Salmophage®: a new preparation for treatment and immune prevention of salmonellosis in fowls

Salmophage® : une nouvelle préparation pour le traitement et la prévention vaccinale de la salmonellose chez les volailles

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SUMMARY

Salmophage® is a new preparation developed against salmonellosis in fowl. It has two components: a dry live vaccine and a bacteriophage. Unlike conventional vaccines which trigger solely an active immune response against the vaccine strain, Salmophage® also has a therapeutic effect due to its phage component.

Experimental inoculation in white mice and chicken demonstrated that Salmophage® is more effective as a preventive agent than the conventional vaccine, especially when Salmonella contamination occurs at a time when animals are unable to develop an active immunity. Therefore, the phage component of the preparation can prevent the death of chicks heavily contaminated just a few days after hatching. A preliminary investigation in Salmonella-infected chicken showed that Salmophage® improves animal survival and helps produce poultry products free of Salmonella enteritidis.

Key words: vaccine, chicken, Salmonella, bacteriophage.

RÉSUMÉ

Salmophage® est une nouvelle préparation développée contre la salmonellose du poulet, et constituée d’un vaccin sec vivant et d’un bactériophage. Au contraire des vaccins conventionnels qui induisent uniquement une immunité active contre la souche vaccinale, Salmophage® a aussi un effet thérapeutique grâce au phage qu’il contient.

L’inoculation expérimentale à des souris blanches et des poulets a démontré que Salmophage® est plus efficace comme agent de prévention que les vaccins traditionnels, particulièrement lorsque la contamination a lieu à des périodes où les animaux ne sont pas capables de développer une immunité active. Ainsi, la composante « phage » peut prévenir la mort de poussins fortement contaminés dans leurs premiers jours de vie.

Une étude préliminaire chez des poulets d’élevage infectés avec Salmonella montre que Salmophage® augmente leur survie et permet d’obtenir des produits indemnes de Salmonella enteritidis.

Mots-clés : vaccin, poulet, salmonella, bactériophage.
Communications

Introduction

Salmonellosis belongs to zoonoses which are widespread in Russia and in many other countries and has no equals among other zoonoses in the abundance of serovars of causative agent and difficulties arising in the process of their control. In the past 15-20 years the pride of place has been taken by serovar *S. enterica*. Enteritidis which causes toxoinfections in humans. Wild and domestic fowl, poultry in particular, constitute a major reservoir of these micro-organisms in nature. Along with its big social importance, salmonellosis causes serious economic damage to poultry farming. Furthermore according to veterinary statistical returns in Russia, salmonellosis is responsible for the death of more than one-third of all fallen chickens.

Methods employed to control salmonellosis in industrial poultry farming are reduced to implementing organizational, veterinary-sanitary measures, exposing by bacteriological or serological methods infected fowl and their eliminating, administering antibiotics and other chemotherapeutical preparations (in *Wray* and *Davies*, 1994). The effectiveness of these measures is inadequate.

In the opinion of many researchers and the WHO Experts Committee on Salmonellosis, the use of antibiotics and chemo-preparations cannot solve this problem (World Health Organization, 1991; *Wray* and *Wray*, 2000). Such treatments of fowl by antibiotics and different chemo-preparations can neither prevent nor eradicate the infection. Their constant application results in the rise of polyresistant clones of bacteria. Contaminated products of poultry farming become a source of genes of multidrug resistance for human pathogens. For this reason, the use of antibiotics in poultry farming is now strictly regulated in the USA and many European countries.

In this connection, development of effective vaccines, application of competing microflora and probiotics are recommended (WHO, 1991).

Many authors have noted the economic effectiveness resulting from the use of vaccines for preventing salmonellosis in fowl in comparison with expenditures on the treatment of fowl by antibiotics and chemotherapeutical preparations (*Wray* and *Wray*, 2000).

