BOVISALM TM AND BOVISALM D VACCINES ARE SAFE AND PREVENT SALMONELLOSIS IN CALVES

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Introduction

Bovine salmonellosis is an important zoonotic infection[1]. Serious outbreaks of clinical disease may occur in cattle of all ages and result in devastating economic loss. The most important serotypes which cause disease in calves are Salmonella typhimurium and Salmonella dublin. Both serotypes can cause fever, anorexia, depression, bacteremia, diarrhoea and death.

Live and dead vaccines have been used to prevent salmonellosis in calves. It is generally believed that cell mediated immunity is necessary to provide longterm protection against salmonellosis. For this reason live attenuated strains have been used because these strains elicit a stronger cell mediated immunity than killed vaccines. Furthermore orally delivered vaccines cause a mucosal immunity, which is important for protection against enteric pathogens.

A number of different types of live vaccines have been developed. Auxotrophic mutants are dependent on metabolites not, or insufficiently present in the vaccinated animal. Recently, a Salmonella typhimurium strain was described which is auxotrophic for histidine and adenine and which also shows a high sensitivity to tensides[2]. Furthermore a Salmonella dublin strain was described which is auxotrophic for thiamine and adenine. These strains have been used for the development of Bovisalm TM (S. typhimurium) and Bovisalm D (S. dublin); lyophilized vaccines for oral immunization of young veal calves to prevent salmonellosis. In this report we present data to show that these strains are safe and effective in preventing salmonellosis in calves.

Material and Methods

Safety experiment

Animals and Housing

Twentyfive female Friesian-Holstein calves 4-7 days of age (35-40 kg) were purchased through a distribution centre. The animals were housed in one unit at an experimental farm in two opposite rows of individual pens. All animals were bucket-fed twice a day with milk-replacer. If they developed diarrhoea, the milk replacer feeding of the calf was replaced by electrolyte solution. The safety test was carried out on 15 animals with the other 10 calves used as unvaccinated controls.

Vaccine and vaccination scheme

The vaccination took place 48 h after arrival on the farm by addition of the appropriate amount of reconstituted Bovisalm TM to the first 0.5 l of milkreplacer. The temperature of the milkreplacer did not exceed 45°C. In all instances the milk was taken freely. After vaccination the calves were fed another liter of milk replacer. The Bovisalm D vaccination took place 14 days after the vaccination with Bovisalm TM. This vaccine was also reconstituted in milkreplacer as described for Bovisalm TM. For the safety experiment higher doses (5 times the recommended dose) than in the efficacy experiment were used. The experimental design of this safety experiment is described in Table 1.

Table 1: Experimental design and amount of vaccine strain of safety experiment.

Group	Bovisalm TM vaccination		Bovisalm D vaccination	
	No. of calves	No. of cfu's	No. of calves	No. of cfu's
I	10	$1x10^{11}$	8	$1x10^{11}$
II	5	$2x10^{11}$	5	$2x10^{11}$
III	10	-	6	-

Clinical observation of calves

Calves were examined twice a day from one day before vaccination up to 13 days after the vaccination. The rectal body temperature, diarrhoea and the general behaviour was recorded.

Efficacy experiment

Animals and housing

Fourtyfive healthy female Friesian-Holstein calves 4-7 days of age (35-40 kg) were puchased through a distribution centre. On arrival the animals were very tired and dehydrated. Thirty calves were housed in one unit in two opposite rows of individual pens at an experimental farm. Another 15 calves were individually housed in a seperate unit and used for unvaccinated control calves in the different challenge tests.

All calves were bucket-fed twice a day with milk-replacer. If diarrhoea developed the milk-replacer was replaced by electrolyte solution.

Vaccination scheme

The first vaccination with Bovisalm TM took place 48 h after arrival on the experimental farm. Twenty calves were vaccinated orally with $2.9x10^{10}$ cfu of the Salmonella typhimurium vaccine strain present in Bovisalm TM. The vaccine was mixed with the first 0.5 l of milk replacer. The Bovisalm D vaccination of the same calves was carried out 14 days later. This vaccine contained $2.3x10^{10}$ cfu of the Salmonella dublin vaccine strain. During both vaccinations the temperature of the milkreplacer did not exceed 42°C. All meals containing the vaccine were taken freely. After the vaccination the calves were given the rest of their ration.

