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Molecular and pathological identification of ovine pulmonary adenomatosis at Mosul city abattoirs

O.Z. Jiad[1] and A.M. Al-Saidya[2]

[1]Veterinarian, Private Sector, [2]Department of Pathology and Poultry Diseases, Faculty of Veterinary, Mosul University, Mosul, Iraq

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Correspondence:
A.M. Al-Saidya
al2011saidya@uomosul.edu.iq

Abstract

Ovine Pulmonary adenomatosis OPA is a frequent condition that affects small ruminants, characterized by inflammation of the lung parenchyma, and causes polyp-like hyperplasia of the epithelium lining the alveoli and interstitial tissue like bronchi. It is frequently brought on by the Jaagsiekte virus. Samples from sheep animals at Mosul's slaughterhouse and butcher shops were collected. The RT-PCR results consider the first molecular identification of the JSRV virus in Mosul city. The Macroscopic examination revealed gray-colored tumor nodules scattered across the surfaces of the lung lobes. The tumor tissue had clear and distinct boundaries from the adjacent pink pneumatic lung. Histological analysis showed nodular tumor areas (proliferative pulmonary nodules) were composed of carcinomatous cells supplementary with an adequate amount of extracellular matrix, characteristic columnar and cuboidal metaplasia, as well as hyperplasia of pulmonary cells, especially the pneumocytes (type II), an epithelial cell (secretory types) in the pulmonary alveoli, Non-ciliated Clara cells as well as the epithelial lining of the terminal bronchioles. Finally, our study sheds light and provides data on the importance of OPA in Mosul abattoir through the PCR diagnosis of the causative agent, and the macroscopic and histological pathological changes.

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Introduction

Ovine pulmonary lesions" are a lung disease that can affect sheep. These diseases can lead to serious health problems, and they can even be fatal. Ovine pulmonary lesions can be challenging to diagnose and can be mistaken for other types of diseases (1,2). Jaagsiekte ovine Retrovirus (JSRV) is a β - retrovirus etiology of ovine pulmonary adenomatosis (OPA) or ovine pulmonary adenocarcinoma, which is a contagious pulmonary and cancerous disease of sheep (3). The virus is present in respiratory discharges and mainly replicates inside the pulmonary alveolar cells (4,5). Jaagsiekte disease spreads primarily through inhaling infected respiratory secretions via airborne transmission and can also spread through milk and colostrum. Ovine pulmonary adenomatosis is confined to the lungs and, in rare instances, the lymph nodes are affected. They appear as hard, white to gray and well-defined nodules of varying sizes, ranging from small nodules to large solid areas encompassing one or more pulmonary lobes (6). The conducting airways contain a significant amount of white, foamy fluid. The diseases are characterized histologically by papillary proliferation of columnar type II pneumocytes and associated cells in the bronchioles (7,8). The current study aimed to demonstrate the pathological and molecular detection of ovine pulmonary adenomatosis in Mosul, Iraq.

Materials and methods

Ethical approval

The Ethical Committee for Animal Experimentation of the Veterinary Faculty of the University of Mosul approved the experimental work U.M.VET.2024.015.

Collection of a lung sample

A total of 11 lung samples were collected from previous work from sheep suspected of OPA, where 25 (16%) Lung samples had been revealed pulmonary adenomatosis lesions at Mosul's slaughterhouse and butcher shops were collected during weekly inspections.

Gross and histopathological evaluation

The collected specimens were examined obviously to record any unusual lesions in the lung that appear by the naked eye or by palpation and observation noting any changes in the lung's consistency, size, and color. Two parts of the samples were taken from the lesion areas. The first part was placed in a buffer solution of formalin at a concentration of 10% for 48 hours or more for fixation. Histopathological sections were carried out through routine paraffin embedding technique, firstly dehydrated with ascending ethanol concentration, clearance with xylol and embedded with paraffin wax (9,10) then sectioned about 5-6 µm in thickness and finally, stain with routine hematoxylin and eosin stain (11). For the second part of the diagnosis, suspected samples were placed in small plastic containers, and tests using polymerase chain reaction (PCR) technology to diagnosis the virus that causes OPA cases.