The existing commercial and experimental vaccines suggested by different authors can be divided into three basic types:

- Inactivated vaccines are represented most widely. Their successful application for preventive effect on salmonellosis in fowl has been reported by many researchers (*Ghosh*, 1989; *Griffin* and *Barrow*, 1993; *Wray* and *Wray*, 2000). The best results have been obtained by use of emulsion (*Timms, Marshall* and *Breslin*, 1990; *Charles*, *Nagaraja* and *Sivanandan*, 1993; *Charles et al.*, 1994; *Timms, Marshall* and *Breslin*, 1994; *Wray* and *Wray*, 2000) and chemical vaccines (*Bouzoubaa et al.*, 1992; *Timms, Marshall* and *Breslin*, 1990; *Wray* and *Wray*, 2000).

- It has been established that live vaccines against salmonellosis produce more pronounced immunity than inactivated ones since they are capable to induce both humoral and cellular immunity as well as a secretary immune response. Besides the immunity caused by live vaccines is characterized by fast onset, intensity and duration (*Gupta* and *Mallick*, 1976, 1977; *Laszlo*, *Csorian* and *Paszti*, 1985; *Linde*, *Fthenakis* and *Fichtner*, 1988; *Cooper et al.*, 1990; *Barrow*, *Lovell* and *Berchieri*, 1991; *Barrow*, 1991, 1992; *Lessard*, *Hutchings* and *Spencer*, 1995; *Wray* and *Wray*, 2000). In the modern world, live vaccine manufacturing uses metabolic mutants, *S. enterica*. Enteritis ar-o-A mutants and double mutants with deletions in genes coding for adenylate-cyclase and *S. Typhimurium* camp receptor (*Barrow*, *Lovell* and *Berchieri*, 1991; *Cooper et al.*, 1992; *Cooper et al.*, 1993; *Griffin* et *Barrow*, 1993), strains with acquired resistance to antibiotics and lowered virulence as a result of mutations in genes coding for ribosomal proteins and RNA polymerase (*Wray* and *Wray*, 2000), and strains obtained by the insertional mutagenesis method based on the property of transposons to transpose in genome of receptive bacteria and thus disturb the functioning of genes modified (*Volozhantsev et al.*, 1997).

There are reports about developing vaccines on the basis of recombinant *Salmonella* strains (*Barrow*, *Lovell* and *Berchieri*, 1991; *Wray* and *Wray*, 2000), and vaccines on the basis of thermosensitive mutants (*Nakamura et al.*, 1994), mutants with lost genes of invasiveness (*Hassan* and *Curtiss*, 1994; *Wray et Wray*, 2000).

- *Ziprin* (1997) et (*Ziprin* and *Kogut*, 1997) have developed a vaccine against salmonellosis in birds on the basis of immune lymphokines belonging to the antidiotypic vaccine type.

As follows from the presented results a considerable experience has been widely accumulated in the field of developing vaccines against salmonellosis in fowl and their effectiveness and the necessity of their application have been proven.

At the same time such vaccines have shortcomings, which limit their effectiveness, and possibilities of their wide application, inactivated and chemical vaccines are most effective when introduced parentally. This makes their use laborintensive and impossible for chickens at their early days of age being most sensitive to salmonellosis, causative agent. Besides vaccination causes stress in fowl (*Nakamura et al.*, 1994).

Antidiotypic vaccines display pronounced effectiveness in laboratory conditions. However, high production cost limits possibilities of their wide practical application.

In the opinion of many researchers application of live and inactivated vaccines cannot prevent vertical transmission of salmonellosis, does not guarantee complete sanitation of fowl from its causative agent and its absence in poultry farming products (Report WHO, 1991; *Wray* and *Wray*, 2000).

