Histopathological study of sheep lung roaming in dump zones

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Abstract

Respiratory system disease is common in sheep, which is frequently difficult to detectable in live animals until it becomes serious and complicated. This study was aimed to describe sheep lung lesions in Mosul city, which roam in dump zones and have been slaughtered at butchers’ shops, fifty male and female sheep 1-5 years were used at the current work from March to December 2021; affected Samples were collected and prepared for standard histopathological analysis. The special stain was also used for carbohydrates demonstrated inside and around lung lesions. The results of the current study showed the presence of gross and histological finding changes, the gross lesion presented by inflammation of the upper right lobe, congestion, and bleeding on all parts of the lobe in addition to different sizes of the nodular lesion. In contrast, the pneumatic histological finding was 12.1%, circulatory disturbance 3.5%, parasitic infections 10.0%, disturbance of growth 7.1%, Necrotic area surround by swirling macrophage 2.0% in addition to the deposition of calcium salt and hemosiderin pigmentation. Organizing Pneumonia (OP) and Masson’s bodies were one of the most detected lesions in the roaming sheep, it was scored by Masson Trichrome from mild, moderate, and severe fibrosis. All lesions were categorized according to the type of lesion, grade, and kind of it. From the current work, we demonstrated that pneumatic disorder represented a considerable and serious problem in the animal livestock industry and leads to economic loss in milk, wool, and sheep meat production. Additional studies are recommended to determine the etiological agent of sheep lung lesions whether it is a fungal, bacterial, or viral agent.

Introduction

Sheep are considering the most important future livestock growth, it has the capability to convert different kinds of forages to beneficial products for population, such as milk, wool and mutton. Sheep is an important source of animal protein in all the world, it is necessary to study the pathological disease, clinical signs and symptoms that affected these animals for enhance their product of milk and meat (1). Respiratory system disease such as bronchitis, pneumonia, fibrosis and lung cancer continue to be an important problem in animals’ flocks (sheep, goat, and bovine) (2), affecting all animals age and species, many factor play a role in the occurrences of these disease such as environmental condition and poor management (3,4). the effects of these diseases in small ruminants have increased in recent years, especially in developing countries (5). Pneumonia is one of the important and common diseases that affect the lower respiratory tract of sheep, especially lambs, which may be acute or chronic or even progressive type, effect on animal production, carcasses downgrading delayed growth and sudden death so it considers a major source of...
economic losses represented by treatment coast, unthriftiness (6). Due to the anatomical and histological properties of the lung as well as the ratio between the alveolar surface and metabolic weight make it more susceptible to diseases (7,8). The current work was aimed to study the more clinic pathological changes that affected sheep which roaming in dump zones including both macroscopic and microscopic changes in the lung tissue.

Materials and methods

Ethical approve
The current work was approved by ethical committee for animal extermination of the forensic DNA center of Al-Nahrain University based on meeting on 5th of April 2022.

Collection of lung sample
Fifty male and female sheep 1-5 years were used in the present study during March to December 2021. The samples were collected from animals that have been slaughter at butcher’s shops.

Gross and Histopathological exam
All specimens were inspected grossly in order to inspect any abnormal lesion in lung which appears by naked eye or by palpation and observation of any changes in the shape, size, color and lung texture after Specimen was collected from the infected part in a clean pack and transported to the lab in a cool pox. Histopathological examination was carried out by trimming the sample with 2 cm in size then fixed in 10%neutral buffered formalin, dehydrated, xylol cleared and blocked with paraffin wax (9,10) and finally sectioned by microtome into 5-6 thickness and stain with routine hematoxylin and eosin stain (11) as well periodic acid Schiff (PAS and Masson’s Trichrome) were used for better clarification of the lesion (11).

Results

Gross pathological lesion
As shown in figure 1, 50 of the total 70 cases had gross lung involvement; the most common infection was pneumonia which was present in 17 cases and accounted for 12.1% for the total cases. Figure 2 also revealed the presence of circulatory disturbances (hemorrhage and pulmonary congestion) in 5cases which accounted 3.5% of the total cases. Infection due to parasitic were represented by hydatid cysts in 15 instances with a percentage of 10.0% (Figures 3 and 4), nodular lesion in 10 cases with 7.1% (Figure 5) and pulmonary necrosis in 3 cases with a percentage of 2.1% (Figure 6).

