The Possible Role of Stress in the Induction of Pneumonic Pasteurellosis

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ABSTRACT

Five groups of range bred calves (four calves per group) were used to investigate the effect of stress on susceptibility to aerosol exposures with bovine herpesvirus-1 or *Pasteurella haemolytica*. Twelve calves were weaned, transported, processed at a commercial feedlot and transported to isolation facilities three days later. An aerosol challenge of either 10^{10} colony forming units of *P. haemolytica* or $10^{7.5}$ plaque forming units of bovine herpesvirus-1 virus was given to two groups of calves and the third group was not challenged. The fourth group was transported directly to the isolation facilities after weaning and aerosol challenged with *P. haemolytica*. The fifth group remained at the farm after weaning and was not challenged. All transported animals had elevated plasma cortisol levels which remained above normal for at least three days postchallenge. The blastogenic response of all calves was depressed after leaving the farm and remained depressed throughout the experiment. The suppression correlated well with elevated serum cortisol levels. Calves processed through the feedlot encountered bovine herpesvirus-1 because eight out of 12 animals seroconverted to this antigen. Most calves seroconverted to *P. haemolytica* whether they were experimentally challenged or not. Where the unchallenged calves encountered *P. haemolytica* is unknown. Calves challenged with bovine herpesvirus-1 but not with *P. haemolytica*, had significant clinical signs of pneumonia and two animals died due to bovine herpesvirus-1 infection. Transportation and handling under the present circumstances was not sufficient to make calves susceptible to *P. haemolytica*, but did make calves more susceptible to bovine herpesvirus-1 virus.

Key words: Stress, pneumonic pasteurellosis, bovine herpesvirus-1, *Pasteurella haemolytica*.

RÉSUMÉ

Cette expérience visait à déterminer l'effet du stress sur la susceptibilité des bovins à l'endroit d'aérosols de l'herpèsvirus bovin du type 1 ou de *Pasteurella haemolytica*. Les auteurs utilisèrent à cette fin cinq groupes de veaux de ranch qui en comptaient chacun quatre. Douze veaux furent sevrés et transportés à un parc d'engraissement où on les entassa dans un couloir, avant de les marquer à chaud, de leur administrer une bactérine contre les maladies à *Clostridium* spp. et de les mélanger à d'autres veaux; trois jours plus tard, on les transporta dans des unités d'isolation. Huit de ces veaux subirent une infection de défi au moyen d'aérosols qui contenaient 10^{10} unités formatrices de colonies de *P. haemolytica* ou $10^{7.5}$ unités formatrices de plages de l'herpèsvirus bovin du type 1; les quatre autres servirent de témoins. Aussitôt après le sevrage, on transporta directement aux unités d'isolement les veaux du quatrième groupe et on les soumit à une infection de défi, au moyen d'aérosols de *P. haemolytica*. Ceux du cinquième groupe restèrent sur la ferme et ne subirent pas d'infection de défi. Tous les veaux transportés affichèrent une élévation de leur cortisol plasmatique qui persista pour au moins trois jours après l'infection de défi. La réponse blastogénique des veaux qu'on transporta de la ferme manifesta une baisse qui dura jusqu'à la fin de l'expérience et s'accompagna d'un taux sérique élevé de cortisol. Les 12 veaux transportés au parc d'engraissement croisèrent l'herpèsvirus bovin du type 1 quelque part, puisque huit d'entre eux développèrent des anticorps à son endroit. La plupart des veaux développèrent des anticorps contre *P. haemolytica*, qu'ils aient ou non subi l'infection de défi. On ignore où les veaux témoins croisèrent *P. haemolytica*. Les veaux soumis à l'infection de défi avec le virus précité, mais non avec *P. haemolytica*, manifestèrent des signes cliniques de pneumonie et en moururent. Le transport et la manipulation impliqués dans l'expérience ne suffirent pas à rendre les veaux susceptibles à *P. haemolytica*, mais il les rendirent plus susceptibles à l'herpèsvirus bovin du type 1.

Mots clés: stress, pasteurellose pulmonaire, herpèsvirus bovin du type 1, *Pasteurella haemolytica*.

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INTRODUCTION

Stress has been associated with increased susceptibility to bacterial pneumonia in both humans and animals (1,2). The exact mechanisms whereby increased susceptibility occurs have not been fully investigated, but it has been suggested that stress may increase the release of adrenocorticotropic hormone (ACTH) which in turn stimulates the synthesis and secretion of cortisol (1). Increased cortisol levels may then alter immune functions and increase susceptibility to bacterial pneumonia (1,2). Stressful conditions which contribute to increased cortisol levels in cattle include weaning (3,4), transportation (3-6), handling (3), castration and dehorning (6), parturition (7,8), forced exercise (9), endotoxin mastitis (10), neonatal diarrhea (11-13) and ambient temperature fluctuations (14).

