Pneumonia in Calves Produced with Aerosols of Bovine Herpesvirus 1 and Pasteurella haemolytica

K. W. F. Jericho and E. V. Langford*

ABSTRACT

In each of 11 experiments, four calves were exposed first to an aerosol of bovine herpesvirus 1 (BHV1, virus of infectious bovine rhinotracheitis) and second to an aerosol of Pasteurella haemolytica. The interval between aerosols was three to five days. In two other experiments, calves were exposed only to a bacterial aerosol. Climate was controlled for all experiments from the day of viral exposure and for eight of the experiments it was also controlled for four to six days before the first aerosol. The concentration of infectious doses of virus in the aerosols and the number of bacteria in the aerosols of each calf were determined. Macroscopically recognizable rhinitis, tonsillitis, laryngitis, tracheitis and pneumonia of lobar distribution in 42 lobes from 11 calves were seen in five experiments in which bacterial aerosol followed the viral aerosol by at least four days. One calf died with marked respiratory disease in each of four experiments within four days of exposure to the bacterial aerosol. Production of pneumonia was dependent on an interval between aerosols of at least four days but not on the condition of controlled climate on the environmental chamber either before or after the viral aerosol nor on the period of habituation allowed calves of some experiments.

RÉSUMÉ

Cette étude comportait 11 expériences au cours de chacune desquelles quatre veaux subirent d'abord une vaporisation avec une suspension du virus de la rhino-trachéite infectieuse bovine, ainsi qu'une vaporisation ultérieure avec une suspension de Pasteurella haemolytica. On sépara ces vaporisations par un intervalle de trois à cinq jours. Au cours de deux autres expériences, des veaux ne subirent qu'une vaporisation bactérienne. Dans toutes ces expériences, on contrôla les conditions climatiques des chambres expérimentales, à partir du moment où on vaporisait la suspension virale; dans huit de ces expériences, on commença à contrôler les conditions climatiques des chambres expérimentales, environ quatre à six jours avant la première vaporisation. On détermina, pour chacun des veaux, la teneur des aérosols en doses virales infectieuses et en bactéries. Ces expériences provoquèrent le développement de lésions macroscopiques de rhinite, d'amygdalite, de laryngite et de trachéite; elles entraînèrent aussi de la pneumonie lobaire dans 42 des lobes pulmonaires des 11 veaux des cinq expériences où on laissa écouler au moins quatre jours entre la vaporisation de la suspension virale et celle de la suspension bactérienne. Au cours de quatre expériences, un veau de chacun des quatre groupes correspondants mourut de troubles respiratoires marqués, dans les quatre jours qui suivirent la vaporisation bactérienne. La production de pneumonie dépendait d'un intervalle d'au moins quatre jours entre la vaporisation de la suspension virale et celle de la suspension bactérienne; elle ne semblait cependant pas reliée au contrôle des conditions climatiques des chambres expérimentales, avant ou après la vaporisation de la suspension virale, pas plus qu'à la période d'acclimatation accordée aux veaux de certaines des expériences.

INTRODUCTION

The experimental production of lobar pneumonia in calves has been reported previously. Hamdy *et al* (8) reported the irregular production of macroscopically recognizable pneumonia using myxovirus parainfluenza 3 (PI-3), *Pasteurella multo-*

*Animal Pathology Division, Health of Animals Branch, Agriculture Canada, Animal Diseases Research Institute (Western), P. O. Box 640, Lethbridge, Alberta, Canada T1J 3Z4.

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cida and *P. haemolytica,* pleuropneumonia-like organisms (PPLO) and climatic stress, in various combinations. All calves were inoculated intratracheally and by aerosol. Similarly, Baldwin *et al* (1) reported pneumonic lesions in some calves exposed to *P. haemolytica* intratracheally and/or to PI-3 aerosol. Macroscopically recognizable pneumonia was also produced in colostrum-deprived calves given intranasal and intratracheal inoculations of the virus of PI-3 (5).

