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Diagnostic studies for enterotoxaemia in rabbits

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Abstract. The relevance of this study is highlighted by the occurrence of enterotoxaemia in rabbits, particularly caused by *Clostridium perfringens*, in the context of industrial rabbit farming. The research aimed to determine the causes of morbidity and increased mortality in young rabbits during the growing period. The study involved a stepwise analysis of feed samples,

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clinical examination of rabbits reared in an industrial rabbit farm, and laboratory examination of the obtained biological material. Additionally, to establish a diagnosis and provide the farm with appropriate recommendations regarding the maintenance and prevention of rabbit diseases, the health status of the rabbits, the causes of digestive disorders with symptoms of diarrhoea and increased mortality under production conditions were analysed. Clinical, haematological, pathological, microbiological, and statistical research methods were used. In diseased rabbits, an increase in rectal body temperature, symptoms of diarrhoea, and in some animals, seizures were established. Several animals succumbed to the disease. During laboratory blood tests of the rabbits, moderate anaemia was detected, with a decrease in the number of erythrocytes and a reduction in haemoglobin content. There was also a disturbance in the qualitative composition of erythrocytes, including the presence of poikilocytosis and altered erythrocyte forms: acanthocytes, echinocytes, schistocytes, dacryocytes, keratocytes, and drepanocytes. A decrease in the immune status of the rabbits was characterised by a reduction in the total number of leukocytes and lymphocytes in the blood and a low neutrophil-to-lymphocyte ratio. Biochemical analysis of rabbit serum revealed a decrease in glucose levels below the physiological range and an increase in the activity of the enzymes alanine aminotransferase, alkaline phosphatase, and gamma-glutamyl transferase. Results of post-mortem examinations of the deceased rabbits indicated the presence of gas-filled small intestines, with a fluid content and no macroscopic signs of inflammation. The feed provided to the rabbits on the farm was found to contain sulphite-reducing clostridia, specifically *Clostridium perfringens*. Identifying the causes of morbidity and mortality in rabbits will enable the selection of appropriate methods for preventing deaths within specific farming conditions

Keywords: enterotoxin; anaemia; digestive disorders; morphological blood studies; biochemical blood studies

Introduction

Clostridia are widespread in nature and found in soil, water bodies, bedding, and feed. They are part of the normal microflora in the gastrointestinal tract of animals. These are highly resistant spores capable of surviving in the environment for extended periods. Not all species of clostridia cause disease, but those that do are usually fatal (Clostridiosis on a dairy…, 2024).

According to A. Camargo *et al*. (2024), *Clostridium perfringens* bacteria, which have an etiological significance in the infectious pathology of both animals and humans, produce a range of toxins and cause various types of diseases. The Clostridia that synthesise toxins of types A, B, C, D, and E can be both an etiological factor in the development of foodborne illnesses in calves, lambs, and piglets and a causative agent of infectious diseases characteristic of each animal species. According to M. Hoonakker *et al*. (2024), a common feature of all *Clostridium perfringens* is their impact on their hosts through the secretion of very potent exotoxins, some of which are spore-forming toxins. Clostridia do not pose a threat to the animal's body until favourable conditions arise. Typically, clostridial infections are very severe and manifest acutely (Clostridiosis on a dairy…, 2024).

Moreover, A. Camargo *et al.* (2024), expressed that the etiological role of clostridia in animal pathology is often controversial, as most species of clostridia can be part of the microbiota of the intestines of healthy animals and

humans, and from there enter and accumulate in the environment, feed, water, and soil. Therefore, infectious diseases can be endogenous in nature, when unfavourable conditions enhance the multiplication and accumulation of *Clostridium perfringens* in the intestine. For example, as a result of poor feeding hygiene (incomplete and sudden changes in diet) or poor animal husbandry conditions, or in the case of using feed contaminated with *Clostridium perfringens*, several different antibiotics and disinfectants are naturally ineffective, especially against their spore forms.

According to research by G.G. Alves *et al*. (2024), the beta-toxin produced by *Clostridium perfringens* types B and C causes necrotic enteritis, characterised by deep segmental necrosis of the mucosa with pronounced haemorrhage in the small intestine, the occurrence of neurological symptoms, and sudden death in animals, and rarely, humans. M. Hoonakker *et al*. (2024) indicated that while *C. perfringens* type C secretes several exotoxins, the beta-toxin is the primary virulence factor for inducing necrohaemorrhagic intestinal lesions. B. Tarek *et al*. (2021) established that beta-toxin plays a pivotal role in the pathogenesis of necrohaemorrhagic enteritis in young animals and humans by targeting endothelial cells in the intestine. M. Hoonakker *et al*. (2024) noted that beta-toxin primarily affects the endothelial cells of blood vessels in the mucosa and may also suppress platelet function. The beta-toxin is produced as a monomeric protein with a molecular weight of 34.86 kDa. *Clostridium perfringens* secrete it in the form of a water-soluble monomer that binds to membrane receptors on target cells, making it one of the most potent clostridial toxins known. Binding to receptors in the plasma membrane of target cells results in local concentration, oligomerisation, and the formation of a multimeric prepore that undergoes conformational changes and inserts into the lipid bilayer, where it creates a functional transmembrane pore. These pores disrupt membrane permeability by allowing ions to pass through, which, according to B. Tarek *et al*. (2021) and G.G. Alves *et al*. (2024), lead to changes in intracellular concentration and the responses of affected cells. Furthermore, A. Camargo *et al*. (2024) discovered that *Clostridium perfringens* produces other clinically significant supplementary toxins, such as *perfringolysin O*, tetanotoxin, and *Clostridium perfringens* beta 2 toxin. Although these toxins are not used for toxinotyping, they can act synergistically with extracellular toxins, influencing the expression of other toxins, their production levels, and virulence factors, thereby contributing to the overall progression of the disease.

