

MORPHOLOGICAL LUNG LESIONS IN AN OUTBREAK OF *PASTEURELLA MULTOCIDA* IN RABBITS RAISED IN A HOUSEHOLD SYSTEM

LEZIUNI MORFOLOGICE PULMONARE IN FOCARE DE *PASTEURELLA MULTOCIDA* LA IEPURI CRESCUȚI IN SISTEM GOSPODARESC

Marioara NICULA NEAGU¹⁾, I. PET¹⁾, T. IANCU²⁾,
Ionica IANCU^{3),*}, Iasmina LUCA³⁾,
V. IORGONI³⁾, A. GLIGOR³⁾,
V. HERMAN³⁾

ABSTRACT | REZUMAT

Pasteurella multocida is a conditionally pathogenic bacterium that is part of the normal microflora of the oral cavity, nasopharynx, and upper respiratory tract of birds, mammals, and other species. The investigations were carried out on 11 females, 6 males, and 41 young rabbits of different ages and breeds, coming from two locations in the counties of Arad and Timiș. Lung and long bone samples were taken from the rabbit carcasses from the two locations, and bacteriological and histopathological examinations were performed on these samples. *Pasteurella multocida* was the main etiological agent of respiratory infections in rabbits from the disease outbreaks studied. 11 strains of *Pasteurella multocida* were isolated, which were sensitive to penicillin (81.8%), ciprofloxacin and florfenicol (63.6%), gentamicin and trimethoprim/sulfamethoxazole (54.5%), and enrofloxacin (45.5%). The highest resistance of *Pasteurella multocida* strains was found to doxycycline, tetracycline and tylosin (72.7%), and amoxicillin (63.6%), antibiotics commonly used in the treatment of rabbits.

Keywords: *Pasteurella multocida*, rabbit, lesions

Pasteurella multocida este o bacterie condiționat patogenă, care face parte din microflora normală a cavității orale, nazofaringe și tractul respirator superior la păsări, mamifere și alte specii. Investigațiile au fost efectuate pe 11 femele, 6 masculi și 41 de pui de iepuri de vârste și rase diferite, provenind din două locații din județele Arad și Timiș. De la cadavrele de iepuri, din cele două locații au fost prelevate probe de pulmoni și os lung, din aceste probe au fost efectuate examene bacteriologice și histopatologice. *Pasteurella multocida* a fost principalul agent etiologic al infecțiilor respiratorii la iepurii din focarele de boală luate în studiu. Au fost izolate 11 tulpini de *Pasteurella multocida* care au fost sensibile la penicilină (81,8%), ciprofloxacină și florfenicol (63,6%), gentamicină și trimetoprim/sulfametoxazolă (54,5%) și enrofloxacină (45,5%). Rezistența cea mai mare a tulpinilor de *Pasteurella multocida* a fost găsită la doxicilină, tetracicline și tylosin (72,7%), respectiv amoxicilină (63,6%), antibiotice utilizate în mod obișnuit în tratamentul iepurilor.

Cuvinte cheie: *Pasteurella multocida*, iepuri, leziuni

Respiratory infections in rabbits cause considerable economic losses in large production facilities worldwide (21), consisting of mortalities, costs of prophylaxis, and their control. The main bacterial agent incriminated is *Pasteurella multocida*, which produces not only respiratory infections but also subcutaneous pyogenic infections and is localized in various organs and is one of the main causes of morbidity in rabbits (7, 9, 16). *Pasteurella multocida* is a conditionally pathogenic bacterium, that is part of the normal microflora of the oral cavity, nasopharynx, and upper respiratory

tract of birds, mammals, and other species (1, 2, 4, 18). *Pasteurella multocida* produces infections with clinical manifestations in farm species: atrophic rhinitis in pigs, cholera in birds, and haemorrhagic septicaemia in buffaloes and cattle (10, 11, 12, 15, 17, 20).

