Bovine Para-influenza 3 Vaccine Studies

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SUMMARY

A bovine para-influenza 3 modified live virus vaccine was studied in colostrum-deprived calves. A single dose of vaccine induced antibody responses and afforded protection against experimental challenge. This was evidenced by reduced clinical disease and virus excretion as compared to unvaccinated control calves. The vaccine virus did not spread from vaccinated calves to contact controls.

INTRODUCTION

Myxovirus para-influenza 3 (PI-3) was isolated in 1959 from cattle showing symptoms of respiratory disease (4). Subsequent serological and virological studies have indicated that the virus is widespread in cattle populations and have implicated the virus in the etiology of the shipping fever complex (2). There is evidence that the virus may be associated with other clinical entities in cattle, including pneumoenteritis (1) and abortions (5). These findings suggest that a vaccine against bovine para-influenza 3 would be of value to the livestock industry.

Tests of para-influenza 3 vaccines for safety and efficacy present problems, and many investigations involving experimental immunization and natural or artificial exposure have been reported (3). In some cases, the results have been inconclusive, due to variability in the susceptibility of the test calves to PI-3 virus.

In this study, colostrum-deprived calves seronegative to PI-3 received single intramuscular (i.m.) injections of PI-3 modified, live virus vaccine. After two weeks, they, together with the non-vaccinated control calves, were exposed to virulent PI-3 virus. The subsequent observation period revealed significant differences in febrile response, virus recovery, and final serum antibody levels in the two groups of animals.

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MATERIALS AND METHODS

Cell Cultures

Primary bovine kidney cell cultures (BK) were prepared from cell suspensions obtained by trypsinization of fetal bovine kidneys. Cells were grown in Earle's balanced salt solution with 5% lamb serum, changed to Eagle's medium with 1% lamb serum for maintenance. Cells were changed to Eagle's medium with no serum at the time of inoculation.

Test Animals

Calves were obtained from local farms within 12 hours after birth. The calves were not allowed to nurse and were raised in isolation, deprived of colostrum. Consequently, the calves were seronegative to PI-3.

Vaccine Strain

The vaccine strain tested was a live virus vaccine, derived from the SF-4 strain of bovine para-influenza 3 virus and propagated in bovine kidney tissue culture.

Vaccination Procedures

Calves were vaccinated intramuscularly and housed together with unvaccinated controls to determine if the vaccine virus spread to unvaccinated contact control animals. Temperatures of both vaccinated and control calves were followed to determine vaccine safety.

Experimental Challenge

Two ml of the virus challenge were administered both intranasally and intratracheally. Intranasal challenge was performed by aerosolizing the virus with a DeVilbiss atomizer No. 152 into a plastic bag held over the calf's head. Intratracheal injection was by syringe and 18 gauge needle at a point well below the larynx.

TABLE I — Serological Responses of Calves to PI 3 Vaccination and Challenge

Vaccinated Calf No.	Age at Vaccination	HI Titer 2 Weeks Post Vaccination	HI Titer 2 Weeks Post Challenge	Unvaccinated Calf No.	Age at Challenge	HI Titer 2 Weeks Post Challenge
15	6 months	64	256	14	6 months	1024
42	3 weeks	128	512	43	4 weeks	256
48	3 weeks	128	1024	49	4 weeks	64
53	3 weeks	16	256	55	4 weeks	32
36	2 weeks	16	256	69	4 weeks	16
57	2 weeks	32	1024	60	3 weeks	256
65	2 weeks	32	512	70	3 weeks	8
67	2 weeks	64	512	71	3 weeks	32
61	3 days	8	512	39	2 weeks	8
62	1 day	8	512	63	2 weeks	64
				72	5 days	16
Arithmetic Mean Titer		53	538			160

Three pairs of calves were challenged with a mixture of a strain of PI-3 isolated from a field outbreak in Ontario[1] and a low-passage SF-4 strain[2], while the remaining calves were challenged with a triple plaqued clone of the SF-4 strain. The titer of both virus pools was approximately $10^{7.5}$ $TCID_{50}$/ml.

Serological Methods

Antibody titers in calves' sera were determined by a hemagglutination inhibition (HI) microtest with para-influenza 3 strain Ohio 54[3] and guinea pig erythrocytes. Sera were inactivated by heating at 56°C for 30 minutes, then diluted 1:4 in phosphate-buffered saline (PBS) and treated for 20 minutes with an equal amount of 25% kaolin in PBS to remove non-specific inhibitors of viral hemagglutination. The starting serum dilution was 1:8.

Clinical Observations

Rectal temperatures of vaccinated and control animals were recorded daily both before and after vaccination and after challenge. Animals were also observed for clinical manifestations such as abnormal respiratory signs, anorexia, depression, etc.

