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PRODUCTION AND APPLICATION OF A LIVE *SALMONELLA GALLINARUM* VACCINE

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ABSTRACT

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The production and application of a freeze-dried *Salmonella gallinarum* vaccine are described in this report. The vaccine is stable when kept at 4 °C and a single injection elicits a good immunity for 2 months, though its effect gradually diminishes. Immunity is neither enhanced nor depressed by repeated injections of the live vaccine, and no interference effect was observed in experimentally infected chickens.

Furazolidone therapy jeopardizes the immunogenicity of a live vaccine, but its effect can be countered by the administration of either an inactivated or a live vaccine when medication is commenced and this is followed by the application of live vaccine 6 days after cessation of medication.

Résumé

PRODUCTION ET APPLICATION D'UN VACCIN VIVANT DE SALMONELLA GALLINARUM

On décrit dans ce rapport la production et l'application d'un vaccin lyophilizé de Salmonella gallinarum. *Le vaccin reste stable quand on le conserve à 4 °C et une seule injection fournit une bonne immunité pour deux mois, bien que ses effets diminuent graduellement. Des injections répétées de vaccin vivant n'augmentent ni ne diminuent l'immunité et l'on n'a pas observé de réactions d'interférence chez des poulets infectés expérimentalement.*

La thérapeutique à la furazolidone met en danger le pouvoir immunogène d'un vaccin vivant, mais on peut contrecarrer cette action en administrant un vaccin soit inactivé soit vivant au début de la médication et en administruant de vaccin vivant 6 jours après la fin de la médication.

INTRODUCTION

Live vaccines prepared from rough mutants of *Salmonella gallinarum* have been effectively used to protect chickens against fowl typhoid (Cameron, Fuls & Van Reenen, 1972; Smith 1956b). Certain aspects of the mass production of vaccine and its practical application, however, need elucidation and experiments were therefore undertaken to determine (a) whether sedimentation of bacteria with polyethyline glycol (p.e.g.) (Cameron & Weiss, 1974) affects their immunogenicity, (b) the keeping quality of such a vaccine, and (c) the duration of immunity it produces.

A serious drawback of a live vaccine is that, should an active outbreak of fowl typhoid occur, it cannot be used simultaneously with furazolidone therapy, since this drug interferes with the immunizing properties of the vaccine (H. W. Botes, personal communication, 1965). The possibility of solving this problem by the use of inactivated vaccines was consequently also investigated.

MATERIALS AND METHODS

Experimental animals

Immunization trials were conducted in eight 10-week-old New Hampshire chickens. They were housed in wire cages and fed a commercial broiler mash *ad libitum*.

Medication of feed

Furazolidone ('Neftin' 20% active ingredient)* was thoroughly mixed with the feed to give a final concentration of 0,04% of the active ingredient.

Vaccine production

Live vaccine.—Live lyophilized vaccine was produced by growing *S. gallinarum* strain 5503 (2) in D15 medium (Schlecht & Westphal, 1966) in a fermenter at 37 °C for 24 h. The culture was continuously stirred by means of an impeller revolving at 150 rpm and thoroughly aerated in the process by means of a sparger. At the end of the growth period the culture was harvested and 80 g of p.e.g., made up in 100 mℓ of distilled water, was added to 20 ℓ culture material to give a final concentration of 4,0% p.e.g. The culture was mixed thoroughly and allowed to stand at room temperature for 2–3 h, after which 17–18 ℓ of the supernatant fluid was siphoned off to produce an approximate tenfold concentration of bacteria (Cameron & Weiss, 1974).

The cell suspension was then mixed with an equal volume of half-strength buffer lactose peptone (BLP)*, distributed in 2 mℓ quantities in rubber-capped bottles, freeze-dried, and sealed *in vacuo*. The shelf temperature was raised from −30 °C to 15 °C over a period of 18 h and kept at 35 °C for 2 h. The final product contained less than 3% moisture and the number of viable organisms was assessed by plate counts.

Live vaccine was also prepared without p.e.g. sedimentation, in which case the culture was concentrated tenfold by centrifugation.

Live vaccines were reconstituted for use to give $1–2 \times 10^8$ live organisms/mℓ, the dosage which was used in all the experiments, unless otherwise specified.

