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# Data on the evolution of the resistance phenomenon to anti-infectives in 16 pig breeding units from Banat

# Date despre evoluția fenomenului rezistenței la antiinfecțioase în 16 unitati de cresterea porcului din Banat

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**Cuvinte cheie:** *rezistență, antiinfecțioase, suine, afecțiuni digestive, afecțiuni respiratorii, Banat*

**Abstract**

**Respiratory and digestive diseases of bacterial origin in pigs can cause severe symptoms with major economic impact on farms. Out of all the pathogens, *E. coli* represents an important factor in the occurrence of enteric syndrome, producing significant material damage by decreasing productivity, associated with increased production costs associated with low profitability indices. In this sense, the present study proposes highlighting the main epidemiological aspects and etiopathogenic in pigs around the weaning age from 16 pig breeding units located in the western part of Romania. The objectives of the study were: the identification of specific anatomical-pathological lesions, bacteriological examination, and biochemical identification of pathogenic germs using the API 20E method. From the biological material taken from the pigs, 139 samples were collected from the lungs, portions of the liver, small intestine, spleen and mesenteric lymph nodes, which were later microbiologically analyzed. In descending order, *E. coli* was identified in most samples, followed by *Streptococcus suis* and *β-haemolytic E.coli* equally. With a much lower incidence, the microbiological examination showed Salmonella spp and Pasteurella multocida relevant, and pathogens from the genus *Klebsiella spp, Serratia marcescens, Morganella morganii, Actinobacillus pleuropneumoniae* and *Mannhemia haemolytica* had a sporadic incidence. In conclusion, the diseases identified in the 16 farms in Banat studied were predominantly digestive and were mostly caused by *E. coli*, which had high incidence rates, especially in the pig youth categories. The most common anatomic-pathological changes identified were: abdominal distension, loops of the small intestine full of gas, the contents of the colon having a pasty-watery consistency with much thinned walls, proliferative ileitis, enlarged mesenteric lymph nodes and a distended stomach with a gelatinous content.**

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**Rezumat**

**Afecțiunile respiratorii și digestive de origine bacteriană la specia suine pot determina apariția unor simptome severe cu impact major economic asupra fermelor. Din totalitatea agenților patogeni, *E. coli,* reprezintă un factor important în apariția sindromului enteric, producând pagube materiale însemnate prin scăderea productivității, asociate cu creșterea costurilor de producție asociați cu indici scăzuți ai profitabilității În acest sens, prezentul studiu propune evidențierea principalelor aspecte epidemiologice și etiopatogenice la suinele aflate în jurul vârstei de înțărcare din 16 unități de creștere a porcului situate în zona de vest a României. Obiectivele studiului au fost: evidențierea leziunilor anatomo-patologice specific, examinare bacteriologică, identificarea biochimică a germenilor patogeni pin metoda API 20E. Din materialul biologic prelevat de la suine s-au recoltat 139 de probe din pulmoni, porțiuni ficat, intestin subțire, splină și limfonoduri mezenterice, care ulterior au fost analizate microbiologic. În ordine descrescătoare, *E. coli* a fost identificat în cele mai multe probe, urmat de *Streptococcus suis* și *E.coli β-hemolitic* în egală măsură. Cu o incidență mult mai mică examenul microbiologic a relevant *Salmonella spp* și *Pasteurella multocida,* iar ca agenți patogeni din genul *Klebsiella spp, Serratia marcescens, Morganella morganii, Actinobacillus pleuropneumoniae și Mannhemia hoemolytica* au avut o incidență sporadică. În concluzie, afecțiunile identificate în cele 16 fermele din Banat luate în studiu au fost preponderant digestive și au fost produse, în mare parte de *E. coli,* care a avut rate crescute ale incidenței, în special la categoriile de tineret suin. Modificările anatomo-patologice cele mai frecvente identificate au fost: distensia abdominală, ansele intestinului subțire pline cu gaze, conținutul colonului având o consistență păstos-**

**apoasă cu pereții mult subțiați, ileita proliferativă, limfonodurile mezenterice mărite în volum și stomacul destins cu un conținut gelatinos.**

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## Introduction

Bacterial respiratory and digestive diseases in pigs can cause severe symptoms with a major impact on average daily growth rate, influence meat quality, alter profitability indicators, and decrease farm efficiency (2, 5, 9, 11, 13, 18).

