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Article

Prevalence and Characterization of Tuberculous Lesions in Imported Beef Cattle Slaughtered at Abu-Simbel City, Aswan Governorate

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Abstract

The number of infected cattle with tuberculosis in Egypt is increasing annually, probably as a result of the importation of live animals from countries where bovine tuberculosis is predominant. Therefore, the current study was conducted to investigate the prevalence of tuberculosis in imported beef cattle. During the period from September 2020 to March 2022, 2150 slaughtered beef cattle were examined. Sixty animals (2.78%) of them showed tuberculous lesions grossly. Depending on the gross morphologic lesions, tuberculous cases were classified into generalized tuberculosis (9 cases, 15%) and localized tuberculosis (51, 85%). Localized cases were subdivided into pulmonary tuberculosis (28, 46.66%) and extra-pulmonary tuberculosis (23, 38.33%). Specimens were collected for histopathological examination, granuloma staging, and Ziehl-Neelsen (ZN) staining. Based on the histopathological examination, the granulomas were classified into: Stage I (158, 21.43%), Stage II (187, 25.37%), Stage III (156, 21.16%), and Stage IV **(**236, 32.02%). In conclusion, our study reveals that imported beef cattle slaughtered in Aswan Governorate have a high incidence of tuberculosis. Accordingly, we recommend strict quarantine measures for imported beef cattle to decrease the possibility of disease transmission.

Keywords: Tuberculosis, Granuloma, Beef cattle, Slaughterhouse, Egypt.

1. Introduction

Animal tuberculosis (TB) is a chronic, progressive infectious disease affecting a variety of domestic and wild animals and humans (Rodriguez-Campos et al. 2014). The disease is caused by bacteria belonging to the *Mycobacterium tuberculosis* complex (MTC). *Mycobacterium bovis*,

the main member of the MTC has become recognized in animal populations all over the world and causes severe disease in numerous species (Mohamed, 2020). Tuberculosis is a major public health issue, with 8-9 million new cases and 3 million deaths worldwide each year. The pathognomonic lesion of the disease is the tuberculous granuloma (Wangoo *et al.*, 2005; Tulu *et al.*, 2020) which is a variable-sized circumscribed yellowish granulomatous inflammatory nodule encapsulated by connective tissue and frequently contains a central core of necrotic tissue with varying degrees of mineralization (Aranday-Cortes *et al.*, 2013; Domingo *et al.* 2014). The location of tuberculous nodules is primarily determined by the route of infection. The association of lungs and their regional lymph nodes in the pathogenesis of tuberculosis has been described many years ago (Ghon, 1912) as a primary complex or Ghon complex. However, infection acquired through ingesting *M. bovis* is more likely to result in non-pulmonary forms of the disease (Domingo *et al.*, 2014). The granuloma comprises a chronic inflammatory reaction with abundant epithelial-like (epithelioid) macrophages (Palmer, Thacker, & Waters, 2015). Lymphocytes, plasma cells, and Langhans-type multi-nucleated giant cells (MNGCs). Trial infection in cattle has permitted the qualitative cataloging of granulomas during the course of bovine tuberculosis infection (Wangoo *et al.*, 2005; Aranday-Cortes *et al.*, 2013). However, others (Tulu *et al.*, 2020; Larenas-Muñoz *et al.*, 2022) also classified the granuloma in naturally infected cattle with mycobacterium tuberculosis complex. Microscopically, granulomas are classified into four different stages of development. Briefly, stage I (initial) granulomas are characterized by abundant epithelioid macrophages with lymphocytes, neutrophils, and at times MNGCs. Stage II (solid) granulomas are characterized by epithelioid macrophages with minimal necrosis and a thin connective tissue capsule. In this stage, neutrophil and lymphocyte infiltrate may coexist with MNGCs. Stage III (necrotic) granulomas have a caseous necrotic center surrounded by an epithelioid macrophage zone with or without MNGCs and lymphocytes, encapsulated by fully formed fibrous connective tissue. Stage IV (necrotic and mineralized) granulomas are characterized by coalescent granulomas with a complete fibrous encapsulation, widespread central caseous necrosis, and calcification surrounded by epithelioid macrophages and MNGCs. Currently available bovine tuberculosis (bTB) diagnostic tests are imperfect due to low Sensitivity (Se) and specificity (Sp) (Byrne *et al.*, 2018). The tuberculin test is the most commonly used method for detecting bovine tuberculosis, because the test's sensitivity is less than 100%, it is unlikely that tuberculosis can be eradicated from a herd with a single tuberculin test. It must be known that when used in chronically infected animals with severe pathologic lesions, the tuberculin test might be unresponsive or give false negative results (Ayman *et al.*, 2014). While, histopathology has the benefit to identify and classify lesions in both reactor and non-reactor animals even when they are unobserved during the gross inspection at the slaughterhouses(Larenas-Muñoz *et al.*, 2022).