Challenge experiments

25 weeks after the Bovisalm TM vaccination, 6 vaccinated calves and 6 control calves were challenged orally with the virulent Salmonella typhimurium phage type 201 strain ($1x10^7$ cfu per calf).

10 weeks after the Bovisalm D vaccination a group of 5 vaccinated and 5 control calves were challenged. 22 weeks after the Bovisalm D vaccination a group of 7 vaccinated and 6 control calves were challenged. Both groups were challenged orally with a virulent Salmonella dublin 98 strain ($2x10^7$ cfu per calf).

Clinical observations

The rectal body temperature was recorded twice daily, prior to vaccination and challenge and for a period thereafter. The character of the freshly produced faeces was recorded twice a day. Diarrhoea was scored as moderate or severe. The general behaviour and especially the appetite of the animals was checked and recorded.

Bacterial sampling of calves.

Faeces were collected from the rectum. Approximately 1 g of faeces was added to 10 ml Muller-Kaufman tetrathionate broth base, supplemented with brilliant green and iodine and incubated at 37°C for 18 h. Approximately 1 g faeces was added to Selenite Brilliant Green-Mannit broth and incubated at 37°C for 18 h. After incubation both broths were subcultured on brilliant green agar plates. These agar plates were incubated for 18 h at 37°C. Colonies with typical Salmonella features were tested for agglutination with polyvalent and O group specific antisera.

Blood samples were inoculated into Trypticase Soy broth and incubated at 37°C for 14 days, after which the broth was subcultured on brilliant green agar plates and examined for the presence of Salmonella as described above.

Results

Safety of the vaccine strains

The mean daily temperatures of the groups after the Bovisalm TM vaccination remained under 40°C. The highest mean daily temperature was recorded in Group II at day 2, 0.8°C higher than the mean of the control group at the same day. At day 3 and day 4 the difference between vaccine Group II and the control group decreased to 0.6 and 0.5°C, respectively. Thereafter the difference further decreased to was less than half a degree Celsius. Vaccine Group I had a 0.5°C temperature difference with the controls only on day 2 after the vaccination.

Individual body temperatures between 40 and 40.5°C were only seen in one animal of Group II on day 2 and day 3 and one animal of Group I on day 5 after vaccination.

The mean daily temperatures of the groups after the Bovisalm D vaccination never exceeded 39.5°C and this was only seen on day 1 and day 2 after vaccination in Group II. On all other observation days the difference between the vaccine groups and the control group was 0.3°C or less. The control animals had sometimes even a higher mean body temperature than the vaccinated animals after the Bovisalm D vaccination. Individual body temperatures of 40°C or a litle higher were seen in all groups.

In Table 2 the the number of calves per group are given showing at least once per observation day watery, thin diarrhoea. From these data it can be seen that around day 5 after the Bovisalm TM vaccination a high percentage of diarrhoea is present in all three groups. Group I has more or less the same pattern as the controls, whereas Group II shows a somewhat higher incidence of diarrhoea which also persists for a longer period. After the Bovisalm D vaccination almost no watery, thin diarrhoea was observed in any of the calves (data not shown).

Table 2: Number of calves with watery, thin diarrhoea after Bovisalm TM vaccination.

Group	Calves with watery, thin diarrhoea after vaccination with Bovisalm TM														
	-1	0*	1	2	3	4	5	6	7	8	9	10	11	12	13
I	0	1	0	1	3	2	5	5	2	2	0	1	0	0	0
II	0	0	0	1	2	3	5	2	4	4	2	2	1	1	1
III	0	0	0	1	3	0	6	3	0	1	1	0	0	0	0

*: day of vaccination with Bovisalm TM

Efficacy experiment

Salmonella dublin challenge 10 weeks after vaccination

On day 2 after the challenge all control animals had a substantial fever (>41°C). From 3-6 days after challenge all control animals showed a temperature of >40°C. In the vaccinated group 3 out of 5 calves showed a temperature of >40°C, but only for 2 days.

