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VACCINATION OF CALVES WITH LIVE MODIFIED AND LIVE VIRULENT STRAIN OF SALMONELLA TYPHIMURIUM

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SUMMARY

Sixteen normal colostrum-fed -Friesian and Holestein-Friesian X Angus crossbred calves, ranging in age from 2 to 7 weeks were divided into 4 vaccinated groups and 1 nonvaccinated (control) group. Group I consisted of 2 calves vaccinated orally twice at a two week interval with small doses of organisms (10^4 than 10^6 live virulent Salmonella typhimurium, strain 108-11). Group II consisted of 2 calves vaccinated orally twice at two weeks interval with 10^{11} organisms (modified Salmonella typhimurium, strain SL 3261). Group III consisted of 2 calves vaccinated IM twice at two weeks interval with small doses of organisms (10^4 than 10^6 virulent Salmonella typhimurium, strain 108-11). Group IV consisted of 3 calves which were vaccinated IM twice a two weeks interval with 10^9 live modified strain 108-11(SL 1479). Group V consisted of 7 calves which served as nonvaccinated controls. One to two weeks after the final vaccine dose all calves were orally challenge exposed with 1.5×10^{11} viable Salmonella typhimurium, strain 108-11.

The results of vaccination with different preparation of vaccine indicated that a modified live vaccine strain designated as SL 1479 with complete, nonreverting blocks in aromatic biosynthesis may be

appropriate for protecting domestic animals against Salmonella infection.

INTRODUCTION

Bovine Salmonellosis is an economically important disease as well as public health concern in most countries. Salmonella typhimurium and Salmonella dublin appear to be the most common serotypes isolated from cattle, although the distribution of these 2 serotypes differs between countries (1). Because the disease has been difficult to treat, a means of prevaention by vaccination has been sought.

It is now generally agreed that killed bacterial vaccines are less efficient than living vaccines in inducing anti-Salmonella resistant. Smith et al. (2) has been demonstrated that bacterins given parenterally or orally to calves or given parenternally to the dams of calves did not decrease morbidity and mortality, whereas, small numbers of live organisms given orally to calves were effective in lessening morbidity and mortality following oral challenge exposure with virulent Salmonella typhimurium. The reason for conducting this experiment was to determine the efficacy of different preparations of antigens (virulent versus modified) to be used as vaccines , because live virulent vaccines, although quite effective initiating acceptable level of immunity, have certain limitation which restrict their application under field conditions.

MATERIALS AND METHODS

Sixteen male Holstein-Friesian or Holstein Friesian X Angus Cross-breed calves , ranging in age from 2 to 7 weeks were used. Only healthy , colestrum fed calves with normal serum immunoglobulins concentration (as measured by zinc sulfate turbidity test were selected. All calves had negative fecal culture for Salmonella and a normal complete blood count before immunization and challenge. Calves were fasted for 36 hours to 48 hours and water

deprived for 24 hours before challenge as form of stress . Calves were housed in a maximum isolation facility and fed commercial milk replacer twice daily plus alfalfa hay and a solid high protein supplement daily. Calves that died after challenge exposure were necropsied and selected tissues were cultured for Salmonella . Calves that survive the challenge exposure for 14 days were euthanatized at varying periods to culture intestinal contents and tissues for Salmonella. Materials cultured directly on B.G. agar and also in selenite enrichment broth included duodenal contents and lymph nodes ileal contents and lymph nodes, colon contents and lymph nodes, spleen, liver, bile (gall bladder) , bone marrow from the femure or tibia and lung.

Vaccination:

Group I -Calves in group I were given 10^4 live virulent organisms (S.typhimurium, strain 108-11) orally and 7 to 14 days later, another dose 10^6 virulent organisms were given.

Group II- Calves in this group were orally vaccinated with 10^{11} modified organisms (strain SL 3261) which was kindly supplied by Dr.Bruce A.Stocker (Department of Medical Microbiology, Stanford University). The strain was obtained by genetic manipulation (3). This defected microorganism prevents the Salmonella from multiplication in the tissues of the host and thus makes them safe for use as live vaccines. Seven days later another 10^{11} organisms were given. In this group the vaccine strain was not homologous with the challenge strain.