Microscopic lesion
Table 1 showed the lung microscopic lesions which revealed the presences of different type of inflammation such as supplicative bronchopneumonia represented by infiltration of multinucleated inflammatory cell and bronchiectasis of the lung (Figures 7 and 8), also showed fibrinous bronchopneumonia which appeared as accumulation of fibrin filament inside the bronchi wall and infiltration of lymphocytes and nodule image (Figures 9 and 10), there was also oedema which was associated with vascular changes, bleeding within the alveoli and bronchi in additional to blood clots within the blood vessels image (Figures 11 and 12) Parasitic infections was evident with eggs and larval stage of the parasite inside the lung tissue in additional to presences of hydatid cysts with heavy infiltration of inflammatory cell within the lung tissue and adjacent to the hydatid cysts image (Figures 13 and 14). Disturbance of growth was seen and it was represented by demonstrated hyperplasia of the bronchi epithelial cell, atrophy of the mucous gland image (Figure 15), necrosis of the cartilage tissue and degeneration the bronchi and cell lining the alveoli was also observed image (Figure 16 and 17). There was also deposition of calcium salt with hemosiderin pigment inside lung tissue image (Figures 18 and 19).

Figure 1: Showed gross finding kind, number of cases and percentage of it.

Figure 2: Macroscopic appearances of affected sheep lung showing pulmonary pneumonia with inflammation in the upper right lobe (arrow).
Figure 3: Macroscopic appearances of affected sheep lung showing bleeding and congestion in all part of the left lobe (arrow).

Figure 4: Macroscopic appearances of affected sheep lung showing large hydatid cyst in the lower right lobe.

Figure 5: Macroscopic appearances of affected sheep lung (A) showing the spread of nodular lesion with various shape and size inside the pulmonary lobe (B) nodular lesion in the additional lobe of the right lung.

Figure 6: Macroscopic appearances of affected sheep lung showed necrotic granulomatous inflammatory foci in the posterior lobe of the lung (arrow).

Table 1: Categories of lung lesion including description, grade of it and type of lesion

<table>
<thead>
<tr>
<th>Categories</th>
<th>Lesion description</th>
<th>Grade</th>
<th>Type of lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td>Inflammation with infiltration of lymphocytes inside bronchi wall, bronchiectasis in additional to multiple nodules inside lung tissue</td>
<td>Mild</td>
<td>Progressive</td>
</tr>
<tr>
<td>Disturbance of circulation</td>
<td>Blood clots within the blood vessels &amp;bleeding within the alveoli and bronchi</td>
<td>Mild</td>
<td>Progressive</td>
</tr>
<tr>
<td>Parasitic infections</td>
<td>Larva stage, eggs &amp; hydatid cyst inside lung tissue</td>
<td>Moderate</td>
<td>Progressive</td>
</tr>
<tr>
<td>Disturbance of growth</td>
<td>Hyperplasia of the epithelial cells lining the bronchi as well as there is atrophy of the mucous gland</td>
<td>Moderate and sever</td>
<td>Non-progressive</td>
</tr>
<tr>
<td>Necrosis</td>
<td>Necrosis in the cartilage surrounding the bronchi &amp;degeneration inside the cell lining the alveoli</td>
<td>Sever</td>
<td>Non-progressive</td>
</tr>
<tr>
<td>Calcification</td>
<td>Deposition of calcium salt in the tissue and presence of hemosiderin pigmentation inside the lung</td>
<td>Sever</td>
<td>Non-progressive</td>
</tr>
</tbody>
</table>
Figure 7: Micrograph of sheep lung showed infiltration of inflammatory cell with inflammatory exudate. H&E, 100x.

Figure 8: Micrograph of sheep lung showed bronchiectasis of the lung. H&E, 100x.

Figure 9: Micrograph of sheep lung showed multiple nodules inside the tissue. H&E, 100x.

Figure 10: Micrograph of sheep lung showed infiltration of lymphocytes with accumulation of fibrin filaments inside bronchi wall and emphysema (arrow). H&E, 100x.
Figure 11: Micrograph of sheep lung showed thrombus with infiltration of inflammatory cell (arrow). H&E, 100x.

Figure 12: Micrograph of sheep lung showed thrombus with infiltration of inflammatory cell (arrow). H&E, 400x.

Figure 13: Micrograph of sheep lung with parasitic pneumonia showed egg and variable stage of parasites. H&E, 400x.

