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IMMUNIZATION OF MICE AND CALVES AGAINST *SALMONELLA DUBLIN* WITH ATTENUATED LIVE AND INACTIVATED VACCINES

C. M. CAMERON and W. J. P. FULS, Veterinary Research Institute, Onderstepoort

Abstract

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Previous findings, viz. that mice can be successfully immunized against infection with *Salmonella dublin* with either live or inactivated vaccine, were confirmed. Immunity lasted for at least 12 weeks in mice which had been immunized with inactivated alum-precipitated vaccine. The immunogenicity of inactivated vaccine gradually decreased on storage at 4 °C, but this was only detectable if a single injection was used for immunization: 2 injections virtually eliminated this phenomenon.

The immunogenicity of live vaccine in mice was not enhanced by levamizole or the simultaneous injection of inactivated organisms.

Both live and inactivated vaccines provided immunity in calves. A single injection of lyophilized vaccine, prepared from live rough *Salmonella dublin* strain (HB 1/17), protected 3 out of 6 calves, while 2 injections of a formalin-inactivated, alum-precipitated vaccine, containing 1% packed cells of *S. dublin* strain 2652 V, protected 5 out of 6 calves against intraduodenal challenge with 2×10^9 *S. dublin* strain 2652 V. Two calves which had been immunized with an inactivated oil adjuvant vaccine were also solidly immune to this challenge.

Serum antibody response in calves was poor when measured by the tube agglutination and the haemagglutination tests. Similarly, the sera had only marginal protective values when tested by means of a passive protection test in mice. Antibody titres alone are not a valid measure, therefore, for the immune status of immunized animals.

Résumé

L'IMMUNIZATION CONTRE SALMONELLA DUBLIN *AUX VACCINS VIVANTS ATTENUÉS ET VACCINS INACTIVÉS CHEZ LA SOURIS ET LE VEAU*

Les auteurs ont pu confirmer des conclusions antérieures que la souris peut être immunisée avec succès contre Salmonella dublin *en employant soit un vaccin vivant soit un vaccin inactivé. La durée de l'immunité chez souris immunisées à un vaccin inactivé et précipité à alun a été de* 12 *semaines. Après stockage à* 4°C *l'immunogenicité du vaccin inactivé a diminué, phénomène appréciable seulement après l'immunisation à une seule injection et presque mis à l'écart avec* 2 *injections.*

Chez souris ni l'addition de levamizole ni l'injection simultanée de bactéries inactivées n'a exalté l'immunogenicité d'un vaccin vivant.

Les vaccins soit vivants soit inactivés assurent une immunité chez le veau. Une seule injection d'un vaccin lyophilisé et préparé d'une souche "rough" (HB 1/17) *de* S. dublin *a conféré une immunité à* 3 *sur* 6 *veaux, tandis que* 2 *injections d'un vaccin inactivé à formol, précipité à alun et renfermant* 1*p* 100 *cellules entassées de* S. dublin *souche* 2652 *V ont conféré une immunité à* 5 *sur* 6 *veaux contre une épreuve de* 2×10^9 S. dublin *souche* 2652 *V. Deux veaux immunisés avec un vaccin inactivé et auquel un adjuvant de huile à été ajonté, ont également fait preuve d'une bonne immunité contre cette épreuve.*

Chez veaux la réponse immunitaire en anticorps sériques appreciée à l'aide du test d'agglutination en tube et d'hémagglutination a été faible. En plus, la valeur protectrice des sérums apprecié chez souris à l'aide d'un test de protection passive a été petite. Il s'ensuit donc que les taux d'anticorps à eux seuls ne sont pas une mesure valable de l'état immunitaire d'animaux immunisés.

Introduction

Despite the application of vaccine and the availability of therapeutic agents, salmonellosis in cattle remains a serious and widespread problem (Richardson, 1974; Richardson, 1975; Sojka & Wray, 1975). Salmonellosis is common in South Africa and is responsible for considerable losses in calves (Botes, 1965).