I.M. Gohari *et al*. (2021) demonstrated that most of the toxin genes in *C. perfringens* are encoded in conjugative plasmids, including pCW3-like and recently discovered pCP13-like families of plasmids. The production of *C. perfringens* toxins is tightly regulated through processes involving two-component regulatory systems, quorum sensing, and/or alternative sigma factors associated with sporulation. Non-toxin factors, such as degradative enzymes and sialidases, are also involved in the pathogenicity of this bacterium. These factors may contribute to the action of toxins *in vitro* and possibly *in vivo*, and may also enhance intestinal colonisation by *C. perfringens*. For example, sialidase NanI increases the adherence of *C. perfringens* to intestinal tissue and generates nutrients for its growth, at least *in vitro*.

This study aimed to determine the health status of rabbits, taking into account the results of clinical, haematological, pathological, and microbiological examinations, and to elucidate the causes of digestive disorders with diarrhoeal syndrome and increased mortality in these animals under production conditions.

Literature Review

Modern industrial rabbit farming typically involves slaughtering rabbits at the age of three months. By this time, rabbits should have gained approximately 2.5-3 kg of body weight. Although rabbits are considered fast-growing animals, such results can only be achieved with a complete and balanced diet (Abdelazeem *et al*., 2019a; 2019b) and a healthy gastrointestinal tract, which allows the animal's body to be provided with everything necessary for its growth.

Digestive disorders are one of the primary causes of mortality in young rabbits. C. Romero *et al.* (2011) noted that *Clostridium perfringens* is a widespread pathogenic bacterium associated with intestinal diseases in domestic animals, and its pathogenicity is linked to the production of potent exotoxins. A. Shrestha *et al*. (2022) indicated that *Clostridium perfringens* enterotoxin (CPE) induces gastrointestinal symptoms of enterotoxaemia in animals and is one of the most common bacterial foodborne diseases.

Enterotoxaemia is characterised by symptoms of diarrhoea, primarily in rabbits aged between 4 and 8 weeks, although according to M.E. Ensminger *et al*. (1990), it can affect rabbits of any age. The main symptoms of this disease in rabbits are severe diarrhoea, loss of appetite, and the presence of a rough coat. Death of the animal occurs within the first forty-eight hours of the onset of symptoms. This disease is caused by several microorganisms: *Clostridium spiroforme*, *Clostridium perfringens*, and *Escherichia coli*.

According to C. Romero *et al*. (2011), a rabbit exhibiting mild diarrhoea, distension of the foregut with gaseous fluid content, and lesions of the caecum without macroscopic signs of inflammation is considered to have epizootic rabbit enteropathy (ERE). I.M. Gohari *et al*. (2021) demonstrated that during gastrointestinal tract lesions, *C. perfringens* produces enterotoxin in the intestine. *Clostridium perfringens* enterotoxin (CPE) is a primary cause of food poisoning and antibiotic-associated diarrhoea. D.C. Briggs *et al*. (2011) noted that this enterotoxin structurally belongs to the family of aerolysin pore-forming toxins. CPE binds to receptors belonging to the claudin family of proteins (Jorge *et al*., 2014; Shrestha *et al.,* 2022). Claudins are a family of integral membrane proteins that mediate interactions between cells of the mammalian intestinal epithelium by localising and stimulating the formation of intercellular tight junctions (Anderson & Van Itallie, 2009). *Clostridium perfringens* causes widespread gastrointestinal disorders in mammals due to the action of enterotoxin, which affects claudins. CPE binds to claudins at or near tight junctions in the intestine and disrupts their barrier function (Ogbu *et al.*, 2022). CPE widens β-hairpins in the cell membranes of enterocytes, forming active pores (Jorge *et al*., 2014; Shrestha *et al*., 2022). The formation of this pore causes an influx of free calcium into intestinal enterocytes, killing these cells in a dose-dependent manner. Specifically, according to M.I. Gohari *et al*. (2021), even low doses of CPE induce classical apoptosis. In contrast, according to A. Shrestha *et al*. (2022), low doses of CPE form only a small number of CPE pores, leading to a limited influx of free calcium and moderate activation of calpain. This, in turn, leads to cell death via caspase-3-mediated classical apoptosis. Higher concentrations of CPE induce the formation of many CPE pores, greater influx of free calcium into enterocytes, and stronger activation of calpain, which induces the death of these cells via necroptosis. Furthermore, according to C. Ogbu *et al*. (2022), CPE ultimately destroys cells expressing claudin through the formation of a cytotoxic membrane penetrating β-barrel.

A. Shrestha *et al*. (2022) noted that in the loops of the rabbit small intestine, the cytotoxicity of CPE leads to fluid accumulation in

the intestinal lumen and histological damage to the epithelium, including villus shortening and epithelial desquamation. Considering the data of previous authors and the information from M.I. Gohari *et al*. (2021), it was found that CPE is a significant pathogenicity factor of *C. perfringens* type F strains. Type F strains cause 5-10% of cases of antibiotic-associated diarrhoea in animals.

