



Ovine and Caprine Pulmonary Adenomatosis, At Duhok Abattoir, Iraq, First Prevalence and Pathological Study



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THE current study aimed to investigate the prevalence and pathological observation of ovine pulmonary adenocarcinoma (OPA) in sheep and goats at Duhok abattoir of Iraq. A total of 4061 lung samples from small ruminants (3667 sheep and 394 goats) were selected randomly from slaughtered sheep and goats. All samples were examined macroscopically involving color, consistency, texture, adhesion, pattern, the number of the lesion, distribution, the nature of exudates and lobe of the lung are involved. Histopathological examinations were performed from 55 affected lungs. The results showed that the prevalence of disease in the Duhok abattoir was 1.35% based on macroscopical features while results found 45.45% percentages of affected lung with OPA according to histopathological features, the gross lesions of OPA in sheep and goats found that the upper respiratory part was filled with excessive frothy material fluid and the lungs were heavy, enlargement, and edematous appearance. While the important histopathological observations of the affected parts was varied from small discrete nodules that appears white in color and are involves both lungs, measuring around 0.5–2 cm of the diaphragmatic and other lobes were recorded, and classified as classical and atypical form. Histologically results showed the presence of multiple proliferations foci of neoplastic cells in the epithelial cell of alveoli and bronchi, these proliferations appear either papillary or as the impression of solid growths appearance or acinar shape. In conclusion our study gives important data about the prevalence and pathological changes of OPA in sheep and goats at the Duhok abattoir of Iraq.

Keywords: Lung, Adenocarcinoma, Pathology

Introduction

Ovine pulmonary adenocarcinoma (OPA) is regarded an important neoplastic disease caused by a virus and affects the alveolar cells lining of alveoli of the lower respiratory system of sheep and less commonly affected goats. The disease is reported from different areas of the world such as Europe, Asia, Africa South, North America and also Iraq. The diseases resulting from an infection of a virus belonging to a beta retrovirus called Jaagsiekte sheep retrovirus (JSRV) [1]. OPA was first recorded in South Africa in the 19th century where it was called Jaagsiekte which is derived

from the Afrikaans for (chasing sicknesses) and disease has been noticed in wide types of breeds of sheep and in different countries over the world [2-5]. The characteristic of tumor cells in OPA thought to be originated from type two of alveolar epithelial cells or Clara cells, which is recorded as secretory cells and lining the epithelium of alveoli of the lung therefore, the disease is characterized by excessive secretion fluids by the tumor cells and these fluids are accumulated in the lung, which be sources of infectious virus and causes important economic and animal welfare problems [6]. The disease is classified pathologically into two forms either classical or atypical forms. In the

first from classical, the neoplastic foci occurs in the cranioventral area of all lung lobes, while in the second form atypical, tend to be more granular in both early and late tumors [7, 8]. Histopathologically, the tumor cells characterized by a papillary or acinar adenocarcinoma, which is appears as cuboidal or columnar cells are lining of bronchi and alveoli, the tumor area are surrounded by a zone of connective tissue which is infiltrated with mononuclear inflammatory cells, while the atypical form the tumor cells appears as solitary or multiple nodules in the lung and usually located in the diaphragmatic lobe, histopathologically tumor cells characterized by same features of classical form except severally infiltrated by mononuclear inflammatory cells and zone of fibrous connective tissue [1,9]

Because little information has been carried out OPA in Iraq especially in the Kurdistan region, therefore, our current study was carried out to the study of the prevalence and pathological changes of OPA in sheep and goats at Duhok abattoir in Kurdistan region of Iraq.

Material and Methods

Sample collection

This research was conducted at Duhok abattoir from October 2020 to October 2021, this abattoir is one and biggest abattoir at Kurdistan region of Iraq, it's located in Semel region of Duhok governorate. The most of animals per day are slaughtered during this abattoir sheep and goats are regarded one of important animals are slaughtered compared to other animals, and all animals were male, local breeds, and aged was around between 1-2 years, a total of 4061 lung samples from small ruminants (3667 sheep and 394 goats) were collected in the study. And the samplings were collected twice a day each week. The routine meat inspection ways were carried out to detect the observation of the pathological

changes macroscopically diagnosed by visualization, palpation, and some cut incisions with specific macroscopical criteria are used as color, consistency, texture, adhesion, pattern, number of the lesion, distribution, the nature of exudates and lobe of the lung are involved, all of these criteria was photographed and recorded.

