Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (6): 809-814, 2024
DOI: 10.9775/kvfd.2024.32702

Research Article

# Molecular and Pathological Detection of Jaagsiekte Sheep Retrovirus in Lung Tissues of Sheep

Nuvit COSKUN [1 (*)] Volkan YILMAZ [1] Emin KARAKURT [2] Enver BEYTUT [2]
Hilmi NUHOGLU [2] Mehmet Ozkan TIMURKAN [3]

[1] Kafkas University, Faculty of Veterinary Medicine, Department of Virology, TR-36300 Kars-TÜRKİYE
[2] Kafkas University Faculty of Veterinary Medicine, Department of Pathology, TR-36300 Kars-TÜRKİYE
[3] Atatürk University, Faculty of Veterinary Medicine, Department of Virology, TR-25200 Erzurum-TÜRKİYE

(*) **Corresponding authors:**
Nüvit COŞKUN

Cellular phone: +90 474 242 6836/5155;
Cellular phone: +90 555 714 6344;
Fax: +90 474 242 6853

E-mail: nuvitcoskun@gmail.com

How to cite this article?

**Coskun N, Yilmaz V, Karakurt E, Beytut E, Nuhoglu H, Timurkan MO:** Molecular and pathological detection of Jaagsiekte sheep retrovirus in lung tissues of sheep. *Kafkas Univ Vet Fak Derg,* 30 (6): 809-814, 2024.
DOI: 10.9775/kvfd.2024.32702

**Article ID:** KVFD-2024-32702
**Received:** 24.07.2024
**Accepted:** 04.10.2024
**Published Online:** 15.10.2024

**Abstract**

Ovine pulmonary adenocarcinoma (OPA) is a disease of sheep that is caused by a *Betaretrovirus* named exogenous Jaagsiekte sheep retrovirus (exJSRV). This virus causes oncogenic transformation in lungs, and symptoms develop related to the growing tumors. Disease develops slowly with a long incubation time ranging 2-4 years. Currently there is no serological test to evaluate the presence in the flock and also disease can mostly be diagnosed post mortem. The aim of this study is to determine characterization and the molecular presence exJSRV types in circulation. In this study lung tissues of 25 suspected cases were investigated. Initial diagnosis is made by histopathological (HP) and immunohistochemical (IHC) methods. Hematoxylin & Eosin (H&E) staining was used for examining histopathological changes. Anti JSRV capsid antibody was used with Streptavidin-Biotin peroxidase method. Slides were examined under light microscope and photographs were taken. All 25 cases were diagnosed as OPA with these methods. Lung tissues embedded in paraffin were used as material for nucleic acid extraction. Envelope gene of JSRV nucleic acid was chosen for investigating with reverse transcription polymerase chain reaction (RT-PCR). Since paraffinized tissue blocks were used, sensitivity was not high and only 10/25 tissues were deemed positive. Positive amplicons were sent to sequencing. A phylogenetic tree was constructed after analyzing the sequences. Also predicted amino acid sequences were analyzed. In conclusion we found both type 1 and type 2 exJSRV have been circulating in the region and changes in amino acids were detected which could lead to possible differentiation in pathogenesis.

**Keywords:** Histopathology, Immunohistochemistry, Jaagsiekte sheep retrovirus, Phylogenetic analysis, Polymerase chain reaction, Sheep

## Introduction

Ovine pulmonary adenocarcinoma (OPA) or Jaagsiekte sheep retrovirus (JSRV) is a neoplastic disease characterized by tumor lesions in the lung. This condition is caused by the exogenous form of JSRV (exJSRV) [1,2]. JSRV is the only known virus for naturally causing lung cancer in animals [3]. Sheep is the natural host of the virus and there are studies that report the disease in goats. Causative agent is classified in *Retroviridae* under genus *Betaretrovirus* and species *Betaretrovirus ovijaa* which has single stranded, positive sense RNA genome consisting of approximately 7400 bases. JSRV also has an endogenous form (enJSRV) which is integrated in DNA of many sheep but not associated with the disease [1,4-6]. JRSV genome codes four viral proteins namely *env*, *gag*, *pol*, *pro.* Among these proteins *env* is responsible for inducing oncogenic transformation [6,7]. exJSRV is suggested to have two types. Type 1 is based on South African, Kenyan origin and type 2 is United Kingdom or American origin [1,5]. However, these two types are not reported to have any differences in pathogenicity. Enzootic nasal tumor virus (ENTV) is also another *Betaretrovirus* that is closely related to JSRV and cause a disease with similar course [3,7]. Other *Lentiviruses* (such as Visna-Maedi disease) may also cause coinfection in some cases which may mask diagnosis or worsen the outcome of the disease [8].

