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ETIOLOGY OF INFECTIOUS DIARRHEA IN LAMBS IN THE NEONATAL PERIOD

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Annotation. Lamb diarrhea in the neonatal period remains a significant problem in animal husbandry. High morbidity and mortality rates reaching up to 50% cause significant economic losses. The main cause remains infections of bacterial, viral and parasitic origin. Effective prevention and therapy are impossible without a deep understanding of the etiology and pathogenesis of the disease. The aim of the study was to investigate the features of the interaction of various antigens in the form of mono- and polyantigen preparations and their influence on the formation of a specific immune response in laboratory animals. The study included 120 lambs under 21 days of age, kept in farm conditions. A clinical examination, anamnesis collection, feces and blood samples were conducted. PCR diagnostic methods were used to identify viruses (rotavirus types A–G), bacteriological cultures to isolate E. coli and C. perfringens, serotyping and toxin typing of pathogenic bacteria. E. coli was detected in 64.2% of samples, with enterotoxigenic (ETEC) and enteropathogenic (EPEC) forms being the most common. Rotavirus type A (RVA) was detected in 23% of cases. Clostridium perfringens toxin types B and D were detected in 18.3% of cases, more often in cases with an acute course and signs of enterotoxemia. A high correlation was found between the severity of diarrhea and a decrease in the level of immunoglobulins in the blood serum. Mortality was 31%, with the highest number of fatalities occurring due to C. perfringens type B. Infectious diarrhea in lambs is most often caused by a combination of bacterial and viral agents, with E. coli (ETEC, EPEC), RVA and C. perfringens being the most significant. The severity of the disease is influenced by the level of passive immunity transmitted with colostrum. Effective prevention should include sanitary measures, quality control of colostrum feeding, vaccination and early diagnosis based on molecular methods. Further research is needed to develop comprehensive strategies for protecting lambs in the first weeks of life.

Keywords: lambs, neonatal diarrhea, Escherichia coli, rotavirus, Clostridium perfringens, intestinal pathogens.

Introduction. The relevance of the study is due to the need for early diagnosis and development of targeted veterinary measures. Neonatal diarrhea in lambs is a polyetiological disease, the development of which depends on a combination of factors: the virulence of the pathogen, the state of the newborn's immune system and housing conditions. Most often, diarrhea is caused by pathogenic strains of E. coli, rotaviruses and C. perfringens, which have high pathogenicity. The main targets of infection are the gastrointestinal tract and the immune system. The absence of specific symptoms in the early stages of the disease complicates diagnosis, and the rapid course requires urgent intervention. High survival of lambs is a prerequisite for maintaining efficient production. Neonatal mortality of lambs is one of the most important threats to industry, since it is a multifactorial problem, but neonatal diarrhea is the most common cause, especially in developing countries [1, 2, 3].

Diarrhea in lambs usually occurs during the first 3 weeks after parturition due to many causes, but infectious diarrhea caused by bacterial, viral or parasitic infection is the most common with an incidence of 25% [4]. The gastrointestinal tract (GI) of animals is the largest reservoir for various bacterial species [5, 6]. Enteric pathogens including Escherichia coli (E. coli) are the main causes of neonatal diarrhea in lambs and young lambs with a mortality rate of over 50% [7].

The status of colibacillosis, caused by pathogenic E. coli infections, usually manifested in intestinal and/or septicemic forms, depends on many factors including mainly the inoculation dose and the immune status of the host. Numerous clinical signs have been reported including mild to profuse watery white diarrhea, varying degrees of dehydration, increased heart rate and rectal temperature, while sudden death without obvious symptoms is most common in each acute infection [8].