Pathological study

For histopathological examination, the affected lungs from sheep and goats were collected, and examination was done at histopathology laboratory at College of Veterinary Medicine/ University of Duhok, all samples of lungs with typical lesions were fixed in 10% neutral buffered formalin. Then the samples were dehydrated in ascending concentration of alcohol, and then cleared in xylene, for preparation of paraffin block tissues are embedded in a pure white paraffin wax at melting point 54-56°C. The processed and embedded tissue sections were cut at 4-5 µm with a rotary microtome (Leica, Germany). In the end the slides were stained by using hematoxylin and eosin (H &E) stain [10]. The stained sections will be examined under field microscope and photographed by using digital computerized camera canon (Leica, Germany). The results of gross and histopathology will be analyzed and interpreted

Results

Prevalence of OPA

Out of 4061 small ruminants (3667 sheep and 394 goats) examined of slaughtered animals, 55 cases were suspected of OPA after gross examination 35 samples in sheep and 20 samples in goats. The microscopical examination confirmed that 35 cases are involved with OPA in 35 of lung samples; the study found that 1.35% of the prevalence of the disease in Duhok abattoir. The data of sheep and goats included in this study and confirmation of OPA are summarized in Table 1

TABLE 1. Percentage ratio of samples examination and forms of tumor.

| Animal species | Number of lung samples | Percentage rate of gross examination | Percentage rate of Histological examination | Pathological form of OPA | Histological type of OPA |
|----------------|------------------------|--------------------------------------|---|--------------------------|--------------------------|
| Sheep | 3667 | 35(0.95%) | 29(82.8%) | 27 | Classical |
| | | | | 12 | Atypical |
| Goat | 394 | 20(5%) | 16(80%) | 11 | Papillary |
| | | | | 5 | Atypical |
| Total | 4061 | 55(1.35%) | 35(45.45%) | | |

Gross Pathology

The gross lesions of OPA in sheep and goats showed presence of fluid of trachea and lungs, and appears edematous in textures, enlargement in size and heavy, and in texture. On palpation an affected part appeared consolidated foci and was darker than the adjacent normal part. Furthermore, the results show the regional lymph nodes were often enlargement and edematous as shown in (Figs. 1, 2). The result showed the histopathological changes were observed from affected areas and was vary from small discrete white pale nodules involving both lungs and measuring around 0.5–2 cm of affected of lobes were recorded, during the structure of neoplastic area results showed to from either classified and atypical form. In classical form the structure of neoplastic foci is appears as

diffuse or nodular depending on the extent of the tumor and generally appears in the cranioventral parts of all lung lobes but any part of the lungs may be involved and not protrude to the surface of the lung as shown in (Fig. 3)

While in the atypical form the affected part appears more nodular and these nodules were very hard, pearly white in color, the consistency may be solitary or multiple and sometimes they look like scars. When the tumor nodules are cut, they appear as white in color, very well distinguished from the surrounding parenchyma, and their surface appears dry in texture while the size area of tumor nodules measured from 0.5–1 cm diameter and distributed throughout the lungs as shown in (Fig. 4).

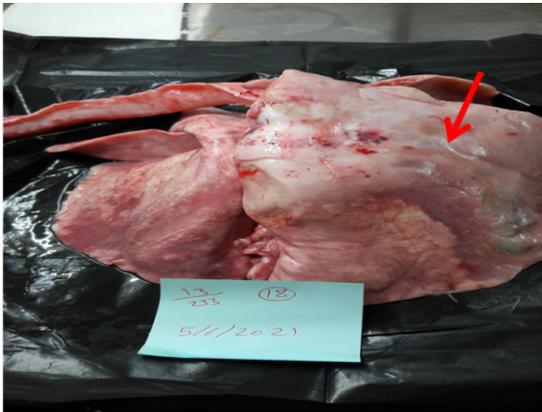


Fig. 1. Macroscopical observation of lung affected with OPA, showed the lung are enlarged, heavy and edematous compared to normal part (Red arrow).