Alum-precipitated vaccine.—Virulent smooth *S. gallinarum* strain 1007 was grown in D15 medium in Pivitski shake flasks for 18 h at 37 °C. The bacteria were killed by adding formalin to a final concentration of 0,5% to the cultures and allowing them to stand at room temperature for 10 days. After the cell density was adjusted to 2,0% packed cell volume, the culture was precipitated by the addition of 10 mℓ of a 10% potassium alum solution and 5 mℓ of a 7,4% potassium hydroxide solution per 100 mℓ of cell suspension without adjustment of the pH. The dosage used for subcutaneous injection was 1,0 mℓ.

Oil adjuvant vaccine.—Bacteria were grown and inactivated as described above and the cell density adjusted to 5,0%. The suspension of cells was

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* BLP: 0,15 M Phosphate buffer pH 7,0—7,1 containing 10% lactose and 2,0% peptone

emulsified in oil according to a procedure recommended for the production of *Haemophilus gallinarum* bacterin (Cameron & Fuls, 1978). The dosage used for subcutaneous injection in all the experiments was 10 mℓ.

Determination of the keeping quality of live vaccine

Bottles of freeze-dried vaccine were kept at temperatures of either 4 °C, 20 °C or 37 °C and viable counts were made after 1 and 2 weeks, and thereafter at monthly intervals. A 4th set was kept initially at 4 °C for 6 months, then at 30 °C for 1 week and finally again at 4 °C to simulate normal storage and transport temperatures.

For each count, 3 bottles of dried vaccine were resuspended in 600 mℓ of distilled water, tenfold dilutions were made and plate counts done. The counts were expressed as the number of organisms per mℓ, which is equivalent to 1 dose.

Immunity experiments

Effect of p.e.g. sedimentation on antigenicity

Separate groups of 24 chickens each were immunized with $2,5\times10^8$ organisms of live vaccine prepared either by p.e.g. sedimentation or by centrifugation (Groups 1A & 2A). Similarly, 3 groups of 8 chickens each were immunized with 5×10^7, 1×10^7 and 4×10^6 organisms of both vaccines, respectively. All the chickens of the latter dosage groups as well as 8 chickens from Groups 1A & 2A were challenged 14 days after immunization. A further 8 chickens of Groups 1A & 2A vaccines were challenged 60 and 120 days after immunization.

Duration of immunity

Groups of 36 chickens were immunized as follows: Group 1 was given a single injection of live vaccine, Group 2 was given 2 injections of live vaccine with an interval of 14 days between the injections and Group 3 was given an injection of oil adjuvant vaccine, followed 14 days later by a single injection of live vaccine. Six chickens from each group and 6 non-immunized controls were challenged 2, 4 and 6 months after immunization. At this stage all the remaining chickens were given a booster injection of live vaccine and 6 chickens of each group again challenged at 2-month intervals.

Effect of furazolidone on the immune response to live vaccine

The design of the experiment is apparent from Table 4. Groups of 16 chickens were fed on mash with or without furazolidone for 10 days. Eight birds from each group were immunized with live vaccine when medication commenced and 8 birds were immunized 6 days after the termination of medication. All the immunized birds as well as non-vaccinated controls were challenged 6 days after the termination of medication.

Effect of priming on subsequent immunization with live vaccine

Groups of 16 chickens were given either live, alum-precipitated or oil emulsion vaccine at the commencement of the experiment. After 14 days, 8 chickens of each group were given an injection of live vaccine and all the birds as well as 8 non-immunized controls were challenged simultaneously with the last injection of vaccine.

Effect of consecutive use of inactivated and live vaccine in furazolidone-treated chickens

Groups of 16 chickens on medicated feed were immunized with either alum-precipitated, oil emulsion or live vaccine. They were all given a 2nd injection of live vaccine 16 days later (i.e. 6 days after cessation of medication) and challenged at the same time. Non-immunized controls, whether medicated or not, were challenged at the same time.

Challenge of chickens

Experimental birds were infected orally with $2–5\times10^8$ virulent *S. gallinarum* strain 1007 as described previously (Cameron *et al.*, 1972), except that a nepholometrically standardized suspension of freshly-grown bacteria was used instead of a dried culture. Deaths were recorded for 14 days after challenge.

