Scientific Works. Series C. Veterinary Medicine. Vol. LXVIII (2), 2022
ISSN 2065-1295; ISSN 2343-9394 (CD-ROM); ISSN 2067-3663 (Online); ISSN-L 2065-1295

ISOLATION AND IDENTIFICATION OF TWO *Pasteurella* STRAINS, RESPONSIBLE FOR AN OUTBREAK OF PNEUMONIA IN SHEEP

George MOGOŞ[1], Anca BULGARU[1], Elena NEGRU[1], Horia DINU[1], Mihai DANEŞ[2], Doina DANEŞ[1]

[1]Faculty of Veterinary Medicine/University of Agronomic Sciences and Veterinary Medicine of Bucharest, Splaiul Independentei 105, District 5, Bucharest, Romania
[2]Faculty of Veterinary Medicine/Spiru Haret University, 256 Basarabia Avenue, District 2, Bucharest, Romania

Corresponding author email: mogoshge@yahoo.com

Abstract

The present study was carried out with the purpose of identifying the etiological agent(s) of a reemmergent outbreak of respiratory syndrome affecting a flock of sheep. The first cases of pneumonia on the farm were recorded during warm season (June - July 2021), affecting mainly lambs, with a few isolated cases among adult sheep. Symptoms included nasal discharge, coughing, dispnea, loss of appetite and severe weight loss, a morbidity of 80% among young animals and 5% mortality. Pasteurella spp. was isolated from lung tissue samples collected during necropsy. The lambs were treated with Enrofloxacin administered orally, and the clinical status improved after 6 days of therapy; however, during the fall of 2021, a recurrence of respiratory distress was reported, this time affecting both young and adult animals. Bacteriological examinations were performed on nasal and palatine tonsil swabs collected from live animals and lung and lymph node tissue collected from slaughtered animals for diagnostic purposes. Two strains of Pasteurella were isolated, Pasteurella multocida and Pasteurella spp. The isolates were characterized biochemically and antibiotic susceptibility tests were performed. Following test results, the entire flock of 2000 sheep was treated with Enrofloxacin for 6 days and complete remission of respiratory symptoms was achieved.

Key words: *Pasteurella multocida, Pasteurella spp., pneumonia, respiratory infection, sheep.*

INTRODUCTION

Small ruminant husbandry plays an important role in worldwide economy. Ovine infectious pathology is complex and diverse, comprising numerous morbid entities, such as anthrax, mycoplasmosis, anaerobiosis and viral diseases, which have to be considered when the prophylactic regime is established for a certain herd (Turcu et al., 2010; Enache et al., 2017; Negru et al., 2021). Diseases caused by bacteria belonging to the *Pasteurella* genus among sheep populations can be a cause of major economic loss, especially due to manifestations such as pneumonia and sepsis in lambs. The main species responsible for ovine pasteurellosis is *Pasteurella* (*Mannheimia*) *haemolytica*; very rarely, *Pasteurella multocida* is the causative agent of pneumonia in sheep. Prevention of the disease is difficult to accomplish, due to the large number of circulating strains and low immunogenicity of the bacteria (Manzat, 2001). *P. multocida* represents a heterogenous group of microorganisms, characterized by antigenic variation, diverse host predilection and pathogenesis. Some of the strains included in the group are primary pathogens, determining severe outbreaks of respiratory infections in various species, and others are ordinary commensals of the respiratory tract, able to multiply and invade tissues, causing respiratory disease, as comorbidity, in immuno-suppressed individuals (Weisser et al., 2003). The purpose of the current study was to identify the etiological agent(s) of an reemergent outbreak of respiratory syndrome affecting a flock of sheep, and to establish a correct course of treatment, in accordance with the antibiotic susceptibility tests.

MATERIALS AND METHODS

The outbreak of pneumonia described in the current study affected a flock counting 2000

sheep, located in the south-eastern region of Romania, in the Danube Meadow. The animals were breeded in the traditional husbandry system, both in enclosed sheds, during cold season, and on pastures during the warm season. Besides the respiratory infections described in the current study, other ovine health issues of the flock, common in the area, include tick infestations and internal parasitic infestations with trematodes (*Fasciola* spp. and *Dicrocoelium* spp.) and cestodes. Also, the ground of the pastures was a flood zone, and during rainy weather, the ground became muddy, acting on the prevalence of foot rot.

The entire flock was subjected to deworming twice every year, during spring and fall, with albendazole, administered orally and ivermectin, injected subcutaneously, and the lambs were also dewormed during summer, in order to maintain parasitic infestations under control. Prophylactic measures included vaccinations against anthrax during spring, against anaerobic diseases during fall, and against contagious agalactia every six months. To prevent anaerobic diseases, lambs were first vaccinated at 4-6 weeks of age, and received a booster after 4 weeks.