Since 1990, we performed the effectiveness of the use of formulated vaccine from *S. Typhimurium* and *S. Dublin* suppressor revertants against salmonellosis in poultry. The
inactivated vaccine was administered orally to chickens in their first two days, two times (10^6 cfu) and one time in 60 and 180 days, intramuscularly (10^6 cfu) in volume 0.5 cm^3. The live vaccine was administered orally during the first days of life two times (10^9 cfu) and additionally, at the age of 100 days, two times (5x10^9 cfu). The frequency of Salmonella detection in group immunized by the inactivated vaccine and the live one constituted 7% and 5.6% respectively; and in controls, 32.1% and 32.6% respectively. Despite the positive results of the application of such preparations insufficient effectiveness of the traditional approach in immune prevention of salmonellosis in poultry was revealed. The main problems consisted in the impossibility of protection from death of already contaminated chickens in first days of life and absence of sanative effect in grown up birds.

Analysis of the litterature and our experience in developing and applying vaccine preparations against salmonellosis in poultry have served as a basis for developing preparations of a new type, Salmophages (our term), possessing both treating and preventive action.

**Materials and Methods**

**Bacteriophages**
We tested our 27 isolates of *S. Enteritidis* phages, 8 phages for phage typing of *S. Enteritidis* from LASZLO Hungarian collection (LASZLO, CSORIAN and PASZTI, 1985), and 10 *S. Gallinarum-pullorum* phages from VGNKI collection of microorganisms.

**Bacteria**
The following *S. Enteritidis* and *S. Gallinarum* strains have been used:
- virulent epizootic strains isolated by us from poultry in different regions of Russia in 1992-2003;
- virulent strains isolated from poultry and human from VGNKI collection of microorganisms; the All-Russia Salmonellosis Centre and the State Research Centre of applied microbiology brought from Russia, Great Britain, France, Germany, Yugoslavia, Bulgaria, Italy and Poland;
- attenuated *S. Enteritidis* strain 204 and R-6 phage-resistant attenuated strain selected;
- for specificity of spectrum of phages, strains belonging to several genera of enterobacteria: *Citrobacter, Escherichia, Klebsiella, Morganella, Proteus, Salmonella, Shigella, Yersinia*.

**Media**
Buffered Pepton Water (CM 509), Rappoport-Vassiliadis (RV) Enrichment Broth (CM 669), Bismuth Sulphite Agar (CM 201), Oxoid; Agar Powder, Bacteriological (RM 026), Nutrient Broth (M 002), Himedia.

**Animals**
White mice (not purebred), 14-16 and 16-18 g body weight. Chickens (1-43-day-old), Smena-2 cross.

**Methods of investigating bacteriophages**
Isolation of phage was conducted from waste waters of salmonellosis-unsafe poultry farms. To raise the probability of phage detecting, the enrichment method described by Adams was performed (ADAMS, 1959). The samples were inoculated on beef extract broth jointly with *S. Enteritidis* strain in exponential phase of growth, incubated for 18-24 hours at 37°C. The tests were filtrated (porosity of 0.22 micron), then the presence of phages was searched in microbefeer filtrates by inoculation in fluid and agar media which preliminary seeded by *S. Enteritidis* indicator strains.

Pure clones were obtained by consecutive cloning of morphologically monotypic negative colonies. A comparative evaluation of morphology of negative colonies of different phages was conducted by using one batch of cultural medium and identical conditions of cultivation.

Electron diffraction patterns of bacteriophages were obtained with JEM 100 CX electron microscope, with accelerating voltage of 80 kilovolts, using Tikhonenko method of negative staining by tungstophosphoric acid (TIKHONENKO, 1968). The bacteriophage T2 was used as model.

Activities of bacteriophages were determined as follows: number of viable particles using Gratia methods and lytic activity using Appelmans method (ADAMS, 1959).

