

Histopathological and Molecular Identification and Characterization of Pneumonic Pasteurellosis in Sheep and Goats from Outbreaks at AS-Sharika Province, Egypt


Amira Saad Helal Hassenin^{1,*} and Akram Dossier Mohdi Khan²

ABSTRACT

Pneumonic Pasteurellosis highly fatal septicemic disease in multi-animal species in bovine, ovine, Poultry and swine. The strain identified from ovine pneumonia were characterized by MLST (Multi-host and RIRDC databases) and virulence-associated gene (VAG) typing and compared with bovine isolates. Recording outbreak of pasteurellosis with high mortality & History of several deaths in different farm and homeowner cases of sheep and goats with severe respiratory illness after shipping them from long distance Elarish and Sinai in period to El-Sharqia province. Most cases that still live are treated with antibiotics after make sensitivity test on sampling from sick cases and most cases recovered and responded to treatments. Histopathological changes of lung tissue from recently dead cases showed the proliferation of pasterulla multicida in the respiratory system. The presence of *Pasteurella multocida* in swap samples collected from per acute & acute cases of sheep & goats was detected by bacteriological examination and Culture.

Submitted: May 28, 2024

Published: July 24, 2024

 10.24018/ejvetmed.2024.4.4.127

¹ Assistant Professor, Faculty of Veterinary Medicine, Zagazig University, Egypt.

² Associate Professor, International Research of Auzbakistan University, Uzbekistan.

*Corresponding Author:
e-mail: amm_microhass@yahoo.com

Keywords: Lung, Pneumonia, Pasteurella, Shipping fever.

1. INTRODUCTION

Hemorrhagic septicemia is a bacterial disease affecting the respiratory of buffalo, and cattle while in sheep & goats diseases called pneumonic pasteurellosis [1]. This is caused by Gram-negative bacteria [2]. These need stress factors as predisposing factors to cause severe illness and often deaths in most cases depending on the severity of stress factors. Shipping animals under uncontrolled conditions is one of the most common deaths and or occurrences hemorrhagic septicemia in small ruminants' sheep and goats and also affects transportation those small animals with large numbers most common stress and concurrent infection with internal parasites as a lower immune system for poor or no treatments to cases for precaution before transportation.

1.1. Clinical Signs

Most clinical signs of *pasteurellosis* are acute and show high mortality and fatality up to 48 hours. Apparent per acute cases showed septicemia, moist mouth with extra salivation, mucopurulent, for short incubation period may

be noticed or not [3], [4]. An acute form of pneumonic pasteurellosis continues for 5 days with complicated symptoms of recumbency, laying in the ground with severe septicemia, petechial hemorrhage, congestion in mouth and extended edematous swelling from neck to belly often legs [5], [6].

Postmortem pneumonic pasteurellosis in dead carcass [7] showed edematous dark reddish fluid in muscle from the diaphragm, subcutaneous peritoneum up to all abdomen, flank region. Cyanosis is, a bluish mucous membrane with severe fibrous tissue in the alveoli and lobes of the lung causing obstructive pneumonia and some cases observed with severe gastrointestinal inflammation with hemorrhagic diarrhea [8].

Accurate identification for detection of different types of *Pasteurella* species and serotypes using hyperimmune sera [9] from rabbits and mice in most world countries but depending on definitive characteristics of *P. multocida* by coagulation test, immunoelectrophoretic, molecular diagnosis combined with histo-pathological lesion [10].

Hygienic measures all over the world using vaccination protocols with the old vaccine which is available to be



used in young animals called inactive vaccine showed a short period of protection and needs to be administrated in two doses every year [11]. So, most world health organizations developed new protocols for attenuated vaccines from local strain-infected regions to be used for protection and increased longevity with prolonged immunity or even solid natural protection [12], [13].

2. MATERIAL & METHODS

From November 2018 to October 2019, 11 lung samples out of 33 carcass cases were under aseptic condition for further lab processing from 55 bacteriological swap cases of sheep & goats from different farms showing clinical signs of hemorrhagic septicemia.

