

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

**UTILIZAREA ANTICORPILOR POLICLONALI SPECIFICI  
ANTI-SALMONELLA IZOLAȚI DIN OUĂ DE GĂINĂ, ÎN  
PROFILAXIA SALMONELOZELOR**

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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,  $1 \times 10^7$  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 ( $1 \times 10^8$  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 $\mu$ l Tiforomvac vaccine, consisting in  $2 \times 10^7$ 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<sub>2</sub>) 9.14.

**Biological material.** 6 experimental groups, with 10 quails (*Coturnix coturnix 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:

Trial	I			II		
Infection dose	10 <sup>8</sup> CFU			10 <sup>7</sup> CFU		
Groups	I Control 1	II Immunoglobulin extract	III Yolk	IV Control 2	V Immunoglobulin extract	VI Yolk antibodies

**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<sup>8</sup> CFU in trial I and a reduced dose 1 x 10<sup>7</sup> CFU in II<sup>nd</sup> 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 disinfestations, 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  $1 \times 10^8$  CFU, after 4 days from the experimental infection  $1 \times 10^6$  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  $1 \times 10^7$  CFU, after 4 days from the experimental infection  $1 \times 10^3$  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

TRIAL I 1 x 108 CFU <i>Salmonella gallinarum</i>									
Day	L I Control			L II Extract			L III Yolk		
	Total egg number	No. of eggs infected.	%	Total egg	No. of eggs infected	%	Total egg number	No. of eggs infected.	%
1	6	5	83.33	5	4	80.00	7	6	85.71
3	12	11	91.66	12	11	91.66	14	13	92.85
5	14	13	92.85	16	14	87.50	12	12	100.00
7	12	12	100.0	14	14	100.0	15	14	93.33
TRIAL II 1 x 107 CFU <i>Salmonella gallinarum</i>									
Day	L I Control			L V Extract			L VI Yolk		
	Total egg number	No. of eggs infected	%	Total egg	No. of eggs infected	%	Total egg number	No. of eggs infected	%
1	5	1	20.00	5	1	20.00	6	1	16.66
3	10	4	40.00	13	4	30.76	14	4	28.57
5	13	6	46.15	16	4	25.00	13	4	30.76
7	16	9	56.25	14	3	21.42	15	4	26.66

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 3<sup>rd</sup> day in group V where the immunoglobulin extract was administered, the same level 30.76%, respectively, but in the 5<sup>th</sup> 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.

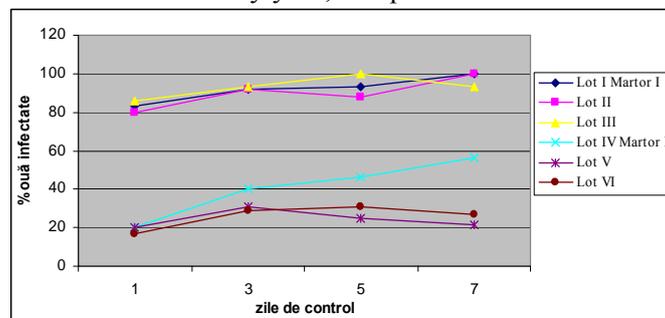


Figure 1. The level of *Salmonella gallinarum* contamination in both experimental variants

## 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 recorded 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.

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# UTILIZAREA ANTICORPILOR POLICLONALI SPECIFICI ANTI-SALMONELLA IZOLAȚI DIN OUĂ DE GĂINĂ, ÎN PROFILAXIA SALMONELOZELOR

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*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 gălbenuș a avut un efect protectiv împotriva multiplicării germinilor. Acest efect nu a fost înregistrat și în cazul administrării unei doze de infecție de 10 ori mai mare ( $1 \times 10^8$  CFU). Efectul profilactic al administrării anticorpilor policlonali a fost astfel demonstrat.*

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