Therefore, based on the analysis of the literature, the impact of enterotoxin on the mechanisms underlying the development of the corresponding symptoms in diseased rabbits has been determined.

Materials and Methods

The study was conducted in 2023 within an industrial farm in the central region of Ukraine, involving rabbits aged between 35 and 68 days. The rabbits were housed in wire mesh cages with wire mesh floors, with 3-5 animals per cage. Each cage was supplied with water through a system equipped with nipple drinkers, allowing free access to water for each animal. The rabbits were fed a granulated feed produced on the farm.

Blood samples were collected from the jugular vein of diseased rabbits into vacuum tubes containing ethylenediaminetetraacetic acid (EDTA). Blood smears were immediately prepared during venipuncture to prevent changes in blood cell morphology. The blood samples were transported in a thermally insulated bag with ice, separated by a layer of cotton, to inhibit the processes of lactic acid formation from glucose in the erythrocytes. For post-mortem examination, carcasses of animals that had just died were collected. Post-mortem examinations of the rabbits were carried out immediately on the rabbit farm in a separate, equipped room. During the experiments on the rabbit farm, all bioethical requirements for animals were adhered to, by the Law of Ukraine No. 249 (2012) and the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (1986)

The clinical condition of the animals was assessed using standard methods. During the study, attention was paid to the animal's general appearance, visible mucous membranes, condition of the coat and skin, lymph nodes, organ systems, and individual organs. Body temperature was measured rectally using a mercury thermometer manufactured by Tetafarm LLC (Ukraine). Quantitative analysis of the morphological composition of the blood was performed using a haematology analyser "Mindray animal care" BC – 5000 Vet (China). The haematocrit value was determined using a haematology analyser "VetAutoread", manufactured by "IDEXX Laboratories" (USA). The qualitative composition of erythrocytes was examined in blood smears stained with haematological dyes "Leucodif 200", manufactured by "Erba lachema" (Czech Republic).

Biochemical parameters of rabbit serum, namely: total protein, albumin, glucose, urea, creatinine, inorganic calcium, total phosphorus, total bilirubin, and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl transferase (GGT), and alkaline phosphatase (ALP), were determined using standard methods with a photometric biochemical analyser "Lab Line 010" (Austria). Reagents from the company SpinaLab (USA) were used for the biochemical studies.

Microbiological analysis of the feed was conducted following the following testing methods: determination of the number of yeasts and mould fungi – DSTU ISO 7954:2006 (2006) Microbiology of food and animal feed. General guidelines for counting yeasts and microscopic fungi. Colony counting technique cultivated at a temperature of 25°C (DSTU ISO 7954:1997,

IDT); determination of *Bacillus cereus* – DSTU ISO 7932:2007 (2009); Microbiology of food products and animal feeds. Horizontal method for the enumeration of presumptive *Bacillus cereus*. Counting technique at a temperature of 30°C (ISO 7932:2004, IDT); determination of the number of sulphite-reducing clostridia *Clostridium perfringens* – (6) DSTU ISO 7937:2006 (State standard..., 2006); Microbiology of food products and animal feeds. Horizontal method for the enumeration of *Clostridium perfringens*. Colony counting technique.

Samples of pathological material from rabbits (heart blood, liver, kidneys, stomach, and intestine) were examined for the presence of pathogens of pasteurellosis (Pavlenko *et al.,* 1995), streptococcosis (Methodological guidelines..., 1983), staphylococcosis (Methodological recommendations..., 1999), salmonellosis (DSTU 4769:2007, 2009), yersiniosis (Melnychuk *et al.,* 2013), listeriosis (Bondar *et al.,* 2007), and clostridial diseases (DSTU 8492:2015, 2017). Statistical analysis of the research results was performed using the computer program Microsoft Excel 2016.

Results and Discussion

The results of the clinical examination of the animals indicated that a portion of the rabbit population experienced periodic digestive disorders during the period from 35 days of age (the weaning period from the female rabbit) until slaughter at 68 days. These disorders included loose stools and soiling of the fur around the anal region with faeces. At the onset of diarrhoea, the rectal temperature increased to 40-41°C. Subsequently, the body temperature decreased to within physiological ranges (38.5-39.5°C), but diarrhoea persisted. The rabbits exhibited stunted growth and poor weight gain, and some individuals displayed seizure-like symptoms with backward head tilting A portion of the rabbits succumbed to the condition. Palpation of the abdomen revealed signs of intestinal gas, but no signs of pain were observed.

According to the results of the morphological study of rabbit blood, moderate anaemia was recorded, as indicated by a haemoglobin level lower than the physiological range, a reduced erythrocyte count, and a decreased haematocrit value (Table 1).

Table 1. Morphological blood parameters of rabbits, M ± m, n = 5

	Parameter	Research results	Physiological range*
%	Ht, Haematocrit	33.0 ± 0.15	36.6-47.4
g/L	HB, Haemoglobin,	104.0 ± 2.0	115-151
10^{12}/L	RBC, Erythrocytes	5.05 ± 0.18	5.2-6.8
pg	MCH, Haemoglobin content in an erythrocyte	20.5 ± 0.44	21.1-24.5
%	MCHC, Haemoglobin concentration in an erythrocyte	31.7 ± 0.5	29.5-33.9
fl	MCV, Erythrocyte volume	65.34 ± 1.16	64.6-76.2
10^9/L	WBC, Leukocytes	4.2 ± 0.6	6.30-10.06
10^9/L	Basophils	0	0.06-0.36
10^9/L	Eosinophils	0.05 ± 0.13	0.01-0.15
10^9/L	Band Neutrophils	0	0
10^9/L	Segmented Neutrophils	1.47 ± 0.06	1.49-3.21
10^9/L	Lymphocytes	2.48 ± 0.34	3.36-7.00
10^9/L	Monocytes	0.21 ± 0.05	0.05-0.45

