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# Necrotic enteritis of birds

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**Abstract.** Poultry clostridia are a serious problem for the domestic poultry industry, which is stated in a number of works by foreign and Russian scientists. In modern conditions, the epizootic situation of clostridia is becoming increasingly tense - the number of outbreaks of necrotic enteritis caused by clostridia increases significantly in a number of poultry farms in Russia, but knowledge of clostridium poultry infection is rather limited. This phenomenon has a tendency to aggravation also due to the fact that many enterprises according to the order of the Government of the Russian Federation No 2045-p of September 25, 2017 “On the strategy for preventing the spread of antimicrobial resistance in the Russian Federation for the period up to 2030” are already preparing to reduce use of antibiotics administered through feed and water. In this case, the producers of the poultry industry, striving to solve one social problem, receive a number of new production and economic problems that require fundamental scientific and applied research to solve them. The solution to this issue may be an alternative approach to the control of poultry clostridia, based on vaccine prevention, the issues of which are currently not studied. Additionally, it is worth noting that the group of poultry diseases caused by microorganisms of the Clostridiaceae family (clostridium) has not only epizootic significance, but also epidemiological one, since it is dangerous for people due to the toxic infection through processed products. In view of the above, the relevance of scientific research on the topic of clostridia of agricultural poultry species is considered obvious.

## 1. Introduction

In recent years, throughout the World, and in particular in Russia, the incidence of infectious diseases of the poultry digestive system associated with anaerobic bacteria - Clostridiums is increasing. Poultry clostridia, necrotizing enteritis, clostridia enteritis, anaerobic enterotoxaemia of birds are all names for one and the same disease caused by bacteria of the Clostridium perfringens species of various types of toxins. At present, the spread and expansion of areas unfavourable for bird clostridiosis occurs exponentially, which is primarily associated with the forced minimization or complete abandonment of the use of antibacterial drugs originally used for the treatment of pathologies of non-anaerobic etymology. According to a number of Russian and foreign scientific authors [1-2], poultry clostridia causes serious economic damage not only for poultry farms, but also has a negative epidemiological significance, as it leads to people infection and, consequently, the increases the cost of their treatment. So, the team of V N Afonushkin and others in 2015, found out by molecular biological methods that the infection rate of broiler chickens aged from 19 to 35 day ranges from 8.3 to 80% [3].

According to J Apajalahti, the microflora of the poultry’s digestive system affects the digestive function of the microorganism itself, and in the case of its normal development, prevents colonization of the intestine by pathogenic bacteria, since normal flora enters into symbiotic relationships with the



carrier [4]. In other words, the authors emphasize the existence of a connection between various representatives of microorganisms that are in the poultry digestive system, which it needs for the digestive activity. In the case of disruption of the normal flora of the digestive system, associated with a change in species diversity or a change in the quantitative composition of the microbiome, we can observe dysbacteriosis or other gastrointestinal lesions. As an example of changes in the species composition of microbiome, we can consider cases of the development of pathogenesis of colibacillosis or pullorosis in birds, when livestock are infected with enteropathogenic *Escherichia* serotypes or *Salmonella*, which are not originally representatives of normal flora [5]. It should be noted that the microflora of the digestive system itself consists of more than 400 species of cultured bacteria, which in turn amounts to only 40% of the total background. Changes in the quantitative composition of the digestive microbiome should be associated with the use of antibacterial drugs when ABP has a selective effect thereby freeing a certain niche, or in the case of increased reproduction of representatives of the normal flora, which include clostridia associated with the appearance of predisposing factors [6]. Changes in the quantitative composition of the microbiome should be attributed to the constant intake of microorganisms with feed.

Long J R indicates that among chickens the most susceptible to the disease is a bird at the age of 2-6 weeks. The same author noted the possibility of necrotic enteritis (NE) outbreaks in poultry enterprises several times a year [7]. The period of the disease development has no definite boundaries, according to J R Long. In Canada, the problems with NE start in the period July-October, while in the UK and Norway, according to M Kaldhusdal and E Skjerve, the peak of NE happens in winter. Therefore, it is worth making the assumption that the pathology under consideration is not seasonal [8].

