerosol exposure, some bacilli pass beyond the upper respiratory tract and are deposited in terminal bronchioles and alveoli where they are phagocytosed by resident alveolar macrophages causing primary tuberculosis and the development of the disease's granuloma or tubercle (Yadav and Prakash, 2017).

Mycobacteria-infected macrophages can then enter the circulatory and lymphatic systems, inducing lymphocytic sensitization to mycobacterial antigens. At that point, lymphocytes and macrophages form granulomas around the mycobacteria, effectively "walling off" the mycobacteria. Mycobacteria enter a dormant state in order to survive in a variety of environments, including lung tissue, lymph nodes, bone, and other organs. The mycobacteria circulate further in animals leading to multi-organ system infections (Jemberu *et al*., 2015).

The two stages by which bovine tuberculosis spreads in the body are the primary complex and post primary dissemination. The primary complex composed of the original lesion at the site of invasion and the adjacent lymph node, Infection by the oral route can lead to a primary complex in the pharynx or mesenteric lymph nodes. Bacilli within the lesion are unable to proliferate and may lay dormant for years, resulting in a latent infection. This is referred to as the infection's non-replicative persistence phase (Domingo *et al*., 2014).

The previously pathogen is reactivated in certain animals, tissue destruction develops, and the tuberculous lesion enlarges resulting in a caseated, necrotic, mineralized, and fibrosed lesion that can be chronic or secondary in nature. When the immune response is inadequate, mycobacteria spreads through the blood or lymphatic system, resulting in the generalization form (acute miliary tuberculosis) (Constable *et al*., 2016).

Caseous necrosis results from the cellular hypersensitivity that follows, which causes cell death and tissue damage. In some cases, liquefaction and cavity development are brought on by enzymatic effect on lipids and proteins, and the bacteria grows uncontrollably within these holes. By rupturing the bronchi's cavities, bacteria disseminated as aerosols. Like acute miliary TB, bacteria-containing macrophages can migrate via systemic pathways causing lesions in a variety of tissues including the pleura, peritoneum, liver, kidney, spleen, skeleton, mammary glands, reproductive tract, and CNS (Flynn *et al*., 2011).

Diagnosis

Case history, clinical and post-mortem observations, intradermal tuberculin skin tests, meat inspection at the abattoir, and histological examination are commonly used to diagnose the disease in exposed animals, primarily cattle, buffalo, and sheep. Polymerase chain reaction, culture, and biochemical assays are used to conclusively diagnose members of the Mycobacterium genus (Oreiby et al., 2015).

Ante-Mortem diagnosis of bovine tuberculosis

A) Field test (Tuberculin skin test) (TST)

The single intradermal tuberculin skin test (SITT) comprises intradermal injection of tuberculous antigen (mainly by purified protein derivatives) and measuring skin thickness after 72 hours. A different kind of tuberculin test is the single comparative intradermal tuberculin skin test (SCITT), that increases tuberculin skin test specificity by utilizing both avian and bovine purified protein derivatives for preventing non-specific Mycobacterium avium complex reactions (Jaroso *et al*., 2010). Egypt's general veterinary organizations do not use SCITT because they receive insufficient resources. The owners of certain herds are the only ones that use it privately (Klepp *et al*., 2019).

The purified protein derivatives (PPD) are administered to the caudal fold of the tail in North America, whereas it is administrated into the mid-cervical region in many other countries, including Egypt (cervical intradermal test). The PPD injection causes localized vascular dilatation, deposition of fibrin network, and the accumulation of various inflammatory cells, which results in local skin swelling by stimulating the cell mediated immunity and sensitizing T lymphocytes (Vordermeier *et al*., 2016).

OIE (2005) established the standard tuberculin skin test methods. (1) Shaving and clipping are done at the injection site. (2) Measure a fold of skin in the area where the hair was trimmed using a calliper. (3) The dosage is given by obliquely inserting a graded tuberculin syringe with a tiny needle that has a bevel edge into the skin, the highest dose of bovine PPD that is suggested is 2000 IU. (4) Correct intradermal injection is indicated by the feeling of a pea-sized expansion at the injection site. (5) There should be a 12 to 15 cm gap between each injection. (6) The size of the skin folds is determined again after 72 hours by the same operator. When the swelling is higher than 4 mm, it is considered positive; when it is less than 3 mm, it is regarded negative, and suspected when it ranges from 3 - 4 mm (Elnaggar *et al*., 2017).

Tuberculin skin test, despite its extensive use, has substantial drawbacks: (1) Difficulty level. (2) Interpretation is subjective and depends on the operator. (3) Non-Visible Lesions (NVL) are false positive reactions that occur when animals test positive for TST but do not display PM lesions or positive culture. (4) False negative reactions especially when administered late in the course of the disease (In serious and extensive cases), Furthermore, until 3-6 weeks after infection, false negative results are given. This is referred to the pre-allergic phase (Broughan *et al*., 2016). The strength, purity, and dose of the PPD, the location of the injection, incorrect data interpretation, and the genetic make-up of the animal are all factors that influence TST accuracy (Borham *et al*., 2021).

Gamma interferon assay is an in vitro test performed on a heparinized blood sample from the tested animal. It has advantages over the single intradermal tuberculin test, being more sensitive and could be repeated frequently without the need to wait for a desensitization period. In addition, it is not used only for tuberculosis, but also for tuberculosis-like diseases such as pseudotuberculosis of sheep (Oreiby and Hegazy, 2016).

B) Clinical signs

Clinical symptoms may differ based on the location of the infection, the amount of infection, the severity of the infection, the host's immune capacity, and external stimuli (El-Sawalhy, 2012).

Many animals with tuberculosis appear clinically normal and there are few early clinical signs of the disease. Emaciation over time, a low-grade fluctuating temperature, weakness, and appetite loss are among late-stage signs. As well as the bronchial LN enlargement can cause dyspnea due to airway constriction, whereas involvement of the retropharyngeal LN resulting in eructation, dysphagia and noisy breathing. Additionally, ruminal tympani, both recurring and permanent, is frequently associated with mediastinal LN enlargement. As a result of gastrointestinal system involvement, there may be diarrhea or constipation (Belinda and Erin, 2018).

Mammary tuberculosis has been seen in a variety of animals and accompanied by severe induration and chronic mastitis, which often starts in the upper udder and progresses to the supra mammary

LN. Infertility can be caused by tuberculous metritis, which is characterized by suppurative vaginal discharge, vaginitis, and, in rare cases, tuberculous orchitis. Additionally, the military tuberculosis can cause abrupt or subacute death (Thoen *et al.,* 2016).

Postmortem diagnosis of bovine tuberculosis

In endemic locations, postmortem inspection is an important component of BTB management efforts, allowing infection to be found in either routinely slaughtered animals or tuberculin test reactors (Pascual-Linaza *et al*., 2017). Aboukhassib *et al*. (2016) reported that only about 47% of presumptive lesions were discovered during standard postmortem meat inspection. The lymph nodes, organs, and the seven lobes of the two lungs were all thoroughly checked. Under bright light, the sliced surfaces were evaluated for evidence of a caseated material, pus and nodules. If any of the organs had obvious BTB lesions, the entire carcass was labeled and rejected.

