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**Case Report**
Olgu Sunumu

# Pathomorphological and immunohistochemical evaluation of chronic (isolated) organ tuberculosis in a cow

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**Abstract:** In this study, it was aimed to present a case of chronic organ tuberculosis in a cattle pathomorphologically and immunohistochemically. General findings of chronic isolated organ tuberculosis in cattle in the late period were presented in this report. The 2-year-old cattle died as a result of respiratory distress and was brought to the pathology laboratory to determine the cause of death. In necropsy, it was detected that encapsulated caseous foci localized in the caudal lobe of the right lung and mediastinal lymph nodes were inactive.Histopathologically, secondary caseous necrosis, mineralized, peripheral mononuclear cell infiltration, and fibrous capsule were detected. Immunohistologically, macrophages and neutrophils were found to be positive for *Mycobacterium bovis*. However, regional lymph nodes were negative. Also, there was no histopathological reaction in other parenchymatous organs. As a result, the postprimary form of tuberculosis is not always detectable when compared to other forms. Therefore, we believe that this case report may be useful.

**Keywords:** Cow, post-primary tuberculosis, immunohistochemical diagnose, pathomorphology.

## İneklerde kronik (izole) organ tüberkülozunun patomorfolojik ve immünohistokimyasal değerlendirilmesi

**Özet:** Bu çalışmada bir sığırda kronik organ tüberkülozu vakasının patomorfolojik ve immunohistokimyasal olarak sunulması amaçlandı. Bu raporda geç dönemde bir sığırda gelişen kronik, izole organ tüberkülozuna ait genel bulgular sunuldu. Solunum sıkıntısı sonucu yaşamını yitiren 2 yaşındaki sığır, ölüm nedeninin belirlenmesi için patoloji laboratuvarına getirildi. Nekropside, sağ akciğerin kaudal lobunda lokalize kapsüllenmiş kazeöz odaklar ile mediastinal lenf düğümlerinin inaktif olduğu tespit edildi.Histopatolojik olarak sekonder kazeöz nekroz, mineralize, periferal mononükleer hücre infiltrasyonu ve fibröz kapsül tespit edildi. İmmünohistolojik olarak, makrofajlar ve nötrofillerin *Mycobacterium bovis* açısından pozitif olduğu görüldü. Ancak bölgesel lenf düğümleri negatifti. Ayrıca, diğer parankimatöz organlarda histopatolojik reaksiyon yoktu. Sonuç olarak, postprimer tüberküloz formu, diğer formlarla karşılaştırıldığında her zaman tespit edilememektedir. Bu nedenle, bu olgu raporunun yararlı olabileceğine inanıyoruz.

**Anahtar kelimeler:** İnek, post-primer tüberkülozu, immunohistokimyasal tanı, patomorfoloji.

## Introduction

Bovine tuberculosis (bTB) is a contagious bacterial disease in cattle. The infection can affect a variety of animals, and also humans. The etiologic agent is *Mycobacterium bovis* (O'Reilly Daborn. 1995). Transmission is by droplet infection, sputum, feces, inhalation of dust contaminated with the urine of infected animals (Jemal. 2016; Neill et al. 2001). At this point, innate immunity plays an important role to avoid its spread to other organs through hematogenous and lymphomatous routes, and also by respiratory channels (Arentz Hawn. 2007). Due to the large amount of lipid entering the cell wall of the tubercle bacillus, it resists phagocytosis and multiplies in macrophages. The initial reaction against mycobacteria occurs in the cells of the interalveolar septum and triggers the inflammatory reaction via signaling pathways after phagocytosis by macrophages and neutrophils (Domingo et al. 2014). Accumulated mycobacterial agents stimulate the inflammatory response and cell-mediated hypersensitivity (Admassu et al. 2015). It leads to the production of two main mediators of the immune response, NO and TNF-α, in actived macrophages. Antigens derived from *M. bovis* can also activate bovine γδ T cells in vitro (Askar et al. 2021). In addition, γδ T cells play an important role

