

Intrathoracic and Extrathoracic Metastases in Pirlak Ewes With Ovine Pulmonary Adenocarcinoma (Jaagsiekte)

Key words

extrathoracic metastasis;
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Abstract: Ovine pulmonary adenocarcinoma (OPA) is a contagious neoplastic disease caused by jaagsiekte sheep retrovirus (JSRV) and is characterized by chronic respiratory clinical signs. In this study we describe intrathoracic and extrathoracic metastases in Pirlak ewes naturally infected with JSRV. Two Pirlak sheep flocks brought from two different provinces had progressive respiratory distress, nasal discharge, cough, and emaciation, and nine ewes from these two flocks were necropsied at the request of the owners. Gross findings revealed purple colored and consolidated cranioventral lung lobes with scattered white nodules of various sizes. One ewe had metastasis in the mediastinal lymph node, and another had metastasis in the left kidney. Histopathological examinations of the tumors in the lungs and metastases showed papillary and acinary growth patterns. Immunohistochemically, strong JSRV-Env expression was observed in neoplastic cells.

Introduction

Ovine pulmonary adenocarcinoma (OPA, Jaagsiekte), caused by jaagsiekte sheep retrovirus (JSRV), is an infectious, neoplastic disease characterized by chronic respiratory clinical signs (1). Progressive cases that cause neoplastic pulmonary lesions cause economic losses (decreased milk, meat, and wool production) in sheep-raising in many countries (2). JSRV is an exogenous beta-retrovirus that induces neoplastic transformation of secretory epithelial cells of the respiratory tract (1, 3). JSRV, mainly transmitted by inhalation, can also occur experimentally by intratracheal inoculation with lung secretions (4). Perinatal transmission to lambs through colostrum and milk has also been reported (5). The incidence of OPA in affected flocks is usually around 2-5% effective in the average age group of 2–4 years after a long incubation period (6). Replication of JSRV occurs in epithelial cells of the respiratory system, bronchiolar cells, type II pneumocytes, and Club cells (7).

Classical and atypical OPA forms have been defined in the literature. In classical OPA, the neoplastic lesions occur generally in the cranioventral lobes. There is copious

amount of lung fluid in the bronchi and bronchioles. In atypical OPA, focal to multifocal coalescing white nodules are observed in the lungs (4, 6). The classical form is invasive, while the atypical form is more easily circumscribed. There may not be visible changes in the lymph nodes, or there may sometimes be small metastases in the mediastinal lymph nodes (4). Renal, cardiac (8), liver, spleen, skeletal muscle, and adrenal gland (9) metastases have been reported in different studies. Microscopically, papillary and acinary or myxoid growth patterns have been described in both forms (4). Mornex et al (2003), suggested that these pathological findings observed in OPA are similar to a subtype of lung adenocarcinoma found in humans (10). Due to some clinical and pathological similarities, OPA is considered as a useful animal model for understanding human lung adenocarcinomas (11-13).

Clinical signs of OPA are tachypnea or dyspnea, progressive pneumonia, unresponsiveness to antibiotic treatment, emaciation, and exercise intolerance (4). The wheelbarrow test in affected animals is used as an in vivo diagnostic tool that

facilitates diagnosis, but it has possible consequences for animal welfare and may result in euthanasia (14). Although PCR and ELISA techniques are also used in the diagnosis, no reliable farm-level antemortem diagnostic test exists (3, 4). Therefore, necropsy followed by histopathological examination is the convenient method of diagnosis (15).

In this study, we describe intrathoracic and extrathoracic metastases in Pirlak ewes naturally affected by OPA. The present work reveals that it is essential to evaluate regional lymph nodes and distant organs for metastasis along with the lungs in sheep with OPA.

Materials and methods

Pirlak sheep from two different flocks from two geographically close provinces had severe progressive respiratory distress along with nasal discharge, cough, and emaciation despite a good appetite. The animals were unresponsive to antibiotic treatment, and death followed. The anamnesis stated that similar clinical signs were observed in most of the sheep in these flocks and that they had continued over two years. The first flock was previously diagnosed as pasteurellosis by a veterinarian, and a wheelbarrow test demonstrated severe seromucous nasal discharge in the second flock.