In the current context, special attention should be paid to the young after weaning, due to factors that affect individuals by overstraining the body to new feeding conditions, changing gut flora, and decreasing immune status (3, 6, 25, 26, 28).

The extent of diarrheal and respiratory disease on farms has attracted the attention of many researchers, who argue that these conditions are caused by pathogenic bacteria with multiple antibiotic resistance. This can lead to functional imbalances through the development of pathological processes, an example of which is E. coli strains in the small intestine of piglets (weaning diarrhea) (4, 7, 8, 10, 12, 14).

The present research aimed to highlight some epidemiological and etiological aspects of pigs around weaning age in pig farms located in the western part of Romania. Of all the pathogens, *E. coli* is an important factor in the occurrence of enteric syndrome, causing significant material damage through decreased productivity, associated with increased production costs and low profitability index (6).

## Objectives

- Highlighting of specific pathological lesions
- Bacteriological examination
- Biochemical identification of pathogens by API 20E method

## 2. Materials and methods

Western Romania is a well-known area with a strong development in the agro-food industry, thanks to its favorable relief and geo-strategic position with Western Europe (figure 1) and of course, the rail and road interconnection, facilitating intra-community trade. Thus, investments in the livestock system, particularly in pig breeding and breeding, have shown that farmers focus on the quality of products obtained according to current standards, so improving breeds and obtaining a good yield at slaughter, thus ensuring a high percentage of meat to the weight of the animal, the quality of feed and the modernization of farms are the keys to the success of such establishments.

The farms are set up as an intensive all-in-all-out system and are structured by age and weight categories. The farms are constructed of concrete, and covered with insulated sheet metal panels. The floor of the halls is made of a reinforced concrete frame which serves as a support for the plastic gratings on which the animals sit. The sewage system is organized on a water cushion with channels under the grating, and the manure is discharged by continuous dumping into the manure storage tanks (figure 1).

Feeding of the animals is carried out automatically using plastic feeders on metal or concrete structures, and watering is carried out through the own network equipped with pressure soothers, combined water and feed tanks are located at the entrance to the shelters. Feeding is carried out according to the age of the animals and the breeding technology adopted.

The study was carried out between October 2022 and September 2023 and monitored 16 pig breeding and fattening establishments located in the western part of Romania. In these farms, pigs showing clinical changes were examined and special attention was paid to cases of coli bacillary enteritis and respiratory diseases. These diseases are frequently encountered because they are

rapidly and easily transmitted from one animal to another. They are often caused by different types of pathogenic bacteria which, without a well-optimized and timely treatment, can lead to a reduction in herd size due to high mortality and therefore significant economic losses for farmers.

**Figure 1**. Organization of pig farms (original Doma)

Naturally, maintaining biosecurity, animal health and hygiene and dietetic conditions ensures a high organoleptic quality of pork production. (1, 17, 23, 26).

In this study, we followed the health status of pigs and analyzed macroscopically the anatomic-pathological changes caused by bacterial diseases in pigs in the monitored farms. Subsequently, samples were taken for microbiological laboratory examination to identify pathogens (figure 2).