An association between stress and pneumonic pasteurellosis in cattle has been postulated. Hoerlein and Marsh (15) proposed that stress reduced calf resistance sufficiently to allow infection with viruses, bacteria or both. Although many viruses and bacteria may be associated with bovine respiratory disease and pneumonic pasteurellosis (16), *Pasteurella haemolytica* and *Pasteurella multocida* was isolated from the lungs in 63% of feedlot cattle suffering from this disease (17). This suggests that *P. haemolytica* is the major bacterium involved in this disease. Further support for the role of *P. haemolytica* in pneumonic pasteurellosis is the ability to reproduce the disease experimentally by aerosol challenge with bovine herpesvirus-1 (BHV-1) or parainfluenza-3 (PI-3) and *P. haemolytica* (18,19). The observation that the disease can be reproduced experimentally by the synergistic interaction between viruses and bacteria suggests that the virus may alter the clearance mechanisms in the lungs sufficiently to allow the bacteria to colonize and induce pneumonia (20).

The present study was undertaken to determine whether weaning, transportation and handling increased cortisol concentration in serum, depressed blastogenic response to phytohaemaglutinin (PHA) and worsened clinical signs of pneumonia after challenge exposure to BHV-1 or *P. haemolytica*.

MATERIALS AND METHODS

CALVES, STRESS AND CHALLENGE

The experimental protocol is outlined in Table I. Crossbred Hereford calves (six to eight months old) (n = 20), male or female, from a ranch in southwestern Saskatchewan were weaned and bled. Nasal swabs for isolation of bacteria and viruses were taken also from all animals. Twelve calves (groups 1-3) were transported 420 km to a commercial feedlot. Upon arrival, they were processed as were other calves entering the feedlot. That is, there were unloaded, crowded in the chute, branded and vaccinated with a clostridial bacterin and penned with other calves. No viral vaccines were given. Samples of blood (10 cc clotted, 20 cc citrated) and nasal secretions were taken. Three days later (day 0), they were transported 92 km to the isolation facilities and randomly placed into three groups (1-3): groups 2 and 3 were challenged with an aerosol of $10^{7.5}$ plaque forming units (PFU) of BHV-1 virus or 10^{10} colony forming units (CFU) of *P. haemolytica* respectively, whereas group 1 was not challenged. The calves in these groups are designated throughout the manuscript as "processed".

Calves in groups 4 and 5 were weaned at the same time as groups 1-3. Group 4 was transported 470 km to the isolation facilities and challenged with 10^{10} CFU *P. haemolytica* immediately after arrival. Group 5 remained at the farm. Blood samples were taken from calves in group 4 as indicated in the figures and tables. Calves in group 5 were bled on days -3, 0 and 4. These two groups are designated as "nonprocessed".

WEATHER

During the experiment, ambient outdoor temperatures were below 0° C at night and freezing rain and snow fell.

CLINICAL INVESTIGATION

The degree of respiratory disease in each calf was assigned a numerical value using a method modified from Thomas *et al* (21). The thirteen parameters evaluated and the method of calculation are described in Table II. Clinical assessment of each calf in the isolation rooms was done at approximately the same time each morning on days 2, 3, 4, 5 and 7 after bacterial or viral challenge.

A calf was deemed to be apathetic if it was slow to rise or move during examination. If a calf did not approach the feed bin within ten minutes after feed was given, it was judged to be anorexic. Coughing was recorded as absent or present after palpating the larynx. Temperature was measured by a rectal probe using an electronic digital thermometer (GLA, Agricultural Electronics Div., Montclair, California). The value in the numerator of the "body temperature" parameter (39.3° C) is the arithmetic mean plus two standard deviations of the body temperatures of all calves before stress, and the value of the denominator (0.11° C) is one standard deviation. The lungs of each calf were scored after auscultating three locations on each side of the thorax. Vesicular sounds (normal or completely absent indicating consolidation), rales, and rasping were evaluated and a score from 0 (normal) to 5 (extremely abnormal) was assigned. The daily clinical index is the sum of

TABLE I. Experimental Protocol for the Effect of Stress in the Induction of Pneumonic Pasteurellosis

	Location			
Group	Ranch day -3	Feedlot day -3, 0	Isolation Rooms day 0	Challenge
Processed				
1	+[a]	+	+	none
2	+	+	+	BHV-1
3	+	+	+	*P. haem*
Nonprocessed				
4	+	–	+	*P. haem*
5	+	–	–	none

[a]Samples taken or drawn

TABLE II. Method of Calculation of Daily Clinical Index of Experimental Calves

Variable	Range of Scale or Units	Weight
Apathy	0,1	x 100
Anorexia	0,1	x 100
Nasal discharge	0-3	x 10
Occular discharge	0-3	x 10
Adenitis	0-3	x 10
Conjunctivitis	0-3	x 10
Dyspnea	0,1	x 10
Wasting	0,1	x 10
Cough	0,1	x 10
Respiratory rate	respirations/min	x 1
Temperature	°C - 39.3°C / 0.11°C	x 1
Lung Score	0-10	x 25
Clinical index	0 to 700	(Normal = 20)

these 13 weighted scores. The date of death or euthanasia *in extremis* was recorded.

