The Relationship of Serology and Nasal Microbiology to Pulmonary Lesions in Feedlot Cattle

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ABSTRACT

A group of 62 beef calves, born and raised in an institutional herd, were transferred at nine months of age to a commercial feedlot where they remained until slaughter seven months later. Clinical, immunological and microbiological monitoring was carried out during this period. No serious clinical illness occurred. One hundred percent seroconversion to bovine virus diarrhea virus took place after introduction of the calves into the feedlot as well as almost complete (59/62) seroconversion to bovine herpesvirus 1, a proportion of which could be related to a single vaccination. Significant increases in recoveries of *Mycoplasma* spp. from nasal swabs also occurred in the feedlot. At slaughter, the lungs of all animals were recovered and examined for pathological lesions: 23 were completely normal and 39 showed minor histological changes chiefly characterized by areas of lobular to sublobular atelectasis.

For this group of calves, no relationship was found between the presence of potential pathogens in nasal mucus and the occurrence of lesions in the lung. The serological results are discussed in terms of vaccinations and other known events that occurred during the study period.

RÉSUMÉ

Cette étude portait sur un groupe de 62 veaux de boucherie, nés et élevés dans le troupeau d'une institution, mais transférés, à l'âge de neuf mois, dans un parc d'engraissement commercial où ils demeurèrent jusqu'à ce qu'on les abatte, sept mois plus tard. Au cours de cette période, on exerça sur eux une surveillance clinique, immunologique et bactériologique. On n'enregistra pas de maladie clinique sérieuse mais, après l'introduction des veaux dans le parc d'engraissement, on constata que tous possédaient des anticorps à l'endroit du virus de la diarrhée à virus bovine et que 59 en possédaient en plus à l'endroit de l'herpèsvirus bovin du type #1; une certaine proportion de ces derniers résultait d'une injection de vaccin. On nota également une augmentation appréciable du nombre d'isolements de *Mycoplasma* spp., à partir d'écouvillons nasaux. Lors de l'abattage, on récupéra les poumons de tous les veaux et on y rechercha la présence de lésions. Ceux de 23 d'entre eux s'avérèrent tout à fait normaux, tandis que ceux des 39 autres recelaient de légères lésions microscopiques qui se caractérisaient par de l'atélectasie qui affectait la totalité ou seulement une partie de certains lobules.

Ces veaux ne présentèrent pas de relation entre la présence d'agents pathogènes potentiels dans le mucus nasal et le développement de lésions pulmonaires. Les auteurs commentent les résultats sérologiques précités, en tenant compte de la vaccination et des autres événements connus qui eurent lieu au cours de leur étude.

The immunological and microbiological status of calves used in respiratory disease research is considered important, whether the studies are experimental or epidemiological in design. The most common methods employed are serological tests and the culturing of nasal mucus. An opportunity arose to examine the relationship between several common measures of health status and the pulmonary changes found at slaughter, when a group of calves born at this Institute was finished in a commercial feedlot.

The subject group consisted of 62 male and female crossbred Hereford calves born between April and June of 1980. Some were involved in a colostrum force-feeding experiment, and all were weaned in mid-summer at six to eight weeks of age. The calves were then kept in corrals at the Institute on a ration of alfalfa hay and calf starter, with gradual conversion to alfalfa hay *ad libitum*. The males (n = 29) were castrated in January of 1981 and all were vaccinated

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against clostridial diseases[1] in February, using a separate needle for each calf. All calves were shipped to the commercial feedlot 190 km away on March 10, and on arrival were given an intranasal vaccine[2] containing bovine herpesvirus 1 (BHV1) and parainfluenze type 3 (PI-3) virus. At the feedlot the calves were started on a hay ration, with gradual introduction of concentrate (containing monensin) starting on March 15. They were mixed with 62 calves from another ranch on April 3 and slaughtered in mid-October. Males and females were fed together from weaning to slaughter.