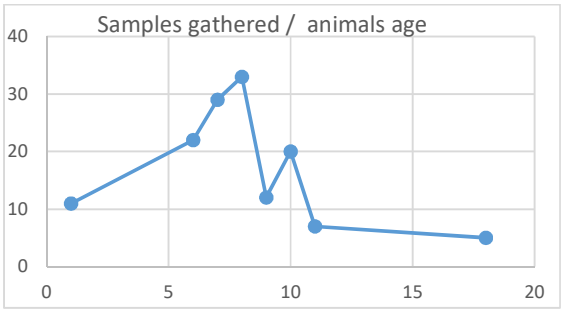


**Figure 2.** Number and age of animals examined

**Figure 3.** Bowel and liver sampling (original Doma)

From the biological material taken from the pigs, 139 samples were collected from the lungs, portions of the liver, small intestine, spleen, and mesenteric lymph nodes, which were then placed in broth, and from there, using the Drigalsky loop, transferred to the usual and selective media: nutrient agar, broth, 5% blood agar, McConkey, and Levin medium and poured into Petri dishes (Figure 3).

Samples from the intestines were collected on the farm at the time of necropsy examination using test tubes provided with collection loops and nutrient medium for bacterial culture, and from the portions of the organs, which required more complex sampling procedures, were transported to the infectious disease laboratory under optimal temperature conditions as required by law for collection.

All samples collected from pigs showing clinical manifestations and morph pathological changes were subjected to microbiological

testing and for the growth of microorganisms it was necessary to thermostat the samples for 24 hours at 37°C (Figure 4).

**Figure 4.** Sample preparation for seeding on selective media and incubation of samples at 37°C (original Doma)

Pathogens identification was performed using the API 20E test, this system consists of two components made of plastic:

1. A plastic tray, which ensures a moist environment during the test;
2. A rack on which there are 20 separate compartments, each compartment being represented by a well and a tube containing a specific dehydrated nutrient medium.

Depending on the presence or absence of the pathogen under study, colorimetric changes may be observed by adding additional reagents to the 20 compartments (Figure 5).

The system allows 23 different biochemical tests to be performed simultaneously (15, 16, 20).

**Figure 5.** API 20E kits (Biomerieux) used in our testing (original Doma)

Compared to traditional methods, the use of multi-test identification systems has some advantages:

- obtaining results in a short time, in some cases within 5 hours;
- uniformity of results;
- accuracy/safety of results;
- simplicity of working techniques;
- minimal consumption of materials and culture media (15, 16, 20).

The final evaluation of the test involves recording all positive (+) or negative (-) results, and species identification is done by consulting the Index provided by the manufacturing company (15, 16, 20).

The working technique of the API 20E multi-test system is shown in Table 1.

**Table 1. Working technique of the API 20E multi-test system**

| Stage I - Inoculation | Stage II - Test evaluation |
|---|---|
| Isolation in pure culture of the test microorganism; | After the thermosetting period of 18-24 hours, the result in the GLU compartment is negative, the tested microorganism does not belong to the family *Enterobacteriaceae*, an additional incubation period is necessary for its identification; |

| | |
|---|---|
| The membership of the tested microorganism in the Gram-negative and oxidase-negative category is determined using the Gram stain and oxidase test | The results obtained are not relevant if less than three positive results appear on the test plate and the GLU compartment is negative; |
| The API test is noted with the number of the microorganism to be tested and the name of the worker performing the identification; | The test will continue if the GLU is yellow (positive) and the minimum number of positive reactions on the plate is exceeded. |
| To prevent contamination of test samples with germs from the environment (air or work furniture), aseptic handling conditions should be observed; | Add the following reagents: |
| The microbial colony selected to continue the procedure is transferred to a 0.85% NaCl solution (saline). | Add a drop of 10% ferric chloride in the TDA tube to the positive (red-brown) or negative (yellow) reaction; |
| Using a Vortex, the inoculated saline is thoroughly homogenized. | A drop of Barritt solution A or B is added to the VP tube and after 10 minutes the positive (dark pink or red) or negative (colorless) result is evaluated; |
| With a pipette add 5 ml of distilled water to the API system tray; | A drop of Kovacs reagent is added to the IND tube and after two minutes the result is read: positive (red ring) or negative (yellow to tan ring); |
| If gas bubbles appear in the GLU tube, indicating the formation of gaseous N2 and reducing | If gas bubbles appear in the GLU tube, indicating formation of gaseous N2 and reduction of NO2-(nitrite) |
| Using a sterile Pasteur pipette, dispense the inoculated NaCl solution into the 20 compartments of the API gallery; | Add two drops of nitrite reagent to the GLU compartment, wait 2-3 minutes, then read the result: positive reaction, confirming nitrate reduction (red). |
| The ADH, LDC, ODC, H2S and URE compartments will be partially filled; | To confirm the negative result of the test add zinc powder, wait 10 minutes and then examine the sample: positive reaction - reduction of nitrite and appearance of pink-orange color, negative reaction - presence of N2 and appearance of yellow color; |
| The CIT, VP and GEL compartments will be completely filled; | A drop of hydrogen peroxide (hydrogen peroxide) is added to the MAN, INO and SOR compartments and the result is read after 2 minutes: positive reaction - bubbles appear or negative reaction. |
| With another Pasteur pipette add sterile mineral oil to the liquid surface of the ADH, LDC, ODC, H2S and URE compartments to ensure anaerobic conditions, then cover the API 20E with a plastic lid and incubate at 37°C for 18-24 hours. | |