World Organisation of Animal Health (WOAH) has reported [8] OPA is widespread around the world where there is sheep livestock, causing economic loss. Affected animals have symptoms related to lung lesions; dyspnea,

cough, nasal discharge is most common in the early stages of the disease [6]. As the disease progresses, symptoms become more apparent, animals have difficulty moving and excess (mucous) fluid is discharged from nares when hind legs are elevated. This is called wheelbarrow test and it is the main clinical symptom leading to diagnosis. Clinical symptoms are worsened by secondary infections and death is commonly caused the secondary infectious agents. Bacterial or verminous bronchopneumonia is common with OPA cases and can be seen solely or in combination in necropsy [6-8].

Disease has a long incubation period, ranging from months to years. The time disease is most prevalent is between ages 2 to 4, although it can rarely be seen younger animals [8,9]. Transmission of the virus occurs mostly with aerosols and lamb can also be infected with the virus by milk/colostrum. Transplacental crossing of the virus does not have a significant role in transmission. Virus is present in lung fluid, tumors, regional lymphoid tissues and sometimes in peripheral blood [6-8].

Although virus genome has been extensively studied molecularly and many strains are sequenced, no permissive cell culture has been found to propagate the virus. Only some tumor cells from young lambs can be used to maintain the virus for a short period [8]. No circulating antibodies develop after OPA infection [5]. Viral proteins can be found only in tumor cells but not anywhere else [6,10]. There are some related problems caused by aforementioned information; firstly, no vaccine is available, secondly no routine early diagnostic test detection is present and thirdly no seroprevalence test can be applied to monitor the disease in a flock. Isolation or compulsory slaughter of the infected animals can be considered the only method of prevention [6,8].

The aim of this study is to investigate the presence of exJRSV from paraffin embedded tissue blocks and determine the molecular characterization of circulating isolates. Data such as exJSRV types that is in circulation cannot be identified without molecular analyses, for this reason sequencing and phylogenetical analyses were mandatory.

## Material and Methods

### Ethical Approval

The study was approved by Kafkas University Animal Experiments Local Ethics Committee (Decision Number: KAÜ-HADYEK/2021-180.)

### Material

Material of the study consists of 25 sheep lung tissues embedded in paraffin which belong to Pathology Department's tissue archive. Lung tissues were collected between years 2010-2018. These tissues belong to animals suspected of the disease.

### Histopathological (HP) Examinations

Lung tissues were fixed in 10% formalin and routine procedures were followed for paraffin embedding. Sections were taken into slides and Hematoxylin & Eosin (H&E) staining was used for examining histopathological changes. Detailed examinations and photographing were done under light microscope.

### Immunohistochemical (IHC) Examinations

Streptavidin-Biotin peroxidase method was used for immunostaining according to manufacturer instructions (Thermo Scientific Histostain-Plus IHC Kit, HRP, broad spectrum, REF: TP-125-HL). Anti JSRV capsid (CA) antibody (Supplied by Professor Massimo Palmarini, used with dilution 1/1500 and incubated overnight at 4ºC) was applied after antigen retrieval and blocking of non-specific proteins. Reactions were generated using aminoethyl carbazole (AEC) as chromogen (Thermo Scientific, TA-125-HA). Contrast staining was done with hematoxylin. Slides were mounted with AEC specific mounting media. Phosphate Buffer Saline (PBS) was used instead of primary antibody for control slides.