BACTERIOLOGICAL CULTURE OF NASAL SWABS

Sterile cotton-tipped swabs (Culturette, Marion Scientific Corp., Kansas City, Missouri) were introduced 2 cm into the nasal cavity of calves, and replaced in their plastic container. The ampule of transport medium was crushed and the unit was taken to the laboratory for processing. Longer, protected swabs (Teigland swabs (modified), Haver-Lockhart, Shawnee, Kansas) were passed through the nasal cavity (20 cm) and the pharynx was touched with the swab. These swabs were taken to the laboratory without transport medium.

In the laboratory, the swabs were plated on 5% sheep blood agar and incubated at 37° C for 24 h for a qualitative assessment of bacterial growth. Hemolytic reactions were noted. Gray colonies (2-5 mm diameter) were tested on conventional media for the following biochemical reactions: indole, urease, oxidase, and catalase production; nitrate utilization; motility; and change in triple sugar iron (TSI) medium. Isolated producing positive indole, oxidase and catalase reactions and producing acid over neutral reaction in TSI were identified as *P. multocida*. Those producing oxidase, catalase, hemolysis, and acid over neutral TSI reactions were identified as *P. hemolytica* (22).

CORTICOSTEROID LEVELS

Plasma corticosteroid concentrations were measured by a competitive radioimmunoassay employing the Amerlex cortisol kit (Amersham, Oakville, Ontario). Cortisol levels were measured at various times during the experiment. Before shipping, all processed and nonprocessed calves were bled at the ranch. Blood samples were taken when calves arrived at the feedlot, before and after shipment to the isolation rooms, and three days after arriving at the isolation rooms. Except for samples corresponding to the time of arrival at the feedlot, corresponding samples were taken from calves left at the farm.

MEASUREMENT OF THE BLASTOGENIC AND ANTIBODY RESPONSES

The mitogenic, blastogenic and antibody responses were performed as described by Filion *et al* (23). The blastogenic response to the mitogen PHA was assayed on the days indicated in Fig. 1. The antibody response was measured on days 0, 4, 21 and 33.

STATISTICAL ANALYSIS

The blastogenic response data were analysed for statistical significance using two-tailed Student t test. The clinical index scores were evaluated for statistical significance using ranks in one-criterion variance analysis (24).

RESULTS

CORTICOSTEROID LEVELS AND ANTIBODY RESPONSES

Corticosteroid levels were elevated at the time calves arrived at the feedlot (Tables III and IV). These levels were maintained for at least three days after arrival at the isolation rooms. The elevation of cortisol was not detected in nonprocessed calves, and these calves had greater variation in cortisol levels making their higher values statistically nonsignificant. Furthermore, weaning did not significantly increase cortisol levels, at least not at the time of sampling (Table IV, day -3 and 0).

Calves were seronegative for BHV-1 and *P. haemolytica* on day 0 with one exception, which was seropositive for BHV-1 (Tables V and VI). Five out of six calves in groups 1 and 3 processed through the feedlot and not challenged with BHV-1, seroconverted to this agent by day 33. Nonprocessed calves did not seroconvert to BHV-1. An anti-*P. haemolytica* antibody response was detected eventually in all processed and nonprocessed calves after assembly and weaning.

EFFECT OF STRESS ON BLASTOGENIC RESPONSE

Calves transported to the feedlot had a lower but not significantly different blastogenic response upon arrival at the feedlot as compared to the response detected prior to leaving the ranch (Fig. 1). The standard deviation of the mitogen induced blastogenic response declined as the experiment continued. The blastogenic response

TABLE III. Plasma Cortisol Levels (μg/100 mL) of Processed Calves

Days (Location)	Challenge Group[a] (x ± SD)[b] 1	2	3
-3 (Leaving ranch)	0.62 ± 0.21	0.40 ± 0.14	0.70 ± 0.032
-3 (Arriving feedlot)	3.17 ± 1.50[c]	2.30 ± 0.37[c]	3.45 ± 1.75[c]
0 (Leaving feedlot)	1.47 ± 0.43[c]	1.98 ± 0.89[c]	2.60 ± 1.25[c]
0 (Arriving at isolation rooms)	1.53 ± 0.50[c]	1.88 ± 0.76[c]	2.00 ± 0.45[c]
3 (3 days after challenge)	0.73 ± 0.73	0.70 ± 0.77	0.50 ± 0.56

[a]For each group n = 4
[b]Mean ± SD
[c]Greater than sample leaving ranch ($p < 0.02$)