Confirmation of the infection development in humans after eating meat products affected by anaerobic pathogens is reflected in the work of medical specialists, in particular V G Novoselov and V I Sergevnnin [9]. According to P Gazdzinski and R J Julian, in addition to chickens, turkeys, quails, and ravens are susceptible to necrotic enteritis [10]. The age groups of birds of 2-6 weeks are the most susceptible to the disease. N Kozhemyaka associates it with the presence of a large amount of poor-quality soybean meal and fish meal in their diet, which the bird does not digest well [11]. The parental population of laying hens after the start of egg laying, and cocks after increasing the diet during the laying of chickens are subject to the disease.

Outbreaks of necrotic enteritis among birds are sporadic, but due to high mortality and productivity losses, they lead to significant economic losses.

According to M Kaldhusdal and M Hofshagen, the main pathological change in this disease is the development of necrotic lesions of the mucous membranes of the small intestine, especially the jejunum and ileum, but sometimes in the duodenal ulcer. The disease can occur both clinically, mainly from 14 days of life, and subclinically, in older age groups of birds, which is reflected in the works of F Al-Sheikhly and R B Truscott [12]. W Van der Sluis in his scientific works notes that the subclinical course of necrotic enteritis is most typical for birds of 21 days old [13]. According to C F Helmboldt with co-authors, the clinical signs of necrotic enteritis manifest as severe depression, dehydration, decreased mobility, diarrhea, ruffled plumage, reduced consumption. Death in the clinical manifestation of the disease occurs quite quickly, up to several hours, due to the toxic effect of the pathogen [14]. L Timbermont with co-authors described the cases of mass mortality of young birds from NE without the development of clinical signs of the disease [15]. The subclinical course of infection in birds is accompanied by an increase in feed conversion ratio and the detection of gross necrotic lesions in the mucous membranes of the small intestine, which is emphasized by M Kaldhusdal and M Hofshagen [16]. J T B Skinner and others clearly indicate that the subclinical form of necrotic enteritis leads to greater losses for the industry than the clinical form, which in turn is due to the fact that with this form of loss is associated with loss of productivity, increased costs of health and medical events, etc. [17]. This opinion is confirmed in the works of L Timbermont with co-authors, who determined that a tendency of the prevalence of subclinical manifestations over clinical ones is observed, while the disease proceeds without a high mortality rate [18]. The death of a bird according to T Fukata etc. is accompanied by diarrhea and necrosis of the mucous membrane of the small intestine [19].

According to M Kaldhusdal, O Evensen and T Landsverk the clinical manifestation of NE is a reduction in bird mobility, anorexia, and ruffled plumage [20]. Subsequently, a diseased bird has such

symptoms as limb lesions, manifested in the form of lameness; the bird constantly lies on the litter, which is noted in the works of R B Williams [21].

## **2. Pathogen**

The causative agent of necrotic enteritis in birds is bacteria of the *Clostridium perfringens* species producing toxins of A and C type. Anaerobic microorganisms of the genus *Clostridium* may not in all cases be considered as a pathogenic microorganism. In particular, it should be noted that clostridia are normal representatives of the poultry digestive system, into which they penetrate from the first days of life, and vice versa contribute to the development of digestive activity [22, 23].

According to some reports, bacteria of *Clostridium sordellii* species may be involved into the development of NE pathogenesis, which is described in detail in the work of G Rimoldi et al [24, 25].

## **3. Ways of the pathogen transmission**

A sick bird, the litter on which it is located, as well as feed should be considered the main source of the pathogen spread, which is reported in a number of scientific papers of D D Frame and A A Bickford [26]. Interesting conclusions were made by S M Shane et al, who established the possibility of transmitting the pathogen in a vertical way [27]. A S Dhillon with co-authors, in turn, were able to establish the possibility of pathogen transmission by flies [28].

