THE USE OF THE SPECIFIC ANTI-SALMONELLA POLYCLONAL ANTIBODIES ISOLATED FROM HEN EGGS, IN SALMONELLOSIS PROPHYLAXIS

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The administration of increased doses of antibodies in groups experimentally infected with Salmonella gallinarum, in order to record the efficiency of their administration in salmonellosis prophylaxis was the aim of our research. When a low infection dose, 1x10^7 CFU Salmonella gallinarum, was used the administration of IgY polyclonal antibodies as immunoglobulin extract, or even yolk administration had a protective effect against germs invasion. This effect was not recorded when a 10 folds higher dose was administered (1x10^8 CFU). The prophylactic effect of the administration of polyclonal antibodies is demonstrated.

Key words: IgY, immuno-prophylaxis, Salmonella gallinarum

Material and Method

The administration of high antibody doses in hens groups experimentally infected with Salmonella gallinarum in order to record the efficiency of their administration in salmonellosis prophylaxis.

Antibody production. Laying hens submitted to hiper-immunization with Salmonella gallinarum 9R during 10 weeks, which consisted in 4 immunizations, were used for polyclonal anti-Salmonella antibody production. The hens were intramuscular inoculated with cu 200 μl Tiforomvac vaccine, consisting in 2 x 10^7 CFU/Salmonella gallinarum - 9R strain, vaccine dose, and aluminum hydroxide as adjuvant. After 2 weeks interval, 3 rappel immunizations were performed. After 42 days from the first immunization, eggs from immunized hens were collected in order to test the prophylactic and therapeutic effect of the antibodies. The determination of the IgY total quantity was performed after isolation with water and ammonia sulfate. The resuspension of the antibodies was realized in distilled water within yolk initial volume. The total antibody quantity was photometrically determined at 280 nm wave length. Values of 21.70 mg IgY/ml yolk were recorded. The specific antibodies were determined on the same immunoglobulin
extract using the micromethod of slow agglutination reaction in tubes, and titre was of \((\log_2) 9.14\).

**Biological material.** 6 experimental groups, with 10 quails (Coturnix conturnix japonica) aged of 70 days each, were performed in order to record the prophylactic effect specific anti-salmonella antibodies. The groups consisted of 8 females and 2 males, sheltered in special cages. Concentrate forage was administered. Because both prophylactic and therapeutic effects of antibody administration were studied, the 6 experimental groups were divided in two trials as follows:

<table>
<thead>
<tr>
<th>Trial</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Infection dose</td>
<td>10^8 CFU</td>
</tr>
<tr>
<td>Groups</td>
<td></td>
<td>I Control I</td>
</tr>
</tbody>
</table>

**Biological material used as inoculate**

In order to obtain inoculates, a *Salmonella gallinarum* strain from the collection of DSV Cluj was used as antigen. The culture was seed on SS-agar Difco differentiation media. Passages in nutritive bullion were performed from well differentiated colonies from SS media in order to obtain a germ rich germ mass. After incubation, the number of the germs was determined. The extinction was red at 600 nm with Eppendorf photometer. The infection was performed with two different doses, 1 x 10^8 CFU in trial I and a reduced dose 1 x 10^7 CFU in IIth trial.

The administration of the *Salmonella gallinarum* suspension was orally performed with 1 mL microbial suspension/poultry.

**Experimental protocol**

The poultry were divided by groups and disinfested with Albendazol. After one week from disinfections, antibodies were administered. They were orally administered, 1 mL/poultry, during 14 days, after previous isolation in water and ammonia sulfate, or by direct yolk administration. Beginning with the initial moment of the trial, before the administration of the *Salmonella gallinarum* suspension, and 4 days after infection doses, faces samples were harvested in order to emphasize the *Salmonella gallinarum* infection. In order to record the prophylactic effect of administration of IgY antibodies, the identification of the infection with Salmonella gallinarum was performed by analyzing the eggs from experimentally infected quails. The *Salmonella gallinarum* germs were identified from the egg shell, albumen, and yolk.

**Isolation and identification of Salmonella gallinarum germs.**

*The quantitative determination of Salmonella germs*

The following stages must be accomplished for salmonella identification, even in small quantities:

- sample preparation; in order to identify salmonella on egg shell, the samples are prelevated using a blotter with NaCl 1% solution. In order to identify salmonella from different egg components, the egg shell was previously washed with ethanol
(96%). The eggs were opened with a sterile lancet, albumen separated from yolk, and placed on sterile plate. The yolk was sterile prelevated with the siring.
- previous enrich in non selective liquid media
- enriching on selective media
- selective isolation on SS media. The Salmonella developed as transparent colonies black in center
- the serologic identification – the use of rapid serum agglutination technique on the blade and specific serum for anti-Salmonella group D antibodies.