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both in the early stages of *M. bovis* infection and in lesion formation (Cassidy et al. 2001). Mycobacterial factors enable CD4+ T cells to differentiate into Th1 cells (Hunter. 2020). CD4+ T cells attempt to arrest intracellular mycobacterial growth, while CD8 T cells lyse *M. bovis*-infected macrophages (Skinner et al. 2003). In addition, activated T cells, cytokine, and chemokine signals at the site of infection attract and activate monocytes and macrophages, and granulomas form as cells accumulate (Palmer et al. 2021). Granulomas are pathognomonic lesions of tuberculosis (Carrisoza-Urbina et al. 2019). The typical tuberculoid granuloma microscopic structure is surrounded by a central region with caseous necrosis, epithelioid macrophages, and a region of multinucleated giant cells, the outermost region containing an increased number of lymphocytes and rarely plasma cells (Carrisoza-Urbina et al. 2019; Palmer et al. 2021). As the infection persists, it may be surrounded by a partial or complete fibrous capsule (Palmer et al. 2021).

The tubercle is usually about 2-20 mm in diameter and whitish to yellowish in color and is more or less encapsulated by connective tissue and often contains central caseous necrosis and mineralization (Admassu et al. 2015; Domingo et al. 2014; Neill et al. 2001). Tuberculosis lesions are usually found in the bronchial and/or mediastinal lymph nodes and are generally thought to be the first to be affected (Stamp. 1948). In the lung, it is characterized by extension along the bronchiole and bronchial tree with multifocal confluent caseous necrosis in chronic organ tuberculosis (Domingo et al. 2014). Ulcerative lesions can be seen in the bronchi and trachea.

In this case, we have shown some specific lesions developed against *Mycobacterium bovis* in chronic organ tuberculosis, which is a type of post-primary tuberculosis.

## Case History

The material of the case was provided from lung specimens obtained in the autopsy of a 2-year-old cow. First of all, tissue specimens were taken from all lesions localized in the caudal lobe of the right lung. They were fixed at 10% neutral buffered formalin (NBF) solution for 48 hours. The specimens were trimmed and processed in automatic tissue processor (Leica ASP300S). The tissues were solidified in degraded alcohol series, xylol and paraffin wax. After being embedded in paraffin (Shandon Histocentre2), blocks were serially trimmed at 4 µm thickness. Glass slides were stained with routine hematoxylin-eosin (H&E) and coverslips were mounted using Entellan® mounting medium.

Sections taken on poly-lysine slides were passed through xylol and alcohol series. Peroxidase immunostaining was performed according to the Novocastra, RE7120-K (Leica, United Kingdom) kit procedure to detect the antigen in the tissue. As the primary antibody, monoclonal anti *Mycobacterium bovis* (1/1000 dilution, *M. bovis*, VMRD, 280-5, USA) was used. Proteinase K (ab64220) was dripped onto paraffin block sections and then incubated in 3% $H_2O_2$ peroxidase block solution. Then, the protein block solution was poured. The sections 1:1000 anti-*M. bovis* were dropped and left at room temperature for 1 hour. After the primary antibody, Biotinylated Secondary Antibody was dripped and kept at room temperature for 30 minutes. Sections were dripped with Streptavidin-HRP and incubated for 30 minutes. Diaminobenzidine (DAB) was used to show antigen-antibody complex and Hematoxylin was used to counterstain. After dehydrated alcohol series and xylols, the glass slides were covered with coverslip by using Entellan® mounting medium.

The owner stated it showed a number of symptoms in the period of terminal illness such as respiratory distress. In autopsy, caseous lesions had spherical shape and reached 1 to 3 cm in diameter in right lobe of the lung (Fig 1a). After histopathology examination, it was confirmed that other lobes and regional lymph nodes were not affected by the lesions (Fig 1b). Other organs were intact. However, primary and secondary caseous necrosis as well as predominant mononuclear cell infiltration including predominantly macrophage, lymphocyte and less epitheloid histiocytes, multinucleated Langhans giant cell, plasma cell as well as neutrophil leucoytes were observed by collecting in foci of lung (Fig 1 a-d). In addition, encapsulation and mineralization were not observed in some lesions.