Slides were examined under light microscope (Olympus Bx53) and photographs were taken with Cell^P software (Olympus Soft Imaging Solutions GmbH, 3,4). Detailed analyzes were done with Image J software (1.51j8).

### Nucleic Acid Extraction

Five to ten sections of 10 microns were taken from paraffin embedded tissue blocks and put to 1.5 mL tubes for each case. The extraction was carried out according to Pikor et al.[11]. The method described yields in total DNA and RNA if no RNase is added. For this reason, no RNase was added to yield RNA as much as possible. This step is the only modification of the aforementioned method.

### Reverse Transcription Polymerase Chain Reaction (RT-PCR)

Since exJSRV is RNA form it was necessary to perform reverse transcription before submitting samples to polymerase chain reaction (PCR). This was achieved using onestep RT-PCR kit by Hibrigen (Türkiye). The primer pair (targeted for envelope KT279065.1 gene), was used as reported by Mansour et al.[9]. The conditions were modified, for reverse transcription 40 min at 55ºC, 95ºC5 min, was used for initial denaturation, 35 cycles of 95ºC denaturation 1 min, 58ºC 1 min of annealing and 72ºC 1 min for extension followed by final extension at 72ºC for 10 min. Expected amplicon size was 398 base pairs.

### Phylogenetic Analysis

Positive amplicons (10 in total) were sent to a commercial company (BM Laboratuvar Sistemleri, Ankara) for Sanger sequencing. Sequence assembly and editing were performed using Bioedit (Version 7.0.5.3) and Clustal W [12], before comparing with the GenBank nucleotide sequence database for sequence similarities using the Basic Length Alignment Search Tool (BLAST) software of the National Center for Biotechnology Information (NCBI) [13]. Phylogenetic analysis was performed using MEGA7 software's neighbour-joining method [14] and the evolutionary distances between different sequences were calculated by the Kimura two-parameter model. The confidence level of the NJ tree was assessed by bootstrapping using 1000 replicates. A table of amino acid comparison was also prepared according to the sequence data to examine the differences.

## Results

### Histopathological Results

Several widespread tumor foci of varying dimensions were observed in lung tissues. These foci consisted mostly of papillary extensions or acinar structures originating from bronchiolar or alveolar cells. Tumoral cells were in the shape of cuboidal or columnar. Mitotic figures were very few. Stroma layers of tumors were thickened because of the increased count of mononuclear cells and extension of the connective tissue. Alveolar macrophages and mononuclear cells were present around the neoplastic foci. In some cases, parasitic agents were also identified in addition to OPA findings and there was neutrophil leukocyte infiltration in some cases, indicating secondary bacterial infection *(Fig. 1-a,b)*.

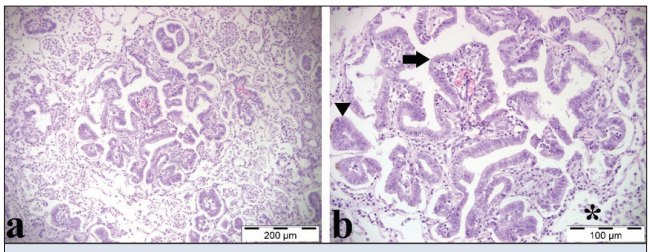


**Fig 1. a:** Lung, Tumoral foci, **b:** Papillary *(arrow)* and acinar *(arrowhead)* proliferations that extend to the lumen, alveolar macrophages *(asterisk)* in the tumoral vicinity

### Immunohistochemical Results

JSRV positive reactions were mostly observed in cytoplasms of cuboidal or columnar cells that form papillary or acinar structures. There was also positivity in cytoplasms of alveolar macrophages in some areas surrounding tumoral region *(Fig. 2-a,b)*.