**Salmonella Investigation**
- *S. Enteritidis* attenuated strain 204 was obtained by insertional mutagenesis from highly virulent *S. enteritidis* strain 126 from human source (VOLOZHANTSEV et al., 1997);
  - Tn7 transposone which is an integral part of the vector plasmid with a ts replication DNA pVD3::Tn7 (Tc Ap Km Sm Tp) was used for insertional mutagenesis. The plasmid transfer to recipient *S. Enteritidis* 126-Rif strain was effected at the conjugation cross: E.coli JC 1553 (pVD3::Tn7) x *S. Enteritidis* 126-Rif. Transconjugates *S. Enteritidis* 126-Rif (pVD3::Tn7) with phenotype Rifr (determined by the rif-mutation of strain) Tcr Apr Km (markers of pVD3 plasmid) Tpr Smr (markers of Tn7 transposone) were selected.

For the selection of insertional mutants of the *S. Enteritidis* 126-Rif (pVD3::Tn7) bacteria grown on meat infusion at 31°C were sifted on nutrient medium which contains streptomycin (50 microgram/ml) and were incubated at 43°C which make plasmid unable to replicate autonomously. The grown clones were subjected to the verification of the presence of non-selective markers of upper plasmid (Tc Km Ap) and to sampling of the variants with only Tn7 markers (Sm Tp), i.e. the clones with the insertions Tn7.

- From inoculated mice (intraperitoneally 5x10^5 cfu), *Salmonella* mutants giving 100% animals survival were selected. From such clones, we selected new with the highest immunogenicity. Then mice were immunized by cell suspension of attenuated mutant (one time, subcutaneously 1x10^5 cfu). After 19-21 days, immunized and control animals were infected by 10 LD_{50} (2x10^2 cfu) of virulent strain 126.
- Phage-resistant mutant of the strain 204 was selected by growth with equal-volume mixture of *S. Enteritidis* and *S. Gallinarum-pullorum* phages and control.

- Detection of *Salmonella* in mice and chickens was examined as follows: shredded pieces of organs (liver, spleen (mice), liver, heart, kidney, intestines, ovaries (chickens)) in ratio 1:10 were placed in buffered pepton water, then reinc inoculated in Rappoport-Vassiliadis (RV) enrichment broth, and final cultures were performed on bismuth Sulphite agar. Each step was obtained during 18-24 hours at 37°C, but 43°C for RV medium. Isolates were identified by biochemical and antigenic procedures. In the order of attribution of the isolates selected to *S.*enterica subsp. enterica, we determined their ability to glucose, lactose, saccharose, mannitol, rhamnose, sorbitol, arabinose, xylose fermentation. We also verified their ability to the hydrogen sulphide production, indole production, acetoin production, revealed in Voges-Proskauer reaction, to urea hydrolysis, to use of citrate as carbon source, as well as the presence of lysine decarboxylase and ornithine decarboxylase. We performed the identification of Salmonella isolates to serovar in the reaction of agglutination with adsorbed monoreceptor Salmonella sera « O » and « H », produced by Krasnodar biological plant (Russia). In the order of differentiation of virulent strains from the attenuated strain *S. Enteritidis* R-6, we used its ability to grown on the mediums containing rifampicin (200 µg/ml), streptomycin (200 µg/ml) and trimethoprim (200 µg/ml).

The presence of antibodies to D group Salmonella in chickens was determined with the help of blood-dropping indirect agglutination reaction with pullorum erythrocytic antigen. We considered as positive the reaction, in which the agglutinate formation became clear in the equivoluminar mixture of erythrocytic antigen and birds’ blood within 2 minutes. The reaction was considered as negative in the case of formation in the drop of blood with antigen of the stable suspension of erythrocytes.

- From 89 poultry farm waste waters, 27 bacteriophage were selected against indicator strains *S. Enteritidis* S-6. The composition of bacteriophage populations was heterogeneous and was represented by several types of negative colonies (Figure 1).

After cloning and 5 consecutive passages on host strain, 8 most active phages were selected (F1 to F8). From 8.4.10^9 to 2.0.10^11 viable phage particles accumulated in their phage lysates during 3-5 hours of culture in stationary conditions.

