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*Pathology*

# Pathological Study on Bovine Tuberculosis: A Survey Among Slaughtered Cattle and Buffalo in Menofiya Governorate During 2021-2022.

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## ABSTRACT

Bovine tuberculosis (bTB) is a chronic granulomatous disease that mainly affects the lungs and their lymph nodes. The proportion and pathological studies of bovine tuberculosis among slaughtered cattle and buffalo were determined in this study. Tissue samples from lymph nodes, lungs, heart, pleura, liver, and intestine were collected and rapidly fixed in 10% formalin for histopathology. This study was conducted on 91 cattle and 68 buffalo that revealed visible lesions of tuberculosis. The Gross lesion showed greyish-yellow cheese-like nodules in lymph nodes and internal organs. Microscopically, bTB is classified into miliary TB (9.37%) and localized TB (90.62%) in which granulomas were graded in four stages: Stage I (6.25%), Stage II (8.59%), Stage III (25%), and Stage IV (50.78%). Staining with Ziehl-Neelsen demonstrates acid-fast bacilli within the cytoplasm of macrophages, and giant cells and in the necrotic masses of TB granuloma. The higher proportion of bTB was recorded in cattle (59.34%) and buffalo (58.82%) aged 4-6 years. The sex-based proportion was higher in female buffalo (67.64%) than in males (32.35%), While in cattle, males (58.24%) had a greater frequency than females (41.75%). Data obtained from the Directorate of Veterinary Medicine, Shebin El Koom, Menofiya Governorate, revealed that the seasonal proportion of bTB was high during spring (8.5% cattle and 5% buffalo). The highest annual proportion of bTB was observed in 2022 (15.8% cattle and 9.8% buffalo) compared to 2021. Ashmon abattoir (76.5%) revealed a higher proportion, while El-Batanon (3.6%) had a lower proportion of bTB.

**Keywords:** Bovine tuberculosis, *Mycobacteria bovis*, Histopathology, Proportion, Ziehl-Neelsen.

## INTRODUCTION:

Bovine tuberculosis (bTB) manifests as a chronic granulomatous disease caused by *Mycobacterium bovis* (*M. bovis*), which is one of the most serious animal health problems in Egypt. It primarily affects the lungs and their lymph nodes but also affects other organs according to the route of the infection. The infection stays latent for months or years until the organ involvement is severe enough to induce functional impairment. The lesions may remain localized or may generalize to other tissues and organs (Domingo et al., 2014). About 51.6% and 33.2% of the indigenous output of red meat in Egypt comes from cattle and buffalo, respectively. The purchase of live animals from nations where bTB is common and endemic has

raised the annual frequency of bTB in cattle and buffalo, according to the General Organization of Veterinary Services' (GOVs, Egypt) official report (Abdellrazeq et al., 2016).

The important risk factors associated with bTB were herd size, poor husbandry and sanitary practices, the introduction of a new animal from an unknown source, season, sex, age, and animals having close contact with other animals (Abdul Basit et al., 2018).

Abattoir surveillance for bovine tuberculosis is crucial for early diagnosis of diseased animals prior to tuberculin planned dates, allowing for early control of disease spreading within and across herds (Shittu et al., 2013). The second arm of the surveillance system is post-mortem inspection, which is based on sufficient visual observation, palpation, and identification of suspected gross tuberculous lesions (McKinley et al., 2018). The hallmarks of classic TB lesions are characterized by different-sized, superficially or deeply located, caseous or calcified nodules protruding from the mucous or serous surface (Tulu et al., 2020).

The histomorphological feature of tuberculous granuloma is characterized by a central area of caseous necrosis, dystrophic calcification, intermediate zone of epithelioid cells, macrophages, Langhans cells, and peripheral fibrosis (Goswami et al., 2014). Histopathology is an early, rapid, and economical method to identify and classify microscopic lesions of bTB. As well as it demonstrates the presence of mycobacteria bacilli by Ziehl-Neelsen (Larenas-Muñoz et al., 2022).

The present study investigates statistical analysis and gross and histopathological alterations of bTB in various organs among slaughtered cattle and buffalo in Menofiya Governorate during 2021-2022.

## MATERIAL AND METHODS:

### ***Animals:***

The present study was conducted on 159 animals (91 cattle, 68 buffalo) over two years from January 2021 to December 2022 as grossly suspected positive cases from the total 5610 animals (3067 cattle, and 2543 buffalo) slaughtered during this period.

### ***Localities*:**

Examination and sampling were carried out at the abattoirs of Shebin El-Koom, Tala, EL Batanoun, El-Shohadaa, EL. Bagour, Ashmon, and Menouf in a survey in EL- Menofiya Governorate.

**Table (1):** Showing grossly suspected positive tuberculous cases from different slaughterhouses of EL- Menofiya Governorate from January 2021 to December 2022.

| Locality | Number of positive suspected tuberculous cases | |
|---|---|---|
| | Cattle | Buffalo |
| Shebin EL-Koom | 1 | 2 |
| Tala | 23 | 11 |
| EL Batanon | 1 | 1 |
| El-Shohada | 9 | 7 |
| EL. Baghour | 20 | 18 |
| Ashmoun | 25 | 19 |
| Menouf | 12 | 10 |
| **Total** | 91 | 68 |

***Sampling*:**

The samples were carefully inspected post-mortem in order to recognize apparent lesions suggestive of tuberculosis. They were obtained at the time of slaughter using a septic approach.

The tissue samples showing typical lesions of bTB collected from lungs, pleura, heart, liver, Intestine, mesentery, and lymph nodes (bronchial, mediastinal, hepatic, mesenteric, retropharyngeal, pre scapular, pre femoral, submandibular, parotid and supra mammary) were preserved in 10% formalin solution for histopathological investigation. All samples were given a serial number and detailed information such as the type of sample, date of sampling, clinical manifestation, and postmortem lesion.

***Histopathological investigation:***

Formalin-fixed tissue samples were routinely processed, embedded in paraffin wax, sectioned with a microtome (3-5µm thicknesses), and stained with hematoxylin and eosin (H&E) stain for histopathological investigation according to (Suvarna et al., 2018) and photographed by using Leica DMLB microscopes and a Leica EC3 digital camera.

***Ziehl-Neelsen, Acid Fast Staining Technique:***

The BTB-suggestive lesions were collected and stained with Ziehl-Neelsen's stain, as described by (Ahmad et al., 2017). The stained slides were viewed under an x100 objective lens of a light microscope to determine the presence and morphology of acid-fast bacilli. The presence of typical pinkish rod-shaped organisms under a blue background (when methylene blue was used as a counter stain) indicated acid-fast positivity and, hence, confirmatory of bTB.

***Statistical analysis:***

Frequency tables with the obtained data were analyzed based on abattoir records of tuberculosis lesions from January 2021 to December 2022. The association between bTB infection and season, as well as the year, sex, and age of cattle and buffalo, was determined.

**RESULTS:**

***I-Post mortem finding:***

***1-Lymph nodes*** *(Figure 1)*

In this study, most of the slaughtered animals were in the late stage of tuberculosis and showed typical visible lesions (Stage IV of bTB granuloma). Animals showing severe emaciation with serous atrophy of fat and bulging of ribs (Fig.1a). The mediastinal lymph node of buffalo showing severe granulomatous lymphadenitis with multifocal granuloma on the cut surface (Fig. 1b). Additionally, the retropharyngeal lymph node of cattle showing enlargement with focal yellowish solid caseous nodule (Fig. 1c).

Following the incision of the affected mesenteric lymph nodes of buffalo, hemorrhage, caseation, and calcification replace the center of the sectioned node (Fig. 1d). As well as, White to yellow caseous material was also found in the pre-scapular lymph node of cattle (Fig. 1e).

***2-Other organs*** *(Figure 2)*

The thoracic cavity of slaughtered cattle carcass shows multifocal to coalescing pale yellow firm nodules of different sizes that are dispersed and adhered to the internal surface of the thoracic wall (Fig. 2a). The lungs of cattle had small localized granulomatous tubercles and the cut sections revealed a creamy white caseous material whose consistency

ranged from thick cream to crumbly cheese-like (Fig. 2b). Also, the heart of a tuberculous cow revealed small calcified granulomatous tubercle on cut surface (Fig. 2c).

Cut section of liver of cattle showing greyish yellow caseated nodule in the liver parenchyma (Fig. 2d). Additionally, the buffalo lung displayed miliary tuberculosis (Pearl disease) with numerous small tubercles of the same size that resembled millet seeds scattered throughout the surface (Fig. 2e). As well as the liver of buffalo showing miliary tuberculosis with diffuse multifocal whitish nodules of uniform size and shape on the surface (Fig. 2f).

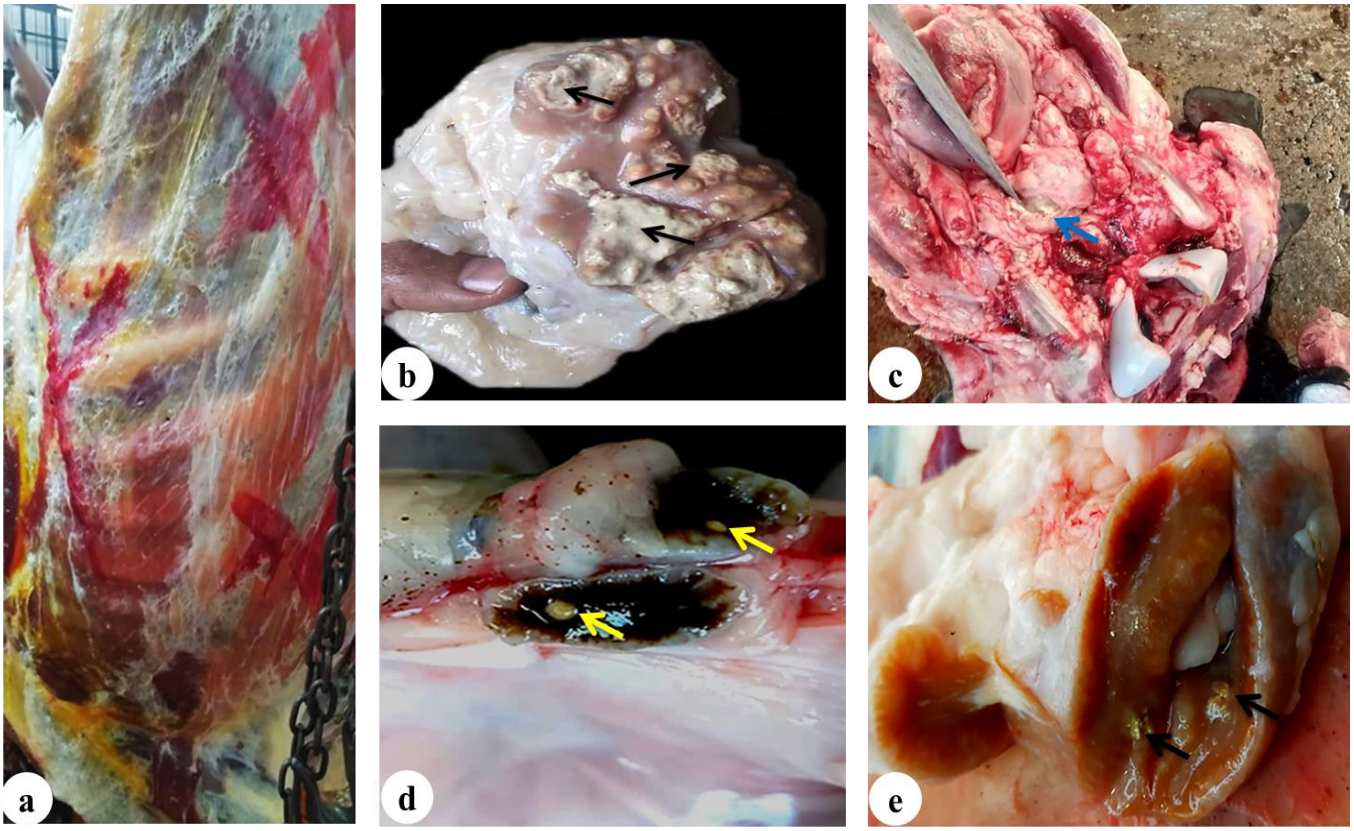



**Fig. 1a)** Slaughtered buffalo carcass: Showing severe emaciation with serous atrophy of fat and bulging of ribs. **Fig.1b)** Mediastinal LN, Buffalo: Showing severe granulomatous lymphadenitis with multifocal granuloma on the cut surface (arrows). **Fig. 1c)** Retropharyngeal LN, cattle: Showing enlargement with focal yellowish solid caseous nodule (blue arrow). **Fig. 1d)** Mesenteric LN, Buffalo: Showing hemorrhage, caseated and calcified tubercle that replaces the center of the sectioned node (yellow arrows). **Fig. 1e)** Pre-scapular lymph node, Cattle: Showing whitish to yellow caseous material during cut surface (arrows).

