Epizootiologic Studies Of Shipping Fever Of Cattle
I. The Microbial Agents Isolated

by J. R. Saunders*, David T. Berman and Merwin L. Frey

ABSTRACT

Nineteen strains of Pasteurella spp., but no viruses cytopathogenic for bovine embryonic kidney cells were isolated from pneumonic lesions present in "normal" veal calves at slaughter.

In studies on two herds of native cattle and six lots of western feeder calves, Pasteurella spp. were isolated from nasal swabs from healthy cattle and those with shipping fever. Viruses of the psittacosis-lymphogranuloma group were isolated from nasal swabs from animals in five groups. Viruses provisionally identified as bovine enteroviruses were isolated from nasal swabs of calves in two lots.

There was serologic evidence of a temporal association of myxovirus para-influenza 3 (PI3) with shipping fever in three lots of calves. From two of these three lots, strains of PI3 were isolated from ten animals, four of which had clinical shipping fever at the time of virus isolation.

Introduction

Shipping Fever (SF) is an acute respiratory infection which primarily affects young cattle during or after shipment. The theory that SF is a stress-induced pasteurellosis has not been upheld experimentally.[1] A recently advanced concept[2] is that stressors associated with transit predispose calves harboring viruses in their respiratory tracts to secondary bacterial infections. In 1959 Reisinger *et al.*[3] reported the association of myxovirus para-influenza 3 (PI3) with SF in calves.

This project was initiated to study the epizootiology of SF with the emphasis on the role of viruses. In 1959, Laxson, working in this laboratory, isolated PI3 from nasal swabs obtained from three healthy calves and one with SF[4]. Further epizootiologic studies are described herein, while the results of experiments in which calves were exposed to isolates of PI3, alone or in combination with other agents, are reported in another paper.[5]

Materials and Methods

Specimens from apparently healthy cattle and those with respiratory infections were collected for microbiologic, serologic, and histopathologic studies. Bovine embryonic kidney (BK) tissue cultures and, in some cases, chicken embryos were used for the isolation of viruses.

Abattoir studies

Six lots, totalling 105 samples of veal-calf lungs with pneumonic lesions were collected at an abattoir at different seasons of the year. For the isolation of bacteria a piece of tissue 10 mm^3 was ground in ten ml of nutrient broth in a tissue grinder; after centrifugation the supernatant fluid was plated onto Albimi brucella agar and brucella agar containing 1.5 μg Neomycin per ml. Laxson[4] described the processing of tissues for the inoculation of BK cell cultures. For histologic examination, the tissues were fixed in neutral formalin, embedded in paraffin, sectioned at six microns and stained with hematoxylin and eosin.

Field studies

In field investigations two native herds, in which epizootics of respiratory disease occurred, and six lots of western feeder calves were studied.

The two native herds and four lots of feeder calves were followed in horizontal surveys in which the primary purpose was the isolation of microbial agents. Samples were obtained from cattle selected at ran-

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*Present address: College of Veterinary Science and Medicine, Purdue University, West Lafayette, Indiana.

dom on the day of their arrival at the farm or when the herd was visited for the first time. Rectal temperatures were taken and any clinical observations were made; two or three nasal swabs, and heparinized and unheparinized blood samples were collected. Similar samples were obtained from animals with fever or signs of SF on subsequent visits. Paired serum samples could be secured only occasionally in these six groups.

More complete longitudinal studies were made in the two remaining lots of feeder calves. Each of the calves was identified and samples were taken from each when they were unloaded at the farm. Clinical observations and rectal temperatures were recorded daily over a two to three week period and samples were collected from all calves with temperatures over 104 F, as well as from some normal calves. Sera were collected from all the calves at the end of the observation period.

Sterile cotton-tipped six inch applicator sticks were used to swab the nasal passages of the cattle. For the isolation of viruses in tissue culture, the swabs were put into tubes of Hank's balanced salt solution (HBSS) containing 500 units of penicillin and 500 μg of streptomycin per ml and stored at -65 C until inoculated into BK cultures. For attempted isolations of viruses of the psittacosis-lymphogranuloma (PL) group, the swabs were put into tubes of HBSS containing 1000 μg of dihydrostreptomymic per ml.[6] The tubes were shaken, left at room temperature for two hours and the suspensions were inoculated into the yolk sacs of embryonating chicken eggs or stored at -65 C for later inoculation into eggs. For the isolation of bacteria, the swabs were put into vials of physiological saline at 4 C and plated onto agar media within a few hours.