The specificity range of phages is limited to *S. Enteritidis* and *S. Gallinarum-pullorum* belonging to serogroup D and considerably smaller sensitivity for strains belonging to serogroups B (*S. Typhimurium*) and D (*S. Dublin*). The F6 phage strain had the widest spectrum of lytic action in relation to the main causative agents of

### Table 1: Lytic spectrum and specificity range of *Salmonella Enteritidis* bacteriophages.

<table>
<thead>
<tr>
<th>Bacteriophage</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F6</th>
<th>F7</th>
<th>F8</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. Enteritidis</em></td>
<td>49/60***</td>
<td>51/60</td>
<td>55/60</td>
<td>56/60</td>
<td>51/60</td>
<td>58/60</td>
<td>54/60</td>
<td>51/60</td>
</tr>
<tr>
<td><em>S. Gallinarum</em></td>
<td>24/34</td>
<td>30/34</td>
<td>28/34</td>
<td>30/34</td>
<td>26/34</td>
<td>31/34</td>
<td>29/34</td>
<td>28/34</td>
</tr>
<tr>
<td><em>S. Choleraesuis</em></td>
<td>1/15</td>
<td>1/15</td>
<td>4/15</td>
<td>2/15</td>
<td>1/15</td>
<td>1/15</td>
<td>0/15</td>
<td>1/15</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>2/5</td>
<td>3/5</td>
<td>3/5</td>
<td>2/5</td>
<td>1/5</td>
</tr>
<tr>
<td><em>Y. pseudotuberculosis</em></td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
<td>0/2</td>
</tr>
<tr>
<td><em>Proteus sp.</em></td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
<tr>
<td><em>Morganella sp.</em></td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td><em>Klebsiella sp.</em></td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td><em>Citrobacter sp.</em></td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
</tr>
<tr>
<td><em>Shigella sp.</em></td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
<td>0/3</td>
</tr>
</tbody>
</table>

* from museums ** strains from poultry farms *** ratio: number of strains lysed/ number of strains tested

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**Results**

From 89 poultry farm waste waters, 27 bacteriophage were selected against indicator strains *S. Enteritidis* S-6. The composition of bacteriophage populations was heterogeneous and was represented by several types of negative colonies (Figure 1).

After cloning and 5 consecutive passages on host strain, 8 most active phages were selected (F1 to F8). From 8.4.10^9 to 2.0.10^11 viable phage particles accumulated in their phage lysates during 3-5 hours of culture in stationary conditions. The lytic action and specificity of bacteriophages (F1 - F8) were determined against *Salmonella* strains of different serovars and some enterobacteria are represented in Table 1.

The specificity range of phages is limited to *S. Enteritidis* and *S. Gallinarum-pullorum* belonging to serogroup D and considerably smaller sensitivity for strains belonging to serogroups B (*S. Typhimurium*) and D (*S. Dublin*). The F6 phage strain had the widest spectrum of lytic action in relation to the main causative agents of...
salmonellosis in poultry. *S. Enteritidis* and *S. Gallinarum-pullorum*. The F3, F5 and F7 bacteriophages lysed a considerable number of strains from museums and *Salmonella* isolates of these serovars.

- Electron microscopic investigation of phages showed that all of them have a bival type of symmetry, consist of equilateral tiny head in the form of an isometric polyhedron having a form of an icosahedron, 50-70 nm in diameter, a long uncontractable appendage with transversal streakness 105-150 nm long, have a basal lamina and short fibrils. Phages of this type belong to the Siphoviridae family, relate to B1 morphological type according to classification by ACKERMAN and DUBOV (1987).

- The most immunogenic *S. Enteritidis* strain 204 was revealed by analysis of 54 insertional mutants. Immunization of mice by 10^7 cfu of this attenuated strain prevented the death of 89.3 ± 0.5% of animals challenged against the high virulent strain. Its LD50 was 5x10^5 ± 2x10^7 cfu and the virulence of the initial *S. Enteritidis* strain 126 constituted 18 ± 5 cfu.