Note: **Physiological ranges according to M.A. Thral et al. (2012)*
Source*: authors' development*

M.I. Tsvilikhovskyi *et al*. (2023) demonstrated that a decrease in haematocrit can occur in anaemia of various origins or due to hyperhydration. With a normal concentration of total plasma protein, a low haematocrit can be a result of increased erythrocyte destruction, decreased erythrocyte production, or chronic blood loss. These authors note that the causes of decreased haemoglobin in the blood can include iron deficiency anaemia, anaemia due to acute blood loss, hypoplastic anaemia, haemolytic anaemia after a haemolytic crisis, B_{12}-deficiency anaemia, anaemia associated with neoplasms or leukaemia, and hyperhydration. The development of anaemia in an animal, according to A.H. Rebar *et al*. (1999), is associated with the development of hypoxia, which, in turn, causes damage to the cell membranes of parenchymal organs (such as the liver) and an increase in the activity of cytoplasmic enzymes.

After centrifugation, the blood plasma had a red colour (Fig. 1), which may indicate intravascular haemolysis and the development of hemoglobinemia and, consequently, haemoglobinuria in the experimental rabbits.

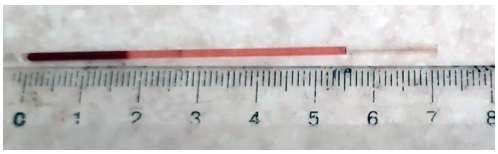

Figure 1. Haematocrit content determination
***Sourse**: authors' material*

According to L.M. Perry-Clark & L.D. Meunier (1991), this could also be due to the fact that rabbit blood readily haemolyzes and clots quickly. However, colour indices did not indicate a deficiency of iron and copper in the experimental rabbits. The size of the erythrocytes was not reduced. The authors of this article believe that the decrease in the number of erythrocytes occurred as a result of haemolytic processes, i.e., the development of haemolytic anaemia. This is confirmed by polychromasia (an increase in the number of polychromatophils greater than 4/100 erythrocytes) (Figs. 2-5). According to M. Suckow *et al*. (2002), in rabbits, 1-4% of circulating erythrocytes can be polychromatophils. The presence of a few nucleated erythrocytes (1-2×100 leukocytes) should be considered within the normal reference range for rabbits, rather than an indicator of cell regeneration (Melillo, 2007). However, according to A. Bodnariu (2022), in rabbits up to two months of age, the percentage of reticulocytes can be as high as 12% of the number of erythrocytes. Polychromasia and the presence of polychromatophils in rabbit blood smears are explained by the short lifespan of the animals and the intensity of erythropoiesis (Suckow *et al*., 2002). The increased number of polychromatophils in the blood of the examined rabbits indicates that the red bone marrow of the animals is undamaged and actively restores erythrocytes lost due to haemolysis. A. Bodnariu (2022) found that erythrocyte regeneration is characterised by increased anisocytosis and polychromasia. The authors of this article believe that a likely cause of haemolysis in rabbits may be the influence of toxins with a haemolytic effect (feed toxins, bacterial toxins, in particular alpha-toxin of *Clostridium perfringens*, etc.).

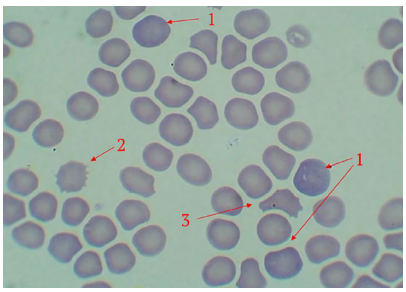

Figure 2. Blood smear from an experimental rabbit. Marked polychromasia
***Note**: Polychromatophils – 1, echinocyte – 2, and acanthocyte – 3 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

During the examination of blood smears from the rabbits, no nucleated erythrocytes or Howell-Jolly bodies were detected. Erythrocytes in the blood smear exhibited marked poikilocytosis. The presence of the latter indicates the development of processes in the rabbits' bodies that lead to a disruption of the erythrocyte membrane structure due to changes in lipid and protein metabolism. Poikilocytosis can be caused by both a disruption of the erythrocyte membrane structure and a disruption of redox processes in the erythrocyte and at the level of the whole organism. Almost all erythrocytes had an altered shape: many acanthocytes (Figs. 2-6), echinocytes (Figs. 2-6), schistocytes (Fig. 3), dacryocytes (Fig. 4), keratocytes (Fig. 5), and drepanocytes (Fig. 6). In the literature presented above, the appearance of acanthocytes in the blood of experimental rabbits is associated with an increase in the concentration of cholesterol relative to phospholipids in erythrocyte membranes, which occurs with an increase in the level of cholesterol in the blood plasma or the presence of abnormal lipoprotein in it. In blood smears of humans, acanthocytes are found with impaired lipid metabolism, which manifests itself in liver pathology. However, in the blood of dogs, acanthocytes are found quite rarely with hepatopathology. As a rule, acanthocytes are recorded in blood smears of domestic cats with hepatosteatosis, and in the blood of dogs with hemangiosarcoma. Echinocytes, like acanthocytes, appear in the blood of animals with uraemia, vitamin E deficiency, and hyperlipidaemia caused by liver dysfunction. J.S. Owen *et al*. (1985) proved that plasma membrane receptors of erythrocytes bind to high-density lipoproteins and this causes a change in their shape. According to M.I. Tsvilikhovskyi *et al*. (2023), the presence of schistocytes in rabbit blood can be attributed to intravascular damage, particularly in cases of disseminated intravascular coagulation (DIC). During DIC, fibrin strands trap and fragment erythrocytes, leading to the formation of schistocytes. Animals experiencing DIC may also exhibit thrombocytopenia. In calves, the appearance of schistocytes is widely recognised as a characteristic feature of DIC. In the blood of horses and cats, schistocytes are rarely observed, and their presence is associated with hepatopathy (Fig. 4).