In addition to zoo-sanitary control measures (Hartwigk, 1970; Richardson, 1975), prophylactic immunization plays an important role in the control of salmonellosis. Earlier workers commonly employed inactivated vaccines (Henning, 1953a), but these fell into disfavour when live vaccines prepared from avirulent mutants (Botes, 1964; Botes, 1965; Smith, 1965) became available. More recently, however, renewed interest has been shown in inactivated vaccines (Rudge, Cooper & Jull, 1968). In fact, Cameron & Fuls (1974) showed that in mice an excellent immunity to *Salmonella typhimurium* infection could be established by means of an inactivated vaccine. Furthermore, in a subsequent investigation, Cameron & Fuls (1975) showed that a formalin-inactivated alum-precipitated vaccine protected 75% of immunized mice against *S. dublin*, though it was ineffective in protecting guinea-pigs against infection with *S. dublin*.

These findings suggested that inactivated vaccines may have a practical use, and experiments were conducted in mice to determine the heat stability of such vaccines and to ascertain whether the duration of immunity was satisfactory.

Furthermore, in addition to studies on the efficacy of inactivated vaccines in calves, the possibility of enhancing the immunogenicity of live vaccine by levamizole (Renoux & Renoux, 1973; Woods, Fliegelman & Chirigos, 1975) was investigated.

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Materials and Methods

Experimental animals

Mice. Conventionally-reared male albino mice from the colony maintained at the Institute were used throughout. They were fed a balanced, pelleted ration and housed in plastic cages.

Calves. All the calves used in this study were either Afrikaner or Afrikaner-Hereford-Simmenthaler crosses. They were obtained from a herd maintained by the Institute and were allowed to run with their dams, each in a separate pen, throughout the course of the experiment.

Bacterial strains

The strains of *S. dublin* and *S. typhimurium* used in this study have already been described and characterized (Cameron & Fuls, 1974; 1975) *S. dublin* strain HB 1/17 is an avirulent rough strain, while *S. dublin* strain 2652V and *S. typhimurium* 2656V are virulent smooth strains.

Preparation of experimental vaccines

Live vaccines

For the experiments in mice, fresh live vaccine was prepared from *S. dublin* strain HB 1/17, as described previously (Cameron & Fuls, 1975), and contained approximately $2,5 \times 10^8$ live bacteria/ml.

Lyophilized live vaccine. Lyophilized live vaccine was used in calves and was prepared from *S. dublin* strain HB 1/17. The organisms were grown in D15 medium (Schlecht & Westphal, 1966) in shake cultures for 24 h at 37°C. The growth was collected by centrifugation, re-suspended in a buffer-lactose-peptone solution (BLP) to half the original culture volume and lyophilized in 1 ml quantities. The vials were stored at 4°C and counts for viability were done at monthly intervals. The dried vaccine was reconstituted for use in distilled water, and diluted in tryptone water to give the desired number of viable organisms/ml required for each particular experiment.

Oil emulsion live vaccine. In one experiment in mice, the live vaccine was emulsified in oil, as follows: lyophilized *S. dublin* HB 1/17 was reconstituted in distilled water to contain $2,5 \times 10^9$ organisms/ml; 19 ml of this suspension was mixed with 0,5 ml Tween 80* and emulsified in a mixture of 72 ml Bayol 72** and 8 ml Cirrasol***. The final vaccine thus contained approximately 5×10^8 bacteria/ml.

Inactivated vaccine

Formalin-inactivated, alum-precipitated vaccines were prepared from *S. dublin* strains 2652V or HB 1/17 and *S. typhimurium* strain 2656V, essentially as described previously (Cameron & Fuls, 1974), except that 0,5% and 0,7% instead of 0,3% formalin was used for inactivating *S. dublin* and *S. typhimurium*, respectively. Monovalent vaccines contained either 2% or 5% packed cells. When combined inactivated vaccines were used, equal volumes of each strain were mixed to give either 1% or 2,5% packed cells/strain, 1% packed cells of *S. dublin* being equal to approximately 10^{10} organisms/ml.

Inactivated oil emulsion vaccine was prepared from *S. dublin* strain 2652V. The bacteria were inactivated with 0,5% formalin and the density of the suspension adjusted to contain 10% packed cells. This suspension was emulsified in oil as described above and the final product thus contained 2% packed cells.

Immunization of experimental animals

Mice

The experiments in mice were designed and performed as described previously (Cameron & Fuls, 1975).

Simultaneous injection of live and inactivated vaccine. In the first experiment, different groups of mice were immunized with live *S. dublin* strain HB 1/17 vaccine alone, inactivated *S. dublin* strain HB 1/17 vaccine alone or with both live and inactivated vaccines simultaneously. Details of the dosages employed and the concentration of the vaccines are shown in Table 2.