The resistance markers of this strain were as follows: rifampicin (200 µg/ml), streptomycin (200 µg/ml) and trimethoprim (200 µg/ml) which were stable *in vitro* after 10 consecutive cultures in fluid medium and *in vivo* after 10 passages on mice and chicken. The strain phagotyping showed that it belongs to one of the most widespread phagotypes in the world, phagotype IV in Wald’s scheme.

A phage-resistant mutant of the attenuated strain 204 was obtained after 15 mixed cultures of different suspensions of 8 *S. Enteritidis* phages and 10 *S. Gallinarum-pullorum* phages. The phage-resistant mutant, *S. Enteritidis* R-6, kept all former properties (immunogenecity, resistance markers), but the LD50 in mice was different, 8,0x10^6 ± 1,6x10^6 cfu, ie, its residual virulence declined by 15-16 fold-times.

- The study of the phage-resistant strain has demonstrated that the tolerable dose for 3-day-old chickens constituted 4x10^9 cfu and the ED50 was 10^9 ± 4 x. 10^7 microbial cells. The strains persistence time in chicken was 10-12 days, and in white mice, 24-28 days. The attenuated strain stayed longest before its elimination in the liver and spleen of immunized animals.

The salmophage consisted of two components: a dry live vaccine made of the phage-resistant attenuated R-6 strain and a bacteriophage (F6). This phage possesses the highest activity and a wide range of lytic action among the examined phages.

- Preventive effectiveness was preliminarily studied on a *in vivo* model. White mice were immunized subcutaneously by a preparation containing 10^5 cfu of the attenuated strain and 5x10^8 F6 particles. Each group consisted of 20 animals. Other groups were either only treated by the vaccine either control. Finally the preparations were also administered twice with a 3-day interval. The experimental and control animals were contaminated at various times (1, 3, 14 days) by 5 LD50 of the virulent *S. Enteritidis* strain. The results of experiments are given in Table 2.

The best protective effect was observed in the group of mice immunized by Salmophage twice. A lower protective effect was observed when mice were infected during periods unable to obtain specific immunity (1 and 3 days). The introduction of the preparation Salmophage was again more effective than the use of the vaccine alone.

- The treating and preventive effectiveness of the phage component of the Salmophage was studied on chicken in their first days of life. With the aim of evaluating the treating effectiveness the 2-day-old chickens preliminarily were contaminated perorally by 5 LD50 (5x10^6 cfu) of the *S. Enteritidis* virulent strain and 4 hours later, they were administered perorally 5x10^6 phage particles. The phage treatment was repeated when the chickens were 5-day-old. Furthermore the 5-day-old chickens were analogously treated by the phage, and infected by 10 LD50 (8x10^6 cfu) of the *S. Enteritidis* virulent strain. Experimental and control groups consisted of 24 chickens observed during 15 days after the challenge.

Bacteriophage introduced to chickens before immunization (3 days) saved from death 100% fowl both in the experimental and control lots.

The preliminary evaluation of effectiveness of the Salmophage was conducted on broiler chickens of the Smena-2 cross in the conditions of natural contamination at

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**Table 2:** In vivo preventive effectiveness of Salmophage in mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Challenge Time*</th>
<th>Number mice**</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaccine</td>
<td>1</td>
<td>60</td>
<td>40.0 ± 0.35</td>
</tr>
<tr>
<td>Salmophage</td>
<td>1</td>
<td>60</td>
<td>71.7 ± 0.41</td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Vaccine</td>
<td>3</td>
<td>60</td>
<td>53.3 ± 0.52</td>
</tr>
<tr>
<td>Salmophage</td>
<td>3</td>
<td>60</td>
<td>75.0 ± 0.28</td>
</tr>
<tr>
<td>Control</td>
<td>3</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td>Vaccine</td>
<td>14</td>
<td>60</td>
<td>91.7 ± 0.55</td>
</tr>
<tr>
<td>Salmophage</td>
<td>14</td>
<td>60</td>
<td>93.3 ± 0.12</td>
</tr>
<tr>
<td>Control</td>
<td>14</td>
<td>60</td>
<td>0</td>
</tr>
</tbody>
</table>