Heparinized blood samples with antibiotics added were stored at -65 C for later inoculation into BK tissue cultures. Sera from clotted samples were stored at -10 C.

BACTERIOLOGIC STUDIES

Stock cultures of reference* strains of *P. hemolytica and P. multocida,* types A, B, C and D, were maintained on brucella agar slants and were also lyophilized in sterile skim milk. Type specific antisera were prepared in rabbits and chickens.

Brucella agar and brucella agar with neomycin were used routinely for the isolation of pasteurellae from samples. These bacteria were identified by the procedures cited by Breed *et al.*[7] and Carter.[8]

VIRUS STUDIES

Secondary cultures of BK cells in 15 by 125 mm tubes in stationary racks were used in these studies, following the methods of Madin *et al.*[9] and the trypsinization procedure of Youngner.[10] The basic growth medium was HBSS containing ten to 20 per cent bovine or porcine serum and one-half per cent lactalbumin hydrolysate. The maintenance medium differed in that it contained two to five per cent porcine serum. These media contained 100 units of penicillin and 100 μg of streptomycin per ml.

After thawing, 0.1 ml from each sample was inoculated into each of three to five BK cell cultures. The cultures were examined daily for 14 days for cytopathic effects. At the sixth and fourteenth day the fluids from each sample were pooled and stored at -65 C and the cultures were tested for hemadsorption with one ml of a 0.25 per cent suspension of guinea pig erythrocytes in HBSS according to the technique of Shelokov *et al.*[11] The samples were passed three times in BK cultures and were discarded if negative.

Nasal swabs, intended for the inoculation of chicken embryos, were prepared as previously described. One half ml of the suspended material from each swab was inoculated in 0.5 ml amounts into the yolk sacs of six to ten eggs embryonated for six days. The eggs were then candled twice daily for ten days. When an embryo died between days two and ten, the yolk sac was harvested and ground in four volumes of sterile physiological saline in a Ten Broeck tissue grinder. Prior to grinding, an impression smear of the yolk sac was made on a glass slide, heat fixed, stained with Macchiavello's stain and examined for elementary bodies.

These suspensions were then inoculated into additional embryonating eggs. In those groups of eggs in which no deaths had occurred by the end of six days, two of the eggs were chilled, and the yolk sacs were harvested and repassaged by yolk sac inoculation. If no deaths occurred in either the first passage or during the first six days

*Supplied by Dr. G. R. Carter, Ontario Veterinary College, Guelph, Ontario, Canada.

TABLE I — Distribution of Eighty-five Strains of Pasteurellae Isolated from Cattle

Cattle Sampled		Number Positive	Strains of Pasteurellae		
Type	Number		P. multocida	P. hemolytica	Not Typed
Had Symptoms of SF	61	29	14	4	11
Veal Calf Lung Samples	105	19	17	2	0
Exposed Calves*	26	7	5	2	0
Normal Animals	152	30	19	8	0

*Artificially exposed to PI3 and Pasteurellae[5]

in second passage, yolk sacs were removed from two of the second passage eggs and examined for elementary bodies.

SEROLOGIC STUDIES

The majority of serologic tests were concerned with the isolates of cytopathogenic, hemadsorbing (HA) viruses. Antisera were prepared in rabbits by inoculating fluids from infected BK cell cultures. The SF-4 strain of PI3*, two strains of bovine enterovirus,** and the respective antisera were obtained for reference. Homologous antisera as well as a battery of myxovirus hyperimmune sera were used in the identification of the HA viruses by the hemagglutination-inhibition (HI) test and in BK cultures by the neutralization and hemadsorption-inhibition tests with guinea-pig erythrocytes. Cytopathogenic non-hemadsorbing viruses were identified by their general properties and by neutralization tests.[12]

Sera from cattle of five of the eight field groups and from some native Wisconsin cattle were tested by the HI procedure for antibody to PI3. The sera were first treated with acid-washed kaolin and then absorbed with guinea-pig erythrocytes. The techniques of hemagglutination and HI tests were essentially those of Clarke and Casals[13] with modifications adapted from Reisinger *et al.*[5] Guinea-pig erythrocytes were used in these tests.