Immunohistochemistry showed a positive reaction in the cytoplasm of macrophages (Fig 2e) and in the cytoplasm of cells (Fig 2f). The microbial agent were present immunopositively in such areas. However, positive reactions were observed in interalveolar septum cells in some regions (Fig 2g). In contrast to be founded positivity in lung, mediastinal lymph nodes did not react with the specific antibody of *M.bovis*.

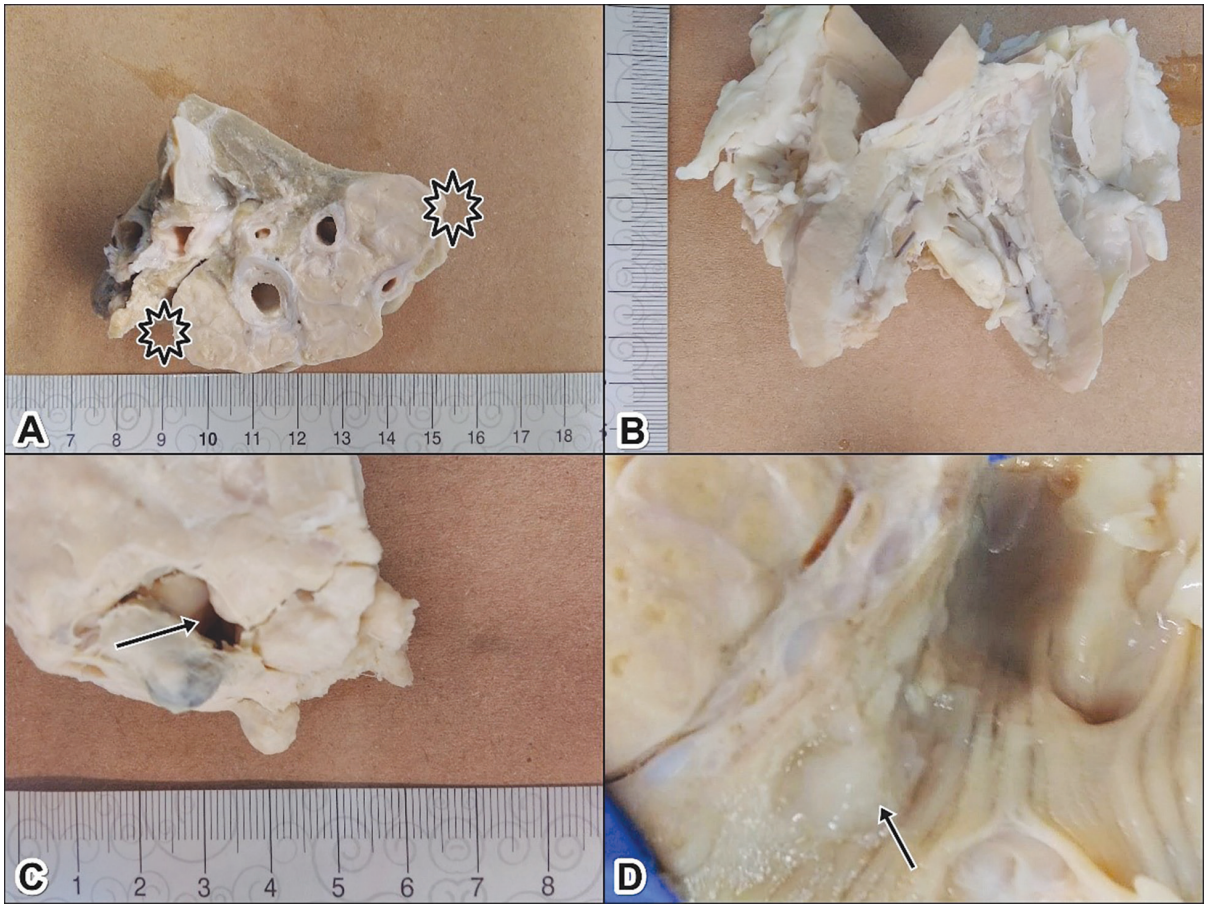


**Figure 1.** Macroscopic appearance of isolated organ tuberculosis. **A.** Acinar foci (stars) general view of lesions at cut section in right lung. **B.** General view of the lung mediastinal lymph node **C.** No lesion in lymph node (black arrow) **D.** Ulcerated mucosa of bronche (black arrow).