Group III- Calves in this group were given (IM) 10^4 live virulent organisms (S. typhimurium, strain 108-11) and 7 days another 10^6 virulent organisms.

Group IV- Calves in this group were given IM with 10^9 modified (S. typhimurium, strain 108-11) SL 1479 followed by another dose of 10^9 organisms IM a week later.

Group V- represented "control group". Calves in this group were not vaccinated and served as challenge controls.

Challenge organism

A highly virulent and drug resistant Salmonella typhimurium. (UCD strain 108-11), isolated from the feces of a calf that died of sever enteritis and septicemia, was selected. Trypticase soy broth was inculated from a nutrient agar slant and incubated at 37 °C for 18 hours. The challenge dose 1.5 x 10^{11} (100 ml of an 18 hour broth culture) was delivered orally by allowing the calves to nurse from a syringe Immediately after challenge , water and alfafa hay were made available and milk replacer was given within a few hours.

Daily monitoring and culture:

Calves were examined each day and rectal temperature appetite, attitude and character of feces were noted. Blood samples were obtained each day for hematologic examination (Packed Cell Volume and total and differential WBC count). Plasma protein and plasma Fibrinogen were measured by refractometer and heat precipitation respectively. Calves were considered to be non shedders after 5 consecutively negative fecal cultures were done on calves during fever spikes, using commercial trypticase soy broth vacutainers (Becton, Dickinson and Co. Cockeysvillo, MD) with CO_2 . Blood culture were incubated until cloudy or for 72 hours and then subcultured on B.G. agar.

Agglutination titers:

Serum tube agglutination titers were determined, using O and H Salmonella anitgens. The H (flagellar) antigens was prepared by standard methods from formalin-killed whole organisms (4). Standard O (Somatic or cell wall) antigens were prepared by heating whole organism at 121 °C for 60 minutes. Antigen and serum were incubated at 37 °C for 16 hours.

RESULTS

Rectal temperature and blood cultures:

The rectal temperature after first vaccination for the groups I,II, III and IV are shown in Fig. (I). The highest temperature was recorded in group II with one positive blood culture on the third day after administration of the vaccine. Group IV showed only one positive blood culture one day after the vaccination.

After the second vaccination the temperature in all four groups was below 40 °C and there were no positive blood culture detected Fig.(2). The temperature after challenge with 1.5×10^{11} organisms of virulent Salmonella typhimurium.strain (UCD 108-11) for the four vaccinated group compared with the temperature of seven control calves are shown in Fig.(3). The highest temperature were seen in group II and the controls.

Clinical Findings

The results of four groups of vaccinated calves are summarized in Table (1) . Vaccinated calves developed less diarrhea compared with non-vaccinated (control) calves. In groups I, II, III and IV , the diarrhoea was less sever and contain less fibrin and blood (clinical observation) compared with the changes seen in controls and group II.

Fecal and Tissue Cultures.

The results of the fecal cultures for each calf in the 4 groups are shown in Table. (2). The results for the nonvaccinated group calves are summarized in Table (3).

The tissues which yielded positive Salmonella culture most often from calves in group I, II, III and IV which survived challenge were ileum, colonic lymph nodes and contents of the colen, duodenum and duedenal lymph node. In none of the surviving calves were Salmonella cultured from the bile, nor were chronic intestinal lesions found. Culture results from tissues of the vaccinated calves are summarized in Table (4). All calves in this group showed positive culture results from tissues except calf (No.1) which survived the

challenge exposure and had negative Salmonella cultures from all the tissues.

Hematologic Findings:

Marked changes in the packed cell volume were not observed except for increases terminally in calves with sever dehydration. Total WBC counts obtained at 24 and 48 hours after challenges exposure varied between 2.700 and 21.300 and neutrophil counts varied between 405 and 14.271 . There was a tendency for both total WBC and neutrophil counts to be within or below normal limits during the first 48 to 72 hours, followed by a return toward normal limits at about 5 days after calves were challenge exposed. Plasma protein tended to decrease , particularly in calves with sever diarrhea. In one calf, for example , plasma protein decreased from 5,9,g/dl to 3.7 g/dl in 6 days. Before death, plasma proteins often increased again due to dehydration. The calves in which plasma Fibrinogen did not increase did not develop sever diarrhea. Serum titers were measured in calves from each group as shown in Table (5). All nonvaccinated control calves tested had negative O and H titers before challenge.