The diameter of cattle lesions can range from 1 mm to more than 10 cm. It is possible to have a single lymph node lesion or a main complex, which involves lesions in both the organ and its associated a lymph nodes. The majority of lesions develop as dry and firm yellowish caseous nodules (Parkale and Kulkarni, 2011). Calcification is common, especially in lymph nodes, and a gritty sensation and grating sound suggest its presence when the lesion is sectioned. Many tubercles can form on the pleural or peritoneal surfaces as a result of one or more neighboring tubercles developing and merging. Metastases produce a huge number of tubercles that are all of the similar size (Paylor, 2014).

Tubercles are found in lymph nodes, notably those in the bronchial, retropharyngeal, and mediastinal regions. They are also common on the surfaces of the lung, spleen, liver, heart, kidney, and body cavities with some exhibiting cheesy material when cut. Certain lesions emerge as abscesses with yellowish pus in general. The pus is creamy to orange in color and fluctuates in viscosity from thick cream to crumbled cheese (Ahmad *et al.,* 2017).

If miliary tuberculosis was found in several lymph nodes, the entire carcass was condemned, Acute miliary tuberculosis is distinguished by the presence of numerous tiny nodules of the same age and size that resemble millet seeds and observed in various organs in disseminated cases. Also, dissemination to the serous membranes with many microscopic tubercles that resemble pearls, may develop (Genzebu *et al*., 2018).

Histopathological examination of bovine tuberculosis

Canal et al. (2017) identified the distinctive histological appearance of granulomatous lesions, that can microscopically divided into four stages (stages I–IV). Lesions of stage I of TB granuloma (non-necrotizing granuloma) revealed focal proliferation of epithelioid macrophages and dispersed lymphocytes with or without langhans cells as well as the absence of necrosis. Stage II of TB granuloma has nodular to irregular accumulations of lymphocytes, plasma cells, macrophages, and giant cells with mild central necrosis. Stage III of TB granuloma (Solid or Necrotic stage) were more regular in structure and appeared as encapsulated granulomas with central eosinophilic necrosis. Stage IV of TB granuloma (Necrosed and mineralized stage) (Caseo calcerous stage) involving calcification and fibrosis.

Chronic tissue damage disrupts the physiological processes of injury and repair, resulting in fibrosis. Granuloma encapsulation can help with infection control, isolating and inhibiting mycobacteria spread, and separating the lesion from healthy tissue (Warsinske *et al.,* 2017).

Dystrophic calcification is a pathogenic process associated with intra- and extracellular calcium salt formation at necrotic sites. On H&E-stained slices, the calcified lesions are dark blue to purple color (Myers *et al*., 2012).

As granulomas grow and time passes after infection, the necrotic caseum can be changed from semisolid to liquid, most likely due to the action of multiple proteases and nucleases. Necrosis can cause holes in the lung parenchyma by infecting the bronchi or bronchioles (Hunter, 2011). Michelet *et al*. (2018) described that the histopathological examination is simple, quick, and inexpensive for detecting mycobacteria, but it lacks specificity because it can also find other bacteria such as Rhodococcus equi, which can cause non-specific TB lesions and has a histological profile that is difficult to distinguish from M. bovis. As a result, Ziehl-Neelsen (ZN) staining in microscopy is used to identify acid-fast microorganisms, most notably mycobacteria, and can improve tuberculosis (TB) diagnosis accuracy.

The Zeihl-Neelsen method is often employed for staining the mycobacterium. The smears are heated in the same way that basic fuchsin exists, soaked with 20% sulphuric acid and left for 1 minute to decolorize the carbol fuchsin stain. Counter stains such as malachite green or methylene blue are regularly utilized. An ordinary light microscope is used to examine the stained slides for the presence of acid-fast bacilli, which appear as red bacillary cells 1-3 microns in length that occur individually or in clumps (Patterson and Grooms, 2000).

Molecular diagnosis for detecting mycobacterial infections

Bovine tuberculosis is frequently diagnosed using direct polymerase chain reaction which have a number of benefits, including rapid, accurate diagnosis, effective management, overcoming the lack of specificity of other conventional methods like histopathology, also showing great promise in epidemiological studies. It has allowed for diagnosis in some species in less than 48 hours, although the presence of inhibitors in tissue samples may restrict its usefulness (Algammal *et al*., 2019).

The first and most important stage in the PCR process is the extraction of DNA from mycobacterial cells, which produces sufficient pure DNA for a successful PCR test. Direct extraction of DNA is possible from growing colonies or tissue samples. Growing colonies have a larger bacterial burden than tissues, which makes DNA extraction easier, Furthermore, developing colonies are frequently less polluted than tissues (Cedeño *et al.,* 2005).

Mycobacterial DNA is extracted using a number of physical and chemical techniques. One physical method used to extract DNA from mycobacteria is boiling. Heating to 100 °C in the appropriate buffer destroys the connections between the lipids in the cell wall, allowing for effective DNA extraction. Furthermore, it is a fast, simple, and low-cost method of extracting DNA from cultures but not clinical specimens. A chemical technique, on the other hand, is phenol extraction. Because of phenol's strong proteolytic, corrosive, and caustic properties, proteins and lipids become solubilized and denaturized, damaging mycobacteria cell walls. Furthermore, because it has the same properties as phenol, the chloroform utilized in this method amplifies the phenol's impact (Ruqaya *et al*., 2014).

Mycobacteria detection in clinical specimens through DNA amplification by PCR with the target gene expressing 16S rRNA. Mycobacterial nucleic acids were amplified genetically, then screened using genus-specific probes, and lastly separated into species using highly discriminating probes or nucleic acid sequencing (Kirschner *et al.,* 1996).

Mycobacterium bovis were discovered using primers targeting at the mpb64, mpb70, hsp65, 16S rRNA, or IS6110 insertion sequences after DNA extraction. Direct identification of mycobacterium bovis in tissue specimens was made possible by employing the PCR method to amplify the essential DNA sequences, which has been shown to give rapid diagnosis of a number of diseases. Mycobacterial nucleic acids were amplified genetically, then screened using genus-specific probes, and lastly separated into species using highly discriminating probes or nucleic acid sequencing (Ramadan *et al*., 2012; Santos *et al*., 2015).

Despite their benefits, PCR approaches have a number of limitations, including the fact that they are limited to MBTC or *M. avium* complex members and are only effective for PM diagnosis (Soares Filho *et al.,* 2019). Furthermore, decreasing DNA amounts and interfering compounds in samples during DNA extraction may degrade the sensitivity of certain PCR tests (Ikuta *et al.,* 2016).