At the request of the owners, nine four-year-old ewes, which were brought to our laboratory as dead at different times, were necropsied. Tissue samples were taken for histopathologic evaluation and fixed in 10% formalin solution. After routine processing, samples were embedded in paraffin, cut 4 μ m thickness, and stained with hematoxylin & eosin (H&E). In addition, the slides of the ewes with the most severe lesions (case 1) and with metastasis (cases 6 and 7) were stained with JSRV-Env antiserum (kindly supplied by Dr. Dusty Miller) by routine immunohistochemistry technique. Briefly, hydrated tissue sections were exposed to tris-buffered saline for 3x10 minutes (TBS; 0.05 M tris HCl, 0.15 M NaCl, pH 7.4-7.6). Sections were incubated with Bloxall™ endogenous peroxidase and alkaline phosphatase blocking solution (Vector Laboratories Inc, Burlingame, California, USA) for 10 minutes to inactivate endogenous peroxidase activity. Slides were then washed in TBS for 3x10 minutes. The slides were then treated for 30 minutes with 2.5% horse serum (Vector Laboratories Inc, 30022) to remove nonspecific tissue antigens. After removing the excess solution, the slides were incubated at 4 °C overnight with primary antibody against JSRV-Env (mouse monoclonal diluted 1:500 in TBS). Diluted normal rabbit serum and TBS replaced primary antibodies for nonspecific reactions and endogenous peroxidase activity. After 3x10 minutes of TBS washes after incubation, slides were coated with ImmPRESS polymer peroxidase (Vector Laboratories Inc, MP-7500) for 30 minutes, then washed for 3x10 minutes with TBS. Sections were then covered with ImmPACT DAB peroxidase substrate (Vector Laboratories Inc, SK-4105)

solution and incubated for 3 minutes. Finally, these sections were counterstained with Carrazi's hematoxylin for 2 minutes, and then were washed with tap water, dehydrated, and mounted with DPX.

Results

Necropsy Findings

In flock I, we noted the classical form of OPA in four animals and the atypical form in one animal (Table 1). Gross examinations of the lungs demonstrated that several lobes (mostly the cranial lobes) were purple in color and were consolidated. In the atypical form, the lung lobes contained white nodules about 0.5 cm in size distributed throughout the lung surface (Fig. 1A). Caudal lobules were more extensively affected, and the cut surface was granular and white in color (Fig. 1B). A purple thin area sharply separated these areas from normal lung tissue. Frothy fluid oozed from the trachea and also from the airways on the cut surface in all ewes. Affected lung lobes were heavier and larger compared to healthy lung lobes.

In flock II, we detected the classical form of OPA in four ewes (Table 1). In one animal, extrathoracic metastasis was found in the left kidney. This was a well-demarcated white nodule approximately 2.5 cm in size (Fig. 1C and 1D). The rest of the kidney showed pale or hemorrhagic areas. In another animal, metastasis was observed as a single well-demarcated white nodule approximately 1 cm in size in the mediastinal lymph node. Additionally, it was observed that there was an abscess in the rest of the mediastinal lymph node. It was noted that the nodules were embedded in the surrounding tissue in both organs.

Histopathological Findings

Histopathological examination revealed neoplastic proliferations of epithelial cells in alveolar and bronchiolar areas (Fig. 2A). These proliferations were comprised of a simple cuboidal to columnar epithelium on a connective tissue stroma, forming acinary, papillary, lepidic, or solid structures. Also, masses formed by neoplastic cells in alveolar lumens were notable. These masses had compressed adjacent alveoli. Similar growth patterns were also present in the bronchi. In some areas, solid growths were observed. The tumor stroma comprised connective tissue and severe lymphocytic infiltration. Also, mild intraalveolar macrophage infiltrations were observed in these areas. Fibrinous bronchopneumonia and nonpurulent interstitial pneumonia were other significant findings.