In Egypt, various studies in different areas have reported the prevalence of bTB; it ranges from 2.1% to 23.9 % in slaughterhouses and dairy farms (Ameni & Erkihun, 2007; E. Nasr, Abdel Twab, Abdel Rahman, & Abdel Aziz, 2008; Nawal, Mohey, Alaa, & Yasser, 2009; El-Sify *et al.*, 2013; E. A. Nasr, Marwah, Melika, Tammam, & Gorge, 2016)**.** However, scarce information is available about the prevalence of tuberculosis in imported beef cattle. Therefore; this study aimed to investigate the prevalence of bTB, and assess the distribution of tuberculous lesions and granuloma scoring among tissues of slaughtered imported beef cattle at Abu-Simple city, Aswan Governorate.

2. Materials and Methods

2.1. Animals

Sudanese-origin beef cattle aged from 2 – 3 years were kept in quarantine for 21 days under the observation and supervision of Egyptian Quarantine Veterinarians in the Sudanese city of Wadi Half (bordering Egypt). These beef cattle were subjected to a tuberculin test and gave a

negative result. Afterward, cattle were shipped down the Nile to the city of Abu Simbel, Aswan Governorate where they were slaughtered at the Middle East and Wadyna abattoirs.

2.2. Postmortem examination and samples collection

The collection of the samples for this study was carried out during the period from September 2020 to March 2022. Routine postmortem examinations of 2150 slaughtered beef cattle were carried out with particular attention to the tuberculous-like lesions. Representative samples of the tissues from lymph nodes (left bronchial, mediastinal), liver, and lungs of 60 animals grossly showing tuberculous-like lesions were collected and all lesions were imaged.

Histopathological examinations

The tissues were fixed in 10% neutral buffered formalin for 24 to 72 h, embedded in paraffin, sectioned into 4-m sections, and stained with hematoxylin-eosin (H&E) and Ziehl-Neelsen acid-fast stain. Granulomas were classified into different stages (stages I to IV) according to the previously described criteria by (Wangoo *et al.*, 2005) and recently by (Larenas-Muñoz *et al.*, 2022).

3. Results

3.1. Pathological examination

In the present study, 2150 slaughtered imported beef cattle aged 2 to 3 years old were examined, from which 60 cases showed gross lesions. Histopathological examination revealed microscopic changes in all cases. Depending on the gross morphologic lesions, the cases were classified into 9(15%) cases diagnosed as generalized tuberculosis and 51 (85%) cases diagnosed as localized tuberculosis. The localized cases were subdivided into 28 (46.66%) cases that were diagnosed as pulmonary tuberculosis and 23 (38.33%) cases diagnosed as extrapulmonary tuberculosis. The main characteristic lesion of tuberculosis is a granuloma. Based on the histopathological lesions of the examined tissue, the granulomas were classified into 158 (21.43%) Stage I, 187 (25.37%) Stage II, 156 (21.16%) Stage III**,** and 236 (32.02%) Stage IV. The results were summarized in Tables (1, 2).

Table 1. Types, number, incidence, and prevalence of tuberculous cases in imported beef cattle.

Type of pathological affection	Number of cases	Incidence %	Prevalence%
Generalized tuberculosis.	9	15 %	0.41 %
Localized tuberculosis Pulmonary tuberculosis	28	46.66 %	1.30 %
Extra-pulmonary tuberculosis	23	38.33 %	1.06 %
Total	60		2.78 %

Table 2. Stages, number, and incidence of each stage of Granuloma in imported beef cattle.

