Miliary Bovine Tuberculosis in Buffaloes in Al-Muthanna Governorate, Iraq

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Abstract: *Background*: *Bubalus bubalis* (river buffaloes) are widely distributed in the southern marshes of Iraq. This study intends to record a case of miliary tuberculosis in buffalo for the first time in Al Muthanna abattoir, Iraq, with its clinical, gross, and histopathological findings and microbiological investigations.

Case Description: Ten years old buffalo showed chronic cough, infertility, emaciation, debilitation, lower milk production, loss of weight, and loss of appetite for 2 months; during meat inspection, thousands of various size typical tubercles, yellowish, granulomatous, and caseous lesions were distributed over all the body.

Results: Microscopically, features of tuberculosis granuloma lesions were observed and revealed oval or round caseous necrosis with irregular central areas. Moreover, mineralization was enclosed by a thin to a broad layer of diverse inflammatory cells and solid collagenous connective tissue sheath. Moreover, a direct smear from the lesion stained by the Ziehl-Neelsen stain showed a slightly curved, red, and straight rod that was seen alone or in clusters, indicating the bacilli of tuberculosis. Additionally, the growing bacteria on the Löwenstein-Jensen media slant revealed flat, smooth, moist, white, not pigmented colonies suggestive of *M. Bovis* that revealed typical results with traditional biochemical tests, including negative reaction to nitrate reduction, niacin test, and deamination of pyrazinamide.

Conclusion: For the author's knowledge, this is the first case report of miliary bovine tuberculosis in buffalo in Iraq. The diagnosis was made according to clinical signs, gross pathology, and histopathological features supported by Ziehl-Neelsen stain and bacterial isolation. The author recommends future epidemiological molecular studies to improve the diagnosis tools of bovine tuberculosis in Iraq and investigate the causative agent *M. Bovis* to establish the roles for disease control that becomes much more challenging.

Keywords: *Bubalus bubalis*, miliary TB, *M. Bovis,* tuberculosis, Löwenstein-Jensen media.

INTRODUCTION

Bubalus bubalis (river buffaloes) are widely distributed in the southern marshes of Iraq. Buffaloes are one of the important domesticated animals and play an important role economically, mainly as dual-purpose animals (milk and meat production) and for draft purposes. A 1978 Iraqi government survey estimated water buffaloes at 170,000 [1]. Genetically different chromosome numbers, including 48 and 50, have been reported in water buffalo with divergent reproductive, productive, and physiological performance.

Buffaloes are affected by various pathogens and can be infected by the same diseases and parasites as bovines, with considerable variations in susceptibility according to country and region. Various external parasites can infest buffaloes, such as *Haematopinus tuberculatus*, with a degree of resistance to ticks [2]. Various diseases like foot and mouth disease, bovine viral diarrhea, brucellosis, leptospirosis, fasciolosis, babesiosis, theileriosis, and tuberculosis are affected by water buffaloes, accompanied by economic influences. Additionally, buffaloes also play important roles in transmitting zoonotic diseases like schistosomiasis [3, 4], human Q fever, *Coxiella burnetii* in milk [5], and tuberculosis. Tuberculosis in bovine is a chronic disease that has an impact on human public health. It is an essential obstacle to achieving the universal control program that aims to end tuberculosis by 2030 [6, 7]. The estimated numbers of zoonotic tuberculosis cases were 147.000, with 12.500 death (WHO, 2017) [8]. Human infection with *M. Bovis* was reported throughout the 19th and early 20th centuries and linked to the consumption of raw milk [9, 10]. The causative agent of bovine tuberculosis is *Mycobacterium Bovis,* which belongs to the Mycobacteriaceae family, genus Mycobacterium. The bacteria are short, non-capsulated, not flagellated, non-mobile, facultative intracellular, and aerobic [11]. It is distributed via air when the infected animal sneeze or coughs. Moreover, the digestive and respiratory systems are the route of infection. Emaciation, chronic cough, and progressive weight loss are the most prominent clinical signs.