In rabbits, infection with *Pasteurella multocida* can also occur subclinically, infected rabbits do not show symptoms as long as the immune system is working effectively, or until the installation of various forms of stress (5). Following the colonization of the nasal cavity, the infection can spread to the sinuses and facial bones, via the Eustachian tube to the ears, through the nasolacrimal duct to the eyes, through the trachea to the lower respiratory tract, and through the blood flow to the joints, bones, and other organ systems. The damage to the nasal cavity can be permanent (atrophic rhinitis), affecting local defence mechanisms and allowing re-infection with *Pasteurella* or other bacteria (3, 6, 13).

1) University of Life Sciences "King Mihai I",
Faculty of Bioengineering of Animal Resources, Timisoara, Romania

2) University of Life Sciences "King Mihai I",
Faculty of Rural Management and Tourism, Timisoara, Romania

3) University of Life Sciences "King Mihai I",
Faculty of Veterinary Medicine, Timisoara, Romania

*) Corresponding author: ionicavet@gmail.com

MATERIALS AND METHODS

The investigations were carried out on a number 20 corpses of rabbits (females and males) of different ages and breeds, coming from 2 locations in the counties of Arad and Timiș. The population from Arad County comprised 4 females, 2 males, and 20 cubs, and that from Timiș county of 7 females, 4 males, and 21 cubs.

Lung and long bone samples were taken from rabbit carcasses from the two locations, and bacteriological and histopathological examinations were performed on these samples.

The bacteriological examination consisted of a bacterioscopic examination, isolation, and typing of the germ, and the performance of the antibiogram.

The media used for the isolation of germs from bone and lung samples were: nutrient broth, heart-brain infusion (BHI) broth, and 5-10% blood or serum agar. The samples were incubated for 24 hours at 37°C, after which they were passaged on a selective medium, depending on the suspected pathogen.

After isolation in pure culture, the identification of bacterial species, and the typing of the etiological agent, were carried out.

Identification of the isolated bacteria was carried out on the basis of morphological characteristics, cultural characters, and biochemical properties (25).

To identify the cultures, the morphological character of the colonies was examined regarding the shape, colour, degree of development and spread on the surface of the medium, the appearance of the colonies, the type of haemolysis, and the size of the haemolysis area. After being obtained in pure culture, the isolated *Pasteurella* strains were characterized based on biochemical properties and sensitivity to antibiotics.

The definitive identification of *Pasteurella* strains was made with the help of diagnostic kits: API 20 NE for the identification of pathogens from the *Enterobacteriaceae* family (27).

Determination of antibiotic resistance of Pasteurella strains was performed by the disc diffusimetric method (Kirby - Bauer method), using the Muller - Hinton medium and biodiscs impregnated with antibiotics, provided by the producing companies, respectively 11 antimicrobial substances: amoxicillin with clavulanic acid (20/10 µg), ciprofloxacin (30 µg), doxycycline (10 µg), enrofloxacin (5 µg), florfenicol (10 µg), gentamicin (10 µg), neomycin (10 µg), trimethoprim/sulfamethoxazole (30 µg), tetracycline (30mg) penicillin (10 µg) and tylosin (30mg).

These were placed on the surface of the inoculated plates using an antimicrobial disc dispenser (Oxoid) and incubated for 24 hours at 37°C.

The interpretation of the results was made according to the document - European Committee on Antimicrobial Susceptibility Testing - EUCAST (26).

In order to thoroughly examine a histological structure, permanent microscopic (histological) preparation was obtained. The staining method used was the HEA method: haematoxylin-eosin-methylene blue.

RESULTS AND DISCUSSION

The necropsy examination performed on the dead rabbits, from the two locations, revealed lesions characteristic of pasteurellosis - the evolutionary peracute and chronic forms, the diagnosis being confirmed by bacteriological examination. In the peracute forms, at necropsy, the lungs showed severe inflammation and haemorrhagic pneumonia.