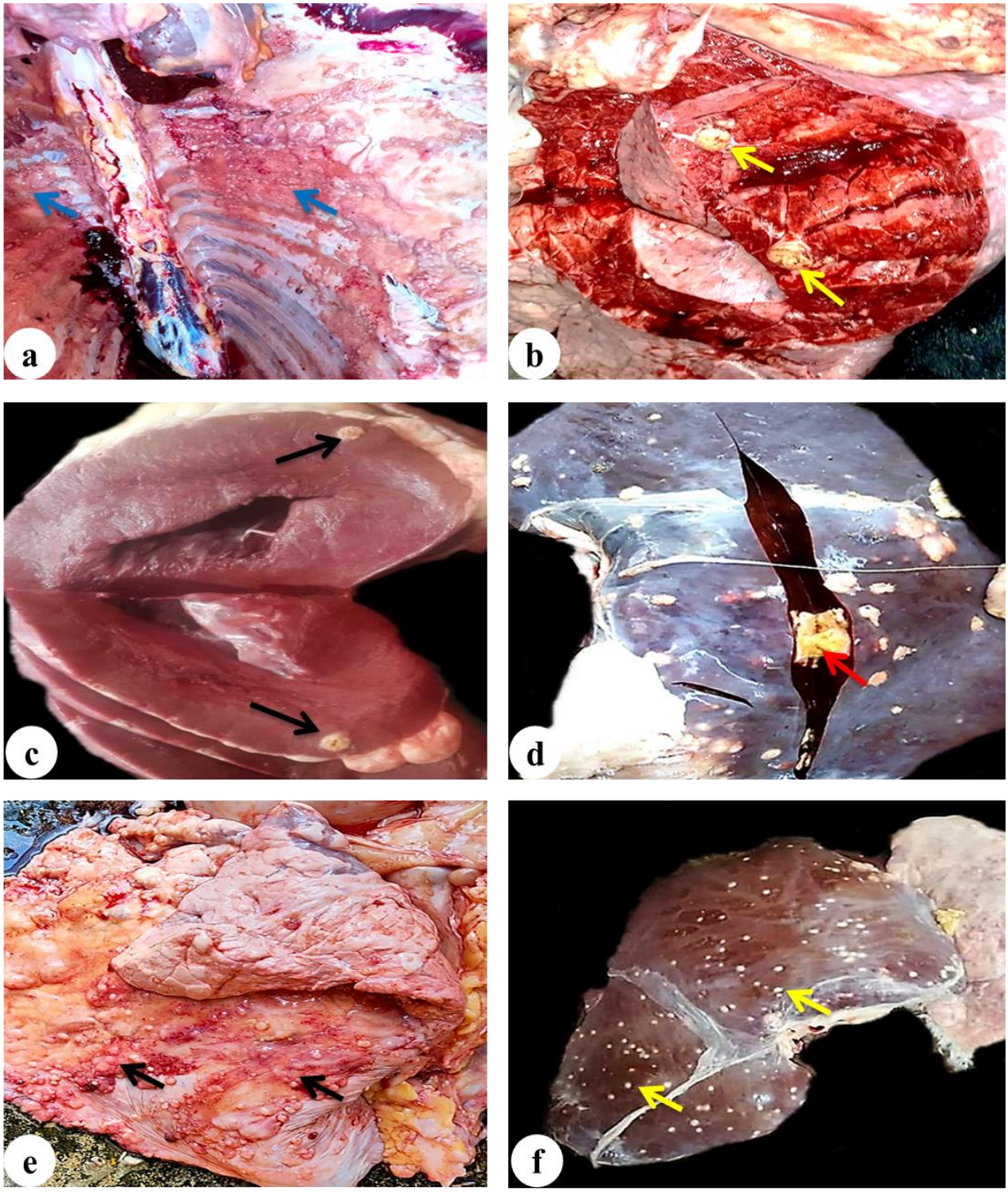



**Fig. 2a)** Thoracic wall, Cattle: Showing multifocal to coalescing pale yellow, firm nodules of different sizes (blue arrows). **Fig. 2b)** Lung, Cattle: Showing small localized granulomatous tubercles and the cut section revealed a creamy white caseous material whose consistency ranged from thick cream to crumbly cheese-like (yellow arrows). **Fig. 2c)** Heart, Cattle: Showing small calcified granulomatous tubercle on cut surface (black arrows). **Fig. 2d)** Liver, Cattle: Showing grayish yellow caseated nodule in the liver parenchyma (red arrow). **Fig. 2e)** Lung, Buffalo: Showing miliary tuberculosis (Pearl disease) with numerous small tubercles of the same size that resembled millet seeds scattered throughout the surface (black arrows). **Fig. 2f)** Liver, Buffalo: Showing diffuse multifocal whitish nodules of uniform size and shape (miliary tuberculosis) on the surface (yellow arrows).

***II- Histopathological findings:***

***1-Lymph nodes*** *(Figure 3)*

Histopathological examination of the right bronchial lymph node of cattle revealed stage I of bTB granuloma (Initial stage) which is characterized by rounded non-capsulated focal proliferation of chronic inflammatory cells mainly lymphocytes, epithelioid cells, macrophages and immature Langhans giant cell with absence of necrosis (Fig. 3a). Also the mesenteric lymph node of cattle exhibited stage II of bTB granuloma that resembles stage I with nodular to irregular accumulations of epithelioid cells, macrophages, lymphocytes and multinucleated giant cells of Langhans type with minimal amounts of central necrosis (Fig. 3b). The necrotic or solid granuloma (Stage III of bTB granuloma) were observed in the hepatic lymph node of buffalo which is more regular in shape and appeared as fully encapsulated granulomas with eosinophilic central caseous necrosis surrounded by a zone of epithelioid macrophages admixed with Langhans cells and lymphocytes with thin layer of fibrous capsule (Fig. 3c). As well as the pre-femoral LN of cattle revealed thick fibrous connective tissue capsule surrounding irregular granulomas with eosinophilic caseous necrosis and several islands of basophilic dystrophic calcification of stage IV of bTB granulomas on the stereo microscope (Fig. 3d). The mediastinal lymph node of cattle showed acute miliary tuberculosis which appeared as irregular numerous small tuberculous nodules distributed in tissue that are infiltrated by chronic inflammatory cells without peripheral fibrosis (Fig. 3e). The pre-femoral lymph node stained with ZN stain demonstrate numerous positive free acid-fast bacilli which is straight or curved arranged singly or in pairs, colored red and cluster on the methylene blue background (Fig. 3f).

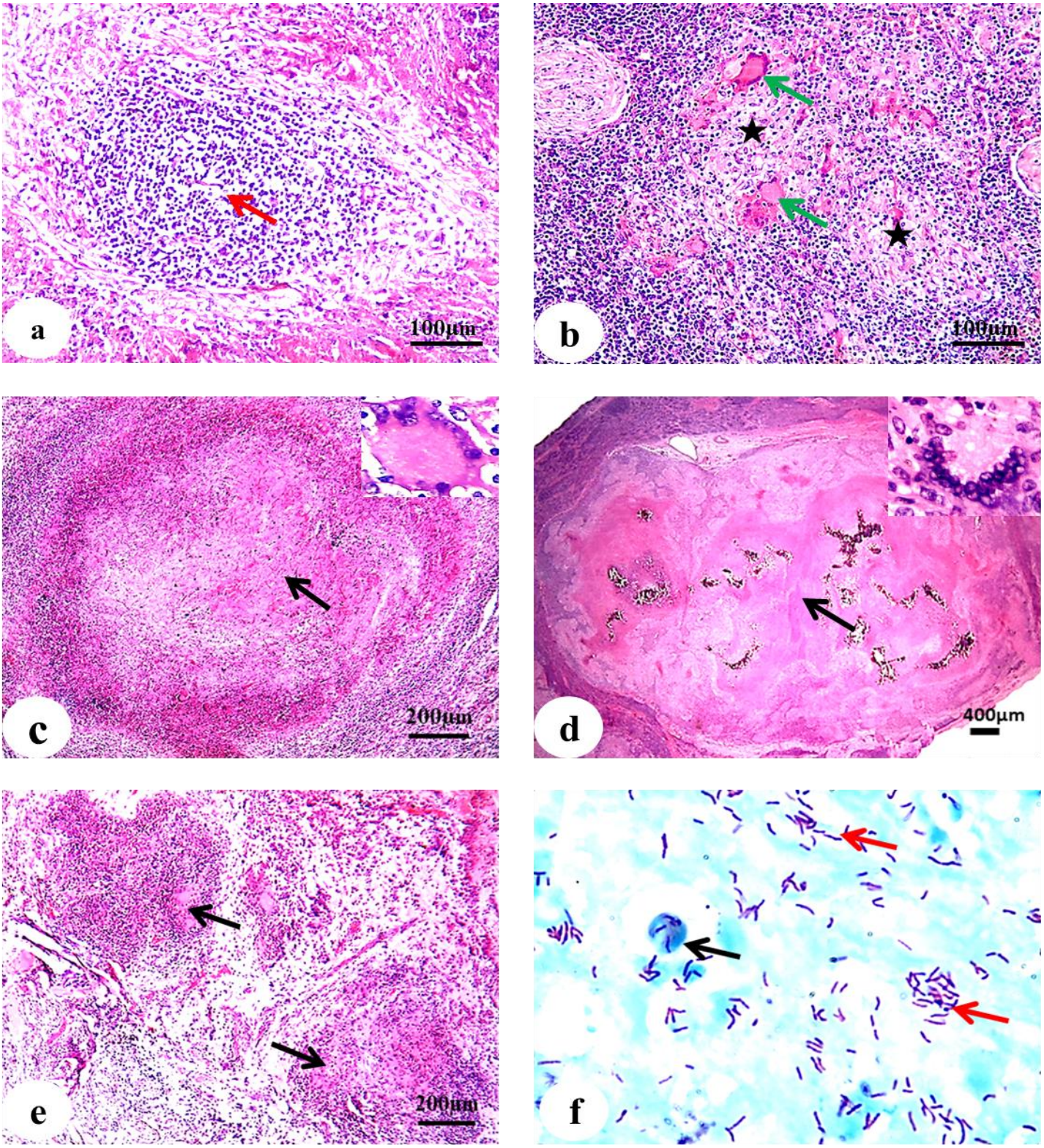



**Fig. 3.** Lymph nodes: a) Right bronchial LN, Cattle: Showing stage I of bTB granuloma (initial stage) (red arrow) with the non-capsulated rounded focal proliferation of chronic inflammatory cells mainly lymphocytes, epithelioid cells, macrophages, and Langhans giant cells with the absence of necrosis, Bar =100µm. **b)** Mesenteric LN, Cattle: Showing stage II of bTB granuloma, which is characterized by nodular to irregular accumulations of epithelioid macrophages, lymphocytes, and Langhans giant cells also present (green arrows) with minimal amounts of central