In tuberculosis, Lungs and mediastinal lymph nodes are also characterized by forming granulomatous

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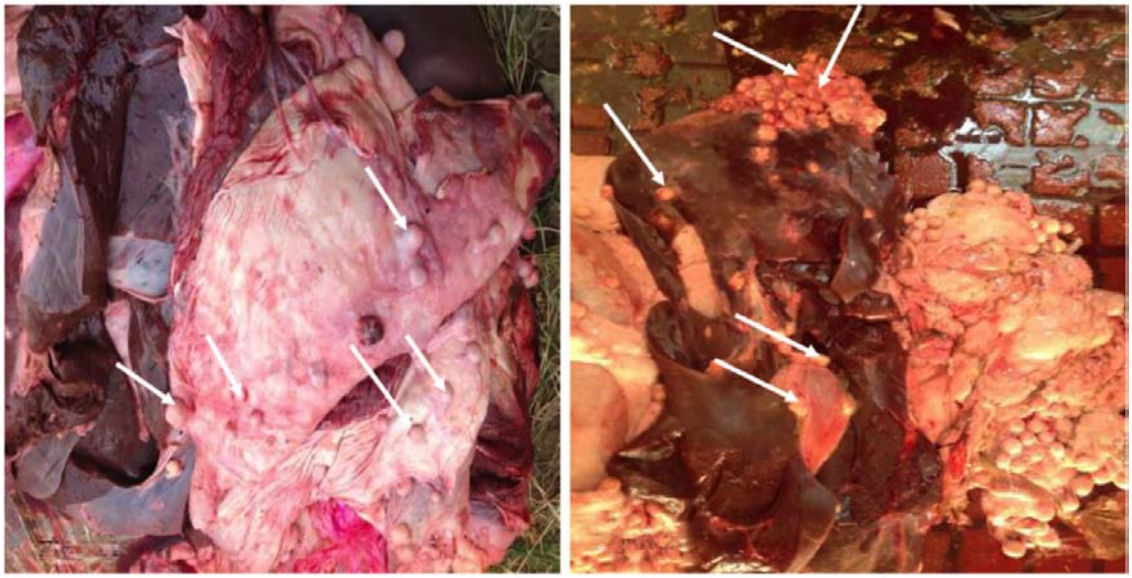

Figure 1: Shows the distribution of tubercles in the internal organs of buffalo.

lesions called tubercles. However, the miliary form of tuberculosis affects different tissues like head and neck lymph nodes, lungs, liver, lymph nodes, spleen, peritoneum, thorax, intestines, mesentery, and the kidney because of the hematogenous spreading [12]. Diagnosis of miliary TB is confusing with other diseases due to presenting different symptoms complicating the precise diagnosis. However, the diagnosis depends on the case history, tuberculin test, isolation of microorganisms, and macro and microscopical examination. According to a survey [13], about 50 million cattle were infected with tuberculosis universally, which caused high economic losses in the bovine industry.

The extension of TB in Iraq has been reported in domesticated animals and human being according to the WHO data (14), associated with 0.63% (938) of total death. The epidemiology and situation of buffalo tuberculosis in Iraq revealed scarce information. Consequently, this study intends to describe the gross pathology and microscopical features of a case of miliary tuberculosis in a buffalo slaughtered in Al Muthanna Abattoir.

CASE DESCRIPTION

According to the owner, 10 years old buffalo was slaughtered at Al Muthanna abattoir because of non-responsive to the treatment of chronic cough, infertility, emaciation, debilitation, lower milk production, loss of weight, and fluctuant appetite for 2 months. During meat inspection of the buffalo, thousands of various size typical tubercula, yellowish, granulomatous, and caseous lesions were distributed over the body (Figure **1**).

Grossly, the lesions varied in size from small (1-5 mm diameter) miliary foci to large tubercles or abscesses ranging from 26 mm to 32 mm in diameter and nearly the size of a small or medium cherry, which was presented as the majority of lesions (Figure **2**).

Figure 2: Shows the variations in the size of tubercle lesions.

In this case study, the affected organs revealed various size lesions that varied from caseated calcified tubercles to thin-walled abscesses formation. The large tubercle showed dispersed small flakes of mineralized substance with yellowish-white to greyish-white sticky exudate. The tubercular lesions appeared as multi-laminated, encapsulated with concentric necrotic

caseated substance. Moreover, most of these tubercles revealed a lamellar arrangement of mineralized substance close to the outer fibrous capsule (Figure **3**).