Effect of levamizole hydrochloride on immunity. In the experiment in mice to determine whether levamizole hydrochloride (7,5% m/v)* would enhance the immune response, 4 groups of 24 mice were immunized with lyophilized live vaccine prepared from *S. dublin* strain HB 1/17, as outlined previously (Cameron & Fuls, 1975). All 4 groups were given simultaneous injections of vaccine and levamizole, the latter by the intramuscular route; two groups received levamizole at a dosage rate of 1,25 mg/kg, whereas the other two groups received 7,5 mg/kg. The levamizole injections were repeated 48 h later.

Duration of immunity. In order to establish the duration of immunity provided by different vaccines in mice, 4 groups of 24 mice were immunized with the following vaccines all administered subcutaneously: Group 1, inactivated alum-precipitated *S. dublin* strain 2652V (2% packed cell volume), Group 2, inactivated oil emulsion of *S. dublin* strain 2652V (2% packed cell volume), Group 3, live lyophilized *S. dublin* strain HB 1/17, and Group 4, live lyophilized *S. dublin* strain HB 1/17 re-suspended in water and emulsified in oil. The dosages used and the administration schedules are given in Table 3.

Effect of storage on potency of inactivated vaccine. In the experiment to determine the effect of storage at different temperatures on combined *S. dublin*: *S. typhimurium* vaccine, bottles of vaccine containing 1% packed cells of each strain were kept at 4°C, 25°C, 37°C and 50°C for 3 months and 6 months, respectively, and their potency assayed according to a standard procedure (Cameron & Fuls, 1975). Since the temperature stability of inactivated *S. typhimurium* vaccine had been established previously (Cameron & Fuls, 1974), the immunized mice were only challenged with *S. dublin* strain 2652V.

Calves

A total of 18 calves was employed in an experiment designed to compare the immunogenicity of various live and inactivated vaccines. Six calves were immunized with a single injection of live vaccine prepared from *S. dublin* strain HB 1/17. Two calves were given inactivated oil emulsion vaccine and 6 calves were immunized with inactivated alum-precipitated vaccine, prepared either from *S. dublin* strain 2652V alone (monovalent vaccine) or combined with inactivated *S. typhimurium* strain 2656V (bivalent vaccine).

Details of the concentration of the vaccines, dosage employed and the immunization schedule are given in Table 1. All the vaccines were injected subcutaneously.

All the calves were bled prior to immunization, and the serum was stored at –20°C until it could be tested for antibodies by the agglutination or haemagglutination test and its protective value for mice be assayed. The calves were bled just prior to challenge as well, and the serum was stored and tested as above in order to determine whether there was any rise in antibody titre.

* N.B.C., Cleveland, Ohio, U.S.A.
** Esso, P.O. Box 78011, Sandton 2146, R.S.A.
*** I.C.I., P.O. Box 3784, Alrode, R.S.A.

* Ripercol-1, Ethnor Laboratories, Johannesburg

TABLE 1 Immunization of calves with live and inactivated vaccines

Calf No.	Vaccine	Concentration	Dosage ml	Immunization schedule
242	Live *S. dublin* strain HB 1/17	1×10^9/ml	1,0	1 injection at 17 days of age
247	Live *S. dublin* strain HB 1/17	1×10^9/ml	1,0	1 injection at 16 days of age
324	Live *S. dublin* strain HB 1/17	1×10^9/ml	2,0	1 injection at 14 days of age
332	Live *S. dublin* strain HB 1/17	1×10^9/ml	2,0	1 injection at 13 days of age
451	Live *S. dublin* strain HB 1/17	1×10^9/ml	2,0	1 injection at 14 days of age
465	Live *S. dublin* strain HB 1/17	1×10^9/ml	2,0	1 injection at 14 days of age
338	Inactivated oil emulsion *S. dublin* 2652V	2% pcv	2,5	1 injection at 14 days of age
346	Inactivated oil emulsion *S. dublin* 2652V	2% pcv	2,5	2 injections at 10 and 23 days of age, respectively
333	Inactivated alum-precipitated *S. dublin* strain 2652V	2% pcv	2,5	1 injection at 17 days of age
377	Inactivated alum-precipitated *S. dublin* strain 2652V	5% pcv	2,5	1 injection at 15 days of age
424	Inactivated alum-precipitated *S. dublin* 2652V plus *S. tm* 2656V	2,5% pcv/ strain	5,0	1 injection at 19 days of age
426	Inactivated alum-precipitated *S. dublin* 2652V plus *S. tm* 2656V	2,5% pcv/ strain	5,0	1 injection at 18 days of age
340	Inactivated alum-precipitated *S. dublin* strain 2652V	2,0 pcv	2,5	2 injections at 10 and 20 days of age
452	Inactivated alum-precipitated *S. dublin* strain 2652V plus *S. tm* 2656V	1% pcv/ strain	5,0	2 injections at 14 and 24 days of age
216	None	—	—	—
219	None	—	—	—
279	None	—	—	—
245	None	—	—	—