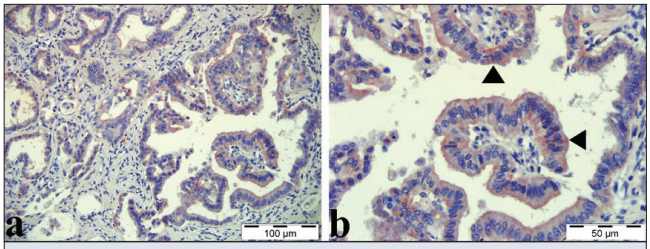


**Fig 2. a-b:** JSRV positive reactions *(arrowheads)* in cytoplasm of the tumoral cells in papillary and acinar tumoral regions

### Molecular Results

Ten of the 25 samples of paraffin embedded tissues were positive with the RT-PCR procedure and had correct size amplicons visualized at 398 bp. 7 amplicons were sequenced and deposited to GenBank with accession numbers OM953765, OM953766, OM953767, OM953768, OM953769, OM953770, OM953771. Other 3 amplicons could not be sequenced due to low nucleic acid concentration.

A phylogenetic tree was constructed with the reference sequences obtained from GenBank. Endogenous forms of JSRV and exogenous JSRV reference sequences of type 1 and type 2 were included. ENTV references were added to indicate the close distance relation and other retroviruses were included for outgrouping. OM953768 and OM953770 were clustered with type 2 exJSRV references and our other remaining 5 isolates were clustered with type 1 exJSRVs *(Fig. 3)*.

Histopathological results belonging to all 25 cases indicated characteristic OPA findings. These results were further

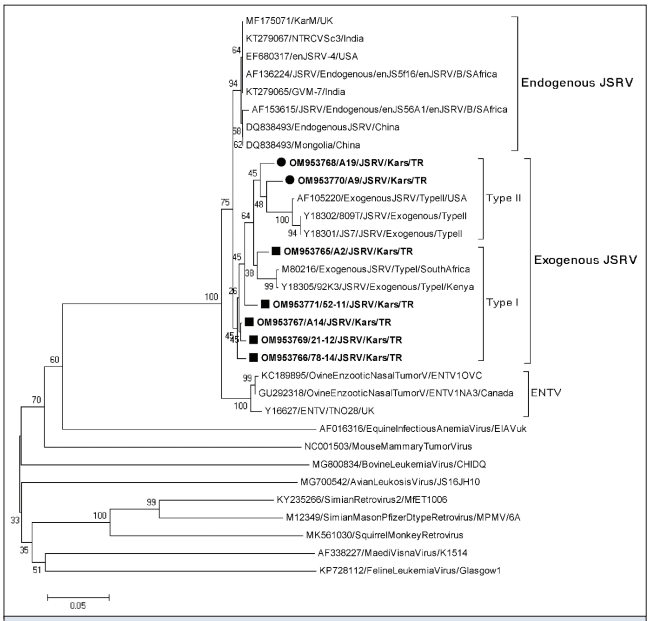


**Fig 3.** Phylogenetic tree constructed using *env* gene reference strains and our isolates. Black circles are type 2 isolates of this study and black squares are type 1 isolates of this study