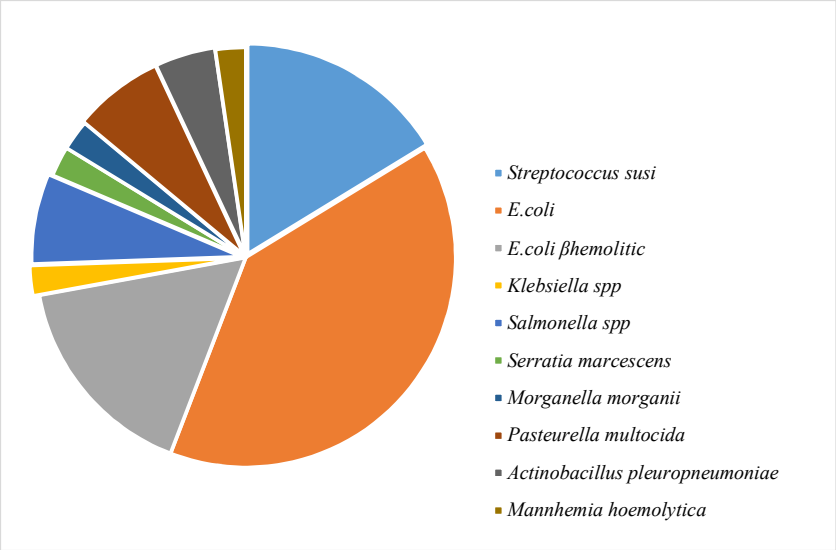


**Figure 6.** Incidence of pathogens on farms

After interpretation of the results, positive samples were subjected to disc-diffusimetric testing to determine the efficacy of antimicrobials and verification of resistance status according to the Clinical and Laboratory Standards Institute 2009 (CLSI-2009) standard (21, 28, 29).

Figure 6 shows the most common diseases in pigs in the units taken in our study, listed in descending order: *E. coli* was identified in most samples, followed by *Streptococcus suis* and β-hemolytic *E. coli* equally for both types of microorganism, with a much lesser consequence compared to pedes we can mention *Salmonella spp* and *Pasteurella multocida*, and the other cases of bacterial diseases caused by pathogens of the genus *Klebsiella spp, Serratia marcescens, Morganella morganii, Actinobacillus pleuropneumoniae* and *Mannhemia hoemolytica* had a sporadic incidence.

From the total number of samples examined, only the 44 positive samples (Figure 7) that were tested by the Kirby-Bauer disc-diffusimetric method, whose operating principle is to determine the sensitivity or resistance of a bacterial culture to an antibiotic, were considered. This method is performed in Petri dishes with solid media, where the pure bacterial culture is seeded (Figure 7).

On the surface of the Petri dishes in which the culture medium has been poured and seeded with the prepared test pathogen, micro compresses with antimicrobials of different concentrations are applied so that a diffusion well based on the local concentration gradient of the antibiotic is formed in close proximity to the disc.