DISCUSSION

In the study reported here, regarding the vaccination of calves with live modified and live virulent strain of Salmonella typhimurium by different routes, some interesting results are emerging. Several different degree of illness occurred in the vaccinated and non-vaccinated groups following oral challenge. Calves in the non-vaccinated group died within 3 to 4 days after challenge. All tissues were positive for Salmonella except one calf which survived challenge and was euthanized later. The deaths could be attributed to septicemia (bactermia) following challenge. Calves in vaccinated groups showed syndremes varying from a transient fever peak with fecal shedding of small numbers of organisms for one day to sever acute diarrhea and septicemia with death in a few days. All calves that died were terminally septicemic, since all tissues examined by

cultural technique were positive (except calf No.9 who died from pneumenia). The tissues which most consistantly yielded a positive Salmonella culture from euthanatized calves after they survived the challenge exposure are duodenum and duodenal lymph nodes , contents of the colonic lymph nodes and contents of the ileum. In non of the surviving calves were Salmonella cultured from the bile , nor were chronic intestinal lesions found. The results seem to indicate that calves rarely become chronic carriers of Salmonella typhimurium after an active infection, whether true chronic cows for Salmonella typhimurium , as they are believed to for Salmonella dublin has not been established (5). Diarrhea was present in most calves and in severely infected calves the diarrheic feces were foul smelling, and contained stringy mucous, chunks of fibrin and flecks of blood. Weight loss and dehydration were apparent in severely affected animals. The inflammation and necrosis are probably responsible for the observed decrease in plasma protein and the plasma fibrinogen. Smith et al (6) suggested that the increase in plasma fibrinogen may be useful determinant in evaluating; the cause of diarrhea in clinical cases compared to calves with diarrhea due to noninvasive organism such as entrotoxigenic E. coli which usually will increased plasma fibrinogen concentration. The WBC total and differential counts were variable and should not be considered diagnostic or bovine Salmonellosis (6).

In group I, calves vaccinated orally with small doses of organism were protected against death. Vaccinated calves were not protected against the development of diarrhea; this result agrees with previous studies performed in other laboratories (2,7,8). This may indicate that the vaccine was able to prevent fetal septicemia but not local invasion and damage to the gastrointestinal tract.

Group III , calves vaccinated IM twice with small doses of organism were protected against death following sever challenge exposure. Diarrhea or increased frequency or defecation usually occurred less in this group. But body temperature reached a peak of 40.6 °C one

to three days after the challenge. All tissue culture were negative for Salmonella except one calf with positive one colon contents and colonic lymph nodes. However, it should be remembered that such trials merely indicate good protection against death following sever challenge exposure compared with that observed in the control group. It is also important to take into consideration the fecal shedding of the viable organisms for variable periods of time. This goal of reducing fecal output of organisms more than justifies the continued search for an improve modified live vaccine for use in the protection of Salmonellosis.

Group II calves were not protected against death following challenge exposure with a hetrologous strain of S. typhimurium. They died from sever diarrhea and septicomia within a few days. All tissues cultured were positive Salmonella except the bone marrow. This death could be attributed to highly virulent strain that was used as an oral challenge exposure. The failure of this modified strain to protect may be because it (SL3261) differs from (UCD 108-11) antigenically , or it -may be- that SL3261 was not a good vaccine antigen.

Experimental results in mice showed that the aro strain SL3261 protected all five mice against i.p. challenge (3) this may suggest that the immune system in mice differs in regard to the response to some antigens from the response seen in calves.

Calves in group IV were protected against death following challenge exposure with the virulent , homologous strain (UCD 108-11). The results indicated that the vaccine prevented such clinical signs as diarrhea , anorexia, depression and weight loss which are caused by injury to the gastrointestinal tract.