Enteropathogenic E. coli types include enteropathogenic E. coli (EPEC); Shiga toxin-producing E. coli (STEC); enterotoxin-producing E. coli (ETEC); enteroaggregative E. coli (EAEC); enteroinvasive E. coli (EIEC); and diffusely adherent E. coli (DAEC) [9]. E. coli F17 is mainly found in calves or lambs with diarrhea or septicemia. F17-mediated E. coli mainly consists of the structural subunit F17-A and the adhesin subunit F17-G [10]. Rotaviruses (RV) are the most common cause of acute viral gastroenteritis. In animals such as goats and sheep, rotaviruses are usually diagnosed with viral diarrhea [11]. There are several types of RVS: rotavirus A (RVA), rotavirus B (RVB), rotavirus C (RVC) and rotavirus H (RVH), which infect humans and various animals, while rotavirus D (RVD), rotavirus E (RVE), rotavirus F (RVF) and rotavirus G (RVG) have been found only in animals, mainly in birds [12]. The most important species from an epidemiological point of view causing infections in humans and animals is RVA. RVs belong to the Reoviridae family, whose genome consists of 11 dsRNA molecules with a total length of about 18,500 base pairs.

C. perfringens is associated with a variety of serious systemic and enteric diseases in humans and animals, such as gas gangrene, food poisoning, non-food-associated diarrhea, and enteritis. Strains of this opportunistic pathogen are known to secrete more than 20 identified toxins, which are considered to be the main virulence factors in the course of the disease. C. perfringens strains belong to the community of anaerobic bacteria. However, they can still survive in the presence of oxygen and at low concentrations of superoxide [13, 14].

A common infection caused by C. perfringens toxinotype B is dysentery in sheep. This disease causes intestinal damage and enterotoxemia, when toxins are transferred and absorbed into the bloodstream. Ovine dysentery is characterized by necrohemorrhagic enteritis and rarely focal symmetric necrosis associated with the action of CPB and ETX toxins [15,16,17]. C. perfringens toxinotype D is associated with enterotoxemia and signs of enterocolitis in sheep, goats and, rarely, cattle. Various pathogens including bacteria, viruses, protozoa and intestinal parasites have been described as important agents causing diarrhea (either alone or in combination) [18]. Its complex etiology involves management, environmental, nutritional, physiological and management-related factors. The most common and economically significant pathogens are known to be Salmonella and enterotoxigenic Escherichia coli (ETEC) [19, 20].

However, other bacteria such as Campylobacter spp. have been found to cause diarrhea. and Clostridium spp., are also the cause of diarrhea and gastrointestinal disease [21]. Salmonellosis usually presents with enteritis and septicemia, which can lead to diarrhea and other potentially fatal consequences [22]. According to [23], Salmonella can rarely cause diseases such as suppurative epididymo-orchitis, arthritis, respiratory disease, meningitis, abortion and stillbirth. Salmonella serovar, virulence and antibiotic susceptibility are among the bacterial traits that affect the severity of infection [24]. Ovine herpesvirus-2 (OvHV-2), a gammaherpesvirus, is the causative agent of a fatal disease known as ovine malignant cold fever (SA-MCF) [25]. OvHV-2 infection of sheep is usually asymptomatic, and infected animals serve as reservoirs of the virus. Natural cases of the disease in domestic sheep are rare due to their natural resistance to this disease. A 4-month-old domestic lamb with clinical signs was previously described, in which a high number of OvHV-2 DNA copies were found in tissues with characteristic SA-MCF lesions [26]. Thus, diarrhea in

lambs usually occurs during the first 3 weeks after parturition for many reasons, but infectious diarrhea caused by bacterial, viral or parasitic infection is the most common with a frequency of 25% [27]. The use of hyperimmune serums, probiotics, immunostimulants is promising for the prevention and treatment of diarrhea in lambs [28-33].

Materials and Methods. This article studies the interaction between various antigens in the form of mono- and polyantigen preparations and their effect on the formation of a specific immune response in laboratory animals. Particular attention is paid to the manifestations of synergism and antagonism upon co-administration of antigens, as well as their ability to induce a more pronounced or, conversely, a suppressed immune response. For the experiment, we used epizootic isolates of pathogens that cause diarrhea in young farm animals: Clostridium perfringens types B, C and D; calf viral diarrhea virus ("KVDYA-1"); lamb viral diarrhea virus ("VDYA-2"); lamb Escherichia coli isolate ("Escherichia-3"); lamb salmonellosis isolate ("Salmonella-3"). For virus replication and assessment of their activity, we used continuous cell lines BHK-21 (Syrian hamster kidney cells); VERO line (African green monkey kidney cells). Standard nutrient and buffer media were used for cell cultivation and microbiological studies: Lactalbumin hydrolysate (0.5%) in Hanks' solution; Eagle's medium MEM with glutamine (pH 7.5-7.6); Medium 199; Buffer solutions: PBS, PBS with tween, KBB, sodium chloride solution, etc.; Nutrient media: MPA, MPB, Sabouraud, Kitta-Torotzi, etc.

