

Pathological Studies on lung affections in sheep and goat at Kalubia Governorate

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Abstract

The gross and histopathological changes in the lungs of small ruminants at Kalubia Governorate were investigated. A total of 159 lung samples (134 sheep and 25 goats) showed gross lesions were collected from February 2018 till November 2019. Bacterial strains were isolated from 26 cases out of 159 (16.35%) including various types where *Staphylococcus aureus* was the most predominant isolated strain from the lungs. Based on histopathological examination, different types of pneumonia (28.9%) including; suppurative pneumonia (10.69%), fibrinous pneumonia (7.54%), interstitial pneumonia (6.28%), granulomatous pneumonia (1.26%) and verminous pneumonia (3.14%) were detected. Bronchitis and bronchiolitis (21.38%); pleural fibrosis (4.4%) were recorded. Pulmonary adenomatosis and fibrosarcoma were detected in lungs of sheep with an incidence of 2.52% and 1.26% respectively. The study concluded that pneumonia is an important lesion in the lungs of small ruminant, and suppurative Pneumonia was the most predominant type. Verminous Pneumonia was detected only in goats and not detected in sheep. Pulmonary adenomatosis were recorded in lungs of sheep but not observed in goat.

Key words: Histopathology, lung affections, small ruminant, Kaluabia

1. Introduction:

Respiratory diseases cause a high mortality rate and economic losses in small ruminants due to the reduction of productivity, treatment costs, vaccination

programs, and organ condemnations in abattoirs (**Goodwin et al., 2004; Lacasta et al., 2008**).

The pathogenesis of the respiratory diseases is multifactorial, and mostly due to the interaction of many infectious agents, host defense, environmental factors, and stress. These predisposing factors decrease the local resistance of the respiratory mucosa allowing the growth of infectious agents in the respiratory tract which leads to the development of different types of Pneumonia (**Yesuf et al., 2012**).

Pneumonia is an inflammatory response of the lung tissue that develops when a certain threshold dose of infective agents is increased coupled with increased host susceptibility and decreased lung defense mechanisms. (**Bruere et al., 2002**). The present work aims to describe the gross and histopathological changes and identify the various bacterial agents in ovine and caprine lung lesions.

2. Materials and Methods:

Sample collection

A total of 159 lung samples showing gross lesions (134 sheep; 25 goats) were collected from both slaughtered and dead animals at Kalubia Governorate from February 2018 to November 2019.

Bacterial isolation

Lung samples were aseptically collected and placed in a sterile plastic bag in an icebox and were submitted to Bacteriology Department, Animal Health Research Institute, Tanta. The outer surface of the pneumonic lungs were first seared with a heated spatula before the cut inner surface of the lungs were inoculated into the nutrient broth and incubated at 37 °C for 24 hours and then subcultured into the following media; 5 % sheep blood agar, Mannitol salt agar,

Eosin methylene blue agar and MacConkey agar at 37 °C for 24-48 hour. The colonies of each strain were identified according to **Quinn et al., (1998)**

Histopathology

Small tissue specimens from lungs were fixed in 10% neutral buffered formalin. After proper fixation, the tissue specimens were trimmed, washed in running tap water, dehydrated in different ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. The paraffin embedding blocks were sectioned at 5 µm thickness then stained by hematoxylin and eosin stain according to **Bancroft et al., (1994)**.

3. Results

3.1. Bacterial isolation:

Various types of bacteria were detected in 26 cases out of 159 cases (16.35%), and these types were *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Klebsiella pneumoniae*, and mixed infection (*Staphylococcus aureus* and *Escherichia coli*). These data were summarized in **Table (1)**.

3.2. Histopathological findings:

The incidence of different pathological lesions in the examined lungs of sheep and goat were illustrated in **Table (2)**.

3.2.1- Abnormalities of inflation

Emphysema:

Grossly, the emphysematous areas appeared pale in color; and plugged above the surrounding lung surface. Microscopically, emphysema was mostly observed in association with bronchitis and different types of pneumonia. The

emphysematous pulmonary alveoli were dilated and distended with air. Some alveoli were ruptured, and the interalveolar septa were destroyed with communication of adjacent alveoli forming syncytium.

Atelectasis:

Grossly, atelectic areas were dark red in color, slightly depressed under the surrounding tissue, and sharply well demarcated from the adjacent pulmonary tissue. Microscopically, the atelectic pulmonary alveoli were collapsed and appeared as parallel slits. Atelectasis may be seen beside large sized abscess or in between large areas of lung fibrosis.

3.2.2- Circulatory disturbances

Passive hyperemia:

Grossly, the affected lung showed dark red colored patches all over their surfaces. Microscopically, the interalveolar blood capillaries were severely dilated and engorged with blood. Moreover, the alveolar lumen was packed with transudate which takes purplish to pinkish coloration

Pulmonary edema:

Pulmonary edema of the non-inflammatory type characterized grossly by dark red lungs that were voluminous, heavy, moist in cut surface, with rib imprints on their surfaces. Microscopically, the pulmonary alveoli showed homogenous faint eosinophilic material in their luminae with focal areas of compensatory alveolar emphysema.

Pulmonary hemorrhages:

Macroscopically, the lungs showed dark red patches on the pulmonary surfaces. Microscopically, scattered red blood cells were seen both in the interalveolar and interlobular septa.

3.2.3-Bronchitis and bronchiolitis

In acute cases, marked congestion in the mucosa of bronchi and bronchioles was seen while in the chronic cases, the bronchial luminae were packed with an abundant amount of mucous exudate and the bronchial walls were thickened. Microscopically, acute bronchitis and bronchiolitis showed congestion of blood vessels and mononuclear leukocytic cellular infiltrations mainly lymphocyte in the peribronchiolar areas. Chronic cases were characterized microscopically by marked hyperplasia and desquamation of the lining epithelium of bronchioles with the presence of a large amount of exudate in their luminae, which occasionally was organized and associated with heavy peribronchial leukocytic cellular infiltration (**Fig.1a**).

3.2.4-Pneumonia

Suppurative bronchopneumonia and lung abscess

Grossly, the affected lungs were consolidated, dark red in color, and showed the presence of grayish-white, varied sized abscesses in the surface of the pulmonary tissue (**Fig.1b**). In cut surfaces; scanty grayish-white pus came out from the bronchial luminae (**Fig.1c**). Microscopically, acute suppurative bronchopneumonia revealed presence of suppurative exudate formed from the aggregation of dead and live neutrophils mixed with few numbers of mononuclear leukocytic infiltrations in the lumen of bronchioles and the alveoli (**Fig.1d**). Meanwhile, abscesses appeared as focal structureless eosinophilic and basophilic

substances infiltrated with neutrophil and surrounded by fibrous connective tissue capsule (**Fig.1e**).

Fibrinous Pneumonia:

Grossly, the affected lungs were hepatized and showed diffuse consolidated dark red areas associated with the thickening of pleura. Microscopically, the luminae of alveoli, bronchi and bronchioles were filled with eosinophilic fibrinous exudate mixed with leukocytic cellular infiltration especially neutrophils (**Fig.1f**).