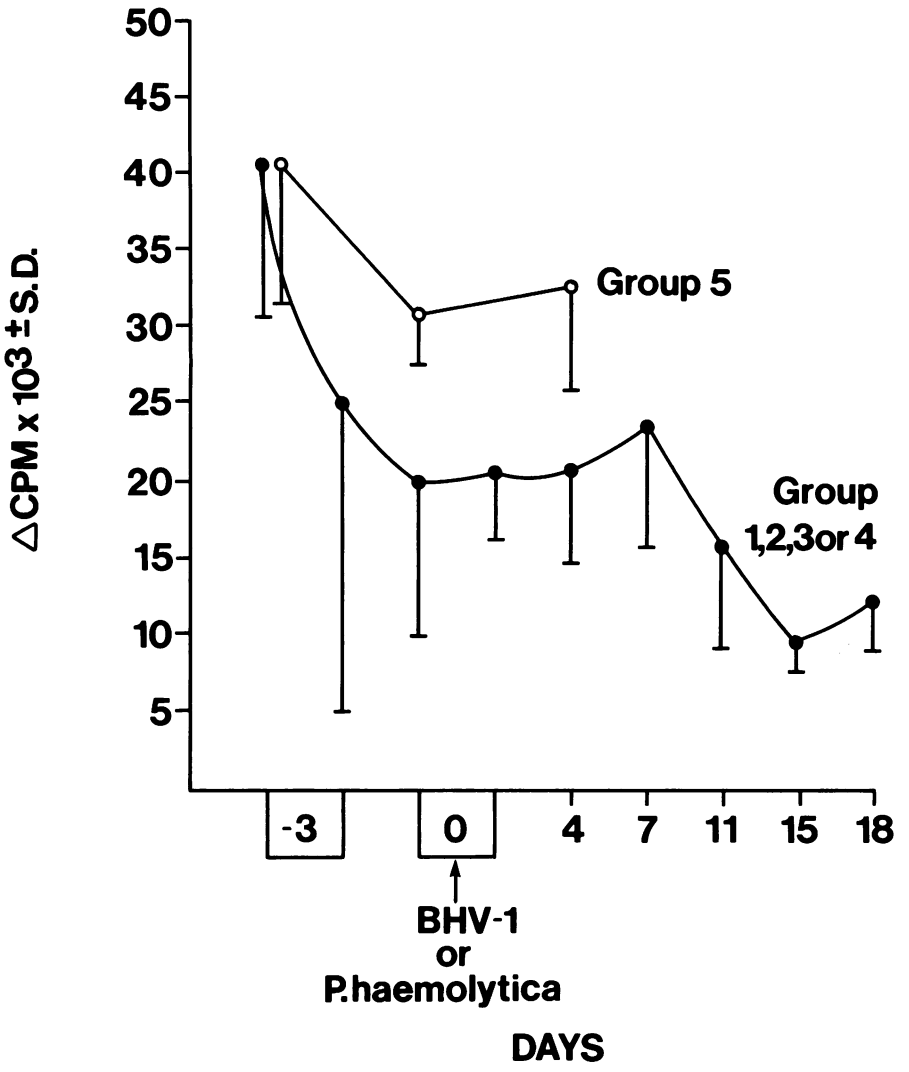

Fig. 1. The mitogenic blastogenic response of stressed and nonstressed calves. The PHA blastogenic response (CPM) was monitored on the days indicated in the figure. The response of groups 1-4 ( ) was not significantly different from each other and are reported as one point. The second blastogenic response on day -3 (blood taken at the feedlot) was not significantly lower than the response of the first bleed taken on this day (blood taken at the ranch). A significant drop ($P < 0.01$) in the blastogenic response was measured from day 0 if this response is compared to the first bleeding day -3 (blood taken at the ranch). Group 5 PHA responses ( ) were measured also and are not significantly different from each other.

TABLE IV. Plasma Cortisol Levels (μg/100 mL) of Nonprocessed Calves

Days (Location)	Challenge Group[a] (x ± SD)[b] 4	5
-3 (Ranch)	0.90 ± 0.20	1.15 ± 0.26
0 (Leaving ranch)	1.70 ± 0.76	1.20 ± 0.66
0 (Arriving isolation rooms)	2.70 ± 1.70	NS[c]
3 (3 days after arrival in isolation rooms)	2.10 ± 2.30	1.45 ± 1.12

[a]For each group n = 4
[b]Mean ± SD
[c]Not sampled

TABLE V. Seroconversion to *P. haemolytica* from Processed and Nonprocessed[a] Calves

Group	Days 0	4	21	33
1	0/4[b]	1/4	2/4	4/4
2	0/4	0/4	0/2	2/2
3	0/4	0/4	3/4	4/4
4	0/4	1/4	1/4	4/4
5	0/4	1/4	NS[c]	NS