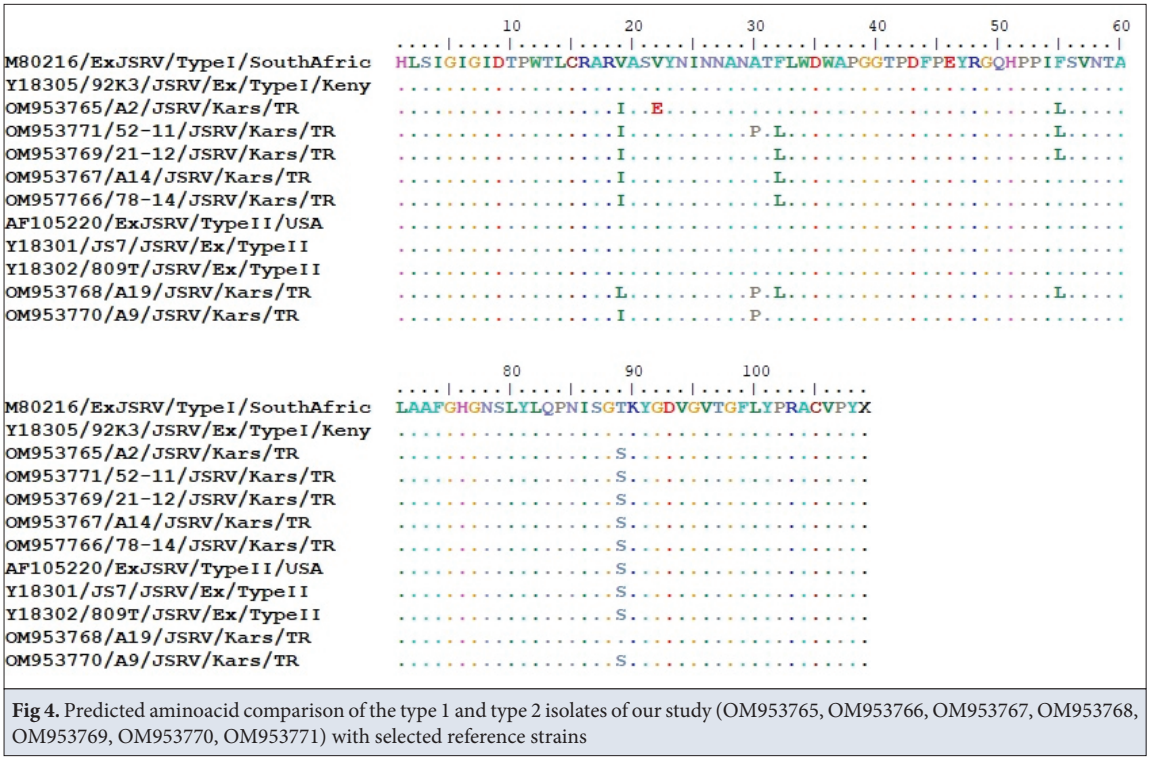


**Fig 4.** Predicted aminoacid comparison of the type 1 and type 2 isolates of our study (OM953765, OM953766, OM953767, OM953768, OM953769, OM953770, OM953771) with selected reference strains

confirmed with immunohistochemical results which were stained with spesific antibody to JSRV envelope. According to these two tests, all tissues were diagnosed as OPA. However, it was not possible to detect exJSRV nucleic acid in all tissues with onestep RT-PCR. RNA of the virus was found in 10 of the 25 tissues. This is not an indication the remaning 15 cases did not contain nucleic acid, only disadvantage of using paraffin embedded tissue blocks. These 15 tissues were regarded as false negatives as histochemical and immunohistochemical findings confirmed presence of OPA.

Predicted amino acid content comparison based on sequence is shown in *Fig. 4*. There are certain changes that is distinctive in our isolates for both type 1 and type 2. There is valine to isoleucine change in 19$^{th}$ position. While all references on this position have valine, our isolates have isoleucine, except one type 2 isolate (OM953768) which has leucine. This change is the most consistent among our isolates although there are some other changes as shown in the *Fig. 4*.

## Discussion

OPA can either be misdiagnosed because of secondary infections or undiagnosed because of early stage. Animals are not admitted to veterinary clinics because prognosis is bad when clinical manifestations are seen. Most studies obtain samples by going to the abattoir to specifically look for OPA [7]. We selected paraffin embedded tissue blocks as material because prevalence of the disease is not known in the region. Before performing RT-PCR, tissue slides were first examined histopathologically. Using only histopathology can lead to false positivity because of the similar lesions [7,10]. This can be prevented using confirming with immunohistochemistry. Considering this fact, all tissues were initially diagnosed as OPA histopathologically and confirmed with immunohistochemistry. This was done because there was no positive control for RT-PCR; hence histopathological and immunohistological results were regarded as positives when performing RT-PCR. While all of the 25 tissues were positive with H&E and IHC, only 10 of them were deemed positive after visualizing amplicons after RT-PCR. This is possibly the result of RNA degrading mainly by the processes of making paraffin blocks, storage time and with the application/incubation time of the extraction procedure and expected [11]. As a result, other 15 tissues can be regarded as false negatives when these conditions are considered. This does not present a problem for the study as we did not investigate prevalence. But this is a general problem in diagnosing OPA prevalence as the most suitable material for early-stage detection is peripheral blood and it is known to give false negative results [7,10].