Previous reports have shown that 63% to 73% of lesions were found in the lung or existed both in lungs and mediastinal lymph nodes. These foci were under 1 cm in diameter (McIlroy et al. 1986; Neill et al. 1988). In our case, the foci were located solely in a lobe of right lung. The mean size of those was almost 1 to 3 cm. It shows us that the lesions may occasionally extend into extraordinary size.

On the other hand, histological presentation of lesions may vary with different cellular architectures in bovine Tuberculosis (bTB). In consequence, Wangoo et al. (2005) have reported granulomatous reaction in four types of granulomas from type I to type IV. For type I granulomas, although there is no necrosis, it is experienced in the initial stage, and shows epithelioid macrophages, multinucleated Langhans-type cells and lymphocytes at the periphery of the lesion. In some instances, neutrophil infiltrations are located at the center. For type II granulomas, aforementioned appearance is still present; but there is also ample amount of central caseous necrosis and peripheral lymphocytes. However, in type III, mineralisation is also added to cellular architecture in addition to a similar appearance. Type IV granulomas contain lymph node lesions that are composed of caseous necrosis with mineralisation, classic cellular architecture and extensive fibrous capsule. In our case, the lesions in the lung mostly belonged to type II and III. It was detected around necrotic foci, in the bronchial, bronchiolar and alveolar epithelium, in the exudate and in the cytoplasm of neutrophils, alveolar macrophages. In previous studies (Adegboye et al. 1995; Radaelli et al. 2008; Yılmaz et al. 2014), it was detected around necrotic foci, in the bronchial, bronchiolar and alveolar epithelium, in the exudate and in the cytoplasm of neutrophils, alveolar macrophages. Similar to the previous study, *M.bovis* antigen was detected in the cytoplasm of interaveolar septum cells, neutrophils and alveololar macrophages.