Molecular detection of ovine pulmonary adenomatosis virus

RNA was extracted from suspected lung tissue samples using (Gena Bioscience, Germany kit) accurately per the manufacturer's instructions. Detection of the JSRV virus using a conventional RT-PCR kit was provided by Bioron GmbH, Germany. One RT-PCR Master Mix is designed for all applications requiring Reverse RNA transcription into cDNA and subsequent PCR amplification. Only primers and sample RNA were added to the Master mix. We used Forward/ Reverse primer 5′ TGGGAGCTCTTTGGCAAAAGCC-3′ 5′-TGATATTTCTGTGAAGCAGTGCC-3, respectively (12,13). The primers were manufactured by Macrogen Corporation, Korea. According to the manufacturer's instructions, the subsequent optimal reaction was at 55 °C for 30 min reverse transcription, RNA denaturation, and inactivation of reverse transcriptase at 95 °C for 3 min. Then, 40 cycles PCR, denaturation at 92 for 10 s, annealing at 62 °C for 10 s, extension at 72 °C for 20 s, and extension at 72 C° for 7 min. The RT-PCR technique was achieved on the MiniAmp PlusTM Thermocycler PCR, USA. Electrophoresis (Cleaver Scientific, England) was prepared on 1.5% agarose gel holding gel-safe stain to separate charged DNA fragments according to size. RT-PCR bands were 176 bp JSRV cDNA.

Results

RT-PCR for Ovine Pulmonary adenomatosis virus

All 11 lungs tissue showing gross and microscopic characteristic features for ovine pulmonary adenomatosis were positive for the JSRV virus and located in lanes 2-12 (Figure 1).

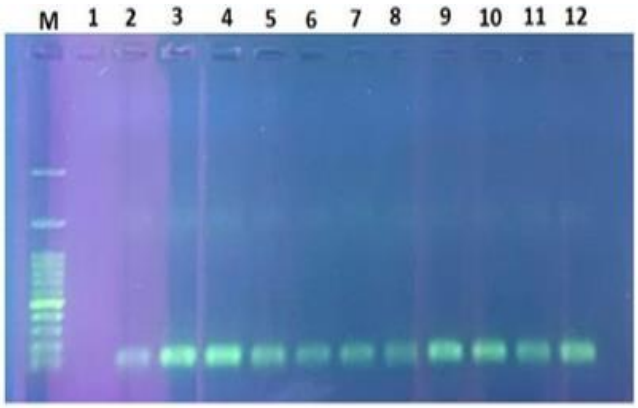

Figure 1: Positive JSRV virus is located in lanes 2-12 (176 bp), whereas negative control is in lane 1. Lane M: 100 bp DNA ladder (Addbio company, Korea).

Pathological characteristic of ovine pulmonary adenomatosis

The Macroscopic examination of lung samples suspected of being infected with OPA revealed gray-colored tumor nodules scattered across the surfaces of the lung lobes. The tumor tissue had clear and distinct boundaries from the adjacent pink pneumatic lung. Additionally, pneumonia lesions showing congestion and consolidation were observed. The Histological analysis of a lung suspected with OPA, showed nodular tumor areas (proliferative pulmonary nodules) were composed of carcinomatous cells supplementary with an adequate amount of extracellular matrix, characterized by hyperplasia of pulmonary cells, especially the pneumocytes (type II), an epithelial cell (secretory types) in the pulmonary alveoli, Non-ciliated Clara cells as well as the epithelial lining of the terminal bronchioles. These tumors cuboidal or columnar types of cells exchange the typical alveolar cells and form glandular acinar, papillary, and mixed architectural arrangements. Some cancer cells were observed shedding and sloughing inside the lumen of alveoli, along with thickening of alveolar walls due to infiltration of inflammatory cells and deposition of collagen fibers (Figures 2-7).