The first cases of respiratory infections on the farm were recorded in June 2020. The symptoms included coughing, nasal discharge, severe weight loss and death, and affected mainly lambs, aged 3 to 6 months. Out of a batch of approximately 200 lambs, 80% expressed the disease, and the recorded mortality rate was 5 %. Only a few cases were recorded among adult animals, with mild symptoms and no mortality. The lambs that died of the disease were subjected to necropsy, and samples were collected from lung tissue and thoracic lymph nodes for bacteriological examination. According to the results of antibiotic susceptibility tests, the affected group was successfully treated with Enrofloxacin oral solution, 5 mg/ kg/ day, for 6 days.

A recurrence of respiratory symptoms was recorded among the animals on the flock, approximately 3 months after the initial treatment, during the fall of 2021. Both young and adult animals were affected, showing signs of respiratory distress, coughing and weight loss. No mortality was recorded during this period; however, a few more severely affected animals were slaughtered for diagnostic purposes. Bacteriological examinations were performed on samples collected from bronchial secretions, lungs, liver, spleen and thoracic lymph nodes during necropsy, and on nasal swab and palatine tonsils swab samples collected from clinically affected live animals. The samples were cultured on Columbia agar with 5% defibrinated sheep blood and incubated at 37°C for 20-24 hours. The morphological features of the isolated strains were examined microscopically on Gram stained slides, and catalase and oxidase tests were performed using conventional methods. The identification of the isolates was performed using the Api 20 E and Api 20 NE biochemical tests (Biomerieux), with the interpretation of the results performed according to the producer's instructions. Antimicrobial susceptibility was investigated by disc diffusion method, using Liofilchem antimicrobial discs, and the results were interpreted using Liofilchem and EUCAST standards. The entire flock of sheep was placed under treatment with Enrofloxacin oral solution, 5 mg/ kg/ day, for 6 days, as indicated by the results of the antibiotic susceptibility tests. All the animals present on the farm were housed in enclosed sheds and the antibiotic was administered via drinking water, limiting the animals' access to any untreated water source in order to ensure the ingestion of the appropriate dose of medication.

RESULTS AND DISCUSSIONS

Post mortem examinations of the carcasses revealed various degrees of pulmonary consolidation, pulmonary edema (Figure 1), congestion, atelectasis, and in some cases, abscesses were present in the lung tissue. Pleural effusion was present in the majority of the examined carcasses.

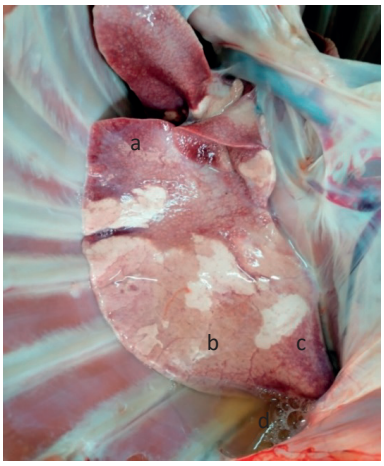

Figure 1. Necropsy examination – lung of an adult sheep, showing congestion (a), edema (b) and marbling (c). A large amount of yellow, serous fluid is present in the pleural space (d).

Bacteriological examinations of palatine tonsil swabs and nasal swabs constantly revealed the presence of medium sized, gray, transparent, non-hemolytic colonies, which appeared as Gram negative cocobacili upon microscopic examination.

The isolated strain was identified as *Pasteurella* spp. via biochemical tests.

The biochemical characteristics of the isolate are detailed in Table 1.

A *Pasteurella* spp. strain, with identical biochemical characteristics, was also isolated in pure culture from the pulmonary abscesses of two lambs, aged 3 and 4 months, which had died during the first outbreak of respiratory infections.

Table 1. Biochemical characteristics of the *Pasteurella* spp. isolated from palatine tonsil swabs and nasal swabs of affected sheep

NO3	TRP	GLU	ADH	URE	ESC	GEL	PNPG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC
+	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-

Legend: NO3 – potassium nitrate, TRP – L-tryptophane, GLU – D-glucose (fermentation), ADH – L-arginine, URE – urea, ESC – esculin ferric citrate, GEL – gelatin, PNPG - 4-nitrophenyl-βD-galactopyranoside, GLU – D-glucose (assimilation), ARA – L-arabinose, MNE – D-mannose, MAN – D-mannitol, NAG – N-acetyl-glucosamine, MAL – D-maltose, GNT – potassium gluconate, CAP – capric acid, ADI – adipic acid, MLT – malic acid, CIT – trisodium citrate, PAC – phenylacetic acid, "+" - positive result, "-" - negative result.