Routes of human infection and zoonotic disease manifestation

Zoonotic tuberculosis is a serious threat, According to WHO Global Tuberculosis Report 2020, 140,000 (1.4%) of the 10 million new cases of active TB are projected to be zoonotic TB in Africa and southeast Asia (WHO, 2020). Zoonotic tuberculosis remains a significant unsolved global concern, particularly with the discovery of new zoonotic mycobacterial strains including *Mycobacterium canetti, Mycobacterium caprae, Mycobacterium microti, Mycobacterium pinnipedii and mycobacterium orygis* (Kock *et al*., 2021). Zoonotic TB complicates animal treatment and recovery since *M. bovis* is naturally resistant to pyrazinamide (PZA), one of the primary medications used to treat TB (WHO, 2017).

Mycobacterium bovis can infect humans via cutaneous penetration, droplet inhalation, or oral ingestion. Fever, nocturnal sweating, and weight loss are all common indicators of sickness. Consuming contaminated products, such as unpasteurized dairy products like raw milk, meat and soft cheeses is the main route for humans to become infected with the microorganism (Deneke *et al*., 2022). Many nations forbid the consumption of contaminated carcasses, greatly reducing the possibility of infection (Oleo-Popelka *et al*., 2017).

Extrapulmonary symptoms of oral consumption sickness include constipation, diarrhea, abdominal pain, intestinal ulcers, and strictures (Gerhardy *et al.,* 2017). Lymph nodes may enlarge, and tumor-like masses can form in the abdomen as a result of the illness, similar symptoms to those of other illnesses, such Crohn's disease, may result in incorrect diagnosis and therapy (Pemartin *et al.,* 2015). People who work in cramped or poorly ventilated conditions are significantly more likely to develop pulmonary M. bovis infections (Vayr *et al*., 2018). Common symptoms include coughing, hemoptysis, caseo-necrotic tubercles in the lymph nodes surrounding the lung and the parotid lymph node. Despite the fact that "Butcher's Warts" were a name for skin illnesses among meat handlers when the prevalence of M. bovis infection in cattle was higher. Furthermore, co-infection with Mycobacterium bovis and Mycobacterium tuberculosis is probable that complicates both diagnosis and therapy (Dias *et al*., 2014).

Prevalence of bovine tuberculosis in Egypt

The main reasons of illness persistence in Africa are weaknesses in preventative or control strategies, insufficient hygiene, inadequate veterinarian and slaughterhouse facilities (Pokam *et al.,* 2019). Tuberculosis has been known in Egypt from the start of humanity more than 5000 years ago. Pott's anomalies in Egyptian mummy skeletons can also be seen in early Egyptian art. TB appears to have been particularly frequent in ancient Egypt because to the large population and dense crowding in cities (Neukamm *et al.,* 2020). Also mycobacterial DNA found in ancient human bone samples indicated that generalized TB was present in those populations, pointing to the systemic dissemination of the mycobacteria in affected persons (Lalremruata *et al.,* 2013).

Robert Koch succeeded in extracting tuberculin from tubercle bacilli in 1890. Initially, the concentrate was studied as a vaccine, but it was later discovered to have clinical advantages in detecting sick animals. After that, the animal tuberculin skin test was developed. In 1907 and 1908, Von Pirquet and Mantoux developed the human skin test (CDC, 1982). Egypt has been doing intradermal tuberculin testing since 1920, when the prevalence of bovine TB in cattle and water buffalo herds was recorded as 2% to 9%. The frequency of bovine TB in these species was 6.9% and 26.2% in the 1980s, despite the fact that the general percentage of tested cattle and water buffalo that were positive reactors in 1981 was 6.2% and 9.4%, respectively. However, once the national bovine TB controlling program was implemented in 1981, the frequency decreased to 2.6% in 1990 (Abdellrazeq *et al*., 2016).

In addition, Ramadan *et al.* (2012) confirmed that the prevalence of 0.96% (32/3347) was determined in cattle from different farms in Egypt tested by tuberculin skin test during the period from November 2010 until December 2011. While abattoir surveillance showed a prevalence percentage of 0.21% (15/7235) for suspected tuberculous carcasses (non-tuberculin tested) slaughtered in El-Basateen abattoir, Cairo, Egypt in the period between December 2010 till March 2011.

Hamed *et al.* (2021) determine the prevalence and identify the risk factors of BTB using the comparative intradermal tuberculin test on 5327 dairy cattle in 16 dairy herds in mid-delta, Alexandria Road and Upper Egypt districts from November 2015 to April 2018. The results of indicated that out of 5327 dairy cattle, 89 (1.67 %) give positive reaction. The highest result for tuberculin skin test reactors was 22 out of 512 tested cattle (4.30%) in mid-delta, while the lowest result was 51out of 4170 tested cattle (1.22%) in Alexandria Road district. Out of 16 tested herd 11 (68.75%) were having at least one positive tuberculin reactor. The highest result was 3 out 4 tested herds (75%) in mid-delta district.

According to Elsayed and Amer (2019), cattle and Egyptian water buffaloes had a 3.5% tuberculin-positive case prevalence in Menoufia, Gharbia, Dakahlia, Beheira, and Cairo as of April 2019.

In August 2019, A total of 7,064 animals belonging to 27 herds from 7 governorates located in the Nile Delta were tested, and only 242 (3.43%) were considered positive TB by the SCITT. However, significant variations in disease prevalence were present within 7 governorates in this study. For example, the prevalence of bTB was 41% at Sharqia but only 0.8% at Kafr EL Sheikh, at the animal-level. At the herd-level (presence of at least one positive reactor in the herd), Gharbia had the highest number of positive herds (4 out of 7 herds). Only 2 herds were examined from Alexandria and only one herd was positive (Abdelaal *et al.,* 2019).

According to new data, 16.4% of dairy cows in Dakahlia, Gharbia, Menoufia, Beheira, and Alexandria tested positive for tuberculin in 2020 (Elsohaby *et al*., 2020).

Table 1. Prevalence of bovine tuberculosis in Egyptian slaughtered cattle and buffalo with regard to age and sex.