2.1. Morphological & Bacteriological Detection

Samples taken from infected cases were bacteriologically identified by cultivation on 3%–5% sheep blood agar (Oxoid Ltd., UK) and MacConkey agar (Oxoid Ltd., UK), followed by incubation at 37°C for 24 hours. The plates were then examined for growth. Next, the colonies were Gram stained and tested, then observed for the following characteristics: no growth on MacConkey agar, then biochemical identification by catalase and oxidase positivity, indole production, with characterization of nonmotility, non-hemolysis on SBA, and acid from glucose [14], [15].

Using the standard method of bacteriological and morphological characterization of growing culture of suspected isolates using gram stain, electron microscope with identification of no hemolysis and biochemical test for *Pasteurella spp* [16].

2.2. Histo-Pathology

Tissue samples taken from the lung were stabilized using the regular method of tissue stained in hematoxylin with iodine after being immersed in formaldehyde with the buffered solution to help slice tissue into small sections of width 3–5 cm.

2.3. Immunohistochemical Staining

Slices of lung lobes, and alveoli that histopathology were immersed in wax and then removed wax from this section and should be truly moist for ready to be stained by avidin-biotin-peroxidase complex (ABC-P) technique (manufactured, USA). and stained by Antigen retrieval was recognized using laboratory-stained oven standard for sample heating up to 20 minutes or less fixed in concentrated buffer solution [17]. Followed by immersed in 0.3% hydrogen peroxide in methanol for 15 minutes, to stop further peroxide reaction should be incubated at 45°C for 15 minutes with regular sera of goat *spp*. Then at room temperature section of tissues was fixed in monoclonal and multi-clonal antibodies of *Pasteurella* species with different dilutions of hyperimmune serum as instructed. results of all used colored labelled ads directed by lab protocols for final indication of samples. Highly specialized electron microscope used by histo-technician for observation and recorded results. All Histo-pathology tissues are examined at 500X resolution using an ultra-light electron microscope at Zagazig University, Faculty of Veterinary, Egypt.

3. RESULTS

3.1. Growth and Hemolysis

Suspected colonies showed growth on Sheep Blood Agar (SBA) and with clear no hemolytic activity. This means that the bacteria did not cause any significant breakdown of red blood cells, resulting in a lack of clearing or discoloration around the colonies on the agar (Fig. 1a). Characteristics identification of bacterial isolates of *Pasteurella Multicoda* in a lab are as follows:

1. **Gram Staining and Cell Structure:** The bacterial cells were Gram-negative bacilli observed not to keep purple color or blue and instead appeared pink/red under electron microscope. They had a coccoid bipolar structure, which means they were spherical in shape with two distinct ends (Fig. 1b).
2. **Additional Characteristics:** The isolates were non-motile, meaning they did not exhibit movement using flagella. They tested positive for catalase and oxidase, indicating the presence of specific enzymes. They were also indole-positive, which means they produced indole when tested. Furthermore, the isolates were able to use glucose for acid production, a characteristic commonly observed in *Pasteurella multocida*.
3. **API® 20NE Test:** Eight isolates did not grow on MacConkey agar, and all isolates positively known as *Pasteurella multocida* using the API® 20NE test.

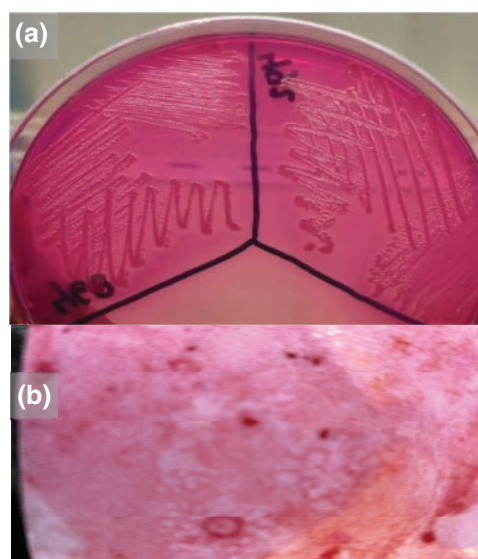


Fig. 1. (a) Sheep Blood Agar (SBA) showed non-hemolysis and (b) Gram-negative coccoid under the electron microscope.