The distribution and extent of respiratory disease in calves exposed to aerosols of bovine herpesvirus 1 (BHV1) and *P. haemolytica* have not been described. One study described the clinical disease only (3) and another study (2) included macroscopically recognizable changes in calves exposed to intratracheal injections of 2 ml of tissue culture of BHV1 and to aerosol of *P. haemolytica.* There is one report of dual infection of calves by PI-3 and/or BHV1 and *P. haemolytica* when exposure was by aerosols (18). These calves were subjected to severe climatic stress during ten days of experimentation. Without specifying which animals were given only BHV1, it was reported that 16% of the calves died of stress alone and 50% died of respiratory disease (18).

The association of stress and respiratory disease in cattle is generally recognized. However, the definition and measurement of stressors which may contribute to disease production are poorly documented. The purpose of our experiments was to study the influence of climatic conditions on the effects of BHV1 and *P. haemolytica* on respiratory tracts of calves. The report describes both failure and success in the production of respiratory disease in groups of calves raised under similar conditions of management, maintained under controlled climatic conditions during experimentation and exposed to aerosols of the virus and the bacterium at different intervals.

MATERIALS AND METHODS

Environmental Chamber

The operation of the chamber was as previously described (15). Calves were not restrained in the chamber and were given alfalfa hay and water *ad lib.* The fresh air input was constant at one changeover every ten minutes. For experiments A, B, C, D and K, temperature and humidity were constant in the chamber. The controlled conditions were different between the hours of 9:00 a.m. to 4:00 p.m. and 4:00 p.m. to 9:00 a.m. (Table II) for the other experiments.

Animals

All calves were derived from the same herd and all except those of experiment H were raised by the same methods as calves described by Jericho and Magwood (15). Calves of experiment H were from the same herd as the others but were raised on an irrigated and fertilized pasture instead of natural prairie grassland. The range of body weights of calves was 86 to 158 kg. Thirty-three males and 15 females, 66-158 days of age, were selected for the experiments. Evaluation of the final data showed no relationship as to the sex of the animals, thus, the gender is ignored in this report. All but one of the 12 experiments were done in either one of two consecutive summer seasons. The mean ambient temperatures for July, August and September were 19°, 18° and 13°C, respectively. Experiment E was started in January at ambient temperatures of -25°C.

Experimental Design

Some experiments began with a period of habituation of the calves to the chamber while it was not in operation (Table I). The time of exposure of the animals to BHV1 and to *P. haemolytica* is also given in Table I.

Aerosol Exposure

A modified Henderson apparatus was used for all of the viral exposures and for bacterial exposures of experiments A and B as previously described (13). The temperature and humidity of the lumen of the brass tube (100 cm x 10 cm) into which all viral aerosols were expelled were recorded. The bacterial aerosols of experi-

TABLE I. Chronological Events and Loss of Weight in kg for all Experiments

Experiment	Day of Control of Climate	Day of Viral Aerosol	Day of Bacterial Aerosol	Day of Killing of Calves	Loss In Weight kg
A	5	—	8	13	5
B	5	6	8	14	52
C	5	6	9	14	49
D	4	5	8	13	0
E	3	4	7	13	—
F	4	5	8	14	—
G	0	0	4	8	39
H	0	0	3	8	48
I	0	0	5	8	89
J	3	3	7	11	55
K	0	0	4	8	164
L	3	3	7	11	120

TABLE II. Temperature and Relative Humidity During Periods of Habituation and Climatic Control of Experiments

	Habituation						Controlled	
	Temperature °C			Humidity %				
Experiment	Max.	Min.	Mean	Max.	Min.	Mean	Temperature °C	Humidity %
A	28	22	24.8	69	59	65.4	2	90
B	28	20	24.0	72	60	64	2	92
C	24	18	22	70	60	66	29	92
D	22	11	16	80	60	68	2	72
E	13	8	10	78	72	74	28-2[a]	48-90
F	29	24	26	81	75	77	30-2	40-90
G	———	———	no habituation	———	———	———	30-2	90-70
H	———	———	no habituation	———	———	———	30-2	40-90
I	———	———	no habituation	———	———	———	30-2	40-90
J	28	14	21	88	68	74	30-2	90-60
K	———	———	no habituation	———	———	———	29	90
L	24	18	21	78	70	72	30-2	90-65