Stage of Granuloma	Number	Incidence %
Stage I	158	21.43%
Stage II	187	25.37%
Stage III	156	21.16%
Stage IV	236	32.02%
total	737	

3.2. Macroscopic observations

The affected lungs and pleura of nine generalized cases showed a large number of small grays to white-yellowish miliary tubercles (Figure1 A). Gross appearance showed also tubercles capsulated by fibrous tissue and containing white to yellowish cheesy calcified material in the liver (Figure1 B). However, most of the macroscopic lesions observed in localized tuberculosis cases were detected at the ribcage level, including the lungs, bronchial, and mediastinal lymph nodes. In the lung, variably sized multiple nodular lesions were detected which when cut revealed cheesy necrotic material (Figure 1 C). In addition, the regional lymph nodes of the af-

fected cases showed diffuse tuberculous lesions, and cut sections revealed cheesy necrotic material as well (Figure 1 D).

3.3. Microscopic Observations

The tuberculous granulomas were microscopically classified into four developmental stages based on criteria previously described for cattle by (Wangoo *et al.*, 2005). Stage I (initial or early lesions) is distinguished by irregular, unencapsulated clusters of primarily epithelioid macrophages, lymphocytes, and multinucleated giant cells that lack necrosis. (Figure 2 A). Stage II (solid granulomas), formed by primarily epithelioid macrophages, Langhans giant cells, and lymphocytes around limited areas of necrosis, was partially or thinly encapsulated. (Figure 2 B). Encapsulated granulomas with central caseous necrosis were present at stage (III). (Figure 2 C). Necrosis and mineralization stage IV consisted of enormous, thickly encapsulated, multicentric granulomas with significant caseous necrosis and highly mineralized areas surrounded by epithelioid cells, giant cells, and lymphocytes. (Figure 2 D).