Factors	Categories	Total Examined animals	Positive Suspected tuberculous Cases	References
Age: total tested animals (1850) in different dairy farms in Egypt	3-5 years	1200	22 (1.83%)	**Mosaad, *et al.*, 2012**
	>5 years	650	14 (2.15%)	
Age : 2935 cattle, Egypt	<3years	1250	22 (1.76%)	**Shereen, *et al.*, 2015**
	>3 years	1685	41 (2.43%)	
Age: Cattle Alexandria, Mid delta, Upper Egypt.	<3years	2038	8 (0.39%)	**Hamed *et al.*, 2021**
	3-6 years	2854	72 (2.52%)	
	>6years	435	9 (2.07%)	
Sex: Cattle and buffalo, Menoufia governorate, Egypt.	Male cattle	61	17 (28%)	**Hasanen, *et al.*, 2017**
	Female, cattle		44 (72%)	
	Male, buffalo	14	10 (71%)	
	Female, buffalo		4 (29%)	
Sex Cattle and buffalo, Qena City, Egypt.	Male cattle	5787	11 (.19%)	**Mahmoud, *et al.*, 2015**
	Female, cattle	123	11 (8.94%)	
	Male, buffalo	306	1 (.33%)	
	Female, buffalo	40	0 (0%)	
Sex Cattle and buffaloes Dakahlia governorate, Egypt	Male cattle	40	16 (40%)	**Amin, *et al.*, 2015**
	Female cattle		24 (60)	
	Male buffalo	12	3 (25%)	
	Female buffalo		9 (75%)	

Table 2. Incidence of bovine tuberculosis in Egyptian slaughtered tuberculin positive cattle based on abattoir inspection and PM findings.

Site of tuberculous lesions	**NO. of Positive PM lesions and percentage**				
References	**Hamdy Hassan, *et al.*, 2016**	**Nasr, *et al.*, 2019**	**Hamed *et al.*, 2021**	**Elagdar, *et al.*, 2022**	**Soliman, *et al.*, 2023**
Generalized	Localized 40 (83.3%) generalized 8 (16.75%)	15 (6.6%)	8 (12.12%)	2 (6.7%)	8 (11.6%)
Respiratory		56 (24.6%)	26 (39.4%)	19 (3.3%)	22 (31.9%)
Digestive		14 (6.1%)	3 (4.54%)	5 (16.7)	6 (8.7%)
Mixed		50 (21.9%)	11 (16.67%)	-	10 (14.5%)
Head		20 (8.8%)	18 (27.27%)	1 (3.3%)	5 (7.2%)
Kidney	-	-	-	1 (3.3%)	-
Udder	-	-	-	2 (6.7%)	-
Total visible lesions	48 (76.2%) out of 63 cattle	155 (67.98%) out of 228 cattle	66 (85.71%) out of 77 cattle	30 positive cattle	51 (%) out of 69 cattle

Table 3: Results of postmortem findings and histopathological examination of BTB in Egyptian slaughtered cattle and buffalo.

Animals and abattoir	**Age**	**Sampling**	**Pm findings**	**Histological examination**	**References**
2150 male beef cattle (Zebu) from Middle East and Wadyna abattoirs of Aswan Governorate	2 - 3 years	tracheobronchial and mediastinal lymph node	60 out of 2150 animals showed variably sized multi nodular TB lesions with cheesy necrotic material or creamy pus when sectioned	Granuloma formations were classified into 4 stages (I, II, III, and IV)	**Hamed, *et al.*, 2023**

750 animals, 569 cows and 181buffalo from Tanta abattoir slaughterhouse , Egypt.	The females were57 years old, while the males were 2–4 years old, in average.	retropharyngeal, bronchial, mediastinal, mesenteric LNs, lungs, liver, spleen and serous membranes	30 animals had tuberculous lesions, including milliary TB in a lung, discrete shot-like tubercles on the pleura, enlarged mediastinum Ln containing thick creamy pus, retropharyngeal Ln containing thick cheesy pus.	Different stages of granulomas were identified in the sampled lesions. in addition to proliferated hyperplastic and metaplastic epithelial lining the bronchioles and thrombus in the blood vessels of a lung sample. the spleen sample showed hyperchromatic pleomorphic undifferentiated cells.	**Borham, *et al.*, 2022**
A case report of one and half year-old Holstein bull from Mansoura abattoir in Dakahlia province, Egypt.	1.5 years old	kidney	Grossly, multiple firm yellowish caseated nodules (0.5 – 3.5 cm in diameter) were Revealed with variable areas of calcification which was detectable by palpation and grittiness. Such nodules were mostly seen throughout the renal medulla of the left kidney	Microscopically, these nodules were focally replaced the medulla of the left kidney and represented by central caseation necrosis and dystrophic calcification which collapsed the adjacent renal tissues The latter were surrounded with neutrophils, epithelioid macrophages, lymphocytes, plasma cells, Langhans giant cells and fibroblasts.	**Awadin, 2012**

Treatment, prevention, and control of bovine TB

Bovine TB is rarely treated in domestic livestock except in rare cases of valuable animals as zoological exhibits. The most effective first-line antituberculous chemotherapy regimens consist of rifampicin, ethambutol (EMB), and streptomycin (SM), and thioacetazone are medications used as a second line of treatment (Waters, 2015).

Control measures have reduced the number of infections. Solutions of phenol, iodine, glutaraldehyde, and formaldehyde have all been found to be effective antibacterial disinfectants, as has exposure to heat of 250°F. The primary BTB control strategies are TST testing of infected animals, isolation of sick animals, regular slaughter monitoring and restricting movement of affected herds (McKinley et al., 2018).

Vaccination against BTB is rarely utilized for disease management because BCG can stimulate an immune reaction in cells that interferes with testing procedures and employ PPD as an antigen (TST). The BCG vaccine proved efficient once provided to newborns, according to recent experimental investigations in cattle (Liao *et al*., 2022).

CONCLUSION

Bovine tuberculosis is an infectious bacterial zoonotic disease transmitted from animals to humans. Besides its health hazard, TB has a large economic impact due to the high cost of eradication programs and a serious consequence for animal and animal- products movements. TB continues to infect animals in Egypt because the traditional and modern diagnostic techniques, as well as prevention, control, and treatment measures, are not enough to eradicate the disease. We urgently require strong strategies to limit the prevalence of bovine tuberculosis and prevent its transmission to the human population in Egypt.

REFERENCES

Abdelaal, H.F., Spalink, D., Amer, A., Steinberg, H., Hashish, E. A., Nasr, E. A., & Talaat, A. M. (2019). Genomic polymorphism associated with the emergence of virulent isolates of Mycobacterium bovis in the Nile Delta. Scientific reports, 9 (1), 11657.

Abdellrazeq, G.S., Elnaggar, M.M., Osman, H.S., Davis, W.C.and Singh, M. (2016): Prevalence of bovine tuberculosis in Egyptian cattle and the standardization of the interferon-gamma assay as an ancillary test.Transbound Emerg Dis 63(5), 497–507. doi: 10.1111/tbed.12291.

Aboukhassib, H., Haraji, M., Bouslikhane, M., Bitar, A. (2016): Bovine tuberculosis: Clinical presentation and diagnosis. J. Bacteriol. Mycol. Open Access, 3(2), 214–217. [Google Scholar]

Ahmad, I., Kudi, C.A., Abdulkadir, A.I., Saidu, S.N.A. (2017): Occurrence and distribution of bovine TB pathology by age, sex, and breed of cattle slaughtered in Gusau Abattoir, Zamfara State Nigeria. Trop. Anim. Health Prod., 49, 583–589.

