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THE PREVALENCE OF TUMOURS AS PART OF THE PULMONARY LESIONS COMPLEX IN SHEEP, IN WESTERN ROMANIA

PREVALENȚA TUMORILOR CA PARTE DIN COMPLEXUL DE LEZIUNI PULMONARE ALE OVINELOR, ÎN VESTUL ROMÂNIEI

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ABSTRACT | REZUMAT

In the period 2019-2021, 276 sheep cadavers were examined, which came from Arad, Caraș-Severin, and Timiș counties. The prevalence of pulmonary lesions was 60.86% (168/276). From the total of 168 lung injuries, the following were identified and classified: 14.28% (24/168) catarrhal-purulent bronchopneumonia, 7.73% (13/168) suppurative gangrenous bronchopneumonia, 76.78% (129/168) parasitic pneumonia, and 1.19% (2/168) tumours. Different types of gastroenteritis prevailed in the other sheep, respectively 108/276 (39.14%). Histopathological examination confirmed and identified two phases of the same neoplastic process: simple adenoma and papillary adenoma (pulmonary adenomatosis).

Keywords: lung, tumours, sheep, necropsy, histopathology

În perioada 2019-2021, au fost examinate 276 de cadavre de ovine, care au provenit din județele Arad, Caraș-Severin și Timiș. Prevalența leziunilor cu localizare pulmonară a fost de 60.86% (168/276). Din totalul de 168 de leziuni pulmonare, au fost identificate și clasificate următoarele: 14,28% (24/168) bronhopneumonii cataral-purulente, 7,73% (13/168) bronhopneumonii supurativ-gangrenoase, 76,78% (129/168) pneumonii parazitare și 1,19% (2/168) tumori. Gastroenteritele de diferite tipuri au predominat la celelalte ovine, respectiv 108/276 (39,14%). Examenul histopatologic a confirmat și identificat două faze ale aceluiași proces neoplazic: adenomul simplu și adenomul papilifer (adenomatoza pulmonară).

Cuvinte cheie: pulmoni, tumori, oaie, necropsie, histopatologie

Pulmonary adenomatosis in sheep and goats belongs to the group of diseases caused by slow viruses, characterized by a long period of incubation and a chronic course, leading to certain death (4, 6, 10, 12, 15, 16, 20). Pneumonia is often over diagnosed in adult sheep because the animal exhibits a high respiratory rate and obvious dyspnoea, even though many infections affecting other systems and organs can develop with such signs, including mastitis, metritis, anaerobic enterotoxaemia, painful conditions, severe lameness, and acute fasciolosis (1, 9, 11, 13, 17). Also, many cases of hypocalcaemia in pregnant ewes are confused with pneumonia because the affected ewes are depressed, anorexic, and show inappetence, with the expression of a liquid in the nostrils. Treatment of respiratory diseases is hampered by a breeder (shepherd) myth that sheep do not respond well to treatment and simply want to die (so therapeutic intervention is often delayed).

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There are three common, major causes of respiratory disease in adult sheep: *Mannheimia haemolytica* respiratory disease, ovine pulmonary adenomatosis (OPA), and suppurative pneumonia/lung abscess (8).

Annual mortality is generally low, about 1-2%, but the incidence of cases can vary from year to year. In some flocks, the losses are higher than 5-10%, sometimes even 20% (10, 14, 15, 16, 18, 19). In the present study, the prevalence of lung tumours, respectively pulmonary adenomatosis, as part of the lesional complex identified at the level of the respiratory system, was monitored in sheep from different flocks in western Romania.

Currently, serological tests to confirm OPA have not been successful. The PCR test used in OPA research is very sensitive and unfortunately fails to detect Jaagsiekte sheep retrovirus in laboratory tests in most infected sheep, only in obvious clinical cases. This is due to the existence of a small number of infected cells in the blood in the early stages of the disease. Bronchoalveolar lavage was used in sedated sheep to collect airway cells, followed by DNA extraction and PCR testing. Although this method is more efficient, it is not recommended on large-scale for routine testing on the farm.

METHODS AND MATERIALS

This study was carried out between 2019 and 2021 on sheep flocks from Arad, Caraș-Severin, and Timiș counties. A total number of 276 sheep, aged between 1 and 5 years, were necropsied in the Forensic Medicine Clinic of the Faculty of Veterinary Medicine (University of Life Sciences "King Mihai I" from Timișoara).