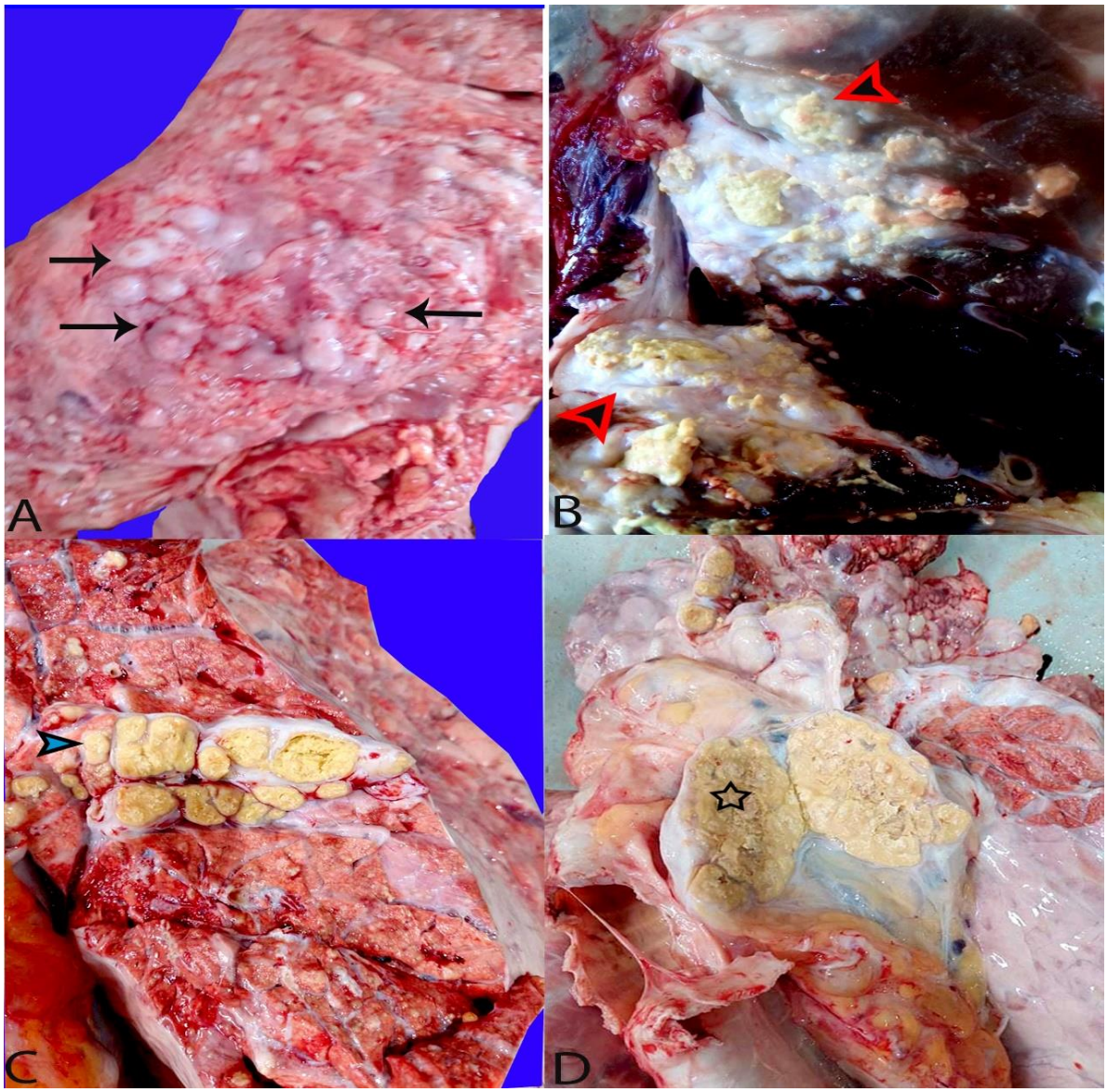

Figure 1. A. Lung showing a large number of small greys to white-yellowish tubercles (Generalization) above the pleura (arrows); B. white, pale, and yellowish cheesy calcified material in the liver, C. Lung of cattle showing multiple localized tubercles; cut sections revealed cheesy necrotic material (arrowhead) with calcification; D. tracheobronchial lymph nodes revealed diffuse caseating, cheesy necrotic materials.