Kidney and mediastinal lymph node metastases were confirmed by histopathological examination in two cases. In the kidney, the neoplastic cells were cuboidal and exhibited lepidic to papillary growth in interstitial areas (Fig. 2B). Besides, the neoplastic proliferations had expanded

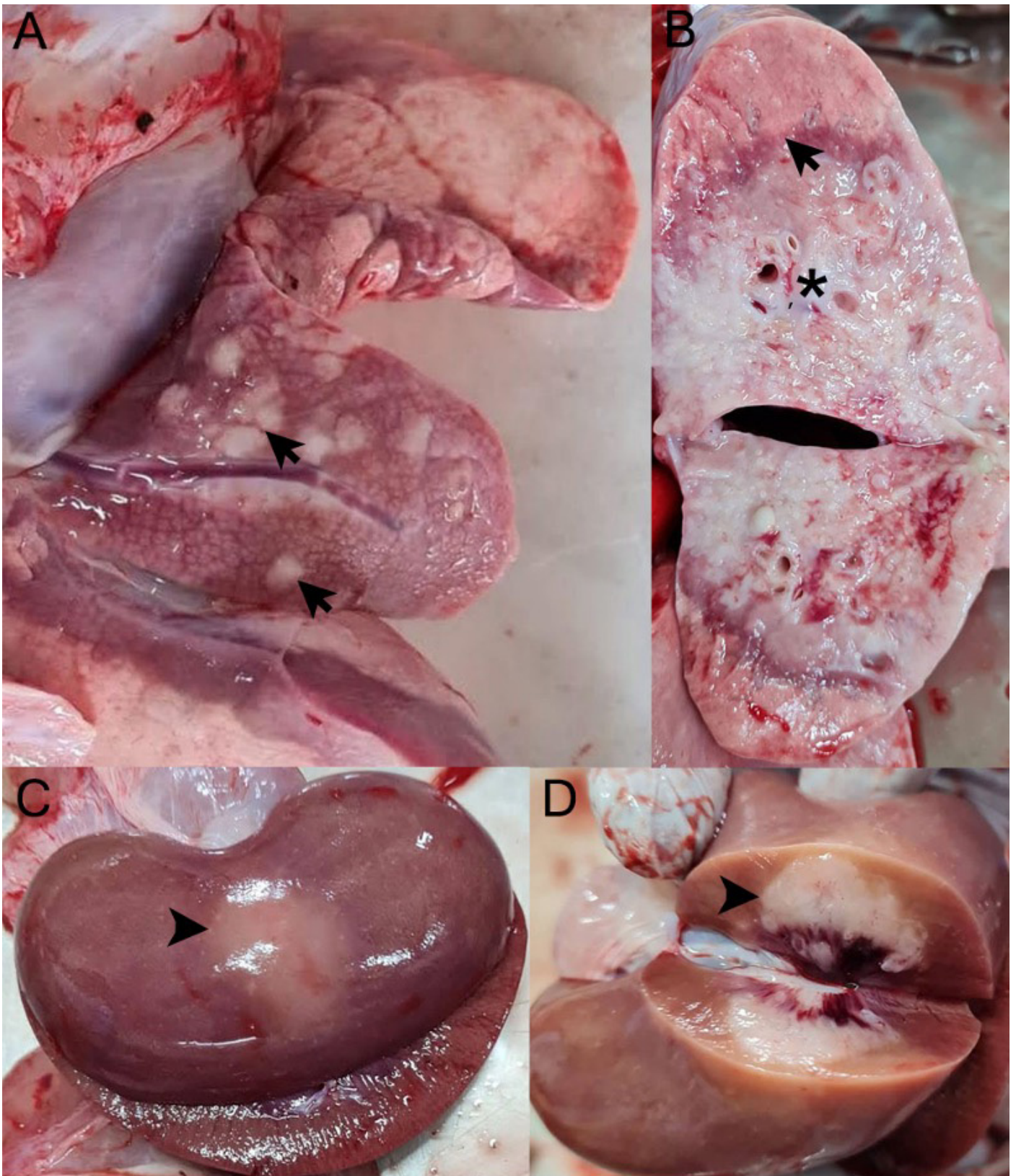


Figure 1: Gross appearance of OPA lesions and extrathoracic metastasis. (A) Multiple white nodules of various sizes on the right lung cranioventral lobe (arrows). (B) Right cranial lung cut surface showing the junction (arrow) between the normal and tumor tissue (star). (C) Neoplastic white nodule (arrowhead) in left kidney. (D) A neoplastic nodule (arrowhead) embedded in the cut surface of the left kidney

