Epizootiologic Studies Of Shipping Fever. II. Exposure Of Calves To Pasteurellae And Para-Influenza 3 Virus.

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ABSTRACT

In four trials, the exposure of six calves to various stressors and an aerosol of pasteurellae did not evoke significant responses. In ten trials, 20 calves were exposed by aerosol or intratracheal injection to myxovirus para-influenza 3 (PI3) alone or combined with pasteurellae. A respiratory syndrome similar to that observed in mild cases of shipping fever was regularly reproduced in calves which had low serum hemagglutination titers to PI3. One calf developed a severe bronchopneumonia, was killed and the virus was reisolated from its lungs. Exposure to PI3 plus pasteurellae usually resulted in an increased nasal discharge. The stressors employed in a few trials did not significantly alter the response.

Introduction

In 1959, Reisinger *et al.*[1] isolated myxovirus para-influenza 3 (PI3) from calves with shipping fever (SF); calves exposed by aerosol to PI3 developed a mild respiratory disease. Similar results were reported in experimental infections of calves by other investigators.[2,3,4] In another study[5], calves exposed by aerosol to PI3 did not develop signs of respiratory illness. Hamdy *et al.*[6] reported the development of severe respiratory disease in calves exposed to PI3 in combination with other agents and stressors but not in calves exposed only to PI3.

As reported in a previous paper[7], strains of PI3 were isolated in 1959 in Wisconsin from nasal swabs collected from feeder calves with SF as well as from apparently healthy ones. This report is concerned with attempts to reproduce SF experimentally by exposing calves to PI3, pasteurellae and stressors.

Materials and Methods

Fourteen trials involving 26 calves were conducted. The calves were Holstein or Holstein crosses and were raised on University of Wisconsin farms. These animals were exposed, by aerosol on intratracheal injection, to *Pasteurella multocida, P. hemolytica,* strains of PI3 or to combinations of these agents.

With the exception of those in trials 3 and 4, all calves were exposed in an isolation building of the Rockefeller Institute type. The calves were placed in the isolation units approximately one week prior to exposure. Clinical observations and rectal temperatures were recorded twice daily. Nasal swabs and heparinized blood samples were collected daily.

These samples were processed as previously described[7] in attempts to isolate *Pasteurella* spp. and viruses cytopathogenic for bovine embryonic kidney (BK) cell cultures. In addition, mean leucocyte values were determined daily from the heparinized blood samples. Pre-exposure serum samples were tested for antibody to PI3 by the hemagglutination-inhibition (HI) test.[7] In some trials, the sera were also tested for anticapsular antibody to *Pasteurella multocida* according to an indirect hemagglutination test described by Carter.[8,9]

In trials 1 through 5, the calves were exposed to aerosols generated by a reflux nebulizer (Vaponefrin No. 16) under 10 to 15 psi of air pressure. The orifice of the nebulizer was fitted into the air inlet hose of a close fitting face mask fastened securely over the muzzle of the calf. In trial 6, the aerosol from the nebulizer was di-

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rected into a small opening in a plastic bag fastened securely over the head of the calf. In trials 7 and 8, exposure was by intratracheal injection. In trials 10 through 14, an atomizer was used to spray the inoculum through the opening in the plastic bag into the calf's nostrils.

Bacterial inocula consisted of suspensions of *P. multocida* types A, B or D, or *P. hemolytica* in physiological saline containing approximately 10^9 cells per ml.* These were prepared by washing the growth from Albimi brucella agar slants, which had been incubated at 37 C for 18 hours, and standardizing the suspension turbidimetrically in relationship to standard plate counts.

In the first four trials where only pasteurellae were given by aerosol, each calf received two to four ml. Where pasteurellae were given by atomizer, the inoculum was increased to 10 to 15 ml and in the one trial, where *P. hemolytica* was given intratracheally, 10 ml was injected.