[a]Day conditions first night conditions last

ments A and B were also passed through this brass tube. The relative humidity was 65% for experiments B and C and 90% for experiments D and E. The temperature for experiment C was 23°C and for experiments D and E it was 19°C. During passage of the bacterial aerosol of experiment B the temperature of the interior of the tube was 19°C. For experiments F to L range of temperature of the air in the exposure room was 20-25°C and the relative humidity was 65-70%. This narrow range ensured a similar rate of respiration of all calves for the time of aerosol exposure. For experiments C to L the bacterial aerosols were blown directly into the face mask. Its total volume was 30 liters including the attached rubberized envelope which encircles the neck of the calf. The aerosol was expelled from the face mask into the chamber air via an opening (6 cm in diameter) at the side of the mask. This opening served as a port for the intake of air and discharge of exhaled air. All exposures to aerosols were of five minutes duration.

Air Sampling

All-glass impingers placed 15 cm from the nostrils of each calf were used for sampling all aerosols for their duration. A total of 30 liters of air were thus sampled for each calf (16). The virus titration was as described by Jericho and Darcel (16). The bacterial aerosols were collected in similar impingers containing 20 ml of 0.1% gelatin saline at room temperature. For experiments A, B, C, D and E these impingers were placed in iced water immediately after the completion of sampling. Impingers for the other experiments were left at room temperature until bacteriological analysis was initiated, usually within two hours of sampling.

Other Technical Procedures

These were essentially the same as reported previously (15). Calves were restrained only during aerosol exposure and feed and water were provided in utensils common to all calves for each experiment. Two types of nasal swabs (15) were taken from left nostrils, one for the isolation of BHV1 and the other for the isolation of *P. haemolytica*. The swabs were taken on four separate days before and on three separate days after bacterial aerosol exposure in experiments A, B, C, D and E. Swabs for the remainder of the experiments were taken on two separate days prior to bacterial aerosol exposure and on the last day of the experiments. However, in experiment F additional swabs were collected one day after exposure to the bacterial aerosol. Sera for the determination of the neutralizing titres to BHV1 were collected from all calves at the same time as the swabs.

Virological Studies

Virus suspensions used to produce aerosols of experiments B, C, D, E, F and H were from the same stock as that described by Jericho and Darcel (16). For the remainder of the experiments the virus and the preparation of its suspension were the same but the virus had been passaged two more times. The procedures used to isolate BHV1 from nasal swabs and from suspensions of tracheal tissue and lung tissue in Eagle's medium were as described previously (16). Viral isolation and titration and serological studies of all experiments were done in three batches. A different tissue culture cell line was used for each batch. Experiments B, C and D were batch 1, experiment E batch 2 and the remainder of the experiments were batch 3. The neutralizing titre of sera for BHV1 were also determined by the method described (16). The concentration of the virus per ml of suspension for each experiment was determined after the last of the four aerosols had been produced. These concentrations ranged from a low of 10^6 (experiments B, C, D) to a high of 10^{10} Tissue Culture Infective $Dose_{50}$ ($TCID_{50}$)/ml (experiment G).

Bacteriological Studies

The suspensions used for the production of the bacterial aerosols of experiments A, B, C and D were four aliquots of the 40 prepared from the suspension described by Jericho *et al* (14). Five percent foetal calf serum (FCS) was added to the aliquot for each of experiments C and D only. A culture of *P. haemolytica* for use in the remainder of the experiments was produced by adding one of the original aliquots to brain heart infusion broth with 5% FCS added and incubation at 37°C for 18 hours. The number of organisms per ml of suspension of each experiment was determined after the last of four aerosols was produced. The concentrations ranged from 5.5×10^5 per ml (experiment B) to 1.8×10^{10} per ml (experiment L). Deep nasal swabs, tracheal swabs and lung tissue were examined for the presence of *P. haemolytica* by the direct inoculation of plates containing trypticase soy agar with 5% bovine blood (BAP). After two days of incubation, colonies resembling those of *P. haemolytica* were transferred onto new BAP and three days later these plates were examined for the presence of colonies producing hemolysis indicative of *P. haemolytica*. Representative colonies were identified by biochemical methods. The method of titration of each bacterial suspension was done as previously described (14). The retropharyngeal lymph nodes of calves of experiment L were also examined for the presence of *P. haemolytica*.

