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The Effectiveness Of The Virus Vaccine Against Infectious Bursal Disease Of Birds From Strain "ST".

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ABSTRACT

The development of a new effective, safe vaccine against infectious bursal disease of birds is one of the urgent tasks of modern biological science. The disease was first described in 1957 in the United States with broiler chickens in the area of Gumboro, Delaware, from where it got its original name. The disease manifests itself in different climatic zones and poultry farms that are different in the direction of activity. Specific factors predisposing to the appearance of the disease other than age, is not installed. The incidence and waste among chickens of egg crosses is higher than meat ones. The purpose of this study was to study the effectiveness of an experimental vaccine against infectious bursal disease in laboratory and industrial conditions. In the studies, the virus vaccine was used from the strain "ST" of the IBB virus with immunological activity of 6.5 TCD50 / ml and the reference virulent strain "52/70". It should be noted that recently a low degree of formation of post-vaccination immunity against IBD is often revealed, resulting in a breakthrough immunity and death of the vaccinated bird, therefore, the lack of the expected effect of vaccinations creates prerequisites for the improvement of existing and development of a new vaccine. Experiments were carried out in the educational farm of Gorsky State Agrarian University and the poultry farms of the Republic of North Ossetia-Alania on the POSS-308 broiler cross-breeding poultry. immunity against the "standard" strains, additional difficulties in the prevention of IBD. The reason for the emergence of variant strains or subtypes of the IBB virus is associated with the large-scale use of vaccines and the occasional appearance of viral reassortants between vaccine and field strains. Highly virulent strains of the IBB virus isolated in Europe and Russia, unlike the variant strains of the IBB virus, do not have antigenic differences, but are characterized by high pathogenicity. Currently, highly virulent strains of the classic first serotype are widespread and cause acute outbreaks of the disease in many countries of the world, including in Russia.

Keywords: antibodies, virus, reactogenicity, passaging, dose, immunogenicity, virulence, prevention.

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INTRODUCTION

Epizootic and economic well-being of poultry enterprises is based largely on timely specific prevention of poultry against viral etiology, the most factorial of which is infectious bursal disease (IBD).

Many authors point to age-related features of infection. In chickens up to the age of 7-19 days, the disease proceeds without clinical signs and is not accompanied by death, however, immunosuppression in recovered chickens in early age is most pronounced. Chickens older than 21 days of age suffer severely, with significant withdrawal, but less pronounced immunosuppression [1,2,3].

The spontaneous manifestation of IBB is found in the 16 and 20 week birds. The death of a bird was 3.5%. [4] An adult bird, although not susceptible to infection, may contribute to the circulation of the virus in the farm. Relapse of the disease in the fallen bird has not been established.

Transferred at an early age IBD leads to a slowdown in the growth and development of chickens and the manifestation of secondary infections, negatively affecting the results of immunization with the introduction of various vaccines [4]. It is generally accepted that the older the chickens, the higher the incidence, the death of birds and, accordingly, higher production costs and economic losses. In this regard, preventive immunization of day-old chicks is particularly important [5].

Currently, the existence of II serotypes of IBBV virus is established: the first was isolated from sick chickens, the second from turkeys. There are 6 subtypes of serotype I. These subtypes are somewhat different in antigen relation from the classical I serotype of the IBB virus, and conventionally they are called “variant strains” [6]. For serotyping of different strains of the IBB virus, a neutralization cross-reaction with monoclonal antibodies is used, the results of which determine the percentage of kinship between the strains under study: homology is less than 10% - differences in serotype; homology 10-32% is a large subtype; homology 33-70% - small subtype; 71-100% homology is a slight difference or lack thereof. Standard and highly virulent strains of IBB serotype I cause immunosuppression [6].

It is known that the molecular basis of the antigenic variability of the IBB virus is the replacement of amino acids in the variable hydrophobic domain of the VP2 viral protein (in the region of 206-350 amino acid residues). Comparison of the structure of the viral protein VP2 (its hydrophobic domain) of known epizootic virus strains made it possible to distribute them, according to antigenic properties, as a phylogenetic tree in order to further study their genetic relatedness [7,8,9,10,11].

Phylogenetic analysis based on the segment showed that the highly virulent strains of the IBB virus form a separate cluster and are more closely associated with classical virulent strains, such as “52/70”, compared to the other groups of strains. But, on the other hand, a comparative analysis of the primary structure of the genomic segment B showed that the VP1 protein of highly virulent strains differs significantly in structure from the VP1 classical virulent, variant, attenuated strains and even serotype II strains.