The size of the halo formed around the pellet is directly proportional to the sensitivity of the pathogen (Figure 7).

Although the minimum inhibitory concentration (MIC) cannot be determined by this method, it provides relevant information on the sensitivity of the tested species to the antimicrobial substances used (22, 29).

After the formation of the well around the tablet with the active substance, its diagonal is measured using a caliper or ruler, and the result is compared with the CLSI / 2009 (Clinical Laboratory Standard Institute USA, 2009) interpretative table (19, 21, 28).

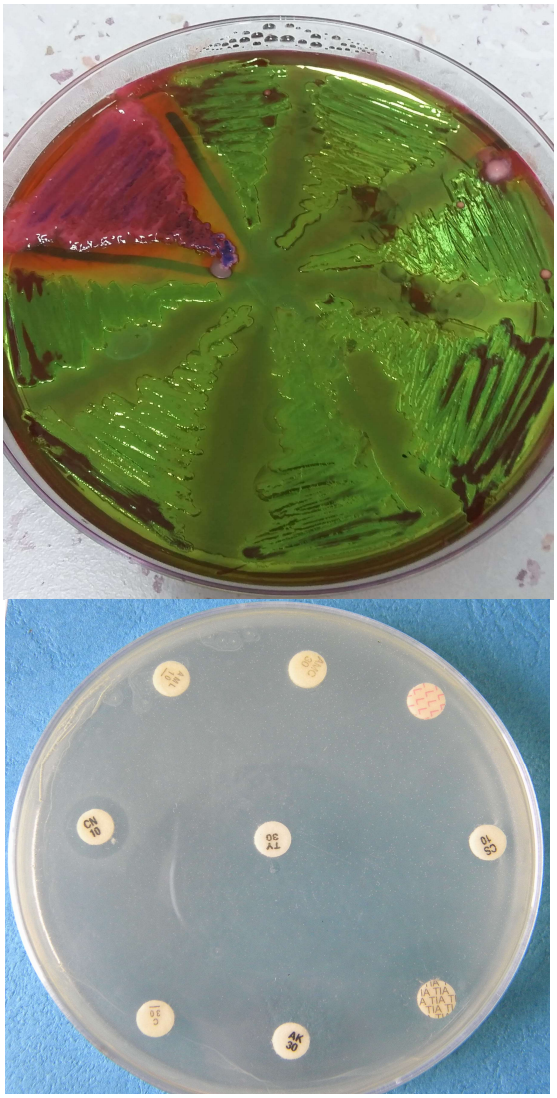

**Figure 7.** Bacterial isolation and bacterial susceptibility testing (original Doma)

Antibiotics are classified according to their action, which is based on their bacterial inhibitory capacity (Table 2).

**Table 2. Antibiotics used**

| Antibiotics used | |
|---|---|
| Amoxicillin | Flunequine |
| Ampicillin | Gentamicin |
| Ceftiofur | Lincomycin |
| Ciprofloxacin | Neomycin |
| Cobactan | Oxytetracycline |
| Colistin sulfate | Penicillin |
| Doxycycline | Spectinomycin |
| Enrofloxacin | Potentiated sulfamides |
| Erythromycin | Tetracycline |
| Florfenicol | Tilozine |

The diameter of the zone of inhibition of each active substance micro-tablet was used to compare the CLSI 2009 recommended breakpoints, thus they can be classified as susceptible, intermediate, or resistant (19, 21, 29).

The results were divided into three categories as follows:

- sensitive (susceptible-S),
- moderately susceptible (intermediate-MS),
- resistant (R),

## 2. Results and discussions

Diagnosis of the pigs was based on clinical signs revealed by the veterinary staff of the units, in conjunction with pathological evaluation of the lesions and laboratory examinations through the identification of pathogens carried out within the disciplines of necropsy and infectious diseases at the Faculty of Veterinary Medicine in Timisoara.