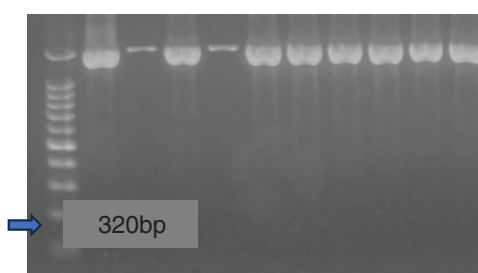


Fig. 2. Molecular identification of *Pasteurella multocida* isolates with specific primers.

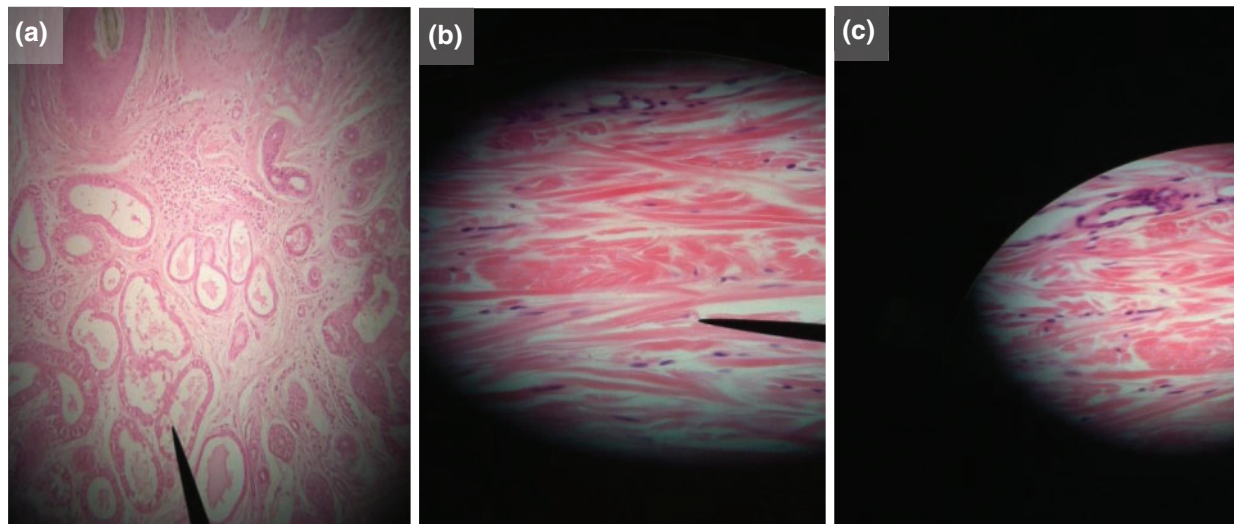


Fig. 3. (a–c) Interstitial pneumonia is characterized by the filtration of interstitial tissue with lymphoid cells and mononuclear cells, smooth destructive muscle cells along fibroid.

This identification method uses a commercial system that determines bacterial identification based on specific biochemical reactions. The numerical profile obtained for these isolates was 3000004, with a per cent identification (% ID) of 96%.

4. *Unusual Isolates*: more than three cultures exhibited the red color of isolates on specialized plate growth, suggesting the ability to ferment lactose. Surprisingly, the API® 20NE test other mixed infections, respectively, with different numerical profiles and per cent identifications. However, subsequent polyclonal using molecular diagnosis with primers, in fact, *Pasteurella multocida* (Fig. 2).

These findings highlight the importance of confirming bacterial identification through molecular techniques like PCR, as they provide more accurate and reliable results compared to solely relying on biochemical tests.

3.2. Histopathology

Gross eye examination and macroscopically found that the right cranial lobe & left cranial lobe highly affected the medial right lobe respectively. Infected tissue and lobes highly showed solidification with severe inflamed enlarged hardly to be manipulated and in texture.

Histo-pathologically, the section taken from lobes of infected lungs and bronchi showed catarrhal and patches of reddish spots mixed with fibrous fluid. Mononuclear cellular infiltration was noted in the bronchioles (Fig. 3).