To date, virulence markers are unknown and attempts to classify viruses on the basis of virulence have failed. Studies of the molecular basis of virulence are carried out quite intensively by many researchers using all known methodological approaches of molecular biology and genetics, such as the polymerase chain reaction (PCR), transfection of cDNA vector in chicken embryo fibroblast cells, cloning of full-sized fragments viral dsRNA, the creation of genetic reassortants, recombinants, mutants. Judging by preliminary data, the virulence factor is not associated with any one heenom, but has a polygenic nature.

A molecular diagnostic method based on the polymerase-valued reverse transcription reaction (RT-PCR) using amplification of the VP2 gene of the IBB virus is widely used in the detection of serotypes and, to a lesser extent, in differentiation of the subtypes of this pathogen. Real-time RT-PCR is used as the primary tool for the diagnosis of IBB. This method allows potentially quickly differentiating classical strains from highly virulent and variant strains of the IBB virus [12,14,15,16.17,18].

At present, antigenic heterogeneity of strains of the IBB virus isolated in Russia and other countries is noted, with which the failure of the use of existing live vaccines in the prevention of disease is attributed. In

this regard, of interest in the study of antigenic differences and the relationship between strains of the pathogen IBB.

Given the above, issues of improving existing and creating new means of specific prevention of IBD remain highly relevant.

The purpose of this study was to study the effectiveness of an experimental vaccine against infectious bursal disease in laboratory and industrial conditions. In the studies, the virus vaccine was used from the strain "ST" of the IBB virus with immunological activity of 6.5 TCD50 / ml and the reference virulent strain "52/70".

MATERIALS AND METHODS

In the experiments, the strain ST of the IBB virus was used, obtained by passaging 1 epizootic isolate obtained from a sick bird at the Vladikavkaz poultry farm of the Republic of North Ossetia-Alania.

The IBD virus was isolated from fabric bags of bird corpses that showed a clinical picture of the disease or were killed for diagnostic purposes. When isolating the IBD virus, attention should be paid to the possibility of contamination by viruses of other taxonomic groups, which are also reproduced in a fabric bag, for example, the leukemia virus and the adenovirus of birds.

Homogenate of fabrication bags (10%) after testing for sterility infected 10-11 days old embryos of SPF chickens in a volume of 0.2 ml per chorioallantoic membrane. Infected embryos were placed in a thermostat at 37.5 ° C and ovoskopirovany twice a day. Embryos that died in the first 48 hours after infection, did not take into account (non-specific death). Embryos that died after 3 days and later, and all remaining alive on the 7th day after infection, were cooled in a refrigerator at 4 ° C for 4-5 hours, then they were opened and lesions were taken into account. The most characteristic of the latter were: the presence of hemorrhage in the head, neck, extremities, edema of the abdominal cavity, as well as lag in growth and development, congestion in the lungs and the decomposition of the kidneys, enlarged liver with many necrotic foci. Chorioallantoic membrane in infected embryos is swollen, with signs of hemorrhagic inflammation. In the absence of specific changes in infected chick embryos, up to 5 passages were performed on embryos of SPF chickens in the first passage, using a suspension from HAO and embryos from the previous passage to infect.

The bioassay was performed on a 21-day-old bird or a 35- to 40-day-old bird obtained from an industrial flock. The test material, prepared in the same way as for infection of embryos, was administered orally to chickens in a volume of 0.5 ml or intranasally - 0.2 ml. Non-infected chickens kept in an isolated room under similar conditions were used as controls. Birds were observed for 7 days.

Experimental infection with IBD of chickens free of passive antibodies leads to the development of infection 36-48 hours after infection with the introduction of even minimal doses of a pathogenic virus. The prerequisites for a successful biological test on chickens are: the age of the bird at the time of the experiment, the lack of maternal antibodies and the presence of a developed factory bag.

Despite the duration of the implementation, significant labor and monetary costs, the neutralization reaction (NR) is the most reliable method for identifying the isolated virus and detecting specific antibodies. For its production, it is necessary to have embryos sensitive to the virus, a known virus, specific and studied sera. The level of neutralizing antibodies depends on the age of the chicks used for infection. A comparative study of the titer of neutralizing antibodies in chickens 3 and 4 weeks of age found that in the second case, their level was higher.