## **4. Pathogenesis**

The pathogenesis of necrotic enteritis has not been fully studied in the opinion of a number of foreign experts, despite the proven role of clostridial toxins and the fact that clostridia themselves are normal representatives of the intestine of a bird [29-30]. According to T Shimizu and co-authors, one of the advantages of anaerobic microorganisms of the genus *Clostridium* is their ability for long-term preservation, as well as the ability of rapid growth [31]. The author notes that in the absence of readily available nutrients, these bacteria are able to survive due to the possibility of transition from the vegetative form to the spore. Toxins, which are factors in the pathogenicity of clostridia, are directly involved in the development of the disease. The most commonly mentioned type of toxin that is associated with NE is alpha toxin. Alpha-toxin has hemolytic, cytotoxic, necrotic properties, and therefore has a direct effect on the metabolism of the macroorganism, including inhibition of neutrophil chemotaxis, vasoconstriction and platelet aggregation [32].

In accordance with the data of foreign experts, the development of NE pathogenesis can be divided into several stages. Thus, according to L Timbermont the initiation of the pathological process occurs with colonization of the intestinal surface by clostridia, followed by degradation of these mucous membranes [33]. In response to colonization of the intestinal epithelium, microorganisms begin to produce mucus, which according to M A McGuckin with co-authors, is the main barrier against the adhesion of bacteria to the intestinal mucosa [34]. The authors note that the barrier function of mucus is caused by the presence in it of a large number of glycoproteins containing various antimicrobial substances. In the works of the group of authors, under the leadership of M Fujita it was determined that *Clostridium perfringens* are capable of producing glycoside hydrolases aimed at destroying specific components of mucus by inactivating them, which is why they are not able to protect intestinal mucous membranes from colonization [35]. At the same time, the authors also note that it is the strains of *Clostridium perfringens* isolated from birds in cases of NE manifestation that are the most active against antibacterial substances in intestinal mucous secretions. The dominant role of colonization and degradation of intestinal mucous membranes by clostridia is also due to the fact that glycoproteins, which are an integral part of mucous secretions, are one of the main components of the membrane surface of epithelial cells, which is reflected in the work of D Lepp with co-authors [36]. According to reports of S Nakjang et al an additional protective factor of the microorganism against colonization, the causative agent of NE is zinc metalloproteinase [37]. The second stage of the NE development according to X-X Yan begins with the fact that clostridia begin to produce NetB toxin, which leads to an increase in the porosity of the cell membranes, with the subsequent release of their contents into the lumen of the intestine, from which the toxin has arrived [38]. The destruction of intestinal mucosa cells leads to its thickening. M L Van Hoek cited the facts of the formation of biofilms of microorganisms, which is a

means of transmitting pathogenicity and antibiotic resistance factors, but in the case of NE development, it has a predominantly protective function for pathogens [39].

## 5. Predisposing factors

The most detailed role of predisposing factors was considered by J G Allaart et al S A and M'Sadeq with co-authors who created the classification of all factors contributing to necrotic enteritis into 4 groups, namely: 1) physical changes in the intestine; 2) changes in the immune status of the bird; 3) violation of the microbiome of the digestive system; 4) proliferation (increase in concentration) of *Clostridium perfringens* [40, 41]. All four classifications of predisposing factors will be discussed in detail below.

### 5.1. Physical changes in the intestines

According to C B Annett with co-authors, the diet of the bird is of great importance in the development of NE. Thus, the authors' team indicates that, for example, whole wheat has a certain effect on provoking infection, by altering the viscosity of the intestinal contents, and due to the presence of complex carbohydrates in the wheat, leads to uncontrolled reproduction of clostridia [42]. Similar data are given in the works of S L Branton et al who emphasize that the bird that eats wheat, barley, oats, rye is more susceptible to necrotic enteritis compared to the bird that eats corn [43]. This phenomenon is explained by the presence in grains of a high level of non-starch polysaccharides (non-starch polysaccharides (NSPs)), as already mentioned above, increasing the viscosity of the intestinal masses, and providing *Clostridia* with nutrients. P G Hermans and K L Morgan explain that due to the presence in the intestinal lumen of a large number of NSPs in birds, water consumption increases, due to which feces become more liquid. And in this state of aggregation, clostridia in feces are able to penetrate deeper into the litter and contaminate it, which means that litter becomes a factor in the spread of the causative agent of NE [44].