Interstitial Pneumonia:

Macroscopically, the affected lungs were firm and dry in cut sections and showed focal pale-colored patches distributed all over their surfaces. Microscopically the lungs showed thickening of the interalveolar septa with the presence of macrophages and lymphocytes in the luminae of alveoli (**Fig.2a**).

Granulomatous Pneumonia:

Granulomatous Pneumonia was recorded only in lungs of sheep. Grossly, the affected lungs showed multiple pale-colored nodules of different sizes scattered all over their surface. Microscopically, the pulmonary granuloma exhibited heavy aggregation of mononuclear leukocytic infiltration around the caseated center and occasionally enclosed with a connective tissue capsule (**Fig.2b**).

Verminous Pneumonia:

Verminous Pneumonia was observed in lungs of goats. Such type of Pneumonia not detected grossly and microscopically it characterized by the presence of cross-section of parasite occluded the bronchial lumen surrounded by

large areas of hemorrhages (**Fig.2c**). Furthermore, the eggs of this parasite were commonly embedded in-between erythrocytes (**Fig.2d**).

3.2.5- Affections of pleura(pleural fibrosis):

Macroscopically, the pleura were thickened, opaque and showed velvety appearance. Microscopically, the affected pleura were thickened due to edema and fibrous connective tissue proliferation accompanied by areas of subpleural emphysema.

3.2.6- Neoplasms:

Pulmonary adenomatosis and fibrosarcoma were recorded in sheep only.

Pulmonary adenomatosis:

Grossly, the affected lungs were voluminous, firm in consistency, with the presence of numerous grayish-white variable-sized nodules containing grayish fluid on the cut section (**Fig.3a**). Microscopically, the neoplastic pulmonary alveoli were lined by proliferating cuboidal to columnar cells forming papillary projections that extended into the alveolar luminae with leukocytic cell infiltration mainly lymphocytes in the peribronchial areas (**Fig.3b**). The bronchioles showed hyperplasia of the lining epithelial cells formed papillary projections with the presence of inflammatory cells, mainly macrophages, lymphocytes, plasma cells, and giant cells with few neutrophils in their luminae (**Fig.3c**).

Fibrosarcoma:

Fibrosarcoma was detected in 2 cases. This secondary neoplasm microscopically was formed from malignant fibroblasts intermixed with collagen fiber in the form of whorls (**Fig.3d**). These neoplastic cells showed high degree of

pleomorphism and anaplasia which taken various shapes and size of nucleus (Fig.3e).

4. Discussion:

The results of bacterial isolation in this study revealed the presence of various types of bacteria isolated from the affected lungs of small ruminants (Table 1). These findings were in partial agreement with **Mahmoud et al., (2005)** who found that, the main bacterial isolates in sheep and goats were *Staphylococcus aureus* (12%), *Streptococcus pneumoniae* (4%), *Escherichia coli* (8%), *Pseudomonas aeruginosa* (4%) and *Klebsiella pneumoniae* (8%).

In this study, emphysema and atelectasis were recorded in 34.1% and 13.2% of the examined lungs respectively. These results were in partial agreement with **Hashemnia et al., (2019)**, who found emphysema in 10.36% and atelectasis in 6.91% in sheep. The gross and microscopic findings of emphysematous and atelectatic alveoli in our study were similar to those reported by **Kumar et al., (2005)** and **Hashemnia et al., (2019)** in sheep.

In the current work, passive hyperemia was detected in 15.7% and characterized microscopically by congestion and dilatation of the interalveolar blood capillaries. These results were in partial agreement with **Hashemnia et al., (2019)**, who found hyperemia in 7.11% and characterized microscopically by the presence of a large number of RBCs and sidrocytes out of the blood vessels.

Our results revealed that; pulmonary edema was observed with an incidence of 15.7%. These results were higher than those recorded by **Priyadarshi et al., (2013)**, and **Obaid and Khudair (2016)**, who found edema in 10.90% and 10% in sheep respectively. In our results, the odematous lungs showed homogenous faint eosinophilic material in the lumen of their alveoli. These finding were in a

complete agreement with **Kumar et al., (2005)** and **Priyadarshi et al., (2013)** in sheep.

In the present study, an incidence of 6.2% of pulmonary hemorrhage was lower than the previous reports by **Priyadarshi et al., (2013)** at 14.55% in sheep and **Rashid et al., (2013)** at 20% in goat.

Bronchitis and bronchiolitis in our study were observed by 44.1%. These results were in partial agreement with **Singh et al., (2017)**, who found bronchitis and bronchiolitis in 2.22% cases in sheep and goats.

Suppurative Pneumonia was observed with an incidence of 15.9%. In contrast to the present investigation, a higher incidence was recorded by **Tijjani et al., (2012)** who found purulent Pneumonia in 48%; **Yesuf et al., (2012)** at 51.22% and **Obaid and Khudair (2016)** at 36.6% in sheep. The isolated bacteria from lungs with suppurative Pneumonia in our study were *Staphylococcus aureus*, *Streptococcus pneumoniae* which was also recorded by **Yesuf et al., (2012)**; and in partial agreement with that recorded by **Chukwuebuka et al., (2017)** who isolates *Staphylococcus aureus*, *klebsiella pneumoniae* and *Escherichia coli* from suppurative cases in sheep lungs. The characteristic features of suppurative Pneumonia and neutrophilic aggregates in this study were in accordance with previous reports by **Azizi et al., (2013)** in sheep.

In the current work, fibrinous Pneumonia was observed with an incidence of 15.5%, which was lower than those reported by **Yesuf et al., (2012)** at 39.02%.

The lesions of fibrinous Pneumonia with the presence of fibrin exudate and few neutrophils in the lumen of bronchi, bronchioles, and alveoli observed in the present work are in line with the previous report by **Hashemnia et al., (2019)**. Moreover, previous studies by **Azizi et al., (2013)**, **Kumar et al., (2014)** and

Singh et al., (2017) reported the presence of multifocal areas of necrosis surrounded by a rim of elongated cells, called 'oat cells' or "swirling macrophages" that formed whorl-like structures in and around the alveoli in cases of fibrinous Pneumonia.

In the present work the incidence of interstitial Pneumonia was 13.9%. This incidence was lower than those reported by **Tijjani et al., (2012)** at 32%, **Yesuf et al., (2012)** at 23.61%, **Azizi et al., (2013)** at 21.43% and **Obaid and Khudair (2016)** at 30%.

In our work, the affected lungs with interstitial Pneumonia exhibited thickening of the interalveolar septa. These results completely agreed with **Oruc (2006)** and **Ugochukwu et al., (2017)**. Meanwhile, our results were in partial agreement with **Azizi et al., (2013)**, who found multinucleated giant cells with spheroidal to ovoid concentrically lamellar corpora amylacea in the alveolar space in some cases of interstitial Pneumonia.