Figure 3: Shows typical tubercula revealing necrotic material organized in concentric lamellar layers.

These granulomatous lesions were widely distributed in the thoracic and abdominal cavities and associated lymph nodes. Additionally, the lesions were also seen on other organs such as the digestive system (rumen) and mesenteric lymph nodes, liver, pleura, peritoneum, kidney, mesentery, bronchial and mediastinal lymph nodes, and mammary glands (Figure **4**).

Suspected tuberculosis lesions from tissue segments were collected from different animal tissue and kept in 10 % formalin directly. Samples were trimmed into small specimens involving tubercles. The samples were sent to the pathology laboratory/ College of the Veterinary Medicine/ University of Baghdad for further processing. All samples were routinely processed by histological technique, sectioned at 4-5µm, stained with Haematoxylin and Eosin, and examined under light microscopy connected with a camera and Image analyzer (Leica). The microscopical examination of the tissue sections confirmed the features of tuberculosis granuloma lesions. The lesions appeared as oval or round with a non-regular central area of mineralized necrotic caseous substance covered by a solid broad layer of collagenous connective tissue invaded by diverse inflammatory cells. The granular necrotic caseous substance stained

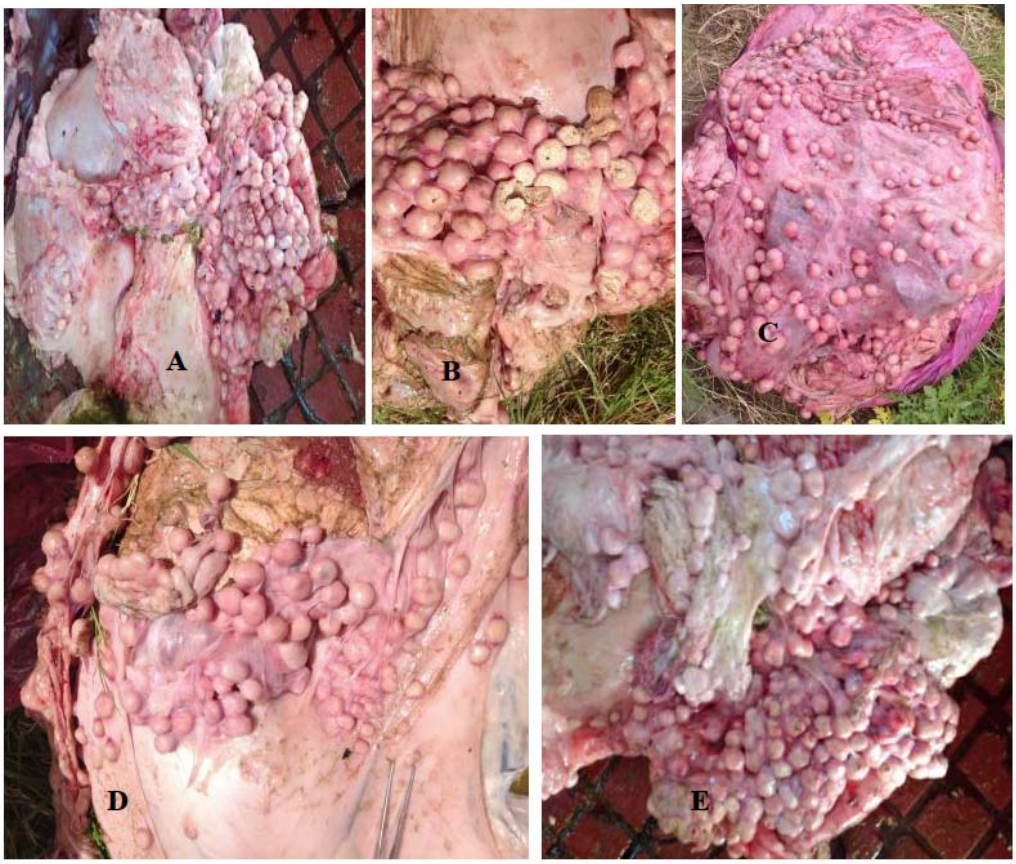

Figure 4: (**A**, **B**, **C**, **D**, **E**) Shows hundreds of tubercles distributed in the buffalo's body.

