Calfhood Immunization Against An Experimental Parainfluenza and Pasteurella Challenge

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SUMMARY

An inactivated vaccine including Parainfluenza-3, PASTEURELLA MULTOCIDA and PASTEURELLA HEMOLYTICA stimulated good immunity in calves against an experimental shipping fever challenge. Calves were first vaccinated when 4 to 10 weeks old and revaccinated at weaning age. There were higher HI titers, lower temperatures, and less lung lesions recorded in the vaccinated group when compared to the control animals.

The exact etiology of the shipping fever complex has been a point of discussion for several years. Early reports in the literature were concerned with the role of Pasteurella species (1,2). That these microorganisms were not the sole etiological agents became more apparent; they were more important as secondary opportunists (3,4). Carter suggested that in some instances a virus might be a forerunner of the Pasteurella infection (3).

In 1959, Reisinger, Heddleston, and Manthei reported on the isolation of a Myxovirus Parainfluenza-3 (PI-3) associated with shipping fever of cattle (5). Based upon serologic, clinical, and experimental studies, this virus has generally been looked upon as the most important viral agent associated with the etiology of bovine shipping fever in the United States (6-9). Consequently, recent investigations of a shipping fever vaccine have included the Myxovirus PI-3 virus as well as Pasteurella species (10-13).

The purpose of this report is to present data on the immunity obtained when calves were vaccinated with a combination inactivated bovine PI-3 vaccine and *Pasteurella hemolytica-Pasteurella multocida* bacterin. Resistance to an experimental PI-3 and Pasteurella challenge as well as hemagglutination inhibition (HI) antibody levels were used to measure the degree of immunity.

Materials and methods

Vaccine — The experimental shipping fever vaccine included Reisinger's isolant of bovine PI-3 (SF-4). This strain had been passed through several calves to maintain infectivity. The virus was cultured on primary bovine embryonic kidney cells and had a titer of 10^{-8} $TCID_{50}$. The Pasteurella portion of the vaccine included Heddleston's[1] strains of *Pasteurella multocida* (P-1062) and *Pasteurella hemolytica* (P-1148) (14). This experimental vaccine was formalin inactivated with adjuvant added.

Test Animals — The 32 calves used in this study were raised from birth at the Greenfield Laboratories, Greenfield, Indiana. Calves and their dams were bled soon after birth for HI titers. Five calves were bled before they had an opportunity to obtain any colostrum.

Vaccination Procedure — When the calves were four to ten weeks old, they were vaccinated with blackleg and malignant edema bacterin and 18 of the 32 calves were vaccinated with the experimental shipping fever vaccine. The calves were 3 to 4½ months old when they were weaned and the previously vaccinated group were revaccinated with the experimental shipping fever vaccine.

Experimental Challenge — Based upon previous findings and results of other in-

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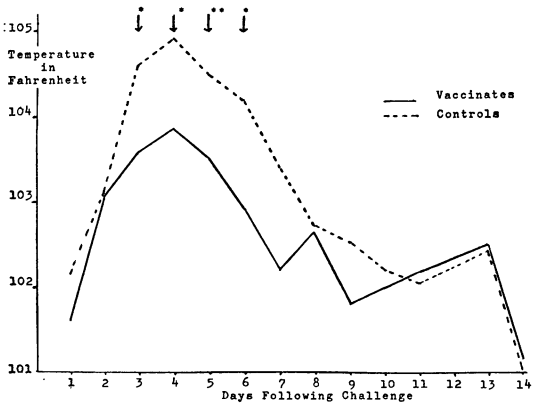
1. Obtained through the courtesy of Mr. K. L. Heddleston, National Animal Disease Laboratories, ARS, USDA, Ames, Iowa.