Our results revealed granulomatous Pneumonia was seen in examined lungs of sheep with an incidence of 1.5%. Earlier studies by **Mira et al., (2014)** found a granulomatous lesion in the lungs collected from goats infected with caseous lymphadenitis. Microscopically, the pulmonary granuloma in our work consists of a heavy aggregation of mononuclear leukocytic infiltration around the caseated center and enclosed with a connective tissue capsule. These results completely agreed with **Mahdi et al., (2015)**.

In the current study, verminous Pneumonia was observed in lungs of goat only which represented by 20% and not detected in the examined lungs of sheep. The lung worms noticed in our work were *Metastrongylus* and *cystocaulus ocreatus*. These findings were completely disagree with that mentioned by **Ayana**

and Chanie (2013) who found that lung-worm infection was recorded in slaughtered sheep with an incidence of 57.55% and named *Dictyocaulus filaria*, *Muellerius capillaris* and *Protostrongylus rufescens*. Pulmonary hydatidosis was another kind of parasitic infection in lungs of sheep as recorded by **Priyadarshi et al., (2013)**, **Mahdi et al., (2015)** and **Singh et al., (2017)**.

Our results revealed that, affections of the pleura were observed by an incidence of 8.5% from examined lungs, which were lower than that recorded by **Obaid and Khudair (2016)** at 10% of pneumonic sheep. The thickened, fibrotic pleura in this study are in accordance with earlier studies by **Amaravathi et al., (2014)**

Pulmonary adenomatosis was represented by 3% of the examined sheep lungs in our work. These findings were lower than those recorded by **Kumar et al., (2005)** and **Sonawane et al., (2016)**, who reported incidences of 7.43% and 8% respectively. The gross lesions and microscopic proliferative changes in the alveolar and bronchiolar epithelium in the present work were in accordance with the earlier reports by **Kumar et al., (2005)** and **Sonawane et al., (2016)**. Earlier studies by **Sayyari and Mohamadian (2012)** reported lesions of Ovine pulmonary adenocarcinoma in goat's lungs that were consistent with our observation in sheep.

5. CONCLUSIONS:

The incidence of bacterial infection in the lungs of sheep and goat almost represents 16.35% and *Staphylococcus aureus* are the most common bacterial pathogens isolated from diseased lungs. Pneumonia is an important lesion in the lungs of small ruminant, and suppurative Pneumonia was the most predominant type. Verminous Pneumonia was detected only in goats and not detected in sheep.

Pulmonary adenomatosis were recorded in lungs of sheep but not observed in goat.

6. Conflict of interested statement:

The author has no competing interests.

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Figures legend

Figure 1:

Lung taken from affected animals, showing:

- (a) Marked hyperplasia and desquamation of the lining epithelium of bronchiole with presence of organized exudate in the lumen (arrow) associated with heavy peribronchial leukocytic cellular infiltration (star). (H&E x200)
- (b) Grayish white varied sized abscesses in the pulmonary surface (arrow).
- (c) Scanty grayish white pus came out from the bronchial lumen in cut section (arrow).
- (d) Aggregation of dead and live neutrophils mixed with few numbers of mononuclear leukocytic infiltrations in the lumen of bronchiole and the alveoli. (H&E x 100)
- (e) Old abscesses formed from focal structurless eosinophilic and basophilic substance infiltrated with leukocytes especially neutrophil and surrounded by fibrous connective tissue capsule. (H&E x100)
- (f) Fibrinous exudate mixed with leukocytes in the lumenae of alveoli, bronchi and bronchiole. (H&E x 200)

Figure 2:

H&E stained section of lung, showing:

- (a) Thickening of the interalveolar septa with presence of macrophages (red arrow) and lymphocytes (black arrow) in the lumen of affected alveoli. (x 400)
- (b) Heavy aggregation of mononuclear leukocytic infiltration around caseated center and enclosed with connective tissue capsule. (x 100)
- (c) Cross sections of the parasite (black arrow) occluded the bronchial lumen surrounded by large areas of hemorrhages (red arrow). (x 100)
- (d) Eggs of this parasite (arrow) embedded in between erythrocytes. (x 400)

Figure 3:

Lung taken from affected animals, showing:

- (a) Numerous grayish white variable sized nodules containing grayish exudate on cut section (arrow).
- (b) Neoplastic pulmonary alveoli lined by proliferating cuboidal to columnar cells forming papillary projections extended into the alveolar lumen (red arrow) with peribronchial leukocytic cellular infiltration (black arrow). (H&E x 100)
- (c) Hyperplasia of the bronchiolar epithelial cells forming papillary projections (brown arrow) with presence of inflammatory cells mainly macrophages (orange arrow), lymphocytes (red arrow), plasma cells (blue arrow) and giant cell (black arrow) with few neutrophils (green arrow) in their lumen (H&E x 400)
- (d) Fibrosarcoma formed from malignant fibroblast intermixed with collagen fiber in the form of whorls. (H&E x 100)
- (e) Neoplastic fibroblasts with high degree of pleomorphism and anaplasia which taken various shapes and size of nucleus. (x 400)