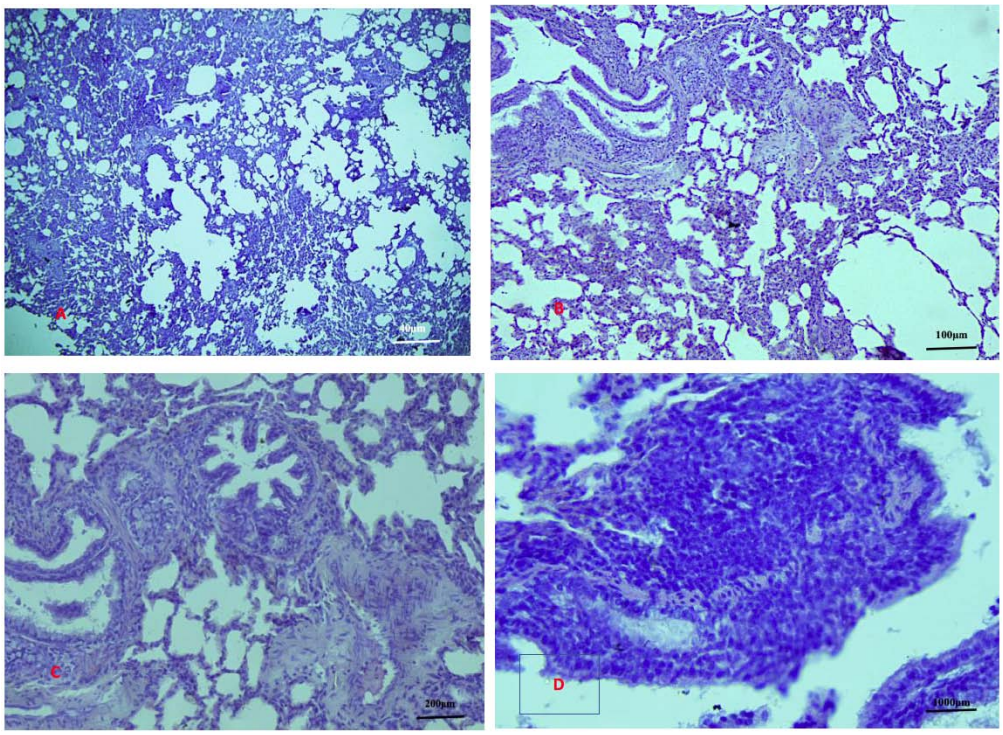

Figure 5: The microscopic features reveal the caseous necrosis and mineralization surrounded by solid connective tissue invaded by various inflammatory cells (**A**. X4; **B**. X10; **C**. X20; **D**. X40).

weakly to moderately eosinophilic, accompanied by the focal accumulation of basophilic chromatin debris that appeared as dotting of diffuse zones. Moreover, numerous neutrophils and degenerated leucocytes were scattered throughout the necrotic foci, accompanied by small or large mineralized foci (Figure **5**). Layers of inflammatory cells circumscribed caseous necrosis lesions were seen that invaded with numerous epithelioid macrophages, multinucleated cells, lymphocytes, macrophages, and Langhan's giant cells that were enclosed completely by solid collagenous connective/ fibrous capsule.

For microbiological investigation, a group of tubercles was collected, kept in a sterile container, and transferred to the clinical pathology laboratory. The sheath of two tubercles was removed, and the contents were homogenized manually using a sterile glass mortar. The homogenized tissue was suspended in 10 ml of 4% NaOH in a test tube, shaken thoroughly, and settled for (10 minutes). Finally, the sample was centrifuged for (10) minutes at 3000 rpm, and then sediment was collected after the upper supernatant layer was discarded. Afterward, sterile normal saline was added and appropriately mixed with sediment and centrifuge at 3000 rpm for 10 minutes. This process was repeated 3 times to neutralize the sample completely.

