CONTROL OF THE STABILITY OF THE RESIDUAL VIRULENCE
OF THE ATTENUATED STRAIN Salmonella dublin 31

Abstract. In recent years, live vaccines based on attenuated strains have been successfully used in agricultural animals and birds to prevent salmonellosis in our country and abroad.

This article presents the results of monitoring the stability of the residual virulence of the attenuated strain Salmonella dublin 31. Our studies showed that the attenuated strain Salmonella dublin 31 retained the typical morphological, tinctorial, cultural, biochemical and antigenic properties that are characteristic of the corresponding serovar. The residual virulence of the vaccine strain Salmonella dublin 31 was tested in comparison with the virulent Salmonella dublin 315/52 culture in white mice (weighing 14-16 g), guinea pigs (weight 240-290) in several repetitions, taking into account their survival, dissemination of the process and timing elimination of attenuated vaccine strain culture. Studies have shown that all mice and guinea pigs infected with culture Salmonella dublin 31 survive 90-100% of cases during 20 days of observation, while control test animals infected with the virulent culture of S.dublin 315/52 at a dose of 104, 105, 106 CFU perished from 60% to 100% of cases.

Keywords: residual virulence, Salmonella dublin 31, biological properties of the strain.

Introduction. Long-term studies have shown that inactivated vaccines form the immunity of insufficient voltage and duration. In this connection, the search for improvements in various areas has been constantly conducted and is still being carried out: improving the methods of preparation of corpuscular vaccines (broth and agar vaccines, inactivated by washing with various physical and chemical agents); selection of vaccine-rich – antigen-bearing strains; use of adjuvants that increase the immunogenic properties of vaccines [1].

In recent years, live vaccines based on attenuated strains have been successfully used in agricultural animals and birds to prevent salmonellosis in our country and abroad [2].

As practice shows, vaccination at young age is not always effective, which is explained by physiological immaturity of the organism and abnormal microclimate of the facilities (low temperature and high humidity). Vaccination at two to five days of age is a strong stress factor, contributing to the occurrence of acute digestive disorders [3].

In recent years, live vaccines based on attenuated strains have been successfully used in agricultural animals and birds to prevent salmonellosis in our country and abroad [4].

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Cellular immune responses are crucial, since salmonellae are capable of intracellular parasitization. The level of antibodies does not reflect the intensity of immunity. In this regard, live vaccines are the most promising for the prevention of salmonella in farm animals [5].

Live vaccines are biological preparations made from attenuated strains of salmonella, having sufficiently high immunogenicity and weak residual virulence, which are safe for the immunized organism and have genetic markers that make it possible to distinguish them from virulent prototypes [6].
The introduction of live attenuated microbes that survive for some time in the host organism, causes a longer antigenic effect on the cells of immunocompetent organs, which is accompanied by the development of a pronounced protective effect.

The mechanism of immunity with the use of live vaccines is made up of factors that increase the activity of phagocytic cells, the formation of specific antibodies and changes in the reactivity of the organism, with the leading role being played by cellular defense factors.

High immunizing activity of live vaccines is explained by the presence in them of a number of antigenic complexes, preservation of the main metabolic pathways, reproduction of the vaccine culture in the body, involvement of large tissue surfaces in the immunogenesis process, the onset of rapid, intense and prolonged immunity with a single administration of vaccines [7, 8].

According to the literature, specific prevention is one of the main means in the fight against salmonellosis in cattle.

Analysis of literature data indicates conflicting information about the specific prevention of bovine salmonellosis using live and inactivated vaccines. However, it should be noted that the literature data of research results of many scientists indicate the significant advantages of live vaccines, because they fully preserve the antigenic set of the pathogen and provide a longer-lasting immunity [9-12].

**Methods.** The work was carried out in the period from 2015 to 2018 in the laboratory of antibacterial biotechnology of the Kazakh National Agrarian University, as well as in a number of Kazakhstani farms.