PI3 strains (WD1-WD4) were used alone or in combination with pasteurellae in trials 6 through 14. In all cases, second passage tissue culture-propagated virus containing 10^6 to 10^7 $TCID_{50}$/ml was used. When PI3 was given by nebulizer, five ml was the size of the inoculum; when it was given by intratracheal injection, five ml of virus was diluted to a volume of 15 ml in Hank's balanced salt solution (HBSS); when PI3 was given by atomizer, 15 ml of stock virus was diluted in HBSS to a final volume of 30 ml.

In some trials, the following methods of stressing the calves were used: the parenteral injection of cortisone and adrenocorticotrophic hormone (ACTH), the intravenous injection of the virus of New York Virus Diarrhea and the trucking of calves for six to 48 hours in inclement weather.

Post-exposure samplings consisted of nasal swabs and heparinized blood samples collected daily for two to three weeks. These were processed in the same manner as those taken during the pre-exposure period. Rectal temperatures and clinical observations were recorded twice daily during this period. Sera were collected at one, two and three weeks post-exposure. In some trials, the calves were slaughtered and tissues were collected for microbiologic and histologic examinations.

Results

In the first four trials six calves were stressed and exposed by aerosol to pasteurellae as outlined in Table 1. These calves did not develop significant signs of respiratory disease, although the two calves in trial 4 did develop characteristic signs of virus diarrhea. Cytopathogenic or hemadsorbing viruses were not isolated from nasal swabs from any of these calves. *P. hemolytica* was isolated from the calf in trial 2 while cortisone was being administered. The calves did not develop measurable anticapsular antibodies to the pasteurellae as a result of exposure. The calves in trials 1 and 2 were later injected subcutaneously with ten ml of a formalinized culture of *P. multocida*, type D and did develop significantly increased titers to this type.

The data on exposure trials 5 through 14, involving PI3 are summarized in Table II. Included is an arbitrary classification of the severity of the response based upon clinical observations.

Trial 5: Calves A and B

Of ten calves, shipped in an open truck for 48 hours in January, two (A and B) were then placed in an isolation unit. These two were exposed to an aerosol of pooled nasal washings from four calves (lot 3D)[7], two of which had spontaneous SF but from none of which PI3 was isolated.

Pasteurellae were not isolated from either calf before or after exposure. Both calves had a slight nasal discharge from day 3 to 5. Calf B had temperatures of 103 F and 105 F on days 1 and 2 and 12 and 13 respectively. A hemadsorbing virus, identified as PI3 was isolated from this calf on day 13. There was a significant increase in the serum HI titers to PI3 in calves A and B.

The eight calves shipped with A and B failed to develop antibodies to PI3. Samples from these eight were negative in BK tissue cultures.

Trial 6: Calves C and D

Two calves were exposed to an aerosol of PI3. There was a slight febrile response and leucopenia in both calves on days 2 to 4 with the fever in calf C persisting at 103 F until day 8. This calf also developed an elevated temperature, leucocytosis and

*Most strains used were supplied by Dr. G. R. Carter, OVC, Guelph, Canada.

TABLE I. — Summary of Four Exposure Trials in Which Six Calves Were Exposed to Aerosols of Pasteurellae

Trial	Calves	Stressors	Species of Pasteurella	Clinical Response
1.	*female 3 mos.	50 mg cortisone /day for five days pre-exposure	**P. multocida** type D	Slight nasal discharge
2.	*female 6 mos.	75 mg cortisone /day for five days pre-exposure	same as trial one	negative
3.	female 3 mos.	trucked 6 hours post-exposure in early March	**P. multocida** type D	inappetance and slight fever for two days
	female 6 mos.		**P. hemolytica**	
4.	twin calves female and male 3 mos.	1 ml NYVD** virus I. V. two days before exposure and trucking as in trial 3	same as trial 3	typical of NYVD- leucopenia, biphasic temperature, diarrhea.