necrosis (star), Bar =100µm. **c)** Hepatic LN, Buffalo: Showing stage III of bTB granulomas (necrotic or solid stage) (black arrow), which are more regular in shape and appear as fully encapsulated granulomas with eosinophilic central necrotic cores surrounded by a zone of chronic inflammatory cells, Bar =200µm. **d)** Pre femoral LN, Cattle: Showing stage IV (necrotic and mineralized) (black arrow) of bTB granulomas, which are characterized by thick fibrous capsule surrounding granulomas with caseous necrosis and several islands of basophilic dystrophic calcification, Stereo microscope, Bar =400µm. **e)** Mediastinal LN, Cattle: Showing acute miliary TB that appeared as irregular numerous small tuberculous nodules distributed in tissue that infiltrated by chronic inflammatory cells without outer fibrosis (black arrows), Bar =200µm, (H&E Stain). **f)** Pre femoral LN, Cattle: Showing numerous positive free acid-fast bacilli that is straight or curved arranged singly or in pairs, Colored red and cluster on the methylene blue background (red arrows) as well as the macrophage containing intracytoplasmic acid-fast bacilli (black arrow), ZN stain, x100 oil immersion.

***2- Lung (Figure 4)***

The buffalo lung exhibited stage I of bTB granuloma, which is characterized by focal proliferation of chronic inflammatory cells with Langhans giant cells and absence of central necrosis (Fig. 4a). Additionally, nodular to irregular accumulations of epithelioid cells, macrophages, lymphocytes, and immature Langhans giant cells were evident and associated with minimal amounts of necrosis of stage II of bTB granuloma (Fig. 4b).

As well as old tubercle was recorded between the lung alveoli with a central area of necrosis, which was surrounded by an intermediate zone of chronic inflammatory cells and thin fibrous connective tissue capsule of the necrosed stage granuloma (stage III of bTB granuloma) (Fig. 4c). The caseo-calcified stage of bTB granuloma which appeared as large old completely encapsulated tubercle between the lung alveoli of buffalo was observed in (Fig. 4d). The lung of cattle demonstrate Langhans giant cell with intracytoplasmic abundant tuberculous acid-fast bacilli (Fig. 4e). Also, the lung of cattle exhibited positive long filamentous free bacilli inside the alveoli which is dark red (Fig. 4f).

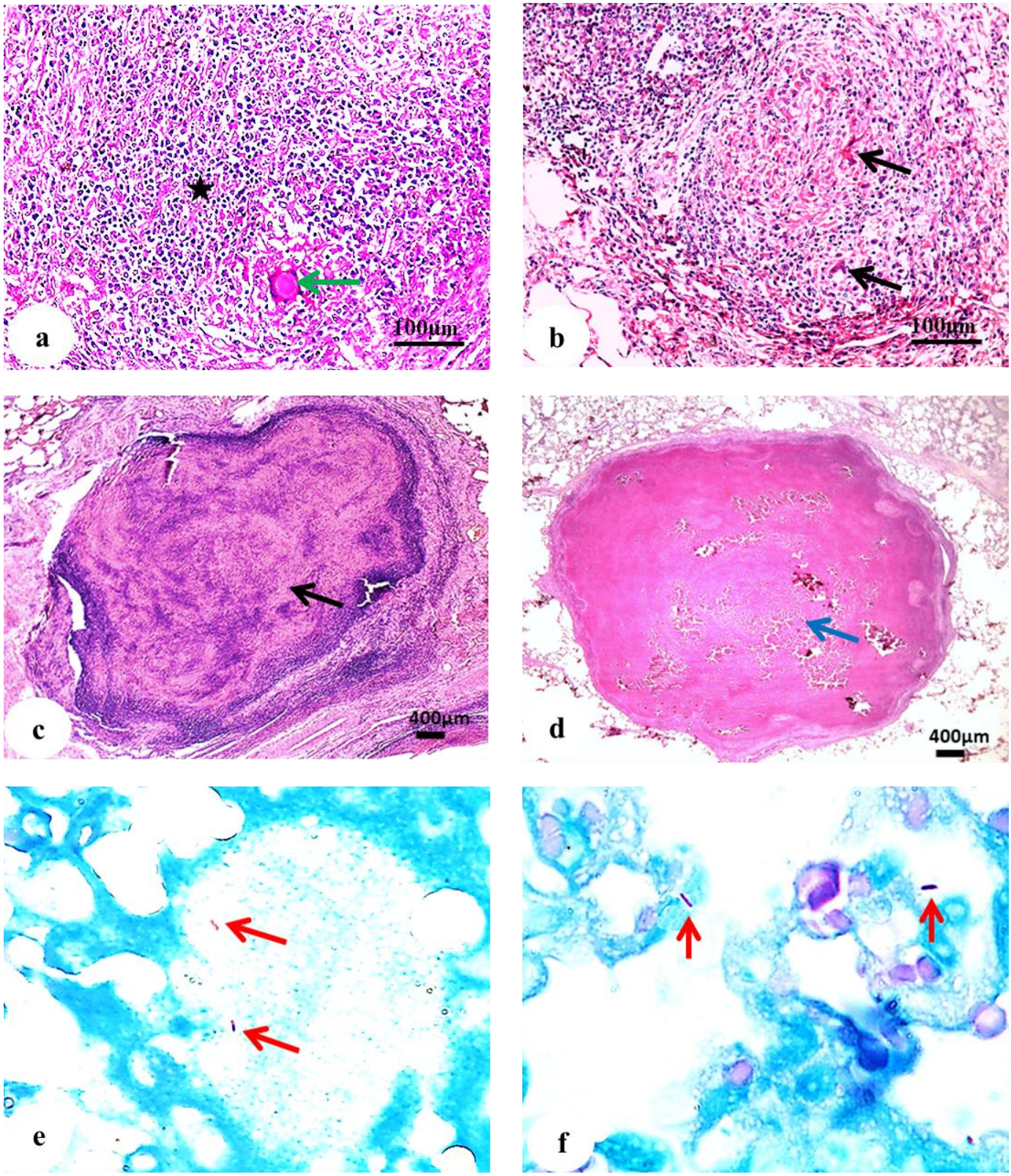



**Fig. 4.** Lung: **a)** Buffalo: Showing stage I of bTB granuloma, which is characterized by focal proliferation of chronic inflammatory cells (star) with Langhans giant cell (green arrow) and absence of central necrosis, Bar =100µm. **b)** Cattle: Showing nodular to irregular accumulations of epithelioid cells, macrophages, lymphocytes, and immature Langhans giant cells (black arrows) and associated with minimal amounts of necrosis of stage II of bTB granuloma, Bar =100µm. **c)** Cattle: Showing

old tubercle between the lung alveoli of stage III of bTB granuloma (black arrow), Stereo microscope, Bar =400µm. **d)** Buffalo: Showing large old completely encapsulated tubercle (caseo-calcified stage) (blue arrow), Stereo microscope, Bar =400µm. **e)** Cattle: Showing Langhans giant cell with intracytoplasmic abundant tuberculous acid-fast bacilli (red arrows), ZN stain, x100 oil immersion. **f)** Cattle: Showing positive long filamentous free bacilli in the lung alveoli that is dark red (red arrows), ZN stain, x100 oil immersion.

***3-Heart and pleura (Figure 5)***

Histopathological examination of the heart of cattle on stereo microscope demonstrates well-formed myocardial stage IV of bTB granuloma with dark purple mineralization, Central eosinophilic caseous necrosis and thin fibrous connective tissue (Fig. 5a). When examined on light microscope exhibited abundant horseshoe shape Langhans giant cell close to an area with calcium deposits and surrounded by zone of chronic inflammatory cells with variable numbers of acid-fast bacilli (inset) (Fig. 5b). The pleura of cattle showing multiple scattered small tubercles on the stereo microscope (Fig. 5c). On the light microscope showing a large basophilic calcified material that surrounded by caseous necrosis and scattered inflammatory cells with acid-fast bacilli are present within the necrotic caseum (inset) (Fig. 5d).

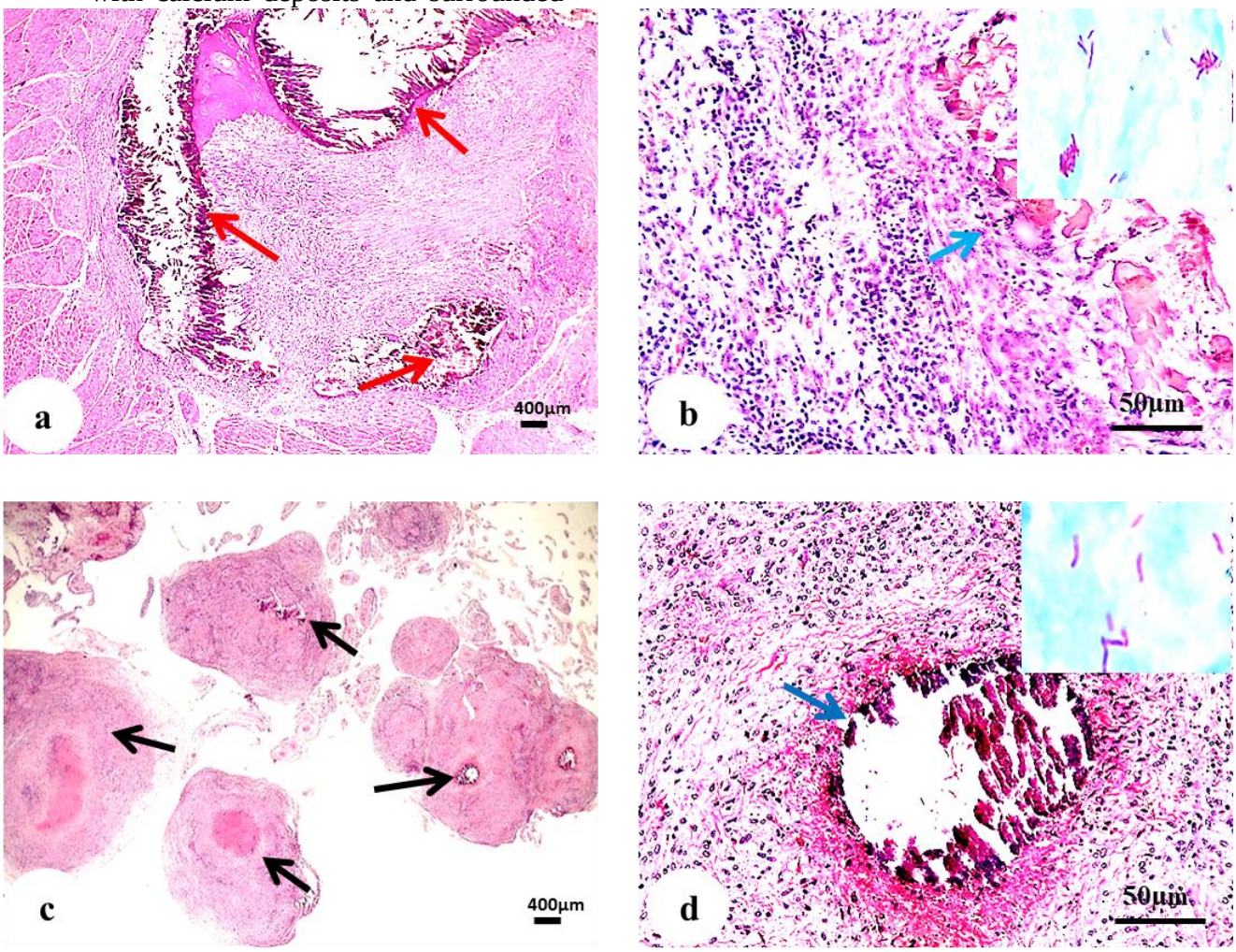



**Fig. 5.** Cattle: **a)** Heart: Showing well-formed myocardial stage IV of bTB granuloma with dark purple mineralization, Central eosinophilic caseous necrosis, and thin