Accumulation of serosanguineous exudate was found in the chest cavity, in both hyperacute and chronic forms (Fig. 1 and 2).

In chronic forms, the observed pathological changes consisted of suppurative pleuropneumonia, the lungs being covered with a film of fibrin (Fig. 3).

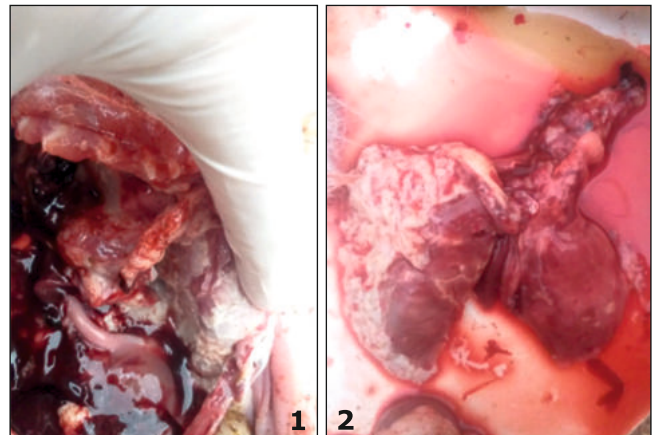


Fig. 1. Serosanguineous exudate in rabbits with pasteurellosis - the peracute form

Fig. 2. Serosanguineous exudate and fibrin on the lung - the chronic form



Fig. 3. Fibrin film on the lung in a rabbit with pasteurellosis - the chronic form

Some specimens showed lung abscesses (Fig. 4), and in the hyperacute form, it prevailed severe inflammation - haemorrhagic pneumonia. The liver of some of the dead specimens of pasteurellosis - the chronic form - was hypertrophied and congested (Fig. 5.) presenting steatosis, and in the case of the peracute form, congestion of the intestinal mass was also observed.

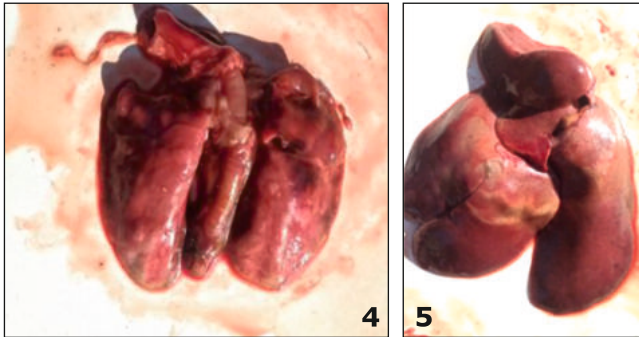


Fig. 4. Pulmonary congestion and abscesses - a chronic form

Fig. 5. Liver - hypertrophy and steatosis

In specialized literature were reported, in addition to lung lesions and pathological changes of the organs in the abdominal cavity, similar to those found by us, congested liver and spleen and petechial haemorrhages on the surface of the gastric mucosa (23).

Histopathological examination of the sections made from the lung tissue revealed abundant fibrin deposits at the pleura and massive interstitial leukocyte infiltration. They also appeared in the microscopic field of the rod-shaped bacterial cells, surrounded by neutrophils and macrophages, and at the level of the bronchioles-necrotic cells (Fig. 6 and Fig. 7).



Fig. 6. Abundant deposits of fibrin on the pleura and massive interstitial leukocyte infiltration (H&E 20x)

Histological changes were similar to those we found - oedema, congestion, necrosis and multifocal to diffuse haemorrhages, inflammatory cellular infiltrates,

and necrotic processes at the level of the bronchioles were reported by Vigneshwar et al., 2020 and Uenoyama et al., 2020 (22, 23).

