Vaccination of pregnant guinea pigs with aromatic dependent ***Salmonella typhimurium*** to protect their newborns

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Summary

Genetically altered stable non reverting aromatic dependent (aro) *Salmonella typhimurium*,strain SL1479 was administrated intramuscularly to healthy pregnant guinea pigs as alive vaccine. Twenty one pregnant guinea pigs were divided into two groups, the first group (15 animals) was vaccinated twice with 1ml containing 17 C.f.U /ml approximately fourth and second week pre-parturition and the second group (6 animals) injected with 1ml trypticase soy broth (TSB) as a control group.

Adverse reaction to vaccination were not observed in the pregnant guinea pigs, which parturated normally. The vaccine induced humoral and cellular immune response as measured by tube agglutination test and delayed type hypersensitivity(DTH)-skin test in the immunized dams and transfer of this response to the newborns, which revealed a high titers of O(somatic)&H(flagller) agglutination titers and positive delayed type hypersensitivity(DTH)- skin test.

The newborn overcome the challenge with virulent *Salmonella typhimurium* at 3,6 &8 weeks of age, compared with the control newborn which died. These results revealed the efficacy of the prenatal vaccination with aro *Salmonella typhimurium* to transfer the passive immunity to the newborn.

تمنيع خنازير غينيا االحوامل بلقاح االسالمونيلا تايفيميوريم المحور وراثياً لحماية مواليدها

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الخلاصة

استخدمت عترة السالمونيلا تايفيميوريم المحورة وراثياكلقاح حي يعطى عضليا لخنازير غينياالحوامل.قسمت الحيوانات الحوامل وعددها واحد وعشرون إلى مجموعتين :المجموعة الأولى وعددها (15) حيوا ن لقحت مرتين في الأسبوعين الرابع والثاني قبل موعد الولادة المتوقع وبجرعة1 مل تحتوى على 10^7 خلية/مل في العضلة، أما المجموعة الثانية (مجموعة السيطرة) وعددها 6 حيوانات فقد حقنت بمرق فول الصويا فقط وبنفس الطريقة. لم تلاحظ أي تأثيرات جانبية على الحيوانات الملقحة وأعطت ولادات طبيعية، حفز اللقاح الاستجابة المناعية الخلطيةوالخلويةوالتي قيست باختبار التلازن الأنبوبي واختبارالحساسية الجلدي المتأخر (DTH) على التوالفي الأمهات الممنعة ثم انتقلت تلك الاستجابة إلى ابنائها حيث اكتسبت معيار عالي من الأضداد ضد المستضدين الجسدي والسوطي O&H للسالمونيلا. وأظهرت تفاعل موجب لفحص الحساسية الجلدي المتأخر (DTH) .قاوم الأبناء من الأمهات الملقحة جرعة التحدي بجرثومة السالمونيلا الضارية وباعمارمختلفة 8,6,3 أسبوع مقارنة بأبناء السيطرة التي هلكت جميعها بعد 3-10أيام بعد الإصابة. أثبت الدراسة كفاءة اللقاح المحضر لتحفيز انتقال المناعة المنفعلة (passive immunity) الخاصة بجرثومة السالمونيلا إلى الأبناء من خلال تمنيع أمهاتها في الأسابيع الأخيرة من الحمل، وخلوه من التأثيرات الجانبية على الحيوانات الملقحة.

Introduction

Salmonellosis is still a major disease in cattle, and infections caused by *Salmonella dublin* and *Salmonella. typhimurium,* in particular, are common all over the world (1 ,2). The disease was clinically manifested by septicaemia and diarrhea, however pneumonia, encephalitis and arthritis were predominant and present in chronic cases. Adult cattle may show similar signs, and pregnant cows often abort. The morbidity and mortality rates in outbreaks of salmonellosis in calves is usually high if treatment is not provided(3).