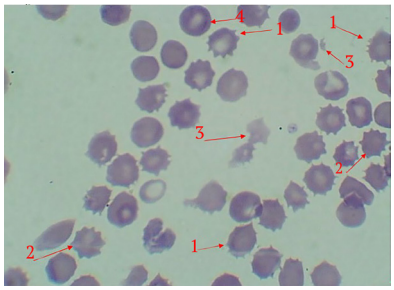

Figure 3. Blood smear from an experimental rabbit. Marked poikilocytosis of erythrocytes
***Note**: Echinocytes – 1, acanthocytes – 2, schistocytes – 3, and polychromatophils – 4 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
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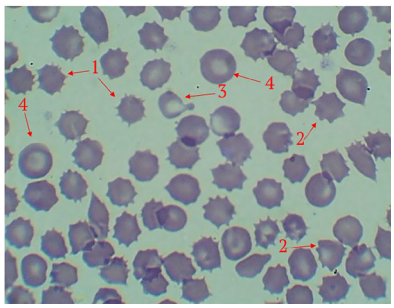

Figure 4. Blood smear from an experimental rabbit. Marked poikilocytosis
***Note**: Echinocytes – 1, acanthocytes – 2, dacryocytes – 3, and polychromatophils – 4 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

The presence of dacryocytes in rabbit blood may indicate haemolytic anaemia, a condition where erythrocytes are destroyed by the body's own immune system. As a result, the bone marrow produces a larger number of blood cells,

including those with structural abnormalities, which are then released into the bloodstream. J.W. Harvey (2012) noted that dacryocytosis is commonly found in the blood of dogs with myelofibrosis, glomerulonephritis, and hypersplenism, as well as in dogs and cats with myeloid neoplasms. In ruminants, dacryocytes are often seen in cases of iron deficiency anaemia.

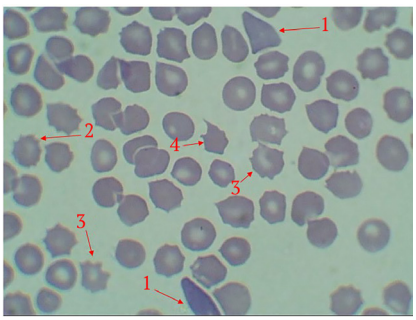

Figure 5. Blood smear of the experimental rabbit
***Note**: Polychromatophils – 1, echinocytes – 2, acanthocytes – 3, and keratocyte – 4 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

The presence of keratocytes in rabbit blood can be indicative of various pathological conditions, including iron deficiency anaemia and liver diseases such as hepatosteatosis. In these cases, damage to the erythrocyte membrane occurs as a result of oxidative stress. For instance, in iron deficiency, a rounded vacuole initially forms within the erythrocyte. This vacuole then moves to the cell's exterior due to oxidative damage to the inner surface of the membrane. According to the literature previously cited, one reason for the formation of this clear zone (vacuole) could be a deficiency of haemoglobin. The same literature mentioned that drepanocytes appear in the blood of animals due to haemoglobin polymerisation. This phenomenon occurs *in vitro* when the partial pressure of oxygen increases and the pH level exceeds 7.4.

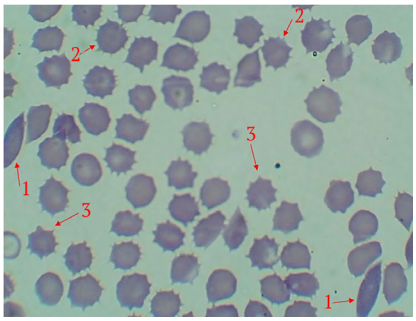

Figure 6. Blood smear of the experimental rabbit
***Note**: Drepanocytes – 1, echinocytes – 2, and acanthocytes – 3 are visible under the microscope. Leucodif 200 staining. Magnification ×1000*
***Sourse**: authors' material*

A reduction in the white blood cell (WBC) count in the experimental rabbits indicated a suppression of their immune system. Absolute lymphopenia was diagnosed in the blood of these animals, further supporting the conclusion of immune suppression. Lymphopenia is more commonly associated with acute or severe inflammatory processes and can be linked to chronic viral, bacterial, and fungal infections. The release of endogenous glucocorticoids in response to severe systemic disturbances may play a major role in the development of lymphopenia, which often accompanies these disturbances. According to M.I. Tsvilikhovskyi *et al*. (2023), glucocorticoids can potentiate apoptosis in susceptible lymphocytes. In rabbits, lymphocytes are primarily found in the blood, spleen, bone marrow, lymph nodes, and gastrointestinal lymphatic tissues. The number of circulating lymphocytes represents a balance between cells entering and leaving the bloodstream, and this does not necessarily reflect changes in lymphopoiesis. A. Melillo (2007) demonstrated that an increase in adrenaline levels in rabbit blood (acute stress) induces lymphocytosis, while an increase in cortisol levels (chronic stress) leads to lymphopenia.