[a]Processed calves were passed through the feedlot, whereas nonprocessed calves were not
[b]Number of calves with ELISA titers greater than 400/calves tested. ELISA titers ranged from 400 to 500 on day 4, 500 to 12,500 on day 21 and 500 to 25,600 on day 33
[c]No sample

TABLE VI. Antibody Titer to BHV-1 Virus of Processed and Nonprocessed[a] Calves

Group	Days 0	33
1	0/4[b]	3/4
2	0/4	2/2[c]
3	0/4	1/4
4	0/4	0/4
5	1/4	NS[d]

[a]Processed calves were passed through the feedlot whereas nonprocessed calves were not
[b]Number of calves with titer
[c]Two animals died prior to the date of bleeding
[d]NS = No sample

of the processed calves was depressed, beginning on day 0 when blood was taken at the feedlot and continuing to the end of the experiment. However, the blastogenic response of calves which remained at the farm was unchanged. All groups of calves which were transported to the isolation facilities had a depressed blastogenic response upon arrival.

CLINICAL SIGNS OF DISEASE

Signs of disease were quantified by the clinical index described in Table II. On days 2, 3, 4 and 5 no significant differences in the clinical indices between groups 1, 3 and 4 were observed, however the clinical index of calves from group 2 (BHV-1 exposed), was significantly higher ($p < 0.05$) than the clinical indices of all other groups (Fig. 2). On day 7, no difference in clinical index ($p > 0.05$) was observed.

MICROBIOLOGY

On the first day of the experiment, day -3, bacteria of various species were isolated from nasal swabs of all processed and nonprocessed calves; however, *P. haemolytica* and *P. multocida* were isolated from only two of the four nonprocessed calves which did not leave the farm (Table VII). After transportation from the feedlot to the isolation rooms, *P. haemolytica* or *P. multocida* was isolated from all processed calves. During the remaining time of the experiment *P. haemolytica* was isolated from three out of eight animals which were not challenged with the bacterium. *Pasteurella multocida* was isolated from ten out of 12 processed calves. Bovine herpesvirus-1 virus was isolated only from animals given this virus.

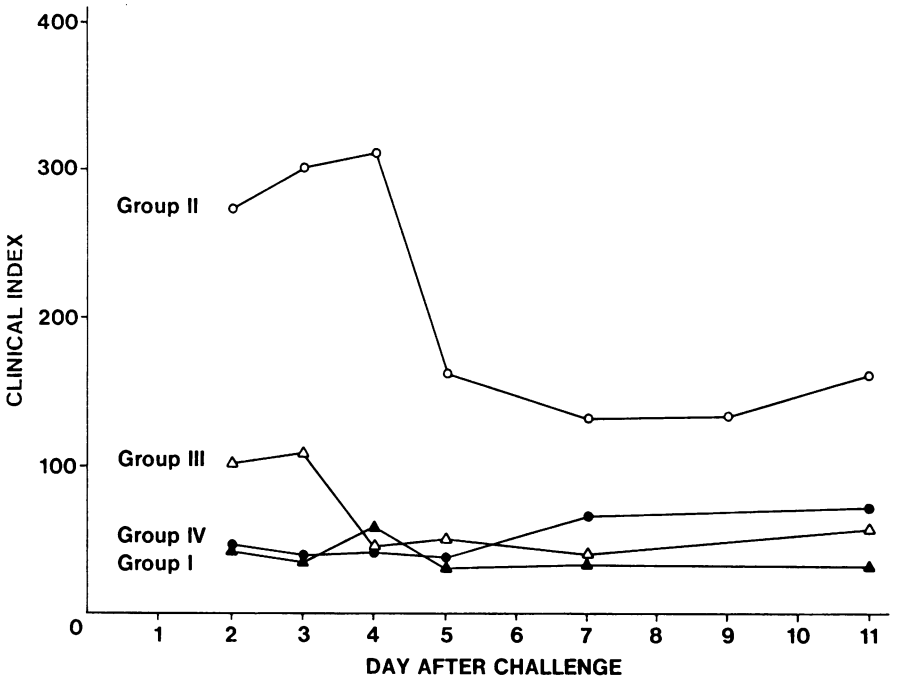

Fig. 2. Summary of clinical index of calves. Clinical signs were recorded and tabulated using the scale or units outlined in Table II. Groups are described in Materials and Methods. Calves were exposed to an aerosol of BHV-1 or *P. haemolytica* on day 0.