Fig 1 a



Fig 1 b

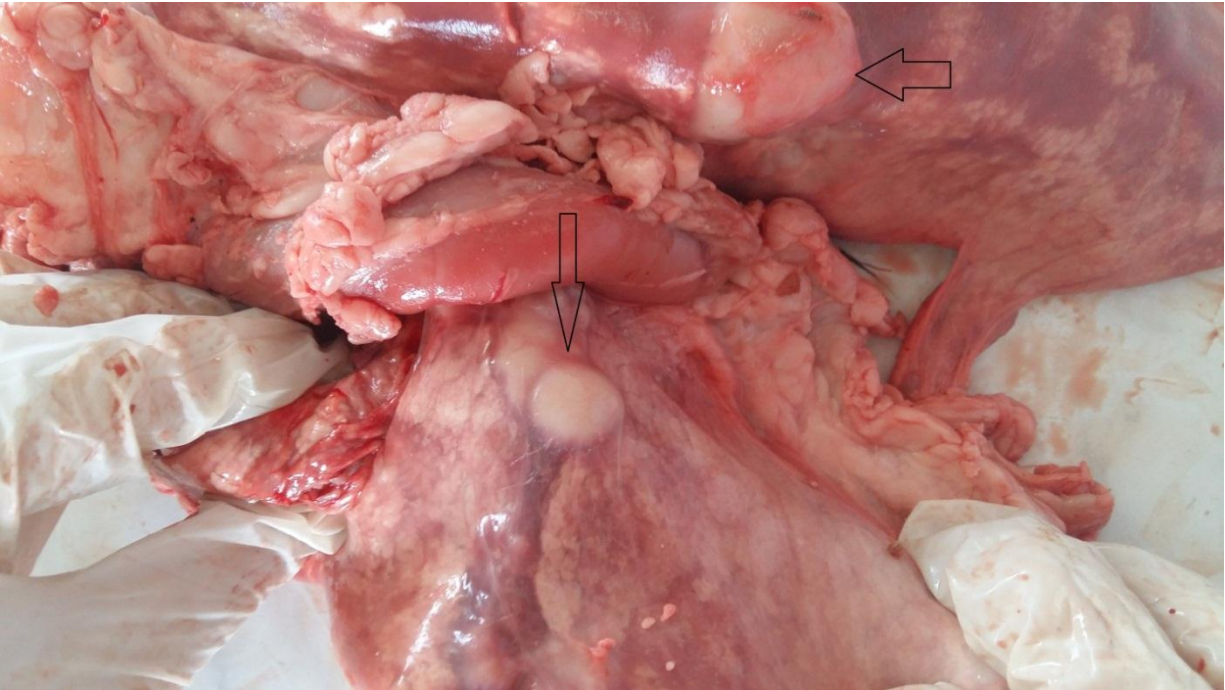


Fig 1 c

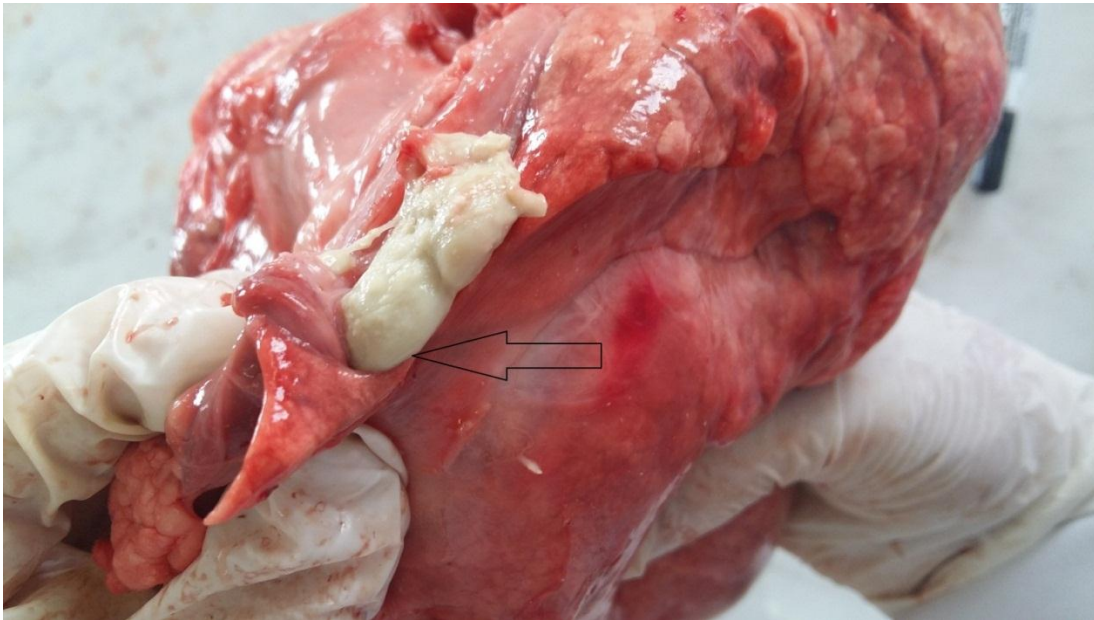


Fig 1 d

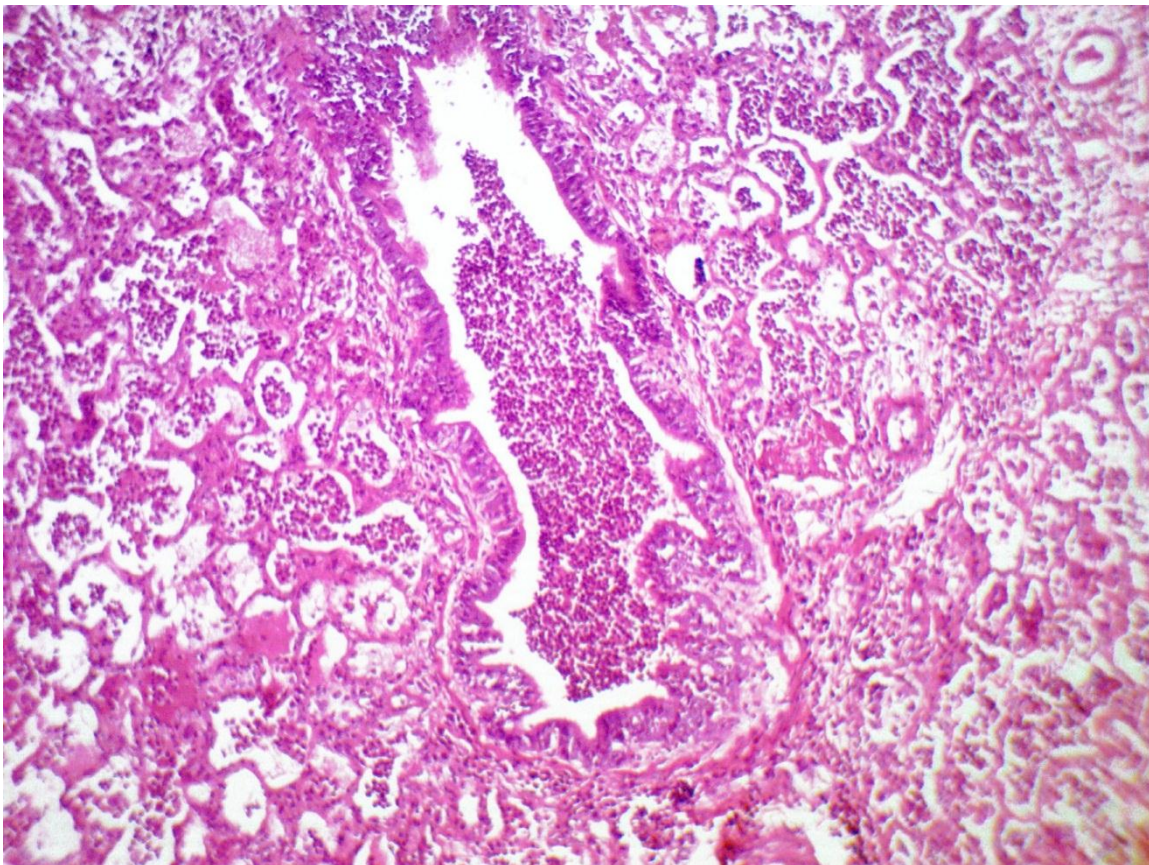


Fig 1 e

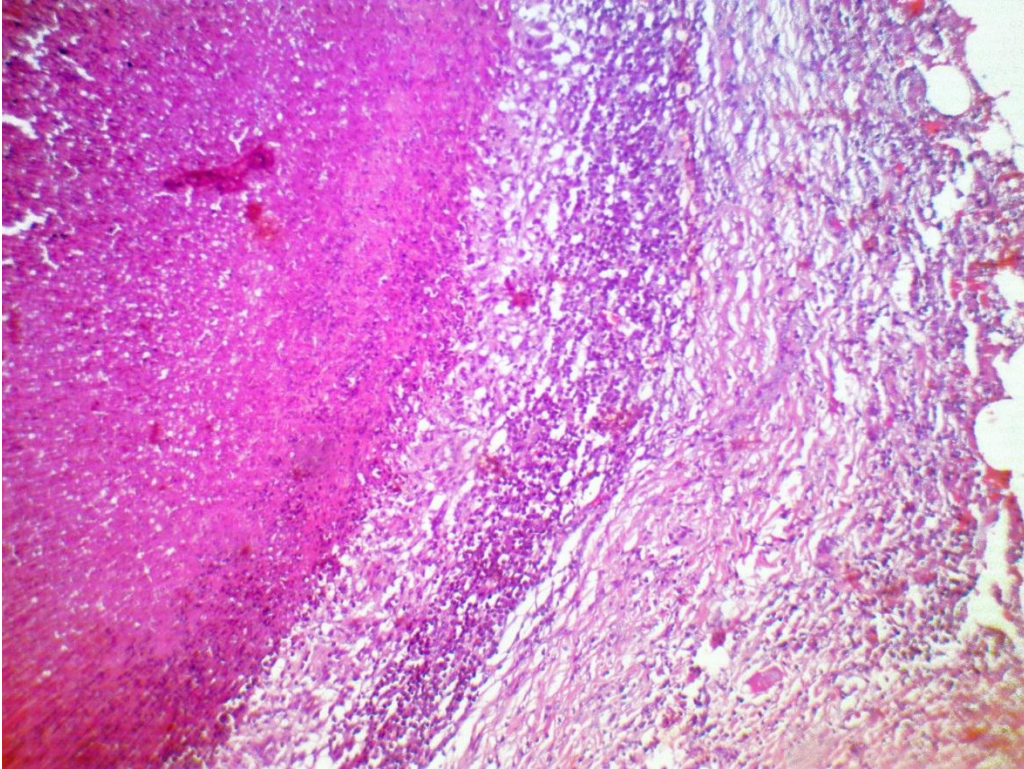


Fig 1 f

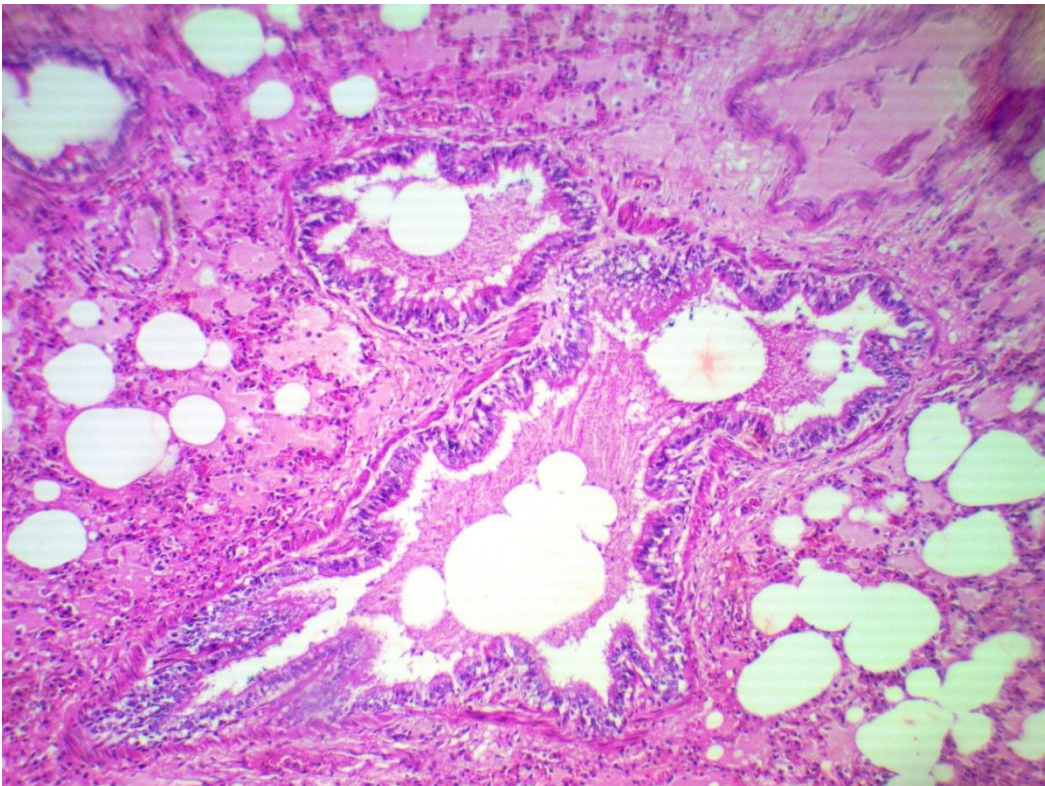


Fig 2 a

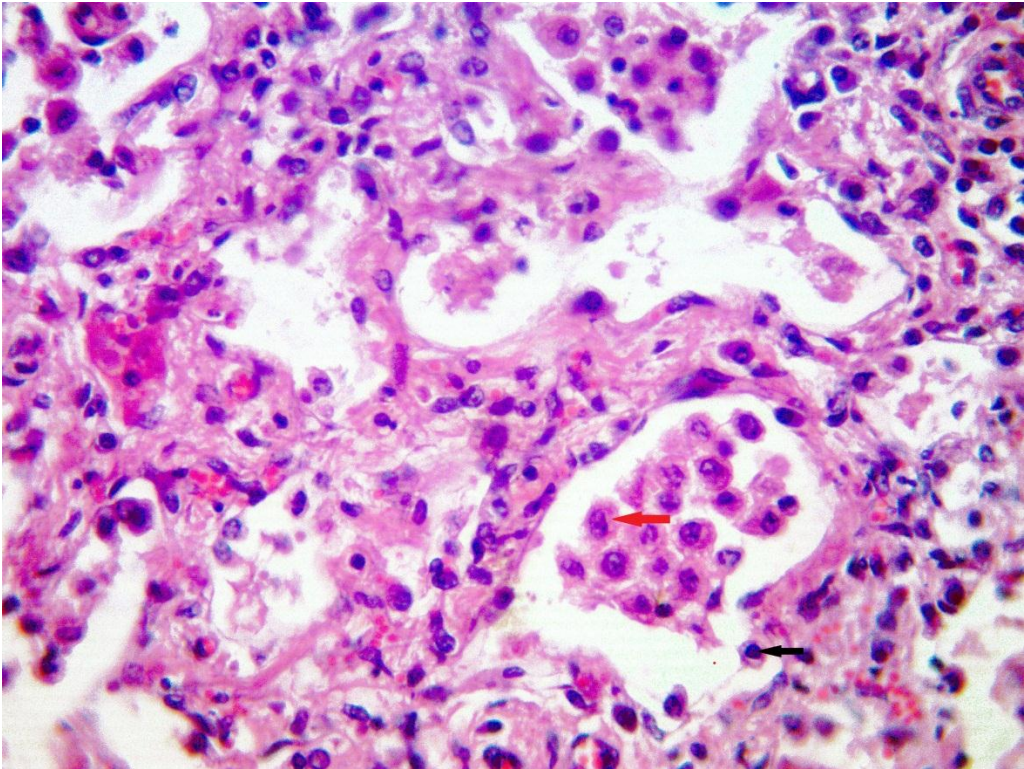


Fig 2 b

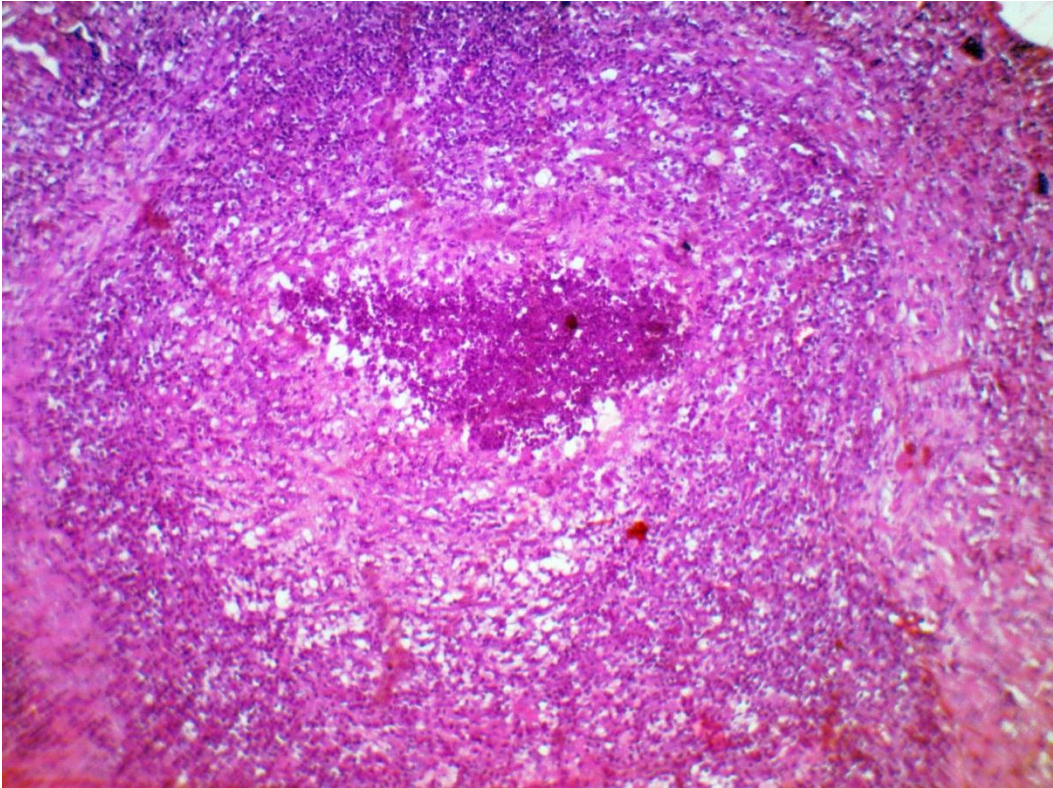


Fig 2 c