In this study, as in previous investigations (2,9) no correlation was found between the protection of a calf and serum antibody titers. Serological test appear to be of limited value in the diagnosis of clinical Salmonellosis. It has recently been shown (4) that the serum agglutination test and indirect haemagglutination test have important disadvantages. The agglutination test is insensitive and nonspecific ,

spontaneous agglutination are frequently observed. Moreover the agglutination techniques only allows for determination of agglutinable antibodies and can not discriminate between the specific immunoglobulines classes, IgG, IgM and IgA. It is obvious from the results of serum agglutination titers in this study, that another test such as ELISA should be used instead of tube agglutination for measuring humoral immunity in future studies. Taken together , the data suggest that live vaccines may be useful for protecting domestic animals against Salmonella infections. The result further suggest that strains with complete , non-reverting blocks in aromatic biosynthesis may be appropriate for such purposes.

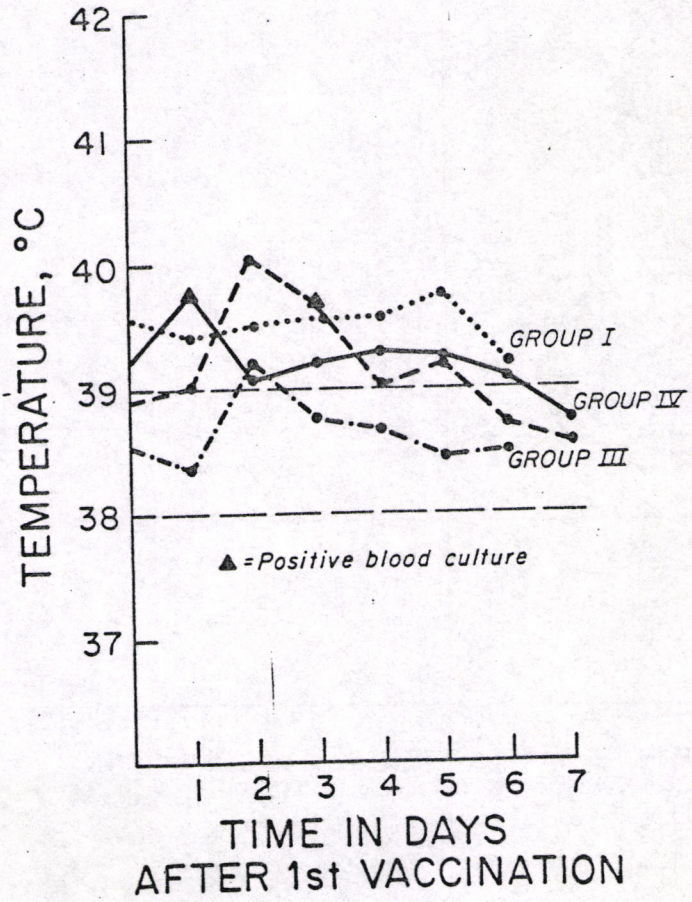

Fig.1 The rectal temperature after the first vaccination.