Pathological Studies

The postmortem procedures and examination of the entire respiratory tracts were conducted as previously described (15). The ventral aspects of all lungs and other sites with pathological changes were photographed. Any macroscopic pathological changes in nose, larynx, trachea and lungs were recorded.

RESULTS

Clinical Observations

Similar clinical observations to experi-

ments with climatic stress (15) and to experiments with climatic stress and virus exposure (16) were observed in these experiments. There were, however, some noteworthy differences. Calves of experiment E were moved from outside temperatures of -25°C to 12°C within the nonoperational chamber. Four hours later one calf had a respiratory rate of 130 per minute whereas the others had a rate of 68 per minute. Two days later all respiratory rates were at expected levels (15). Increases in rectal temperatures were similar to experiments with calves infected with the virus only (16). However, regression of rectal temperature to normal by day 6 postinfection had not occurred in two calves in each of experiments G, I, J, K and L. In these calves, temperatures of up to 41.6°C were recorded. In some calves of all experiments except A and B, mucoid and muco-purulent nasal discharge was evident two days after exposure to the bacterial aerosol. This discharge was usually from the left nostril, the one which was routinely used in the nasal swabbing technique. Two calves raised on an irrigated-fertilized pasture in experiment H developed very severe diarrhea and one of these calves died. One animal died in each of experiments G, I, K and L. All these animals, with one exception, died four days after exposure to the bacterial aerosol. One calf from experiment L died two days after exposure to the bacterial aerosol.

Virological Studies

The ranges of $TCID_{50}$ for BHV1 recovered from 30-liter samples of the four aerosols per experiment were as follows: within experiments B, C and D (batch 1) 4.1×10^2 to 4.0×10^3, the range within experiment E (batch 2) was 4.0×10^6 to 4.0×10^7, the maximum range within any of the other experiments (batch 3) was 5.12×10^4 to 1.02×10^5 (experiment I). Among experiments of batch 3 the maximum range was 1.28×10^4 (experiments I and G) to 3.28×10^6 (experiment J). The means of $TCID_{50}$ of the virus recovered from 30-liter samples of the four aerosols per experiment are given in Table III.

Bovine herpesvirus 1 was isolated from only one nasal swab (F10) taken before the exposure of the calves to the virus. Nasal swabs were negative one day after exposure to the virus in experiments B, C and D but the virus was isolated with increasing frequency from later swabs. All nasal swabs were positive on the last day of all experiments. All nasal swabs from the remainder of the experiments were taken at least three days after virus exposure and all of these yielded BHV1. The virus was isolated from the tracheas of all animals except those in experiments C, E and F. The virus was also isolated from one lung in experiment C, three lungs in experiments B, F and A and from all other lungs. The virus was not isolated from any lungs in experiment E. The sera of 11 animals had neutralizing titres for BHV1, nine (all of experiment F, two of each experiments J and I and one of experiment L) were positive only on the final day. The sera of three calves, one from experiment K and two from experiment E, were positive throughout. The highest titre was 1/12.

Bacteriological Studies

The maximum range of numbers of organisms of *P. haemolytica* in 30-liter samples of four aerosols for all experiments was 4.0×10^5 to 1.0×10^6 in experiment E. The mean bacterial counts in 30-liter samples of four aerosols per experiment are given in Table III. All nasal swabs taken before aerosol exposure were negative for *P. haemolytica*. Of the 20 nasal swabs (experiments A, B, C, D, E) taken immediately after aerosol exposure, all but two (experiment B) were positive. Of 16 nasal swabs taken three or five days after the bacterial aerosol in experiments A and B only one swab was positive for *P. haemolytica*. The remainder of the nasal swabs from all experiments were positive for this organism. *P. haemolytica* was isolated with the following frequency from tracheas: none from experiment A, one from each of experiments B and D, two from experiments C and F, three from experiments E and I and from all tracheas of experiments G, H, J, K and L. All lungs in experiments A, B, C and D were negative for *P. haemolytica*, one was positive in each of experiments E, F, H, J and L. All lungs yielded *P. haemolytica* in experiments G, I and K. One retropharyngeal lymph node from a calf in experiment J was positive.