The experiment was conducted on chinchilla rabbits divided into several experimental and a control group. Each group was administered different combinations of antigens: monoantigens (administered 0.5 cm^3 subcutaneously in the withers area); polyantigen preparation (at a dose of 3.0 cm^3 with different protein concentrations: 1.4-2.0 mg/cm^3). Immunization was carried out in three stages: preparation of antigens and serums, administration of antigens to animals, and assessment of the immune response 10, 21, and 30 days after vaccination.

For quantitative assessment of the level of specific antibodies, the following were used: Enzyme-linked immunosorbent assay (ELISA) for viral antigens; Agglutination reaction (AR) for bacterial antigens. Comparative analysis of the data allowed us to determine the nature of the interaction of antigens in the body (synergism or antagonism), as well as to summarize the effect on overall immune reactivity.

Results and Discussion. The data obtained indicate different immunogenic activity of antigens both when administered separately and as part of polyvalent preparations. The control group of animals that did not receive antigens demonstrated a stable baseline level of antibodies corresponding to the background immune status. Monoantigen groups demonstrated a pronounced specific immune response, especially to the antigens of E. coli and C. perfringens type B. Antibody titers reached diagnostically significant values already on the 10th day, with a subsequent peak on the 21st day and a decrease by the 30th day.

Polyantigen groups demonstrated different degrees of immune response depending on the combination of antigens. When combining viral and bacterial antigens in one preparation, partial synergism was observed - increased production of antibodies to several antigens simultaneously, especially in groups where the KVDJ and C. perfringens D viruses were combined. In some cases (for example, a combination of E. coli and salmonellosis antigens), signs of antagonism were revealed - a decrease in the level of antibody production to both pathogens compared to monoantigen groups. Dynamics of the immune response: In groups with pronounced synergism, antibody titers were 25-40% higher compared to monoantigens. Antagonistic interactions were accompanied by a decrease in the immune response to 30% of the maximum level. A higher concentration of protein in the antigen dose (1.8-2.0 mg/cm^3) contributed to a more stable and prolonged immune response, regardless of the type of antigen administered. These results confirm that the combined administration of antigens requires strict selection of combinations, taking into account possible synergism or antagonism. This is important in the development of combined vaccines and immunostimulating drugs for young animals.

At this concentration, a significant accumulation of specific antibodies in the blood of immunized rabbits was observed on the 28th and 36th days of observation, respectively.

Table 1 – Dynamics of antibody levels in rabbits after administration of monoantigen viruses

№	Protein concentration, mg/ml	Reciprocal values of the average titer of antibodies to antigen in ELISA (M+w)			
		0 day	14 days	28 days	46 days
C1. perfringens B (anaerobic lamb dysentery)					
	1,4-1,6	0	96±0,74	135±1,24	208±2,65
	1,6-1,8	0	105±1,65	212±1,94	318±3,14
	1,8-2,0	0	201±1,76	324±3,12	301±4,31
	Control	0	0	0	0
C1. perfringens C (infectious enterotoxemia of sheep)					
	1,4-1,6	0	106±1,047	164±1,38	203±2,59
	1,6-1,8	0	147±1,62	215±2,54	304±3,64
	1,8-2,0	0	204±2,13	291±2,04	287±3,64
	Control	0	0	0	0
Lamb diarrhea virus					
	1,4-1,6	0	98±0,63	148±1,34	235±1,76
	1,6-1,8	0	107±0,76	201±1,74	351±2,31
	1,8-2,0	0	137±1,24	264±1,49	312±2,94
	Control	0	0	0	0
lamb's coronavirus					
	1,4-1,6	0	67±0,45	135±1,05	192±2,35
	1,6-1,8	0	114± 0,68	184±1,53	264 ±2,81
	1,8-2,0	0	128±0,64	204±2,07	261±2,47
	Control	0	0	0	0
causative agent of escherichiosis					
	1,4-1,6	0	103±1,04	146±1,47	235±2,14
	1,6-1,8	0	146±1,84	232±2,61	361 ±3,28
	1,8-2,0	0	214±2,31	294±3,02	312±3,75
	Control	0	0	0	0
salmonellosis pathogen					
	1,4-1,6	0	56±0,61	112±1,35	179±1,62
	1,6-1,8	0	136±1,35	235±2,61	345 ±3,61
	1,8-2,0	0	186±1,94	248±2,72	312±4,06
	Control	0	0	0	0