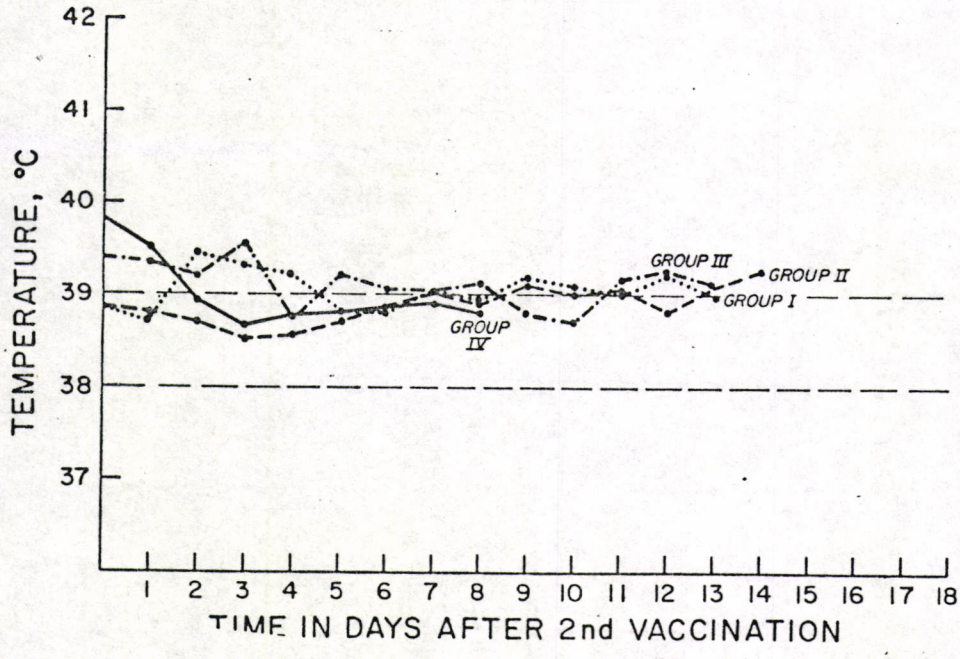

Fig.2 The rectal temperature after the second vaccination.

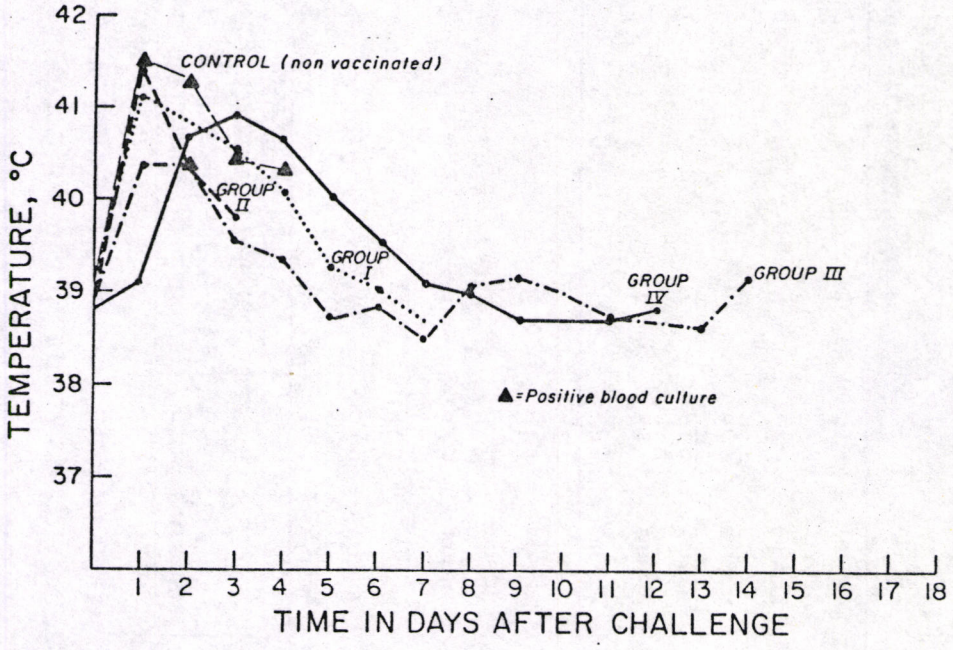

Fig.3 The rectal temperature after challenge of the vaccinated and nonvaccinated (control) calves.

Table 1. Duration of fecal excretion of salmonella and of diarrhea, and the number of positive blood cultures and number of days until death in vaccinated calves.

on Group	Calf No.	Dose	Age in weeks	Duration of Diarrhea (days)	Duration of Positive Selenite (days)	Highest Temp. C°	Anoerxia	Days until Death	Positive Fecal Culture primary	Positive Fecal Culture Selente*	Positive Blood Cultures performed
	8	10^4	3	5	4	40.3	absent	--	2/3	4/3	0/3
		10^6	4	2	16	39.6	absent	--	1/18	4/18	0/0
Group I		10^{11}	6	3	15	41.5	present	sur.	5/15	13/15	0/5
Oral	9	10^4	2	0	6	39.3	absent	--	0/7	6/7	0/2
Virulent		10^6	3	1	14	39.6	absent	--	0/14	12/14	0/1
Strain 108-11		10^{11}	5	2	8	41.1	present	7*	5/8	8/8	0/3
	10	10^{11}	3	0	7	40.5	absent	--	1/14	5/14	1/1
Group II		10^{11}	5	3	1	39.1	absent	--	0/15	0/15	0/0
Oral		10^{11}	7	5	7	41.5	present	8	7/8	7/8	0/4
Modified	11	10^{11}	3	3	12	39.6	absent	--	--	0/14	0/1
Strain SL3261		10^{11}	5	9	2	39.3	absent	--	--	0/15	0/0
		10^{11}	7	3	3	41.4	present	4	4	3/4	1/3

* positive Fecal Culture/number fecals cultured.