Microscopically, lobular and obstructive-pneumonia with fibrinous exudate (n = 11) and catarrhal purulent (n = 22) infiltration of alveoli, lung lobes and internal connective tissue with squamous cell and fibrous mixed by many leukocytes, neutrophil as shown (Figs. 4a–4c).

The lumen of alveoli is filled with necrotic cells with fibrinous tissue adhered to a number of syncytial cells in spindle shape infiltrated with many neutrophils (Fig. 5). Parts of lung lesion showed necrotic coagulation with exudative catarrhal mucous. In certain sections of stained alveoli, the lumen was identified as widespread necrotic, mucopurulent pneumonia with mixed fibrous

tissue and monocytes. more than 11 solidified sections of the pulmonary section showed high perforation with neutrophil, fibroid cells, and sero-edematous fluid with adeno filtrations. On the other hand, more than 5 sections showed interstitial pneumonia characterized by filtration of interstitial tissue with lymphoid cells and mononuclear cells, smooth destructive muscle cells along with fibroid (Figs. 3a–3c).

16 lung tissue sections, interstitial pneumonia was characterized as mononuclear cell infiltration in interstitial areas (n = 16). In 4 lung tissue sections, pulmonary adenomatosis was seen. The immunohistochemical and microbiological results are shown. At the end of the immunohistochemical stainings, pneumonic pasteurellosis was found in 65 of 110 animals. The immunopositive areas were localized in the bronchi, bronchiole alveolar lumen and epithelial.

4. DISCUSSION

Hemorrhagic septicemia is one of the most important causes of losses in shipping animals and the most affected animal in Sharqia province in Egypt. New research and studies have detected big concerns and important infectious disease issues in sheep and goats [18]–[21].

Most affected villages and regions in south climatic areas and Sharqia province identified *pneumonic pasteurellosis* caused by *Pasteurella multocida* [22]–[25] compared to other studies *Pasteurella*.

Pneumonic pasteurellosis in alveoli and lobules was narcotic, fibrinous and mucolytic with obstructive bronchi that make an explanation for severe symptoms of respiratory illness and loss in many sheep and goat flocks. more cases showed in histopathology more severe lobar pneumonia than lobular with severe infection.

The rapid spread of disease through close animal contact spreads mucous of coughing infected cases and mucous in feeding parts and watering supplies makes the situation of *Pasteurella* get worse. Easy growth and contamination of other respiratory bacteria as suppressed immunity of

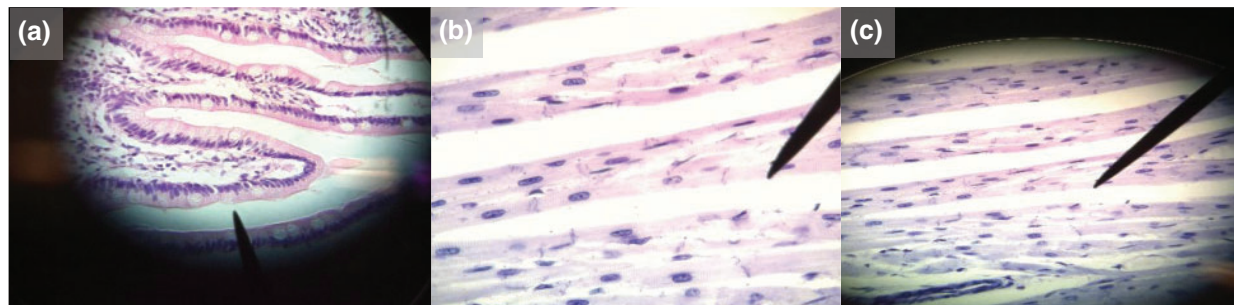


Fig. 4. (a) Infiltration of alveoli, (b) lung lobes and (c) internal connective tissue with squamous cells and fibrous mixed by many leukocytes, and neutrophils.