Very severe diarrhoea was seen in all the control animals which lasted several days. In contrast only one vaccinated animal showed severe diarrhoea whereas the other vaccinated calves produced normal faeces.

The clinical condition of the vaccinated calves was clearly better than that of the control animals during the entire post-challenge observation period. No vaccinated calf was severely ill, whereas 3 out of 5 control calves died from clinical salmonellosis.

The challenge strain was reisolated from the faeces of all calves at day 1 after challenge. The excretion of the challenge strain decreased gradually in the vaccinated animals whereas the control animals mainly excreted until death or until the end of the experiment. Only in one control animal, at day 2 after challenge, was the challenge strain isolated from the blood.

Salmonella dublin challenge 22 weeks after vaccination.

A rise in temperature was observed in the animals two days after the challenge at the end of the fattening period. The fever lasted much longer in the control group and the mean and individual temperatures in the control group were higher than in the vaccinated animals.

The occurrence of severe diarrhoea in the vaccinated animals was half of that in the control animals.

Most of the control animals also showed slight respiratory problems; coughing and nasal discharge. This was hardly noticed in the vaccine group. Although none of the control calves died they were very thin and lean at the end of the observation period.

There was no difference between control and vaccinated animals regarding excretion of the challenge strain. The challenge strain could be reisolated from day 2 till day 6 in almost every animal. Six days after challenge the challenge strain could be reisolated from the blood of two vaccinated calves.

Salmonella typhimurium challenge 25 weeks after vaccination.

Around 40 h after the challenge all control animals showed a substantial fever. The temperature in the vaccinated calves also increased but not as high as in the control animals.

All control calves, bar one, had very severe diarrhoea for 3 to 7 days. Three calves also produced pseudomembranes and four calves showed a lot of blood with their droppings during 3-5 days. In the vaccinated calves thin watery diarrhoea was observed for 6 days in one calf and for 2 days in another.

Four days after challenge all vaccinated animals, except one were healthy again and lively with good appetite. The control animals had recovered 8-9 days after the challenge. On day 12 after the challenge one control animal was found dead. No specific clinical signs were observed during the days prior to death. At post mortem a swollen liver, bilebladder and mesenteric lymphenodes were seen. Only from the lymphenodes Salmonella bacteria could be cultivated.

The challenge strain was reisolated from the faeces of all calves. In the vaccinated calves the percentage of positive animals declined to 33% at day 5 post challenge after which only one animal continued to excrete the challenge strain. 100% of the faecal samples from the control group were positive for 4 days, From day 5 till the end of the observation period the rate of excretion from the controls remained at a level of about 80%.

The challenge strain could not be reisolated from the blood of any animal.

Discussion

Upon arriving at the experimental farm the calves used in the safety experiment were exhausted and suffering from an extreme transportation stress. This resembles the field conditions on veal fattening farms in Western Europe. After the oral vaccination with Bovisalm TM the calves showed a slight rise in body temperature. This rise was most pronounced in Group II which was vaccinated with $2x10^{11}$ cfu, a five fold dose. The high percentage of aspecific diarrhoea in all groups around day 5 is a normal phenomenon in veal calves after having been one week on a milk replacer diet. Group I has almost the same frequency as the control group. Group II showed a somewhat higher incidence and for a longer time. This was mainly caused by one calf which started scouring at day 4 after vaccination for 9 days. It is concluded that oral administration of high doses of Bovisalm TM to transport stressed veal calves is safe.

Sixteen days after arrival at the farm and at the time of the start of the Bovisalm D vaccination the first signs of respiratory disease were seen: some animals were coughing, had dirty noses and increased body temperatures. This resulted in distinct respiratory problems during the observation period in 8 out of the 21 animals. Two animals in Group I were already ill at the time of vaccination, so they were not vaccinated at all. One of the animals in Group II died 7 days post vaccination after 3 days from severe acute lung problems. Post mortem investigation revealed a clear pleuropneumonia due to Pasteurella haemolytica. Bacteriological testing for Salmonella was negative in all investigated tissues.