Jones et al (4) suggested that vaccination of pregnant cattle is a successful method of controlling salmonellosis and may protect calves when they are most vulnerable to infection. There has been much work on development of improved live Salmonella vaccines, such as those attenuated by altration in genes of the aromatic pathway. This gene alteration resulted in a strain unable to synthesize aromatic compound (para-aminobenzoic acid and 2,3 dihydrobenzoic acid (unavailable in mammalian tissues). The bacteria loss virulence, yet retain the capability to persist and to stimulate immune response in host animal (5). Aro vaccines are safe and effective in cattle (6),mice(7) and other animals.

This paper describes the efficacy of vaccination of pregnant G. pigs with aro *S.typhimurium* SL1479 vaccine to stimulate the humoral and cellular immunity to their newborns to protect them against experimental challenge with virulent *Salmonella.*

Materials and Methods

Preparation of Bacterial Strains:

S. typhimurium (aro) SL1479 was used 1-Vaccinal strains:Aromatic dependent as alive vaccine.. The vaccine was prepared according to Smith *et al.,* (6). The number of viable bacteria was determined by plate colony count according to(8).

Vaccines were tested for safety, sterility and potency prior to vaccination according to British pharmacobia (1993).

2-**Challenge strain:** virulent *S. typhimurium* which was isolated from faeces of a calf that had suffered from acute enteric disease was used for experimental challenge. Vaccinal and challenge strains had typical *Salmonella* reaction were confirmed by National center of *Salmonella* in Baghdad (Letter No 3788 dated 19/9/1998).

The lethal dose 50 (LD_{50}) of challenge strain *S.typhimurium* was calculated according to (9),and it has been found equal to 2×10^6.

Animals :-Twenty one adult healthy, Salmonella free, pregnant G. pigs,of similar age were used. Which divided into two groups: -

1- First group (vaccinated group): Fifteen pregnant G.pigs were vaccinated twice, four and two weeks prior to expected parturition intramuscularly with aro *S. typhimurium* SL 1479 vaccine at a dose of 1 ml containing 10^7 c.f.u.

2-Second group (control group):Six pregnant G.pigs were injected similarly with 1 ml TSB as a control group.

Pregnant G.pigs of both groups were examined daily pre and post vaccination. Rectal temperature, pulse, respiration, appetite, attitude, character of feaces was noted. The newborns of both groups were examined daily till day of challenge.

Blood samples were collected from the vaccinated and control groups at 0, 2,4,7 and 9 weeks post vaccination. Similarly samples were obtained from their

newborns weekly from birth till 7th week of age. Sera were separated and kept at –20°C until used. At necropsy, tissue samples from intestine, spleen, liver and kidney were submitted to routine bacteriological examination from dead animals post challenge

The vaccine was evaluated for the humoral immunity by using tube agglutination test (TAT) against O&H antigens which was prepared according to (10).

Soluble antigen from *S.typhimurium &S.dublin* organisms were prepared according to Mitov (11),used for DTH- skin test which was described by Robertsson (12).