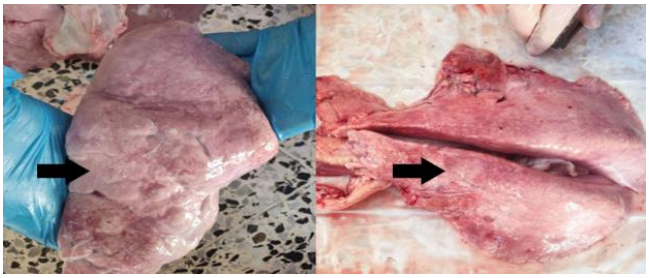

Figure 2: Macroscopic images of Ovine lungs with OPA suspected revealed signs of pneumonia like congestion and consolidation as well as the presence of gray nodules with clear and distinct boundaries between the tumor tissue and the adjacent airy pink lung.

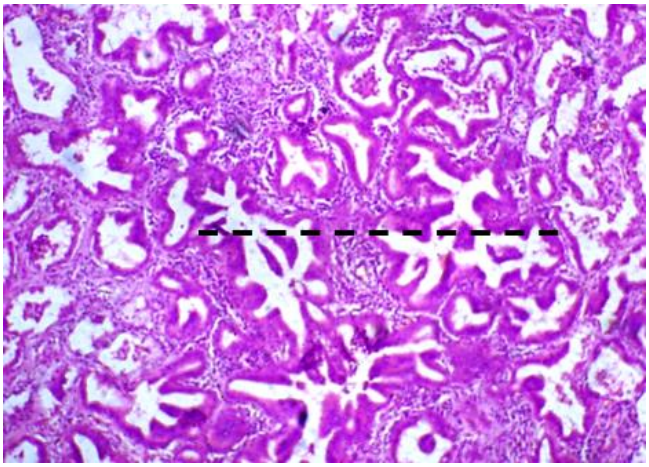

Figure 3: Histological section of ovine lung showing nodular tumor areas (proliferative pulmonary nodules) were composed of carcinomatous cells (H&E stain, 40x).

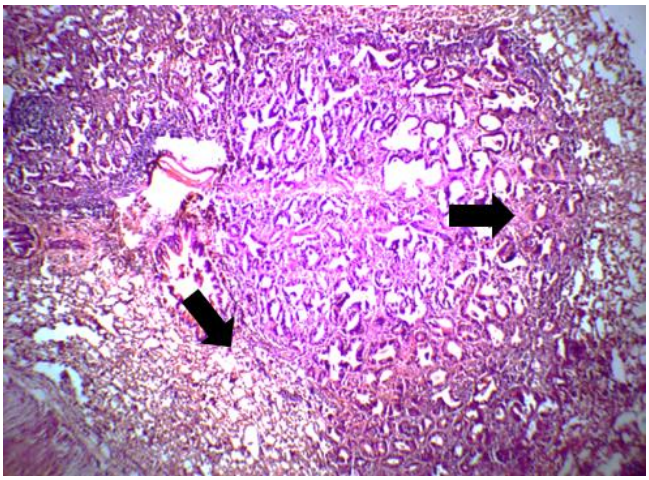

Figure 4: Histological section of ovine lung showing nodular tumor areas (proliferative pulmonary nodules) were composed of carcinomatous cells (H&E stain, 40x).

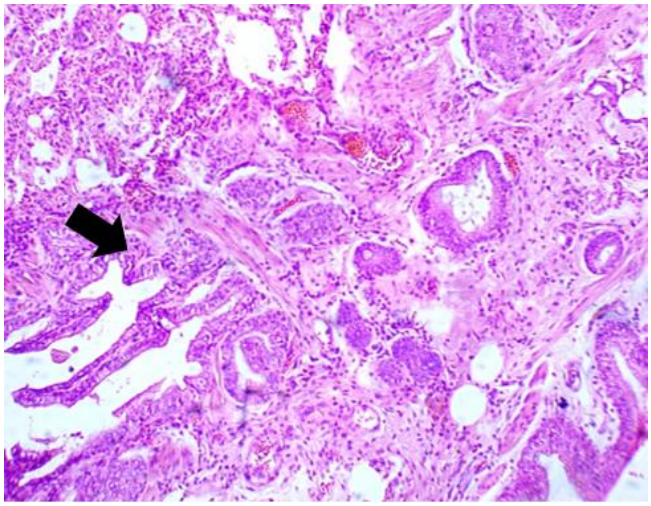

Figure 5: Histological section of ovine lung showing hyperplasia of epithelial cells lining the alveoli and bronchi, forming a glandular acinar, papillary and mixed architectural arrangements, with Some cancer cells were observed shedding and sloughing inside the lumen of alveoli, along with thickening of alveolar walls
(H&E stain, 100x).