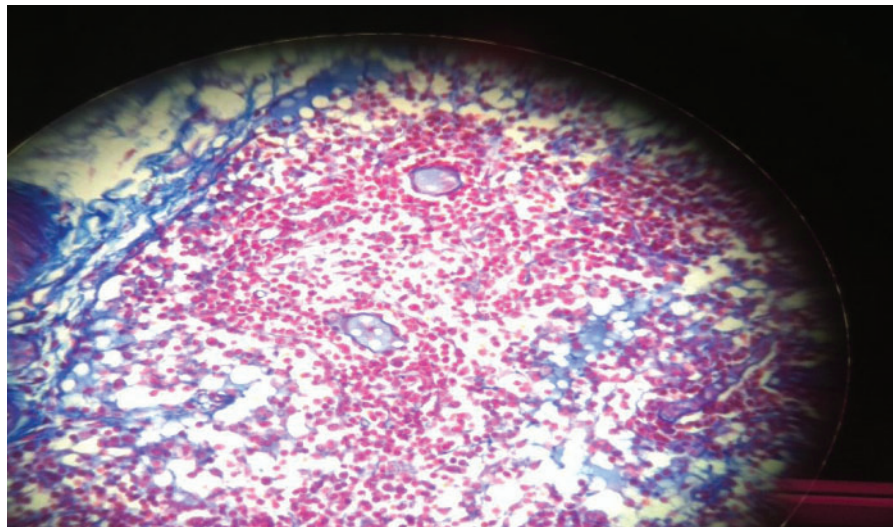


Fig. 5. Necrotic coagulation with exudative catarrhal mucous infiltrated with neutrophilic, mononuclear cells in and around the bronchus and bronchioles.

ill cases and shown mucopurulent bronchopneumonia in the most pathological lesion [26]. The mixed bacterial infection gets conferred using molecular identification to *pastruella multicoda* causes of *pneumonic pasteurellosis* by PCR [27]. Bacteriological isolation with the microscopical examination was completed and confirmed with highly sensitive and more accurate using histochemical for histopathological identification of *pasteurellosis* [28]–[30]. Using hygienic measures and precautions to control and prevent infection is an important way before shipping or moving animals for long distances using specific antibiotics is a good way to standardize protocol.

Sever losses in herd flocks of sheep and goats and deaths fatalities in many cases caused by hemorrhagic septicemia with the most virulent respiratory bacteria called *Pasteurella multocida* will affect animal production and breeding [31]. *Pneumonic pasteurellosis* causes outbreaks in many regions all over the world need together close attention and take all precautions using the most accurate method for diagnosis and control.

Hygienic measures, good animal sanitation during transmission for long distances through vaccination, and good treatment are the best ways to control diseases in our region.

ACKNOWLEDGEMENT

We are thankful to Zagazig University, the Faculty of Veterinary Medicine and the Serum Department of Dohki research polyclonal *Pasteurella multocida* and *p. haemolytica* hyperimmun serum supply.

CONFLICT OF INTEREST

Authors declare that they do not have any conflict of interest.

REFERENCES

- [1] Satparkash S, Vijendra PS, Pawanjit SC, Maninder S, Rajeev R, Santosh KG, *et al.* Immune response to dna vaccine expressing transferrin binding protein a gene of *Pasteurella multocida*. *Braz J Microbiol.* 2011;42(2):750–60. doi: 10.1590/S1517-838220110002000043.
- [2] Rimler RB, Rhoades KR, Bagley RA. Fowl cholera Epornitic: antigenic characterization and virulence of selected *Pasteurella multocida* isolates. *Avian Dis.* 1992;36(1):84–7. doi: 10.2307/1591720.
- [3] Peng Z, Wang X, Zhou R, Chen H, Wilson BA, Wu B. *Pasteurella multocida*: genotypes and genomics. *Microbiol Mol Biol Rev.* 2019;83(4):14–9. doi: 10.1128/mmbr.00014-19.
- [4] Almoheer R, Abd Wahid ME, Zakaria HA, Jonet MAB, Al-shaibani MM, Al-Gheethi A, *et al.* Spatial, temporal, and demographic patterns in the prevalence of hemorrhagic septicemia in 41 countries in 2005-2019: a systematic analysis with special focus on the potential development of a new-generation vaccine. *Vaccines.* 2022;10(2):315–7. doi: 10.3390/vaccines10020315.