pcv = packed cell volume *S. tm* = *S. typhimurium*

TABLE 2 Immunization of mice with live and inactivated *S. dublin* strain HB 1/17 (alone or in combination) against challenge with *S. dublin* strain 2652V

Group	Vaccine	Dosage	Percentage protection to challenge with		Average percentage protection
			$\pm5\times10^7$	$\pm1\times10^7$	
1	Live *S. dublin* HB 1/17	$\pm2,5\times10^7$	60	80	77,5
2	Live *S. dublin* HB 1/17	$\pm5\times10^7$	100	70	
3	Inactivated *S. dublin* HB 1/17 2% pcv	0,1 ml	50	20	32,5
4	Inactivated *S. dublin* HB 1/17 2% pcv	0,2 ml	20	40	
5	Live *S. dublin* HB 1/17* *plus* Inactivated *S. dublin* HB 1/17 2% pcv	$\pm2,5\times10^7$ + 0,1 ml	90	80	75,0
6	Live *S. dublin* HB 1/17* *plus* Inactivated *S. dublin* HB 1/17 2% pcv	$\pm5\times10^7$ + 0,2 ml	50	80	

* The different vaccines were given simultaneously but injected at different sites

pcv = packed cell volume

Challenge of experimental animals

Mice. Immunized as well as non-immunized control mice were challenged with *S. dublin* strain 2652V, as outlined previously (Cameron & Fuls, 1975), and the percentage protection was calculated according to the formula used by Cameron & Fuls (1974).

Calves. The calves were challenged 14 days after the last injection of vaccine. The animals were sedated with Rompun* and a laparotomy performed under local anaesthesia provided by Planocaine**.

A lyophilized suspension of *S. dublin* strain 2652V was reconstituted in tryptone water to contain 1×10^9 live bacteria/ml. Depending on the dose required, 2,0 (2×10^9 organisms), 1 (1×10^9 organisms) or 0,5 ml (5×10^8 organisms) of the suspension was injected intraduodenally. This route was used because it consistently gives rise to infection.

All the immunized calves were challenged with 2×10^9 bacteria and the control calves with 5×10^8 (No. 216), 1×10^9 (No. 219) and 2×10^9 (No. 279 and 245), respectively.

The calves were observed for 14 days after challenge for diarrhoea and other clinical signs of infection.

Serological tests

Pre- and post-immunization agglutination and haemagglutination titres of the calves' sera were determined, as described previously (Cameron, Fuls & Van Reenen, 1972).

* Bayer, Leverkusen, Germany
** Maybaker, (S.A.) (Pty) Ltd, Port Elizabeth

In order to determine whether the calves' sera had protective properties against infection with *S. dublin*, 0,2 ml of each serum was injected intravenously into each of 10 mice. Five mice of each group were challenged with 5×10^7 live *S. dublin* strain 2652V, and 5 with 1×10^7 bacteria 24 h later (Cameron & Fuls, 1975). Deaths were recorded for 14 days and the percentage protection calculated.

RESULTS

Enhancement of immunity in mice

As shown in Table 2, mice which had been immunized with live *S. dublin* HB 1/17 alone exhibited an average protection of 77,5%, while those which received both live and inactivated vaccine prepared from the same strain were 75% immune. Mice which had been immunized with inactivated vaccine prepared from the rough *S. dublin* strain HB 1 17 alone exhibited an immunity of only 32,5%.

Table 3 shows the effect of levamizole on immunity to *S. dublin* in mice. The animals which received live vaccine only were 64,8% immune, while those which were also given 1,25 mg/kg and 7,5 mg/kg levamizole were 55% and 65% immune, respectively.