The data from this author and D.R. Reavill & R.E. Schmidt (2000) suggest that viral diseases may not affect the lymphocyte count in these animals or may even lead to lymphocytosis. The extremely low neutrophil to lymphocyte ratio (0.59), combined with a low absolute lymphocyte count ($2.48 \pm 0.34 \times 10^9$/L) in the blood of the experimental animals (Table 1), indicates immunosuppression rather than stress. The neutrophil-to-lymphocyte ratio in healthy adult rabbits is approximately 1:1. Changes in this ratio can be associated with stress (heterophilia and lymphopenia). A. Bodnariu (2022) noted that rabbits do not develop significant leukocytosis in response to bacterial infection. However, a change in the neutrophil-to-lymphocyte ratio is typically observed. During this study, band neutrophils were not detected in the rabbit blood. According to A. Melillo (2007), these neutrophils are a rare finding in the blood of rabbits with clinical infection, but the absence of a left shift in the leukocyte profile does not rule out an infectious process.

The researcher demonstrated that four days of fasting did not decrease blood glucose levels in healthy rabbits. Glucose metabolism in rabbits differs from that in dogs or cats. Rabbits not only eat continuously throughout the day but also utilise volatile fatty acids produced by the caecal flora as a primary energy source. Obtaining a fasting blood sample is impossible as rabbits practice coprophagy. A rabbit deprived of food may continue to consume cecotropes.

Biochemical analysis of the blood plasma (Table 2) of the experimental rabbits revealed a decrease in glucose levels below the minimum physiological range. This may indicate a reduced level of energy and fibre in the rabbits' diet, as well as impaired synthesis of this metabolite in the animals' bodies, particularly in the liver.

Table 2. Biochemical analysis of rabbit plasma, M ± m, n = 5

Parameter	Result	Physiological range *
Total protein, g/L	60.9 ± 2.38	54-75
Albumin, g/L	38.72 ± 2.34	27-46
Glucose, mmol/L	5.22 ± 0.21	6.1-15.9
Calcium, mmol/L	3.96 ± 0.14	2.4-4.2
Phosphorus, mmol/L	1.34 ± 0.18	0.6-2.7
Urea, mmol/L	5.47 ± 0.41	2.3-6.6
Creatinine, µmol/L	68.04 ± 5.46	44.2-141.4
Alkaline phosphatase, units/L	174.14 ± 4.28	19-173
ALT, U/L	77.48 ± 4.09	25-60
AST, U/L	27.89 ± 1.87	5-31
GGT, U/L	7.54 ± 0.29	0-7
Total bilirubin, µmol/L	6.34 ± 0.64	3.4-8.5

Note: **Physiological ranges according to M.A. Thral et al. (2012)*
Source*: authors' development*

Hypoglycaemia can be induced by anorexia, starvation, or impaired liver function. Additionally, according to A. Bodnariu (2022), it leads to the mobilisation of free fatty acids from adipose tissue, causing ketoacidosis and hepatic lipidosis. A negative consequence of hypoglycaemia can also be impaired detoxification function of the liver, synthesis of heparin, hyaluronic acid, and chondroitin. Insufficient synthesis of the latter can lead to impaired vascular permeability, resulting in haemorrhages in such animals. R.A. Saunders & R.R. Davies (2005) noted that

hypoglycaemia is a rare occurrence in rabbits. In anorexic animals, it suggests that the rabbit's body is utilising fat stores and is at risk of developing fatty liver disease. Hypoglycaemia can occur in cases of terminal mucoid enteropathy, hepatic insufficiency, or other chronic diseases. Rabbits with acute sepsis may also experience hypoglycaemia.

Elevated levels of alanine aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT) in the blood plasma of the experimental rabbits indicated destructive changes in cell membranes, primarily in the liver, and the development of cholestasis. It is known that ALT activity is lower and less organ-specific in rabbits compared to other mammals. A. Bodnariu (2022) noted that increased ALT activity in rabbits suggests possible hepatocyte damage, however, the degree of elevation does not correlate with the severity of hepatopathy and is not a prognostic indicator. Slightly elevated ALT activity in blood plasma is a common occurrence in healthy rabbits. A slight increase in ALT activity in the blood of healthy rabbits can be explained by the influence of low concentrations of toxic substances, such as resins in wooden bedding or aflatoxins in feed. A. Melillo (2007) demonstrated that increased ALT activity in blood plasma in combination with elevated ALP and GGT can be associated with hepatic lipidosis, and was also observed in rabbits with hepatic coccidiosis (*Eimeria steidae*). Thus, increased ALT activity in rabbit blood can accompany both pathological changes in the liver parenchyma and biliary tract of an inflammatory nature, as well as in dystrophy caused by intoxication of various origins.

Alkaline phosphatase (ALP) is a non-specific enzyme. The highest concentrations of ALP are found in the liver and bones, but it is also abundant in the intestinal epithelium, kidney tubules, and placenta. Similar to other species, according to the two aforementioned authors, higher concentrations of ALP are found in the plasma of young rabbits compared to adults due to increased osteoblastic activity. As a liver enzyme, ALP activity does not increase due to hepatocellular damage but indicates bile stasis (e.g., hepatic coccidiosis, liver abscesses, neoplasia, lipidosis). The authors found that increased GGT activity in rabbit plasma is most often associated with obstructive lesions of the bile ducts (cholestasis) but with less sensitivity than in other species.