Results

ABATTOIR STUDIES

Pneumonic lesion were more numerous and more extensive in the calves slaughtered during the fall and winter months. Cytopathogenic or hemadsorbing viruses were not isolated from any of the 105 specimens. Nineteen strains of *Pasteurella* spp. were isolated, the majority from winter samplings. On histologic examination an interstitial pneumonitis without exudation was the common finding. Lesions from which pasteurellae were isolated were more extensive and were characterized by an acute peri-bronchiolar inflammation with exudate in the bronchioles.

The distribution of 85 strains of *Pasteurella* spp. isolated from the calf lungs, from normal cattle and from calves with SF (field lots) is shown in Table I. Serotyping [8] of 13 isolates of *P. multocida* resulted in the recognition of 12 strains of type A or D and one untypable.

FIELD STUDIES

Lot 1-*R*: Of 30 feeder calves brought from Nebraska onto a University farm in January, 1958, two developed a respiratory infection. No cytopathogenic viruses and only two strains of *Pasteurella* sp. were isolated from the 16 calves sampled. Sera were not collected.

Lot 2-*K*: Also in January, 1958, samples were taken from 16 of 34 calves from North Dakota on arrival at a local feedlot. Despite the prophylactic and therapeutic use of antibiotics and sulfonamides a few calves developed signs of SF. Samples were collected from one affected calf and three weeks later sera were obtained from four convalescent calves. From the 17 samples, five strains of *P. multocida* A or D types and two of *P. hemolytica* were isolated. Cytopathogenic viruses were not isolated.

The results of serum HI tests with PI3 were as follows: 14 early and one late serum had titers of 10 or less (reciprocal of serum dilution), three early and one

*Dr. R. Reisinger, U.S.D.A., A.R.S., Beltsville, Md.

**Dr. T. Moll, Dept. of Vet. Science, State University of Washington, Pullman, Washington.

TABLE II. — Microbial Agents Isolated from Nasal Swabs of Calves of Lot 3D

Shipment Number	Days in Transit	Total Calves	Calves Sampled	Isolation Pasteurellae	PI-3*
A. Sampled on Arrival at Feedlot					
1	4	58	10	2	0
2	6	23	10	1	3
3	7	92	21	3	0
4	4	94	7	0	0
5	6	75	5	2	0
6	7	60	8	2	0
Total		402	61	10	3
B. Sampled in First Week at Feedlot					
23 Calves with Shipping Fever				12	4
4 Normal Calves				3	0
27 Total				15	4

*Para-Influenza 3 Virus (WD-1 to WD-7 strains)

late had titers of 20 and two late sera had titers of 40.

Lot 3-D: During the fall of 1958, 88 animals from Montana were sampled at another feedlot. These included 61 calves in six different groups which were studied on arrival, and 27 calves of which 23 had clinical SF and which were observed at varying times after arrival. During the fall, approximately 1000 cattle were brought into the feedlot and at least 100 treated for SF. At the end of the investigation blood samples were collected from seven convalescent calves.

The microbial agents isolated are shown in Table II and the serologic studies in Table III. Laxson[4] reported initially on the isolation of PI3 (strains WD1-4) from four calves, one being the first sick calf sampled and the other three afebrile, from shipment 2.

Lot 4-D: Samples were taken from 27 feeder calves in two shipments at the same feedlot during the fall of 1959 but pyrexia or other signs of respiratory disease were not noted. Three strains of *Pasteurella* were isolated from the second shipment while isolations were not attempted from the first. Viruses were not isolated in BK cultures although viruses of the PL group were isolated from three calves.

Lot 5-T: A group of 40 Hereford feeder calves was brought to a University farm from Montana in October, 1959. During a three week observation period 12 calves became febrile and had nasal discharge and mild signs of respiratory disease. Only one calf (39) had signs severe and prolonged enough to require treatment. Seven strains of *Pasteurella* were isolated but, again, no cytopathogenic viruses were recovered. Viruses of the PL group were isolated from five of the calves, including calf number 39. The serum HI titers to

TABLE III. — —PI3 Serum Hemagglutination Titers of Calves in Lot 3D

When Sampled	Number of Calves	Number of Calves Having the Following Serum HI Titers (Reciprocal of the Highest Serum Dilution)*				
		10	20	40	80	160
A. On Arrival	61	40	17	3	1	0
B. First Week	27	9	7	4	7	0
C. Convalescent**	7	0	0	2	3	2

*Using 4 HA units of antigen (WD2 strain).
**Had Shipping Fever — Bled at least 3 weeks Post-arrival.