fibrous connective tissue (red arrows), Stereo microscope, Bar =400µm. **b)** Higher magnification of fig. a, showing abundant horseshoe shape Langhans giant cell (blue arrow) close to an area with calcium deposits and surrounded by a zone of chronic inflammatory cells (H&E stain), Bar =50µm. Inset: Showing variable numbers of acid-fast bacilli, ZN stain, x100 oil immersion. **c)** Pleura: Showing multiple scattered tubercles (black arrows), Stereo microscope, Bar =400µm. **d)** Higher magnification of fig. c, showing a large basophilic calcified nodule (blue arrow), (H&E stain), Bar =50µm, Inset: Showing acid-fast bacilli are present within the necrotic caseum, ZN stain, x100 oil immersion.

***4-Liver (Figure 6)***

Histopathological examination of the liver of cattle exhibited 1st stage of bTB granuloma (non-necrotizing granuloma), which is characterized by focal proliferation of inflammatory cells, mainly lymphocytes, macrophages, and Langhans giant cells (Fig. 6a). As well as the liver of buffalo revealed stage II of bTB granuloma that appeared as minimal central necrosis surrounded by intermediated zone of chronic inflammatory cells and in complete outer fibrosis (Fig. 6b).

Additionally, the necrotic stage of bTB granuloma in cattle was recorded in (Fig. 6c) which is characterized by tubercles with variable amounts of caseous necrosis and an increased number of inflammatory cells, abundant Langhans cells surrounded by fibrous capsules, and the granulomas were more regular in shape. Stage IV of bTB granuloma, which was examined in the stereo microscope and appeared as a large old encapsulated calcified tubercle in liver tissue, was observed in (Fig. 6d).

The liver displayed miliary tuberculosis associated with a small nodular tuberculous lesion in liver tissue with the proliferation of solid pink Langhans giant cells in the center, macrophages, lymphocytes and epithelioid cells without fibrous connective tissue capsule and also severe hydropic degeneration of hepatic cells with congested hepatic sinusoids (Fig.6e). Additionally the liver of buffalo stained with ZN staining revealed positive acid-fast rod-shaped bacilli of TB (Fig.6f).

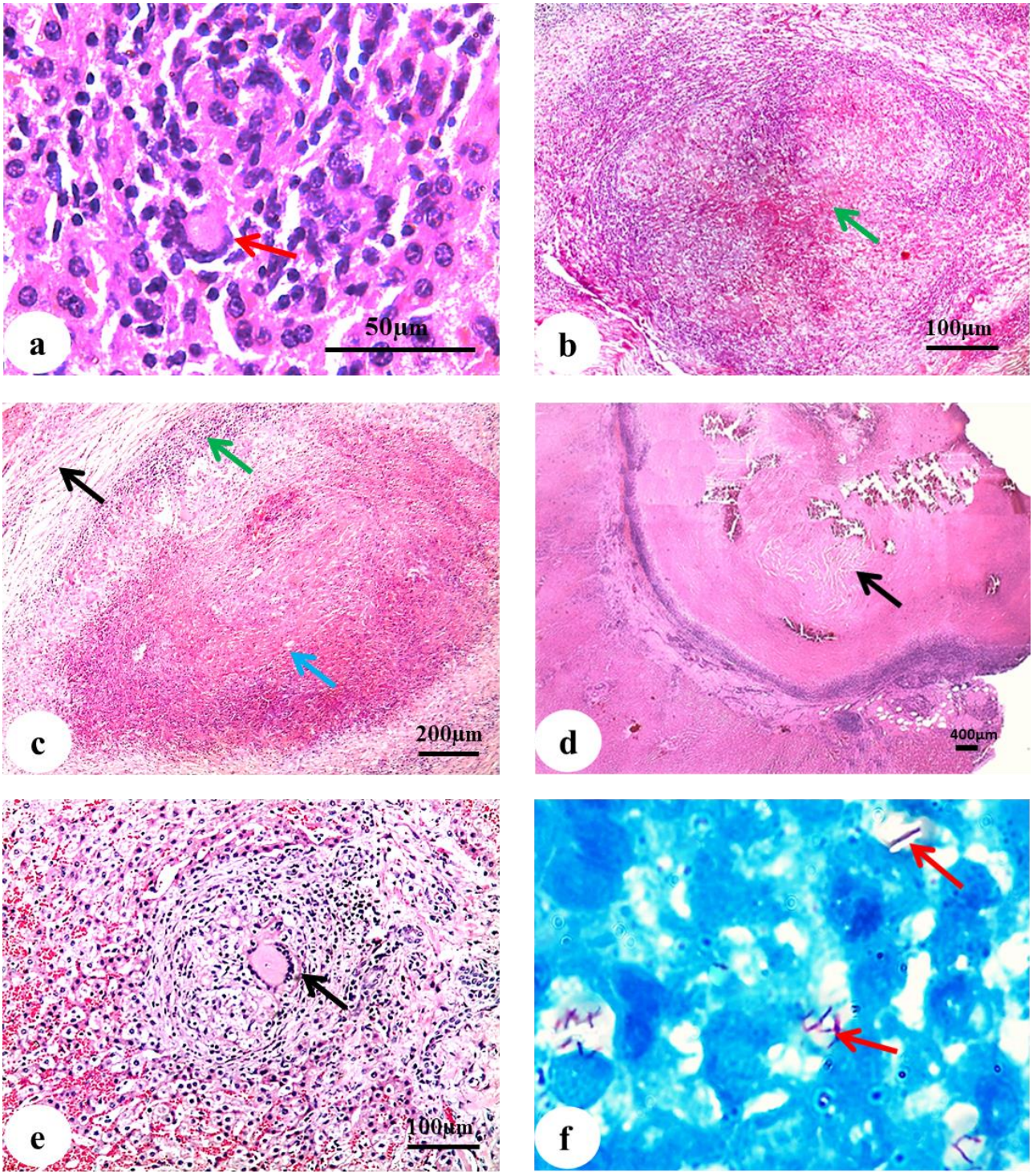



**Fig. 6.** Liver: **a)** Cattle: Showing 1st stage of bTB granuloma with focal proliferation of inflammatory cells, mainly lymphocytes, macrophages, and Langhans giant cell (red arrow), Bar =50µmtubercles with variable amounts of caseous necrosis and an increased number of inflammatory cells, abundant Langhans cells surrounded by fibrous capsules. **b)** Buffalo: Showing stage II of bTB granuloma (green arrow), which is characterized by minimal central necrosis surrounded by an intermediated zone of chronic inflammatory cells and incomplete outer fibrosis, Bar =100µm. **c)** Cattle: Showing 3rd stage of bTB granuloma (blue arrow) which surrounded by intermediate zone of chronic inflammatory cells (green arrow) and fibrous capsule (black arrow), Bar =200µm. **d)** Cattle: Showing caseo-calcified stage (stage IV of

bTB granuloma), which is characterized by large old, encapsulated tubercle in liver tissue (black arrow) (H&E stain), Stereo microscope, Bar =400µm. **e)** Cattle: Showing hepatic miliary tuberculosis (black arrow), Bar =100µm. **f)** Buffalo: Showing acid-fast bacilli of TB (red arrows), ZN stain, x100 oil immersion.

***5-Intestine and mesentery (Figure 7)***

Histopathological investigation of the intestine of buffalo revealed central dystrophic calcification (dark purple color) and eosinophilic caseous necrosis of stage IV of bTB granuloma, which is surrounded by lymphocytes, plasma cells, epithelioid cells, macrophages, and numerous multinucleated giant cells of a Langhans type as showed in (Fig. 7a). As well as another section of intestine exhibited enteritis with thickening and fusion of the intestinal villi, desquamated epithelium in lumen and inflammatory cells infiltration with hemorrhage at the tip of villi was also recorded as seen in (Fig. 7b).

The Intestinal mesentery of buffalo exhibited focal proliferation of chronic inflammatory cells and active Langhans giant cells with dark red dystrophic calcification as seen in (Fig. 7c & d), as well as aggregation of bacterial colonies (dark blue color) as seen in (Fig. 7e). Staining the intestinal mesentery with ZN stain revealed tuberculous bacilli (numerous positive free acid-fast bacilli), the bacilli straight or curved arranged singly or in pairs, colored red and cluster on the methylene blue background (Fig. 7f).