TABLE VII. Isolation of Pasteurellae from Processed and Nonprocessed[a] Calves

	Location			
	Ranch	Feedlot	Isolation Rooms	
Group	Pasteurellae	Pasteurellae	*P. haem*	*P. mult*
1	0/4[b]	0/4	1/4	3/4
2	0/4	0/4	4/4	2/4
3	0/4	0/4	2/4	4/4
4	0/4	ND[c]	4/4	1/4
5	2/4[d]	ND	ND	ND

[a]Processed calves were passed through the feedlot and nonprocessed calves were not
[b]Number of calves from which Pasteurellae were isolated from nares/calves sampled employing short swabs. No *Pasteurella* species were isolated from Tiegland swabs
[c]ND = not done
[d]Both calves had *P. haemolytica* isolated from their nares

PATHOLOGY

Numerous pinpoint necrotic foci in the pharynx and the large bronchi, and scattered areas of atelectasis in the anterior part of the lungs were grossly observed in the calf that died five days postinfection (group 2). Microscopic studies revealed focal caseous necrosis of the epithelium in the major airways. The lung lesions consisted of acute necrotizing bronchiolitis secondarily affecting the surrounding tissue, causing edema, congestion, atelectasis and occasionally hemorrhage and parenchymal necrosis. The lesions were given a score of 15 out of 35 using a scoring system reported previously (25). Bovine herpesvirus-1 and *P. multocida* were isolated from the lung tissue of this calf.

Severe pseudomembraneous, necrotic lesions in the laryngeal mucosa were observed in the calf which was euthanized *in extremis* 11 days after infection with BHV-1 (group 2). Edema and atelectasis affecting more than one third of the apical and cardiac lobes and scattered areas of the diaphragmatic lobes were also observed grossly. The microscopic features of the affected areas were purulent bronchopneumonia with prominent interlobular edema. Necrotizing bronchiolitis appeared scattered in one lobe and there were numerous foci of coagulation necrosis. The lung lesions were given a total score of eight. Bovine herpesvirus-1 was not isolated from the lung of this calf.

DISCUSSION

Calves stressed under field conditions were not susceptible to bacterial infection by aerosol challenge with *P. haemolytica*. However, stressed calves challenged with an aerosol of BHV-1 virus became sick and two out of four either died or were euthanized *in extremis*. The pathological lesions in both animals were attributed to BHV-1 infection. Fibrin observed grossly and necrotising bronchiolitis are characteristics of bacterial pneumonia in calves, and because fibrin was absent in those two cases, bacterial infection did not cause these lesions (26). The other two processed calves challenged with BHV-1 remained lethargic even though they began to eat six days postinfection. The fact that two out of four calves died from BHV-1 alone suggests that stressed calves were more susceptible to BHV-1 infection than nonstressed calves since nonstressed calves very rarely die from BHV-1 infections alone (18,23). Clinical signs usually abate within six days, and virus shedding stops by day 10 postviral challenge (27).

It may be argued that virulence, dose of virus or genetics of the animals used in the present experiment rather than stress due to handling and transportation was responsible for the increased lung damage and subsequent death of the BHV-1 infected animals. Although the virus used in the present experiment is a virulent isolate, the same preparation has been used repeatedly at the same dose for many experiments designed to study the synergistic interactions between BHV-1 and *Pasteurella haemolytica* (23) and none of the virus exposed, nonstressed calves succumbed to the viral infection. Furthermore, these calves were from the same herd as those used in previous experiments when none of the calves succumbed to the virus if they were not stressed or exposed to *Pasteurella* (23). Thus we propose that if calves are sufficiently stressed, as can occur by weaning,

transportation and handling in feedlots, they may die of BHV-1 without significant bacterial complications.

The mechanism whereby transportation increased animal susceptibility to BHV-1 virus infections is not fully understood. However, handling did elevate cortisol levels and suppressed the immune response for an extended period of time (Fig. 1). It is known that corticosteriods can alter herpesvirus replication (28) and allow reactivation of latent BHV-1 virus infection (29). Thus, it is proposed that the corticosteroid may have a twofold effect. First, it can enhance virus replication in individual cells, thereby increasing the virus dosage. Second, due to reduced efficiency of the immune response, the animal cannot cope with the virus infection, therefore sufficient damage occurs to kill the animal.

If stressed, virus-infected animals are exposed to *P. haemolytica* or other bacteria, the reduced mucociliary clearance caused by some viruses (30) combined with reduced neutrophil, monocyte and macrophage functions induced either by the glucocorticoids alone (2, 29-34) or in combination with the virus (23) could allow the bacteria to colonize the lower lung and lead to fibrinous pneumonia as observed under field conditions. The degree of suppression depends upon the intensity of the stressors (35) and influences the severity of the pneumonia.

Transportation and handling alone did not appear to affect resistance to *P. haemolytica* in the absence of a viral infection. Perhaps in the absence of viral infections, the mucociliary clearance mechanisms are capable of preventing large quantities of bacteria from entering the lung. If large quantities of bacteria are introduced into the lung of normal animals by intratracheal installation (36) or by intrathoracic injection directly into the lung (37) pneumonia does occur. Thus the final bacterial load which enters the lung appears to be critical for the induction of pneumonic pasteurellosis (20,38). In our experiment, stress itself does not appear to allow sufficient bacteria to enter the lung of the animal even after aerosol challenge with the bacteria. Those bacteria that do presumably get into the lung are cleared by the local defense mechanisms even though they may be partially suppressed.