The residual virulence of the vaccine strain *Salmonella dublin 31* was tested in comparison with the virulent culture *Salmonella dublin 315/52* in white mice (weighing 14-16 g), guinea pigs (weight 240-290 g) in several repetitions, taking into account their survival, dissemination of the process and timing elimination of attenuated vaccine strain culture.

White mice were infected with the daily culture of the attenuated strain *Salmonella dublin 31* subcutaneously at doses of 5⋅10⁴, 10⁵, 10⁶, 10⁷, 10⁸ CFU and intraperitoneally at a dose of 10⁹, 10⁸, 10⁷ CFU.

A similar experience was put on guinea pigs. An attenuated strain of *Salmonella dublin 31* was injected subcutaneously in doses of 5⋅10⁶, 10⁷, 2⋅10⁶ KOE 10⁹ CFU.

Along with this there was studied the degree of dissemination and the timing of elimination of the vaccine strain from the animal organism.

When white mice infected subcutaneous with a vaccine strain at a dose of 10⁶ CFU, the culture is sown from organs and blood for 15 days, from the inguinal and lymph nodes to 30 days.

**Results of the study.** One of the important requirements for attenuated vaccine strains is the retention of residual virulence, on which the high immunizing ability of the live vaccine depends. In this connection, throughout our experiments, our attention was drawn to the preservation of the initial biological properties and the virulence consistency of the attenuated strain. We conducted such studies with the frequency of 6 months, three times.

Our studies showed that the attenuated strain *Salmonella dublin 31* retained the typical morphological, tinctorial, cultural, biochemical and antigenic properties characteristic of the corresponding serovar.

We paid attention to the possibility of dissociation of the vaccine strain *Salmonella dublin 31*. An evaluation of the degree of dissociation of salmonella was carried out by multiple scatters on plates of Petri with MBP virusulent strains formed stable suspensions in physiological sodium chloride solution at 37 °C during boiling for the above strains did not precipitate. All this gives grounds to believe that all strains (vaccine and virulent) are in a stable S-form.

One of the important requirements for attenuated vaccine strains is the retention of residual virulence, on which the high immunizing ability of the live vaccine depends. In this connection, throughout our experiments, our attention was paid to the virulence consistency.

The results of studies of the residual virulence of the attenuated strain *Salmonella dublin 31* are shown in table 1.

Studies have shown that all mice infected with the culture *Salmonella dublin 31*, survive 90-100% of the cases during the 20 days of observation, while the control test animals infected with the virulent *Salmonella dublin 315/52* culture at a dose of 10⁴, 10⁵, 10⁶ CFU perished from 60 % up to 100% of cases.

A similar experience was put on guinea pigs. When the attenuated *Salmonella dublin 31* strain was injected into the guinea pigs subcutaneously at doses of 2⋅10⁶, 4⋅10⁶, 6⋅10⁶ CFU and intraperitoneally at a
Table 1 – Test of the immunizing activity of an attenuated strain S. dublin 31 in experiments on mice

<table>
<thead>
<tr>
<th>Name of the strains</th>
<th>Number of mice</th>
<th>Infected inoculation method</th>
<th>Infected dose (CFU)</th>
<th>Results died</th>
<th>Results alive</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated strain Salmonella dublin 31</td>
<td>10</td>
<td>Subcutaneously</td>
<td>5·10⁴</td>
<td>–</td>
<td>10</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Subcutaneously</td>
<td>10⁵</td>
<td>–</td>
<td>10</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Subcutaneously</td>
<td>10⁶</td>
<td>–</td>
<td>10</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Intraperitoneally</td>
<td>10⁵</td>
<td>–</td>
<td>10</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>Intraperitoneally</td>
<td>10⁶</td>
<td>–</td>
<td>10</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td>Virulent strain Salmonella dublin 315/52</td>
<td>5</td>
<td>Subcutaneously</td>
<td>10⁴</td>
<td>–</td>
<td>3</td>
<td>Died on the 9th day</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Subcutaneously</td>
<td>10⁵</td>
<td>–</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Subcutaneously</td>
<td>10⁶</td>
<td>–</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>10⁴</td>
<td>–</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>10⁵</td>
<td>–</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>10⁶</td>
<td>–</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Table 2 – Test of residual virulence of the attenuated strain Salmonella dublin 31 on guinea pigs