GRAPH I. Pyrexia Following Challenge in Vaccinates and Controls

(Mean Temperatures)



•Significant differences between groups
* Probability level = 0.01
** Probability level = 0.05

vestigators, stressing of the calves was the first step in the experimental challenge procedure (11, 15, 16). The calves were subjected to an approximate temperature of 38°C for 12 hours in an enclosed room. Stress was continued the next day by trucking the animals in inclement weather and spraying them with water at various intervals throughout the day. After leaving the calves on the truck overnight, they were challenged the next morning with an aerosol[2] containing the PI-3 (SF-4), *P. hemolytica* (P-1148) and *P. multocida* (P-1062). The challenge virus was grown on primary bovine embryonic kidney cells and the challenge Pasteurellas were passed in embryonated eggs to insure good capsule formation. A plastic sack was used over the head of each calf to contain the aerosol. The Pasteurellas were also given intratracheally.

Clinical Observations and Specimen Collection — Temperatures were taken at the time of challenge and daily for two weeks following challenge. Serum samples for HI determinations were collected prior to vaccination, at the time of vaccinations, time of challenge, and 7 and 14 days after challenge. Blood samples and nasal swabs were taken daily for two weeks following challenge. The blood samples were taken for white blood cell counts, hemoglobin and hematocrit determinations. Nasal swabs immersed in Hanks BSS with antibiotics were taken for virus isolations and dry swabs for bacteriologic studies. Except for the blood samples, all other samples were frozen at −20°C until the actual laboratory studies were conducted. All isolants were characterized by cytopathic changes (CPE), serum neutralization tests, and by a hemadsorption technique described by McKercher (17). The serologic conversions were detected by the HI test.

Postmortem Examination — The surviving animals were sacrificed 14 days after challenge and the lungs removed for gross and microscopic examinations.

Results

Clinical signs following vaccination did not occur. Within 5 days after experimental challenge, a few animals showed clinical signs characterized by depression and some coughing, dyspnea, and nasal discharge. Febrile reactions appeared in both groups on approximately the third day post challenge and persisted for approximately 5 days. The daily mean temperature of each group is shown in Graph I. Changes were not significant in leukocyte count or hemoglobin and hematocrit readings.

Table I represents the HI titers of calves before and after vaccination and following challenge. The calves sampled before nursing colostrum did not have a demonstrable HI titer. After nursing, titers rose very markedly and as a group correlated quite well with the HI titers of the dams. This parenteral HI antibody titer was still detectable at the time of the first vaccination. Significant HI titers were not observed three months following the initial vaccination. Seven days following the second vaccination the calves were bled and challenged. The HI geometric mean titer (GMT) was 17.5 for the vaccinated group as compared to 10.1 for the control group. One week following challenge the GMT rose dramatically to 112.9 in the vaccinated animals as compared to 26.8 in the control calves. Final blood samples were taken 14 days after challenge and the GMT had increased to 246.7 in the vaccinated group as compared to 105.4 in the control animals.

Prior to challenge, nasal swab cultures of calves 307, 292, and 286 yielded *P. multocida* whereas swabs from the remaining calves were negative. Attempts to isolate PI-3 virus from nasal swabs were negative. Following challenge, *P. multocida* and *P. hemolytica* were isolated from both vaccinated and control animals with no significant differences noted between the two groups. Twelve of 17 vaccinated animals