TABLE IV. — Changes in PI3 Serum HI Titers of Calves (Lot 5T) which Developed Temperatures of over 104 F.

Calf Number	Serum HI Titers* Against PI3	
	On Arrival	23 Days Later
2, 4, 10, 35....	0	80
1, 16.....	10	80
26.....	0	40
8, 39.....	10	20
12.....	20	20
27.....	20	40
7.....	80	160

*Reciprocal of Highest Serum Dilution using 4 HA units of antigen (WD2).

TABLE V. — PI3 Serum HI Titers of Forty Calves in Lot 5T on Day of Arrival and 23 Days Later.

Serum HI Titers* (WD2 strain of PI3)	No. Calves with titer on arrival	No. Calves with titer on day 23.
0	9	0
10	17	2
20	9	5
40	3	7
80	1	11
160	0	14

1 Calf, serum lost
*Reciprocal of highest serum dilution using 4 HA units of antigen.

PI3 are shown in Table IV. Thirty three of the animals had a four-fold or greater rise in titer.

Lot 6-A: In November, 1959, 16 pairs of twin Hereford calves, on a feeding trial conducted by the Animal Husbandry Department, developed a chronic respiratory infection characterized by dull cough, slight temperature elevation, nasal discharge and poor response to treatment. This epizootic was not associated directly with shipment or the addition of animals. Nasal swabs, taken from 30 calves did not yield cytopathogenic viruses. Viruses of the PL group were isolated from five calves. A full month was required before the calves recovered completely from the disease.

Lot 7-P: In December, 1959, after the addition of six cows, 15 cows in a herd of 80 Jerseys experienced a severe respiratory infection. Samples were collected from ten animals at this time, and four months later, from ten animals, including a goat, seven cows previously sampled, a healthy calf and a sick calf. In the time between samplings 12 young calves died, in most cases from a severe respiratory infection. Strains of *Pasteurella* were isolated from four animals at the first sampling. Cytopathogenic, non-hemadsorbing viruses were isolated from four nasal swabs from the first sampling but from none of the second. Viruses of the PL group were isolated from two nasal swabs at the first sampling and from nasal swabs from a cow and the two calves at the second. There were no significant rises in serum HI titers to PI3.

Lot 8-L: The procedures used in the study of calves in lot 5-T were also employed with a group of 32 calves trucked to a University farm in January, 1960. Only two calves were febrile on the day of arrival, but by the end of the first week, half of the calves had nasal discharge and were coughing. These findings and the results of attempts to isolate microbial agents are summarized in Table VI. The serum HI titers to PI3 are presented in Table VII. Twenty two animals had a four-fold or greater increase in titer.

VIRUS STUDIES

The hemadsorbing viruses isolated from calves of lots 3-D (strains WD1-7) and 8-L (strains WL1-3) were identified as strains of PI3 by reciprocal neutralization tests with the SF-4 strain and respective antisera. Their properties in BK cultures were similar to those of the SF-4 strain. These isolates were also ether sensitive, produced intranuclear inclusions in BK cells and did not produce hemagglutinins in chicken embryos inoculated via the allantois.

The non-hemadsorbing viruses from lots 7-P and 8-L were tentatively identified as bovine enteroviruses on the basis of ether resistance, rapid cytopathic effect and reciprocal neutralization tests with the BE 180 virus.[12] The agents have been characterized more completely by Bahnemann.[14]

The results of attempts to differentiate the isolates of PL virus from other known strains of PL virus by the experimental inoculation of calves, guinea-pigs and mice will be reported elsewhere.[15]

Discussion

The greater incidence of pneumonia in the calves slaughtered in the fall and winter is undoubtedly associated with the seasonal pattern of respiratory infections. The failure to isolate cytopathogenic vi-

TABLE VI. — Infectious Agents Isolated from Nasal Swabs of Calves of Lot 8L

Day	Barn Numbers of Calves over 104 F	Calves from which the following were isolated			
		PI_3	P-L	B-E	Pae
1	9, 80	12, 17, 83	25	9	2, 3, 5
3	9, 83, 96				
4	2, 5, 8, 80		1		
5	2, 13, 16			14	14, 86
6	2, 13, 16, 19, 23			2, 13, 23	
7	2, 13, 19, 23				12
8	12				
9	80, 95			93	
10	93			83	83, 93
15	93		9, 80	2, 9, 20	9