<table>
<thead>
<tr>
<th>Name of the strains</th>
<th>Number of mice</th>
<th>Infected inoculation method</th>
<th>Infected dose (CFU)</th>
<th>Results died</th>
<th>Results alive</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>Attenuated strain Salmonella dublin 31</td>
<td>5</td>
<td>Subcutaneously</td>
<td>2·10⁵</td>
<td>–</td>
<td>5</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Subcutaneously</td>
<td>4·10⁵</td>
<td>–</td>
<td>5</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Subcutaneously</td>
<td>6·10⁵</td>
<td>–</td>
<td>5</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>5·10⁴</td>
<td>–</td>
<td>5</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>10⁵</td>
<td>–</td>
<td>5</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>2·10⁶</td>
<td>–</td>
<td>5</td>
<td>Didn’t sick</td>
</tr>
<tr>
<td>Virulent strain Salmonella dublin 315/52</td>
<td>5</td>
<td>Intraperitoneally</td>
<td>10⁵</td>
<td>–</td>
<td>4</td>
<td>Died on the 9th day</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>2·10⁵</td>
<td>–</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Intraperitoneally</td>
<td>4·10⁵</td>
<td>–</td>
<td>5</td>
<td></td>
</tr>
</tbody>
</table>

Note. The observation period is 20 days after infection.

dose of 5·10⁴; 10⁵; 2·10⁶ CFU, the experimental animals survived in the 80-100% of cases, and control guinea pigs infected with the virulent Salmonella dublin 315/52 culture at doses of 10⁵, 2·10⁶, 4·10⁷ CFU survive in 20% of cases.

The results of the experiments showed that the attenuated strain Salmonella dublin 31 does not have a weak residual virulence.

Along with this, the degree of dissemination and the timing of elimination of the vaccine strain from the animal organism was studied. At subcutaneous infection of white mice with a vaccine strain at a dose of 10⁵ cfu, the isolates were cultured from organs and blood during 15 days, from inguinal lymph nodes during 30 days.

In calves subcutaneously infected with a vaccine strain at a dose of 2·10⁶ cfu, generalized vaccine infection was noted in the first three days. After 7 days the culture was well isolated from the lymph nodes and spleen, weak culturing from the liver and bone marrow; After 14 days, the culture in the form of single colonies was isolated from spleen, pre-lobed, mediastinal and mesenteric lymph nodes.

Thus, in experiments on laboratory animals and calves, the inability of the attenuated strain Salmonella dublin 31 to cause a typical infectious process was established.
The persistence of the biological properties of the vaccine strain has been studied during long-term storage (for 5–6 years) and repeated crossings on semi-liquid and solid nutrient media, after freeze-drying of the Salmonella dublin strain 31, and also after 10-fold passage on white mice and three times through the bodies of calves.

Passage of Salmonella dublin strain 31 was carried out on white mice by intraperitoneal fatal infection of mice at a dose of 3·10⁷ CFU.

Passage of Salmonella dublin strain 31 was carried out on 8-10 day-old calves by intraperitoneal infection in a dose of 2·10⁸ CFU. Calves responded to infection with significant oppression, fever, digestive disorders, but did not die.

On the 3rd and 5th day, the experimental white mice and calves were killed for bacteriological examination.

A total of 10 passages were made on white mice and calves.

All passivated strains were controlled by the nature of growth and agglutinability. In addition, they were tested for virulence, by subcutaneous infection of white mice at doses of 10⁷ and 10⁸ CFU, the experimental animals remained alive.

The conducted studies testify to the constancy of the properties of vaccine strain Salmonella dublin 31 when grown on artificial nutrient media and when passaging on susceptible animals.

The absence of reversion of the vaccine strain is indicated by numerous immunological experiments in laboratory animals and calves, as well as immunization with an experimental vaccine from the same strain of more than 2,000 heads of cattle in farms infected with salmonellosis.

