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Immunity level in the chickens and hens inoculated with various dosages of the viral vaccine against the newcastle disease

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Abstract

Currently, there is a wide variety of vaccines capable of causing an intense immune response in poultry to the Newcastle disease. The work is aimed at studying the immune response in chickens and hens, depending on the dosage of the virus, and determining the optimal dosage for immunization with vaccines created at Joint-Stock Company (JSC) "Pokrovsky Plant of Biological Products".

Keywords: immunity; antibody titer; virus; dosage; immunization; specific antibodies.

INTRODUCTION

Industrial poultry growing and its intensification have revealed complex practical and scientific problems, one of which is ensuring lasting infectious safety at poultry farms with the aim of obtaining food products of high sanitary quality. Viral diseases in poultry are widespread in many countries, including Russia. In the current tense epizootic situation, preventing these infections is one of the most effective ways of fighting them.

Therefore, poultry breeding needs high-quality immunobiological preparations and schemes of their administration. For poultry breeding, the most important is the prevention of zoonotic infections, Newcastle Disease (ND) in particular, with vaccines. Development and production of vaccines is one of the most important branches of biotechnology. The wide use of vaccines aimed at preventing and treating infectious diseases determines the constantly increasing requirements to their quality, which, according to the international requirements, is ensured by three key indicators: safety, efficiency, and stability [1, 2].

ND is a highly contagious disease of poultry, which is included in the list of quarantine and particularly dangerous diseases supervised by the International Epizootic Bureau (IEB). According to the IEB, outbreaks of the disease have been observed in the recent years in over 60 countries, including the areas bordering Russia in China, Finland, Mongolia, Turkey, and Kazakhstan [1, 3-5].

In Russia the ND epizootic situation remains tense; new outbreaks of the disease are registered every year; the outbreaks of the disease are observed in both previously unfavorable areas and new regions. Besides, there is a constant threat of virus introduction into the country with wild birds. Import of hatching eggs and day-old hybrid chickens to Russia from 14 countries also poses a threat to the epizootic well-being of domestic poultry [4-13]. IEB experts believe that for preventing ND, it is necessary to constantly maintain a high level of vaccine coverage of the entire population of poultry.

The above data necessitate constant monitoring of the ND welfare in the Russian Federation, and constant vaccination of the entire poultry at livestock breeding farms and at private farms of citizens in the areas located near poultry farms, for creating immune buffer zones. It means that vaccination remains the main measure of preventing and fighting ND both in Russia and around the world.

Separate literary sources say that the dosage of the vaccine for preventing viral diseases with live vaccines has no significant effect on the immunity level, since after getting into the animal organism, the vaccine virus starts multiplying and penetrates (dissimulates) into the organs in the quantities sufficient for full-fledged immunogenesis. According to some researchers, the amount of the immunizing dosage can significantly affect the time of immunity onset [3, 14, 15]. Some publications testify that the duration of immunity against pox in chickens is interrelated (correlates) with the amount of the vaccine dosage: with the increase in the dosage of vaccine virus, the duration of immunity increases accordingly.

Based on the above, it would be interesting to study immunity level in chickens and hens, depending on the dosage of the vaccine virus injected into the body of poultry, and, based on the research results, to substantiate the optimal immunizing dosage of ND viral vaccine from strains "La-Sota" and "Bor 74".

MATERIALS AND METHODS

Strains of ND "La - Sota" and "Bor 74" produced at JSC "Pokrovsky Plant of Biological Products", series No. 15, made on 10.2017, were used in the experiments. Vaccine activity was determined by virus titration on 9-day-old chicken SPF embryos.

Tenfold dilutions of the virus from 10^{-1} to 10^{-10} were made in sterile glassware in a buffered physiological solution. The diluted virus was injected into the allantoic cavity of chicken embryos in the amount of 0.1 ml. Four embryos were used for each dilution. After seven days' incubation at 37 °C, the presence of the virus was assessed by the number of dead embryos in the period from the third to the seventh day. The titer of the virus was calculated according to the Karber's method modified by Ashmarin, and expressed in lg EID_{50}/cm3.

With regard to the obtained vaccines' activity against ND of strains of "La - Sota" and "Bor 74", experimental samples were prepared, containing the vaccinating dosage of the vaccine from strain "La - Sota" — 10 thousand, 100 thousand, 5 million and 50 million lg EID_{50}/0.5 cm3, and from strain "Bor 74" — 10 thousand, 100 thousand, 1 million and 5 million lg EID_{50}/0.5 cm3.

The efficiency of the prepared experimental samples of the vaccine was tested on 20-day-old chickens without specific antibodies to the virus against ND.