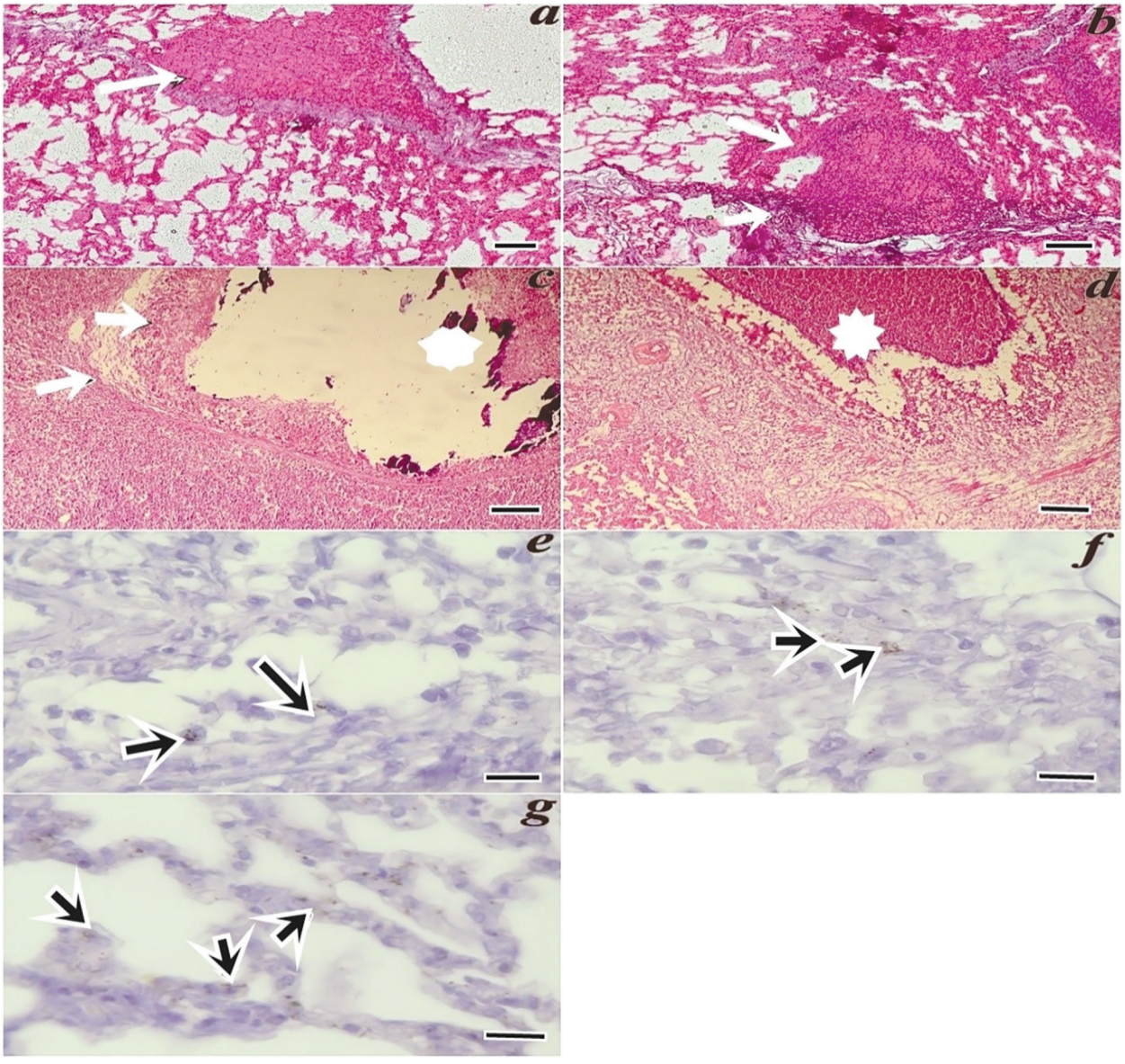


**Figure 2.** Histopathological and immunohistochemical findings. **a.** Histopathological appearance of primary caseous necrosis (arrow). Bar: 250 µm. H &E. **b.** Caseous necrosis (white arrow) and fibrous encapsulation. Bar: 250 µm. H&E. **c.** Mineralization (asterisk), macrophage, lymphocyte, Langhans giant cell, epiteloid histiocytes (arrow) and fibrous encapsulation (white arrow). Bar: 250 µm. H&E. **d.** Caseous necrosis opening into the bronchiole (asterisk). Bar: 250 µm. H&E. **e-f.** *M. bovis* positive staining in the cytoplasm of macrophages (black arrows) and in the cytoplasm of cell (black arrows). Bar :150 µm. ABC-P. **g.** In interalveolar septum cells *M.bovis* positive staining (black arrows). Bar :150 µm. ABC-P.

This inflammatory progression may be associated with microbial burden. For this situation, Phillips et al. (2003) have reported that $10^3$–$10^5$ *Colony Forming Unit* (CFU) might trigger natural bTB, resulting in the initial lesions of disease. However, in another study, it was demonstrated that intranasal administration of 92 CFU of *M. bovis* did not lead to lesion formation (Neill et al. 1988). Buddle et al. (1994) and Buddle et al. (1995) have reported that intratracheal inoculation of 500–800 CFU of *M. bovis* culminated in small lesions in the lungs and regional lymph nodes. For the generalization of such lesions, it was suggested that $5x10^5$ CFU or higher may be effective. In this natural case, we have considered that ba-

cterial load of *M. bovis* infection could be lower than $10^5$ CFU since we did not observe any generalization into regional lymph nodes and other organs.

In the result, the lesions in this case pertained to chronic or isolated organ tuberculosis. The tubercles were often in middle stage of progression. Hence, we did not believe there was any immunosupression or re/super-infection that may change the microorganism burden in tissue environment. Therefore, no lymph nodes were affected in this post-primary stage of bTB.

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