The following microbiological and immunological examinations were carried out prior to transport (March 9) and three times during the feeding period (April, July, September): *Mycoplasma* spp. (other than *M. dispar*) and *Pasteurella* spp. cultures from nasal swabs; BHV1 and bovine virus diarrhea (BVD) virus serum neutralization (SN) tests; PI-3 virus hemagglutination-inhibition (HI) tests; and complement fixation (CF) tests for *Pasteurella* spp. For mycoplasma isolation, cotton swabs were placed in 1.8 mL of noninhibitory horse serum (NIHS) broth, which is composed of Hayflick's medium (1) with thallium acetate and penicillin omitted. Hayflick's medium with 1% purified agar was then used for isolation of *Acholeplasma* and *Mycoplasma* spp. Tubes containing swabs were shaken on a vibromixer for 10 s; then the swabs were discarded. Broths were plated directly onto agar and carried through two tenfold dilutions which were plated three times at 2-d intervals. All plates and broths were incubated at 37°C, the plates under 5% CO_2. Colonies were identified by fluorescent antibody staining.

Ureaplasma broth (2) was inoculated with 10% inoculum and incubated at 37°C. Broths were plated to A-7 agar (3) at the first sign of color change. Plates were incubated under H_2 and CO_2 (Gaspak).[3] Ureaplasma colonies were identified by their black color and characteristic morphology.

Pasteurella spp. antibodies were detected by the modified complement fixation test of Cho (4) whereas *Pasteurella* spp. isolations were done by standard aerobic techniques. Previously described methods were used for the PI-3 HI test (5) and the BHV1 SN test (6).

For the bovine virus diarrhea (BVD) SN test, heat inactivated sera were tested against 100 $TCID_{50}$ of the NADL strain of virus at dilutions of 1/3, 1/9, 1/27, 1/81, 1/243, 1/729, 1/2187 and 1/6561. After 1 h at room temperature the mixtures were transferred to tissue cultures of bovine fetal spleen cells and incubated for 7 d. At this time they were fixed and stained for examination using the formalin-crystal violet solution of Witte *et al* (7).

At slaughter, all lungs were examined and the left cranial lobe was sampled in each case. When abnormalities were present, an additional tissue specimen was taken from a typical lesion. Both normal and abnormal tissues were examined histologically and cultured for *Pasteurella* and *Mycoplasma* spp. For the latter, specimens were collected in NIHS broth after which they were minced with scissors and shaken in fresh NIHS broth. The supernatant was then cultured in the same way as were the nasal swabs.

Counterimmunoelectrophoresis, using undiluted goat anti-BVD serum[4] in an electrophoresis chamber,[5] was used in an attempt to detect the presence of BVD antigen in the lungs. A 50% homogenate of lung was prepared in borate-buffered saline at pH 9.0 and clarified at 2000 rpm for 20 min. The supernatant was used as crude antigen in the test. All samples were negative.

The results of the serological and cultural tests are given in Tables I and II. No SN antibodies against BHV1 were detectable prior to vaccination on March 10. Subsequently, 20/62 were positive by April 10 and 29/62 by July 15. This is taken to indicate that about 1/3 of the calves responded to the vaccine since detectable serum neutralizing antibodies to BHV1 after intranasal exposure occur about

TABLE I. Results of Serological Tests on 62 Calves Before and During the Period in the Feedlot

	Number of Calves Positive[a]			
	Pre-transport	Commercial Feedlot		
Test[b]	March 9	April 10	July 15	Sept. 24
IBR-SN	0/62	20/62 (1/2-1/16)[c]	29/62 (1/4-1/64)	59/62 (1/4-≥1/128)
PI-3-HI	10/62 (1/100-1/200)	15/62 (1/100-1/200)	13/62 (1/100-1/3200)	7/62 (1/200-1/1600)
BVD-SN	0/62	3/62 (1/27-1/729)	61/61 (1/27-≥1/6561)	62/62 (1/81-≥1/6561)
P. haem.-CF	21/22 (trace-≥1/64)	48/48 (trace-≥1/64)	61/61 (trace-≥1/64)	48/48 (trace-≥1/64)
P. mult.-CF	8/22 (trace-1/16)	29/48 (trace-≥1/64)	49/61 (trace-≥1/64)	46/48 (trace-≥1/64)