For this purpose, eight groups of five chickens were formed, which were inoculated once with the experimental vaccine samples by watering. According to the instructions for using vaccine of strain "La - Sota", the immunizing dosage for chickens, when administered by watering, should be 50 million EID_{50} per chicken, and of strain "Bor 74" — 5 million EID_{50} per chicken. Blood was sampled from the axillary vein before the experiment (background) and on day 14 after vaccination. Specific antibodies against ND were determined in the serum of chickens in the hemagglutination-inhibition test (HI test) [16].

RESULTS AND DISCUSSION

The results of studying the dynamics of antibody formation in chickens after vaccination with experimental samples of live vaccines against ND from strain "La - Sota" are shown in Table 1.

Based on the data in Table 1, a conclusion may be made that 14 days after the vaccination, specific antibodies against ND

are found in the serum of chicken. In the chickens inoculated with vaccine at the dosage of 10 thousand EID50, the antibodies' titer in all inoculated chickens was 256 log_2. With the increase in the inoculating dosage of the vaccine, a tendency to decreasing antibody titers was observed in the inoculated chickens. Thus, in the animals inoculated with the vaccine containing 100 thousand, 5 million and 50 million EID_{50}, the average antibodies' titer was 192, 166 and 140 log_2.

In the next series of experiments, the viral vaccine from strain "Bor 74" was used as a vaccine for immunizing chickens against ND. The results of studying the dynamics of antibody formation in chickens after vaccination with experimental samples of live vaccines against ND from strain " Bor 74" are shown in Table 2.

Studying the dynamics of antibody production after immunizing chickens with experimental samples of the vaccine against ND from strain "Bor 74" showed the same results as when the vaccine of strain "La - Sota" was used. The average antibody titers in the inoculated chickens immunized with the vaccine containing vaccination dose of 10 thousand, 100 thousand, 1 million and 10 million EID_{50} decreased with the increase in the dosages of the vaccine, and amounted to 256, 205, 154, and 140 log_2, respectively.

Thus, the vaccination of 20-day-old chickens with experimental samples of the vaccines against ND made from strains "La - Sota" and "Bor 74", containing increasing dosages of the virus, revealed specific antibodies as early as on day 14 after vaccination. A higher immune response was observed in chickens to the viral vaccine at the inoculating dosage containing 10 to 100 thousand EID_{50}. Increasing the dosage of the vaccine to 1 million, 10 million, and 50 million EID_{50} did not create more intense immunity in the inoculated chickens. With the increase in the inoculating dosages of the vaccine, the titer of the specific antibodies decreased rather than increased [17]. Obviously, higher dosages of the live vaccine cause more intense multiplication of the vaccine virus in chickens, which results in the suppression of the immune system in the first period of humoral immunity formation.

In the subsequent experiments, the formation of the immune system to the experimental vaccines with various content of the vaccine virus was tested on 90-day-old chickens without specific antibodies against the ND virus. The vaccines for immunizing chickens against ND were prepared using the viral vaccine from strains "La - Sota" and "Bor 74". The results of studying the dynamics of antibody formation in the chickens after the vaccination with experimental samples of live vaccines against ND from strain "La - Sota" with the inoculating dosage containing 10 thousand, 100 thousand, 5 million, and 50 million EID50 are shown in Table 3.

Table 1. *Antibody titers in the chickens immunized with various dosages of the viral vaccine against ND from strain "La - Sota" on day 14 after vaccination.*

No. chicken	Name of the strain	Titer of antibodies in HI test log_2			
		10 thousand EID_{50}	100 thousand EID_{50}	5 million EID_{50}	50 million EID_{50}
1	"La - Sota"	256	64	64	128
2	"La - Sota"	256	256	128	128
3	"La - Sota"	256	256	256	128
4	"La - Sota"	256	256	128	64
5	"La - Sota"	256	128	256	256
Average antibodies' titer		**256**	**192**	**166**	**140**

Table 2. *Antibody titers in the chickens immunized with the viral vaccine against ND from strain "Bor 74" containing 10 thousand, 100 thousand, 1 million, and 10 million EID_{50}.*

No. of chicken	Name of the strain	Titer of antibodies in HI test log_2			
		10 thousand EID_{50}	100 thousand EID_{50}	1 million EID_{50}	10 million EID_{50}
1	"Bor 74"	256	128	64	128
2	"Bor 74"	256	128	128	64
3	"Bor 74"	256	256	256	256
4	"Bor 74"	256	256	64	128
5	"Bor 74"	256	256	256	128
Average antibodies' titer		**256**	**205**	**154**	**140**

Table 3. *Antibody titers in the chickens immunized with a viral vaccine against ND from strain "La -Sota" containing 10 thousand, 100 thousand, 5 million and 50 million lg EID50/0.5 cm3.*