For the histopathological examination, 168 lung samples were selected, which were processed by the classical method of embedding in paraffin, with sectioning at 6 µm and staining by the H.E.A. method.

Thus, the general prevalence of lung lesions was established, along with their classification and the identification of the prevalence of adenomatosis, as part of the lung lesion complex.

RESULTS AND DISCUSSIONS

Following the necropsies performed on the 276 sheep, a general prevalence of lung lesions of 60.86% (168/276) was identified. The rest of the percentage of 39.14% was represented by multiple types of gastroenteritis. Most of the sheep cadavers that showed lesions in the respiratory system came from the herd of Arad County, respectively 60/168 (35.71%). The general prevalence of lung lesions in sheep from the flocks of Caraș-Severin and Timiș counties was 32.73% (55/168), respectively 31.54% (53/168). In Fig. 1, the general prevalence of lung injury cases can be seen for each year, 2019, 2020, and 2021.

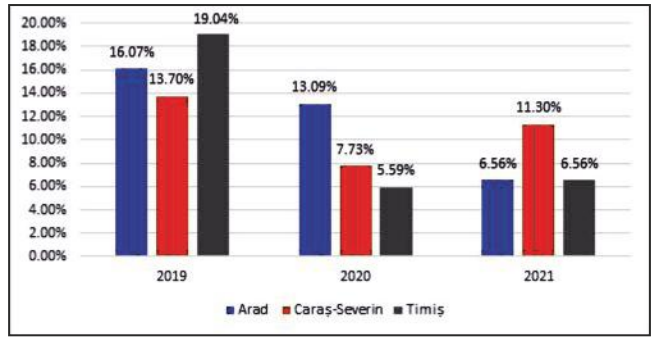

Fig. 1. Prevalence of lung lesions in sheep from western Romania

Table 1

Classification of lung lesions identified between 2019-2021, in each county

Lesion		County	n/N (%)		
			2019	2020	2021
Catarrhal-purulent bronchopneumonia (24/168)		AR	6/168 (**3.57**)	1/168 (**0.59**)	4/168 (**2.38**)
		CS	2/168 (**1.19**)	3/168 (**1.78**)	1/168 (**0.59**)
		TM	3/168 (**1.78**)	3/168 (**1.78**)	1/168 (**0.59**)
Suppurative gangrenous bronchopneumonia (13/168)		AR	-	2/168 (**1.19**)	1/168 (**0.59**)
		CS	1/168 (**0.59**)	1/168 (**0.59**)	4/168 (**2.38**)
		TM	2/168 (**1.19**)	1/168 (**0.59**)	1/168 (**0.59**)
Parasitic pneumonia (129/168)	Hydatid cyst (100/168)	AR	16/168 (**9.52**)	10/168 (**5.95**)	4/168 (**2.38**)
		CS	17/168 (**10.11**)	9/168 (**5.35**)	9/168 (**5.35**)
		TM	23/168 (**13.69**)	5/168 (**2.97**)	7/168 (**4.16**)
	Verminous pneumonia (29/168)	AR	5/168 (**2.97**)	8/168 (**4.76**)	2/168 (**1.19**)
		CS	3/168 (**1.78**)	-	5/168 (**2.97**)
		TM	4/168 (**2.38**)	1/168 (**0.59**)	1/168 (**0.59**)
Pulmonary adenomatosis (2/168)		AR	-	1/168 (**0.59**)	-
		CS	-	-	-
		TM	-	-	1/168 (**0.59**)

Abbreviations: AR-Arad, CS-Caraș-Severin, TM-Timiș, n-number of positive cases, N-total number of cases

The classification and prevalence of lung lesions observed in the 3 years, in each of the 3 counties, can be found in Table 1. Pneumonia of parasitic origin was the most encountered lesion, with an overall prevalence of 76.78% (129/168), followed by catarrhal-purulent bronchopneumonia (14.28%, 24/168) and suppurative gangrenous bronchopneumonia (7.73%, 13/168). In the case of tumors, pulmonary adenomatosis was diagnosed in two sheep from Arad and Timiș counties, with a prevalence of 1.19% (2/168).