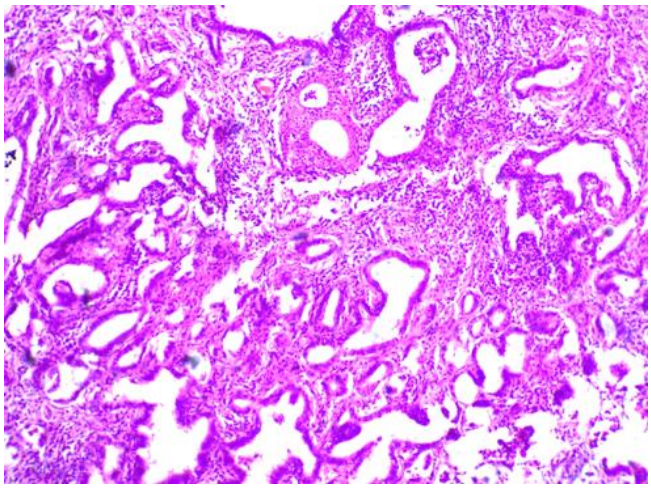

Figure 6: Histological section of ovine lung showing a glandular acinar, papillary and mixed architectural arrangements, with Some cancer cells, along with thickening of alveolar walls
(H&E stain, 100x).

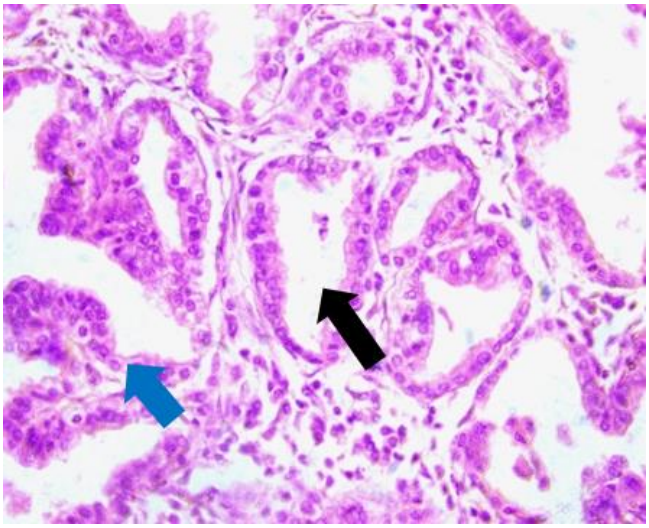

Figure 7: Histological section of ovine lung showing hyperplasia of epithelial cells lining the alveoli and bronchi, forming a glandular acinar, papillary and mixed architectural arrangements, as well as cuboidal and columnar metaplasia of the squamous epithelium lining the alveoli.

Discussion

Ovine pulmonary adenomatosis (OPA) has been documented in various countries globally, such as Iraq (13,14). Also known as Jaagsiekte, it's a contagious lung tumor in sheep caused by Jaagsiekte sheep retrovirus. JSRV can infect and transform the alveolar and bronchiolar secretory epithelial cells, causing the formation of tumors that may occupy significant portions of the lung. Furthermore, tumor growth frequently disrupts normal respiration leading to increased fluid production in the lung. This study is dedicated to detecting and identifying pathological, gross, and microscopic characterization and the definitive diagnosis of the viral causative factor of OPA using the PCR reaction in sheep at Mosul city abattoirs.

The results indicated that ovine lungs with characteristic macro and micro lesions were positive for the JSRV virus, this agreed with different previous researchers (15,16). PCR analysis can detect the JSRV virus from blood, lung samples, mediastinal lymph nodes and lung fluid (17,18). Our RT-PCR results consider the first molecular identification of the JSRV virus in Mosul city (19,20). Different studies categorized the Pathological findings of OPA into Classic and Atypical gross and microscopic features (21). The Classical type of the suspected OPA lung samples revealed a gray-colored tumor nodule scattered across the surfaces of the lung lobes. The tumor tissue had clear and distinct boundaries from the adjacent pink, pneumatic lung (22).