Alemu, J., Mamo, G., Ameni, G. and Pal, M. (2016): Molecular epidemiology of bovine tuberculosis in cattle and its public health implications in Gambella region, Ethiopia. Molecular Microbiology Res. 6(1): 1-15. doi: 10.5376/mmr.2016.06.0001.

Algammal, A. M., Wahdan, A., & Elhaig, M. M. (2019): Potential efficiency of conventional and advanced approaches used to detect Mycobacterium bovis in cattle. Microbial Pathogenesis, 134, 103574. https://doi.org/10.1016/j.micpath.2019.103574.

Aliraqi, O.M., Al-Jammaly, M., AL-Hankawi, O., Al-Farwachi, M.I., Dahl, M.O. (2020): Preliminary Prevalence and Risk Factors of Mycobacterium bovis in Local and Imported Breeds of Cattle and Buffaloes in Mosul city, Iraq. Egyptian J Vet Med.; 51(1): 83-88. doi: 10.21608/EJVS.2019.17753.1102.

Amin, R. A., Nasr, E. A., El-Gaml, A. M., & Saafan, E. S. M. (2015): Detection of Tuberculosis in slaughtered food animals by using recent technique. *Benha Veterinary Medical Journal*, *28*(2), 129.431

Ashbolt, N.J. (2015): Environmental (saprozoic) pathogens of engineered water systems: Understanding their ecology for risk assessment and management. *Pathogens*, *4(2)*, 390–405. https://doi.org/10.3390/pathogens4020390.

Awadin, W. (2012): Unilateral tuberculous nephritis in a bovine bull in Egypt (case report), pp 39-47.

Batt, S.M., Minnikin, D.E., Besra, G.S. (2020): "The thick waxy coat of Mycobacterium, a protective layer against antibiotic and the host immune system". The Biochemical Journal. 477(10): 1983-2006. Doi:10. 1042/BCJ20200194. PMC 7261415. PMID 32470138.

Belinda, S.T. and Erin, L.G. (2018): Miscellaneous Infectious Diseases. In Rebhun's Diseases of Dairy Cattle, 3rd ed.; Elsevier: Amsterdam, The Netherlands; pp. 745-746. [Google Scholar].

Bhembe, N. L., Jaja, I. F., Nwodo, U. U., Okoh, A. I., & Green, E. (2017): Prevalence of tuberculous lymphadenitis in slaughtered cattle in Eastern Cape, South Africa. International Journal of Infectious Diseases, 61, 27–37. https://doi.org/10.1016/j.ijid.2017.05.005.

Borham, M., Oreiby, A., El-Gedawy, A., Hegazy, Y., Al-Gaabary, M. (2021): Tuberculin test errors and its effect on detection of bovine tuberculosis. *J. Hell. Vet. Med*, *72(4)*, 3263– 3270. https://doi.org/10.12681/jhvms.29357.

Borham, M., Oreiby, A., El-Gedawy, A., Hegazy, Y., Hemedan, A., & Al-Gaabary, M. (2022): Abattoir survey of bovine tuberculosis in tanta, centre of the Nile delta, with in silico analysis of gene mutations and protein–protein interactions of the involved mycobacteria. Transboundary and Emerging Diseases, 69(2), 434-450.

Borham, M., Oreiby, A., El-Gedawy, A., Hegazy, Y., Khalifa, H.O., Al-Gaabary, M., Matsumoto, T. (2022): Review on Bovine Tuberculosis: An Emerging Disease Associated with Multidrug-Resistant Mycobacterium Species. Pathogens, 11(7), 715. https://doi.org/10.3390/pathogens11070715.

Broughan, J.M., Judge, J., Ely, E., Delahay, R.J., Wilson, G., Clifton-Hadley, R.S., Goodchild, A.V., Bishop, H., Parry, J.E., Downs, S.H. (2016): A review of risk factors for bovine tuberculosis infection in cattle in the UK and Ireland. *Epidemiol. Infect*, *144(14)*, 2899–2926. https://doi.org/10.1017/S095026881600131X.

Canal, A.M., Pezzone, N., Cataldi, A., Zumarraga, M., Larzabal, M., Garbaccio, S., Fernandez, A., Dominguez, L., Aranaz, A., Rodriguez-Bertos, A. (2017): Immunohistochemical detection of pro-inflammatory and anti-inflammatory cytokines in granulomas in cattle with natural *Mycobacterium bovis* infection. *Res. Vet. Sci. 110*, 34–39. https://doi.org/10.1016/j.rvsc.2016.10.006.

Cedeño, I., de Obaldía, R., Sanjur, O., Bayard, V., Ortega-Barría, E., Escobar, C. (2005): Use of the polymerase chain reaction for diagnosing bovine tuberculosis in Panama. Rev Sci Technol 24(3):1067-1075.

Centre for Disease Control (CDC). (1982): Historical Perspectives Centennial: Koch's Discovery of the Tubercle Bacillus. https://www.cdc.gov/mmwr/preview/mmwrhtml/00000222.htm.

Choi, S.R., Britigan, B.E., Switzer, B., et al. (2018): In Vitro Efficacy of Free and Nanoparticle Formulations of Gallium (III) meso-Tetraphenylporphyrine against Mycobacterium avium and Mycobacterium abscessus and Gallium Biodistribution in Mice. Mol Pharm; 15(3): 1215–1225. https://doi.org/10.1021/acs.molpharmaceut.7b01036.

Constable, P.D., Hinchliff, K.W., Done, S., Gruenberg, W. (2016): Veterinary medicine: a textbook of the diseases of cattle, horses, sheep, pigs, and goats. 11th Edition, (Saunders, U.S.A) BrazJ Microbiol (2021) 52:431–438.

Constable, P. D., Hinchcliff, K. W., Done, S. H., Grunberge, W. (2017): Systemic and Multi-organ diseases. In Veterinary Medicine (PP. 2002- 2214), https:/doi.org/10-1016/B978-0-7020-52470,00021-8.

Deneke, T., Bekele, A., Moore, H. L., Mamo, T., Almaw, G., Mekonnen, G. A., Mihret, A., Tschopp, R., Yeheyis, L., Hodge, C., Wood, J. L. N., & Berg, S. (2022): Milk and meat consumption patterns and the potential risk of zoonotic disease transmission among urban and peri-urban dairy farmers in Ethiopia. *BMC Public Health*, 22(1), 222.

Dias, M.F., Bernardes Filho, F., Quaresma, M.V., Nascimento, L.V., Nery, J.A., Azulay, D.R. (2014): Update on cutaneous tuberculosis. A Bras Dermatol;89(6):925–938. https://doi.org/10.1590/abd1806-4841.20142998.

Didkowska, A., Orłowska, B., Krajewska-Wędzina, M., Augustynowicz-Kopeć, E., Brzezińska, S.,

Zygowska, M., Wiśniewski, J., Kaczor, S., Welz, M., Olech, W., et al. (2021): Microbiological and molecular monitoring for bovine tuberculosis in the Polish population of European bison (Bison bonasus). *Ann. Agric. Environ. Med.*, *28*, 575–578.