P. multocida was isolated only from lung tissue samples from lambs and adult sheep.

On Columbia blood agar, the *P. multocida* colonies appeared grayish and non-hemolytic, slightly smaller and more transparent than the *Pasteurella* spp. colonies (Figure 2).

Both bacterial strains were catalase positive and oxidase negative.

The identity of the *P. multocida* isolate was confirmed by the results of two biochemical tests, Api 20 E and Api 20 NE, the results of which are presented in Table 2.

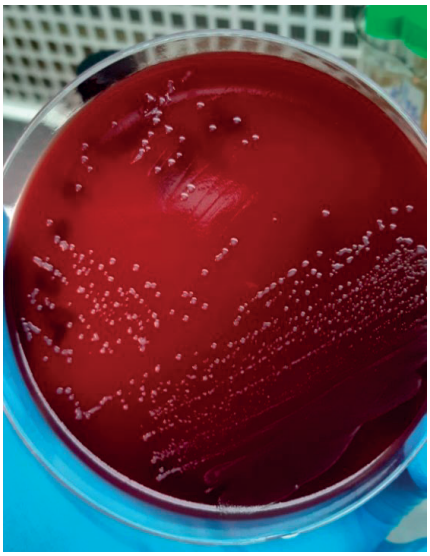

Figure 2. *Pasteurella multocida* colonies on Columbia blood agar

Table 2. Biochemical characteristics of the *Pasteurella multocida* isolated from lung tissue samples of an adult sheep

API 20 NE	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNG	GLU	ARA	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC
	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
API 20 E	ONPG	ADH	LDC	ODC	CIT	H2S	URE	TDA	IND	VP	GEL	GLU	MAN	INO	SOR	RHA	SAC	MEL	AMY	ARA
	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-

Legend: Legend: NO3 – potassium nitrate, TRP – L-tryptophane, GLU – D-glucose (fermentation), ADH – L-arginine, URE – urea, ESC – esculin ferric citrate, GEL – gelatin, PNPG - 4-nitrophenyl-βD-galactopyranoside, GLU – D-glucose (assimilation), ARA – L-arabinose, MNE – D-mannose, MAN – D-mannitol, NAG – N-acetyl-glucosamine, MAL – D-maltose, GNT – potassium gluconate, CAP – capric acid, ADI – adipic acid, MLT – malic acid, CIT – trisodium citrate, PAC – phenylacetic acid, ONPG - 2-nitrophenyl-βD-galactopyranoside, LDC – L-lysine, ODC – L-ornithine, H2S – H_2S production, TDA – L-tryptophane, IND – indole production, VP – acetoin production, INO – inositol, SOR – D-sorbitol, RHA – L-rhamnose, SAC – D-saccharose, MEL – D-melibiose, AMY – amygdaline, "+" - positive result, "-" - negative result.

Antibiotic susceptibility tests revealed that both isolates were sensitive to the majority of the antimicrobials used in the essay (Table 3), including enrofloxacin, the selected antibiotic for the treatment of the flock.

Table 3. Antibiotic susceptibility results for *Pasteurella* spp. and *Pasteurella multocida*

Antibiotic	***Pasteurella spp.***	***Pasteurella multocida***
Enrofloxacin	Susceptible	Susceptible
Trimethoprim + Sulfametoxazole	Susceptible	Susceptible
Gentamycin	Susceptible	Susceptible
Norfloxacin	Susceptible	Susceptible
Spectinomycin	Susceptible	Susceptible
Doxycycline	Susceptible	Susceptible
Ampicillin	Susceptible	Susceptible
Amoxicillin	Susceptible	Susceptible
Erythromycin	Intermediately susceptible	Intermediately susceptible
Tetracycline	Intermediately susceptible	Susceptible
Lincomycin	Resistant	Resistant
Colistin sulfate	Resistant	Resistant

The initial therapeutic approach, which targeted only the affected animals, was successful at the time in improving the clinical status of the lambs, and limiting mortality. However, the disease was not eradicated from the flock, possibly due to subclinically infected animals, which continued to spread the bacteria. After the second course of treatment, which included all of the animals on the farm, a complete remission of the respiratory symptoms was achieved.