No. of chicken	Name of the strain	Titer of antibodies in HI test log_2				
		10 thousand EID_{50}	100 thousand EID_{50}	5 million EID_{50}	20 million EID_{50}	50 million EID_{50}
1	"La - Sota"	64	64	64	128	256
2	"La - Sota"	32	128	128	128	256
3	"La - Sota"	32	64	128	64	128
4	"La - Sota"	64	64	128	128	128
5	"La - Sota"	64	32	128	256	128
Average antibodies' titer		**51**	**70**	**115**	**140**	**179**

Table 4. *Antibody titers in the chickens immunized with a viral vaccine against ND from strain "Bor 74" containing 10 thousand, 100 thousand, 1 million, 5 million, and 20 million EID_{50}.*

No. chicken	Name of the strain	Titer of antibodies in HI test log_2				
		10 thousand EID_{50}	100 thousand EID_{50}	1 million EID_{50}	5 million EID_{50}	20 million EID_{50}
1	"Bor 74"	64	128	32	64	256
2	"Bor 74"	32	32	128	256	128
3	"Bor 74"	32	64	64	128	128
4	"Bor 74"	32	64	128	128	64
5	"Bor 74"	64	64	128	32	128
Average antibodies' titer		**45**	**70**	**96**	**121**	**140**

Table 3 shows that 14 days after the vaccination, specific antibodies against ND are found in the serum of chicken. The lowest titer of specific antibodies was detected in the chickens inoculated with the vaccine at the dosage of 10 thousand EID_{50}, the average antibodies' titer in the inoculated chickens being 51 log_2. With increasing the inoculating dosage of the vaccine for chickens, a positive tendency to increasing antibody titers was observed in the inoculated chickens. Thus, in the chickens inoculated with the vaccine containing 100 thousand, 5 million, 20 million, and 50 million EID_{50} the average antibodies' titer was 70, 115, 140, and 179 log_2, respectively.

In the next series of experiments, the viral vaccine against ND from strain "Bor 74", containing an increased amount of virus in the inoculating dosage, was used for 90-day-old chickens. The results of studying the dynamics of antibody formation in the chickens after the vaccination with experimental samples of live vaccines against ND from strain "Bor 74" with the inoculating dosage containing 10 thousand, 100 thousand, 5 million, and 50 million EID_{50} are shown in Table 4.

Studying the dynamics of antibody production after immunizing the chickens with experimental samples of the vaccine against ND from strain "BOR 74" showed the growing dynamics of increasing antibody titers with the increased content of the virus in the inoculating dosage of the vaccine. The average antibody titers in the chickens inoculated with the experimental vaccine samples increased with the increase in the content of the viral vaccine in the inoculating dosage from 10 thousand to 100 thousand, 1 million, 5 million and 20 million EID_{50} from 70 to 83, 96, 121 and 140 log_2.

CONCLUSION

Thus, as a result of the immunization with live vaccines against the ND made from strains "La - Sota and "Bor-74", antibodies were detected in 20-day-old chickens and 90-day-old hens as early as on day 14 after the vaccination. Comparison of the antibody titers in blood serum of chickens after the immunization with the vaccines against ND made from strain "La - Sota", containing an increasing amount of vaccine virus from 10 thousand to 100 thousand, 1 million, 5 million, and 50 million EID_{50}, shows that on day 14 after the vaccination, the highest antibodies' titer was in the groups inoculated with the vaccine containing 10 and 100 thousand EID_{50}. Further increase of the virus to 5 and 50 million EID_{50} was accompanied by decreasing titers of specific antibodies.

Similar results on 20-day-old chickens were obtained after the immunization with the vaccine from strain "BOR-74", where the most pronounced humoral immunity was also obtained in response to the vaccine containing 10 thousand and 100 thousand EID_{50}. In the chickens immunized with the vaccines containing 1 million and 10 million EID_{50} in the inoculating dosage, the number of specific antibodies was lower. Apparently, this may be explained by the fact that the increased levels of virus in the vaccine lead to the rapid multiplication of the vaccine virus, resulting in overloading of the immune cells of chickens, which is accompanied by a delay in the specific humoral response. Therefore, for the chickens being several days old, in administering the vaccine against ND made from strains "La - Sota" and "Bor 74" via watering, the optimal dosage should be 10 thousand to 1 million EID_{50}.

The results of studying the immunity level in the adult birds in response to increasing dosages of the vaccine testify that with the increase in the content of vaccine virus in the inoculating dosage, the immunity level increases both to the vaccine made from strain "La - Sota" and the vaccine made from strain "Bor 74". In this case, one should consider the immune system of the adult birds as fully formed and adequately responding to higher dosages of the virus. Given the fact that protection of the organism from the pathogenic isolate of ND virus occurs if the organism contains antibodies with the titer of 1:32, the best dosage should be considered the dosage of at least 100 thousand EID_{50}.

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Abbreviations

ND – Newcastle disease
IEB – International Epizootic Bureau
RF – Russian Federation
PRC – People's Republic of China
SPF – Specific Pathogen Free
EID – Effective Infectious Dosage