Globally, Hashemnia et al. (2019) described several lung lesions observed in 1.200 sheep from an abattoir, in Iran. Bronchopneumonia was in the proportion of 32.52%, and lung abscesses were in the proportion of 8.13%. Pneumonia of parasitic origin, respectively verminous pneumonia, and hydatid cysts were reported in a proportion of 2.23% and 10.16%, respectively, much lower percentages compared to those identified in the present study. Regarding the prevalence of lung tumours, two types of tumours, adenoma, and melanoma were diagnosed with a proportion of 0.6% each (7). Higher rates of lung adenocarcinoma of 8% were reported in 2016 in sheep from Rajasthan, India (17). In Egypt, in addition to pulmonary adenomatosis (2.52%), fibrosarcoma (1.26%) was also described in necropsied sheep. Suppurative pneumonia was diagnosed in a similar proportion to that in our study, namely 10.69%, and verminous pneumonia, in lower percentages (3.14%) (5). In a study in Bolivar, Colombia, no lung tumours were found. A much lower prevalence of 3% has been reported for suppurative and verminous bronchopneumonia (3). Higher percentages of suppurative bronchopneumonia, 25.7%, and also of pulmonary adenomatosis (3.4%), were described by Mekibib et al. (2019), in Ethiopia (2).

The two suspected cases of pulmonary neoplasms on macroscopic examination were confirmed by histopathological examination in the Pathological Anatomy laboratory. One case came from Arad County, and the other from Timiș County.

Macroscopically, in both cases, the lungs increased in volume and weight. In the first case (3-year-old sheep), nodules were detected that did not exceed the size of a pea, grey in colour, slightly prominent subpleural, dense to palpation, being disseminated mainly in the cranial and middle lobes. On the surface of the bronchus section, an abundant foamy liquid, greyish-whitish in colour was spilled, and the foci had a rough appearance on the section (Fig. 2, 3).

In the second case (5-year-old sheep), in addition, pearly white confluent lesions with a higher consistency were reported. On the section, the amount of liquid that leaked from the bronchi was reduced, being responsible only for the moist and glossy appearance of the bronchial mucosa. Vicarious alveolar emphysema was consistently observed around the grey-whitish nodules, regardless of the presentation of the lesions and their sizes.

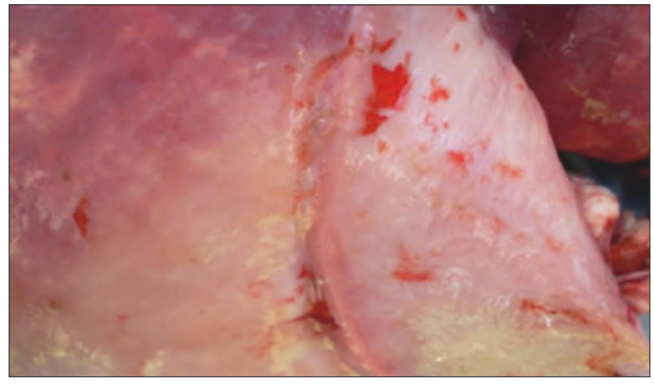

Fig. 2. Lung – the macroscopic appearance of the affected lobes

Fig. 3. Lung – section view of affected lobes

At necropsy, macroscopically, degenerative lesions affecting the large parenchyma (liver, kidney, and/or myocardium) were noted.

Microscopically, with the objective of 4x, in the sections performed, a proliferation of the epithelium of the terminal bronchioles and the alveolar endothelium was observed, with the complete filling of the lumens.

Following the examination with higher objectives (10x, 20x, 40x), it was noted that tumoral hyperplasia of the bronchiolar epithelium and alveolar pneumocytes, in the form of territories with an acinar appearance, or through papillary hyperplastic areas.

The territories with acinar appearance are the result of moderate tumoral hyperplasia of the bronchoalveolar epithelium, which imprints the image of alveolar epithelialization. The alveolar epithelium consists of cubic or cylindrical, tachromatic pneumocytes with oval nuclei and clear cytoplasm. They outline the alveoli, which, on superficial examination, can be confused with an acinar lung tissue (simple adenoma). Alveolar lumens are proportionally smaller, depending on the intensity of pneumocyte hyperplasia.

Papillary hyperplastic areas are predominant from

the first appearance and unevenly dispersed in the microscopic field. As the neoplastic process intensifies, the tumour cells no longer fit on the basement membranes and come out into the lumen of the alveoli, in the form of pedicled papillae. Initially, the papillae are not pedicled in the form of protrusions that slightly penetrate the lumens of the alveoli.