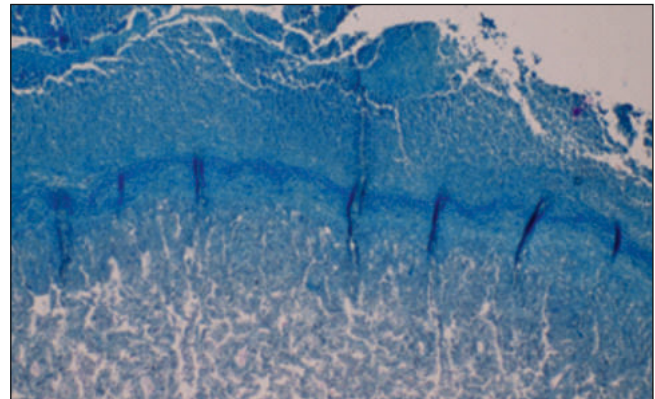


Fig. 7. Massive interstitial leukocyte infiltration (H&E 20x)

Following the bacteriological examination, the etiological agent was isolated, *Pasteurella multocida*, it having the characteristic morphological and biochemical characters. Large, mucoid, nonhemolytic, gray to gray-white colonies were identified on blood agar plates after 48 h of incubation. But the definitive identification was made with the help of the API 20 NE kits.

The analysis of antibiotic resistance highlighted the fact that the tested *Pasteurella* strains had a different behaviour towards antibiotics. *Pasteurella multocida* strains were sensitive to the following antibiotics: amoxicillin (AML), ciprofloxacin (CIP), doxycycline (DO), enrofloxacin (ENR), florfenicol (FFC), gentamicin (CN), neomycin (N), trimethoprim/sulfamethoxazole (SXT), tetracycline (TE) and penicillin (P). Instead, they showed resistance to tylosin (Ty) (Fig. 8).



Fig. 8. Multiple antibiotic susceptibilities of *Pasteurella multocida* strains

According to the synopsis of antibiotic sensitivity (Table 1), the 11 strains of *Pasteurella multocida* were sensitive, as follows: penicillin (81.8%), florfenicol

Table 1
Antibiotic susceptibility synopsis for 11 strains of *Pasteurella multocida*

Antibiotics	Sensitive (S)		Intermediate (I)		Resistant (R)	
	No stranger	(%)	No stranger	(%)	No stranger	(%)
Amoxicillin	2	18.2	2	18.2	7	63.6
Ciprofloxacin	7	63.6	3	27.3	1	9.1
Doxycycline	2	18.2	1	9.1	8	72.7
Enrofloxacin	5	45.5	4	36.4	2	18.2
Florfenicol	7	63.6	3	27.3	1	9.1
Gentamicin	6	54.5	4	36.4	1	9.1
Neomycin	1	9.1	5	45.5	5	45.5
Penicillin	9	81.8	1	9.1	1	9.1
Tetracyclines	1	9.1	2	18.2	8	72.7
Trimethoprim/sulfamethoxazole	6	54.5	3	27.3	2	18.2
Tylosin	1	9.1	2	18.2	8	72.7

(63.6%), ciprofloxacin (63.6%), gentamicin (54.5%) trimethoprim/sulfamethoxazole (54.5%) and enrofloxacin (45.5%). The highest resistance of *Pasteurella multocida* strains was found to doxycycline, tetracycline, and tylosin (72.7%), amoxicillin (63.6%), antibiotics commonly used for treating rabbits (Fig. 9).

Values very close to those recorded in this study

were published by Iorgoni et al. (2022) for 13 strains of *Pasteurella multocida* isolated from rabbit carcasses or rabbits slaughtered of necessity who were raised in the extensive system (14).

In another study conducted in Brazil, 47.8% (22/46) of the rabbit *Pasteurella multocida* strains were resistant to at least one of the drugs tested (8).

