THE IMMUNITY TO *SALMONELLA GALLINARUM* INFECTION IN CHICKENS PRODUCED BY LIVE CULTURES OF MEMBERS OF THE *SALMONELLA* GENUS

By H. WILLIAMS SMITH

*The Animal Health Trust, Houghton Grange, Huntingdon**

Dead vaccines have been used in most studies on the immunological relationships that exist between members of the *Salmonella* genus. Greenwood, Topley & Wilson (1931), for example, compared the immunity produced by dead vaccines of several *Salmonella* species against *Salm. typhi-murium* infection in mice, and because the immunity produced by the dead vaccines, even of *Salm. typhi-murium*, was of a low order, assessed their results by a consideration of survival times. Since live, but not dead, vaccines of *Salm. gallinarum* produced a complete immunity in chickens against oral infection with virulent strains of this organism, it was decided to re-investigate some aspects of cross-immunity within the *Salmonella* genus using live cultures as immunizing agents and *Salm. gallinarum* infection in chickens as the test system.

MATERIALS AND METHODS

The breed of chickens employed, their housing, diet and general management, have been described previously (Smith, 1956); so have the techniques of infecting them orally with *Salm. gallinarum* and examining their faeces for salmonellae and their blood for agglutinins to *Salm. gallinarum*.

Vaccination with live cultures. Different groups of 6-week-old chickens were injected subcutaneously with various species of salmonellae and other bacteria, the dose employed being 1 ml. of a 24 hr. broth culture. Variations of this technique were practised in the case of some bacteria mainly because of their lethal nature. These are listed below.

(1) *Salm. typhi-murium* and *Salm. thompson*. The dose was reduced to 0·1 ml.

(2) *Pasteurella septica*. Chickens were injected intramuscularly with 0·1 ml. of a 24 hr. broth culture of a strain of *Past. septica*, N.C.T.C. 2479 (Smith, 1955*c*), and 18 hr. later, when 15% of them had died, were given terramycin, 25 mg./kg., intramuscularly; a further 10% died.

(3) *Staphylococcus aureus*. This group of chickens was injected intravenously with 0·1 ml. of a 24 hr. broth culture of *Staph. aureus*, strain 9 (Smith, 1954). On the third and fourth day after injection, when they were all obviously ill, they were given 30,000 units of procaine penicillin intramuscularly. None of them died.

Tests carried out on a few of the chickens in the *Past. septica* and *Staph. aureus* groups 2 weeks later showed that they were immune to re-infection.

All the chickens used in these experiments appeared perfectly healthy at the time their immunity was challenged by the oral administration of *Salm. gallinarum*

* Now at Lilystone Hall, Stock, Essex.

3 weeks after vaccination. After challenge, chickens that died were examined to determine whether they had died from either the acute or the chronic form of *Salm. gallinarum* infection, the lesions of which have been described previously (Smith, 1955*a*); in unvaccinated chickens, deaths from the acute form usually occur within 14 days of infection, those occurring after this time being of the chronic form.

Table 1. *The immunity to* Salm. gallinarum *infection of groups of* 30 *chickens injected* 3 *weeks previously with live cultures of* Salm. gallinarum (*attenuated*), pullorum, enteritidis *and* dublin (*rough*)

Vaccinating culture	No. of chickens excreting vaccinal culture in faeces at 7 and/or 14 days	Average 'O' agglutination titre produced*	Total no. of 30 chickens that died when challenged from		No. of survivors			
			Acute form	Chronic form	Total	With severe lesions	With mild lesions	Faecal positive
Smooth *Salm. gallinarum*, 9S	0	1/1800	0	0	30	0	0	1
Smooth *Salm. gallinarum*, M	10	1/800	0	0	30	2†	4†	5†
Smooth *Salm. pullorum*	—	1/400	0	0	30	0	3	0
Smooth *Salm. enteritidis* var. *essen* N.C.T.C. 4777	3	1/150	0	3	27	10	6	18
Rough *Salm. gallinarum*, 9R	0	0	0	0	30	2	8	1
Rough *Salm. dublin*	—	1/250	12	5	13	4	4	9
None	—	0	16	4	10	4	6	7

* Titre to smooth *Salm. gallinarum* antigen. † Probably vaccinal in origin.

Three of the chickens originally included in the *Salm. gallinarum* M and *enteritidis* groups died from vaccination. 9S and 9R were attenuated strains prepared from the strain of *Salm. gallinarum*, 9, used for challenge (Smith, 1956).

Experiments were terminated 21 days after infection since deaths seldom occur after this time. All surviving chickens were killed, their faeces examined for *Salm. gallinarum* and their organs for lesions of the chronic disease. A number of chickens that had been vaccinated with live cultures of different *Salmonella* species, but had not been infected subsequently with *Salm. gallinarum*, were also killed at this time to confirm that no lesions similar to those of *Salm. gallinarum* infection were present.