- [5] Naz SA, Hanif A, Maqbool S, Ahmed MK. Isolation, characterization and monitoring of antibiotic resistance in *Pasteurella multocida* isolates from buffalo (*Bubalus bubalis*) herds around Lahore. *J Anim Plant Sci.* 2012;22(Sup 3):242–5.
- [6] Townsend KM, Dawkins HJS, Papadimitriou JM. Analysis of haemorrhagic septicaemia-causing isolates of *Pasteurella multocida* by ribotyping and field alternation gel electrophoresis (FAGE). *Vet Microbiol.* 1997;57(4):383–95. doi: 10.1016/S0378-1135(97)00121-1.
- [7] Choi KH, Maheswaran SK, Felice LJ. Characterization of outer membrane protein-enriched extracts from *Pasteurella multocida* isolated from turkeys. *Am J Vet Res.* 1989;50(5):676–83.
- [8] Dabo SM, Taylor JD, Confer AW. *Pasteurella multocida* and bovine respiratory disease. *Anim Health Res Rev.* 2008;8(2):129–50. doi: 10.1017/s1466252307001399.
- [9] Boyce JD, Harper M, Wilkie IW, Adler B, Prescott JF, Songer JG, et al. *Pathogenesis of Bacterial Infections in Animals*. Hoboken, NJ, USA: Blackwell Publishing; 2010, pp. 325–46.
- [10] Merquior VLC, Peralta JM, Facklam RR, Teixeira LM. Analysis of electrophoretic whole-cell protein profiles as a tool for characterization of *Enterococcus* species. *Curr Microbiol.* 1994;28(3):149–53. doi: 10.1007/bf01571056.
- [11] Santos OD, De Resende MCC, De Mello AL, Frazzon A.P. G, D'Azevedo PA. The use of whole-cell protein profile analysis by SDS-PAGE as an accurate tool to identify species and subspecies of coagulase-negative staphylococci. *Acta Pathol, Microbiol et Immunol Scand.* 2011;120(1):39–46. doi: 10.1111/j.1600-0463.2011.02809.x.
- [12] Lee CY, Hong Y, Ryu J, Kim Y-R, Oh S-S, Lee SH, et al. Detection and identification of *Vibrio* species using whole-cell protein pattern analysis. *J Microbiol Biotechnol.* 2012;22(8):1107–12. doi: 10.4014/jmb.1201.01001.
- [13] Dziva F, Christensen H, Olsen JE, Mohan K. Random amplification of polymorphic DNA and phenotypic typing of Zimbabwean isolates of *Pasteurella multocida*. *Vet Microbiol.* 2001;82(4):361–72. doi: 10.1016/s0378-1135(01)00406-0.
- [14] Dziva F, Christensen H, van Leengoed LA, Mohan K, Olsen JE. Differentiation of *Pasteurella multocida* isolates from cases of atrophic rhinitis in pigs from Zimbabwe by RAPD and ribotyping. *Vet Microbiol.* 2004;102(1–2):117–22. doi: 10.1016/j.vetmic.2004.06.006.
- [15] Ozbey G, Kilic A, Ertas HB, Muz A. Random amplified polymorphic DNA (RAPD) analysis of *Pasteurella multocida* and *Manheimia haemolytica* strains isolated from cattle, sheep and goats. *Vet Med.* 2004;49(3):65–9. doi: 10.17221/5678-vetmed.
- [16] Shirzad-Aski H, Tabatabaei M. Molecular characterization of *Pasteurella multocida* isolates obtained from poultry, ruminant, cats and dogs using RAPD and REP-PCR analysis. *Mol Biol Res Commun.* 2016;5(3):123–32.
- [17] Supardiyono EK, Smith D. Microbial diversity: ex situ conservation of Indonesian microorganisms. *World J Microbiol Biotechnol.* 1997;13(3):359–61. doi: 10.1023/a:1018559729803.
- [18] Confer AW. Immunogens of *Pasteurella*. *Vet Microbiol.* 1993;37(3–4):353–68. doi: 10.1016/0378-1135(93)90034-5.