RESULTS

Following vaccination, no increase in temperature or other clinical signs were observed, in vaccinated animals or their contact controls. Vaccinated animals developed positive HI titers within two weeks after vaccination. None of the contact controls developed HI titers during this period. Table I records the approximate age of the animals at vaccination and the arithmetically averaged HI antibody titers after vaccination and challenge.

Vaccinated animals and their contact controls were challenged two weeks after vaccination. A febrile response occurred in both vaccinated and control animals on the second day after challenge. In the control group, the febrile response was significantly higher than the vaccinated group on days 3, 4 and 5 after challenge, when analyzed by Student's t Distribution. Mean temperatures of the vaccinated and control groups following challenge are recorded in Fig. 1.

Clinical symptoms, including depression and coughing, were observed in about half the animals in the control group and in two animals in the vaccinated group.

Nasal swabs for virus isolation were collected from vaccinated and control animals at various intervals prior to and following challenge. No virus was isolated from any of the calves prior to challenge. In Fig. 2, the percentages of calves shedding virus after challenge in the vaccinated and non-vaccinated group are compared. Virus isolations were made from the vaccinated animals up to day 6 after challenge, whereas from the control group, isolations were made as late as day 9 after challenge. The percentage of nasal swabs from the vaccinated group positive for PI-3 was 38% as opposed to 63% for the control group.

[1]Obtained through the courtesy of Dr. W. J. B. Ditchfield, Ontario Veterinary College, Guelph, Ontario.
[2]Obtained through the courtesy of Dr. R. Sweat, Department of Veterinary Science, University of Nebraska.
[3]Obtained through the courtesy of Dr. A. H. Hamdy, Ohio Agriculture R & D Center.

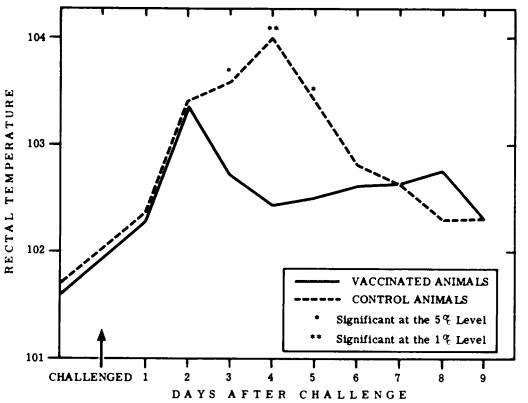

Fig. 1. Temperature response following challenge.

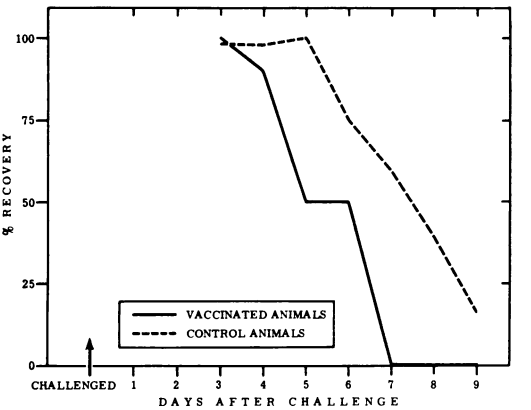

Fig. 2. Virus recovery following challenge.

DISCUSSION

Experiments previously reported in the literature were mainly concerned with prophylaxis by combinations of bovine para-influenza 3 and other microbial agents, both bacterial and viral. Animals were subsequently challenged by exposure to the same combinations of agents, sometimes with the addition of stress.

In this preliminary study, bovine para-influenza 3 alone was used for prophylaxis and challenge. The experiments were done using colostrum-deprived calves, a highly sensitive test system.

A single dose of para-influenza 3 live virus vaccine afforded protection against experimental challenge: although clinical symptoms following challenge were not severe, the temperature responses differed significantly, and virus excretion among the vaccinated animals was reduced. No apparent differences were observed between the two strains of virus used for challenging.

No virus was isolated from calves following vaccination or from their contact controls. None of the controls developed antibody titers during the post-vaccination period. These observations indicate that the vaccine virus is not shed by vaccinated animals.

Virus was detected in the nasal secretions of both the vaccinated and unvaccinated calves after challenge. However, more animals from the unvaccinated group excreted virus and for a longer period of time. Virus excretion from the vaccinated animals may have been due to the large challenge dose of virus given soon after vaccination. The excretion occurred for onlyl a short time. Presumably the vaccine virus had primed the sites of local antibody production so that nasal antibody appeared earlier and in large amounts.

The vaccine produced a rapid antibody response in young susceptible calves. Following challenge with virulent virus, the vaccinated calves exhibited a higher antibody response than the unvaccinated calves and had fewer clinical symptoms.

Para-influenza 3 virus has been implicated as one of the agents involved in the bovine respiratory disease complex. A modified live virus vaccine, either singly or in combination with other components, should be of value in control.

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