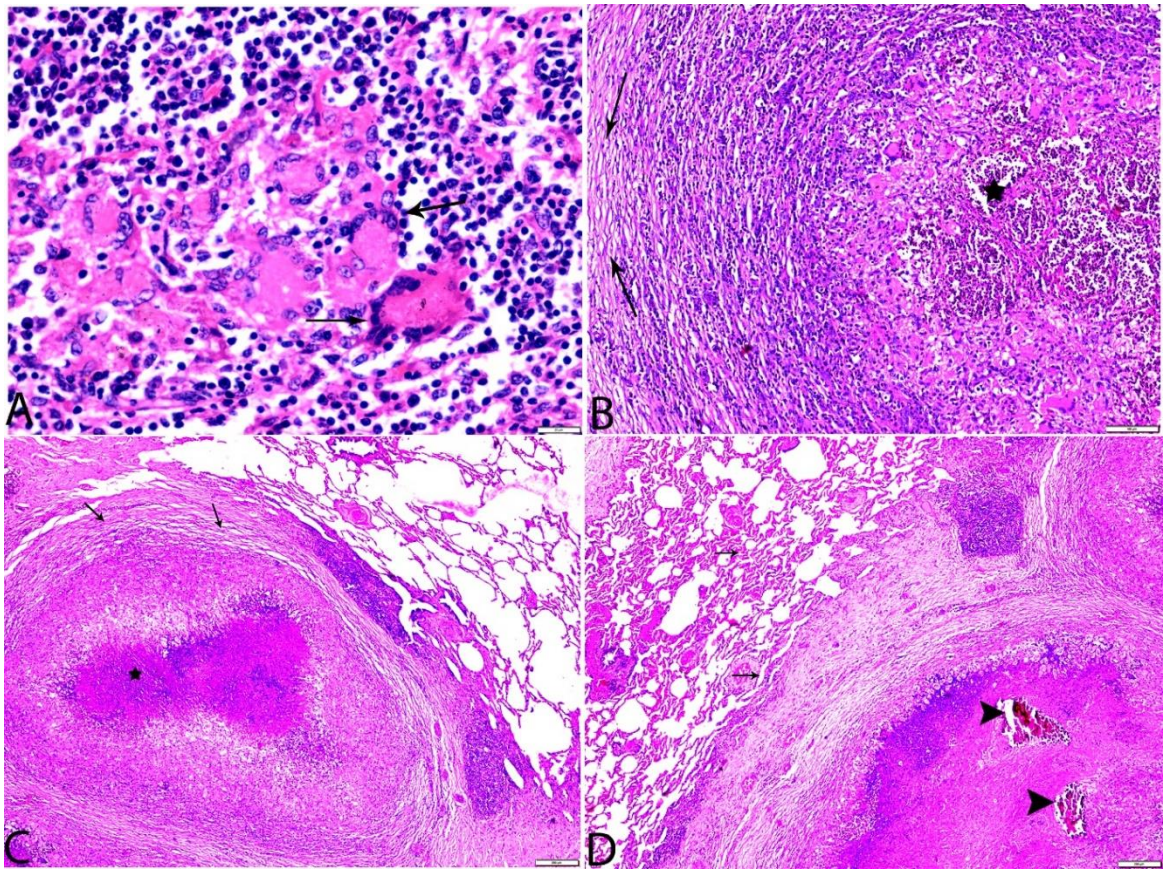

Figure 2. Histological sections show granulomas' four stages from naturally infected beef cattle with bovine tuberculosis. A. Stage I granuloma, epithelioid cell, and multinucleated giant cells (arrows) surrounded by lymphocytes. (Hx & eosin. Bar = 50 µm), B. Stage II granuloma (solid). Increased numbers of epithelioid macrophages can be seen, encircled by a thin layer of C.T. (arrows) and minimal caseous necrosis (Star) (Hx & E, Bar = 100 µm), C. Stage III granuloma showing fully formed C.T. (arrows) and central caseated area (star)with little mineralization (Hx& E, Bar = 200 µm), D. Stage IV granuloma with the extensive central caseated area, mature C.T capsule, and highly multiple mineralizations (arrows head), the surrounding alveoli showed atelectasis (arrows) (Hx& E, Bar = 200 µm)

3.4. Acid-fast bacilli (AFB) identification (ZN):

A sample was considered positive for ZN if at least one high-power field magnification revealed the presence of one or more AFB. In this investigation, only 42 (70%) of 60 bTB probable tuberculous cases were found to be acid-fast bacilli (AFB) positive using the Ziehl-Neelsen staining method. ZN+ cases were categorized as either paucibacillary (1–10 AFB) or paucibacillary (if ≥11 AFB) bacilli in high-power fields (HPF)(García-Jiménez *et al.*, 2013). A paucibacillary form was present in 35 of the total ZN+ cases (Figure 3 A), whereas a multibacillary form was present in 7 of the cases (Figure 3 B) Acid fast bacteria (AFB) has been found inside macrophages, multinucleated giant cells, and the necrotic center of granulomas