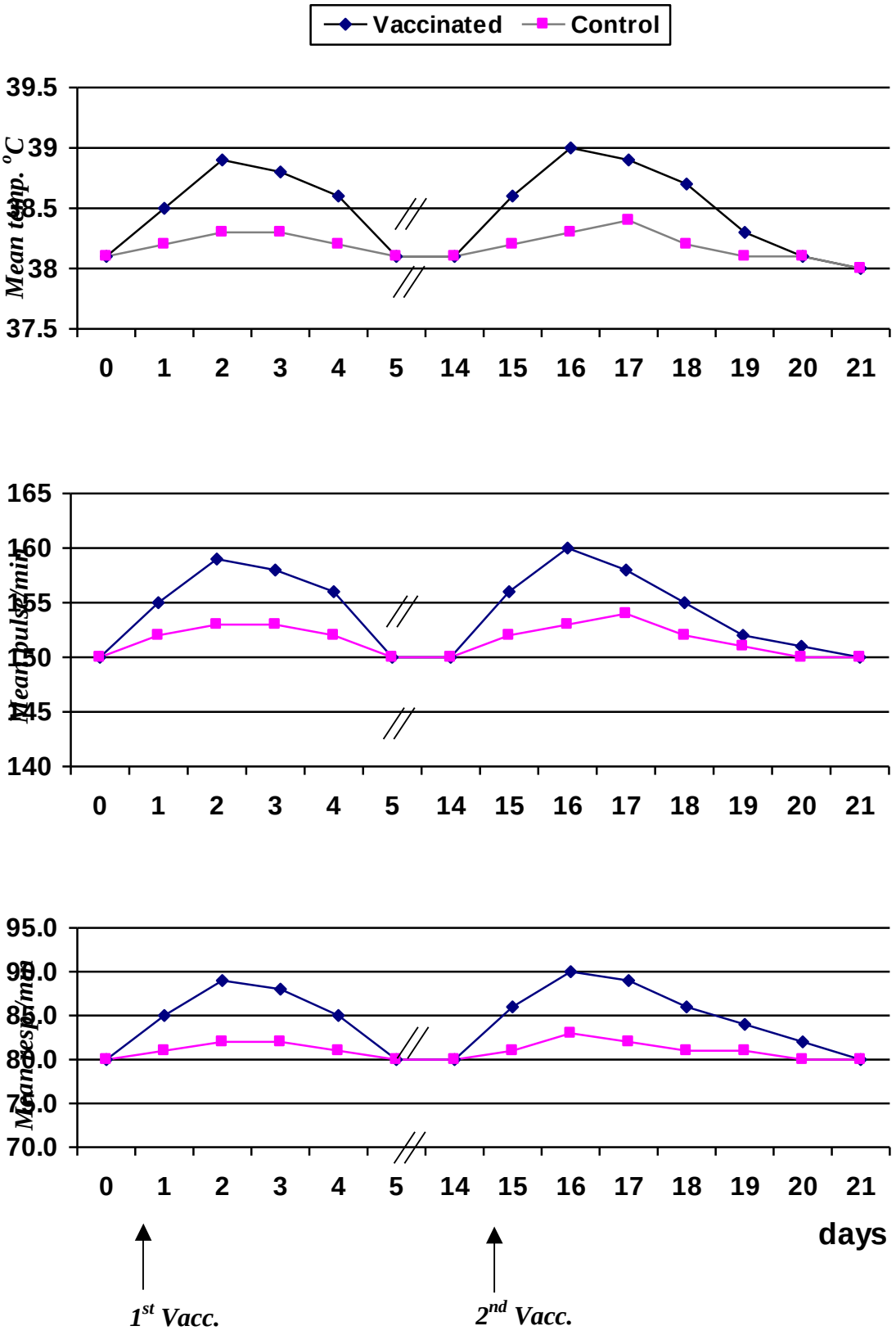
Experimental challenge:

Twenty one newborns of vaccinated dams and twelve newborns of control dams were divided into three groups, these groups were challenged orally with 100 LD_{50} of virulent *S. typhimurium* at different age, 3 rd, 6th and 8th weeks of age respectively.Post challenge the newborns from both group were examined daily, and temperature, pulse, respiration. Faeces were submitted for bacteriological culture.

RESULTS

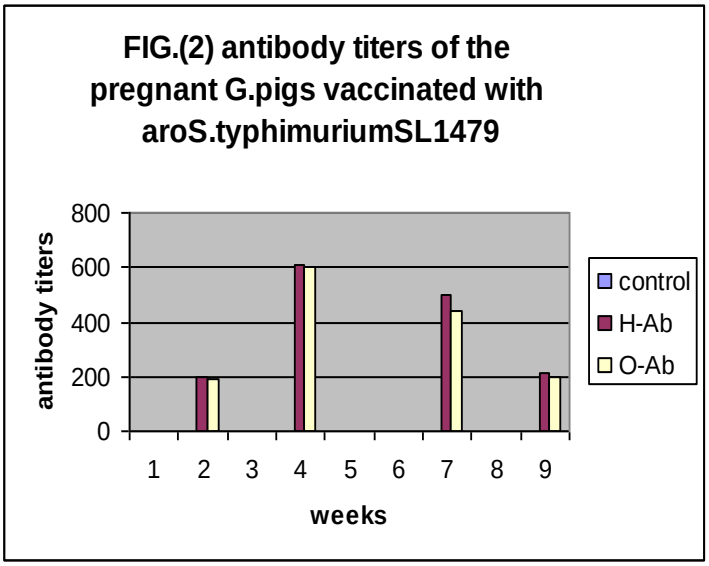
Clinical response to vaccination: The vaccinated group revealed transit increases in mean temperature (38.9 ± 45°C), mean pulse rate (160 ± 6.4 min) and mean respiratory rate (90 ± 5.4min) persisting about four days and returned to normal range in the fifth day (Fig I).They were slightly depressed during the first 72 hours post vaccination but still had normal appetite and passed normal feaces. A localized swelling was detected at the site of vaccination after 48 hours. Adverse reactions