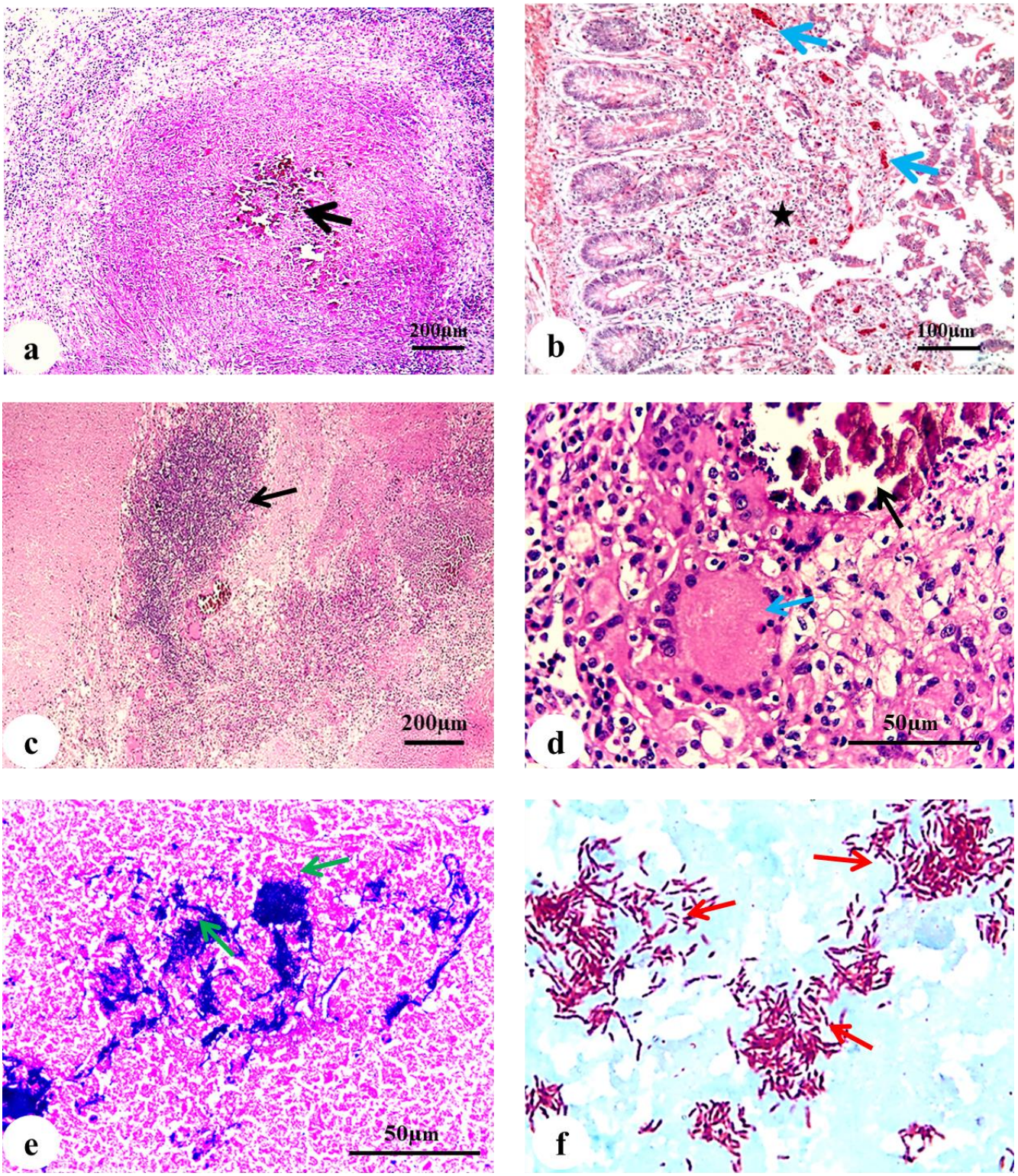



**Fig. 7.** Buffalo: **a)** Intestine: Showing central dystrophic calcification (dark purple color) and eosinophilic caseous necrosis of stage IV of bTB granuloma (black arrow), which is surrounded by chronic inflammatory cells and peripheral fibrosis, Bar =200µm. **b)** Intestine: Showing enteritis, thickening, a fusion of the intestinal villi (star) with hemorrhage at the tip of villi (blue arrows), desquamated epithelium in the lumen, and inflammatory cells infiltration, Bar =100µm. **c)** Intestinal mesentery: Showing focal proliferation of chronic inflammatory cells (black arrow) and

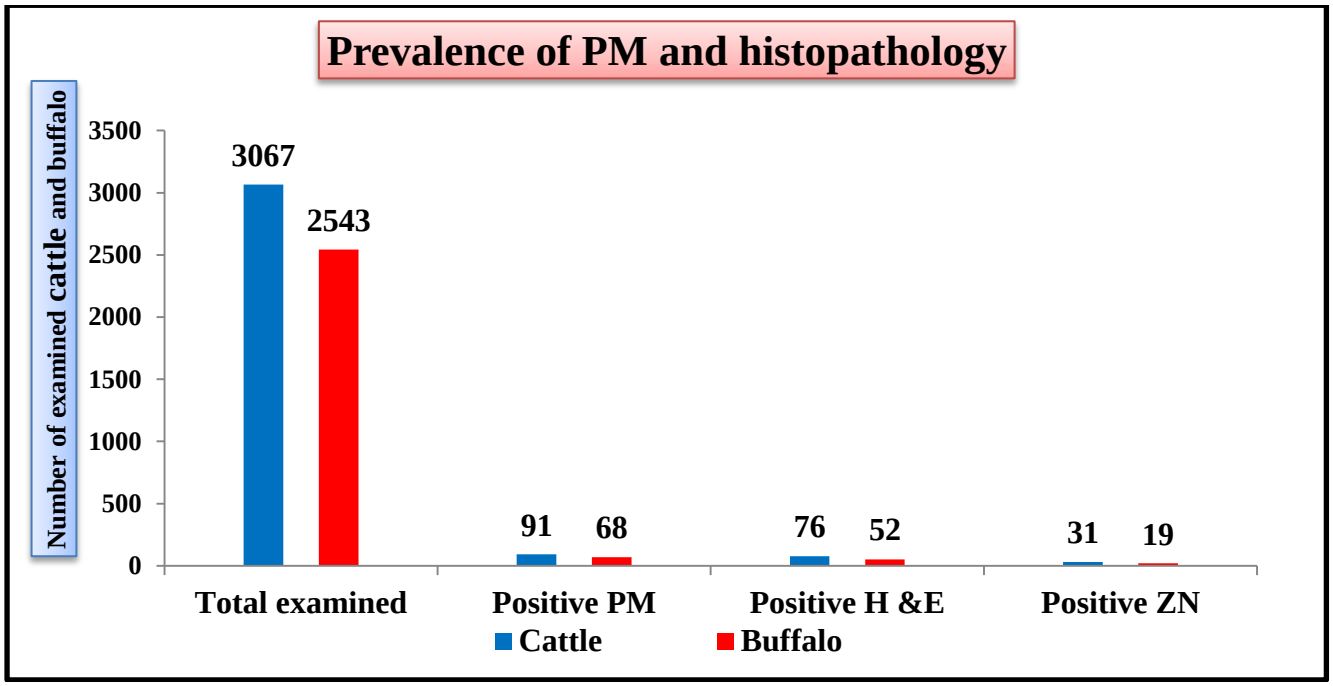
numerous multinucleated giant cells of a Langhans type with dark red dystrophic calcification, Bar =200µm. **d)** Higher magnification of fig. c, showing dystrophic calcification (black arrow) and active Langhans giant cell (blue arrow), in which phagocytosed lymphoid cells suffered from ballooning degeneration, Bar =50µm. **e)** Intestinal mesentery: Showing aggregation of bacterial colonies (dark blue color) (green arrows), H&E Stain, Bar =50µm. **f)** Intestinal mesentery: Showing numerous positive free acid-fast bacilli (red arrows), ZN stain, x100 oil immersion.

***III- bBT data analysis:***

Table (2) and Histogram (1): Revealed the proportion of bovine tuberculosis according to histopathology and Ziehl Neelsen staining of lymph nodes and organ tissues. Of the total positive PM cases (159 positive PM cases [91 cattle and 68 buffalo]), only 76 (83.51%) cattle and 52 (76.47%) buffalo carcasses were positive by H&E stain. In addition, 31 out of 76 cattle and 19 out of 52 buffalo carcasses were positive for ZN, with a percentage of 40.78% and 36.53%, respectively.

**Table (2):** Showing the proportion of bovine tuberculosis according to PM and histopathology of lymph nodes and organ tissues from total examined slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021-2022.

| Animal | Total examined | PM Positive | % | H & E stain Positive | % | ZN stain Positive | % |
|---|---|---|---|---|---|---|---|
| **Cattle** | 3067 | 91 | 2.96% | 76 | 83.51% | 31 | 40.78% |
| **Buffalo** | 2543 | 68 | 2.67% | 52 | 76.47% | 19 | 36.53% |
| **Total** | 5610 | 159 | 2.83% | 128 | 80.5% | 50 | 39.06% |

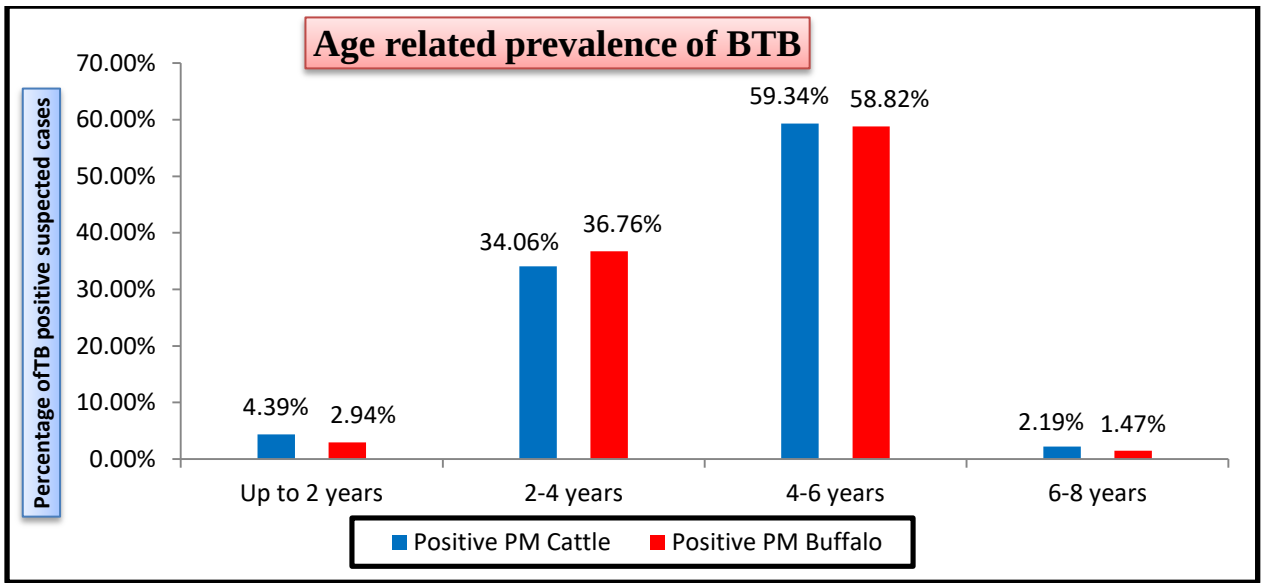



**Histogram (1):** Showing the proportion of bovine tuberculosis according to PM and histopathology of lymph nodes and organ tissues from total examined slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021-2022.

Table (3) and Histogram (2): Showing the relationship between age, rate of infection, and PM findings of bTB in slaughtered cattle and water buffalo. Our research on the relationship between risk variables and bovine tuberculosis determined that age was a significant risk factor for bTB in cattle and buffalo. Risk increased significantly with age, increasing to 6 years, and then declined. Out of 159 animal carcasses, 91 cattle and 68 buffalo carcasses presenting visible lesions of TB, only 4 (4.39%) cattle (up to 2 years old) and 2 (2.94%) water buffalo at the same age demonstrate visible lesions. In addition, 31 (34.06%) cattle and 25 (36.76%) buffalo carcasses aged 2-4 years display visible lesions. Meanwhile, 54 (59.34%) cattle and 40 (58.82%) water buffalo aged 4-6 years revealed apparent lesions. Additionally, 2 (2.19%) cattle and 1 (1.47%) water buffalo aged 6-8 years tested positive for postmortem examination and displaying visible lesions.