Domingo, M., Vidal, E. N. R. I. C., & Marco, A. (2014): Pathology of bovine tuberculosis. Research in veterinary science, 97, S20-S29. https://doi.org/10.1016/j.rvsc.2014.03.017.

Donoghue, H.D., Taylor, G.M., Stewart, G.R., Lee, O.Y.C., Wu, H.H.T., Besra, G.S., Minnikin, D.E. (2017): Positive diagnosis of ancient leprosy and tuberculosis using ancient DNA and lipid biomarkers. Diversity 9:1–30. https://doi.org/10.3390/d9040046.

Elagdar, A., Edris, A., Moustafa, S. M., Heikal, G. I. (2022): Prevalence of tuberculosis in bovine slaughtered animals and suspected patients in Gharbia governorate. Benha Veterinary Medical Journal, 43(1), 95-99.

Elnaggar, M.M., Abdellrazeq, G.S., Elsisy, A., Mahmoud, A.H., Shyboub, A., Sester, M., Davis, W.C. (2017): Evaluation of antigen specific interleukin-1β as a biomarker to detect cattle infected with Mycobacterium bovis. Tuberculosis, 105, pp 53–59. https://doi.org/10.1016/j.tube.2017.04.009.

El-Sawalhy, A. (2012): Veterinary Infectious Diseases in Domestic Animals, 3rd ed.; Vetbook: Cairo, Egypt; pp. 305–308.

Elsayed, M.S.A.E., Amer, A. (2019): The rapid detection and differentiation of Mycobacterium tuberculosis complex members from cattle and water buffaloes in the Delta area of Egypt, using a combination of real-time and conventional PCR. Mol Biol Rep;46(4):3909–3919. https://doi.org/10.1007/s11033-019-04834-3.

Elsohaby, I., Ahmed, H.A., El-Diasty, M.M., Elgedawy, A.A., Mahrous, E., El Hofy, F.I. (2020): Serological and molecular evidence of Mycobacterium bovis in dairy cattle and dairy farm workers under the intensive dairy production system in Egypt. J. Appl. Microbiol.;129(5):1207– 1219. https://doi.org/10.1111/jam.14734.

Fitzgerald, ScottD., Hollinger, C., Mullaney, T. P., Bruning-Fann, C. S., Tilden, J., Smith, R., Kaneene, J. B. (2016): Herd outbreak of bovine tuberculosis illustrates that route of infection correlates with anatomic distribution of lesions of cattle and cats. Journal of Veterinary Diagnostic Investigation. Official Publication of the American Association of Veterinary Laboratory Diagnosticians, Inc, 28(2), 129- 132. https:/doi.org/10.1177/1040638715626484.

Flynn, J.L., Chan, J., Lin, P.L. (2011): Macrophages and control of granulomatous inflammation in tuberculosis. Mucosal Immunology;4(3), pp 271-278. DOI: 10.1038/mi.2011.14.

Genzebu, A.T., Menghistu, H.T., Weldearegay, Y.H. (2018): A case of miliary tuberculosis in a Holestein Fresian dairy cow, Mekelle, Tigray, Ethiopia. Ethiop. Vet. J., 22(1), 121–129.

Gerhardy, B., Nagel, R.A., Sellars, R., Andreou, A. (2017): An unusual pain in the gut. Med. J. Aust;207(4):145–146. https://doi.org/10.5694/mja16.01196.

Ghodbane, R., Medie, F.M., Lepidi, H., Nappez, C., Drancourt, M. (2014): Long-term survival of tuberculosis complex mycobacteria in soil. *Microbiol.*;160(3):496–501. https://doi.org/10.1099/mic.0.073379-0.

Guta, S., Casal, J., Napp, S., Saez, J.L., Garcia-Saenz, A., Perez de Val, B., Romero, B., Alvarez, J., Allepuz, A. (2014): Epidemiological investigation of bovine tuberculosis herd breakdowns in Spain 2009/2011. PLoS One 9 (8), e104383. https://doi.org/10.1371/journal.pone.0104383.

Hamdy Hassan, W., Amin Nasr, E., & Mohamed Moussa, H. (2016): Direct detection and identification of mycobacteria in bovine samples by PCR assay. *Journal of Veterinary Medical Research*, *23*(2), 184-190.

Hamed, Y.K., Nasr, E.A., Azooz, M.F., Youssef, H.M. (2021): Prevalence and risk factors of bovine tuberculosis in dairy cattle farms in Egypt. Iraqi J. Vet. Sci;35(2):351–359. https://doi.org/10.33899/ijvs.2020.126850.1399.

Hamed, M. G., Gómez-Laguna, J., Larenas-Muñoz, F., Mahmoud, A. Z., Ali, F. A. Z., & AbdElghaffar, S. K. (2023): Monitoring the immune response of macrophages in tuberculous granuloma through the expression of CD68, iNOS and HLA-DR in naturally infected beef cattle. BMC Veterinary Research, 19(1), 220.

Hasanen, F. S., Hassan, M. A., & Moawad, E. (2017): Studies on Tuberculosis in Slaughtered Animals at Menufia Governorate. Benha Journal of Applied Sciences, 2(2), 99-102.

Hunter, R.L. (2011): Pathology of post primary tuberculosis of the lung: An illustrated critical review. *Tuberculosis*, *91(6)*, 497–509. https://doi.org/10.1016/j.tube.2011.03.007.

Ikuta, C.Y., Oliveira, D.C.R., de Souza, G.O., de Souza Filho, A.F., Grisi-Filho, J.H.H., Heinemann, M.B., Neto, J.S.F. (2016): Comparison of DNA extraction protocols to detect Mycobacterium bovis in bovine tissue by PCR. Semin. Cienc. Agrar., 37(5), 3709–3718. http://www.redalyc.org/articulo.oa?id=445748364028.

Jaroso, R., Vicente, J., Fernandez-de-Mera, I.G., Aranaz, A., Gortázar, C. (2010): Eurasian wild boar response to skin-testing with mycobacterial and non-mycobacterial antigens. Prev Vet Med 96(3-4):211–217. https://doi.org/10.1016/j.prevetmed.2010.06.011.

Jemberu, A., G. M., Ameni, G., and Pal, M. (2015): Molecular epidemiology of bovine tuberculosis in cattle and its public health implications in Gambella town and its surroundings, Gambella Regional State, Ethiopia. Global Journal of Medical Research, 15, 2249-4618.

Jolma, E.R., R.J. Delahay, F. Smith, and J.A. Drewe. (2021): Serologic responses correlate with current but not future bacterial shedding in badgers naturally infected with *Mycobacterium bovis .Transbound. Emerg. Dis.* n/a, 69(4), 1922-1932. DOI: https://doi.org/10.1111/tbed.14181.