NRformulation is carried out in two ways:

a) with a constant dose of the virus and different dilutions of serum;
b) with a constant concentration of serum and a different dose of the virus.

Usually, the option "a" is used. NR is considered positive if the embryos do not die and they do not see changes in the IBB virus when they are dissected.

At present, a simplified method of setting NR in cell cultures is widely used.

First, 0.05 ml of the virus in a dose of 100 TCD50 / ml was added to the culture medium in the wells on a plate. Pre-investigated sy-cranks were inactivated at 56 ° C for 30 minutes. Of these, a series of two-fold dilutions was prepared and added to the suspension of the virus in the wells. After 30 minutes of incubation at room temperature, 0.2 ml of fibroblasts of embryos of SPF chickens with a cell concentration allowing to obtain a monolayer after 24 hours of incubation were introduced into the wells on the plates. The plates were closed and placed in a thermostat at 37 ° C in an atmosphere with 5% carbon dioxide for 4-5 days, after which they were carefully examined under a microscope for the presence of cytopathogenic action (CPD). The limiting dilution of serum, in which the JRS did not appear, was taken for its titer. In the control, an immune serum with a known titer was used.

Enzyme-linked immunosorbent assay (ELISA) is the most sensitive diagnostic method, far superior to other immunological reactions.

The results were considered positive if the solution in the wells with the test antigen acquired a brown color. The solution in the wells with a negative antigen, the control of the conjugate and the control of the substrate should be colorless.

In the study of blood sera of chickens in ELISA and RDP, ELISA was the most sensitive. The geometric mean ELISA titer ranged from 1 to 7.4 log, in the RDP - 2.7-3.8 log. The number of positive samples was also higher in the ELISA than in the RDP, 78.3 and 54.7%, respectively.

Computer software has been developed that allows both to quickly and statistically reliably process the data.

RESULTS AND DISCUSSION

It was established that the use of a lyophilized culture vaccine from the “ST” strain possesses a biological activity of 6.5 TCD50 / ml.

The study of three epizootic isolates isolated by us in the poultry farms of North Ossetia-Alania: (No. 1 - Vladikavkaz; No. 2 - Mikhailovsky; No. 3 - Nogirsky), and the reference 52/70 virus strain was performed in a cross-section RDP with strain-specific sera , which showed that all these strains of the virus have the same precipitogenic activity and form the same precipitation lines. The activity of immune sera in the cross-section RDP was almost the same with respect to homo- and heterologous antigens, which indicates the presence of common virus-specific antigenic determinants. Strains of IBD virus have a common group antigen, which is involved in the RDP, immunofluorescence reaction and enzyme-linked immunosorbent assay. The capsid proteins VP2 and VP3 contain epitopes that are responsible for the group antigenicity of the virus.

In addition to the common group antigen, the IBD virus contains antigen or antigens responsible for the specificity associated with a particular serotype. For serotyping of the virus, an in vitro neutralization reaction was used with a specific immune system for each serotype or invivo.

Given the above data, we conducted an experiment to study the cross-resistance of chickens to the studied strains of IBB virus (Table 1). For this, 4 groups were formed, 60 chickens each. Chickens in groups 2-4 were injected intramuscularly with inactivated antigens of epizootic strains; in the 1st group, strain 52/70-M, after inactivation, in a volume of 0.5 ml. The protective activity of the IBP anti ¬ genes was assessed on the 28th day of the experiment serologically (in the RDP and ELISA) and infection of 10 chickens from each group with different virus strains at a dose of 103IDad / ml.

Table 1: Cross-resistance of chickens to different strains of IBB virus

Antigen	Strain of IBB virus taken for infection			
	52/70-M	Isolate 1	Isolate2	Isolate 3
52/70-M	2/10	1/10	0/10	1/10

Isolate 1	1/10	1/10	1/10	0/10
Isolate2	2/10	1/10	0/10	1/10
Isolate3	1/10	0/10	2/10	1/10

Note: in the numerator - the number of cases, in the denominator - infected, in brackets - the number of dead.

From the data of table 1 it follows that the epizootic strains circulating in the poultry farms of the RNO-Alania do not have antigenic differences and are related to the known strain of IBB virus. The death of chickens during infection with epizootic strains was 2-2.5 times higher than in the group of birds infected with the reference strain 52/70-M, which indicates a high degree of pathogenicity of newly detected isolates compared with the previously isolated strain.