The severity of the disease depends on whether clostridiosis develops as a primary disease, or whether it develops against the background of a primary pathology, such as coccidiosis [45, 46]. Several authors' teams came to this conclusion at the same time, when in 2010, J A Smyth, and T G Martin, who, during the experimental infection, were able to find out and prove that in the form of a combined infection caused by *ameri* and clostridia, necrotic enteritis developed more likely than with the disease in monoinfection. But at the same time, these same authors emphasize the fact that in industrial conditions the disease can develop without the presence of *ameri*, but at the expense of other predisposing factors that are difficult to design in laboratory conditions [47]. According to F Al-Sheikhly et al, reliable in vitro reproduction of poultry necrotic enteritis due to a combined infection with eimeriosis is caused by physical damage to the intestinal mucous membranes, which opens up direct access of the pathogen to the basal layer of the intestine, and provoking immunosuppression [48]. The research team of F Van Immerseel, J D Buc, F Pasmans, G Huyghebaert, F Haesebrouck and R Ducatelle designates that one of the predisposing factors for NE development related to physical causes is intestinal blood with gastric bleeding, since blood is a rich source of nutrients for clostridia [49]. The group of authors of D Stanley describe the role of fish content in the diet for the development of NE, which provokes an increase in the level of nutrients for the growth of clostridia with a subsequent change in the microbiota profile [50].

### 5.2. Changes in the bird immune status

A Lovland et al found that a change in the bird immune status can lead to an increase in incidents of NE development [51]. The same authors found out that the peak of NE development associated with a change in immune status is 3 weeks of life, which is caused by the period of termination of maternal immunity, which is why a 3-week-old bird is most susceptible to NE [52].

In the works of F J Hoerr the dependence of the development of necrotic enteritis against the background of primary viral pathologies, in particular, Marek's disease, infectious bursal disease, and viral chicken anemia, leading to immunosuppression of the poultry organism, is clearly tracked [53]. Having the data, the group of authors, led by J L McReynolds etc. it became possible to reproduce the experimental infection with NE due to the use of birds previously infected with viruses [54].

Examples of NE development on the background of physiological stresses, for example, caused by overcoupling, vaccinations, etc., are referred to non-infectious factors of immunosuppression of the organism by V Tsiouris with co-authors. [55].

### 5.3. *Impaired digestive microbiome*

Until recently, the model of NE development implied that in the microbiome of the poultry digestive system there exists a basic set of microorganisms, including representatives of *Clostridium perfringens*, which under certain conditions multiplied uncontrollably, as mentioned by K K Cooper and J G Songer [56]. But thanks to a detailed study of the virulence factors of clostridia, it became clear that the mechanism of NE development is more complicated, since it was not possible to prove that *Clostridium perfringens* is always pathogenic for the bird.

Nevertheless, Y Feng et al and D Stanley et al during the experimental infection of NE, were able to establish that the microbiome of the digestive system of an infected bird was different from the microbiome of the control group not susceptible to infection [57-58].

We have previously mentioned the effect of coccidiosis as a predisposing factor of NE development, due to the fact that coccidiosis is involved as a physical factor in violation of the intestinal wall, as well as an immunosuppression factor, but do not forget that the effects of coccidiosis do not have local significance. In other words, it cannot be assumed that the consequences of coccidiosis contribute to the development of exclusively clostridiosis, since this is not the correct conclusion. Coccidiosis can activate any gastrointestinal bacterial infections, which in turn can lead to changes in the structural or quantitative state of the microbiome, which is described in a number of scientific papers of S Wu and others [59].

As evidence of the influence of the poultry digestive system normal microbiota for NE development R M La Ragione etc. demonstrated the role of probiotic preparations with competitive bacterial cultures in the fight against chicken necrotic enteritis [60-62].

### 5.4. *Clostridium perfringens proliferation*

Thanks to modern molecular biological methods research team of A J Barbara with collaborators could determine that in the digestive system of the bird affected by NE there is one type of toxin, *Clostridium perfringens* prevailing, while in healthy birds there are more types of toxins [63]. In view of this, it may be considered that there are clostridium strains capable of producing substances that inhibit toxins. These substances were described in the work of L Timbermont L with co-authors. They are perfrins, antimicrobial proteins, which are only found in strains carrying the netB gene [64]. Under laboratory conditions, the expression of perfrins of clostridia was able to inhibit non-pathogenic isolates of clostridia that do not have this protein, despite the fact that the growth of pathogenic cultures is enhanced by the inhibition of non-pathogenic. B Nauerby et al proved that in healthy birds, the genetic diversity of microorganisms of the *Clostridium perfringens* species is higher than in sick birds, which can also be associated with the inhibiting properties of clostridia that cause NE [65].