TABLE 3 Effect of levamizole on immunity to *S. dublin* 2656 V in mice immunized with lyophilized live *S. dublin* HB 1/17

Group	Vaccine dosage	Levamizole (mg/kg)	Percentage protection to challenge with		Average percentage protection
			$\pm5\times10^7$	$\pm1\times10^7$	
1	2×10^7	1,25	50	40	55,0
2	1×10^8	1,25	50	80	
3	2×10^7	7,5	50	50	65,0
4	1×10^8	7,5	80	80	
5	2×10^7	—	50	40	64,8
6	1×10^8	—	90	79	

TABLE 4 Duration of immunity in mice immunized with various *S. dublin* vaccines

Weeks after immunization			2		4		8		12	
Group	Vaccine	Dosage & routine	Percentage protection to challenge with							
			$\pm5\times10^7$	$\pm1\times10^7$	$\pm5\times10^7$	$\pm1\times10^7$	$\pm5\times10^7$	$\pm1\times10^7$	$\pm5\times10^7$	$\pm1\times10^7$
1	Inactivated alum-precipitated *S. dublin* 2652V 2% pcv	2 injections of 0,1 ml s.c. at 2 week intervals	70	70	70	90	90	60	70	70
2	Inactivated alum-precipitated *S. dublin* 2652V 2% pcv	1 injection of 0,1 ml s.c.	60	60	50	80	70	60	70	70
3	Inactivated oil emulsion of *S. dublin* 2652V 2% pcv	1 injection of 0,1 ml s.c.	50	50	60	90	70	60	60	60
4	Live *S. dublin* HB 1 17 lyophilized	1 injection of 5×10^7 bacteria s.c.	60	70	70	90	70	70	80	60
5	Live *S. dublin* HB 1/17 oil emulsion	1 injection of 0,1 ml s.c.	70	30	60	80	30	70	50	70

pcv = packed cell volume

TABLE 5 Potency for mice of combined inactivated *S. dublin* and *S. typhimurium* alum-precipitated vaccine stored for various periods at different temperatures

Storage temperature			4° C		25° C		37° C		50° C	
Storage time (months)	Vaccine dosage (ml)	No. of injections	Percentage protection with challenge to							
			5×10^7	1×10^7	5×10^7	1×10^7	5×10^7	1×10^7	5×10^7	1×10^7
3	0,1	2	100	80	90	90	50	80	50	90
	0,2	1	50	60	70	30	30	40	40	40
		2	80	70	70	80	80	80	30	70
6	0,1	1	30	50	10	40	0	0	0	0
		2	50	70	50	30	80	50	20	50
	0,2	1	40	30	30	0	0	30	0	10
		2	90	80	40	50	50	80	40	80

Duration of immunity in mice

The presistence of immunity induced by 4 different vaccines was compared over a period of 12 weeks (Table 4). That provided by inactivated alum-precipitated vaccine prepared from *S. dublin* strain 2656V, remained at 70% irrespective of whether 1 or 2 injections were given. Inactivated oil emulsion vaccines, as well as live oil emulsion vaccines, were very slightly poorer than alum-precipitated vaccine, but the level of immunity they provided also lasted for 12 weeks. Live vaccine prepared from *S. dublin* strain HB 1/17 was essentially as good as alum-precipitated vaccine, and also gave an average immunity of 70%, 12 weeks after immunization.

Effect of storage on the potency of inactivated alum-precipitated vaccine for mice

As shown in Table 5, vaccine stored for 6 months at 4°C retained its potency provided that 2 injections were used for immunization. When only 1 injection was used, the deterioration was more marked, and vaccine stored at 37°C or 50°C was virtually inert after six months storage, especially when only 1 injection of vaccine was used.

Immunity in calves

The experimental data relating to the immunization of calves are shown in Table 6.

The results show that 3 of the 6 calves which were immunized with live *S. dublin* strain HB 1/17 at dosages of $1–2 \times 10^9$ bacteria survived challenge.

The sera from calves immunized with live vaccine exhibited no increase in serum antibody titre nor did their serum possess any protective activity for mice.

The 2 calves (338 and 346) which had been immunized with 2 injections of oil emulsion vaccine were solidly immune (Table 6). While no rise in serum antibodies could be detected, their sera protected 40% and 30% of mice, respectively.