Fig. (1) Temperature, pulse, and respiratory rates in control and vaccinated pregnant G.pigs with aro *S.typhimurium* SL 1479

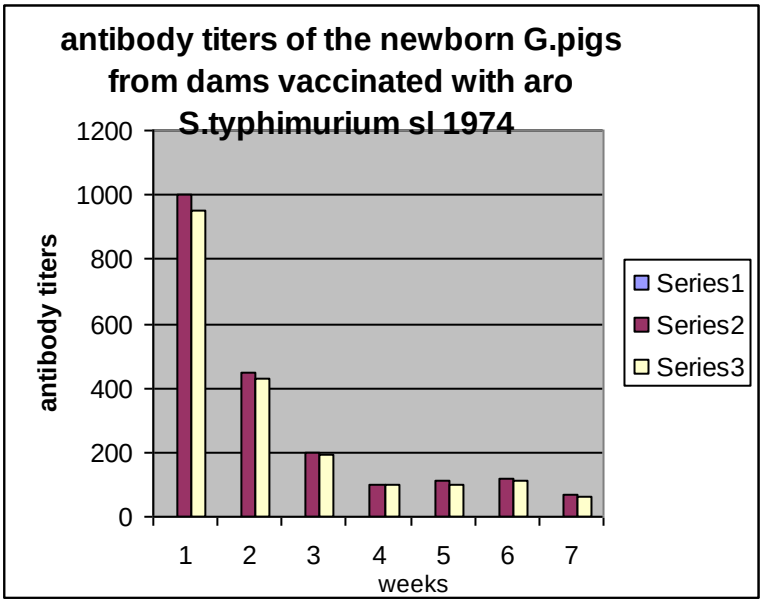


Were not observed in the vaccinated pregnant G.pigs and normal healthy newborns were born. The control group showed normal ranges of body temperature, pulse and respiratory rates, however a small localized swelling was detected at the injection site.

Humoral immune response:- The vaccinated group developed O and H antibody titers after two weeks from the first vaccination with a range between (80-320) and (160-320). After boosting, levels of both O and H antibody titers were higher two weeks latter to reach a range between (320-1280) for both O and H antibody titers, then declined gradually by the 7th and 9th weeks. Antibody titers were not detected in the control group (Fig 2).



TheOand H antibody levels in the sera of newborns from vaccinated G.pigs are shown in Fig.(3) The maximum O and H antibody titers appeared in the first week of age with a range between (320-1280 and 640-1280) respectively, then declined gradually.



Antibody titer can not be detected in the control newborns from non-vaccinated group before challenge, and the titers could not be estimated after challenge as all animals died within 10 days.

Cellular immune response: The vaccinated group developed positive skin reactions characterized by erythematous and induration area at site of inoculation, at 24, 48 and persisted to 72 hours (Table 1). While the control Newborns from vaccinated group group showed no reaction at injecting sites. developed skin reaction (erythematous and indurated) against both soluble antigens at 24, 48, 72 hours, in the second week of age,While the newborns from the control group didn't show any reaction (Table 2).

Clinical response to challenge:

After oral challenge the three groups of immunized newborns at different ages exhibited moderate elevation in the means of body temperature (38.8 ± 0.4°C), pulse rate (158 ± 2.942 min) and respiratory rate (85 + 3.76 min). They were slightly depressed with normal appetite and normal feaces. Animals of the three groups survived the challenge exposure, and only during the first week post challenge the feacal cultures were positive to *S. typhimurium*.