The Word Typical form of OPA, is described by multiple nodules in the lung lobes especially the diaphragmatic lobe, as well as large volumes of frothy fluid inside the conducting airways (23,24). Microscopically, The Classical OPA included hyperplasia of pulmonary cells, especially the pneumocytes (type II), an epithelial cell (secretory types) in the pulmonary alveoli, Non-ciliated Clara cells as well as the epithelial lining of the terminal bronchioles (25,26). The OPA virus (JSRV) replicates inside these types of pulmonary cells, which express a particular type of cellular receptor called Hyal-2 considered the primary receptor for JSRV (27,28). JSRV viral envelope glycoprotein, surface protein attached to the Hyal-2 receptor, leading to enter the virus by the endocytic process. The main multiplying cancerous cell type, signifying as 60-80% of cell residents, were epithelial cells in alveoli type II (Pneumocytes) with papillary or acinar like structures noted. as well as the proliferative Clara cells as 15- 20% (29,30). All lung samples with OPA lesions also show multiple degrees of inflammatory lesions, like inflammation of bronchitis, bronchiolitis, interstitial pneumonitis, and inflammatory cell reaction as a pulmonary tissue defense mechanism (31,32). One of the significant causes described by OPA as a malignant type of tumor is metastasis. Unusually, most metastases of the primary tumor are microscopically found in the mediastinal lymph nodes but in only 10% of the ovine Pulmonary adenocarcinoma (OPC) cases (33,34). R-PCR and immunohistochemical studies for JSRV capsid protein markers were used to identify the metastasis of OPC in other tissues and organs (35,36).

Conclusions

The current study concluded that JSRV was identified molecularly using R-PCR. This was further confirmed by the fact that a significant percentage of the Pulmonary samples displayed the typical macroscopic lookand all the typical histological abnormalities associated with the OPA characteristic lesions.

Acknowledgment

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Conflict of interest

The investigator announces there is no conflict of interest.

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الكشف الجزيئي والمرضي للورم الغداني الرئوي في الأغنام في مجازر مدينة الموصل

أسامة زياد جياد[1] و أحمد محمد علي السيدية[2]

[1]طبيب بيطري، قطاع خاص، [2]فرع الأمراض وأمراض الدواجن، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

الورم الغداني الرئوي في الأغنام هو من الحالات المرضية المتكررة والتي تصيب المجترات الصغيرة والذي يتميز بالتهاب النسيج الرئوي مسبباً فرط تنسج للخلايا الطلائية المبطنة للحويصلات والهوائية والقصيبات الهوائية والمسبب هو فايروس الريترو. تم جمع عينات من الأغنام في مسالخ الموصل ومحلات الجزارة. يعد التشخيص تعتبر نتائج اختبار تفاعل البوليميراز المتسلسل أول تحديد جزيئي لفيروس الريترو في مدينة الموصل. اظهر الفحص العياني وجود عقيدات ورمية رمادية اللون منتشرة على أسطح فصوص الرئة وكان للنسيج الورمي حدود واضحة ومتميزة عن الرئة الطبيعية المجاورة. نسيجياً لوحظ أن مناطق الورم العقيدي (العقيدات الرئوية التكاثرية) تتكون من خلايا سرطانية التي كانت تعاني من حؤول عمودي ومكعبي مميز بالإضافة إلى تضخم الخلايا الرئوية وخاصة الخلايا الرئوية (النوع الثاني)، والخلايا الظهارية (أنواع إفرازية) في الحويصلات الرئوية وخلايا كلارا غير الهدبية وكذلك البطانة الظهارية للقصيبات الهوائية. أخيرًا سلطت الدراسة الحالية الضوء على أهمية مرض الورم الغداني الرئوي في الأغنام في مدينة الموصل ومن خلال التشخيص الجزيئي للعامل المسبب للمرض عن طريق تفاعل البوليميراز المتسلسل، وكذلك دراسة التغيرات المرضية العيانية والنسيجية.

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