During necropsy of the rabbit carcasses, the presence of gas-filled loops of small intestine with fluid contents and no macroscopic signs of inflammation was diagnosed (Fig. 7).

Figure 7. Meteorism of the intestine in an experimental rabbit
***Note**: The arrow indicates the location of the pathological process*
***Sourse**: authors' material*

Samples of pathological material from rabbits (heart blood, liver, kidneys, stomach, intestine) were examined for the presence of the causative agents of pasteurellosis, streptococcosis, staphylococcosis, salmonellosis, yersiniosis, listeriosis, and clostridial diseases. No pathogens were detected. Given the clinical symptoms, blood test results, and post-mortem findings, it was necessary to determine the cause of these changes. To this end, feed samples were collected and subjected to microbiological analysis.

Samples of plant-based rabbit feed (grist, compound feed, bran, premix, sunflower seed hulls) were examined for microbiological indicators (number of yeasts and mould fungi; quantity of *Bacillus cereus* and sulphite-reducing clostridia *Clostridium perfringens*; detection of *Enterobacteriaceae*, pathogenic *Yersinia*, *Salmonella* spp., coagulase-positive *Staphylococcus aureus*, and *Listeria monocytogenes*). The results of the studies are presented in Table 3.

Table 3. Results of the microbiological analysis of rabbit feed samples

Indicator assessed	Rabbit feed samples								[(2)] Norms according to regulatory documentation
	10/1	10/2	10/3	10/4	10/5	10/6	10/7	10/8	
Yeast count, [(1)] CFU/g	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	Not more than 5×10^5
Mould fungi count, [(1)] CFU/g	4.5×10^1	<10 (not detected)	3.5×10^4	1.4×10^1	<10 (not detected)	3.2×10^2	9.0×10^2	<10 (not detected)	
Bacillus cereus count, [(1)] CFU/g	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	<10 (not detected)	Not established
Sulphite-reducing clostridia *Clostridium perfringens* count, [(1)] CFU/g	<10 (not detected)	3.6×10^1	1.4×10^1	<10 (not detected)	1.8×10^1	<10 (not detected)	<10 (not detected)	<10 (not detected)	Not permitted in 1 g
Coagulase-positive staphylococci *Staphylococcus aureus* count, [(1)] CFU/g	not detected								Not permitted in 1 g
Presence of bacteria from the family *Enterobacteriaceae*, [(3)] MPN in 1.0 g	No *Enterobacteriaceae bacteria* detected (The actual number of microorganisms in 1 g is within 0.0-9.4 at a 95% confidence level)								Not more than 300 CFU in 1 g
Pathogenic *Yersinia*, in 25 g	not detected								Not more than 300 CFU in 1 g
Pathogenic microorganisms, including *Salmonella* spp., in 25 g	not detected								
Presence of Listeria monocytogenes, in 25 g	not detected								Not established

Note: *[(1)]CFU – Colony-forming units. [(2)]List of maximum permissible levels of undesirable substances in animal feeds and feed raw materials. [(3)]MPN – Most probable number*
Source*: authors' development*

No yeasts, *Enterobacteriaceae*, pathogenic *Yersinia*, *Salmonella* spp., coagulase-positive *Staphylococcus aureus*, or *Listeria monocytogenes* were detected in any of the feed samples examined. According to the test results, samples 10/1 – Grist (No. 2); 10/4 – Bran; 10/6 – Grist (No. 1); 10/7 – Bran; and 10/8 – Compound feed PK-90 Kremiks complied with the requirements of the normative documentation (ND) (On the approval..., 2012).

Samples that did not meet the requirements of the ND were: 10/2 – Compound feed (due to "Quantity of sulphite-reducing clostridia *Clostridium perfringens*"); 10/3 – Sunflower seed hulls (due to "Mould fungi count" and "Quantity of sulphite-reducing clostridia *Clostridium perfringens*"); and 10/5 – Premix "Kremiks" (due to "Quantity of sulphite-reducing clostridia *Clostridium perfringens*").

Based on the research conducted, the most probable diagnosis is enterotoxaemia in rabbits caused by *Clostridium perfringens*. Due to the epidemiological data, clinical symptoms, and post-mortem findings, it is quite difficult to establish a definitive diagnosis for this disease. Therefore, the results of bacteriological and toxicological studies are crucial for confirming the diagnosis. The absence of positive results from culturing the pathological material from rabbit tissues and the inability to detect the presence of toxins cannot confirm but also cannot refute this diagnosis. R.M.C. Guedes (2016) suggested that certain strains of *Clostridium perfringens* are pathogenic to piglets and can cause diarrhoea and reduced growth rates in these animals, but how to identify them is still unknown. This, of course, poses a significant challenge in combating this disease. Some of the lesions observed in the experimental rabbits may partially reflect the effects of α-toxin on the organism, specifically causing gas production and tissue damage.