The experiment was guided by data according to which viruses in the inactivated state do not create cross-protection even for strains that have undergone minor antigenic variability, and they are used for serotyping of the virus in vivo, which served as an additional argument in favor of a high degree of antigenic relatedness of the strains studied IBB virus.

It should also be noted that recently a low degree of the formation of post-vaccination immunity against IBD is often detected, as a result of which breakthroughs in immunity and death of the vaccinated bird are observed. The lack of the expected effect of vaccinations creates prerequisites for the improvement of methods and virus vaccines.

It should be noted that the lack of effectiveness of specific prophylaxis of IBD is due to the high level of maternal immunity and the degree of attenuation of vaccine strains and is not associated with antigenic differences of virus strains.

Taking into account that the half-life of maternal antibodies varies within 3-4 days, virtually all herds are susceptible to the virus causing the clinical form of IBB from 21 days. Given that the herd has a “mosaic” of immunity and the widest range of coefficient of variation in the average antibody titer, the probability of being infected with IBD can be large.

It should be noted that these strains have increased reactogenicity cause pathology in the structure of the fabric bag and negatively affect the immunological status of the bird. Based on the above, our research was focused on the attenuation of the first isolate designated with consequences by the “ST” symbol.

Subsequently, the CT strain was cloned by 20 blind passions in a cell culture from a factory bag of an embryo of chickens, plaque-cleaned and attenuated by a series of passages in a chicken embryo fibroblast culture (FEC) with a high multiplicity of infection. After attenuation, the virus was less likely to cause bursal atrophy and splenomegaly than the original isolate, and remained immunogenic. However, even at high passages of attenuation, the virus tended to persist for a long time in the bursa and tears of birds. Antibodies to the studied variant, passaged with a low and high multiplicity of infection, weakly neutralized the virus isolated from the bursa on the 28th day after infection. The virus is not detected from the 3rd to the 7th day after infection in the spleen of the chickens, and on the 14th and 28th day, high virus titers were established. Interference was observed in a culture of cells simultaneously infected with a constant amount of virus passaged with a low multiplicity of infection, and serial dilutions of virus virus passaged with a high multiplicity. The interference intensified as the number of passages in FEC culture increased and, to a greater extent, with the virus passaged with a high multiplicity of infection.

There was an inverse relationship between the levels of interference and damage to the bursa and spleen on the 7th day after infection, which indicates the participation of defective virus particles in the process of attenuation. A direct link is marked by inter ¬ interference and viral persistence in the bursa and spleen on the 28th day after infection. After incubation of the studied variant with a specific antiserum, an increase in its non-political replication in FEC culture was noted, with which the mechanism of the emergence of new strains of the infectious bursal disease virus was allegedly associated. In this connection, we studied the effectiveness of the experimental vaccine against IBB in laboratory and production conditions.

The vaccine strain was titrated in parallel — by cytopathogenic action and by the plaque method. As a result, it was found that the infectiousness of the virus, determined by the plaque method, was 10 7.0-10 7.5.

The safety and reactogenicity of the vaccine was assessed by administering a 10-fold immunizing dose to 1-, 25-, and 60-day-old chickens free of specific antibodies. . The reversibility of the ST vaccine strain was studied by successive passages on 7- and 14-day SPF chickens, 20 heads for each of 10 passages, by enteral administration in a volume of 1 ml after each passage. The minimum immunizing dose (ImD50) was determined according to the method of Reed and Mench on 120 SPF-chickens of 14 days of age in 6 groups in two replications (n = 2). A series of consecutive 10-fold dilutions were prepared, and each dilution was inoculated with 20 chickens at the rate of 1 ml of virus per chicken. The presence of immunity was determined by antibody titer in the reaction of diffusion precipitation (RDP) and the results of the control of infection of birds with the reference virulent strain 52/70 of IBB virus (100 ID 50 / ml) 14 and 21 days after vaccination.

In order to determine the immunogenicity of the vaccine virus, single and double vaccination of meat and egg chickens was carried out using various methods: oral, intranasal and ocular in laboratory and production conditions. In addition, laboratory studies were conducted aimed at identifying the immunosuppressive effect of IBB virus on chickens during their vaccination against Newcastle disease.