KEY

PI3 = Para-Influenza 3
B-E = Bovine Enterovirus
—— = Correlation with Temperature
P-L = Virus of the Psittacosis Group
Pae = Pasteurellae

ruses may have been due to the chronicity of the lesions or to the presence of neutralizing antibodies. Viruses of the PL group would not be demonstrable by the tissue culture methods which were employed. Abinanti *et al.*[16] isolated PI3 from nasal swabs rather than the lungs of veal calves.

In some lots of feeder calves, SF did develop even though antibiotics and sulfonamides were administered. However, the generally favorable response to treatment with antibiotics indicated that bacteria were important in the pathogenesis of the disease. The fact that *Pasteurella* spp. were isolated oftener from cattle with SF than from normal ones also indicated that these bacteria were important secondary invaders. The results of typing strains of *P. multocida* agrees with Carter's observations[17] on the serotypes associated with SF in Canada.

The data on the PI3 isolates are in general agreement with the findings of other investigators. [5,16,18,19] In lots 3-D, 5-T and 8-L, there was definite serologic evidence of a temporal association of PI3 with SF and in two lots the virus was isolated from ten calves. Five calves had signs of disease at the time of isolation of the virus and *Pasteurella* spp. were isolated from three of these five either from the same sampling or a later one.

PI3 was isolated from six calves with HI titers of ten or less as well as from four with titers of 20 to 40. Hoerlein *et al.*[18] demonstrated that sera with an HI titer of 20 or greater always had neutralizing capacity in tissue culture. In the calves exposed experimentally to PI3[5], there was a positive correlation between the pre-exposure HI titer and the response.

In lot 5-T, SF was not a problem. The serologic evidence for the presence of PI3 in calves of this lot indicates that inapparent infection can be associated with this virus. The reasons for the failure of development of apparent disease can only be speculative. A comparison of lots 3-D and 4-D illustrates the great variation in incidence of SF from year to year.

Positive HI tests on sera of native cows and calves in 1960 indicated that PI3 was present in Wisconsin cattle. It is therefore postulated that the majority of incoming feeder calves receive an antigenic stimulus sufficient for antibody formation sometime in the two week period preceding and following their arrival in Wisconsin.

The results of this study suggest that PI3 may be associated with many epizootics of SF. It is realized that other viruses

TABLE VII. — PI3 Serum HI Titers of 32 Calves of Lot 8L on Day of Arrival and 45 Days Later

Serum HI Titers* (WD2 strain of PI3)	No. Calves with titer on arrival	No. Calves with titer at 45 days
0	9	0
10	10	0
20	4	0
40	6	6
80	3	16
160	0	10

*Reciprocal of highest serum dilution using 4 HA units of antigen.

may also be involved in such epizootics as pointed out by this study and by others.[5,12,19] Viruses of the PL group were isolated from a few cattle in all five lots in which isolations were attempted. Bovine enteroviruses were isolated from two lots. The experimental exposure of calves with these enteroviruses did not result in respiratory disease.[14] Further studies on the response of calves to exposure with combinations of bacteria, these viruses and stressors are indicated. Field trials with various combinations of bacterins and vaccines are needed to supplement previous ones[20,21] and should give indications of the relative importance of the various agents in the naturally occurring disease.

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Predicting the Time of Parturition in the Normal Cow.

Rectal temperatures were taken twice daily for the 10 days prior to parturition of 25 Ayrshire cows. During this period no signs of disease processes that could have influenced temperature were observed. It was found that body temperature variations were of little value in predicting the time of onset of parturition. The highest rectal temperatures were usually seen between the 2nd and 4th days before calving. An average fall of 11°F. commenced at an average time of 54 hours before parturition.

It was suggested that, even though signs of imminent parturition were present, such as slackening of the sacrosciatic ligament, relaxation of the perineum and vulva, distension of the udder and discharge of mucus from the vagina, a healthy cow is unlikely to calve in the succeeding 12 hours if its rectal temperature is 102°F. or above.

R. Vet. Rec. 75: 367, 1963.