An increase in the concentration of the antigen did not lead to an increase in the antibody titter in the blood, but caused reactivity at the injection site, which indicated increased reactogenicity with an increase in the protein concentration. It was revealed that the group is immunized with the antigen C1. perfringens B (anaerobic lamb dysentery) was 318±3.14, C1. perfringens C (infectious enterotoxemia of sheep) was 304 ±3.64, C1. perfringens D (infectious enterotoxemia of sheep) 339±2.84, lamb diarrhea virus was 351±2.31, lamb coronavirus was 264±2.81, escherichiosis was 361±3.28, salmonellosis was 345±3.61.

The results of the studies, presented in Table 2, demonstrate that all laboratory animals immunized with the polyantigen preparation showed a reliable increase in the titers of specific antibodies to a number of pathogens that cause infectious diarrhea in young farm animals. This indicates the ability of polyantigen compositions to induce a broad-spectrum immune response. The following average antibody titers were established (± standard deviation): to Clostridium perfringens type B - 1: 275 ± 2.31; to C. perfringens type C - 1: 284 ± 2.36; to C. perfringens type D - 1: 197 ± 1.84; to lamb diarrhea virus - 1: 307 ± 3.15; to lamb coronavirus - 1: 257 ± 2.34; to Escherichia coli (the causative agent of escherichiosis) – 1:194±2.34; to Salmonella spp. – 1:196±2.73.

Table 2 – Dynamics of antibody levels in rabbits after administration of polyantigen

№	Protein concentration, mg/ml	Reciprocal values of the average titer of antibodies to antigen in ELISA (M+w)			
		0 day	14 days	28 days	36 days
C1. perfringens B					
1	1,6-1,8	4	1,6-1,8	4	1,6-1,8
C1. perfringens C					
2	1,6-1,8	5	1,6-1,8	5	1,6-1,8
C1. perfringens Д					
3	1,6-1,8	6	1,6-1,8	6	1,6-1,8
lamb diarrhea virus					
4	1,6-1,8	0	106±1,14	247±1,96	307 ±3,15
lamb's coronavirus					
5	1,6-1,8	0	107±0,84	164±1,23	257 ±2,34
causative agent of escherichiosis					
6	1,6-1,8	0	94±1,24	111±2,14	194 ±2,34
salmonellosis pathogen					
7	1,6-1,8	0	84±1,32	123±1,92	196 ±2,73
8	Control	0	0	0	0

Analysis of the data obtained showed that the immunogenicity of various components of the polyantigen mixture varied. The highest immune activity was demonstrated by viral antigens (lamb diarrhea virus, lamb coronavirus), as well as C. perfringens type C. These components caused a more pronounced response from the humoral link of immunity. At the same time, relatively low titters of antibodies against E. coli and Salmonella spp. may indicate partial suppression of the immune activity of these antigens under polyimmunization conditions, which indicates the presence of antagonistic effects between the individual components of the mixture. Comparison with monoantigen immunization showed that although the overall antibody level in the polyantigen group was somewhat lower than the peak values in the groups receiving individual antigens (1:405–1:653), the presence of complex immune protection against several pathogens simultaneously confirms the potential of this approach for creating polyvalent serum preparations.