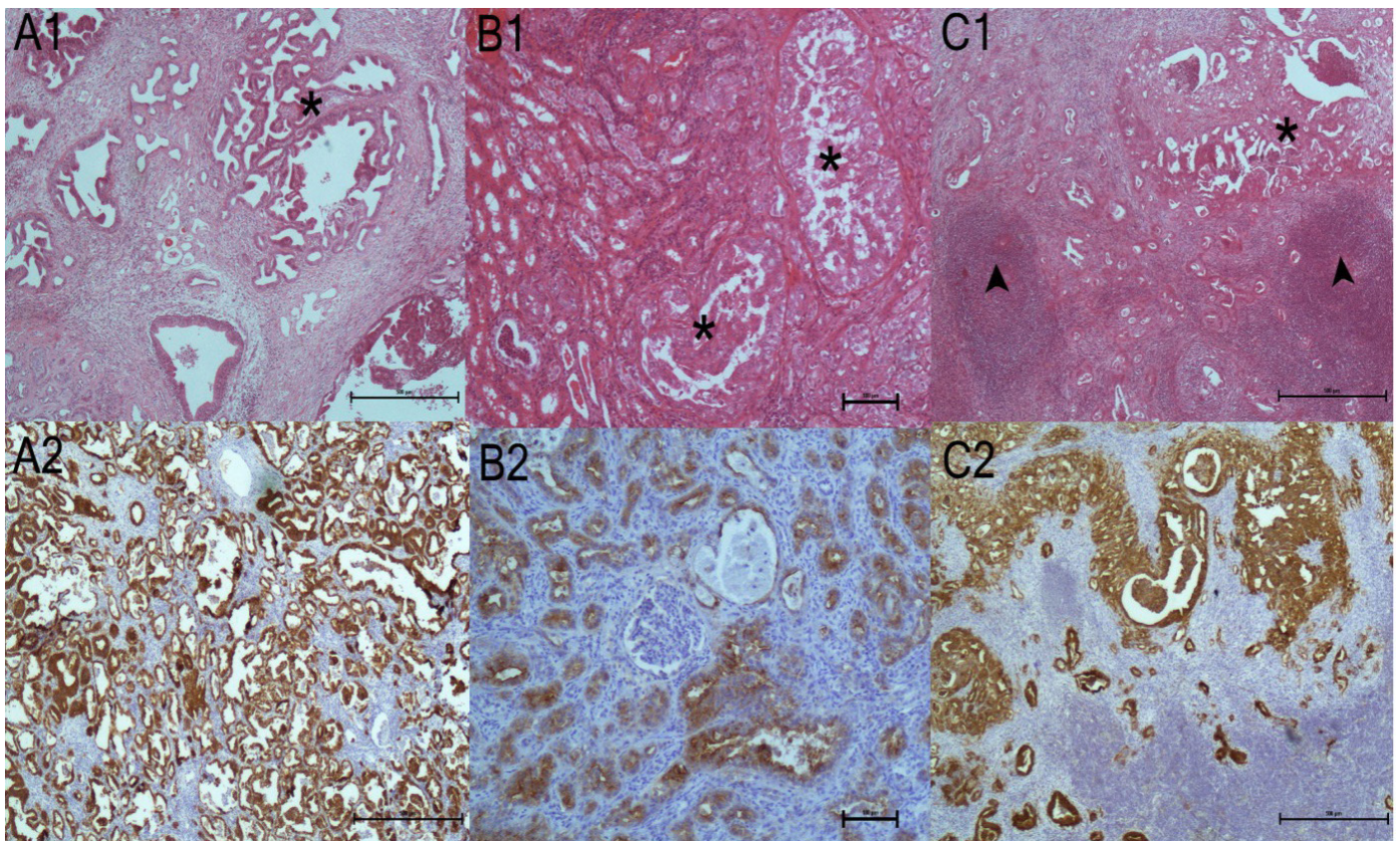


Figure 2: Histopathological appearance and immunopositive reactions of OPA. (A1) Lung, neoplastic proliferations in the alveoli and bronchi (star), H&E, bar=500µm. (A2) Expression of JSRV-Env protein in the cytoplasm of neoplastic cells in the lungs, bar=500µm. (B1) Metastasis in the kidney. Neoplastic proliferations (stars), degenerative and necrotic changes in the tubules, H&E, bar=100 µm. (B2) Moderate expression of JSRV-Env in the kidney, bar=100 µm. (C1) Metastasis in the mediastinal lymph node (star), lymphoid follicles (arrowheads), H&E, bar=500 µm. (C2) Severe positive reaction against JSRV-Env antibody in the lymph node, bar=500µm

Table 1: Details of flocks and OPA forms

Flock	Total number of sheep	Total cases	Age/sex range of affected sheep	Pathologic form	Total intrathoracic metastasis	Total extrathoracic metastasis
Flock I (Afyonkarahisar Province)	133	5	4 year old/female	4 classical 1 atypical	-	-
Flock II (Denizli Province)	200	4	4 year old/female	4 classical	1 mediastinal lymph node (case 7)	1 kidney (case 6)

into adjacent structures. The tumor stroma consisted of connective tissue and moderate lymphocytic infiltration. Degenerative and necrotic changes were also observed. In the lymph node, acinar growths were dominant in some areas, while papillary growths were prominent in others (Fig. 2C). Moreover, depletion of cortical lymphoid follicles and necrosis with numerous neutrophilic leukocytes in the abscess was noted.

IHC Findings

Immunohistochemically, strong positive expression of JSRV-Env was characterized by granular dark brown staining in the membranes and cytoplasm of neoplastic cells.

Moderate staining of tumor cells were observed in the kidney (Fig. 2E), while the tumor cells in the mediastinal lymph node showed severe positive reaction consistent with the presence of intracytoplasmic JSRV antigen (Fig. 2F).

Discussion

OPA is contagious lung cancer caused by JSRV in sheep (1, 3). According to the World Organisation for Animal Health (OIE), it is considered a significant disease in the international sheep trade (16, 17). The disease, reported in many regions with sheep-raising worldwide, causes significant economic losses due to difficulties in control and