RESULTS

The immunizing effect of live cultures of Salm. gallinarum, pullorum, enteritidis *and* dublin

The results of infecting with *Salm. gallinarum* groups of 30 chickens that had been vaccinated either with live attenuated cultures of *Salm. gallinarum*, smooth and rough, or with live cultures of smooth strains of *Salm. pullorum* or *Salm. enteritidis*

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var. *essen* or with a rough culture of *Salm. dublin* are illustrated in Table 1. The characteristics of the attenuated *Salm. gallinarum* cultures 9S and 9R, which had been prepared from the strain used for challenge, have been described previously (Smith, 1956). The heterologous *Salm. gallinarum* strain, M, had not been fully attenuated and produced a vaccinal mortality of 10% as did the *Salm. enteritidis* culture. The culture of *Salm. dublin*, although judged to be completely rough by the acriflavine test, produced agglutinins to smooth *Salm. gallinarum* antigen in the chickens vaccinated with it; this was not the case with the rough *Salm. gallinarum* culture, 9R. The chickens vaccinated with *Salm. pullorum* possessed an immunity as complete as that produced by the smooth *Salm. gallinarum* vaccine 9S, although three showed slight lesions in the myocardium. It is probable that the immunity evoked by the heterologous *Salm. gallinarum* vaccine, M, was equally as complete; the appearance and age of the lesions found in 6 of the chickens after challenge suggested that they were due to the vaccine itself. Although the smooth *Salm. enteritidis* culture gave a complete protection against death from the acute form of *Salm. gallinarum* infection, it gave much less protection against the chronic form; 3 of the 30 chickens died from it and 16 of the survivors, 10 with extensive lesions, were affected with it. Unlike the rough *Salm. gallinarum* vaccine, 9R, the rough *Salm. dublin* culture gave little or no protection against either the acute or the chronic form of the disease.

The immunizing effect of live cultures of the Salmonella *O-groups B, C, D and E and* Bacterium coli

The results of infecting groups of 18 chickens with *Salm. gallinarum* 3 weeks after they had been injected subcutaneously with live cultures of different *Salmonella* species and an avirulent strain of *B. coli* are illustrated in Table 2. The rough cultures had been prepared by growing some of the smooth strains in broth containing anti-serum to smooth *Salm. gallinarum*. Chickens that had been injected with 11 of the 12 members of group D salmonellae possessed a high immunity against death from the acute form of *Salm. gallinarum* infection, the greatest number that died from this form in any batch being 2, compared with 11 in the control group. The majority, however, were affected with the chronic form of the disease, some chickens in most batches dying from it and many developing severe lesions; the disease was mildest in chickens injected with *Salm. javiana*. One smooth strain of *Salm. dar-es-salaam*, N.C.T.C. 5773, produced little or no immunity to *Salm. gallinarum* infection, thus differing markedly from another culture of the same species, N.C.T.C. 2206, and the other group D salmonellae. When different groups and different individual chickens within a group that had been vaccinated with the smooth group D salmonellae were compared, there appeared to be no correlation between the anti-O agglutination titre resulting from the injection of the vaccinal strain and susceptibility to the challenge infection with *Salm. gallinarum*.

Little or no immunity was produced by injecting chickens with rough cultures prepared from either *Salm. eastbourne* or *dublin*. A more effective immunity was produced by the rough *Salm. enteritidis* var. *essen* culture. In contrast to the

chickens injected with the other two cultures, 7 of the chickens injected with this culture were found 7 or 14 days later to be excreting it in their faeces; 6 of them also had detectable anti-O agglutinins in their sera.

Table 2. *The immunity to* Salm. gallinarum *infection of groups of* 18 *chickens injected* 3 *weeks previously with live cultures of different* Salmonella *species and* B. coli