Our location is situated in the Northeastern region of Türkiye and animal husbandry is the main sources of its income. There are 500.000-600.000 small ruminant bred as livestock [15,16]. Mean altitude is 2000 meters, region has large meadows which is used for extensive breeding of small ruminants. Climate of the region has short summer time and harsh winter conditions. Small ruminant breeding is mostly done pastoral/extensively, but harsh

winter conditions limit this time [15]. Animals are reared in closed spaces wintertime, for this reason diseases which spread by aerosols and contact have higher incidences, including OPA [7]. Unfortunately, there is not enough data to determine the prevalence of the disease. But the disease has been previously reported from our country and also our province. Initial reports of the disease focused on histopathological means [17,18]. In the following years, reports on the presence of the disease were published from the Eastern region of the country [19-23]. Currently, disease has been studied in our province [24-28] and neighbouring province [29].

When predicted amino acid sequence table *(Fig. 4)* is examined, there are changes among our regional isolates and reference strains. We can also see some changes in reference strains like in 89$^{th}$ position as some has threonine and others serine. These changes can be of importance as *env* is the gene that is responsible for oncogenic transformation [6,7]. Further research needs to be performed in this regard.

We were able to find both type 1 and type 2 exJSRV from the material we used. This proves both types are in circulation in our country. ExJSRV type 1 is suggested to be of South African origin and type 2 is United Kingdom or American origin [1,5]. Considering this fact, both types were possibly come into our country from an animal movement. Earlier reports of OPA, while some used molecular techniques [21,22,26], do not contain information about types. Because of this we cannot make any assumptions about previous existence of type 1 and type 2. Our findings suggest the need to design further studies for investigating possible differences in pathogenesis of both types as evidence of circulating types are revealed.

OPA can be considered endemic where clinical manifestations are seen, as virus would spread to the flock before symptoms appear. There is currently no vaccine or routine diagnostic test for early-stage disease. Due to lack of early detection diagnostic tests; serosurveys or eradication programmes cannot be planned/executed. Development of a reliable diagnostic test which can be applied ante mortem easily, to crowded sheep flocks and populations is still needed to establish preventive measures.

In conclusion two different types of the exJSRV were discovered with molecular characterization using paraffin embedded lung tissues. We believe our results will contribute to the literature and draw further investigations on OPA.

## Declarations

**Financial Support:** The authors declare that this study has received no financial support.

**Ethical Statement:** The study was approved by Kafkas University Animal Experiments Local Ethics Committee (Decision Number: KAÜ-HADYEK/2021-180)

**Availability of Data and Materials:** The datasets used and/or analyzed during the current study are available within the manuscript.

**Conflict of Interest:** The authors declared that there is no conflict of interest.

**Generative Artificial Intelligence:** No kind of artificial intelligence tool was used in preparing this manuscript.

**Author Contributions:** NC, EK: Idea and study design. EB, NH: Sample collection; NC, EB HN: Laboratory analyses; VY, MOT, NC: Analysis of data; NC, EK: Manuscript preparation. All authors have read and approved the final manuscript.

## References

**1. Bai J, Zhu RY, Stedman K, Cousens C, Carlson J, Sharp JM, DeMartini JC:** Unique long terminal repeat U3 sequences distinguish exogenous Jaagsiekte sheep retroviruses associated with ovine pulmonary carcinoma from endogenous loci in the sheep genome. *J Virol,* 70 (5): 3159-3168, 1996. DOI: 10.1128/JVI.70.5.3159-3168.1996

**2. Palmarini M, Cousens C, Dalziel RG, Bai J, Stedman K, DeMartini JC, Sharp JM:** The exogenous form of Jaagsiekte retrovirus is specifically associated with a contagious lung cancer of sheep. *J Virol,* 70 (3): 1618-1623, 1996. DOI: 10.1128/JVI.70.3.1618-1623.1996

**3. Palmarini M, Mura M, Spencer, TE:** Endogenous betaretroviruses of sheep: teaching new lessons in retroviral interference and adaptation. *J Gen Virol,* 85 (1): 1-13, 2004. DOI: 10.1099/vir.0.19547-0

**4. Palmarini M, Sharp JM, de las Heras M, Fan H:** Jaagsiekte sheep retrovirus is necessary and sufficient to induce a contagious lung cancer in sheep. *J Virol,* 73 (8): 6964-6972, 1999. DOI: 10.1128/JVI.73.8.6964-6972.1999

**5. DeMartini JC, Carlson JO, Leroux C, Spencer T, Palmarini M:** Endogenous retroviruses related to Jaagsiekte sheep retrovirus. **In,** Fan HY, Fan H (Eds): Jaagsiekte Sheep Retrovirus and Lung Cancer. 117-137, Springer, Berlin, 2003.