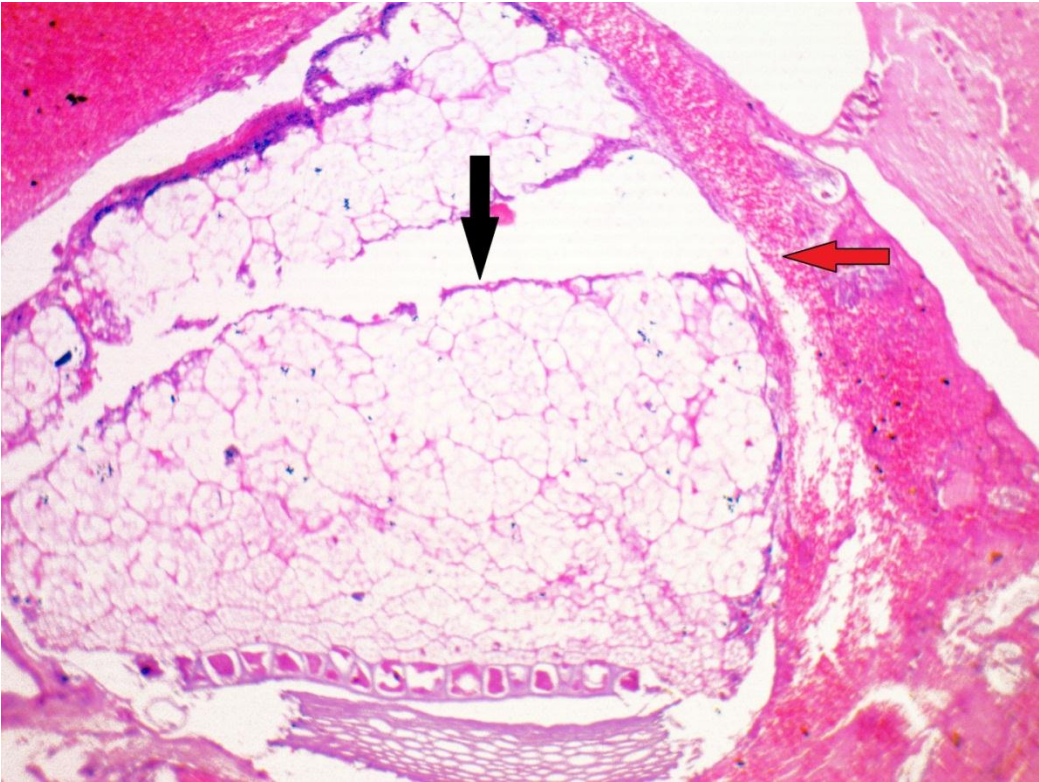


Fig 2 d

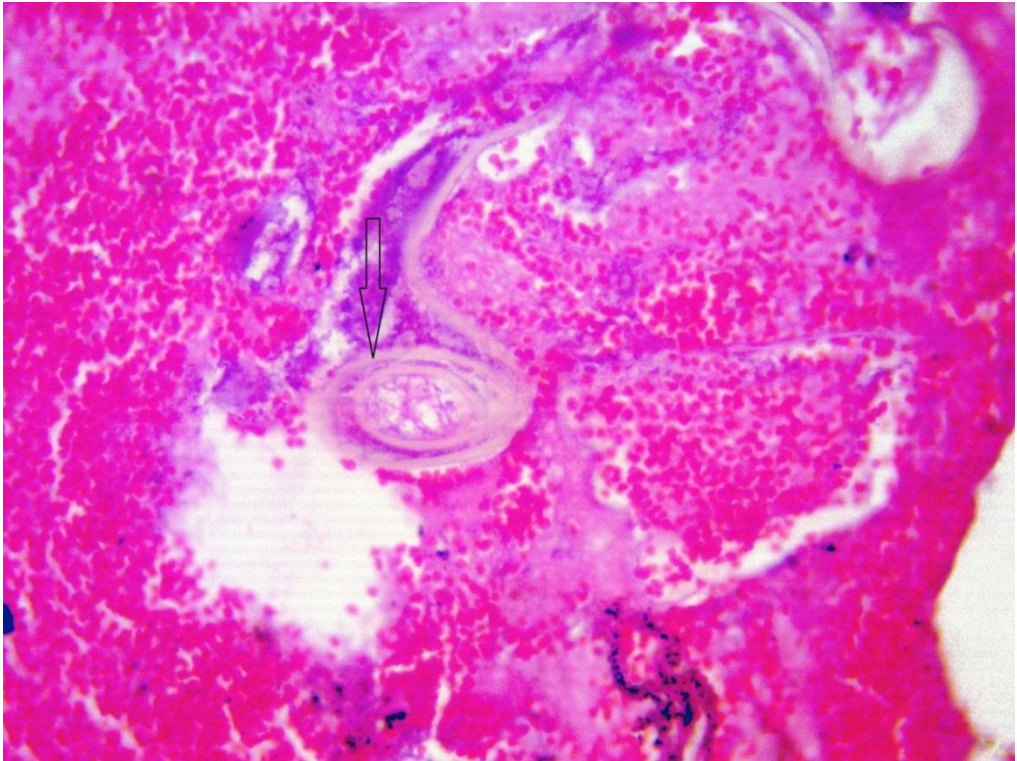


Fig 3 a



Fig 3 b

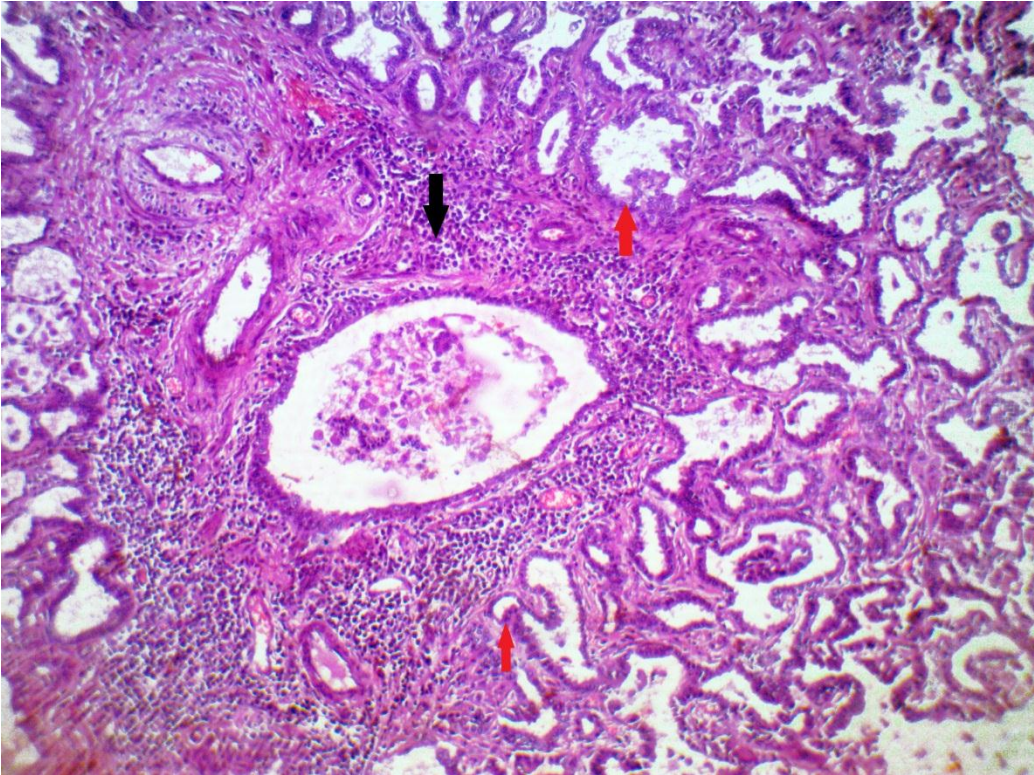


Fig 3 c

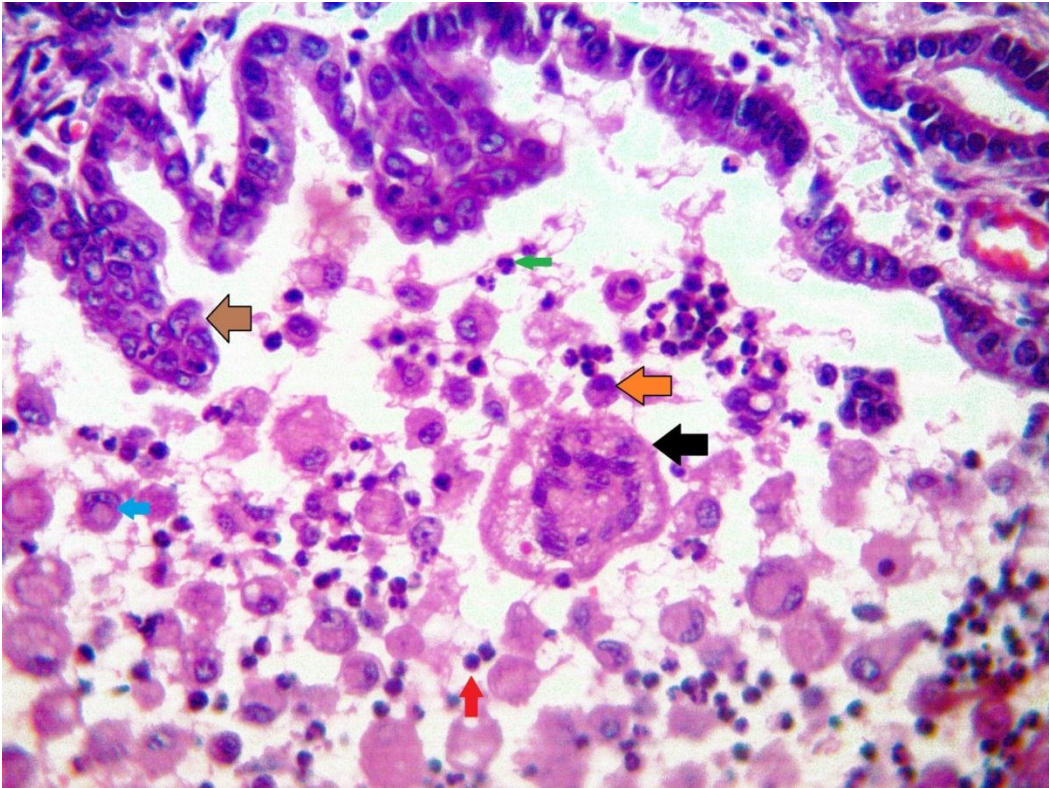


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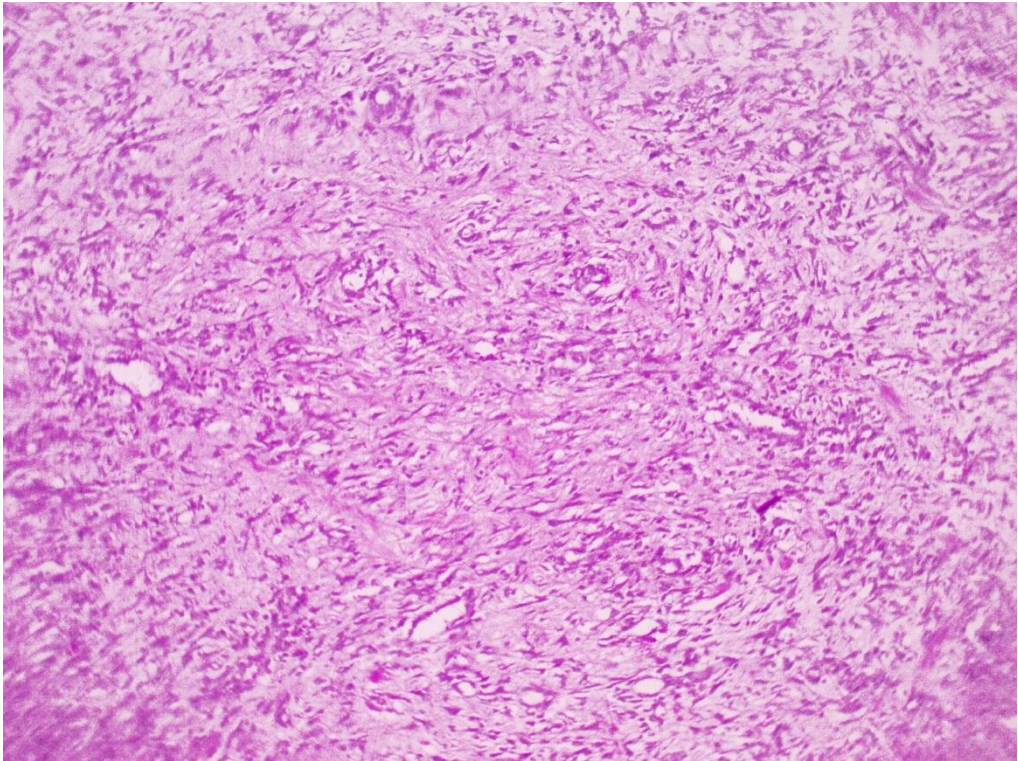


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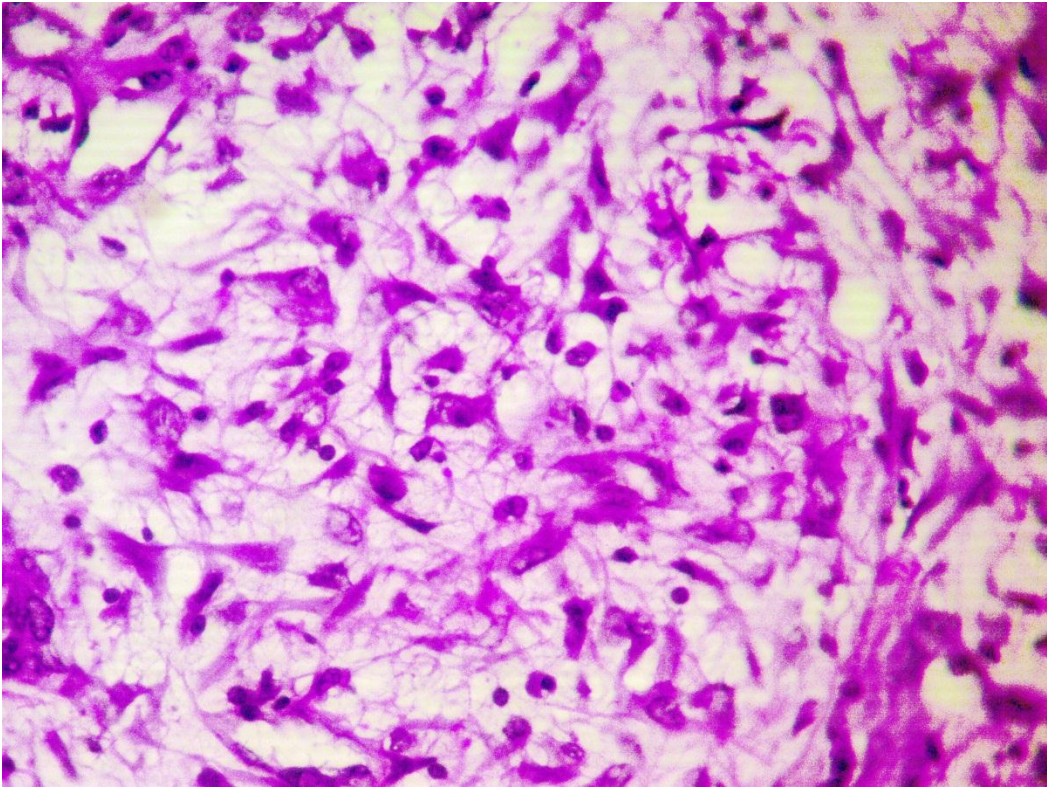