Vaccinal culture	No. of chickens that died from vaccination	No. of chickens excreting vaccinal culture in faeces at 7 and/or 14 days	Average 'O' agglutination titre produced*	No. of chickens that died when challenged from		No. of survivors			
				Acute form	Chronic form	Total	With severe lesions	With mild lesions	Faecal positive
Group D smooth									
Salm. javiana, N.C.T.C. 6495	1	10	1/600 (2)	1	0	16	1	6	6
Salm. rostock, N.C.T.C. 5767	0	5	1/370 (3)	0	2	16	5	8	7
Salm. moscow, N.C.T.C. 5768	0	2	1/250 (2)	1	1	16	5	6	5
Salm. enteritidis var. *jena*, N.C.T.C. 5760	0	1	1/150 (8)	1	1	16	5	10	6
Salm. enteritidis var. *danysz*, N.C.T.C. 5694	0	2	1/180 (2)	2	0	16	2	8	4
Salm. dublin, N.C.T.C. 5766	1	5	1/350 (2)	2	1	14	7	4	4
Salm. durban, N.C.T.C. 6235	0	4	1/400 (1)	0	4	14	5	2	8
Salm. miami, N.C.T.C. 7112	3	0	1/100 (2)	1	3	11	5	3	7
Salm. eastbourne, N.C.T.C. 5771	1	0	1/130 (1)	1	3	13	4	5	9
Salm. onarimon, N.C.T.C. 6259	0	0	1/750 (0)	2	2	14	4	6	4
Salm. dar-es-salaam, N.C.T.C. 2256	0	10	1/70 (1)	2	2	14	2	9	8
Salm. dar-es-salaam, N.C.T.C. 5773	0	0	1/40 (5)	8	3	7	1	2	5
Group D rough									
Salm. enteritidis var. *essen*, ex. N.C.T.C. 4777	0	7	1/25 (12)	2	1	15	1	10	7
Salm. eastbourne, ex. N.C.T.C. 5771	0	0	0	8	2	8	3	4	3
Salm. dublin, ex N.C.T.C. 5766	0	0	0	11	1	6	2	2	4
Group B smooth									
Salm. typhi-murium	50%†	6	1/30 (9)	3	0	15	2	6	8
Salm. chester, N.C.T.C. 5718	40%†	6	1/50 (5)	3	2	13	2	6	7
Group C smooth									
Salm. cholerae-suis, N.C.T.C. 5737	0	1	0	6	2	10	3	3	6
Salm. thompson	70%†	3	0	6	3	9	3	4	6
Group E smooth									
Salm. anatum, N.C.T.C. 5779	0	5	0	5	2	11	3	3	6
Salm. senftenburg, N.C.T.C. 5788	0	3	0	8	1	9	2	4	5
Avirulent *B. coli*	0	—	0	10	3	5	1	5	4
None	0	—	0	11	2	5	2	2	3

* Titre to smooth *Salm. gallinarum* antigen. Figures in parentheses represent number of chickens without demonstrable agglutinins in sera.

† Sufficient chickens were vaccinated to provide 18 survivors.

The course of *Salm. gallinarum* infection in chickens vaccinated with *Salm. typhi-murium* and possibly in those vaccinated with *Salm. chester*, the two members of the B group, resembled that in the chickens vaccinated with group D salmonellae. Many of the chickens originally intended for these experiments with the live group B strains, however, died as a result of vaccination. A high vaccinal mortality was also observed in chickens injected with *Salm. thompson*. The survivors, and the chickens injected with the other group C culture, *Salm. choleraesuis*, exhibited only a slight resistance to *Salm. gallinarum* infection. Similar results were also obtained with chickens vaccinated with two group E strains, *Salm. anatum* and *Salm. senftenberg*. Apart from the chickens vaccinated with avirulent *B. coli*, it was noted, however, that the mortality rate from *Salm. gallinarum* infection in vaccinated chickens was always somewhat lower than in the unvaccinated chickens.

The immunizing effect of live cultures of Pasteurella septica, Staphylococcus aureus *and* Salm. senftenberg

The course of *Salm. gallinarum* infection in groups of 30 chickens that had been infected with either *Past. septica*, *Staph. aureus*, or *Salm. senftenberg* 3 weeks earlier is illustrated in Table 3. As previously stated, it was necessary to treat the chickens after infection with *Past. septica* and *Staph. aureus* with chemothera-

Table 3. *The immunity to* Salm. gallinarum *infection of groups of* 30 *chickens infected* 3 *weeks previously with* Staphylococcus aureus, Pasteurella septica *and* Salm. senftenberg

Previous infection	Total number of 30 chickens dead by the following days after challenge with *Salm. gallinarum*											No. of survivors			
	7	8	9	10	11	12	13	14	15	16	16+	Total	With severe lesions	With mild lesions	Faecal positive
*Pasteurella septica**	0	0	2	4	6	8	9	11	12	12	12	18	6	11	12
Staph. aureus	0	2	4	4	7	9	10	10	12	13	14	16	2	11	8
Salm. senftenberg	1	4	4	6	8	8	11	13	14	14	16	14	4	7	9
None	3	6	11	15	18	20	20	20	21	21	22	8	3	5	5

* Sufficient chickens were infected to provide 30 survivors. The mortality rate was 25 %, occurring mainly before terramycin treatment. Owing to penicillin treatment, none of the chickens infected with *Staph. aureus* died.