[a]Minimum titers considered positive on various tests: IBR-SN, 1/4; PI-3-HI, 1/100; BVD-SN, 1/3
[b]Serological tests for viral antibodies: SN = serum neutralization; HI = hemagglutination inhibition
[c]Range of titers

[1]Covexin-8, Combined Clostridial Bacterin-Toxoid, Burroughs Wellcome Ltd., Kirkland, Quebec.
[2]Contravac, Connaught Laboratories Ltd., Willowdale, Ontario.
[3]BBL Microbiological Systems, Becton, Dickinson & Co. (Canada), Mississauga, Ontario.
[4]Institut Armand-Frappier, Box 100, Laval-des-Rapides, Québec H7N 4Z3.
[5]Gelman Electrophoresis Chamber, Gelman Instrument Company.

TABLE II. Microbiological Isolations from 62 Calves

	Nasal Swabs				Lung
	Pre-transport	Commercial Feedlot			Slaughter
	March 9	April 10	July 15	Sept 24	October 20
P. haemolytica	1	12	8	1	0
P. multocida	0	5	1	12	0
M. bovis	0	17	28	7	1[a]
M. bovirhinis	3	58	26	38	0
Ureaplasma	0	9	8	11	0
M. arginini	2	0	15	2	1[b]
A. laidlawii	0	0	23	3	0

[a,b]Isolated from morphologically normal lung of one animal

day 8 or later (8-11) and the calves were not mixed with those from the other ranch until April 3, 7 d before the March 10 sampling. The fact that 59/62 were positive on September 24, with titers as high as $\geqslant 1/128$, is suggestive of natural exposure during the feeding period despite a lack of clinical IBR during that time.

The number of animals with titers to PI-3 virus did not vary markedly over the study period (Table I). Titers first appeared concurrent with slight coughing during the fall of 1980, preceding shipment to the feedlot, but there was no evidence of any booster effect of vaccination or exposure during the monitoring period.

Lacrimation and conjunctivitis occurred in the original group of calves after those from the other ranch were mixed with them on April 3. This was followed by a marked increase in the number of animals showing titers to BVD virus, from 3/62 on April 10 to 61/61 on July 15 (Table I). Seventeen of these calves were thought by the feedlot attendants to be affected severely enough with conjunctivitis to require antibiotic treatment between April 15 and May 29. In addition, one animal was treated for footrot (infectious pododermatitis) and one for sexual riding during this time period. The only treatment in the 62 calves between May 29 and the time of slaughter was one animal for sexual riding.[6] Twenty of the 62 calves from the other ranch were sampled on July 15 and all had BVD titers, eight of which were $\geqslant 1/729$. However, only one of the latter calves was deemed sick enough to require antibiotic therapy by the attendants. It is of interest that the clinical disease coincided with marked changes in serum titers to BVD virus but not to BHV1.

The majority of the calves had CF titers to pasteurellae throughout the sampling period. Whether or not this related to the lack of pneumonia found at slaughter is a matter for speculation because it is as yet unknown whether or not naturally occurring serum antibodies are protective against pneumonic pasteurellosis.

Only one animal had detectable pasteurellae in its nasal passages prior to feedlot entry, but several isolations were made thereafter (Table II). The presence of these organisms is expected in calves (12-15), and even animals with negative nasal swabs may harbor pasteurellae in deeper nasal tissues (16).

The number of mycoplasma isolates from nasal swabs was also low before the calves entered the feedlot, but increases were noted for several species thereafter (Table II). *Mycoplasma bovirhinis*, *M. arginini* and *Acholeplasma laidlawii* are not thought to be pathogenic in the bovine respiratory tract (17), but the presence of *Ureaplasma* spp. and *M. bovis*, particularly the latter, was the main stimulus for comparison of microbiological and postmortem findings in this study. In a paper published recently, Springer *et al* (18) found no respiratory tract disease in calves from which *M. bovirhinis*, *M. arginini*, *A. laidlawii* and *M. bovis* were isolated from nasal swabs, and further stated that this was the first reported isolation of *M. arginini* and *M. bovis* from the upper respiratory tract of healthy calves.