Figure 14: Micrograph of sheep lung showed the presence of hydatid cyst inside lung tissue. H&E, 100x.

Figure 15: Micrograph of sheep lung showed hyperplasia of the epithelial cell lining the bronchi with atrophy of the mucous gland. H&E, 100x.

Figure 16: Micrograph of sheep lung showed necrosis inside the bronchi cartilage. H&E, 100x.
stain (Figures 20-22), collagen fiber was prominent as fibrotic foci in addition to mason bodies which distinguish as a proliferation of fibroblast (deposition of collagen) by Masson Trichrome (Figures 23-27).

Figure 17: Micrograph of sheep lung showed degeneration and necrotic lesion inside the bronchi and cell lining the alveoli image. H&E, 100x.

Figure 18: Micrograph of sheep lung showed deposition of calcium salt with coagulate necrosis and infiltration of inflammatory cell. H&E, 100x.

Figure 19: Micrograph of sheep lung showed hemosiderin inside lung tissue. H&E, 100x.

Figure 20: Micrograph of sheep lung showed hyperplasia of gland with mild mucopolysaccharides. PAS stain, 100x.

Special stain
In table 2 the histopathological finding of lung showed the presences of positive neutral mucopolysaccharides (mild - moderated) which demonstrated by periodic acid Schiff stain (Figures 20-22), collagen fiber was prominent as fibrotic foci in addition to mason bodies which distinguish as a proliferation of fibroblast (deposition of collagen) by Masson Trichrome (Figures 23-27).

Table 2: Categories of lung change according to figure number, type of stain, degree and scoring

<table>
<thead>
<tr>
<th>Figure number</th>
<th>Type of stain</th>
<th>Scoring</th>
<th>Degree of lesion</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>PAS</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>19</td>
<td>PAS</td>
<td>++</td>
<td>Moderate and sever</td>
</tr>
<tr>
<td>20</td>
<td>PAS</td>
<td>+++</td>
<td>Sever</td>
</tr>
<tr>
<td>21</td>
<td>PAS</td>
<td>+++</td>
<td>Sever</td>
</tr>
<tr>
<td>22</td>
<td>Masson trichrome</td>
<td>+++</td>
<td>Sever</td>
</tr>
<tr>
<td>23</td>
<td>Masson trichrome</td>
<td>+</td>
<td>Mild</td>
</tr>
<tr>
<td>24</td>
<td>Masson trichrome</td>
<td>++</td>
<td>Moderate</td>
</tr>
<tr>
<td>25</td>
<td>Masson trichrome</td>
<td>+++</td>
<td>Sever</td>
</tr>
</tbody>
</table>
Figure 21: Micrograph of sheep lung showed hyperplasia of gland with moderate and severe mucopolysaccharides. PAS stain, 400x.

Figure 22: Micrograph of sheep lung showed necrosis and emphysema inside alveoli wall. PAS stain, 400x.

Figure 23: Micrograph of sheep lung showed swirling macrophage (oat cell). PAS stain, 100x.

Figure 24: Micrograph of sheep lung showed multi fibrotic foci. Masson’s Trichrome, 100x.

Figure 25: Micrograph of sheep lung showed thinking of alveolar wall and involve bronchiolar lumen. Masson Trichrome. 100x.

Figure 26: Micrograph of sheep lung showed masons bodies are distinguish as proliferation of fibroblast embedded in collagen and has coalescing bundle as papillary like lesion. Masson’s Trichrome, 100x.
Vascular changes represented by congestion, newly formed thrombus and bleeding of the blood vessels within the alveoli and bronchi was found in 10% of the affected cases and these may contribute to trauma-induced contusions, neoplasia specially hemangiosarcoma and bacterial infection (31,32). Furthermore, degeneration and necrosis of the epithelial cells lining the alveoli and the cartilage was observed in 6% and this might be due to the ischemia which occurs due to vascular damage (33). The necrotic area often surrounded by edge of elongated spindle cell known as (swirling macrophages or oat cell) (34-36), and the formation of oat cell in bronchopneumonia has been contributed to the release of toxin from Gram negative bacterial infection which demonstrated in most cases of fibrins bronchopneumonia (37,38). In addition to these finding there was also deposition of calcium salt due to low pH that resulted from chronic infection and parasitic disease (11,39), hemosiderin pigment, represented by the presence of copper-colored granules inside the tissue due to present of hemorrhage inside lung tissue (35,39). The presence of neutral mucopolysaccharides inside lung may be contributed to the bronchi and alveoli inflammation or due to prolonged action of irritative insults (40).