None of the chickens had agglutinins to *Salm. gallinarum* as a result of their initial infection.

peutic agents; otherwise a very high vaccinal mortality would have occurred. The results indicate that these chickens possessed a slight but definite degree of immunity to infection with *Salm. gallinarum*, an immunity at least equal to that produced by *Salm. senftenberg*. The mortality rate from the acute form of *Salm. gallinarum* infection in the chickens previously vaccinated with *Past. septica*, *Staph. aureus* and *Salm. senftenberg* and in the unvaccinated control chickens was 12, 11, 13 and 20 respectively.

DISCUSSION

Any assessment of the immunological relationship of *Salmonella* species to each other based on the present studies has to be considered in the light of the observation that previous infection with either *Past. septica* or *Staph. aureus*, bacteria generally accepted as being unrelated to *Salm. gallinarum*, conferred an appreciable immunity in chickens against fatal infection with this bacterium. Particularly as this immunity was not conferred by the avirulent strain of *B. coli*, it is probable that it is due to a general non-specific stimulus to the defence mechanisms produced by the severe type of infection caused by *Past. septica* and *Staph. aureus*. In view of this, it is conceivable, other things being equal, that a greater immunity would be produced by those bacterial species that produce a greater infective stimulus, i.e. those that are more pathogenic. That a considerable infective stimulus is necessary even in the case of *Salm. gallinarum* itself was noted during studies on the treatment of *Salm. gallinarum* infection with furazolidone (Smith, 1955*b*), the significance of which, from the immunological point of view, has been discussed in the previous paper (Smith, 1956). Thus it is possible that the reason why three of the four rough group D cultures and the smooth *Salm. dar-es-salaam* culture, N.C.T.C. 5773, produced no immunity to *Salm. gallinarum* infection was not necessarily because they lacked some immunogenic component but because they were completely non-pathogenic. By contrast, the fourth rough group D cultures and the other smooth culture of *Salm. dar-es-salaam*, N.C.T.C. 2206, both of which produced an appreciable degree of immunity, produced a definite reaction in the host as judged by the fact that many of the chickens vaccinated with these cultures were found to be excreting them in their faeces 7 or 14 days later. That specific immunogenic components also play a very important part is obvious from the results, but the probability that an infective stimulus is also necessary makes any attempt to classify salmonellae on immunological grounds from the results of the present studies very difficult.

Bearing in mind the considerations referred to above, it would appear that the group D salmonellae, as a whole, produced a similar degree of immunity which, although by no means as complete as that produced by *Salm. gallinarum* itself, was quite substantial. *Salm. pullorum* was unique amongst them in that it provoked a complete immunity to *Salm. gallinarum*, a fact that supports the view that *Salm. pullorum* should be considered as a variant of *Salm. gallinarum* rather than as a species distinct from it as are the other group D salmonellae. For the reasons given previously, the exact position of the rough group D strains is difficult to assess. It is noteworthy, however, that all of a number of rough cultures of *Salm. gallinarum* prepared in a variety of ways produced an observable immunity (Smith, 1956), whereas three of the four rough group D cultures did not.

Although the immunity conferred by *Salm. typhi-murium* and, possibly, *Salm. chester* was of the same order as that conferred by the group D strains, it is not possible to draw any definite conclusions from these observations as to the status of the group B salmonellae since a high vaccinal mortality occurred in the chickens injected with these two cultures. This may have resulted in the elimination from

the challenge experiment of the chickens that were the most susceptible to *Salm. gallinarum* infection. Since the immunity conferred by the group C and E cultures was similar to that conferred by *Staph. aureus* and *Past. septica* it is highly probable that it was of a non-specific character and that these *Salmonella* cultures bear no specific immunological relationship to *Salm. gallinarum*. It is advisable, however, that the terms 'specific' and 'non-specific' be used cautiously since many factors operate in *Salm. gallinarum* immunity (Smith, 1956).

SUMMARY

1. The immunity to *Salm. gallinarum* infection produced by injecting chickens with live cultures of different species of *Salmonella* and other bacteria has been studied.

2. *Salm. pullorum* produced a complete immunity equal to that produced by *Salm. gallinarum* itself. The immunity evoked by 11 of 12 other *Salmonella* species belonging to group D, although substantial, was less complete. Three of four rough group D strains and an avirulent strain of *B. coli* produced no immunity. Although a considerable degree of immunity was produced by the fourth rough strain it was not equal to that produced by rough strains of *Salm. gallinarum*.

3. Owing to their lethal nature, it was not possible to be definite about the extent of the immunity produced by two group B strains, *Salm. typhi-murium* and *Salm. chester*, although it may have been equal to that produced by the group D strains.

4. A slight but definite degree of immunity was produced by two group C and two group E *Salmonella* cultures. Since this immunity was no better than that possessed by chickens previously infected with either *Pasteurella septica* or *Staphylococcus aureus*, it was considered to be non-specific in character.

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