**Table (3):** Showing the relationship between age, PM findings, and histopathology of bTB in slaughtered cattle and buffalo in a survey in Menofiya Governorate between 2021 and 2022.

| Places of collected samples | Age | Animal | +ve PM Finding | % | +ve H&E stain | % | +ve ZN stain | % |
|---|---|---|---|---|---|---|---|---|
| Menofiya Governorate (within two years) | Up to 2 years | Cattle | 4 | 4.39% | 3 | 75.00% | 2 | 66.66% |
| | | Buffalo | 2 | 2.94% | 1 | 50.00% | 1 | 100% |
| | (2-4 years) | Cattle | 31 | 34.06% | 23 | 74.19% | 8 | 34.78% |
| | | Buffalo | 25 | 36.76% | 16 | 64% | 7 | 43.75% |
| | (4-6 years) | Cattle | 54 | 59.34% | 48 | 88.88% | 20 | 41.66% |
| | | Buffalo | 40 | 58.82% | 35 | 87.50% | 11 | 31.42% |
| | (6-8 years) | Cattle | 2 | 2.19% | 2 | 100% | 1 | 50% |
| | | Buffalo | 1 | 1.47% | 0 | 0.00% | 0 | 0.00% |
| | | Total | 159 (91 cattle) (68 buffalo) | 100% | 128 (76 cattle) (52 buffalo | 80.5% | 50 (31 cattle) (19 buffalo) | 39.06% |

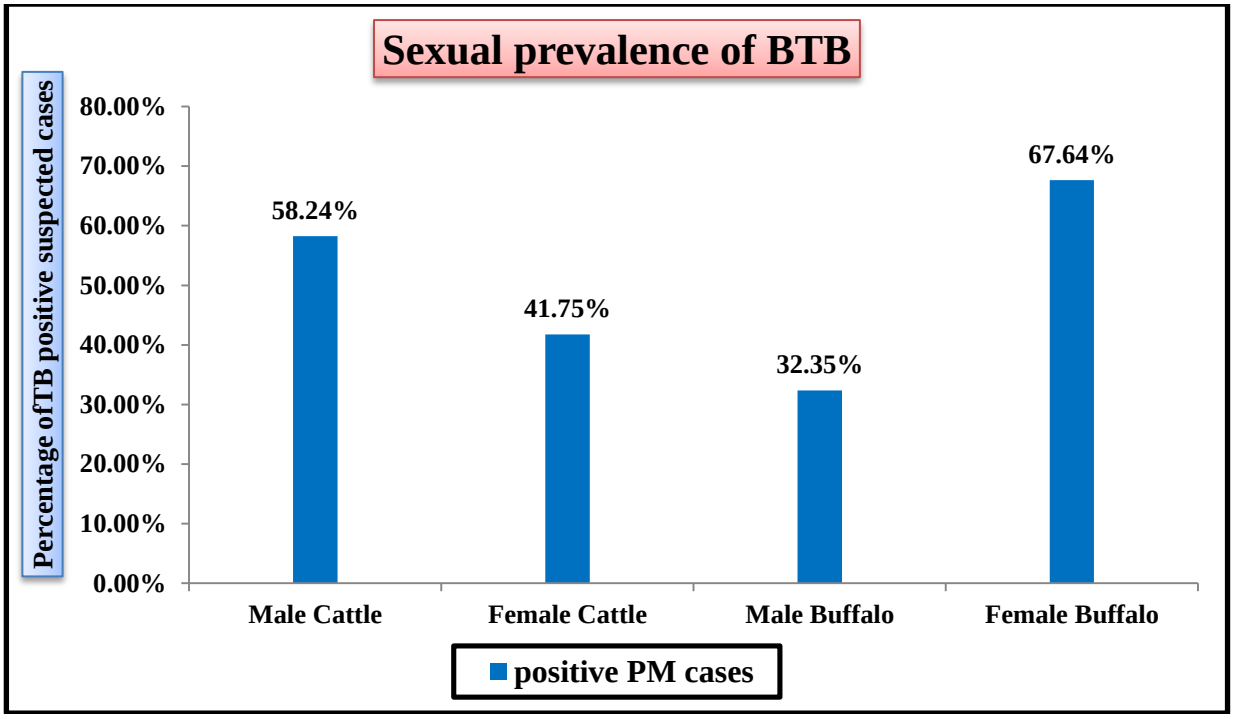



**Histogram (2):** Showing the relationship between age, PM findings, and histopathology of bTB in slaughtered cattle and buffalo in a survey at El-Menofiya Governorate between 2021 and 2022.

Table (4) and Histogram (3): Indicate The frequency of bovine tuberculosis among examined slaughtered cattle and water buffalo with respect to sex. Among 159 carcasses with visible TB lesions, the proportion of bovine tuberculosis in females buffalo 46 (67.64%) was higher than the proportion in the males 22 (32.35%), While in cattle, males 53 (58.24%) had a considerably greater frequency of bovine tuberculosis than females 38 (41.75%).

**Table (4):** Showing sexual proportion of bovine tuberculosis in slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021-2022.

| Places of collected samples | Sex | Animal | +ve PM finding | % | +ve H&E stain | % | +ve ZN stain | % |
|---|---|---|---|---|---|---|---|---|
| Menofiya Governorate (within two years) | Female | Cattle | 38 | 41.75% | 29 | 76.31% | 8 | 27.58% |
| | | Buffalo | 46 | 67.64% | 40 | 86.95% | 14 | 35.00% |
| | Male | Cattle | 53 | 58.24% | 47 | 88.67% | 23 | 48.93% |
| | | Buffalo | 22 | 32.35% | 12 | 54.54% | 5 | 41.66% |
| Total | | | 159 (91 cattle) (68 buffalo) | 100% | 128 | 80.5% | 50 | 39.06% |




**Histogram (3):** Showing sexual proportion of bovine tuberculosis in slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021-2022.

Table (5): Indicate the distribution and occurrence of the tuberculous gross lesions among different lymph nodes and organ tissue in positive 91 cattle and 68 buffalo carcasses on the tissue section level. TB grossly lesions were mostly detected in the thoracic cavity, with a high proportion in the lung and its lymph nodes 79 (49.68%). Additionally, bTB lesions were observed in other LN and organs, as shown in (Table 5).

**Table (5):** Showing the distribution of the tuberculous lesions among various organs and lymph nodes of slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021-2022.

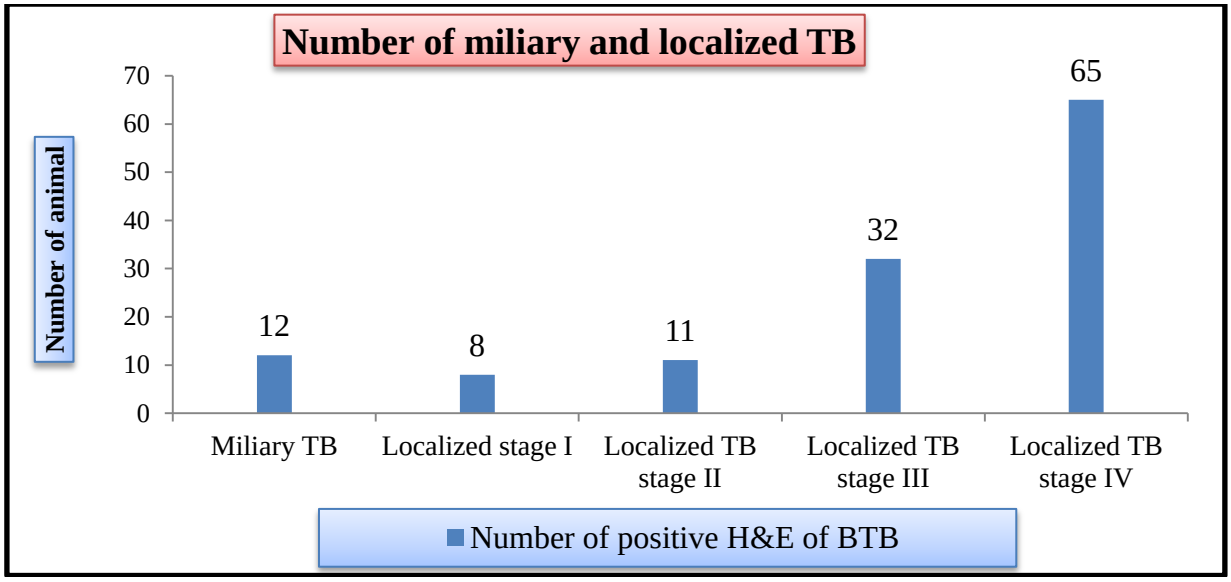
| Organs | Positive PM finding | | | Microscopic examination (+ve H&E Stain) | | | | +ve ZN Stain | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cattle | Buffalo | Total | Cattle | Buffalo | Total | | Cattle | Buffalo | Total | |
| | | | | | | NO | % | | | NO | % |
| **Lung** | 17 | 14 | 31 | 13 | 12 | 25 | 80.64% | 6 | 5 | 11 | 44% |
| **Bronchial LN** | 12 | 10 | 22 | 9 | 8 | 17 | 77.27% | 2 | 2 | 4 | 23.52% |
| **Mediastinal LN** | 15 | 11 | 26 | 11 | 10 | 21 | 80.76% | 2 | 3 | 5 | 23.81% |
| **Hepatic LN** | 8 | 7 | 15 | 6 | 6 | 12 | 80% | 2 | 1 | 3 | 25% |
| **Liver** | 9 | 7 | 16 | 8 | 5 | 13 | 81.25% | 3 | 2 | 5 | 38.46% |
| **Mesenteric LN** | 4 | 3 | 7 | 4 | 2 | 6 | 85.71% | 1 | 1 | 2 | 33.33% |
| **Intestine and mesentery** | 3 | 2 | 5 | 3 | 2 | 5 | 100% | 2 | 1 | 3 | 60% |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Heart** | 2 | 1 | 3 | 1 | 0 | 1 | 33.33% | 1 | 0 | 1 | 100% |
| **Skeletal muscle** | 1 | 2 | 3 | 1 | 0 | 1 | 33.33% | 1 | 0 | 1 | 100% |
| **Pre-scapular LN** | 3 | 2 | 5 | 3 | 1 | 4 | 80% | 2 | 1 | 3 | 75% |
| **Pre-femoral LN** | 2 | 2 | 4 | 2 | 1 | 3 | 75% | 1 | 0 | 1 | 33.33% |
| **Retropharyngea LN** | 1 | 3 | 4 | 2 | 2 | 4 | 100% | 1 | 1 | 2 | 50% |
| **Supra mammary LN and M.G** | 2 | 1 | 3 | 1 | 0 | 1 | 33.33% | 1 | 0 | 1 | 100% |
| **Sub-mandibular LN** | 1 | 0 | 1 | 1 | 0 | 1 | 100% | 0 | 0 | 0 | 0% |
| **Parotid LN** | 1 | 1 | 2 | 1 | 1 | 2 | 100% | 0 | 0 | 0 | 0% |
| **Miliary TB** | 10 | 2 | 12 | 10 | 2 | 12 | 100% | 6 | 2 | 8 | 66.66% |
| **Total** | 91 | 68 | 159 | 76 | 52 | 128 | 80.5% | 31 | 19 | 50 | 39.06% |

Table (6) and histogram (4): Showing the number and incidence of military and localized tuberculosis in slaughtered cattle and buffalo based on the histopathological assessment of tissue sections in this study. The bTB was classified into miliary (generalized TB) 12 (9.37%) and localized (chronic organ TB), in which the granulomas were categorized into: stage I 8 (6.25%), stage II 11(8.59%), stage III 32 (25%), and stage IV 65 (50.78%).

**Table (6):** Showing the number and incidence of miliary and localized tuberculosis in slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021-2022.

| **Classification of bovine tuberculosis (microscopically)** | | **Number** | **Incidence (%)** |
|---|---|---|---|
| **Miliary tuberculosis** | | 12 | 9.37% |
| **Localized tuberculosis (Chronic organ tuberculosis** | **Stage I** | 8 | 6.25% |
| | **Stage II** | 11 | 8.59% |
| | **Stage III** | 32 | 25% |
| | **Stage IV** | 65 | 50.78% |
| **Total** | | 128 | 100% |




**Histogram (4):** showing the number of miliary and localized tuberculosis in slaughtered cattle and buffalo in Menofiya Governorate during 2021-2022.