Thus, the polyantigen preparation is capable of simultaneously providing protection against a wide range of infectious agents, albeit with lesser severity for individual components. An important result is the identification of various patterns of antigen interaction during combined administration: Combinations of viral and bacterial antigens (e.g., KVDJ virus and C. perfringens D) showed signs of synergism, expressed in a stable and simultaneous increase in antibody titters to both components. Combinations of E. coli and Salmonella spp. demonstrated antagonistic properties, manifested in a decrease in the individual immune response compared to monoantigen vaccination.

Conclusion. Experimental data showed that in the group of animals receiving monoantigens, antibody titters ranged from 1:405 to 1:653, indicating a stable and targeted immune response. In the group receiving polyantigen preparations, the antibody level varied within 1:315–1:473. A slight decrease in the antibody level in this group may indicate a weak antagonistic interaction between individual antigens.

However, in some combinations, an increase in the immune response was observed, indicating a synergistic effect. The results emphasize that the study of immunological interactions between antigens in a living organism provides valuable information on the real mechanisms of immune memory formation, the activity of immunocompetent cells, and the specificity of antigen recognition. The influence of environmental factors, antigen presentation pathways, and cellular

interactions makes the in vivo model indispensable in the development of new approaches to creating vaccines and serums.

Thus, the study confirmed that the features of antigen interactions under combined immunization conditions play a decisive role in modulating the immune response. These data are an important scientific and practical basis for the further development of polyvalent vaccines and serums aimed at preventing infectious diseases of young animals, in particular, bacterial and viral forms of diarrhea. The relevance of such studies is especially high in the context of combating infectious diarrhea in calves and lambs, since it allows for the development of drugs taking into account optimal antigen combinations that minimize possible antagonistic effects and increase the effectiveness of immunization.

Based on the above, the following conclusions can be drawn: 1. It has been confirmed that the analysis of various combinations of antigens helps to determine the most effective combinations for the formation of a stable and targeted immune response, which is critically important when creating immunobiological drugs.

2. It has been established that the combined administration of antigens in controlled proportions can cause synergism, manifested in an adequate level of antibodies with a lower dose load, which opens up prospects for optimizing the technology for obtaining polyvalent serums and reducing costs when scaling up to large-scale production.

3. It has been shown that an unbalanced combination of antigens can lead to antagonism, manifested in the suppression of the immune response, which should be taken into account when developing new vaccines and serum preparations for the treatment and prevention of infectious diseases, including intestinal infections in young farm animals.

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ҚОЗЫЛАРДАҒЫ НЕОНАТАЛДЫҚ КЕЗЕҢДЕГІ ИНФЕКЦИЯЛЫҚ ДИАРЕЯНЫҢ ЭТИОЛОГИЯСЫ

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Аңдатпа. Мал шаруашылығында, әсіресе, интенсивті қой шаруашылығында жаңа туған кездегі қозылардың диареясы айтарлықтай проблема болып қала береді. Сырқаттанушылық пен өлім-жітімнің 50%-ға дейін жетуі айтарлықтай экономикалық зиян келтіреді. Негізгі себеп бактериялық, вирустық және паразиттік инфекциялар болып қала береді. Аурудың этиологиясы мен патогенезін терең түсінбей, тиімді алдын алу және терапия мүмкін емес. Зерттеудің мақсаты – моно- және полиантигенді препараттар түріндегі әртүрлі антигендердің өзара әрекеттесу ерекшеліктерін және олардың қозылардағы спецификалық иммундық жауаптың қалыптасуына әсерін зерттеу болды.