Fig. 2. Macroscopical observation of lung affected with OPA, showed the lung with neoplastic areas was vary from small discrete white nodules involves both lobes of lungs, mearing around 0.5-0.2cm (Red arrow).

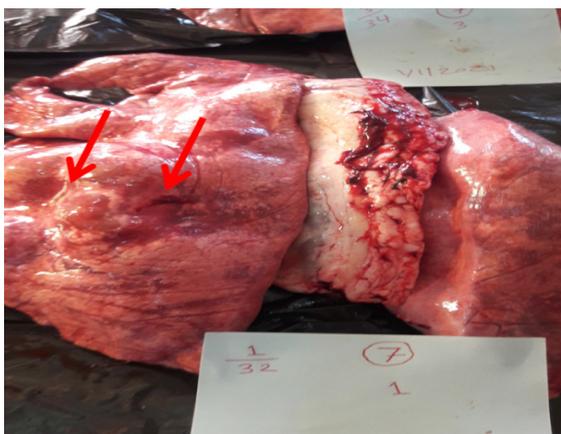


Fig. 3. Macroscopical observation of lung affected with OPA classical form showed parts of all lung lobe involved nodular, light purple to light grey in color, and not protrude to the surface of the lung (Red arrow).



Fig. 4. Macroscopical observation of lung affected with OPA atypical form showed parts of all lung lobe involved multiple nodules very hard, pearly white in color, the consistency may be solitary or multiple and sometimes the look like scars shape (Red arrow).

Histopathology

The result shows the histological changes of OPA from the lung specimens from nodular area by the presence of several proliferation of multiple foci of neoplastic cells in the epithelial cell lining of both alveoli and bronchi, and these proliferations appears either as papillary or as impression of solid growths appearance or acinar shape as shown in (Fig. 5, 6). Furthermore, some neoplastic cells are sufficiently pronounced to give the alveolar neoplastic nodules pressure to the adjacent alveoli and leading to collapse with increase in size. The normal alveolar epithelial cells replace cuboidal or columnar cells, while the general structure of the alveolar wall is preserved as normal and the nuclei of the tumor cells are located in the basement region of the cell with mitotic figures are not abundant, while the

cytoplasm of tumor cells appears homogeneous eosinophilic in cuboidal cells, clear spaces as vacuole within columnar ones and stroma of the tumor cells of OPA is and infiltrated by variable number of inflammatory cells mononuclear types as plasma cells, macrophages lymphocytes cells and connective tissue fibers, around the neoplastic nodules and filling the lumen of alveoli, macrophages, vacuolated cytoplasm and are continually present with variable numbers. The result showed the histological characteristic changes of atypical form of OPA are similar to classical form of OPA, the structure of pattern growing neoplastic cells appears more acinar than papillary structure and the stroma is heavily infiltrated by mononuclear inflammatory cells as well as fibers connective tissue as shown in (Fig. 7, 8, 9,10)

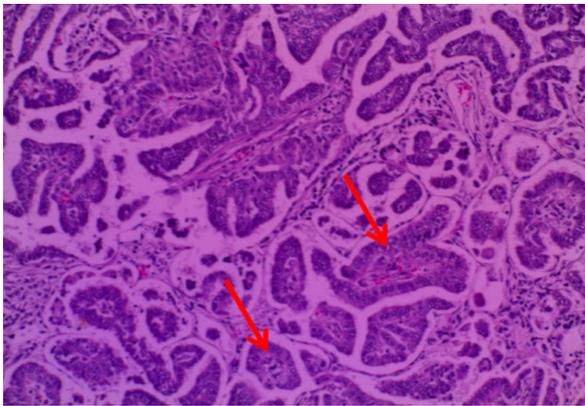


Fig. 5. Histopathological features of classical Papillary OPA, the neoplastic alveoli are lined by cuboidal or columnar cells red arrow and the nuclei of the tumor cells are located in the basal region of the cell with mitotic Fig. (black arrow). H&E, 20x.