Although 3 of the 4 calves (333, 337, 424 and 426) which had been immunized with a single injection of inactivated alum-precipitated vaccine survived, they were not solidly immune. They all developed a severe, persistent diarrhoea. No rise in their serum antibody levels or passive protection values could be demonstrated.

Only calves 340 and 452 received 2 injections of alum-precipitated vaccine. Both, however, survived challenge, showed mild transient diarrhoea and generally remained healthy. The serum of 1 calf protected 50% of mice, but that of the other was ineffective. The serum of neither had any detectable rise in antibody titre.

All 4 non-immunized control calves became severely ill after challenge. Although only 2 died, the other 2 developed a severe persistent diarrhoea, and were extremely emaciated and weak at the termination of the experiment.

Discussion

Previous results (Cameron & Fuls, 1975) that mice can be effectively immunized against *S. dublin* infection by means of a live vaccine prepared from an avirulent rough mutant were confirmed in this investigation. These findings were extended to calves and agree with the results of Botes (1964) that they can be adequately immunized with such strains.

TABLE 6 Serological response and immunity to *S. dublin* of calves given live and inactivated vaccines

Calf No.	Vaccine*	No. of injections*	Agglutination		Haemagglutination		Mouse protection percentage		Outcome of challenge
			At immunization	At challenge	At immunization	At challenge	At immunization	At challenge	
242	Live HB 1/17	1	0	0	1:2	1:2	20	10	No diarrhoea; survived
247	Live HB 1/17	1	0	0	1:2	1:2	0	0	Died 7th day
324	Live HB 1/17	1	0	0	0	0	10	20	Died 5th day
332	Live HB 1/17	1	0	0	1:2	1:2	0	0	Died 4th day
451	Live HB 1/17	1	0	0	1:4	1:2	0	0	No diarrhoea; survived
465	Live HB 1/17	1	0	0	1:8	1:16	10	0	Mild diarrhoea; survived
338	Inactivated oil emulsion	1	0	0	1:2	1:2	10	40	No diarrhoea; survived
346	Inactivated oil emulsion	2	0	0	0	0	0	30	No diarrhoea; survived
333	Inactivated alum-ppt.	1	0	0	0	0	40	40	Severe diarrhoea plus fever; recovered
337	Inactivated alum-ppt.	1	0	0	0	0	0	10	Died 6th day
424	Inactivated alum-ppt.	1	0	0	1:2	1:2	20	0	Severe diarrhoea; recovered
426	Inactivated alum-ppt.	1	0	0	0	0	0	20	Severe persistent diarrhoea; survived for 14 days
340	Inactivated alum-ppt.	2	0	0	1:2	0	0	50	No diarrhoea; survived
452	Inactivated alum-ppt.	2	0	0	1:2	1:2	10	0	Mild diarrhoea; survived
216	None		0	nt	1:2	nt	30	nt	Severe diarrhoea: prostrate 3 days; recovered
219	None		0	nt	1:2	nt	0	nt	Died 5th day
279	None		0	nt	0	nt	0	nt	Died 3rd day
245	None		0	nt	1:2	nt	0	nt	Severely ill with diarrhoea; recovered

* For details see Table 1
nt = not tested ppt = precipitated

Attempts to enhance the immunogenicity of a live *S. dublin* vaccine in mice, either by non-specific immunostimulation with levamizole hydrochloride or the simultaneous injection of inactivated bacteria of the vaccine strain (*S. dublin* HB 1/17), were ineffective.

It is also established that inactivated vaccine prepared from the rough *S. dublin* strain HB 1/17 was ineffective, while the effectiveness of inactivated vaccine prepared from a virulent smooth strain (*S. dublin* 2625V) was confirmed. It must therefore be stressed that the use of a highly virulent strain for preparing inactivated vaccine is essential. Certain strains of *S. dublin*, such as 5765, 5565 (K1), 6792 and 6785 (Cameron & Fuls, 1975), are not typical rough strains. Their colonies can only be distinguished from virulent smooth strains after prolonged incubation on agar when their atypical morphology becomes apparent. They possess a deficient "O" antigen structure and it is conceivable that their immunogenicity would be poor when used in the form of an inactivated vaccine. The inadvertent inclusion of such strains in inactivated vaccines may account for the breakdowns in immunity reported by Henning (1953a) and Botes (1964).