**6. Quintas H, Pires I, Garcês A, Prada J, Silva F, Alegria N:** The diagnostic challenges of ovine pulmonary adenocarcinoma. *Ruminants,* 1 (1): 58-71, 2021. DOI: 10.3390/ruminants1010005

**7. Toma C, Bâlteanu VA, Tripon S, Trifa A, Rema A, Amorim I, Pop RM, Popa R, Catoi C, Taulescu M:** Exogenous Jaagsiekte sheep retrovirus type 2 (exJSRV2) related to ovine pulmonary adenocarcinoma (OPA) in Romania: Prevalence, anatomical forms, pathological description, immunophenotyping and virus identification. *BMC Vet Res,* 16 (1): 296, 2020. DOI: 10.1186/s12917-020-02521-1

**8. WOAH:** World Organisation of Animal Health in Manual of Diagnostic Tests and Vaccines for Terrestrial Animals, Chapter 3.8.8 Ovine Pulmonary Adenocarcinoma. 2021. https://www.woah.org/fileadmin/Home/fr/Health_standards/tahm/3.08.08_OPA.pdf; *Accessed:* 10.10.2023.

**9. Mansour KA, Al-Husseiny SH, Kshash QH, Jassim A:** Clinical-histopathological and molecular study of ovine pulmonary adenocarcinoma in Awassi sheep in Al-Qadisiyah Province, Iraq. *Vet World,* 12 (3): 454-458, 2019. DOI: 10.14202/vetworld.2019.454-458

**10. De Las Heras M, Ortín A, Salvatori D, Pérez de Villareal M, Cousens C, Miguel Ferrer L, Miguel Cebrián L, García de Jalón JA, Gonzalez L, Michael Sharp J:** A PCR technique for the detection of Jaagsiekte sheep retrovirus in the blood suitable for the screening of ovine pulmonary adenocarcinoma in field conditions. *Res Vet Sci,* 79 (3): 259-264, 2005. DOI: 10.1016/j.rvsc.2005.02.003

**11. Pikor LA, Enfield KS, Cameron H, Lam WL:** DNA extraction from paraffin embedded material for genetic and epigenetic analyses. *J Vis Exp,* (49):e2763, 2011. DOI: 10.3791/2763

**12. Hall TA:** BioEdit: A user-friendly biological sequence align-ment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symp Ser,* 41, 95-98, 1999.

**13. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ:** Gapped BLAST and PSI-BLAST: A new generation of protein database search programs. *Nucleic Acids Res,* 25 (17): 3389-3402, 1997. DOI: 10.1093/nar/25.17.3389

**14. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S:** MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol,* 28 (10): 2731-2739, 2011. DOI: 10.1093/molbev/msr121

**15. Demir M:** Kars ilinde büyük ve küçükbaş hayvancılık; Livestock breeding in Kars province. *Dogu Cogr Derg,* 21 (35): 39-62, 2016. DOI: 10.17295/dcd.33180

**16. TUIK:** Turkish Institute of Statistics in Yearly Report. 2016. http://www.turkiyekoyunkeci.org/tr/HaberIcerikDetay/kucukbas-hayvan-sayimiz-44-milyon-34-bin-basa-yukseldi/36760; *Accessed:* 18.01.2023.