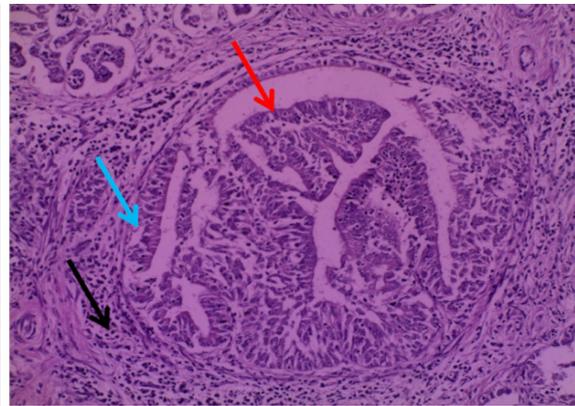


Fig. 6. Histopathological features of classical acinar OPA, the bronchiole showing polypoid in growths red arrow lined by columnar cells with vacuolated cytoplasm blue arrow and sustained by connective tissue core with lymphoid aggregation (black arrow). H&E, 20x.

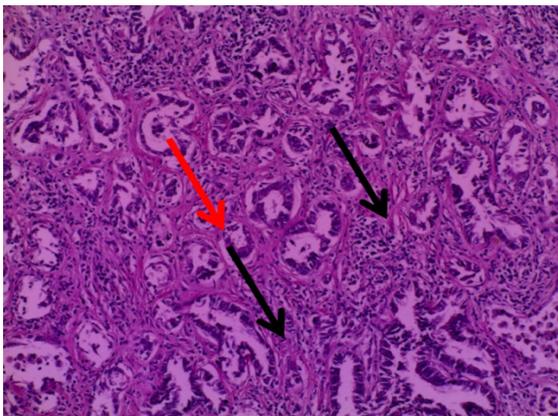


Fig. 7. Histopathological features of Atypical acinar OPA, columnar epithelial cells lining affected alveoli with acinar red arrow, in which the stroma appears infiltrated by mononuclear cells and connective tissue (black arrow). H&E, 20x.

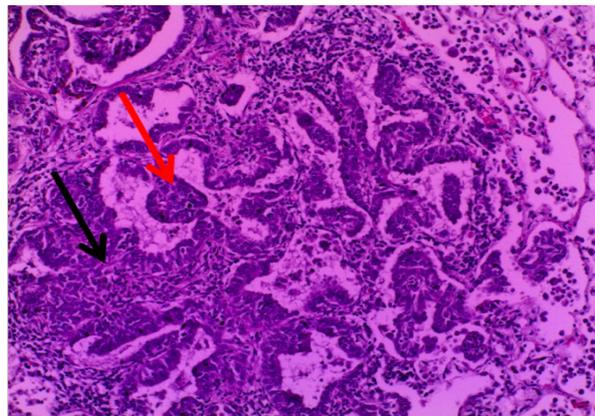


Fig. 8. Histopathological features of typical Papillary OPA, tumor cells polypoid in growth lined by columnar cells red arrow sustained by connective tissue core and severe infiltration of mononuclear inflammatory cells (black arrow). H&E, 20x.

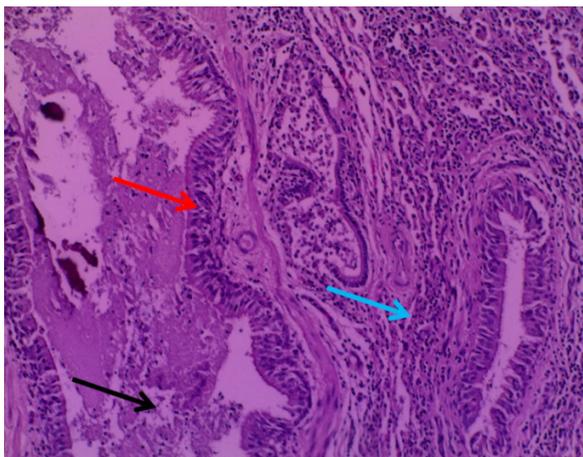


Fig. 9. Histopathological features of classical acinar OPA, hyperplasia of epithelial cells lining of bronchi red arrow, the lumen filled with frothy discharge material black arrow, and peribronchial lymphoid aggregation (blue arrow). H&E 20x.