Following the evaluation of the clinical status and anatomo-pathological changes (Table 3) of the pigs from the farms taken in our study, the presence of common lesions of hemorrhagic type in foci located in the small intestine, mainly in the anterior third, along the whole duodenum and a small portion of the jejunum was found, hemorrhagic enteritis, hemorrhagic colitis, enlarged and hemorrhagic mesenteric lymph nodes, hepatic congestion, exudate in the pericardial cavity, renal congestion, pulmonary congestion, the mosaic appearance of lungs, well-defined interlobular edema, distended stomach filled with undigested feed, blackish portions of the gastric mucosa.

In addition to hemorrhagic chromatic changes, intestinal distention by gas accumulation was observed.

**Table 3. Pathological changes**

| Farm | Farm type | Pathological changes reported |
|---|---|---|
| 1 | F1 | Abdominal distention, gas-filled small bowel loops, colon contents with a slimy consistency, much-thinned walls, proliferative ileitis, diffuse hemorrhagic bronchopneumonia, enlarged mesenteric lymph nodes, liver congestion and hyperplasia, distended stomach with gelatinous-mucus contents. |
| 2 | F1 | Hemorrhagic enteritis, hemorrhagic colitis, enlarged and hemorrhagic mesenteric lymph nodes, hepatic congestion, exudate in the pericardial cavity, renal congestion, pulmonary congestion, mosaic appearance of lungs, well evident interlobular edema, distended stomach filled with undigested feed, blackish portions of gastric mucosa. |
| 3 | F1 | Per hepatitis, hepatic and splenic hyperplasia, hepatic congestion, gall bladder greatly enlarged in volume, hemorrhagic bronchopneumonia, with mosaic appearance, presence of edema, stomach greatly distended with a yellowish content and few undigested feeds remains, localized proliferative ileitis, gas-filled jejuna loops, hemorrhagic enteritis, mesenteric lymph nodes enlarged in volume and congested. |
| 4 | F1 | Cyanosis of the rostrum, abdominal distention, fibrinous poly-serositis in the abdominal cavity, (liver, spleen, intestinal loops), extensive hemorrhagic enteritis, hemorrhagic colitis, abundant serosanguinous exudate in the abdominal cavity, bronchopneumonia in various phases with localization on the apical and cardiac lobes, enlarged heart with sub-epicardia spots and suffusions, hepatic dystrophy, peritonitis. |
| 5 | F1 | Anemic corpses, sub-epicardia petechial, and suffusions, hepatic hyperplasia, and congestion, distended stomach filled with undigested coagulated milk, catarrhal hemorrhagic enteritis, gelatinous edema of the meso-colon, gas-filled small bowel contents, yellow in color, liquid in consistency, mesenteric lymph nodes congested and enlarged in volume. |
| 6 | F1 | Caecal corpses, anemia, posterior train soiled with yellowish feces, sub-epicardia pets, and suffusions, stomach full of undigested coagulated milk, catarrhal enteritis, intestinal contents with a liquid, yellowish consistency, mesenteric lymph nodes enlarged in volume, mild splenic hyperplasia, hepatic dystrophy, discrete edema of the spiral colon. |
| 7 | F2 | Cyanosis of the rumen, abdominal distention, watery stomach with very little feed, hemorrhagic gastritis, yellowish diarrhea, hemorrhagic enteritis, proliferative hemorrhagic ileitis in small portions, and hemorrhagic meningitis. |
| 8 | F2 | Abdominal distention, gas-filled small bowel loops, colonic contents with a slimy-sticky consistency, much-thinned walls, proliferative ileitis, diffuse hemorrhagic |