The supernatant was removed, and direct smears were prepared from the sediment on a cleaned glass slide, stained with conventional acid-fast stain (Ziehl-Neelsen staining). The slides were allowed to air-dry and examined by a light microscope under an oil immersion objective lens (X100) to investigate the presence of acid-fast bacilli, considered a primary diagnostic tool. The examination of slides revealed somewhat curved and red rods that were seen alone or aggregated as clusters, indicating the tuberculosis bacilli.

Later, a loopful of sediment was cultured in quadruplicate into Löwenstein-Jensen media/ Slant in screw-capped bottles according to [14], incubated at 37 o C for 8-10 weeks, and examined weekly to verify the bacterial growth. In the fourth week, the examination of the Löwenstein-Jensen media slant revealed flat, smooth, moist, white, not pigmented colonies that break up easily, suggestive of *M. Bovis* (Figure **6**). Traditionally, nitrate reduction and deamination of pyrazinamide and niacin test were used to identify the bacteria. The isolated bacteria revealed negative results for nitrate reduction, niacin test, and deamination of pyrazinamide that identified *M. Bovis*.

DISCUSSION

Water buffalo is one of the important livestock in Iraq, especially in the marshes of southern Iraq.

Figure 6: Shows the Löwenstein-Jensen media slant revealed flat, smooth, moist, white, not pigmented colonies suggestive of *M. Bovis.*

According to a national survey, the census of Iraqi buffaloes was 285.537 [17]. *Bubalus bubalis* is the second most crucial milky animal and is produced by more than 95% of South Asia [16]. The *Bubalus bubalis* is recognized for its ability to live in different natural climatic conditions and soil issues, even though *Bubalus bubalis* has reported its susceptibility to different infectious diseases that significantly impacted production performances.

Like other domestic and wild animals, buffaloes are susceptible to infectious diseases during their contact with ecosystems [18]. Numerous diseases of buffaloes are widely spread in many countries. The epidemiology of these diseases is thoroughly studied and showed a significant economic impact because it leads to high animal mortality, decreased production, and risk to the healthy population, trade, and tourism [19]. Water buffalo also acts as a reservoir host for various zoonotic diseases like brucellosis, rotavirus, leptospirosis, and tuberculosis [20, 21]. The cause of bovine tuberculosis is *Mycobacterium Bovis*, which affects many organisms, including humans, and is considered a chronic zoonotic disease. The disease is responsible for livestock's economic losses, including body condemnation and low milk production. In addition to serious public health problems and costs of the eradication programs [21-24].

Mycobacterium Bovis belongs to a group of microorganisms of dominant clinical importance called the *Mycobacterium tuberculosis* complex that causes tuberculosis in humans and animals. *Mycobacterium tuberculosis* is the cause of human tuberculosis and contains a massive range of genetic lineages. Zoonotic tuberculosis is caused by *M. Bovis*, *M. suricattae*, *M. orygis, M. caprae, M. Canetti*, *M. pinnipedii*, *M. mungi*, and *M. microti*, and they are less well-understood animal- associated subspecies.

In the current study, the milk-productive buffalo was slaughtered due to infertility, emaciation, debilitation, lower milk production, loss of weight, and fluctuated appetite for 2 months. All these clinical signs are compatible with previously reported research [25, 26]. In this case study, the obvious clinical signs were related to the postmortem and histopathological findings. These notes are confirmed that this case is miliary tuberculosis in buffalo. The gross pathology of this case showed thousands of various size typical tubercula, ranging from small miliary foci to large tubercles or abscesses.

Additionally, these lesions were seen as caseated calcified tubercles surrounded by solid connective tissue invaded by various inflammatory cells. The tubercles revealed mineralized material near the outer fibrous capsule in a lamellar arrangement. These granulomatous lesions were extensively distributed in the thoracic and abdominal cavities and associated lymph nodes, other organs such as the digestive system (rumen) and mesenteric lymph nodes, liver, pleura, peritoneum, kidney, mesentery, and bronchial and mediastinal lymph nodes and mammary glands. This gross pathological appearance is suggestive of tuberculosis. This gross appearance agrees with previous studies [12, 27, 28].