Results

Stability of live vaccine

A previous comparison of 10 freeze-drying media indicated that the best results were obtained with a 1:1 dilution of BLP (B. H. J. Smit, personal communication, 1977).

From the results shown in Table 1 it is evident that *S. gallinarum* can be satisfactorily dried in BLP and that the resulting product is stable when kept at 4 °C. Higher temperatures, however, are harmful, and storage at 20 °C, and particularly at 37 °C, results in a marked drop in the viable count.

TABLE 1 Temperature stability of *S. gallinarum* freeze-dried vaccine

Storage time in months	Viable counts/mℓ			
	Storage temperature			
	4 °C	20 °C	37 °C	4 °C for 6 months and 30 °C for 7 days
0	$4,6\times10^8$	$3,5\times10^8$	$4,6\times10^8$	$4,6\times10^8$
¼	nd	nd	$1,7\times10^8$	nd
½	$6,9\times10^8$	$4,0\times10^8$	$4,0\times10^6$	$6,9\times10^8$
1	$5,2\times10^6$	$4,0\times10^8$	$3,0\times10^4$	$5,2\times10^8$
2	$2,1\times10^8$	$1,4\times10^8$	0	$2,1\times10^8$
3	$4,0\times10^8$	6×10^7	0	$4,0\times10^8$
4	$2,8\times10^8$	3×10^7	0	$2,8\times10^8$
5	$3,2\times10^8$	2×10^2	0	$3,2\times10^8$
6	$2,9\times10^8$	0	0	$2,9\times10^8$
6¼	nd	0	0	$2,0\times10^8$
6½	nd	0	0	$1,5\times10^8$
7	$2,7\times10^8$	0	0	$0,8\times10^8$
7½	nd	0	0	$1,0\times10^8$
8,0	$2,2\times10^8$	0	0	$1,0\times10^8$

nd = not determined

Immunogenicity of p.e.g. sedimented bacteria

The results of an experiment in which both the duration of immunity and the effect of dosage on immunity afforded by dried vaccine prepared from either p.e.g. sedimented bacteria or bacteria concentrated by centrifugation were compared, are given in Table 2. Both products gave a good immunity with as few as 1×10^7 bacteria and at a dosage of $2,5\times10^8$ bacteria per chicken the immunity persisted for 2 months. By 4 months, however, the protection afforded by both vaccines had appreciably decreased.

TABLE 2 Comparison of immunity induced by polyethylene glycol-sedimented bacteria and bacteria collected by centrifugation

Vaccine	Group	Bacteria per dose	Days after immunization		
			14	60	120
			Survivors/8,14 days after challenge		
P.e.g. sedimented bacteria	1A	$2,5\times10^8$	7	7	4
	1B	5×10^7	7	nd	nd
	1C	1×10^7	7	nd	nd
	1D	4×10^6	—	—	—
Centrifuged bacteria	2A	$2,5\times10^8$	8	8	5
	2B	5×10^7	7	nd	nd
	2C	1×10^7	7	nd	nd
	2D	4×10^6	7	nd	nd
Controls	3	None	4	2	0

nd=not determined

TABLE 3 Duration of immunity afforded by different immunization regimens

Group	Immunization schedule	Months after initial immunization					
		2	4	6	8	10	12
		Survivors/6,14 days after challenge					
1	Single injection of live vaccine	6	3	1	2	4	3
2	2 injections of live vaccine at 14-day-intervals	5	3	2	4	4	3
3	Oil emulsion vaccine followed 14 days later by live vaccine	6	3	3	3	4	3
4	None	2	0	0	0	1	1

TABLE 4 Effect of furazolidone therapy on development of immunity induced by a single injection of live vaccine

Group	Treatment with furazolidone for 10 days	Immunization regimen		Total survivors/8,14 days after challenge
		1st immunization simultaneous with commencement of treatment	2nd immunization 6 days after cessation of treatment and simultaneous with challenge	
1	Given	Live vaccine	—	3
2	Not given	Live vaccine	—	6
3	Given	—	Live vaccine	1
4	Not given	—	Live vaccine	1
5	Given	—	—	1
6	Not given	—	—	2

Duration of immunity

Chickens given either a single injection of live vaccine, 2 injections of live vaccine, or an injection of oil emulsion vaccine followed by an injection of live vaccine, all showed a comparable level of immunity when challenged either 2 or 4 months after immunization (Table 3). By 6 months the immunity had waned, but a booster injection of live vaccine given at this stage restored the level of immunity in all 3 groups to approximately 50%, a level which persisted for a further 6 months.