The following data were obtained from the Directorate of Veterinary Medicine, slaughterhouse meat inspection, Shebin El Koom, Menofiya Governorate, from January 2021 to December 2022.

**Table (7):** Showing the proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo in Menofiya Governorate during 2021.

| The proportion of suspected bovine tuberculosis-positive cases during 2021 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Slaughterhouse** | **Total NO. of slaughtered cattle** | **NO. of suspected positive bTB in cattle** | **%** | **Total NO. of slaughtered buffalo** | **NO. of suspected positive bTB in buffalo** | **%** | **Total NO of slaughtered animals** | **Total NO of +ve bTB cases** | **%** |
| **Shebin EL Kom** | 1685 | 13 | .77% | 5822 | 42 | 0.72% | 7507 | 55 | 0.73% |
| **Tala** | 1492 | 31 | 2.07% | 1284 | 22 | 1.71% | 2776 | 53 | 1.91% |
| **EL Batanon** | 323 | 2 | .61% | 2458 | 17 | .69% | 2781 | 19 | 0.68% |
| **EL Shohada** | 4667 | 66 | 1.41% | 1201 | 22 | 1.83% | 5868 | 88 | 1.49% |
| **EL Baghour** | 6362 | 313 | 4.91% | 673 | 34 | 5.05% | 7035 | 347 | 4.93% |
| **Ashmon** | 2348 | 267 | 11.37% | 819 | 45 | 5.49% | 3167 | 312 | 9.85% |
| **Menouf** | 5117 | 130 | 2.54% | 788 | 27 | 3.42% | 5905 | 157 | 2.65% |
| **Total** | 21994 | 822 | 3.73% | 13045 | 209 | 1.60% | 35039 | 103 | 2.94% |

**Table (8):** Showing the proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo in Menofiya Governorate during 2022.

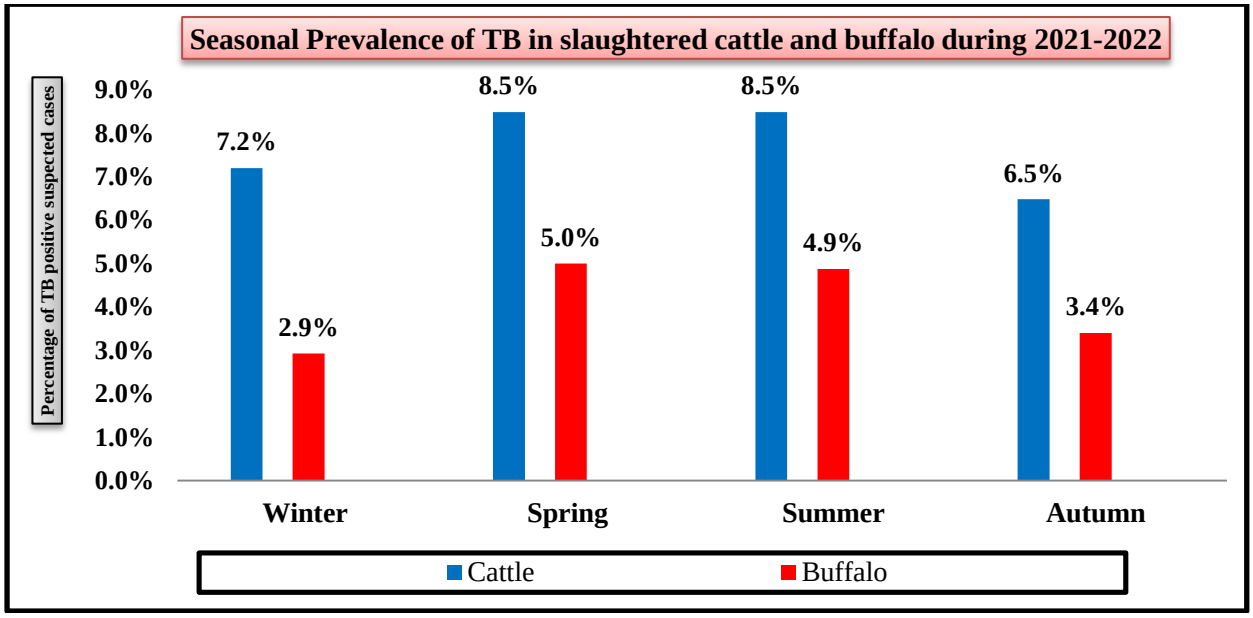
| The proportion of suspected bovine tuberculosis-positive cases during 2022 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Slaughterhouse** | **Total NO. of slaughtered cattle** | **NO. of suspected positive bTB in cattle** | **%** | **Total NO. of slaughtered buffalo** | **NO. of suspected positive bTB in buffalo** | **%** | **Total NO of slaughtered animals** | **Total NO of +ve bTB cases** | **%** |
| **Shebin EL Kom** | 1431 | 1 | 0.06% | 5567 | 18 | .32% | 6998 | 19 | 0.27% |
| **Tala** | 1050 | 51 | 4.85% | 1202 | 79 | 6.57% | 2252 | 130 | 5.77% |
| **EL Batanon** | 349 | 0 | 0% | 2162 | 6 | .27% | 2511 | 6 | 0.23% |
| **EL Shohada** | 3814 | 69 | 1.81% | 1380 | 30 | 2.17% | 5194 | 99 | 1.91% |
| **EL Baghour** | 5567 | 287 | 5.15% | 719 | 70 | 9.73% | 6286 | 357 | 5.67% |
| **Ashmon** | 1993 | 177 | 8.88% | 658 | 66 | 10.03% | 2651 | 243 | 9.16% |
| **Menouf** | 4990 | 175 | 3.51% | 665 | 37 | 5.56% | 5655 | 212 | 3.74% |
| **Total** | 19194 | 760 | 3.95% | 12353 | 306 | 2.47% | 31547 | 1066 | 3.37% |

Table (9) and Histogram (5): Indicate the seasonal proportion of suspected tuberculosis-positive cases in slaughtered cattle and buffalo. The seasonal variation of bovine tuberculous lesions was assessed based on abattoir records of tuberculosis lesions from January 2021 to December 2022. This study results revealed that bTB is present during all seasons, but with high frequency during spring (8.5% cattle and 5% buffalo) and summer (8.5% cattle and 4.9% buffalo) and lower frequency in winter (7.2% cattle and 2.9% buffalo) and autumn (6.5% cattle and 3.4% buffalo).

**Table (9):** Showing the seasonal proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo in L-Menofiya Governorate during 2021-2022.

| Seasonal proportion of suspected tuberculosis-positive cases during 2021-2022 | | | | | | |
|---|---|---|---|---|---|---|
| **Season** | **Percentage of bTB-positive suspected cases** | | | | | |
| | **Cattle** | | | **Buffalo** | | |
| | **2021** | **2022** | **Total** | **2021** | **2022** | **Total** |
| **Winter** | 3.2% | 4.0% | 7.2% | 1.5% | 1.4% | 2.9% |
| **Spring** | 4.1% | 4.4% | 8.5% | 1.5% | 3.5% | 5.0% |
| **Summer** | 4.3% | 4.2% | 8.5% | 2.2% | 2.7% | 4.9% |
| **Autumn** | 3.3% | 3.2% | 6.5% | 1.2% | 2.2% | 3.4% |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Total** | 14.8% | 15.8% | 30.6% | 6.4% | 9.8% | 16.2% |

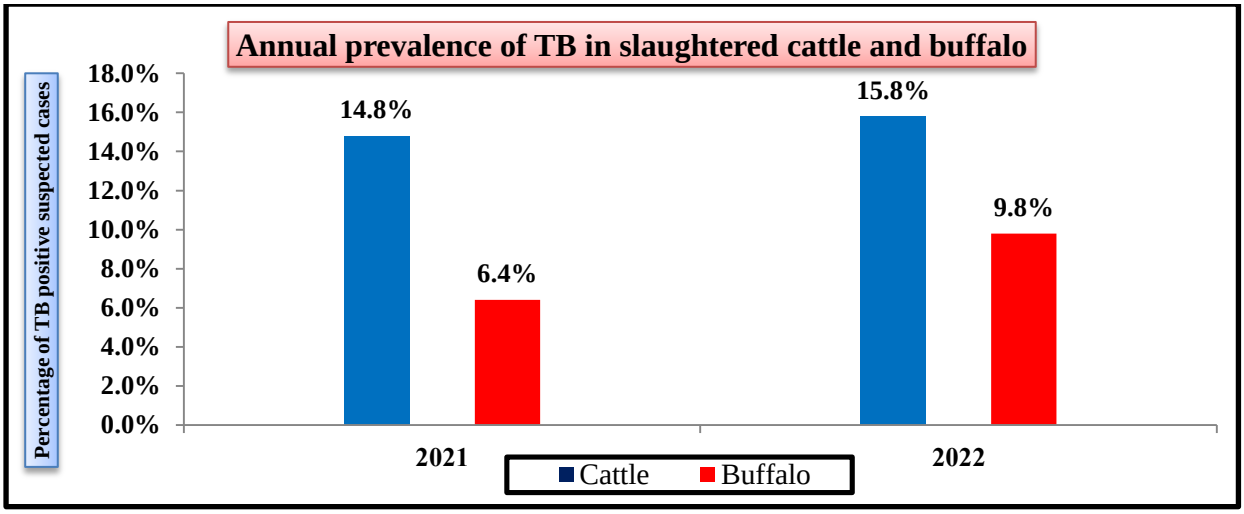



**Histogram (5):** Showing the seasonal proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo in a survey in Menofiya Governorate during 2021- 2022.

Table (10) and Histogram (6): Showing the annual proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo. The present study clarified that the highest yearly proportion of bTB-positive cases was observed in 2022 (15.8% cattle and 9.8% buffalo) than in 2021 (14.8% cattle and 6.4% buffalo). The overall annual proportion of TB for 2 years was significantly higher in cattle (30.6%) compared to buffalo (16.2%).