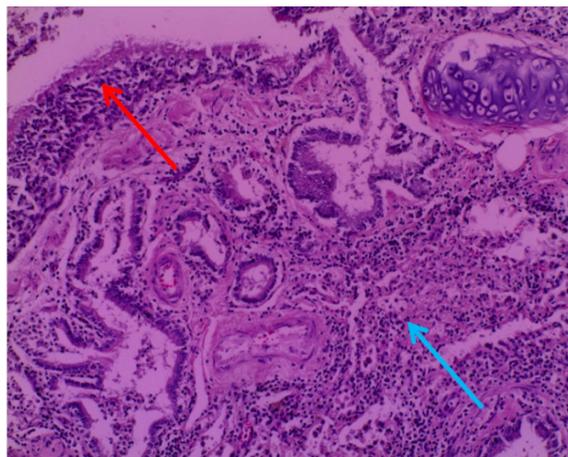


Fig. 10. Histopathological features of classical papillary OPA, showed hyperplasia of epithelial cells lining of bronchi red arrow, and diffuse infiltration of mononuclear inflammatory cells (black arrow). H&E 20x.

Discussion

The disease OPA has been studied in different countries of the world, involving Iraq [3-5,9]. The occurrence of disease throughout the world is either sporadic or endemic and it is characterized as important disease of sheep and goats [11]. There is a lack of quiet data information about the occurrence of disease at Duhok city of Iraq. Therefore, our study investigated the prevalence and Histopathological alterations of disease investigated in slaughtered sheep and goats at Duhok abattoir. Our results showed that 1.35% of sheep and goats are affected after gross examination of lung samples, while around 45.45% of samples were improved via histopathological investigation. This data in disagreement with some authors [9, 12] and this is variation could belong to group or herds of animals, housing, breed, sex, age, management conditions, source of material, location, nature of dissemination, season, and research time, environment and cultural breeding of animals [13, 14], However, to obtain quite information about the prevalence of OPA of the world is difficult because OPA is not appropriate disease and therefore very few countries collected data around the diagnosis of a number of cases of disease also Singh et al. [15] recorded that the most of sheep affected with disease is in belong to housing system mainly during the cold weather, may have a substantial impact on the prevalence of OPA and the time during when animals are maintained closely [16]. Macroscopically our

study showed that the lesions of OPA are varied from structure which is either classical or typical form and growth of tumor cells appears as multifocal nodules or complete consolidation of the entire lungs tissues and in the color which was varied from white to grayish in color [6,7,17] as well as the study showed excessive amounts of fluids in the trachea and lungs of affected samples which was an outstanding finding to distinguish the OPA from others disease of respiratory system and these findings have been investigated in other researches as important point for diagnosis of OPA, these researches are in agreement that the characteristic clinical diagnostic finding of OPA is accumulation of amount fluid in lungs and these fluids is pass through out of nostrils of affected sheep and goats when the animal lowering its head to down [18].

In our current study, involved histopathological changes between forms of OPA were found in the lungs of sheep and goats. As well as proliferation of tumor cells in the alveolar or bronchial epithelial cells giving variation patterns of growth of tumor cells either as papillary form or acinar form. Our study shows little observation has been shown between the macroscopical and microscopical lung changes in sheep and goats. So far, the researchers [19, 20, 21] recorded similar histopathological observation as nodular foci, projection of tumor as papilla, acinar form, infiltration of inflammatory cells and peribronchiolar lymphoid aggregation.

Histopathological observation was shown to have different growth of neoplastic as proliferated foci of cuboidal or columnar cancer cells rising in both the alveoli or from the wall of bronchioles, which were observed in tissue sections from the nodules areas of the affected parts. The microscopical changes which were investigated in all OPA affected lungs in this study were confirmed by other previous studies [22, 23]. There were mainly two forms of OPA and the developmental of tumor growth found as structure acinar, glandular and papillary. The wall of alveoliis enlarged in a papillary projection revealing the epithelial cells and supported by zone of connective tissue, the proliferation of neoplastic cells are filled both the alveolar and bronchial lumen either partially or totally and these observation is in agreement with previous studies [14,24]. The explained lesions in the affected lung tissues, beside the infiltration of inflammatory cells in the areas nearing the neoplastic area, So far, depend to the nature of the causative agents replication and its effects on type two pneumocyte cells as well as the proliferation of these cells and the establishment of malignant tumors, and these findings were also similar to the observations made by some authors [17].