TABLE 5 Effect of priming on subsequent immunization with live vaccine

Group	First immunizations	Second immunization	Total survivors/8, 14 days after challenge
1A	Live vaccine	—	7
1B	Live vaccine	Live vaccine	7
2A	Alum vaccine	—	2
2B	Alum vaccine	Live vaccine	8
3A	Oil vaccine	—	4
3B	Oil vaccine	Live vaccine	8
4	—	—	1

TABLE 6 Effect of consecutive use of inactivated and live vaccine in furazolidone-treated chickens

Group	Treatment with furazolidone for 10 days	Immunization regimen		Total survivors/16, 14 days after challenge
		1st immunization simultaneous with commencement of treatment	2nd immunization 6 days after cessation of treatment and simultaneous with challenge	
1	Given	Alum vaccine	Live vaccine	9
2	Given	Oil vaccine	Live vaccine	12
3	Given	Live vaccine	Live vaccine	9
4	Given	—	—	5
5	Not given	—	—	3

Effect of furazolidone medication on the development of immunity induced by live vaccine

Although, admittedly, the number of experimental birds was small, the results given in Table 4 indicate that birds which were treated with furazolidone and immunized simultaneously (Group 2) were less resistant than those which were immunized only (Group 1). When vaccine was given simultaneously with challenge (Groups 3 & 4), no immunity was established irrespective of whether the chickens had been medicated or not. No so-called 'interference effect' could thus be demonstrated.

Effect of priming on development of immunity

Since the application of live vaccine during medication proved unsatisfactory, the possibility of using an inactivated vaccine at this stage arose, provided that it did not jeopardize the subsequent application of live vaccine. The results given in Table 5 show that inactivated vaccines alone do not give a good immunity (Groups 2A & 3A) and that subsequent application of a live vaccine results in solid protection (Groups 2B & 3B) which is comparable to the immunity afforded by a single injection of live vaccine (Group 1A). Furthermore, a 2nd application of live vaccine did not adversely affect the immunity afforded by a single injection of live vaccine (Group 1B).

Effect of the consecutive use of inactivated vaccines and live vaccine in furazolidone-treated chickens

The data presented in Table 6 show that, when chickens are primed during medication, the application of live vaccine 6 days after cessation of medication produces a good immunity. Although there is no significant difference in immunity between the 3 groups, slightly better results were obtained with the oil emulsion vaccine.

Discussion

The results presented in this paper show that an effective live *S. gallinarum* vaccine can be prepared from p.e.g. sedimented bacteria. The resultant product, though heat labile, maintains its potency for at least 8 months provided that it is stored at 4 °C.

The immunity stimulated by the vaccine is initially good and remains at a high level for 2 months, but it then decreases to a level of approximately 50% at 3–4 months and, by 6 months, it is poor. Comparable results were obtained both when immunization with live vaccine was preceded by the application of inactivated vaccines and when 2 injections of live vaccine were used. This latter finding is in disagreement with the contention that 2 consecutive injections result in depressed immunity (Botes, personal communication, 1965). Thus chickens immunized at the recommended age of 2 months should be re-immunized at the age of 8 months if they are still exposed to conditions which favour the occurrence of fowl typhoid. Under normal circumstances, however, the exposure level would be far lower than that used experimentally and it is rarely neccessary to re-immunize chickens.

Medication of chickens with furazolidone at therapeutic levels clearly jeopardizes immunization. As it was also shown that immunization simultaneous with challenge was ineffective, the so-called 'interference effect' reported by Smith (1956a) could not be substantiated.

Chickens given either an inactivated vaccine or live vaccine during medication are apparently immunologically primed and respond very well to subsequent immunization with live vaccine. The secondary response is such that they are well protected even when they are challenged simultaneously with the application of the booster injection. It can, therefore, be generally recommended that, should an outbreak

of fowl typhoid occur, chemotherapy and immunization should commence immediately, followed by the application of live vaccine 6 days after cessation of medication.

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