All 62 animals survived the feeding period. At slaughter, 23 had no gross pulmonary lesions while 39 had minor lesions. The latter consisted of a single case of multifocal abscessation, two cases of fibrous pleural adhesions (both negative for pasteurellae, *Mycoplasma* spp. and *Haemophilus* spp.), several with one or more fibrous strands causing adherence of lobes to one another or to the parietal pleura, but the majority showed only areas of lobular to sublobular atelectasis. Lung parenchyma immediately dorsal to the cardiac notch was so frequently affected by mild atelectasis as to suggest that this area was predisposed to such a change either anatomically or due to processing at the packing plant.

Lung tissue that appeared grossly normal also lacked histological lesions. The areas described as atelectatic on gross inspection consisted of collapsed alveoli only, with no accompanying inflammatory changes or occlusive lesions of the related airways. Aggregates of lymphoid tissue were present in most sections in association with bronchi and some bronchioles. The amount of this lymphoid tissue was variable but in general the nodules were larger when associated with larger airways. The one animal from which *M. bovis* and *M. arginini* were isolated from lung tissue did not have lymphoid tissue in excess of that seen in other calves. In general, the several species of mycoplasma isolated from many of the calves over the feeding period were not related to any significant pulmonary lesions.

These data suggest that neither

[6]Treatments, all diagnosed and administered by feedlot personnel, consisted of: 1) for conjunctivitis, 2 cc, Derapen (Ayerst Laboratories, Montreal, Quebec) + 1 cc Azium (Schering Canada Inc., Pointe Claire, Quebec) subconjunctivally plus 20 cc Derapen intramuscularly (IM), 2) for footrot, 20 cc Derapen IM and 3) for sexual riding, 20 cc Derapen IM.

the isolation of various bacteria (including *Mycoplasma* spp.) from nasal swabs, nor the detection of serum antibodies to respiratory viruses, provide *ipso facto* evidence of internal lesions. Furthermore, they raise the question of whether such testing is even relevant to calf status and selection, at least from the standpoint of whether the calves have a background of preexisting pulmonary lesions or are fully susceptible to experimental exposures. Such tests do, however, provide information on vaccine responses and on the natural antigens to which cattle are exposed, and as such may provide data useful for feedlot management decisions.

These results do not, of course, resolve the important question of whether the *Mycoplasma* spp. isolated are capable of causing pneumonia. *Mycoplasma bovis* was present within one lung which was free of lesions and several *Mycoplasma* spp. were present in the nasal passages without any associated pneumonia. However, this was also true of *P. haemolytica* and *P. multocida*, which are known pulmonary pathogens in cattle. The question of whether the presence of some *Mycoplasma* spp. can adversely influence host defenses against other pathogens without causing any morphologically evident damage themselves is beyond the scope of this report, but there was no indication that such an effect was operative.

Experimental studies involving cattle are usually limited to small numbers of animals for economic reasons. This has led to the justifiable assumption that the immunological and microbiological status of the animals used is vitally important. The results reported here, however, suggest that traditional tests probably provide little *a priori* information on the presence or absence of pulmonary lesions, although they may provide information on immune status which should be considered in light of any proposed experimental exposures. Until improved methods are available it would seem appropriate to document carefully the microorganisms and antibodies present during a study, so that these data are on hand for future comparison and comment.

This study also demonstrates that many of the factors associated with bovine respiratory disease (19) can be present (shipping, mixing of groups, vaccinating, exposure to various viruses and bacteria) without causing a pneumonia problem. Such an observation points to the present inadequacies in our understanding of the pathogenesis of the bovine respiratory disease complex.

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