| | | |
|---|---|---|
| | | bronchopneumonia, enlarged mesenteric lymph nodes, hepatic congestion and hyperplasia, distended stomach with gelatinous-mucus contents. |
| **9** | **F2** | Cyanosis of the rostrum, abdominal distention, watery stomach with little feed, hemorrhagic gastritis, yellowish diarrhea, hemorrhagic enteritis, proliferative hemorrhagic ileitis in small portions, hemorrhagic meningitis. |
| **10** | **F1** | Enlarged and congested mesenteric lymph nodes, gelatinous edema of the mesentery of the spiral colon, edema of the people, in the abdominal cavity presence of a discrete fibrin network, stomach full of feed, regional ileitis, the marked proliferation of the ileum mucosa with the reduction in size of the intestinal lumen, serous arthritis. |
| **11** | **F1** | Gelatinous edema of the mesocolon, gas-filled small bowel contents, yellow in color, liquid in consistency, mesenteric lymph nodes congested and enlarged in volume, abundant serosanguinous exudate in the abdominal cavity, bronchopneumonia and cardiac, enlarged heart with subepicardial petechiae and suffusions, hepatic dystrophy, peritonitis |
| **12** | **F2** | Stomach enlarged in volume, blackish gastric mucosa in certain portions, hemorrhagic enteritis, hepatic congestion, exudate in the pericardial cavity, renal congestion, pulmonary congestion. |
| **13** | **F2** | Stomach full of undigested clotted milk, gas-filled small bowel contents, catarrhal-hemorrhagic enteritis, gelatinous edema of the mesocolon, yellow with a liquid consistency, mesenteric lymph nodes congested and enlarged in volume, hyperplasia, and hepatic congestion. |
| **14** | **F2** | Abdomen enlarged in volume, gas-filled intestinal loops, proliferative ileitis, thinned intestinal walls, mesenteric lymph nodes enlarged in volume, diffuse hemorrhagic bronchopneumonia, hepatic and splenic congestion, and hyperplasia, distended stomach with gelatinous-mucus content. |
| **15** | **F2** | Hemorrhagic enteritis, hepatic congestion, exudate in the pericardial cavity, enlarged mesenteric lymph nodes, renal congestion, pulmonary congestion, the mosaic appearance of lungs, distended stomach full of undigested feed, |
| **16** | **F1** | Fibro-adhesive pleurisy, pronounced interlobular edema, hemorrhagic-necrotic bronchopneumonia in foci, bronchopneumonia in different phases of hepatization, the mosaic appearance of lungs in apical lobes, presence of encapsulated microabscesses, enlarged tracheobronchial lymph nodes and haemorrhagic infiltrates. |

****F1 - mixed farm; F2- youth farm**

## 3. Conclusions

From the research carried out we can state that:

1. Digestive diseases in pigs from the farms studied are mainly caused by E. coli with an increased incidence rate, especially in young pigs, with the characteristic anatomo-pathological changes of abdominal distension, gas-filled small intestine loops, colon contents with a slimy consistency, much-thinned walls, proliferative ileitis, and mesenteric lymph nodes increased in volume, distended stomach with a gelatinous-mucus content.
2. In the second category *Streptococcus suis* and hemolytic *E. coli* are found less frequently than in the first category, and the most obvious macroscopic changes are in the gastric mucosa in some parts with a blackish nun chuck, hemorrhagic enteritis, exudate in the pericardial cavity, enlarged heart with sub-epicardial petechial and suffusions, ulcerous-vegetating endocarditis - the presence of yellowish-white, friable deposits.
3. *Pasteurella multocida* and *Salmonella spp*. two diseases producing respiratory and digestive changes are classified in the third category according to the level of pathogenicity revealed in our research. It is important to mention that the presence of these microorganisms is associated with cyanosis of the throat, rhinitis, diffuse hemorrhagic bronchopneumonia, abdominal distension, fibrinous polyserositis in the abdominal cavity, and avoidable pulmonary congestion, these changes may vary depending on the strain, stage of the disease and specific response of the host organism.
4. In the case of generated conditions *Klebsiella spp, Serratia marcescens, Morganella morganii, Actinobacillus pleuropneumoniae* and *Mannhemia hoemolytica* have a sporadic incidence rate.
5. The varied course of digestive and respiratory diseases in pigs are common

problems in pig establishments and may have various causes. Excluding the causative micro-organisms, genetic, environmental, management, and nutritional factors may influence their development.

6. Thus, implementing biosecurity measures can significantly contribute to maintaining pig health on farms.

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