In fact, J.G. Songer (1996) and C. Romero *et al.* (2011) noted that this toxin hydrolyses lecithin and sphingomyelin, causing membrane destruction. According to these authors, some rabbits in which α-toxin was not detected had lesions similar to those caused by high concentrations of α-toxin. D. Marlier *et al.* (2006) were also unable to detect the presence of α-toxin in the intestinal contents of rabbits that died from anaerobic enterotoxaemia or other digestive disorders. *Clostridium* type C produces a potent toxin – leukocidin – which almost completely blocks the immune system of the diseased animal. This makes it vulnerable to other diseases and leads to the development of new, non-specific clinical symptoms for enterotoxaemia (Moraru, 2020). Additionally, according to M.E. Fernandez-Miyakawa *et al.* (2007), isolates of *C. perfringens* type B also produce epsilon toxin, which exhibits potent neurotoxic activity. In their opinion, there is currently limited information on the pathogenesis of diseases associated with type B, although these same authors indicated that both CPE and epsilon toxin can contribute to lethality.

As a result of enterotoxaemia, the death of a rabbit occurs quite rapidly; therefore, treatment is rarely employed. The choice of feed has a significant impact on the development of the disease – the higher the fibre content in the diet, the lower the incidence of enterotoxaemia. M.E. Ensminger *et al.* (1990) have demonstrated that a diet consisting of hay, straw, and oats significantly reduces the risk of developing this disease. Therefore, preventive measures to prevent the development and spread of enterotoxaemia in rabbit populations must be considered when formulating diets and preparing feed for feeding.

Conclusions

A comprehensive study of the health status of the experimental rabbits, including clinical, morphological, and biochemical analyses of blood samples, as well as microscopic examination of feed components and tissues from deceased animals, revealed changes characteristic of enterotoxaemia. Affected rabbits exhibited digestive disturbances, watery faeces, and intermittent fever of 40-41°C. These rabbits demonstrated stunted growth, poor weight gain, and, in some cases, experienced convulsions; several of them succumbed to the condition. Blood analysis revealed a decrease in the number of erythrocytes, leukocytes, neutrophils, and lymphocytes, as well as low haemoglobin levels and

reduced haematocrit. The blood plasma had a red colour, indicating intravascular haemolysis and the development of hemoglobinemia and, consequently, haemoglobinuria. The obtained colour indices refuted the deficiency of iron and copper in the experimental rabbits. Erythrocyte size was not reduced. An increase in the number of polychromatophils to more than 4/100 erythrocytes confirmed the presence of haemolytic processes in the sick rabbits. Rabbit erythrocytes exhibited altered morphology, which also indicated the development of anaemia and a decreased immune status in the diseased animals. The sick rabbits developed hypoglycaemia, suggesting an energy imbalance in the body. Simultaneously, an increase in the activity of liver-specific enzymes – alkaline phosphatase, alanine aminotransferase, and gamma-glutamyl transferase – was detected in the blood serum of these animals, indicating destructive changes in hepatocyte membranes and the development of cholestasis. During the post-mortem examination, the anterior loops of the small intestine were found to be distended with gas and fluid content, but macroscopic signs of inflammation were absent. Samples of the feed components did not meet the requirements of the normative documentation regarding the quantity of sulphite-reducing clostridia – *Clostridium perfringens*, suggesting the possible influence of their toxins on the rabbit organism.

A promising avenue for future research involves identifying the specific types and quantities of *Clostridium perfringens* toxins that cause mortality in rabbits, as well as developing effective preventive measures.

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None.

Conflict of Interest

None.

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Діагностичні дослідження за ентеротоксемії кролів

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Анотація. Актуальність дослідження зумовлена проблемами ентеротоксемії у кролів, що викликається *Clostridium perfringens* в умовах промислового кролівництва. Мета дослідження – визначення причин захворюваності та підвищеної летальності в молодняку кролів у період дорощування. У роботі поетапно проведено аналіз зразків корму, клінічне обстеження кролів, вирощених в умовах промислової кролеферми, та лабораторне дослідження отриманого від них біологічного матеріалу. Крім того, для встановлення діагнозу та надання господарству відповідних рекомендацій щодо утримання та профілактики захворювань кролів аналізували стан їх здоров'я, причини розладів травлення з симптомами діареї та підвищеної смертності в умовах виробництва. Використовували клінічні, гематологічні, патологоанатомічні, мікробіологічні та статистичні методи дослідження. У хворих кролів встановлено підвищення ректальної температури тіла, симптоми діареї, в окремих тварин – напади судом. Частина тварин загинула. Під час лабораторного дослідження крові кролів виявили помірну анемію зі зменшенням кількості еритроцитів і зниженням вмісту гемоглобіну та порушення якісного складу еритроцитів, зокрема наявність пойкілоцитозу, змінених форм еритроцитів: акантоцитів, ехіноцитів, шистоцитів, дакріоцитів, кератоцитів і дрепаноцитів. Зниження імунного статусу

організму кролів характеризувалося зменшенням у крові загальної кількості лейкоцитів, лімфоцитів, низьким співвідношенням нейтрофілів до лімфоцитів. Під час біохімічного аналізу сироватки крові кролів встановлено зниження рівня глюкози нижче межі фізіологічних коливань та підвищення активності ферментів – аланінамінотрансферази, лужної фосфатази і гамма-глутамілтранспептидази. Результати патологічного розтину кролів, що загинули, свідчили про наявність переповнених газами передніх петель тонкого кишечнику, з рідким умістом та без макроскопічних ознак запалення. У кормі, яким годували кролів у господарстві, виявлено сульфітредукуючі клостридії – *Clostridium perfringens*. Визначення причин захворюваності та летальності кролів надасть можливість підібрати належні методи профілактики падежу в умовах конкретного господарства

Ключові слова: ентеротоксин; анемія; розлади травлення; морфологічні дослідження крові; біохімічні дослідження крові