Another possible explanation of the discrepancy between the satisfactory results obtained with inactivated vaccine and the variable to poor results reported by Henning (1953a) and Botes (1965), concerns the question of concentration. It was previously shown that, whereas a vaccine containing 0,1% packed cells was capable of protecting mice against *S. typhimurium* infection (Cameron & Fuls, 1974), a concentration of 0,5% packed cells was required in the case of *S. dublin*. In the experiments in calves reported in this paper, vaccines containing 1%, 2% or 5% packed cells were used. A 1% pcv corresponds to approximately 10^{10} bacteria/ml which is about 10 times higher than the concentration used by Henning (1953a).

Botes (1964 and 1965) primarily denounced inactivated *S. dublin* vaccine on account of its poor stability. Whereas inactivated *S. typhimurium* is very stable (Cameron & Fuls, 1974), this investigation showed that *S. dublin* vaccine deteriorated on storage, a deterioration which is only detectable, however, when single doses of vaccine are used for immunization. Two injections of vaccine which has been stored for 6 months at 4°C virtually eliminated this defect.

Furthermore, it should be pointed out that the trials in calves also indicated that a more substantial immunity was induced when two injections (5 ml) of vaccine containing 1,0% packed cells per strain were administered. Similar observations were reported by Cooper & MacFarlane (1974). In the light of these findings, therefore, two injections are recommended for routine immunization.

It is commonly believed that young calves are not amenable to immunization, but, whereas Botes (1964) failed, we were able to establish an adequate immunity in calves by the time that they were 4 or 5 weeks old. Our results are in agreement with those of Husband & Lascelles (1975), who showed that neonatal calves are quite immunocompetent. Age has a minimal influence on their capacity to respond immunologically, but passively-acquired maternal antibody does have a depressive effect. Even if very young calves do not manifest a high antibody response, there is evidence that they are immunologically primed and will readily mount a secondary response when re-exposed to antigen, or become artificially infected (Kerr, 1956).

A poor antibody response in the calves was a feature of our experiments and corresponds with the findings of Henning (1953a) and Botes (1964). The very low levels of antibodies which could be detected by conventional serological tests, as well as the virtual absence of mouse protection by the sera of the calves indicate that serum antibodies are probably not the exclusive or even primary mediators of protection to infection. In fact, this observation lends strength to the contention that immunity to *Salmonella* infections is largely cell-mediated (Cameron, *et al*, 1972; Cameron & Van Rensburg, 1975). On the other hand, the possible contribution of serum antibodies cannot be ignored. The evidence that serum antibodies may nevertheless play a role in protection has been reviewed previously (Cameron & Fuls, 1974), and Jonas & Pulford (1970) have been able to protect mice with immune serum. The relative significance of cellular mechanisms and humoral mechanisms possibly varies with the type of vaccine used, the stage of the immune response, the species concerned and other as yet undefined factors.

We did not investigate the possibility of protecting calves against *S. dublin* infection by immunizing their dams. Live vaccine is ineffective in this respect (Deans-Rankin & Taylor, 1970), but on the other hand Henning (1953b) obtained fair results with inactivated vaccine, and Royal, Robinson & Duganzich (1968) found that calves, receiving colostrum from cows vaccinated with an inactivated *Salmonella* vaccine, showed a significant reduction in faecal excretion of *S. typhimurium* after challenge. The immunization of cows with inactivated vaccine may thus have some merit when very young calves have to be protected. Since colostral immunity may jeopardize the immune response of the calf to active immunization, however, this procedure should be avoided or only be resorted to where calves under the age of 2 weeks are at risk.

In conclusion it can be stated that, although a live *S. dublin* vaccine gives a satisfactory degree of protection and is probably adequate for routine immunization, it has certain shortcomings. The chief disadvantage is that, because no suitable strain of *S. typhimurium* has been found with which it can be combined (Cameron & Fuls, 1974), its spectrum is resctricted to paratyphoid caused by *S. dublin* only. Further, the fact that live vaccine cannot be used concomitantly with antibiotic therapy is a major drawback in intensive calf rearing establishments or when active outbreaks of the disease have to be controlled.

An inactivated *S. dublin* vaccine, therefore, has certain advantages, despite the fact that two injections are required to establish solid immunity. It can be readily combined with other serotypes, such as *S. typhimurium* and *S. bovis-morbificans* (Rudge, *et al*, 1968), and it may be used in conjunction with antibiotics when required.

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