In conclusion, the pathogenesis of pneumonic pasteurellosis may involve at least three components. First, stress makes the calves susceptible to infection by inducing a state of immunological unresponsiveness through the elevation of cortisol levels. Second, a viral infection may further immunosuppress and/or create lung lesions to make the animal susceptible to superinfection by bacteria. Third, the bacteria overwhelm the weakened specific and nonspecific immunological defense mechanisms of the lung and establish a pulmonary infection. Perhaps all three components are not required to induce pneumonic pasteurellosis. However, the experimental induction usually requires aerosol challenge with both virus and *P. haemolytica* unless the bacteria are injected directly into the trachea or lung. These two latter techniques used for the induction of disease may not accurately reflect what is actually seen in the field situation.

Further studies are needed to elucidate the mechanism(s) by which stress predisposes calves to viral infection, to determine whether the LD_{50} of BHV-1 or the combination of BHV-1 and *P. haemolytica* is lower in stressed calves, as well as to determine the relationship between stress and susceptibility to viral-bacterial synergistic interactions.

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REFERENCES

1. **STEPHENS DB.** Stress and its measurement in domestic animals: a review of behavioral and physiological studies under field and laboratory situations. Adv Vet Sci Comp Med 1980; 24: 179-210.
2. **ROTH JA, KAEBERLE ML.** Effect of glucocorticoids on the bovine immune system. J Am Vet Med Assoc 1982; 180: 894-901.
3. **GWAZDAUSKAS FC, GROSS WB, BIBB TL, McGILLIARD ML.** Antibody titers and plasma glucocorticoid concentrations near weaning in steer and heifer calves. Can Vet J 1978; 19: 150-154.
4. **CROOKSANK HR, ELISSALDE MH, WHITE RG, CLANTON DC, SMALLEY HE.** Effect of transportation and handling of calves upon blood serum composition. J Anim Sci 1977; 48: 430-435.
5. **SHAW KE, NICHOLS RE.** Plasma 17-hydroxycorticosteroids in calves: the effect of shipping. Am J Vet Res 1964; 25: 252-253.
6. **JOHNSTON JD, BUCKLAND RB.** Response of male Holstein calves from seven sires to four management stresses as measured by plasma corticoid levels. Can J Anim Sci 1976; 56: 727-732.
7. **HUDSON S, MULLFORD M, WHITTLESTONE WG.** Bovine plasma corticoids during parturition. J Dairy Sci 1975; 59: 744-746.
8. **GUIDRY AJ, PAAPE MJ, PEARSON RE.** Effects of parturition and lactation on blood and milk cell concentrations, corticosteroids, and neutrophil phagocytosis in the cow. Am J Vet Res 1976; 37: 1195-1200.
9. **ARAVE CW, WALTERS JL, LAMB RC.** Effect of exercise on glucocorticoids and other cellular components of blood. J Dairy Sci 1978; 61: 1567-1572.
10. **PAAPE MJ, SCHULTZE WD, DESJARDINS C.** Plasma corticosteroid, circulating leukocyte and milk somatic cell responses to *Escherichia coli* endotoxin-induced mastitis. Proc Soc Exp Biol Med 1974; 145: 553-559.
11. **DVORAK M.** Plasma 17-hydroxycorticosteroid levels in healthy and diarrheic calves. Br Vet J 1971; 127: 372-377.
12. **MASSAP A.** Haematocrit, biochemical and plasma cortisol changes associated with diarrhea in the calf. Br Vet J 1979; 135: 600-605.
13. **CABELLO G.** Plasma cortisol and aldosterone levels in healthy and diarrheic calves. Br Vet J 1980; 136: 160-167.
14. **KELLEY KW, RANDALL E, GREENFIELD BS, EVERMANN JF, PARISH SM, PERRYMAN LE.** Delayed-type hypersensitivity, contact sensitivity and phytohemagglutinin skin test response of heat and cold stressed calves. Am J Vet Res 1982; 43: 775-779.
15. **HOERLEIN AB, MARSH GL.** Studies on the epizootiology of shipping fever in calves. J Am Vet Med Assoc 1957; 131: 123-127.
16. **JENSEN R, PIERSON RE, BRADDY PM, SAARI DA, LAUERMAN LH, ENGLAND JJ, KEYVANFER H, COLLIER JR, HORTON DP, McCHESNEY AE, BENITEZ A, CHRISTIE RM.** Shipping fever pneumonia in yearling feedlot cattle. J Am Vet Med Assoc 1976; 169: 500-506.
17. **ROTHWELL BW, MILLS JH, DOIGE CE.** Necropsies on feedlot cattle. First Western Canadian Veterinary Conference, Saskatoon, Saskatchewan, 1979.