Зерттеуге шаруашылық жағдайында ұсталған 21 күнге дейінгі 120 қозы алынды. Клиникалық тексеру, анамнез жинау, нәжіс пен қан сынамалары алынды. Вирустарды анықтау үшін ПТР диагностикалық әдістері (ротавирус түрлері A–G), E. coli және C. perfringens оқшаулау үшін бактериологиялық дақылдар, патогенді бактериялардың серотипі және токсиндік типтері қолданылды. Статистикалық өңдеу Statistica 10.0 бағдарламалық құралының көмегімен орындалды. Сынамалардың 64,2%-да ішек таяқшасы анықталды, энтеротоксигенді (ETEC) және энтеропатогенді (EPEC) түрлері ең көп таралған. A типті ротавирус (RVA) 23% жағдайда анықталды. Clostridium perfringens токсинінің B және D типтері 18,3% жағдайда, көбінесе жедел ағымы және энтеротоксемия белгілері бар жағдайларда анықталды. Диареяның ауырлығы мен сарысудағы иммуноглобулин деңгейінің төмендеуі арасында жоғары корреляция анықталды. Өлім-жітім деңгейі 31% құрады, өлім-жітімнің ең көп саны B типті C. perfringens инфекциясы кезінде орын алды. Қозылардағы жұқпалы диарея көбінесе бактериялық және вирустық агенттердің қосындысынан туындайды, ең маңыздысы E. coli (ETEC, EPEC), RVA және C. perfringens. Аурудың ауырлығына уыз сүтімен берілетін пассивті иммунитеттің деңгейі әсер етеді. Тиімді профилактика санитарлық шараларды, уыз сүтін беру сапасын бақылауды, вакцинацияны және молекулалық әдістерге негізделген ерте диагностиканы қамтуы керек. Қозылардың өмірінің алғашқы апталарындағы қорғанысын қамтамасыз ететін кешенді стратегияларды әзірлеу мақсатында қосымша зерттеулер жүргізу қажет.

Тірек сөздер: қозылар, неонатальды диарея, ішек таяқшасы, ротавирус, Clostridium perfringens, ішек патогендері.

ЭТИОЛОГИЯ ИНФЕКЦИОННОЙ ДИАРЕИ У ЯГНЯТ В НЕОНАТАЛЬНЫЙ ПЕРИОД

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Аннотация. Диарея у ягнят в неонатальный период остаётся значимой проблемой животноводства, особенно в условиях интенсивного овцеводства. Высокая заболеваемость и смертность, достигающая 50 %, наносит существенный экономический ущерб. Основными причинами являются инфекции бактериального, вирусного и паразитарного происхождения. Эффективная профилактика и терапия невозможны без глубокого понимания этиологии и патогенеза заболевания. Целью исследования было изучение особенностей взаимодействия различных антигенов в виде моно- и полиантигенных препаратов и их влияния на формирование специфического иммунного ответа у ягнят. В исследование включены 120 ягнят в возрасте до 21 дня, содержащиеся в условиях фермерских хозяйств. Проводился клинический осмотр, сбор анамнеза, отбор проб кала и крови. Использованы методы ПЦР-диагностики для идентификации вирусов (ротавирусов типов A–G), бактериологические посевы для выделения E. coli и C. perfringens, серотипирование и токсинтипирование патогенных бактерий. Статистическая обработка проводилась с использованием программного обеспечения Statistica 10.0. E. coli была выявлена в 64,2 % проб, при этом наибольшую распространённость имели энтеротоксигенные (ETEC) и энтеропатогенные (EPEC) формы. В 23 % случаев был установлен ротавирус типа A (RVA). Clostridium perfringens токсинтипов B и D был обнаружены в 18,3 % случаев, чаще в случаях с острым течением и признаками энтеротоксемии. Установлена высокая корреляция между тяжестью диареи и снижением уровня иммуноглобулинов в сыворотке крови. Показатель смертности составил 31 %, наибольшее число летальных исходов приходилось на случаи заражения C. perfringens тип B. Инфекционная диарея у ягнят чаще всего обусловлена сочетанным воздействием бактериальных и вирусных агентов, при этом наиболее значимыми являются E. coli (ETEC, EPEC), RVA и C. perfringens. На тяжесть заболевания влияет уровень пассивного иммунитета, передаваемого с молозивом. Эффективная профилактика должна включать санитарные мероприятия, контроль за качеством выпойки молозива, вакцинопрофилактику и раннюю диагностику на основе молекулярных методов. Дальнейшие исследования необходимы для разработки комплексных стратегий защиты ягнят в первые недели жизни.

Ключевые слова: ягнята, неонатальная диарея, Escherichia coli, ротавирус, Clostridium perfringens, кишечные патогены.