Individual body temperatures of >40°C could be a consequence of the respiratory problems. Diarrhoea was not observed after the Bovisalm D vaccination in any of the three groups. From these results we conclude that high doses of Bovisalm D given orally to calves 14 days after they were vaccinated with Bovisalm TM cause no adverse clinical symptoms related to the vaccination. Two days before and after the Bovisalm TM or D vaccination it is recommended not to use any antibiotics except the antibiotics already present in the milk replacer. In this safety experiment the calves with the respiratory problems were not treated with antibiotics after the antibiotic-free vaccination period. This was to prevent any influence of antibiotics on the Salmonella vaccine strains. In practice antibiotic treatment of the calves with the respiratory disease would have solved these respiratory problems.

The efficacy experiment clearly demonstrated that 10 weeks after the Bovisalm D vaccination the calves are protected against a severe S. dublin challenge. There was a distinct difference in fever, diarrhoea and mortality between control and vaccinated animals. From these results we conclude that Bovisalm D gives very good protection against S. dublin challenge 10 weeks after vaccination.

At the end of the fattening period (22 weeks post vaccination) there was a distinct difference between vaccinated and control animals after S. dublin challenge. The fever of the control animals lasted twice as long as in the vaccinated animals whereas the number of observations of watery thin faeces in the control animals was twice as high as in the vaccinated animals. There was no difference in faecal excretion of the challenge strain between the vaccinated and control animals. From two vaccinated calves the challenge strain was isolated from the blood. One of these calves showed a second fever period after day 6 but the other animal was the most healthy calf throughout the trial with only 2 fever days and no diarrhoea at all, except for day 9. So the importance of these isolations is difficult to judge.

At the end of the fattening period vaccinated animals recovered quickly; four days after a S. typhimurium challenge, whereas the control animals were severely ill. One control animal died caused probably by the S. typhimurium challenge. The excretion of the S. typhimurium challenge was reduced in the vaccine group compared with the control group. We conclude that Bovisalm TM confers a high degree of protection right up to the end of the fattening period.

In general it can be concluded that Bovisalm TM and Bovisalm D are safe for young calves, induce a good protection until the end of the fattening period and reduce the excretion of pathogenic Salmonella strains.

Summary

Bovisalm TM and Bovisalm D are lyophilized vaccines for oral immunization of young veal calves to prevent salmonellosis. Bovisalm TM and Bovisalm D contain live auxotrophic S. typhimurium and S. dublin strains, respectively. The safety of both vaccines was determined with a high dose. A transient slight rise in body temperature and a temporary increase of diarrhoea incidence was seen after vaccination with a high dose of Bovisalm TM. After the administration of high dose of Bovisalm D no adverse clinical signs due to the vaccination were observed.

The efficacy of both vaccines was determined with a normal dose and was measured by challenge infection of different calves at the end of the fattening period. Both vaccines induce a good protection until the end of the fattening period and reduce the excretion of the Salmonella challenge strains.

Resumé

Bovisalm TM et Bovisalm D sont des vaccins lyophilisés dèstinés à la prevention de la salmonellose par une immunisation par voie orale des jeunes veaux. Bovisalm TM et Bovisalm D contiennent respectivement les souches vivantes auxotrophiques Salmonella typhimurium et Salmonella dublin. L'inocuité des deux vaccins a été déterminée par injection de doses élevées. Une augmentation legère et de courté durée de la température corporelle ainsi qu'une augmentation temporaire de l'incidence de la diarrhée ont étés observée après administration d'une dose elevée de Bovisalm TM. Après administration d'une dose elevée de Bovisalm D, aucun effet secondaire clinique due à la vaccination n'a pu être observé.

L'efficacité des deux vaccins a été determinée à dose normale et mesurée par épreuve sur differents veaux en fin de periode d'engraissenment. Les deux vaccins ont induit une bonne protection jusqu'à la fin de la periode d'engraissement et reduit l'excrétion de la souche (Salmonella) d'épreuve.

References

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