Ovine pathology caused by members of the *Pasteurella* genus has been reported in a number of other studies. A study carried out in Iran reported a prevalence of 3.71% of *P. multocida* infections among pneumonia cases in sheep and goats (Valadan et al., 2014). In Ethiopia and Iraq researchers detected the presence of *P. multocida* and *Mannheimia haemolytica* in nasal swab samples and lung tissue specimens of pneumonic sheep, using the polymerase chain reaction (Deressa et al., 2010; Othman et al., 2014). The results of a study preformed on Icelandic sheep suggest that at least two groups of *P. multocida* coexist in sheep: a genetically homogenous group consisting of upper respiratory tract commensals, and a genetically heterogeneous group representing the cause of ovine pneumonia (Einarsdottir et al., 2016). Regarding antibiotic susceptibility, studies on *P. multocida* and *M. haemolytica* strains isolated from small ruminants revealed the majority of the isolates to be multidrug resistant; however, most strains were susceptible to enrofloxacin (Sarangi et al., 2015).

Further research is required to assess the pathogenicity and immunogenic properties of the isolated *Pasteurella* strains and whether they could be considered as candidates for the production of an auto-vaccine.

CONCLUSIONS

The current study presents an etiological approach over an emerging respiratory infection in a sheep flock. Based on clinical signs and post-mortem examinations, a suspicion of *Pasteurella* induced pneumonia was issued. Samples collected from the flock were subjected to bacteriological examinations, and two strains belonging to the *Pasteurella* genus were isolated. The two isolated were identified as P. spp. and *P. multocida.* Both strains were susceptible to enrofloxacin, and the antibiotic was used successfully for therapeutic purposes. Given the history of the respiratory disease in the herd, induced by *Pasteurella* species, the health of the animals remains under threat of recurrence, whenever the associated risk factors will intervene.

REFERENCES

Deressa, A., Asfaw, Y., Lubke, B., Kyule, M. W., Tefera, G., Zessin, K. H. (2010). Molecular Detection of P*asteurella multocida* and *Mannheimia haemolytica* in Sheep Respiratory Infections in Ethiopia. *Intern J Appl Res Vet Med. 2*(8): 101 – 108.

Einarsdottir, T., Gunnarsson, E., Sigurdardottir, O. G., Jorundsson, E., Fridriksdottir, V., Thorarinsdottir, G. E., Hjartardottir, S. (2016). Variability of *Pasteurella multocida* isolated from Icelandic sheep and detection of the *toxA* gene. *Journal of Medical Microbiology, 65*: 897–904.

Enache, D. A., Baraitareanu, S., Dan, M., Gurau, M. R., Otelea, F., Danes, D. (2017). Preliminary results of MVV and CAEV seroprevalence in Romanian sheep and goats. *Scientific Works Series C. Veterinary Medicine Volume LXIII* (1): 95-100.

Manzat R. M., (2001) *Infectious Diseases of the Animals - Bacteriosis*, Timisoara, Brumar Publishing House. 125 - 127.

Negru, E., Dinu, H., Bulgaru, A., Lupu, D., Danes, M., Gurau, M. R., Danes, D. (2021). Increasing the immunizing value of a *Clostridium septicum* strain. *AgroLife Scientific Journal 10*(2): 136 - 141.

Othman, R. M., Ibraheim, H. K., Sayhood, M. H. (2014). Conventional and molecular detection of *Pasteurella multocida* in outbreak of respiratory tract infection of sheep and goats in Basrah Province. *Bas.J.Vet.Res. 1* (1): 157 – 165.

Sarangi, L. N., Thomas, P., Gupta, S. K., Priyadarshini, A., Kumar, S., Nagaleekar, V. K., Kumar, A., Singh, V. P (2015). Virulence gene profiling and antibiotic resistance pattern of Indian isolates of *Pasteurella multocida* of small ruminant origin. *Comparative Immunology, Microbiology and Infectious Diseases 38*: 33-39.

Turcu, D., Tusose, A., Oporanu, M., Condur, D., Grigorescu P., Barboi, G. (2010). Studies concerning the humoral immune response in sheep inoculated against contagious agalaxie. *Scientific Works Series C. Veterinary Medicine, Vol. LVI* (2).

Valadan, M., Jabbari, A., Niroumand, M., Tahamtan, Y., Bani Hashemi, S. (2014). Isolation and Identification of Pasteurella multocida from Sheep & Goat in Iran. *Archives of Razi Institute*, 69(1): 47 – 55.

Weiser, G. C., DeLong, W. J., Paz, J. L., Shafii, B., Price, W. J., Ward, A. C. S. (2003). Characterization of *Pasteurella multocida* associated with pneumonia in Bighorn sheep. *Journal of Wildlife Diseases,* 39(3): 536–544.