18. **JERICHO KWF, LANGFORD EV.** Pneumonia in calves produced by aerosols of bovine herpesvirus-1 and *Pasteurella haemolytica*. Can J Comp Med 1978; 42: 269-277.
19. **JERICHO KWF, DARCEL CleQ, LANGFORD EV.** Respiratory disease in cattle produced by aerosols of parainfluenza-3 virus and *Pasteurella haemolytica*. Can J Comp Med 1982; 46: 293-301.
20. **YATES WDG, JERICHO KWF, DOIGE CE.** Effect of bacterial dose on pneumonia induced by aerosol exposure of calves to bovine herpesvirus-1 and *Pasteurella haemolytica*. Am J Vet Res 1983; 44: 238-243.
21. **THOMAS LH, STOTT EJ, COLLINS AP, JEBBETT NJ, STARK AJ.** Evaluation of respiratory disease in calves: comparison of disease response to different viruses. Res Vet Sci 1977; 23: 157-164.
22. **KILIAN M, EREDERIKSEN W, BIBERSTEIN EL** (eds). Haemophilus, Pasteurella and Actinobaccillus. New York: Academic Press, 1981.
23. **FILION LG, McGUIRE RL, BABIUK LA.** The nonspecific effect of BHV-1 on bovine leukocyte functions. Infect Immun 1983; 42: 106-112.
24. **KRUSKAL WH, WALLIS WA.** Use of ranks in one-criterion variance analysis. J Am Stat Assoc 1952; 47: 583-621.
25. **THOMSON RG, CHANDER GS, SAVAN M, FOX ML.** Investigations of factors of probable significance in the pathogenesis of pneumonic pasteurellosis of cattle. Can J Comp Med 1975; 39: 194-207.
26. **THOMSON RG.** The pathogenesis and lesions of pneumonia in cattle. Comp Cont Educ Pract Vet 1981; 3: 5403-5411.
27. **JERICHO KWF, DARCEL CleQ.** Response of the respiratory tract of calves kept at controlled climatic conditions to bovine herpesvirus 1 in aerosol. Can J Comp Med 1978; 42: 156-167.
28. **PASTORET PP, JETTEUR P, AGUILARSETIEN A, LEROY P, SCHWERS A, GODART M, SCHOENAERS F.** Effet de la dexamethasone sur le titre et la taille des plaques du virus de la rhinotrachéite infectieuse bovine. Ann Med Vet 1979; 123: 397-402.
29. **SHEFFEY BE, DAVIES DH.** Reactivation of a bovine herpesvirus after corticosteroid treatment. Proc Soc Exp Biol Med 1972; 140: 974-976.
30. **DEGRE M, SOLBERG LA.** Synergistic effect in viral-bacterial infection 3: Histopathological changes in the trachea of mice following viral and bacterial infection. Acta Pathol Microbiol Scand 1971 [B]; 79: 137-141.
31. **NUGENT KM, PESANTI EL.** Chronic glucocorticosteroid therapy impairs staphylococcal clearance from murine lungs. Infect Immun 1982; 38: 1033-1036.
32. **LANDI MS, KREIDER JW, LANG CM, BULLOCK LP.** Effects of shipping on the immune function of mice. Am J Vet Res 1982; 43: 1654-1657.
33. **ROTH JA, KAEBERLE ML, HSU WH.** Effects of ACTH administration on bovine polymorphonuclear leukocyte function and lymphocyte blastogenesis. Am J Vet Res 1982; 43: 412-416.
34. **ROTH JA, KAEBERLE ML.** Effects of *in vivo* dexamethasone administration on *in vitro* bovine polymorophonuclear leukocyte function. Infect Immun 1981; 33: 434-441.
35. **KELLER SE, WEISS JM, SCHLEIFER SJ, MILLER NE, STEIN M.** Suppression of immunity by stress: effect of a graded series of stressors on lymphocyte stimulation in the rat. Sci 1981; 213: 1397-1400.
36. **FRIEND SCE, WILKIE BN, THOMSON RG, BARNUM DA.** Bovine pneumonic pasteurellosis: experimental induction in vaccinated and nonvaccinated calves. Can J Comp Med 1977; 41: 77-83.
37. **NEWMAN PR, CORSTVET RE, PANCIERA PJ.** Distribution of *Pasteurella haemolytica* and *Pasteurella multocida* in the bovine lung following vaccination and challenge exposure as an indicator of lung resistance. Am J Vet Res 1982; 43: 417-422.
38. **YATES WDG.** A review of infectious bovine rhinotracheitis, shipping fever pneumonia and viral-bacterial synergism in respiratory disease of cattle. Can J Comp Med 1982; 46: 225-263.