Moreover, the histopathological features manifested by the presence of necrotic, caseated mineralized, round, and non-regular central area enclosed with a thin to a broad layer of diverse inflammatory cells and a sheet of dense collagenous connective tissue accompanied with small or large mineralized foci infiltrated by inflammatory cells including neutrophils and epithelioid macrophages. The lesions were also revealed numerous lymphocytes (small and middle size) and Langhan's giant cells that enclosed completely by a solid collagenous connective/ fibrous capsule. All these features confirmed the tuberculosis granuloma lesions and agreed with previously reported results [12, 23, 29, 30, 32, 33]. In the current case, the clinical, gross, and histopathological diagnosis of tuberculosis was supported by the results of a direct smear from a lesion stained by Ziehl-Neelsen stain that showed red, straight, and slightly curved rod, seen singly or in clusters indicating the tubercle bacilli that compatible with the previous researcher [34]. In addition, the growing bacteria on the Löwenstein-

Jensen media slant revealed flat, smooth, moist, white, not pigmented colonies suggestive of *M. Bovis* that revealed typical results for nitrate reduction, niacin test, and deamination of pyrazinamide. Therefore, according to the current study's results and to the author's knowledge, this is the first miliary tuberculosis case reported in water buffalo Bubalus bubalis in Al Muthanna province in Iraq.

Miliary TB is rare in animals, and the clinical signs of TB manifest themselves in several clinical types depending on the infection route. However, lungs and bronchial lymph nodes are considered the more common form of pulmonary tuberculosis. The pathogenesis of *M. Bovis* initiate by adherence of inhaled bacilli to the lung alveolar surface and is phagocytosed by macrophages. The *M. Bovis* phagocytized antigen is exposed to T-lymphocyte, an essential immune system element. Miliary TB is more common in humans and relates to the hematogenic invasion of the bacillus, resulting in the establishment of diffuse small and granulomatous lesions affecting all organs, not only the lungs.

Miliary tuberculosis in buffaloes was reported previously in an abattoir in Pará, Brazil, in only five slaughtered buffaloes out of 1735 [35], which led the author to conclude the rare occurrence of this form of the disease. However, the author also considered this occurrence for the first time in the state of Sergipe.

According to Roex *et al*. [36], the classical tuberculosis lesions in buffalo are the masses of the lungs and lymph nodes. However, lesions have been seen to be located in distal sites [31].

Moreover, the susceptibility or resistance of animals to tuberculosis is mediated by adaptive and innate immunity. Consequently, the candidate genes related to either of these processes are called the bovine tuberculosis susceptibility loci [36]—most studies and publications reported on bovine tuberculosis and the wildlife of African buffalo. At the same time, only a few peer-reviewed journals reported bovine tuberculosis in water buffalo [37, 40].

The previous studies on African buffalo bovine tuberculosis showed clinical signs that appeared only in the late stages of the disease course, including coughing, debilitation, emaciation, and lagging when chased [30, 38]. The researcher mentioned that the incubation period for bovine tuberculosis in African buffalos was 9 -12 months. At the same time, the infections occur in the latent form for an extended period and revitalize during stress or in old age [39]. However, Corner [39] reported the bovine tuberculosis occurrence at 25.3 and 21.6% in cattle and buffaloes, respectively; nonetheless, the occurrence varies among countries [40, 41].

In conclusion, this study reported the miliary bovine tuberculosis in buffalo for the first time in Iraq. The diagnosis was made according to clinical signs, gross pathology, and histopathological features supported by the Ziehl-Neelsen stain and bacterial growth on the Löwenstein-Jensen media slant. The authors recommend future epidemiological molecular studies to improve the diagnosis tools of bovine tuberculosis in Iraq and investigate the causative agent *M. Bovis* to establish the roles for disease control that becomes much more challenging.

ETHICAL STATEMENT

Research and Animal Ethical committee, College of veterinary medicine, Al Muthanna University, Iraq approved this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest in publishing this article.

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CONTRIBUTIONS OF AUTHORS

The authors of this study contributed equally to this research.

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