**17. Kıran MM:** Pathological investigations on sheep pulmonary adenomatosis. *Eurasian J Vet Sci,* 9 (1): 41-45, 1993.

**18. Kıran MM, Çiftçi MK, Erer H, Ortatatlı M, Hatipoğlu F, Tuzcu M:** Koyun pulmoner adenomatozisinin eksfoliyatif sitoloji ve bronkoalveolar lavaj yöntemleri ile tanısı ve sonuçların histopatolojik bulgularla karşılaştırılması. *Eurasian J Vet Sci,* 16 (1): 59-68, 2000.

**19. Beytut E, Sözmen M, Erginsoy S:** Immunohistochemical detection of pulmonary surfactant proteins and retroviral antigens in the lungs of sheep with pulmonary adenomatosis. *J Comp Pathol,* 140 (1): 43-53, 2009. DOI: 10.1016/j.jcpa.2008.10.003

**20. Sozmen M, Beytut E:** An investigation of growth factors and lactoferrin in naturally occurring ovine pulmonary adenomatosis. *J Comp Pathol,* 147 (4): 441-451, 2012. DOI: 10.1016/j.jcpa.2012.04.004

**21. Can-Sahna K, Eroksuz Y, Berber E, Sozdutmaz I:** Detection of exogenous Jaagsiekte sheep retrovirus in Turkey. *Indian J Anim Res,* 49 (4): 498-502, 2015. DOI: 10.5958/0976-0555.2015.00053.9

**22. İlhan F, Vural SA, Yıldırım S, Sözdutmaz İ, Alcigir ME:** Expression of p53 protein, Jaagsiekte sheep retrovirus matrix protein, and surfactant protein in the lungs of sheep with pulmonary adenomatosis. *J Vet Diagn Invest,* 28 (3): 249-256, 2016. DOI: 10.1177/1040638716636939

**23. Özkan C, Yıldırım S, Huyut Z, Özbek M:** Selected tumour biomarker levels in sheep with pulmonary adenomatosis. *J Vet Res,* 64 (1): 39-44, 2020. DOI: 10.2478/jvetres-2020-0017

**24. Karakurt E, Beytut E, Dağ S, Nuhoğlu H, Yıldız A, Kurtbaş, E:** Assessment of MDA and 8-OHdG expressions in ovine pulmonary adenocarcinomas by immunohistochemical and immunofluorescence methods. *Acta Vet Brno,* 91 (3): 235-241, 2022. DOI: 10.2754/avb202291030235

**25. Karakurt E, Beytut E, Dağ S, Nuhoğlu H, Yıldız A, Kurtbaş E:** The relationship between metastasis and MMP-9 in sheep with pulmonary adenocarcinomas. *Kafkas Univ Vet Fak Derg,* 28 (2): 211-216, 2022. DOI: 10.9775/kvfd.2021.26678

**26. Karakurt E, Coşkun N, Beytut E, Keleş ÖF, Dağ S, Yılmaz V, Nuhoğlu H, Yıldız A, Kurtbaş E:** Evaluation of the relationship between inflammatory reaction and interleukins in ovine pulmonary adenocarcinomas. *Vet Res Forum,* 14 (1): 1-6, 2023. DOI: 10.30466/vrf.2022.542311.3279

**27. Karakurt E, Beytut E, Dağ S, Nuhoğlu H, Yıldız A, Kurtbaş E:** Immunohistochemical detection of pro-inflammatory and anti-inflammatory interleukins in the lungs of sheep with Jaagsiekte. *TJVR,* 6 (1): 9-14, 2022. DOI: 10.47748/tjvr.1037041

**28. Karakurt E, Beytut E, Dağ S, Nuhoğlu H, Yıldız A, Kurtbaş E:** Immunohistochemical detection of TNF-α and IFN-γ expressions in the lungs of sheep with pulmonary adenocarcinomas. *Acta Vet Eurasia,* 48 (3): 161-166, 2022. DOI: 10.5152/actavet.2022.21124

**29. Bolat İ, Yildirim S, Sağlam YS, Çomakli S, Kiliçlioğlu M, Dereli E:** Investigation of the effects of pulmonary adenomatosis on oxidative DNA damage, inflammation, apoptosis, and autophagy in lung tissues in sheep. *Small Ruminant Res,* 23010717:1, 2024. DOI: 10.1016/j.smallrumres.2023.107171