2. DeVilbiss #40 glass nebulizer.

TABLE I. HI Titers

Calf No.	Pre-Nurse	1st	2nd 10/27[a]	3rd 1/20[b]	4th 1/27[c]	5th 2/2[d]	6th 2/9[e]	Cow No.	HI Titer
VACCINATES									
278	—	20	N	10	Died	—	—		
280	—	160	10	—	20	160	160	611	20
282	—	N	10	10	10	80	320	539	20
283	N	80	20	20	10	160	640	643	40
285	—	40	10	10	10	160	640	566	20
287	—	>320	40	—	N	—	320	587	80
290	N	10	10	—	10	160	320	575	40
292	—	N	10	10	20	320	640	541	N
293	—	80	20	20	20	—	320	553	40
295	—	160	20	20	40	160	640	577	40
298	—	40	20	20	20	160	80	617	20
300	—	N	N	—	10	40	80	547	20
301	—	40	80	40	40	80	40	594	20
302	—	10	40	40	40	Died	—	604	80
304	—	>320	160	80	40	80	80	608	160
305	—	10	10	N	10	20	640	613	10
308	—	—	N	N	160	640	1280	—	—
309	—	80	20	80	20	40	40	582	40
HI Geometric Mean		30.5	12.6	14.3	17.5	112.9	246.7		26.7
CONTROLS									
279	—	40	N	20	10	N	40	611	20
281	—	80	10	—	—	20	—	602	40
284	—	N	10	N	N	20	80	583	10
286	—	80	20	40	20	Died	—	607	20
288	—	40	10	20	10	40	40	627	10
289	—	10	N	—	10	20	320	615	80
291	N	20	10	10	10	—	80	578	20
294	—	80	20	—	20	40	80	593	40
296	—	80	20	20	40	40	320	551	40
297	N	160	40	40	20	40	80	567	40
299	—	N	10	—	10	20	Died	580	20
303	N	10	20	—	20	20	20	612	20
306	—	N	N	N	N	160	1280	624	10
307	—	20	20	20	20	—	Died	625	N
HI Geometric Mean		18.1	8.6	10.2	10.1	26.8	105.4		18.73

[a]First vaccination
[b]Second vaccination
[c]Pre-challenge bleeding
[d]7 days post challenge
[e]14 days post challenge

and 11 of 14 controls shed PI-3 following challenge (Table II). However, shedding of the virus persisted longer in the unvaccinated animals than in the vaccinated calves. Of the 12 isolations made in the vaccinated group, 8 were made for one day only; while in the control group, all 11 isolations were made for more than one day.

Six calves (2 vaccinates and 4 controls) died during the course of the experiment. Vaccinated Calf 278 died during the stress period before challenge, and necropsy examination revealed an extensive hepatic abscess. Calf 302 died 6 days post-challenge. Gross lesions and histopathological changes were not observed.

Control calves 286, 299, 307, and 296 died 7, 8, 12, and 14 days after challenge respectively. All four showed varying degrees of pneumonia upon necropsy. Severe lung lesions characterized by consolidation, fibrin formation and emphysema were seen in calves 286 and 299. Microscopic examination of lung material from these animals revealed extensive cellular infiltration with loss of alveolar structure, evidence of emphysema and edema and some focal areas of abscessation.

Table III shows the postmortem examination results of lungs from calves sacrificed 14 days after challenge. An arbitrary system of grading lung lesions was used to categorize each lung. A lung was considered to be normal if it did not show any lung involvement or had very few isolated, small

TABLE II. PI-3 Isolation Following Challenge I. % of Calves Shedding PI-3

VACCINATES 71% (12/17)
CONTROLS 79% (11/14)

II. Days PI-3 shed

	1 day	2 days	3 days	4 days	5 days
Vaccinates	8/17	1/17	1/17	0/17	2/17
Controls	0/14	1/14	6/14	3/14	1/14

focal areas of consolidation. A moderately affected lung had consolidation in one or two apical or cardiac lobes. Lung involvement was considered to be severe when several lobes showed consolidation, abscessation, fibrin formation, edema and/or emphysema.

Thirteen of 17 calves (76%) in the vaccinated group had normal lungs as compared to 5 of 14 calves (36%) in the control group. Lung lesions were observed in the other 4 calves (24%) in the vaccinated group, with only one of them considered severely affected. In the control animals, 9 of 14 (64%) showed definite lesions of pneumonia and four of these were judged to be affected severely.

Lung sections were prepared for microscopic examination from all the calves. For matter of comparison, those sections which showed normal to mild cellular infiltration without loss of alveolar structure were considered normal. Lung sections showing extensive cellular infiltration with loss of alveolar structure and evidence of abscessation, emphysema and/or edema were considered to be severely affected. The results of this classification are shown in Table IV. Fourteen lung sections from the 17 vaccinated calves (82%) were normal or only slightly changed, while the other three had lesions typical of severe pneumonia. In comparison, 6 of 13 control calves (46%) had normal or slightly changed lungs while 7 (54%) were considered severely affected.