Kirschner, p., Rosenau, J., Pringer, B., Teschner, K., Feldmann, K, and Bottger, E.C. (1996): Diagnosis of Mycobacterial Infections by Nucleic Acid Amplification: 18-Month Prospective Study. Jclin. Microbiol., 34(2): 304-312. https://doi.org/10.1128/jcm.34.2.304-312.1996.

Klepp, L.I., Eirin, M.E., Garbaccio, S., Soria, M., Bigi, F., Blanco, F.C. (2019): Identification of bovine tuberculosis biomarkers to detect tuberculin skin test and IFNγ release assay false negative cattle. *Res. Vet. Sci*, *122*, 7–14. https://doi.org/10.1016/j.rvsc.2018.10.016.

Koch, R. (1882): Die aetiologie der tuberkulose. Berl. Klin. Wochenschr., 19, 221–230.

Kock, R., Michel, A.L., Yeboah-Manu, D., Azhar, E.I., Torrelles, J.B., Cadmus, S.I., Zumla, A. (2021): Zoonotic tuberculosis–

the changing landscape. Int. J. Infect. Dis., 113(1), S68–S72. https://doi.org/10.1016/j.ijid.2021.02.091.

Kuria, J.K. (2019): Diseases Caused by Bacteria in Cattle: Tuberculosis. In *Bacterial Cattle Diseases*; IntechOpen: London, UK. DOI: 10.5772/intechopen.82051.

Lalremruata, A., Ball, M., Bianucci, R., Welte, B., Nerlich, A.G., Kun, J.F. and Pusch, C.M. (2013): Molecular identification of falciparum malaria and human tuberculosis co-infections in mummies from the Fayum depression (lower Egypt). PLoS One, 8(4), e60307. https://doi.org/10.1371/journal.pone.0060307.

Liao, Q., Zheng, Y., Wang, Y., Ye, L., Liu, X., Jiao, W., Wan, C. (2022): Effectiveness of Bacillus Calmette–Guérin vaccination against severe childhood tuberculosis in China: A case-based, multicenter retrospective study. Int. J. Infect Dis., 121, 113–119. https://doi.org/10.1016/j.ijid.2022.04.023.

Mahmoud, Y.A.H., Ali, A.O., Abdel Alim, A.A. (2015): Prevalence of tuberculosis in cattle and buffalo in Qena, Egypt. World Vet. J. 5(3):42–45.

McKinley, T. J., Lipschutz-Powell, D., Mitchell, A.P., Wood, J.L., Conlan, A.J. (2018): Risk factors and variations in detection of new bovine tuberculosis breakdowns via slaughterhouse surveillance in Great Britain. PLoS ONE, 13, e0198760. [Google Scholar] [CrossRef]

Michelet, L., de Cruz, K., Karoui, C., Tambosco, J., Moyen, J. L., Hénault, S., & Boschiroli, M. L. (2018): Second line molecular diagnosis for bovine tuberculosis to improve diagnostic schemes. PLoS One, 13(11), e0207614. https://doi.org/10.1371/journal.pone.0207614.

Mosaad, A. A., Abdel-Hamed, A. S., Fathalla, S. I., Ghazy, A. A., Elballal, S., Elbagory, A., ... & Abdelrahman, H. A. (2012): Sensitive and specific diagnostic assay for detection of tuberculosis in cattle. Glob Vet, 8 (6), 555-564.

Mukherjee, F., Kanani, A. N., Sharma, G. K., Srinivasan, V. A., Bahekar, V. S., Pasha, S. Y., Kannan, P., Prasad, A., Rana, S. K., & Premalatha, D. (2018): Isolation and analysis of the molecular epidemiology and zoonotic significance of Mycobacterium tuberculosis in domestic and wildlife ruminants from three states in India. Revue Scientifique et Technique (International Office of Epizootics), 37(3), 999–1012. https:// doi.org/10.20506/rst.37.3.2902.

Myers, R.K., McGavin, M.D., Zachary, J.F. (2012): Cellular adaptations, injury, and death: Morphologic, biochemical, and genetic bases. In Pathologic Basis of Veterinary Disease, 5th ed.; Zachary, J.F., McGavin, M.D., Eds.; Elsevier Mosby: St. Louis, MO, USA; pp. 122–124.

Nasr, Essam A., Saleeb, O.R., Shereen, A.M. (2019): "Prevalence Of Bovine Tuberculosis In Different Cattle Breeds In Dairy Farm In Egypt." Journal of Applied Veterinary Sciences 4(2), 45-51.

Neukamm, J., Pfrengle, S., Molak, M., Seitz, A., Francken, M., Eppenberger, P., Avanzi, C., Reiter, E., Urban, C., Welte, B. et al. (2020): 2000-year-old pathogen genomes reconstructed from metagenomic analysis of Egyptian mummified individuals. BMC Biol., 18 (1), pp 1-18.

OIE Stear, M. (2005): Manual of Diagnostic Tests and Vaccines for Terrestrial Animals (Mammals, Birds and Bees) 5th Edn. Volumes 1 and 2. World Organization for Animal Health 2004. ISBN 92 9044 622 6. € 140. *Parasitology*, *130* (6), pp, 727. DOI: https://doi.org/10.1017/S0031182005007699.

Oleo-Popelka, F., Muwong, A., Perera, A., Dean, A.S., Mumford, E., Erlacher-Vindel, E., Fujiwara, P.I. (2017): Zoonotic tuberculosis in humans begins caused by mycobacterium bovis- a call for action. The lancet. Infectious diseases, 17(1) e21-e25.https://doi.org/10.1016/S14733099(16)30139-6.

Oreiby, A.F., Hegazy, Y.M., Al-Gaabary, M.H., Osman, S.A., Marzok, M.A., Abushhiwaa, M. (2015): Studies on clinical identification, elisa, bacteriological isolation, PCR and x-ray radiography for diagnosis of ovine caseous lymphadenitis. J. Anim. Vet. Adv., 14(9), 250–253.

Oreiby, A. F., & Hegazy, Y. M. (2016): Diagnosis of ovine caseous lymphadenitis by blood and milk gamma interferon assays. Small Ruminant Research, 144, 109-112.

Parkale, D.D., and Kulkarni, A. (2011): Bovine Tuberculosis and Its Control. Livestock Development Officer WRDDL, DIS, PUNE, 7: 1-9.

Pascual-Linaza, A.V., Gordon, A.W., Stringer, L.A., Menzies, F.D. (2017): Efficiency of slaughterhouse surveillance for the detection of bovine tuberculosis in cattle in Northern Ireland. Epidemiol. Infect, 145(5), 995–1005. DOI: https://doi.org/10.1017/S0950268816003095.

Patterson, J. and D. Grooms, (2000): Diagnosis of bovine tuberculosis: Gross necropsy, histopathology and acid fast staining. Michigan State University Extension, 35: 1-2.

Paylor, R. (2014): Bovine tuberculosis: Michigan State University Veterinary Student, Newsletter page 1-3- edited by Dr. Pam Mouser, Graduate Student.