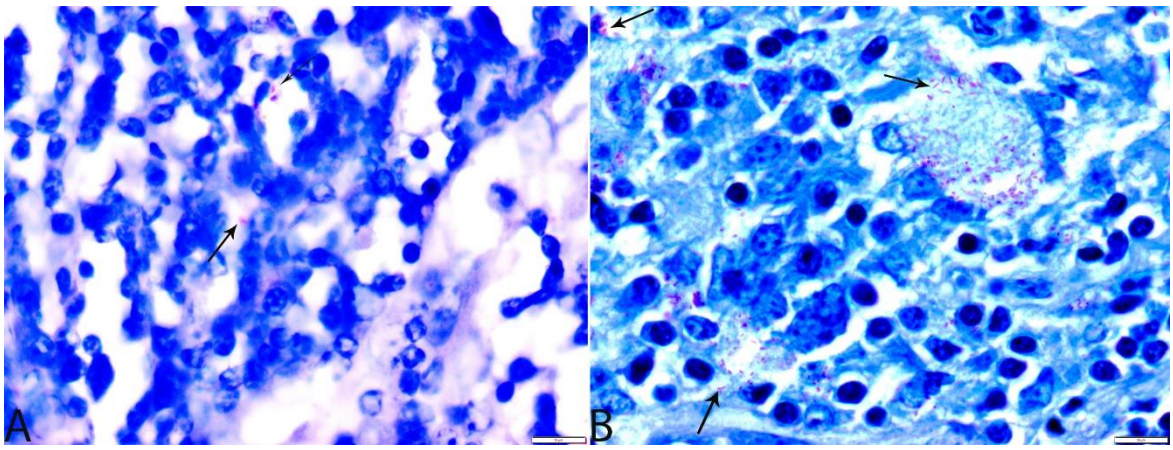

Figure 3. A. paucibacillary; Intracellular acid-fast bacilli present inside the macrophages, ZN (arrows); B. Pluribacillay form; many acid-fast bacilli (arrows) are detected in the multinucleated giant cell and macrophages.

4. Discussion

The importance of meat inspection as a primary diagnostic and preventive tool in our local slaughterhouses cannot be overemphasized. Apart from the provision of epidemiological information on life-threatening zoonotic diseases of meat-borne origins, such as bovine tuberculosis. Improving the diagnosis of bTB in cattle is a cornerstone in control and eradication programs against this disease. Thus, combining different diagnostic tests, both antemortem and post-mortem, represents a smart approach to improving MTC diagnosis. Histopathology is an early, rapid, and economic technique for identifying and grading microscopic lesions associated with MTC infection. Furthermore, it visualizes the presence of mycobacteria within these lesions using other techniques (ZN staining). The present study was conducted on imported beef cattle (mainly Zebu cattle) where 2150 slaughtered imported beef cattle aged 2 to 3 years old were examined, from which 60 cases showed gross lesions with a prevalence rate (of 2.78%, 60/2150). This nearly agrees with (Moussa *et al.*, 2011), 2.46%; and(E. A. Nasr *et al.*, 2016), 2.6%, but differs from(El-Sabban, Lotfy, Hammam, Dimitri, & Gergis, 1995), 24%; (Nawal *et al.*, 2009), 23.91%; and (Ameni & Erkihun, 2007), 11.6%. This difference is attributable to the variation of examined animals as well as the present study was carried out on imported beef cattle (Zebu cattle) which are relatively resistant to bTB. Despite these animals being subjected to quarantine measures and tuberculin tests, tuberculous lesions were detected on them and confirmed by demonstration of acid-fast bacilli through ZN staining. ZN+ mycobacteria are frequently found in giant cells, macrophages, or necrotic areas. (Cancela & Marín, 1993; Jaime *et al.*, 2010), the presence of at least one mycobacterium per one high-power field was sufficient to consider as positive. In this study, forty-two (42/60; 70%) cases were identified as acid-fast bacilli (AFB) positive. These results are nearly similar to previously described studies (Cancela & Marín, 1993; Larenas-Muñoz *et al.*, 2022). Usually, paucibacillary lesions are observed in cattle and this was detected in the present study as most of the samples (35/42; 83.33%) showed paucibacillary form (Larenas-Muñoz *et al.*, 2022), confirming the usefulness of ZN staining in detecting the presence of mycobacteria within a tuberculous granuloma and importance of this technique as a diagnostic tool should be included in the diagnosis of tuberculosis of these imported animals as these animals were subjected to tuberculin test where yielding a negative result. It should be documented that when used in chronically infected animals with severe pathologic lesions, the tuberculin test may be unresponsive or give false negative results (Ayman *et al.*, 2014). Depending on the gross morphologic lesions, the cases were classified into (9/60;15%) cases were diagnosed as generalized tuberculosis, and (51/60; 85%) cases were diagnosed as localized tuberculosis. The localized cases were subdivided into (28/51; 46.66%) cases diagnosed as pulmonary tuberculosis and (23/51; 38.33%) cases diagnosed as extrap-