* positive Blood Culture/performed.

* Calf (No. 9) died from pneumonia 7 days after the challenge and had normal feces for 5 days before death.

Table 1 . Cont.

Vaccination Group	Calf No.	Dose	Age in weeks	Duration of Diarrhea (days)	Duration of Positive Selenite (days)	Highest Temp. C°	Anoerxia	Days until Death	Positive Fecal Culture Primary	Positive Fecal Culture Selente*	Positive Blood Cultures Performed
	12	10^4	2	1	0	39.1	absent	--	0/16	0/6	0/2
Group III		10^6	3	0	11	39.8	absent	--	0/14	6/14	0/3
IM Modified		10^{11}	5	0	18	40.5	absent	surv.	6/23	16/23	0/3
Strain SL1479	13	10^4	2	0	0	39.3	absent	--	0/6	0/6	0/2
		10^6	3	0	3	39.7	absent	--	0/14	2/14	0/3
		10^{11}	5	1	19	40.6	absent	surv.	1/23	19/23	0/3
	14	10^9	2	0	0	39.8	absent	--	0/7	0/7	1/3
Group IV		10^9	3	0	0	39.7	absent	--	0/14	0/14	0/3
IM Modified		10^{11}	5	2	14	40.8	present	surv.	6/16	13/16	0/3
Strain SL1479	15	10^9	2	0	0	39.7	absent	--	0/8	0/8	0/2
		10^9	3	1	0	40.3	absent	--	0/10	0/10	0/3
		10^{11}	5	0	15	40.8	present	surv.	7/16	15/16	0/2
	16	10^9	2	0	0	40.0	absent	--	0/8	0/8	0/2
		10^9	3	0	0	40.2	absent	--	0/11	0/11	0/3
		10^{11}	5	1	16	41.2	absent	surv.	7/16	16/16	0/3

* positive Fecal Culture/number fecals cultured.

* positive Blood Culture/performed.

* Calf (No. 9) died from pneumonia 7 days after the challenge and had normal feces for 5 days before death.

Table 2. Duration of fecal exretion of salmonella and diarrhea , and number of positive blood cultures and number of days until death in nonvaccinated calves challenge exposed with 1.5×10^{11} virulent strain Salmonella tyhimurium.

Calf No,	Vaccination Group	Age in weeks	Duration of diarrhea Selenite (days)	Duration of Positive Selenite (days)	Highest Temp. C°	Anoerxia	Days until Death	Positive Fecal Culture Primary	Positive Fecal Culture Selente*	Positive Blood culture
1	nonvaccin.	5	11	12	41.8	Absent	Euthen	0/15	12/15	4/4
2	nonvaccin.	5	4	3	42	Present	3	2/3	3/3	3/3
3	nonvaccin.	5	6	7	41.7	Present	7	2/7	7/7	1/3
4	nonvaccin.	5	4	4	41.3	Present	4	2/4	4/4	3/3
5	nonvaccin.	3	3	4	41.1	Present	4	4/4	3/4	3/3
6	nonvaccin.	3	2	4	41.3	Present	4	3/4	4/4	0/4
7	nonvaccin.	3	3	3	41.4	Present	3	0/3	3/3	2/3

* Number of cultures performed

Table 3 Culture results from tissues of the control (novaccinated) calves following challenge with Salmonella typhinurium.

Organ culture	Calf No. 1	Calf No. 2	Calf No. 3	Calf No. 4	Calf No. 5	Calf No. 6	Calf No. 7
Lung	-	+	+	+	+	+	+
Liver	-	+	+	+	+	+	+
Gall Bladder	-	+	+	-	+	+	+
Spleen	-	+	+	+	+	+	+
Duod	-	+	+	+	+	+	+
Duod. L.N.	-	+	+	+	+	+	+
Ileum	-	+	+	+	+	+	+
Ileum L.N	-	+	+	+	+	+	+
Colon	-	+	+	+	+	+	+
Colon L.N.	-	+	+	+	+	+	+
Bone Marrow	-	+	-	-	-	+	+
Died or Euthanized	E	D	D	D	D	D	D

+ = Salmonella typhimurim
- = Salmonella absent

Table 4 Culture results from tissue of the vaccinated groups following challenge with Salmonella typhimurium strain (UCD 108-11).