Pemartin, B., Portoles Morales, M., Elena Carazo, M., Marco Macian, A., Isabel Piqueras, A. (2015): Mycobacterium bovis abdominal tuberculosis in a young child. Pediatr Infect. Dis. J.;34(10):1133–1135. https://doi.org/10.1097/INF.0000000000000827.

Pfyffer, G. E. (2015): Mycobacterium: general characteristics, laboratory detection, and staining procedures. Manual of clinical microbiology, 536-569.

Pokam, B.D.T., Guemdjom, P.W., Yeboah-Manu, D., Weledji, E.P., Enoh, J.E., Tebid, P.G., Asuquo, A.E. (2019): Challenges of bovine tuberculosis control and genetic distribution in Africa. Biomed. Biotechnol. Res. J. 3(4), 217-227. DOI: 10.4103/bbrj.bbrj_110_19.

Ramadan, H.H., El-Gohary, A.H., Mohamed, A.A., Nasr, E.A. (2012): Detection of Mycobacterium bovis and Mycobacterium tuberculosis from clinical samples by conventional and molecular techniques in Egypt Global Veterinarian. 9(6): pp 648-654. DOI: 10.5829/idosi.gv.2012.9.6.66176.

Refaya, A. K., Bhargavi, G., Mathew, N. C., Rajendran, A., Krishnamoorthy, R., Swaminathan, S., & Palaniyandi, K. (2020): A review on bovine tuberculosis in India. *Tuberculosis (Edinb)*, 122, 101923. https://doi.org/10.1016/j.tube.2020.101923.

Ruqaya, M.A., Amina, N.A., Fahim, H.D. (2014): Comparison between different methods for DNA Extraction for detection of Mycobacterium tuberculosis from sputum using Polymerase chain reaction. Dyaliya journal for pure science;10(1): ISSN: 2222-8373.

Sa'idu, A., Okolocha, E., Dzikwi, A., Gamawa, A., Ibrahim, S., Kwaga, J., Usman, A., Maigari, S. (2015): Public Health Implications and Risk Factors Assessment of Mycobacterium bovis Infections among Abattoir Personnel in Bauchi State, Nigeria. J. Vet. Med. 2015, 1-5. http://dx.doi.org/10.1155/2015/718193.

Santos, N., Santos, C., Valente, T., Gortazar, C., Almeida, V. and Correia-Neves, M. (2015): Widespread environmental contamination with *Mycobacterium tuberculosis* Complex revealed by a molecular detection protocol. *PLoS One* 10 (11), e0142079. https://doi.org/10.1371/journal.pone.0142079.

Santos, N., Richomme, C., Nunes, T., Vicente, J., Alves, P.C., de la Fuente, J., Gortázar, C. (2020): Quantification of the Animal Tuberculosis Multi-Host Community Offers Insights for Control. *Pathogens*, *9(6)*, 421–431. https://doi.org/10.3390/pathogens9060421.

Serrano, M., Sevilla, I. A., Fuertes, M., Geijo, M., Risalde, M. A., Ruiz-Fons, J.F., Garido, J.M. (2018): Different lesions distribution in calves orally or intratracheally challenged with mycobacterium bovis: implications for diagnosis. Veterinary Research, 49(1), 1-10. https:/doi. org/10.1186/s13567-018-0566-2.

Shereen, A. M., Sobhy, G., & El-Maghraby, A. S. (2015): Assessment of routine and detailed inspection of tuberculous lesions in tuberculin reactor cattle. *Inter J Microbiol Res*, *6*(1), 155-159. Pathogens.;8(3):101. https://doi.org/10.3390/pathogens8030101.

Smith, T. (1898): A comparative study of bovine tubercle bacilli and of human bacilli from sputum. J. Exp. Med., 3(4-5), 451–511. doi: 10.1084/jem.3.4-5.451.

Soares Filho, P.M., Ramalho, A.K., de Moura Silva, A., Hodon, M.A., de Azevedo Issa, M., Júnior, A.A.F., Leite, R.C. (2019): Evaluation of post-mortem diagnostic tests' sensitivity and specificity for bovine tuberculosis using Bayesian latent class analysis. Res. Vet. Sci., 125, 14–23.

https://doi.org/10.1016/j.rvsc.2019.04.014.

Soliman, R., Waheeb, N., Nasr, E., El-Hariri, M., Abo-Elyazeed, H., & Aboul-Ella, H. (2023): Critical field appraisal for the use of bovine tuberculosis' antibody detecting-serodiagnostics. *Adv. Anim. Vet. Sci*, *11*(2), 278-287.

Stanford, J., Stanford, C. (2012): Mycobacteria and their world. International Journal of Mycobacteriology;1(1), 3-12. DOI: 10.1016/j.ijmyco.2012.01.001.

Thoen, C.O., Kaplan, B., Thoen, T.C., Gilsdorf, M.J., Shere, J.A. (2016): Zoonotic tuberculosis: A comprehensive one health approach. Medicina (B Aires). 76(3):159-165.

Vayr, F., Martin-Blondel, G., Savall, F., Soulat, J.M., Deffontaines, G., Herin, F. (2018): Occupational exposure to human Mycobacterium bovis infection: a systematic review. PLoS Neglected Trop Dis; 12: e0006208.https://doi.org/10.1371/journal. pntd.0006208.

Vordermeier, H.M., Jones, G.J., Buddle, B.M., Hewinson, R.G., Villarreal-Ramos, B. (2016): Bovine tuberculosis in cattle: Vaccines, DIVA tests, and host biomarker discovery. *Annu. Rev. Anim. Biosci.*, *4*, pp 87–109. https://doi.org/10.1146/annurev-animal-021815-111311.

Warsinske, H.C., DiFazio, R.M., Linderman, J.J., Flynn, J.L., Kirschner, D.E. (2017): Identifying mechanisms driving formation of granuloma-associated fibrosis during Mycobacterium tuberculosis infection. J. Theor Biol., 429: pp 1–17. https://doi.org/10.1016/j.jtbi.2017.06.017.

Waters, W.R. (2015): Bovine Tuberculosis. In Large Animal Internal Medicine-e-Book; Elsevier: Amsterdam, The Netherlands; Chapter 31; pp. 633–636.

WHO, (2017): Food and Agriculture Organization of the United Nations (FAO); World Organization for Animal Health (OIE); IUAT and International Union Against Tuberculosis and Lung Disease (The Union). Road map for zoonotic tuberculosis. In Proceedings of the 48th Union World Conference on Lung Health, Guadalajara, Mexico, 11–14 October 2017; pp. 11–14.

WHO, (2020): Global Tuberculosis Report 2020; World Health Organization: Geneva, Switzerland. [Google Scholar]

Yadav, K., and Prakash, S. (2017): Tuberculosis: An airborne disease. Global Journal of Microbiology Research; 5(5), pp 225-243.