*injected subcutaneously with formalinized culture of **P. multocida,** type D 18 days post-exposure.
**New York Virus Diarrhea

rhinitis on day 17 and at this time *P. multocida* was isolated.

TRIAL 7: CALVES E AND F

Two calves, three and two months of age, were exposed by intratracheal injection to PI3. Both developed an elevated temperature, accompanied by nasal discharge, a cough and slight leucopenia on days 2 and 3. In calf F, the disease progressed with high temperature, rhinitis, pulmonary rales and pneumonia. This calf was killed on the fifth day, when moribund. The virus was recovered from the nasal passages and both lungs but not from the blood, liver or lymph nodes. Pasteurellae were not recovered from the tissues.

Calf E recovered quickly from the mild initial reaction. When it was re-exposed intratracheally, one month later, to PI3, *P. multocida* and *P. hemolytica* no signs of illness were observed.

TRIAL 8: CALVES G AND H

Two calves, three months of age, were exposed by intratracheal injection to PI3 and *P. hemolytica*.. Both developed a temperature of 104 F on the day of exposure but became afebrile by day 2. Calf H had an elevated temperature on day 9. Nasal discharge increased in both from day 2 to 11 during which time PI3 and *P. hemolytica* were recovered from nasal swabs.

TRIAL 9: CALVES J AND K

After being placed in an isolation unit, these two calves developed a spontaneous upper respiratory disease which lasted for a week. *P. multocida,* type A, was recovered from both calves at this time.

One week later the calves were exposed to an aerosol of PI3 but did not develop significant signs. The calves were slaughtered one month after exposure but neither PI3 nor pasteurellae were recovered from the tissues of the respiratory tract.

TRIAL 10: CALVES L AND M

Two calves, five months of age, were exposed to an aerosol of PI3 by atomizer. Pasteurellae were not recovered from pre-exposure nasal swabs but *P. multocida,* type D was recovered from calf L from days 7 to 12. Both calves developed an upper respiratory infection with nasal discharge and elevated temperature. Signs of pulmonary congestion were observed in calf M by auscultation.

At necropsy three weeks post-exposure the lungs of both contained areas of consolidation in the antero-ventral regions but neither pasteurellae or PI3 was recovered.

TABLE II —. Observations and Laboratory Findings on Calves in Exposure Trials 5-14.

Trial No.	Calf No.	Agents	Days Temp over 102.5 F.	Isoln of Pae	Days When PI3 Reisolated	Leuco-penia	Severity of Response	PI3 HI Pre	Serum Titers Post
5	A	truck na-wh	1	neg	neg	—	+−	20	80
	B	aer	1, 2, 8, 12 13, 14	neg	13	—	++	10	160
6	C	PI3 aer	5, 6, 7, 8 17	P.m day 17	0, 6	+− (slight)	+	10	320
	D	aer	4	neg	0, 2, 6	+	+−	20	160
7	E	PI3 IT	3	neg	0	+	+−	10	160
	F	"	1, 2, 3, 4, 5	neg	0, 4, 5, lungs	++	+++	0	10
8	G	PI3, Pae IT	0, 1, 12	neg	0, 3, 4, 5, 7	+− (slight)	+	10	160
	H	11	0. 2, 9	neg	0, 3, 4, 5, 9	+− (slight)	+	10	160
9	J	PI3 aer	none	pre-exp P.m. A	0	neg	neg	10	160
	K	aer	none	P.m, A	0	neg	neg	20	80
10	L	PI3	4, 6, 9, (16, 18, 19, diarh)	P.m. day 7	0, 4, 5 6, 7	+− (slight)	+	10	160
	M	"	1, 3, 4, 5 6, 9, 10	neg	0, 2, 3 5, 11	+− (slight)	++	0	160
11	N	ACTH PI3 aer	3	P.m. pre-exp	0, 1, 2, 4, 5, 10	neg	+	10	160
	P	"	1, 2, 3, 4, 5, 6, 7	neg	0, 2, 5	+− (slight)	++	10	160
12	Q	PI3, Pae aer	2, 3	neg	0	neg	+−	20	320
	R	"	2, 3	neg	0	neg	+−	20	320
13	S	"	2	P.m. pre-exp	0	neg	−	80	320
	T	"	2, 3	neg	0	+− (slight)	+−	40	640
14	U	ACTH PI3 aer	none	neg	0	neg	−	40	320
	V**	"	1-12	P.m. pre-exp	0, 1, 2	+	(?)	10	160