With the intensity of the neoplastic process, the papillae penetrate deeper into the alveolar lumens, thus reducing the respiratory space. The papillae can be observed in several aspects: mono - bi - tri or tetralobate.

At other times, "free papillae" can be observed, which are not directly attached to the alveolar epithelium, which "seem to float" in the alveolar lumen, due to their oblique sectioning.

Regardless of the shape and size of the papillae, they are formed by a row of tumour cells, arranged peripherally, and the centre of each formation is made up of a connective-vascular tissue (papillary adenoma). The leukocyte hyperplasia that can be observed produces local "alveolar instability", manifested by changes in pulmonary surfactant. In the alveolar and perivascular walls, there is a mesenchymal proliferation, with a lymphohistiocytic appearance.

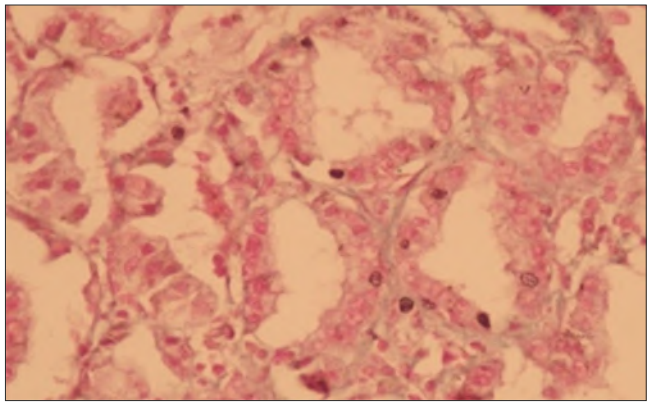

Fig. 4. Simple adenoma (col. HEA 40x)

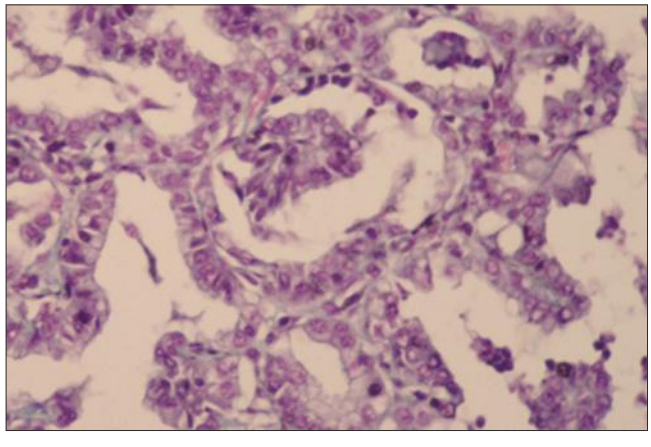

Fig. 5. Papillary adenoma (col. HEA 40x)

The proliferation of the bronchoalveolar epithelium is accompanied in many territories by stromal hyperplasia, initially evidenced by the growth of the fundamental substance, and later, by a conjunctival neogenesis, at first predominantly cellular, and gradually, it becomes predominantly fibrillar.

The obtained results highlight two lesion aspects: simple adenoma and papillary adenoma, characteristic lesions of pulmonary adenomatosis in sheep and goats (Fig. 4, 5).

CONCLUSIONS

The prevalence of tumours in sheep flocks from western Romania was low, at a rate of 1.19%. Only pulmonary adenoma was identified, in the two evolutionary forms: simple and papillary. This indicates a sporadic occurrence of cases in the sheep population studied. Parasitic pneumonia was the most common lesion diagnosed in the sheep included in the study.

REFERENCES

1. *Bai J., Zhu R.Y., Stedman K., Cousens C., Carlson J., Sharp J.M., DeMartini J.C.,* (1996), 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
2. *Berhanu M., Tadesse M., Amene F., Rahmeto A.,* (2019), Prevalence of pneumonia in sheep and goats slaughtered at elfora bishoftu export abattoir, Ethiopia: a pathological investigation. J Vet Med, 2019:5169040
3. *Caicedo-Martínez J.A., Ávila-Rubiano M.A., Orellano-Badillo H., Sanjuanelo-Corredor D.W.,* (2017), Patología pulmonar en ovinos faenados del norte del departamento de Bolívar, Colombia. Cienc y Tecnol Agropec, 18(3):555-569
4. *DeMartini J.C., Bishop J.V., Allen T.E., Jassim F.A., Sharp J.M., de las Heras M., Voelker D.R., Carlson J.O.,* (2001), Jaagsiekte sheep retrovirus proviral clone JSRV(JS7), derived from the JS7 lung tumor cell line, induces ovine pulmonary carcinoma and is integrated into the surfactant protein A gene. J Virol, 75(9):4239-4246
5. *El-Mashad A., Moustafa S., Amin A., Samy E.,* (2020), Pathological studies on lung affections in sheep and goat at Kalubia Governorate. *BVMJ*, *38*(1):17-23
6. *González L., García-Goti M., Cousens C., Dewar P., Cortabarría N., Extramiana A.B., Ortín A., De Las Heras M., Sharp J.M.,* (2001), Jaagsiekte sheep retrovirus can be detected in the peripheral blood during the pre-clinical period of sheep pulmonary adenomatosis. J Gen Virol, 82(6):1355-1358