**Table (10):** Showing the annual proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo in Menofiya Governorate during 2021- 2022.

| **Annual proportion of TB-positive suspected cases** | | |
|---|---|---|
| **Year** | **Percentage of TB-positive suspected cases** | |
| | **Cattle** | **Buffalo** |
| 2021 | 14.8% | 6.4% |
| 2022 | 15.8% | 9.8% |
| Total | 30.6% | 16.2% |

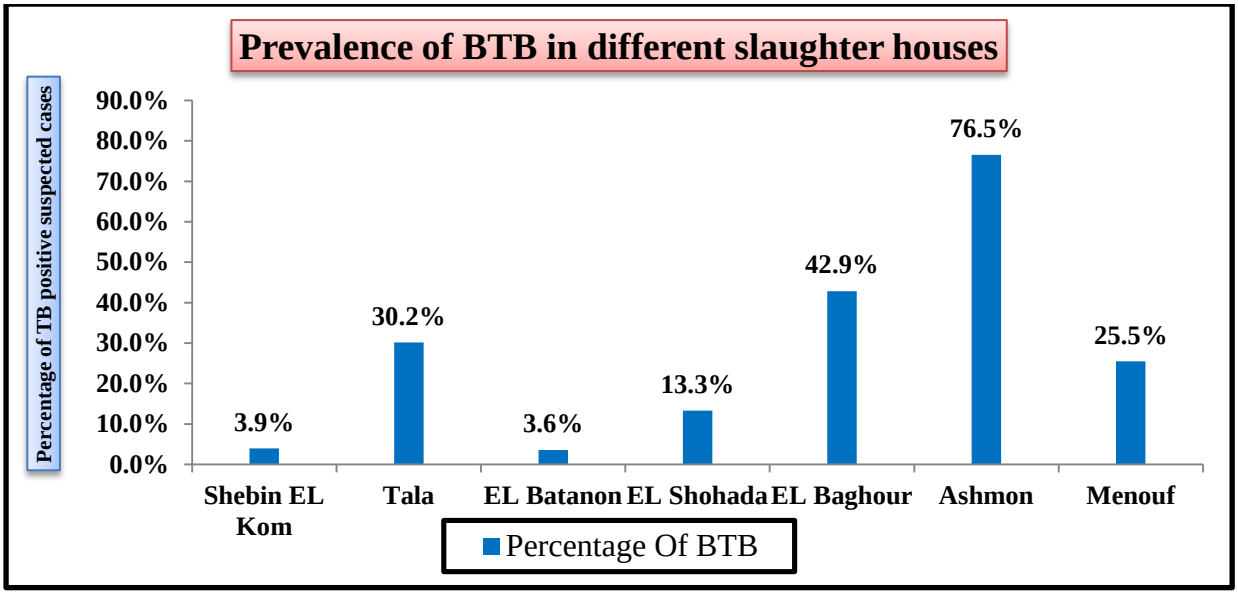



**Histogram (6):** Showing the annual proportion of suspected bovine tuberculosis-positive cases in slaughtered cattle and buffalo in Menofiya Governorate during 2021- 2022.

Table (11) and Histogram (7): Describe the proportion and distribution of bTB in different slaughterhouses of Menofiya Governorate. This study results revealed the highest proportion of bTB (76.5%) in Ashmon abattoir, followed by Elbagour abattoir (42.9%) and Tala (30.2 %), while El-Batanon (3.6%) and Shebin EL koom (3.9%) had lower proportion of bTB.

**Table (11):** Showing the proportion of bTB in different slaughterhouses in a survey in Menofiya Governorate during 2021-2022.

| **Proportion of bTB in different slaughterhouses in Menofiya Governorate** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Slaughterhouses** | **Shebin EL Kom** | **Tala** | **EL-Batanon** | **EL-Shohada** | **El-Baghour** | **Ashmon** | **Menouf** |
| **Percentage o bTB** | 3.9% | 30.2% | 3.6% | 13.3% | 42.9% | 76.5% | 25.5% |




**Histogram (7):** Showing proportion of bTB in different slaughterhouses in Menofiya Governorate during 2021-2022.

## DISCUSSION

Bovine tuberculosis is a chronic granulomatous disease caused by *Mycobacterium bovis* that is one of the most serious animal health problems affecting animal owners, farmers, slaughterhouse employees, and veterinary professionals in Egypt (Hamed *et al*., 2021).

In the current investigation, the lungs and its lymph nodes were the most common organs with tuberculous lesions (79 out of 159 animal carcasses (49.68%). This finding agrees with other research (Aylate *et al*., 2013; Gizaw *et al*., 2017; Youssef & Ahmed, 2014) Who found that most TB-like lesions were scattered throughout the lymph nodes of the lungs in slaughtered cattle and buffalo.

These positive findings suggest that the majority of bTB infections are transmitted through aerosol transmission, and that inhalation may be the primary method of infection in animals, While TB presence in the other tissues could be due to ingestion of the causative organism and subsequent spread to these tissues via the hematogenous route (Ibrahim *et al*., 2021; Pokam *et al*., 2019; Sa'idu *et al*., 2015).

In this study, most of the slaughtered animals were in the late stages of tuberculosis and showed typical visible lesions. Our finding noted severe emaciation of slaughtered animal carcasses during necropsy with serous atrophy of fat and bulging of ribs. Gross lesions in the present study included areas of multifocal consolidation in the pulmonary lobes, enlargement, and focal to multifocal greyish yellow caseated cheesy-like nodules in lymph nodes and internal organs with central caseous mass containing firm white material consistent with mineralization. This finding agrees with (Souza *et al*., 2016; Zimpel *et al*., 2017).

In this study, microscopic morphologic characteristics, including cellular composition and presence or absence of necrosis and fibrosis, are used to categorize granulomas of localized TB into four (I-IV) stages. In the present study, stage IV of bTB granulomas is the most frequently found lesion in slaughtered animals, indicating a chronic process that can also be related

to the fibrosis that is observed surrounding lesions. Our finding agreed with previously reported results (Canal *et al.*, 2017; Fitzgerald & Kaneene, 2013; Nascimento *et al*., 2021; Oliveira *et al*., 2012; Tsairidou *et al.*, 2014; Villarreal-Ramos *et al*., 2018; Wangoo *et al*., 2005).

In the current study, the gross and histopathological diagnosis of tuberculosis was supported by the Ziehl-Neelsen stain. Mycobacteria stained by ZN and appeared as thin, slightly curved, single or even pairs, colored reds within a blue background, indicating the tubercle bacilli that are compatible with the previous researcher (Dametto *et al*., 2020; Santos *et al*., 2021; Silva *et al.*, 2018).

Other authors (Ulrichs *et al*., 2005; Watrelot-Virieux *et al.*, 2006) Assumed a poor stain uptake due to an altered structure of the bacterial cell wall, associated with transition into a state of dormancy, was described as a reason for the lack of staining.

The relationship between risk variables and the proportion of bovine tuberculosis determined that age is a significant risk factor for bTB in cattle and buffalo. Out of 159 carcasses, 91 cattle and 68 buffalo present visible lesions of TB; only four cattle (up to 2 years old) and two water buffalo at the same age demonstrate visible lesions. Similar investigations have been reported by (Moiane *et al*., 2014; Salgado *et al.*, 2009) Who discovered that the low incidence of positive cases in young animals may be due to the predominance of gamma delta T cells in young, which play an important role in anti-mycobacterial immunity.

In addition, cattle (34.06%) and buffalo (36.76%) carcasses aged 2-4 years display visible lesions. Meanwhile, cattle (59.34%) and water buffalo (58.82%) aged 4-6 years revealed apparent lesions. Additionally, cattle (2.19%) and water buffalo (1.47%) aged 6-8 years tested positive for postmortem examination and displayed visible lesions. These results agreed with (Dejene *et al*., 2016; Mekonnen *et al.*, 2019; Milne *et al.*, 2020; Nemomsa *et al.*, 2014), who found that there was a significant increase in risk with increasing age up to 6 years then decline, which could be connected to the fact that older animals are exposed to Mycobacterial infection for more extended periods of time and more frequently. Still, it may also related to the reactivation of latent infections in old stressed animals or the physiological decline of immunity. Also, it may be due to the development of an anergic state or increased mortality of diseased animals.

The proportion of bovine tuberculosis in female buffalo (67.64%) was significantly higher than in males (32.35%), Which agrees with (Arshad *et al.*, 2012; Trangadia *et al*., 2013), who found that females had greater infection rates, which could be related to physiological and immunological changes during pregnancy and lactation.

The reason could be that these females are more retained as they help in building the herd size and are used as replacement animals while the males are usually sent to market for slaughter after reaching a certain age; also, for breeding purposes, females remain in the herd longer than males, increasing their exposure to Mycobacterium species. This study results were in accordance with (Itah & Udofia, 2005) and disagree with (Ameen *et al*., 2008) Who reported that there was no clear connection between sex and tuberculous lesions identified in slaughtered animals.

While in cattle, males (58.24%) had a considerably greater frequency of

bovine tuberculosis than females (41.75%). Our findings are in agreement with the findings of (Kazwala *et al.*, 2001), who stated that male cattle were significantly more affected by bTB than female animals. In this study, the reasons of high percentage of male cattle infection are unclear and may need more investigation. These results differ from (Nalapa *et al.*, 2017) in Uganda and (Nuru *et al.*, 2015) In Ethiopia, who explains that females had a higher proportion of bTB compared to males.

From the data obtained from the Directorate of Veterinary Medicine, Slaughterhouse Meat Inspection, Shebin El Koom, Menofiya Governorate from January 2021 to December 2022, the seasonal variation of bTB lesions was assessed based on abattoir records of tuberculosis lesions and revealed that bTB is present during all seasons, but with high frequency during spring and summer and lower frequency in winter and autumn. In a similar study in Egypt, (Ahmed *et al*., 2013) Found a significant difference between season and pathological finding of TB in slaughtered cattle in Ismailia abattoir.

This study results disagree with (Ameen *et al.*, 2008; Awah Ndukum *et al*., 2010) Who found that the detection of TB lesions was not influenced by season. As well as our results disagree with those results obtained by (Boukary *et al*., 2012; JA *et al*., 2011; Raufu & Ameh, 2010; Sa'idu *et al*., 2017) from Nigeria reported that the frequency of tuberculous lesions was higher at the beginning of the rainy season which showed that variation in seasonal proportion was not significant.

The proportion of TB lesions in summer was significantly higher than in winter because animals were more stressed in the hotter and summer months. In addition, the days are longer than the nights in summer, giving animals more opportunity to come into contact with sick animals (Kemal *et al*., 2019; Yahyaoui Azami *et al*., 2018).

As well as, the high frequency in summer could be related to the extensive destocking of animals by farmers during this season, and the large number of cattle presented for slaughter, particularly during summer, and thus a high demand for meat. During winter, animals are restricted to their farms due to rain. Close contact between animals increases the spread of tuberculosis infection. (Silk *et al*., 2017).

This study's results clarified a higher proportion rate of bTB-positive cases in 2022 compared to 2021. Ejeh et al. (2014) determined that an annual factor does not have a significant role in the proportion of bTB. This may be attributed to the chronic nature of the disease, as tuberculous lesions may persist for months and years in infected bovine. Animals with recent infections may coexist with animals with old infections in the same month or year, which makes it difficult to determine the effect of year on bTB proportion based on carcass inspection. The overall annual proportion of TB during 2 years in cattle was 30.6%, and in buffalo was 16.2%. This finding reported that the proportion of bTB was significantly higher in cattle when compared to buffalo.

These results may be due to the fact that buffalos are more adapted to defend themselves from environmental circumstances than cattle. Moreover, genetic variations between cattle and buffalo were also in consideration for *M. bovis* occurrence (Carneiro *et al.*, 2019).

This study results revealed the highest proportion of bTB in Ashmon abattoir, followed by Elbagour abattoir and Tala, while El-Batanon and Shebin EL kom had a lower proportion of bTB. These may be due to densely populated areas, where the farm families keeping a few animals inside their houses, poor hygiene and muddy soil may allow *M. bovis* to remain for a more extended period and potentially proliferate (Karolemeas *et al.*, 2011).

## CONCLUSION:

It was inferred from the study that Post-mortem examination of lymph nodes and thoracic and abdominal organs can be performed to assess the occurrence of tuberculous lesions that include nodular lesions containing caseous material, which may be mineralized in the center and encapsulated by fibrous tissue. Based on the microscopic observations of tissue sections, the granulomas of localized TB can be graded as stage I, II, III & IV. The stage IV of bTB granuloma was observed with maximum frequency. Staining with the Ziehl-Neelsen technique provides a presumptive identification of bovine tuberculosis; additionally, season, sex, and age were recognized as important risk factors associated with TB infection. The proportion of TB lesions in summer was significantly higher than in winter. Male cattle were found to be more susceptible to infection than females. In comparison, female buffalo revealed a higher proportion. There was a significant increase in risk with increasing age up to 6 years, then decreased.

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