Organ culture	Group I		Group II		Group III		Group IV		
	Calf No. 8	Calf No. 9*	Calf No. 10	Calf No. 11	Calf No. 12	Calf No. 13	Calf No. 14	Calf No. 15	Calf No. 16
Lung	-	-	+	+	-	-	-	-	-
Liver	-	-	+	+	-	-	-	-	-
Gall Bladder	-	-	+	+	-	-	-	-	-
Spleen	-	+	+	+	-	-	-	-	-
Duod	-	-	+	+	-	-	-	-	+
Duod. LN.	-	-	+	+	-	-	+	-	-
Ileum	-	+	+	+	-	-	-	-	-
Ileum L.N.	-	+	+	+	-	-	-	-	-
Colon	-	+	+	+	+	-	-	+	-
Colon L.N.	-	+	+	+	+	-	-	-	-
Bone Marrow	-	-	-	-	-	-	-	-	-
Died or Euthanized	E	D	D	D	D	E	E	E	E

+ = Salmonella typhimurim

- = Salmonella absent

* Calf (No. 9) died from pneumonia 7 days after the challenge and had had normal feces for 5 days before death.

Table 5 : results of agglutinating antibody response of nonvaccinated and vaccinated calves. O and H titers were measured using the tube agglutination test with Salmonella typhimurium

Group VI nonvaccinated calves	Serum Titers			
	Pre-Challenge		Post- Challenge	
	"O"	"H"	"O"	"H"
Calf No.1	0	0	0	1:4
Calf No.2	0	0	1:16	0
Calf No.3	0	0	0	0
Calf No.4	0	0	1:8	0
Calf No.5	0	0	0	0
Calf No.6	0	0	1:16	0
Calf No.7	0	0	ND*	ND*
Group I				
Calf No.8	1:4	1:4	1:4	1:16
Calf No.9	1:16	1:16	1:16	1:32
Group II				
Calf No.10	1:8	0	1:32	1:16
Calf No11.	0	0	1:8	0
Group III				
Calf No.12	0	1:16	ND*	ND*
Calf No.13	1:16	0	ND*	ND*
Group IV				
Calf No.14	1:8	1:16	1:8	1:16
Calf No.15	1:4	1:16	1:4	1:16
Calf No.16	1:4	1:32	1:16	1:32

* ND = Not done

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تلقيح العجول بلقاح حي وذات ضراوة عالية ولقاح حي محور من جراثيم السالمونيلا تايفيموريم

فيصل غازي حبشة

فرع الطب الباطني والوقائي البيطري-كلية الطب البيطري

جامعة بغداد

الخلاصة

ستة عشر عجلا مضربة من سلالة الفريزيان تراوحت اعمارها بين 2 - 7 أسابيع قسمت الى اربعة مجاميع ملقحة وأخرى غير ملقحة استخدمت كسيطرة. المجموعة الاولى ضمت عجلين لقحت مرتين خلال اسبوعين بجرعة صغيرة من جراثيم السالمونيلا تايفيموريم 10^{6}-10^{4}عن طريق الفم. المجموعة الثانية وضمت عجلين لقحت عن طريق الفم مرتين خلال اسبوعين بـ (SL 3261) 10^{11}من جراثيم السالمونيلا المحورة. المجموعة الثالثة وضمت عجلين لقحت عن طريق الحقن بالعضلة مرتين خلال اسبوعين بجرع صغيرة (10^{6}-10^{4}) من جراثيم السالمونيلا تايفيموريم الحية وذات ضراوة عالية. المجموعة الرابعة وضمت ثلاثة عجول لقحت عن طريق العضل مرتين خلال اسبوعين بـ 10^{9} من الجراثيم الحية المحورة (SL 1479). والمجموعة الخامسة ضمت 7 عجول استخدمت كحيوانات سيطرة في هذا البحث. بعد اسبوع الى اسبوعين من الجرع النهائية من التلقيح فان