Pae = pasteurellae; na − wh = nasal washings; P.m. = **P. multocida**; aer = aerosol;
IT = intratracheal; pre-exp = pre-exposure; +++ = clinical case of shipping fever.
*Calf E killed on day 5 when moribund.
**Calf V had pyelonephritis when slaughtered.

TRIAL 11: CALVES N AND P

Two calves, six and four months of age, were injected intramuscularly with 50 USP units of ACTH twice daily for five days prior to their exposure to an aerosol of PI3. *P. multocida* type D was isolated from calf P prior to and after exposure. This calf developed pulmonary congestion, a temperature of 103 to 105 F from day 1 to 5 and increased nasal discharge until day 10.

TRIAL 12: CALVES Q AND R

Two bull calves, six months of age, were exposed to an aerosol of PI3 and a freshly isolated strain of *P. multocida*, type A. Both calves had elevated temperatures on days 2 and 3 and accompanying catarrhal rhinitis which lasted until day 4.

TRIAL 13: CALVES S AND T

Two calves, six months of age, were exposed as in the previous trial. *P. multocida* was isolated from calf S before exposure. Both calves developed slightly elevated temperatures on days 2 and 3 with some increase in nasal discharge from day 2 to 5. Each calf had a slight leucopenia from days 2 to 4.

TRIAL 14: CALVES U AND V

Two calves, nine and five months of age, were injected with ACTH as in trial 11 and exposed to an aerosol of PI3. *P. hemolytica* was isolated from Calf V before exposure and until the termination of sampling.

Calf U did not develop respiratory disease while V developed rhinitis, fever and leucopenia for a few days followed by leucocytosis. The significance of the response in V was questionable because of a concurrent pyelonephritis. At necropsy five weeks post-exposure, areas of consolidation and congestion were present in the cardiac lobe of the lung of V. Bacteria or viruses were not isolated from the respiratory tract of either calf at this time.

Discussion

The lack of response in the six calves exposed to pasteurellae is in agreement with the results obtained by other investigators.[10,11] Rises in titer of anticapsular antibody to *Pasteurella multocida* did not occur in these calves after exposure but did in the two subsequently inoculated with formalinized bacterins.

In most of the calves exposed to PI3, there was an inverse positive relationship between the pre-exposure serum HI titer and the response to exposure. The typical response in calves which had low HI titers to PI3 was an elevated temperature at day 2 or 3 accompanied by signs of upper respiratory disease. Recovery occurred within a week. The calves had an increased serum HI titer by 7 to 10 days post-exposure. PI3 could be reisolated at the time of the pyrexia and nasal discharge.

Calves with pre-exposure HI titers of 20 or greater did not develop appreciable signs of respiratory disease. Virus could seldom be reisolated for more than two days after exposure of such calves. Hamparian *et al.*[5] did not reisolate virus postchallenge from calves with prechallenge HI titers greater than 40 but did reisolate virus for up to 4 days postchallenge from calves with pre-exposure titers of 40 and for 7 to 10 days postchallenge from calves with pre-exposure titers less than 20.