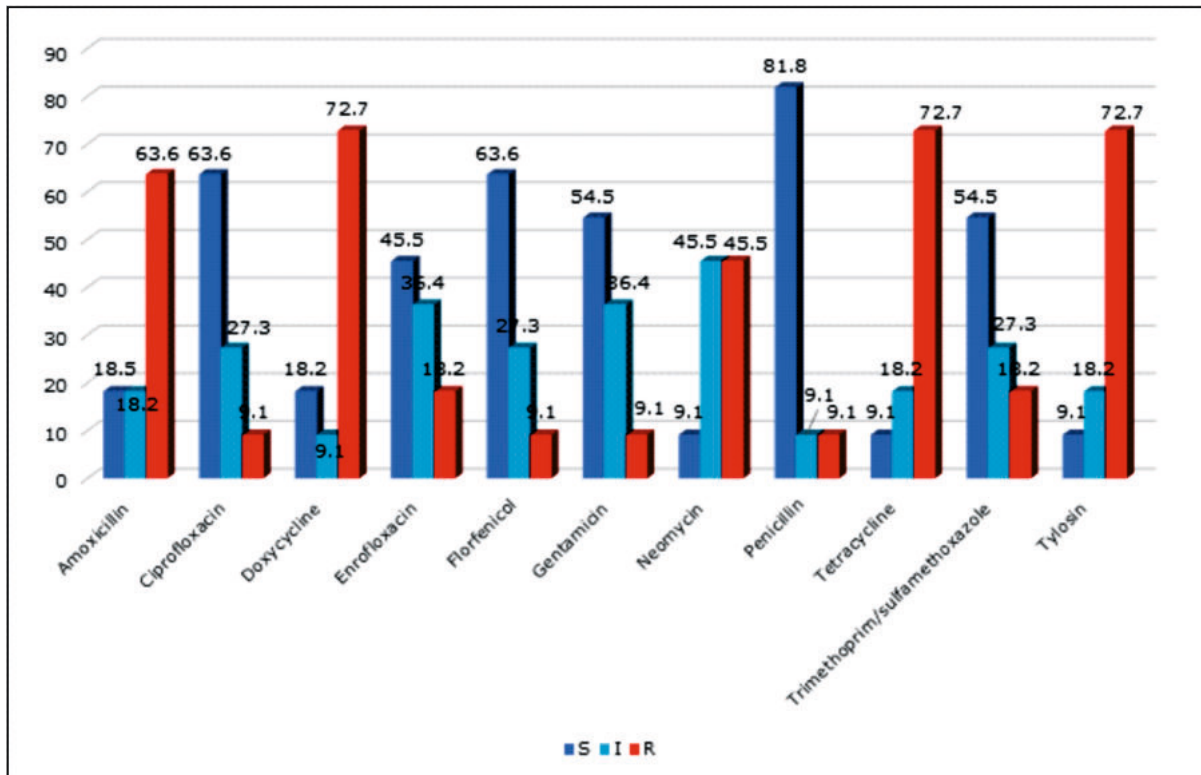


Fig. 9. Antibiotic susceptibility dynamics of *Pasteurella multocida* strains

Contrary to the results obtained in the present research, the specialized literature mentions a predominant resistance to erythromycin and oxytetracycline (90%), followed by kanamycin and ceftriaxone (80%) of *P. multocida* strains isolated both from rabbits and from other species of animals. Instead, they were sensitive to trimethoprim /sulfamethoxazole, gentamicin, amoxicillin, amikacin, ampicillin, and chloramphenicol (19, 24).

CONCLUSIONS

Pasteurellosis was diagnosed in rabbits of different ages and breeds in both studied locations. *Pasteurella multocida* was the main etiological agent of respiratory infections in rabbits from the studied pasteurellosis outbreaks. The 11 strains of *Pasteurella multocida* were susceptible to penicillin (81.8%) ciprofloxacin and florfenicol (63.6%), gentamicin, and trimethoprim /sulfamethoxazole (54.5%), and enrofloxacin (45.5%). The highest resistance of *Pasteurella multocida* strains was found to doxycycline, tetracycline and tylosin (72.7%), and amoxicillin (63.6%), antibiotics commonly used in the treatment of rabbits.

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