eradication, lack of vaccine, and difficulties in determining preclinical stages (6, 18). The determination of the JSRV by molecular methods is limited (19). ELISA or PCR can support the diagnosis, however, it is difficult to identify infected sheep without circulating JSRV-specific antibodies at the preclinical stage (2), and there are no routine tests used for diagnosis at this stage (20). Definitive diagnosis is only possible with postmortem and histopathological examinations made in line with the anamnesis and clinical findings (20). Immunohistochemically identifying neoplastic cells expressing JSRV-related antigens is also of great diagnostic value in the case of OPA (21-24).

JSRV is a slow infection virus with a prolonged incubation period and most infected sheep do not show clinical signs (30). Therefore the cause of the disease is generally not entirely investigated, and the sheep are slaughtered before they are diagnosed. For this reason, the exact number of the animals affected with OPA is unknown (24, 31). Abass and Khudhair (2022), suggested an association between JSRV infection and flock size groups (29). In our study, OPA was detected in five ewes in the first flock of 133 sheep and in four ewes in a flock of 200 sheep. However, it is unknown whether other sheep in the flocks were infected because they were sent to slaughter by the flock owners.

OPA occurs mainly in 2–4 years old animals (27, 28) and the ewes in our study were four years old. Abass and Khudhair (2022), reported no breed and sex susceptibility for OPA (29), whereas Toma et al. (2020), noted that all diagnosed sheep were adult females, ranging from 2 to 6 years (28). In the current study, all nine sheep were of the local Pirlak breed and were female. Pirlak ewes are one of Turkey's most widely grown breeds, and further studies are needed to identify whether female Pirlak sheep are more sensitive to JSRV infection.