*delay between vaccination and challenge; ** Number of mice live the day of challenge and 14 days after; *** vaccination and Salmophage given twice.
a salmonellosis-unsafe poultry farm. The experimental and control group had 200 chickens each and kept in cages. The salmophage was given, together with drinking water, to 3-day-old and 6-day-old chickens (2.5x10^8 cfu of the vaccine strain and 5x10^7 phage particles). Chickens, which died during the experiment or were killed at the end of experiment, were investigated for salmonellosis.

Three chickens from experimental group died during the whole time of experiment (43 days). Salmonella was not detected in them. In the control group, 17 chickens died, 6 of them were infected by *S. Enteritidis*. At the end of the experiment (term of broiler farming) 40 broilers from experimental and control groups were examined to Salmonella carriage. In the control group, 3 broilers were infected by *S. Enteritidis*. In the group of birds, processed by salmophage, there were no revealed Salmonella carriers.

Finally 9 infected chickens were found during the investigation of 57 broilers in the control group. All these isolates were identified as *S. Enteritidis*. Blood analysis of 150 experimental and 150 control chickens in the indirect agglutination reaction with pullorum erythrocytic antigen revealed the presence of antibodies to group D *Salmonella* in 12.2% of control chickens. The presence of antibodies to Salmonella in broilers treated by the Salmophage was not observed.

**Discussion**

The aim of the present work is to try to develop a preparation against salmonellosis in fowl free from shortcomings inherent in the traditional vaccine preparations. As a result, a preparation has been developed and constructed possessing both treating and preventive properties.

Salmophage consists of two components: a dry live vaccine and a bacteriophage. As distinct from the existing preparations, Salmophage besides forming an active immunity under the influence of a vaccine strain possesses a treating and sanitary effect due to the introduction of phage component into the preparation.

The vaccine strain has been obtained by insertional mutagenesis. The introduction into genome of a highly virulent *S. Enteritidis* strain the transposone Tn7 besides reducing virulence resulted in the emergence of stable resistance markers in the attenuated strain. The selection from it of a phage-resistant mutant brought about further considerable reduction of virulence. Reduced virulence in phage-resistant mutants has been described by many authors (ADAMS, 1959).

Experiments on mice have demonstrated that Salmophage owing to the presence in it of the phage component is more effective as a preventive agent than the traditional vaccine especially when animals were contaminated in periods insufficient for forming an active immunity. The phage component in the preparation can save chickens in their first days of life from death when they are infected by high doses of the causative agent.

The preliminary study of the effectiveness of Salmophage in the conditions of an unsafe poultry farm has shown that the application of the preparation allows to enhance the survival of fowl and obtain Salmonella-free poultry-farm products. This served as a basis for conducting large-scale experiments at poultry farms.

A valuable feature, in our opinion, of the Salmophage is the fact that its introduction did not cause the emergence of antibodies in birds’ blood revealed with the help of the indirect agglutination reaction with pullorum erythrocytic antigen. For this reason the application of the preparation does not abolish the use of the most widespread remedy of control salmonellosis in poultry.

**Conclusions**

1. A preparation of new type against salmonellosis in fowl has been suggested. It possesses treating and preventive properties.

2. Salmophage is more effective than the traditional vaccine, especially when it is used in periods insufficient for forming an active immunity.

3. The use of Salmophage does not abolish traditional diagnostics with the help of the indirect agglutination reaction with pullorum erythrocytic antigen.
REFERENCES