Non immunized (control) newborns showed marked increase in temperature with a mean of (40.5±0.22 °C) increase in mean pulse rate (177±4.26 min) and mean respiratory rate of (102±4.66 min), with depression, loss of appetite, severe diarrhea, and positive feacal culture. Before death they showed signs of dehydration, recumbency with shallow respiration and weak pulse. Death occur within 3-10 days post challenge .

At necropsy, control newborns showed congestion of the intestine, enlargement of spleen, kidneys and gall bladder. Cultures from these organs

were positive for *S. typhimurium*. No gross lesions were seen on these organs from newborns of vaccinated group which were sacrificed with the control group.

Table (1) DTH- skin reaction of the vaccinated pregnant G.pigs with aro *S.typhimurium* SL 1479.

Soluble Ag 100 Mg/ml	Diameter of reaction (mm)	Time/hour Vaccinated Group			Control Group
		24	48	72	24-72
S.typhimurium	Range Mean ± SD	12-16 14 ± 0.561	15-20 18 ± 2.549	8-11 8.5 ± 0.666	0
S.dublin	Range Mean ± SD	10-14 11.25 ± 2.549	12-16 14.1 ± 0.565	7-9 8 ± 0.771	0
PBS	0	0	0	0	0

Table (2) DTH- skin reaction of the newborns of vaccinated dams with aro *S.typhimurium* SL 1479.

Soluble Ag 100 Mg/ml	Diameter of reaction (mm)	Time/hours			Control Newborn
		Immunized newborns			
		24	48	72	24-72h
S.typhimurium	Range Mean ± SD	7-11 8.7 ± 1.708	5-8 6.5 ± 1.291	2- 4 3 ± 0.816	0
S.dublin	Range Mean ± SD	6-8 6.2 ± 1.304	3-5 3.2 ± 0.836	0	0
PBS	0	0	0	0	0

Discussion

Vaccination of pregnant G.pigs with aro S.typhimurium SL 1479 with two doses intramuscularly at 4 and 2 weeks pre parturition showed slight elevation in body temperature, pulse, and respiratory rates with slight depression within 4 days post each vaccination, which may be the expression of immunological and inflammatory reactions and was consistent with those observed by (6,13). The vaccination procedure appeared safe for the pregnant G.pigs and without any adverse effects.

The most frequently used serological test for salmonllosis is tube agglutination test, this test is carried out with somatic and flagellar antigen (4). Vaccinated pregnant G.pigs revealed elevated levels of O and H antibody titers, and the maximum elevation was noticed after the booster dose, this is in agreement with the results reported previously (14). Detection of the O and H antibody titers in the newborns may indicate passive transfer of immunity from the vaccinated dams. Higher titers appeared in the first and second weeks and then declined gradually. These results were relied upon to protect newborns in early stage of

life (4,15). Vaccinated group of pregnant G.pigs showed a marked level of cell-mediated immunity, through determination of positive DTH skin reaction, this results in agreement with other studies (7,14).

The indication of positive DTH skin test of newborn G. pigs from vaccinated dams only might prove the passive transfer of cellular immunity to the newborns by colostrums. These findings are in agreement with (16). All newborns from vaccinated dams overcome the challenge exposure. On the contrary the control newborns showed severe systemic reactions with severe diarrhea, depression and loss of appetite. They continued to excrete challenge organism till deaths within ten days, these results were compatible with (4, 6). On postmortem examination of control newborns, the grossly changes in organs, and the isolation of challenge organism from the visceral organs and bile explained the septicemia or bacteria) following challenge. While the newborns from vaccinated dams did not show any gross lesion, the immunization thus elicited a highly protection in these animals. The protective effect exerted by preventing invasion of virulent strain and preventing its intracellular multiplication due to elicited host defense mechanism.
These results seem to indicate that aro live S.typhimurium vaccine strain SL 1479 is safe and effective for pregnant G. pigs which transfer immunity to their newborns and protect them against challenging with the pathogenic strain.

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