The study of Salmonella dublin strain 31 culture properties after freeze-drying showed good survival, which complies with the standard and preservation duration.

Conclusion. Thus, the obtained results on the study of the biological properties of the attenuated Salmonella dublin 31 strain obtained by the genetic method indicate that the strain Salmonella dublin 31 is in stable S form, has stable, typical for Salmonella dublin 31 morphological, cultural, biochemical and antigenic properties, weak residual virulence, is well established in the body, does not reverse during prolonged passage on susceptible animals. The presence of three genetic markers in Salmonella dublin 31 strain is a convincing proof of the stability and safety of an attenuated strain, and also allows it to be differentiated from a natural prototype.

REFERENCES

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АТТЕНУИРЛЕНГЕН SALMONELLA DUBLIN 31 ШТАМЫНЫҢ КЛАДЫТЫҚ УЫТТЫЛЫГЫНЫҢ ТУРАКТЫЛЫГЫН БАҚЫЛГАУ

Аннотация. Сонғы уақытта біздің мемлекеттімізде шетелдерде ауылшаруашылық жаңақтарды мен құстардың салмаселерінің адамды алу ушін тірі вакцина негізінде жасалатын аттенуирленген штаммдардан дайындалатын вакциналар әлі басталады.

Бұл мақалада Salmonella dublin 31 аттенуирленген штаммының кладытқы уыттлығының туралы ізденіс көрсетіледі. Біздің зерттеулеріміз, аттенуирленген Salmonella dublin 31 штаммы морфологиялық, тикторіялық, биохимиялық және антителдік қасиеттері өзіне тәуерлікпен сөз беретін гендалық. Salmonella dublin 31 аттенуирленген штаммының кладытқы уыттлығының нәтижелері ортақталған. Бұлға, егер құстардың құстардың мүмкіндігін мән тәуелді екішілдік. Аттенуирленген вакциналардағы штамының элиминация уақыты мен диссеминация үрдісін, сондықтан, оның әрі бөлінісінен қасиетін ескердік. Зерттеулерінің нәтижесінде, Salmonella dublin 31 есімдісінің арзанында барлық құстардың штаммынан мүмкіндігін 90-100% тірі келеді. Ад. құстардың тобына Salmonella dublin 31/52 штаммынан 10¹, 10², 10⁶ кез келгенде арзанының зерттеп алынған жаңақтарды 60%-дан 100%-ға дейін әлімге ұшырады.

Түкін сөзเดр: кладытқы уыттлық, Salmonella dublin 31, штаммының биологиялық қасиеті.

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КONTРОЛЬ СТАБИЛЬНОСТИ ОСТАТОЧНОЙ ВИРУЛЕНТНОСТИ АТТЕНУИРУЮЩЕГО ШТАММА SALMONELLA DUBLIN 31

Аннотация. В последние годы в нашей стране и за рубежом для профилактики сальмонелеза у сельскохозяйственных животных и птиц с успехом применяют живые вакцины на основе аттенуированных штаммов.

В статье приведены результаты контроля стабильности остаточной вирулентности аттенуированного штамма Salmonelladublin 31. Наши исследования показали, что аттенуированный штамм Salmonelladublin 31, сохраняет типичные морфологические, тикториальные, культуральные, биохимические и антителные свойства, характерные для соответствующего серовара. Остаточная вирулентность вакцинального штамма Salmonelladublin 31 проверялась в сравнении с вирулентной культурой Salmonelladublin 31/52 на белых мышах (весом 14-16 г), морских свинках (массой 240-290 г) в нескольких повторениях. Исследования показали, что все мыши и морские свинки, зараженные культурой Salmonelladublin 31, в течение 20 суток наблюдения, выживают 90-100% случаев, т.е. как контрольные подопытные животные, зараженные вирулентной культурой Salmonelladublin 31/52 в дозе 10⁴, 10⁵, 10⁶ КОЕ, погибали от 60% до 100% случаев.

Ключевые слова: остаточная вирулентность, Salmonelladublin 31, биологические свойства штамма.

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