To establish the optimal dose of the vaccine, providing intense immunity for enteral, intranasal and ocular vaccinations, 10 groups of chickens were formed - 3 groups for each method of administration and 1 control. Chickens were immunized once, while the vaccine was administered in the following doses: 104.0 TCD50 / ml, 103.5 TCD50 / ml and 103.3 TCD50 / ml. After 14 and 21 days, the serum was examined in the RDP, and the young were infected with the virulent strain 52/70 and the birds were observed for two weeks. When assessing the pathogenicity of various strains of IBD virus, the method of determining the “Bursa index” can be used. To assess the degree of pathogenicity of strains "52/70" and "ST", an experiment was conducted on 21-day-old SPF-chickens. For this purpose, three groups of chickens were formed, 30 heads each. The chickens of the first group were infected with the strain “52/70”, the second with the strain “ST”.

The virus for infection was taken in the same dose (104.0 TCD50 / ml) and was administered orally in a volume of 0.5 ml. The third group of chickens, the control, was not infected. Before the experiment, then, after infection, every 7 days, for three weeks, 10 chickens were taken from each group to determine the bursal index and to conduct histological studies of samples from the fabric bag (Table 2).

Table 2: Bursal index (BI) in SPF-chickens infected with IBB virus (M ± m; n = 3)

№	The study period after infection, days.	Age, days	Bursalindex		
			Control	1 group ("52/70")	2 Group ("ST")
1.	0	21	4,62+-0,32	4,60+-0,21	4,60+-0,17
2.	7	28	5,12+-0,11	2,32+-0,15	4,40+-0,19
3.	14	35	5,50+-0,10	1,30+-0,15	4,60+-0,31
4.	21	42	5,25+-0,21	0,82+-0,11	4,25+-0,11

The data in the table show that the bursal index of chickens infected with the strain 52/70 after 7 days was 2.32 + -0.15, which is significantly lower compared to the index in the control and in the group of chickens infected with the strain ST . Subsequently, the difference in indicators of bursa in these groups progressed. Thus, in the first group of chickens, 21 days after infection, the index was 0.82 + -0.11; in the second group-4.25 + -0.11; in the control-5.25 + -0.21.

Thus, it was found that the strain "ST", in contrast to the reference strain, does not possess pathogenic and immunosuppressive properties for chickens.

The vaccine from strain "ST" of IBB virus is harmless and areactogenic for day-old chickens and older. Enteral, intranasal and ocular vaccination 10-fold immunizing dose did not cause any abnormalities in the general condition of the chickens, as well as pathological-anatomical changes in the fabric bag and other internal organs and tissues of the bird.

After 10-fold passaging on SPF-chickens and 20-fold passaging in FEC culture, the strain “CT” of the IBB virus did not cause clinical signs of 10.5-day TCD 50 / ml in parano-oral administration in 7-14 days old pathoanatomical changes inherent to IBD, therefore, passage did not lead to an increase in its virulence.

The minimum immunizing dose for oral vaccination did not exceed 102.7 TCD 50 / ml. The antibody titer was practically independent of the age of the chickens, but differed when using different methods of drug administration. In setting the bioassay, all chickens whose blood serum reacted positively in the RDP with the IBB virus antigen showed 100% resistance.

The results of a single use of the vaccine showed a high level of post-vaccination immunity to highly pathogenic strains of IBB virus, and later on, on the 14-18th day, chickens were resistant to the virulent strain of IBB virus in 96-97% of cases. After a single vaccination, the average antibody titer in the ELISA assay method (ELISA) was 1: 850 and 1: 1020, respectively, the level of specific viral precipitating antibodies in the blood serum did not depend on the vaccine administration method and was 3-4 log.

Therefore, specific prophylaxis of IBD using the vaccine from strain ST is recommended to be performed once, at 7 or 14 days of age, by oral, intranasal and intraocular immunization, and the bursal index values were slightly lower than in the control group.

The clinical condition of the birds vaccinated with the CT strain for the entire observation period was satisfactory, safety increased by 4%, the average weight of the replacement young is 250 g, the yield of business repair young by 10-12% compared to the bird that was planted Winterfield-2512 vaccine.

CONCLUSION

Summing up the study, we note that the vaccine from the strain "ST" of the virus of infectious bursal disease is harmless and are reactive for all ages of the bird. After a single vaccination, the youngsters acquire an intense immunity of at least 6 months.

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