Discussion

Previous aids for prophylaxis against shipping fever were primarily aimed at the Pasteurella species. Bacterins or hyperimmunized bovine serum were used for gaining this protection and results were questionable. One investigation revealed that bacterins used in calves under field conditions did not give full protection against respiratory infections (18). Another report questioned the role of Pasteurella after experimental exposure studies in calves and concluded that Pasteurella alone probably did not cause shipping fever (19). The isolation of PI-3 virus from clinical cases of shipping fever initiated investigations into vaccine possibilities incorporating this virus.

An inactivated vaccine including PI-3, *P. multocida* and *P. hemolytica* was prepared and used for experimental studies. Based upon the results of these experiments and in agreement with other reports (10, 13, 20) two vaccinations given three weeks apart prior to shipment was found necessary to produce good immunity in calves. Since this vaccination schedule may be an undesirable feature from cattle management procedures, this calfhood vaccination study was undertaken to simulate management generally practiced by stockmen. The first vaccination was administered at the time of castration, dehorning, branding etc. then followed with the second vaccination at the time of weaning or shipping. The results indicate that calves can be immunized using this experimental vaccine and following this calfhood vaccination schedule.

Temperature studies gave positive evidence that this vaccine produced immu-

TABLE III. Postmortem Lung Examinations

Vaccinates	Degree of Pneumonia*	Controls	Degree of Pneumonia
280	Normal	279	Normal
282	Very Moderate	281	Normal
283	Normal	284	Moderate
285	Normal	286	Severe - Died
287	Normal	288	Moderate
290	Normal	289	Normal
292	Normal	291	Normal
293	Moderate	294	Severe
295	Normal	296	Severe
298	Normal	297	Moderate
300	Normal	299	Severe - Died
301	Normal	303	Normal
302	Normal -Died	306	Moderate - Died
304	Normal	307	Moderate - Died
305	Moderate		
308	Normal		
309	Severe		
13/17 — Normal		5/14 — Normal	
4/17 — Pneumonia		9/14 — Pneumonia	
1/17 Severe		4/14 Severe	
1/17 — Died (?)		4/14 — Died	

*Normal — no lung involvement or very few isolated, small focal areas of involvement.
Moderate — one or two lobes of the lung involved with some consolidation.
Severe — more than the apical lobes involved with consolidation, abscessation, fibrin formation and/or emphysema.

TABLE IV. Results of Microscopic Lung Examinations

Changes*	Vaccinates	Controls
Normal to mild	14/17 (82%)	6/13 (46%)
Severe	3/17 (18%)	7/13 (54%)

*Based upon degree of cellular infiltration and loss of alveolar structure.

nity against the experimental challenge. The temperatures were higher and persisted longer in the control group when compared to the vaccinated animals. An analysis of variance on the daily mean temperature differences between the two groups were found to be significant at the 0.01 or 0.05 probability levels on the 3rd to 6th day post challenge. There also appeared to be some correlation in animals between persistence of high temperatures and evidence of pneumonia upon postmortem examinations. The numbers of lungs showing lesions and microscopic changes indicative of pneumonia were significantly reduced in the vaccinated calves.

The results of the HI tests indicated that this vaccine stimulated an immune response in calves. Within seven days following experimental challenge, the vaccinated animals had a geometric mean HI titer of 112.9 as compared to 26.8 in the control group. This difference was found to be significant at the 0.05 probability level. This 4-fold increase in titer reflects the anamnestic response elicited in vaccinated animals by the experimental challenge. Another indication of anamnestic response may be interpreted from the PI-3 isolations. Though isolations were made from calves in both groups, it is interesting to note that the PI-3 virus was not isolated from the upper respiratory tract over as long a period of time in the vaccinated animals. This is probably due to the rapid rise in HI antibody titer which in turn correlates with prevention of viral infection.

Based upon the results of the several parameters studied, it would appear that a shipping fever vaccine has been prepared which will induce a satisfactory level of immunity against a severe experimental challenge. Other data, to be published elsewhere, has indicated that this vaccine has given good protection against a natural outbreak of shipping fever (21). Excellent safety data and serologic conversions have been obtained in 923 animals in 9 field trials.

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