ulmonary tuberculosis. The majority of the tuberculous lesions were found in the lung (28/51; 46.66%), followed by lymph nodes (23/51; 38.33%), confirming the respiratory tract is the main route of infection of bovine tuberculosis (Carrisoza-Urbina, Morales-Salinas, Bedolla-Alva, Hernández-Pando, & Gutiérrez-Pabello, 2019), in addition to, our study revealed that presence of a tuberculous lesion in lymph nodes(extrapulmonary tuberculosis) with no lesion detected in lungs(incomplete primary complex). This result was previously described by others (McIlroy, Neill, & McCracken, 1986; O'Garra *et al.*, 2013). Based on the histopathological assessment of tissue sections in this study, the granulomas were classified into: (158/737; 21.43%) Stage I, (187/737; 25.37%) Stage II, (156/737; 21.16%) Stage III and (236/737; 32.02%) Stage IV. Different developmental stages of granuloma formation could be found within the same organ, during a histological assessment of tissue sections in this study. The presence of different microenvironments within the same tissue has been previously suggested in studies with cattle experimentally infected with *M*. *bovis* (Menin *et al.*, 2013; Moiane *et al.*, 2014) or with cattle naturally infected with mycobacterium tuberculosis complex (Tulu *et al.*, 2020; Larenas-Muñoz *et al.*, 2022). In a previous study a total of three-hundred ninety-six microscopically identified granulomas from the adult animal group, were categorized (Wangoo *et al.*, 2005). Most of them were stage IV granulomas. In this study, 236 stage IV granulomas were identified in imported beef cattle with an incidence rate of 32.02% (236/737). The predominance of stage IV granulomas in naturally infected animals indicate a chronic course involving an anti-inflammatory immune response, that can also be correlated to the fibrosis that was seen surrounding lesions.

5. Conclusions

This study decorated that the prevalence of tuberculosis among imported beef cattle slaughtered at Abu-Simple city, Aswan Governorate was (2.78%). A large number of granulomatous lesions in the lymph nodes and lungs of cattle were stage IV granulomas. The predominance of Stage IV granuloma, confirms the chronic nature of the disease that may be given a false negative result when these animals are tested with a tuberculin test. Therefore, we recommend using another diagnostic tool rather than a tuberculin test and subjecting these imported animals to strict quarantine measures to decrease the possibility of disease transmission.

Ethical Considerations

The current study has been approved by the veterinary medical research ethics committee, Faculty of Veterinary Medicine, Sohag University, sohag, Egypt, according to the OIE standards for use of animals in research with number Soh. Un. Vet/0008R.

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مقال

الملخص العربي

معدل انتشار وتوصيف الآفات السلية في عجول التسمين المستوردة التى يتم ذبحها بمدينة أبو سمبل - محافظة أسوان

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يتزايد عدد الماشية المصابة بالسل في مصر سنويًا، ربما نتيجة لاستيراد حيوانات حية من البلدان التي ينتشر فيها مرض السل البقري. لذلك أجريت الدراسة الحالية لتحديد مدى انتشار مرض السل في عجول التسمين المستوردة. خلال الفترة من سبتمبر 2020 إلى مارس 2022، تم فحص عدد 2150 رأسًا من عجول التسمين المذبوحة. 60 (2.78٪) منهم ظهرت عليهم آفات سلية بشكل جسيم من خلال الصفة التشريحية. اعتمادًا على الآفات المورفولوجية الإجمالية، تم تصنيف الحالات إلى 9 (15٪) حالات تم تشخيصها على أنها سل معمم و51 (85٪) حالة تم تشخيصها على أنها سل موضعي. تم تقسيم الحالات الموضعية إلى 28 حالة (46.66٪) تم تشخيصها بمرض السل الرئوي و 23 (38.33٪) حالة تم تشخيصها على أنها سل غير رئوي. تم جمع العينات للفحص الهستوباثولوجى وتحديد مراحل الورم الحبيبي وصباغة الشرئح بصبغة الزيل نيلسون للكشف عن الميكروب المسبب للسل. بناءً على الفحص الهستوباثولوجى، تم تصنيف الأورام الحبيبية إلى: 158 (21.43٪) المرحلة الأولى، 187 (25.37٪) المرحلة الثانية، 156 (21.16٪) المرحلة الثالثة، 236 (32.02٪) المرحلة الرابعة. لذلك، يجب أن تخضع عجول التسمين المستوردة لإجراءات الحجر الصحي الصارمة لتقليل احتمالية انتقال المرض.

الكلمات المفتاحية: السل، الورم الحبيبى، عجول التسمين، المجزر، مصر