- [19] Pati US, Srivastava SK, Roy SC, More T. Immunogenicity of outer membrane protein of *Pasteurella multocida* in buffalo calves. *Vet Microbiol.* 1996;52(3–4):301–11. doi: 10.1016/s0378-1135(96)00066-1.
- [20] Basagoudanavar SH, Singh DK, Varshney BC. Immunization with outer membrane proteins of *Pasteurella multocida* (6:B) provides protection in mice. *J Vet Med Ser A.* 2006;53(10):524–30. doi: 10.1111/j.1439-0442.2006.00900.x.
- [21] Ataei S, Burchmore R, Christopher Hodgson J, Finucane A, Parton R, Coote JG. Identification of immunogenic proteins associated with protection against haemorrhagic septicaemia after vaccination of calves with a live-attenuated aroA derivative of *Pasteurella multocida* B:2. *Res Vet Sci.* 2009;87(2):207–10. doi: 10.1016/j.rvsc.2009.01.007.
- [22] Odugbo MO, Odama LE, Umoh J, Lamorde AG. *Pasteurella multocida* pneumonic infection in sheep: prevalence, clinical and pathological studies. *Small Rumin Res.* 2006;66(1–3):273–7. doi: 10.1016/j.smallrumres.2005.09.003.
- [23] Mifflin JK, Blackall PJ. Development of a 23S rRNA-based PCR assay for the identification of *Pasteurella multocida*. *Lett Appl Microbiol.* 2001;33(3):216–21. doi: 10.1046/j.1472-765x.2001.00985.x.
- [24] Luo Y, Zeng Q, Glisson JR, Jackwood MW, Cheng IH, Wang N, et al. Sequence analysis of *Pasteurella multocida* major outer membrane protein (ompH) and application of synthetic peptides in vaccination of chickens against homologous strain challenge. *Vaccine.* 1999;17(7–8):821–31. doi: 10.1016/s0264-410x(98)00266-7.
- [25] Taylor JD, Fulton RW, Dabo SM, Lehenbauer TW, Confer AW. Comparison of genotypic and phenotypic characterization methods for *Pasteurella multocida* isolates from fatal cases of bovine respiratory disease. *J Vet Diagn Invest.* 2010;22(3):366–75. doi: 10.1177/104063871002200304.
- [26] Rachmawati FR, Wibawan IW, Mayasari NL. Penggunaan antigen *Mycoplasma gallisepticum* freeze-thawing dalam teknik enzyme-linked immunosorbent assay. *Jurnal Sain Veteriner.* 2019;36(2):151–9. doi: 10.22146/jsv.27021.
- [27] Munir R, Shahwar D, Farooq U, Nawaz I, Shahzad I, Khanum A. Outer membrane protein profiling of *Pasteurella multocida*. *Vet J.* 2007;27(1):1–4.
- [28] Vera Lizarazo Y, Rodríguez Ferri E, Gutierrez Martin C. Evaluation of different API systems for identification of porcine *Pasteurella multocida* isolates. *Res Vet Sci.* 2008;85(3):453–6. doi: 10.1016/j.rvsc.2008.02.002.
- [29] Chitarra CS, Silva MI, Brandão LN, Kagueyama FC, Candido SL, Rosa JMA, et al. Molecular characterization of *Pasteurella multocida* isolates from swine lungs by randomly amplified polymorphic DNA. *Ciência Rural.* 2015;46(1):119–25. doi: 10.1590/0103-8478cr20150153.
- [30] Varga Z, Volokhov DV, Stipkovits L, Thuma A, Sellyei B, Magyar T. Characterization of *Pasteurella multocida* strains isolated from geese. *Vet Microbiol.* 2013;163(1–2):149–56. doi: 10.1016/j.vetmic.2012.12.023.
- [31] Blackall PJ, Pahoff JL, Bowles R. Phenotypic characterization of *Pasteurella multocida* isolates from Australian pigs. *Vet Microbiol.* 1997;57(4):355–60. doi: 10.1016/s0378-1135(97)00111-9.