7. *Hashemnia M., Chalechale A., Malmir E.,* (2019), Pulmonary lesions in slaughtered sheep in Western Iran: gross and histopathological findings. Vet Ital, 55(1):47-56
8. *Herman V.*, *Mânzat R.M.*, *Rămneanţu M.*, (2006), Diagnosis in infectious diseases of animals, (Ed.) Mirton, Timisoara, Romania (in Romanian)
9. *Holland M.J., Palmarini M., Garcia-Goti M., Gonzalez L., McKendrick I., de las Heras M., Sharp J.M.,* (1999), Jaagsiekte retrovirus is widely distributed both in T and B lymphocytes and in mononuclear phagocytes of sheep with naturally and experimentally acquired pulmonary adenomatosis. J Virol, 73(5):4004-4008
10. *Mansour K.A., Al-Husseiny S.H., Kshash Q.H., Jassim A.,* (2019), Clinical-histopathological and molecular study of ovine pulmonary adenocarcinoma in Awassi sheep in Al-Qadisiyah Province, Iraq. Vet World, 12(3):454-458
11. *Mesteșanu E., Mesteșanu C., Avram E., Andor C.,* (2018), Study on the antibiotic resistance of strains of *Staphylococcus aureus* isolated from animals with contagious ecthyma. Rev Rom Med Vet, 28(1):5-11
12. *Ortín A., Minguijón E., Dewar P., García M., Ferrer L.M., Palmarini M., Gonzalez L., Sharp J.M., De las Heras M.,* (1998), Lack of a specific immune response against a recombinant capsid protein of Jaagsiekte sheep retrovirus in sheep and goats naturally affected by enzootic nasal tumour or sheep pulmonary adenomatosis. Vet Immunol Immunopathol, 61(2-4):229-237
13. *Özkan C., Yıldırım S., Huyut Z., Özbek M.,* (2020), Selected tumour biomarker levels in sheep with pulmonary adenomatosis. J Vet Res, 64(1):39-44
14. *Palmarini M., Cousens C., Dalziel R.G., Bai J., Stedman K., DeMartini J.C., Sharp J.M.,* (1996), The exogenous form of Jaagsiekte retrovirus is specifically associated with a contagious lung cancer of sheep. J Virol, 70(3):1618-1623
15. *Rosadio R.H., Sharp J.M., Lairmore M.D., Dahlberg J.E., De Martini J.C.,* (1988), Lesions and retroviruses associated with naturally occurring ovine pulmonary carcinoma (sheep pulmonary adenomatosis). Vet Pathol, 25(1):58-66
16. *Shi W., Jia S., Guan X., Yao X., Pan R., Huang X., Ma Y., Wei J., Xu Y.,* (2021), A survey of Jaagsiekte sheep retrovirus (JSRV) infection in sheep in the three northeastern provinces of China. *Arch Virol, 166:*831-840
17. *Sonawane G.G., Tripathi B.N., Kumar R., Kumar J.,* (2016), Diagnosis and prevalence of ovine pulmonary adenocarcinoma in lung tissues of naturally infected farm sheep. Vet World, 9(4):365-370
18. *Stancu A.,* (2014), General veterinary pathological anatomy, Ed. Agroprint, Timișoara, Romania.
19. *Stancu A.,* (2014), Practicum of *veterinary pathological anatomy,* Ed. Agroprint, Timișoara, Romania
20. *Toma C., Bâlteanu V.A., Tripon S., Trifa A., Rema A., Amorim I., Pop R.M., Popa R., Catoi C., Taulescu M.,* (2020), 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.