Table 1: Typing of various bacterial isolates among the total affected cases of sheep and goat lungs.

Affected species	Bacterial infection	No. of isolated bacteria	percentage
Sheep	Staphylococcus aureus	10	38.46%
	Streptococcus pneumoniae	7	26.92%
	Escherichia coli	4	15.38%
	Klebsiella pneumoniae	1	3.84%
Goat	Escherichia coli	2	7.69%
	mixed infection (Staph.aureus and E.coli)	2	7.69%

Table 2: Incidence of different pathological lesions in the lungs of small ruminant collected between February 2018 and November 2019.

Lesions	Isolated bacteria	No. of cases		percentage	
		sheep	goat	sheep	goat
Emphysema	-	35	2	26.1%	8%
Atelectasis	-	7	2	5.2%	8%
Passive hyperemia	-	5	3	3.7%	12%
Pulmonary edema	-	5	3	3.7%	12%
Pulmonary hemorrhage	-	3	1	2.2%	4%
Bronchitis and bronchiolitis	-	31	3	23.1%	12%
Suppurative pneumonia	Staph.aureus	9	1	6.7%	4%
	Strept. pneumoniae	7	-	5.2%	-
Fibrinous pneumonia	-	10	2	7.5%	8%
Interstitial pneumonia	-	8	2	5.9%	8%
Granulomatous pneumonia	Escherichia coli	2	-	1.5%	-
Verminous pneumonia	-	-	5	-	20%
Affection in pleura (pleural fibrosis)	-	6	1	4.5%	4%
Pulmonary adenomatosis	-	4	-	3%	-
Fibrosarcoma	-	2	-	1.5%	-