Discussion

Disorder of the respiratory system are still considering a big problem facing animals’ livestock (12,13), the significant important of sheep respiratory system diseases depended on prevalence, animal value and impact on animal production (14,15). Respiratory disorder are caused by a complicated, multifactorial illness in which viral, fungal, bacterial and mycoplasma infection work in conjunction with stress factor associated with transit, commingling and weaning (16,17), at the current study lung inflammation showed high incidences 33% and divided into three section according to the gross and histopathological examination (purulent bronchopneumonia, fibrous bronchopneumonia and nodular inflammation) this attributed to the lack of weeds and pasturing in hot dry summer which predisposes the animal to nutritional stress, theses result agreement with Dar et al. (18), Abdalla (19) and Naccache et al. (20). The anatomical structures of lung, shortness of the respiratory passage in sheep and their direct branching to the front cranial lobe may play a major role in increase the incidence of lung infection (21,22).

Infection due to parasitic represented by hydatid cysts with 30% of percentage were also demonstrate at the present study and these contributed to importation of animals from different regions (23,24), as well city environmental pollution plays a major role in occurrences of parasitic infection. large percentage of loose doge that indirect contact with other animals (sheep, cow and goat) without any restriction, play role in transmission of larva, cysts and egg of tape worm such as Echinococcus spp which spreads the infection and polluted the soil (25-28)

The histopatholical features of bronchi showed the present of epithelial cell hyperplasia and disturbances of growth inside the epithelial cell lining the mucus gland 12% this could be due to the continuous irritation of the epithelial cell with inflammatory factor (injury, irritation and infection) (29,30).

Acknowledgments

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Conflict of interest

The author declines that there is no conflict of interest

References


الآفات المرضية النسيجية لرئة الأغنام التي ترعى على مختلف المناطق

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الخلاصة

تعتبر اضطرابات الجهاز التنفسي شائعة الحدوث في الأغنام، ويصعب في كثير من الأحيان اكتشافها بسهولة في الحيوانات الحية إلى أن تتطور وتمثل مشكلة خطيرة، ولغرض تحديد الآفات النسيجية في الأغنام في مدينة الموصل والتي ترعى على مختلف المناطق المجزرة في مراكز القضاء، تم فحص 50 عينة لذكور وإناث رئات الأغنام والتي تراوحت أعمارها بين 5-10 سنوات ولفترة من شهرين إلى شهرين في الأستاد الأول خلال سنة 2021 حيث تم جمع العينات المصابة لأجل إجراء الفحص النسيجي لها، أظهرت نتائج الدراسة وجود آفات عيانية ومحجرية عديدة حيث تمتلك الآفات العيانية بوجود الالتهابات الرئوية في الفص الأمين والاعتراضات ونزف بالإضافة إلى آفات مختلفة من العقيدات أما الآفات النسيجية فتمثلت بوجود الالتهابات الرئوية الفييحة وبسبب 42.1 %، فيما كانت نسبة أضرار الرئتين 13.5 %، أما الإصابات الطفيلية فكانت بنسبة 10% كما أظهرت عينات أخرى وجود أضرارات في الفص ونسبة 21.1 %، أما نسبة التهاب والانتكاس الرئوي فكانت 20%، محاطة بخلايا دفاعية تسمى خلايا الشوفان، بالإضافة إلى ترسبة أديم الكالسيوم وصبغة الهيمودرين ويعتبر الالتهاب الرئوي وأجسام ماسون من أكثر الآفات التي تم الكشف عنها في الأغنام التي رعت على مختلف المناطق حيث تراوحت شدتها من الخفيفة إلى المتوسطة والغامضة الضراوة. نستنتج من الدراسة الحالية أن الآفات الرئوية تمثل مشكلة كبيرة وخطيرة في صناعة المواشي الحيوانية وتفوز إلى حدوث الخسائر الاقتصادية في إنتاج الحليب والوصف واللحم للحيوانات، ونوصي بإجراء المزيد من الدراسات لتحديد نوع العامل المسبب للفئات الرئوية في الأغنام سواء كانت عوامل طبيعية أم بكتيرية أم فيروسية.