Calves J and K in trial 9 had pre-exposure HI titers of 10 and 20 but did not respond to exposure to PI3. The spontaneous respiratory disease which these calves developed before exposure was associated with the presence of *P. multocida* but not with cytopathogenic viruses. Other viruses, such as those of the psittacosis group, which would not be detected by the methods used may have been present in the respiratory tracts of these calves. Such agents or *P. multocida* might block or interfere with infection by PI3 of the respiratory epithelial cells as has been demonstrated in other host-parasite systems.[12,13] In contrast, Swedish workers presented evidence to support the idea that a simultaneous infection of cattle with PI3 and virus diarrhea virus produced cases of respiratory-enteric disease in naturally occurring epizootics.[3,4] These workers also exposed four calves to PI3 and observed signs of mild respiratory illness. There was evidence also of concurrent infection with virus diarrhea although this virus was not knowingly included in the inoculum.

The two calves, A and B, which were exposed to pooled nasal washings from which PI3 was not isolated, developed significantly increased HI titers to PI3. Calf B had signs simulating a mild case of SF and PI3 was isolated from it. It appears that these calves were more sensitive than tissue cultures for indicating the presence of small amounts of virus or had concurrently contracted the virus from some other source.

Trucking calves A and B for 48 hours may have influenced their response to the aerosol of nasal washings but this is speculative. Project co-workers,[14,15] who were investigating the role of stressors in the pathogenesis of SF noted that shipped calves often had increased or decreased plasma levels of 17-hydroxy-corticosteroids. Calves in such physiologic states were considered to be possibly more susceptible than normal to virulent microorganisms. Hamdy *et al.*[6] observed that calves exposed to PI3, *Pasteurella* spp. and physical stress developed the most marked clinical signs and lesions of SF. Administration of ACTH did not appear to increase the response to PI3 in trials 11 and 13.

The method of exposure to PI3 did not appear to affect the response except in the case of the youngest calf, F. The pre-exposure antibody level appeared to be more significant. An acute bronchopneumonia in which the cellular reaction was predominantly mononuclear was noted on microscopic examination of the lungs of calf F. In the other calves necropsied, simi-

lar histologic findings were noted, although the process was more chronic and focal.

Leucopenia or a trend in this direction was noted in some calves after exposure to PI3. The most marked decrease was seen in Calf F. When pasteurellae were present with the virus in the nasal passages there was generally little change in the leucocyte count or else a leucocytosis. Hoerlein and Marsh[16] also reported leucopenia in calves with the initial temperature rise with SF. Hamdy *et al.*[6] noted leucopenia on days 2 and 4 in most calves exposed to PI3.

The presence of pasteurellae in the inoculum with PI3 had little influence on the temperature response but was associated with increased nasal discharge. In two calves, C and L, exposure to PI3 seemed to have an enhancing effect on the pasteurellae in the upper respiratory tract because these bacteria were isolated after, but not before exposure to the virus.

Since viruses of the enterovirus and psittacosis groups have also been associated with SF[7] experimental infections of calves with various combinations of these agents, PI3, pasteurellae and stressors should be carried out.

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Observations on the Frequency of Fused Foetal Circulations in Twin-Bearing Cattle.

This report arises from information collected during field studies on the hormonal induction of twin pregnancies in the cow. Cows were examined for evidence of multiple pregnancy six or seven weeks after breeding and the position of twin foetuses within the uterus determined. Blood samples were obtained from 21 sets of like-sexed and unlike-sexed twins and from their parents, and typed for evidence of fused circulations.

In each of nine sets of unicornual twins, evidence of fusion of the foetal vascular system was found. In the 12 sets of bicornual twins, eight gave evidence of fused circulations and four showed individual members with different blood groups.

Eighty-one per cent of the 21 pairs therefore showed good evidence of fusion of their foetal circulations during pregnancy and these included all of the unlike-sexed twins. The results also suggest that foetal vascular systems are more likely to unite in unicornual than in bicornual twin pregnancies.

The four sets of twins which did not show evidence of fusion were all of like-sexed foetuses.

Williams, G. et al. Brit. Vet. J. 119: 467, 1963.