The infiltration of different types inflammatory cells especially mononuclear types as macrophages in the lumen of alveoli and bronchi and this also previously observed by previous studies in both experimentally and natural affected OPA [16, 25]. The role of infiltration of these inflammatory cells especially macrophages cells in OPA has not been clear understand, however, it was suggested that these cells may be have a role to clearance as surfactant system which is secreted by type two pneumocyte cells. It was also observed that the neoplastic cells may have secreted a chemotactic factors that have a role for responsible of the recruitment of inflammatory cells to affected area like macrophages [26]. Besides the result showed the prominent inflammatory reaction observed in the affected lungs were the hyperplasia of the peri-bronchiolar lymphatic tissue accumulates and this due to lymphatic aggregation around bronchiole, and replication of virus in the epithelial cells of bronchi. Therefore, the size of bronchi decreased and hyperplasia produce pressure to the alveoli and bronchi had disappeared. However, also an important signs, was the hyperplasia of smooth muscle cells in the interstitial tissues were observed [27,28]

Moreover the study shows the of stroma atypical form of OPA was infiltrated with mostly with mononuclear inflammatory cells lymphocyte, monocyte, plasma cells, and connective tissue compared than in classical form of OPA as well as lymphoid proliferation around the bronchioles and neoplastic area and this belong inflammatory reaction and tumor events[1,7].

Conclusion

In our study it can be concluded that although the sheep and goats slaughtered are apparently healthy, large percentages were found either to harbor certain pathological lesions or be infected with different diseases. This study showed that one of important disease was diagnosed at slaughtered houses is OPA and results showed there is a difference of the prevalence between sheep and goats and no differences of gross and histopathologic lesions between sheep and goats.

Acknowledgement

The author acknowledges the Duhok research center for preparation of tissue section, and Mrs Balkees Ali Ahmed for helping of collection of samples.

Funding statement

This manuscript was funded by the college of veterinary medicine, University of Duhok, Iraq

Conflict of Interest

This article is no conflict of interest

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أول دراسة امراضية وانتشار الورم الغدي الرئوي في الاغنام والماعز في مجزرة دهوك-العراق

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استهدفت الدراسة الحالية التحقق من انتشار الورم الغدي الرئوي في الاغنام والماعز والتغيرات المرضية في مجزرة محافظة دهوك-العراق. تم اختيار ٤٠٦١ عينة رئوية من المجترات الصغيرة (٣٦٦٧ من الاغنام و ٣٩٤ من الماعز) عشوائياً من الاغنام والماعز المذبوحة. حيث تم فحص جميع العينات عياناً والذي تضمنت اللون، القوام، الملمس، الالتصاق، النمط، عدد الافات، التوزيع، وطبيعة الإفرازات، كذلك تضمن الفحص فصوص الرئة. تم إجراء فحوصات نسيجية مرضية من ٥٥ رئة مصابة وقد أظهرت النتائج أن انتشار المرض في مجزرة دهوك كان ١,٣٥% بناء على السمات العيانية، بينما بينت النتائج ان ٤٥,٤٥% من الرئة كانت مصابة بالورم الغدي الرئوي وفقاً للسمات النسيجية المرضية، وأظهرت الافات أن الجزء التنفسي العلوي كان ممثلاً وبكثرة بسائل ذات طبيعة رغوية وكانت الرئتان ثقيلتان ومتضخمتان ومتورمتان. في حين أن الملاحظات النسيجية المرضية الهامة للأجزاء المصابة كانت متنوعة وبشكل عقيدات صغيرة منفصلة وببضاء اللون وفي كلا الرئتين وكانت حوالي ٢-٠,٥ سم من الحجاب الحاجز والفصوص الأخرى، وصنفت على أنها شكل كلاسيكي وغير نمطي. كما وأظهرت النتائج النسيجية وجود بؤر تكاثر متعددة للخلايا الورمية في الخلايا الظهارية من الحويصلات والشعب الهوائية، وظهرت هذه التكاثرات بشكل حلبي أو كمنو صلب أو بشكل عنبيبي. ختاماً، قدمت دراستنا بيانات هامة حول انتشار الورم الغدي الرئوي إضافة الى التغيرات المرضية في الاغنام والماعز في مجزرة محافظة دهوك-العراق.