The quantitative germ determination:
Previous decimal dilutions are needed for identification of the Salmonella germ number from faeces. 1 mL of obtained dilutions are seed on SS isolation media, incubated for 24 hours at 37°C. The number of the obtained colonies is corrected with the dilution factor and expressed as CFU/g faeces.

Results and Discussions

Regarding the clinic evaluation of the quails after experimental infection. The absence of the salmonella was recorded in faeces samples harvested in 0 day of the trial, before the administration of the Salmonella gallinarum suspension, in all experimental groups.
In trial I, where the infection dose was of 1x10⁸CFU, after 4 days from the experimental infection 1x10⁶ CFU/g faeces were recorded. The oral infection determined slight diarrhea, and decrease of laying percent, but mortality was not recorded.
In trial II, where infection dose was of 1x10⁷CFU, after 4 days from the experimental infection 1x10³ CFU/mg faeces were recorded. The oral infection did not produce specific clinic signs. The decrease of the laying percent and mortalities were not recorded.

Regarding isolation of Salmonella from eggs
Only the qualitative determination of the salmonella was performed in order to identify their presence. The samples prelevated before the administration of the Salmonella gallinarum suspension were negative in all experimental groups, salmonella being absent. After experimental infection, the eggs were processed and results were centralized (table 1). In trial I, a high infection level was recorded in three groups even from the first day after administration of the bacterial suspension, meaning 83.33% in control group, 80.00% in group where immunoglobulin extract was administered, and 85.71% in group where yolk was administered. The tendency of increasing of the egg contamination was constant. After 7 days from experimental infection, all processed eggs from groups I and II were infected with Salmonella, while in group III 93.33 of eggs were infected with Salmonella. The differences between studied groups were statistically not significant (p > 0.05).
### Table 1. Level of *Salmonella gallinarum* contamination in quail eggs

<table>
<thead>
<tr>
<th>Day</th>
<th>TRIAL I 1 x 10^8 CFU <em>Salmonella gallinarum</em></th>
<th>TRIAL II 1 x 10^7 CFU <em>Salmonella gallinarum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LI Control</td>
<td>L II Extract</td>
</tr>
<tr>
<td></td>
<td>Total egg number</td>
<td>No. of eggs infected.</td>
</tr>
<tr>
<td>1</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>5</td>
<td>14</td>
<td>13</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

In trial II, the level of *Salmonella* contamination was 20% in the first day after administration of the suspension, during the following days increased up to 30.76% in the 3rd day in group V where the immunoglobulin extract was administered, the same level 30.76%, respectively, but in the 5th day, in group VI. Later, the number of infected eggs decreased in both groups, when 21.42% *Salmonella* infected eggs were recorded in group V and 26.66 % in group VI, respectively, compared with 56.25% infected eggs recorded after 7 days in control group.

Analyzing the collected data, statistically very significant differences (p < 0.01) were recorded between the experimental groups which received antibodies as immunoglobulin extract or directly yolk, compared to control.

![Figure 1. The level of *Salmonella gallinarum* contamination in both experimental variants](image-url)
Conclusions

In both trials, when a low infection dose was administered, meaning $1 \times 10^7$ CFU *Salmonella gallinarum*, the administration of the IgY polyclonal antibodies as immunoglobulin extract or directly yolk, protective effect was recorded against germ invasion. This effect was not recoded when 10 folds higher dose was administered ($1 \times 10^8$ CFU). The prophylactic effect of the administration of the polyclonal antibodies was demonstrated. The administration of the polyclonal antibodies containing high concentrations of specific anti-*Salmonella* antibodies is an alternative to the use of antibiotics in salmonellosis prophylaxis, reducing the negative impact of antibiotics. From economically and manufacturing point of view, the vaccination of poultry effectives remains the most efficient method.

Bibliography


Cercetările au urmărit administrarea de doze crescute de anticorpi la loturile experimentale infectate cu Salmonella gallinarum, în scopul observării eficienței acestora în profilaxia salmonelozelor. Se poate observa că în cazul dozei mai scăzute de infecție, $1 \times 10^7$ CFU Salmonella gallinarum, administrarea anticorpilor policlonali, sub formă de extract imunoglobulinic sau chiar administrarea de gâlbenuș a avut un efect protectiv împotriva multiplicării germenilor. Acest efect nu a fost înregistrat și în cazul administrării unei doze de infectie de 10 ori mai mari ($1 \times 10^8$ CFU). Efectul profilactic al administrării anticorpilor policlonali a fost astfel demonstarat.

**Key words:** IgY, immunoprofilaxie, Salmonella gallinarum