B Wade and A Keyburn have determined that pathogenic strains of clostridia, provoking the development of NE and possessing the netB gene, are more active than non-pathogenic cultures of this microorganism [66]. A significant contribution to the study of the mechanisms of propagation of pathogenic cultures of clostridia was made by T L Bannam with co-authors, who described the possibility of transferring NetB virulence plasmids from pathogenic strains of clostridia to non-pathogenic [67].

### 5.5. *Specific prophylaxis of necrotic enteritis*

Currently, no specific prophylaxis for necrotizing enteritis has been developed or registered on the territory of the Russian Federation, which, together with the increasing incidence of NE, emphasizes the relevance and necessity of developing such a remedy. Since the prevailing number of diseases caused by clostridia, toxoid biologics are the most effective for immunoprophylaxis, there have already been attempts to create such drugs based on toxins abroad. At the same time, foreign colleagues have already faced the problems of choosing the most effective type of clostridial toxins, for their use as the main component of the vaccine. So, according to R R Kulkarni with co-authors, as well as K K Cooper etc.

many researchers have already conducted tests of immunobiological agents against necrotic enteritis in birds, based on the alpha-toxin *Clostridium perfringens*, during which only their partial protective efficacy was established [68-70]. Similar results were obtained by B T Heier with co-authors who managed to determine that the presence of a high level of antitoxic antibodies in vaccinated blood was less susceptible to infection and death from necrotic enteritis, unlike birds with a low level of antitoxic antibodies.

According to the results of A L Keyburn and co-authors, as well as C G Savva et al NetB necrotic enterotoxin is a pore-forming toxin produced by *Clostridium perfringens*; and it plays an important role in the development of NE in poultry farming [71].

The author group of A Lanckriet et al in 2010 obtained results indicating that biopreparations made from the inactivated supernatant *Clostridium perfringens* possess the best protective efficacy in case of NE [72]. At the same time, the group of authors confirm that specific antigens responsible for the development of the pathogenesis of NE have not yet been established, and the effect of the use of clostridium supernatants is due to the presence of all types of toxins produced by clostridia in this supernatant. Important in the development of immunobiological agents against NE is the use of cultures of clostridia isolated directly from birds in the disease, since the use of other cultures not isolated from birds does not guarantee any protective efficacy.

## 6. Conclusion

Necrotic enteritis of birds is a disease that tends to be widespread in Russia. The lack of control over the infection causes not only obvious economic damage to the poultry industry, but also increases the risk of developing cases of toxic infections in humane medicine. Summarizing this part of the work, it can be considered that factors that change the physical properties of the intestine, damage to the intestinal epithelial lining of the intestinal mucosa due to changes in the size of feed particles, indication of the production of mucus or change in the time of passage of food through the intestine, factors that destroy normal flora digestive system, or alter the immune status of the organism, seasonal factors, changes in diet, both qualitatively and quantitative. Of course, a balanced diet made from high-quality raw materials is important in the prevention of any gastrointestinal diseases, but, unfortunately, the task of ensuring stable feedstock is not feasible for all enterprises. In the development of NE pathogenesis, an important role is played by the ability of clostridia cultures to produce perfrins and carry the NetB gene.

The analysis of the literature, justifies the feasibility of the fight and prevention of necrotic enteritis in birds, through the development and use of immunobiological agents - vaccines. Since the prevention of clostridiosis is widely used in the pig, livestock, sheep industries, and has positive feedback from the use of vaccines, vaccination of birds from clostridial infections is highly relevant [73-76]. This relevance is additionally underlined by a number of foreign specialists whose work is focused on creating new immunobiological agents against NE. The task is currently being carried out by the Russian leading research institute - the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV".

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