In the current study, postmortem examinations of nine ewes in two flocks revealed characteristic macroscopic and histopathologic findings of OPA. Papillary and acinary growths were consistent with the definition of OPA, an adenocarcinoma with different proliferation patterns (25). In addition, the cranioventral lobes were significantly affected, and there was a large amount of fluid in the bronchi and bronchioli. As stated in the literature, these pathological findings were identical to the presentation of classical OPA (4, 6). Besides these pathological findings, multifocal white nodules on the lung surface observed in two cases were similar to atypical OPA. Besides necropsy findings and histopathological evaluations, JSRV-Env expression in the lungs, lymph node, and kidney helped confirm the diagnosis. These expressions were in the membranes and cytoplasm of neoplastic cells. However, we could not use molecular techniques to determine viral DNA.

OPA is generally known to metastasize to mediastinal lymph nodes. It rarely metastasizes to distant organs such as the heart, liver, and kidney (4, 6). Minguignon et al (2013),

intrathoracic metastasis in 0.3-25% of cases (9). In the current study, metastasis to mediastinal lymph node and kidney were observed in two animals. The microscopical features of the metastatic cells in the mediastinal lymph node and kidney were similar to the characteristics of tumor cells in lungs. These findings were consistent with previous studies (9, 26).

Maedi disease is important in the differential diagnosis (32, 33) of OPA because, chronic progressive respiratory problems are observed similarly in Maedi infection (25, 33). OPA can be complicated by respiratory disorders such as secondary bacterial infections, lung abscessation, or other lung lesions (31). Neutrophils in the lungs have been frequently reported in OPA and evaluated as resulting from secondary bacterial infections (4, 34). It should be noted that OPA can cause chronic respiratory problems in sheep and secondary bacterial infections may complicate them (35). In this study, in one case fibrinous pneumonia suggested pasteurellosis/manheimiosis. Besides, in another case the abscess observed in the mediastinal lymph node suggested caseous lymphadenitis. Toma et al (2020), also observed abscesses due to caseous lymphadenitis in their study (28). However, in our cases microbiological examinations could not be performed and the etiology could not be revealed.

Our study provides valuable data about OPA's pathological evaluation and metastasis features. The results have revealed the importance of necropsy findings for the definitive diagnosis of OPA, which to date does not have an effective intravital diagnostic method nor prophylactic tools in live animals. Our study once more showed that OPA can cause distant metastases and for this reason the lungs, regional lymph nodes, and distant organs should be evaluated carefully.

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Intratorakalne in ekstratorakalne metastaze pri ovcah Pırlak z ovčjim pljučnim adenokarcinomom (jaagsiekte)

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Izveček: Pljučni adenokarcinom pri ovcah (OPA) je nalezljiva neoplastična bolezen, ki jo povzroča retrovirus ovca jaagsiekte (JSRV) in za katero so značilni kronični klinični znaki na dihalih. V tej študiji opisujemo intratorakalne in ekstratorakalne metastaze pri ovcah Pırlak, naravno okuženih z JSRV. Pri dveh čredah ovca pasme Pırlak, pripeljanih iz dveh različnih pokrajin, so se pojavili progresivna dihalna stiska, izcedek iz nosu, kašelj in izčrpanost, devet ovca iz teh dveh čred pa je bilo na zahtevo lastnikov evtanaziranih. Grobe ugotovitve so pokazale vijolično obarvane in konsolidirane kranioventralne pljučne lobe z razpršenimi belimi noduli različnih velikosti. Ena ovca je imela metastaze v mediastinalni bezgavki, druga pa v levi ledvici. Histopatološke preiskave tumorjev v pljučih in metastaz so pokazale papilarne in acinarne vzorce rasti. Imunohistokemično je bilo v neoplastičnih celicah ugotovljeno močno izražanje JSRV-Env.

Ključne besede: ekstratorakalne metastaze; intratorakalne metastaze; ovčji retrovirus jaagsiekte; ovca Pırlak; ovčji pljučni adenokarcinom