PATHOLOGICAL STUDIES

It is learned from the data in Tables I and III that the weight loss of the calves in each experiment was not related to the duration of the experimental periods, or to the type of climatic conditions or to the number of lobes with pneumonia.

The number of calves with macroscopically recognizable purulent rhinitis (Fig. 1) of at least one nasal cavity, with purulent tonsillitis, inflammation of the middle trachea, number of lobes with pneumonia of at least one-third of the tissue (henceforth referred to as lobar pneumonia) (Figs. 2 and 3) and the number of calves with lobar pneumonia are given in Table III. Frequently inflammation of the tonsils extended to involvement of the larynx. None of these macroscopically recognizable changes were evident in the animals of the first six experiments. In four experiments (G, J, K and L) all the above changes were seen but not in all calves. In experiment L all sites except the middle trachea were affected. In experiment H, one calf had purulent rhinitis and middle tracheitis. Macroscopically recognizable inflammation of the middle trachea was characterized by either whitish foci of necrosis or by a purulent exudate on the epithelium. At sites of the middle or lower trachea in experiment H, changes occurred independently of tonsillitis, laryngitis or lobar pneumonia. Tonsillitis, laryngitis and lobar pneumonia were present in animals of experiments I, J and K, although tracheitis was absent. However, in experiments G and L, macroscopically recognizable inflammation of the middle trachea was associated with lobar pneumonia, tonsillitis and laryn-

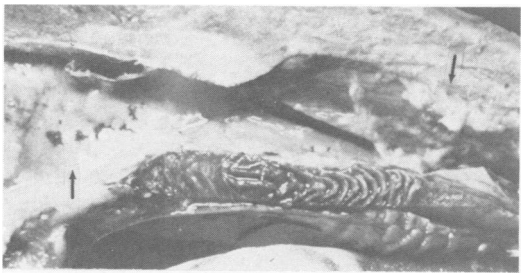

Fig. 1. Purulent rhinitis (arrow, right) with necrosis of epithelium (arrow, left) in calf of experiment K.

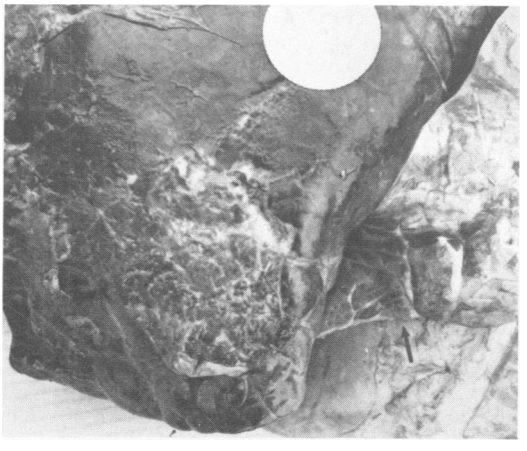

Fig. 2. Pneumonia of cardiac lobe in experiment K. This tissue was severely congested and consolidated and covered by fibrinous adhesions. Lung tissue on the right was affected only at its distal point (arrow).

TABLE III. Mean $TCID_{50}$ of Virus in 30 Liter Samples of Four Aerosols per Experiment, Mean Bacterial Count in 30 Liter Samples of Four Aerosols per Experiment, Number of Calves with Rhinitis, Tonsillitis and Middle Tracheitis and Number of Lobes with Pneumonia in Each Experiment (Number of Calves with Lobar Pneumonia Per Experiment)

Experiment	Average $TCID_{50}$	Average Bacterial Count	Calves with Rhinitis	Calves with Tonsillitis	Calves with Tracheitis	Lobes with Pneumonia
A		1.4×10^3	0	0	0	0
B	1.3×10^3	None recovered	0	0	0	0
C	2.2×10^3	9.0×10^4	0	0	0	0
D	4.0×10^2	9.6×10^4	0	0	0	0
E	2.2×10^7	3.0×10^5	0	0	0	0
F	2.3×10^5	5.2×10^6	0	0	0	0
G	1.9×10^4	5.0×10^4	3	3	2	11(3)
H	4.1×10^5	5.6×10^5	2	0	1	0
I	4.8×10^4	1.1×10^6	2	2	0	6(2)
J	1.84×10^6	4.0×10^5	4	4	0	3(1)
K	1.0×10^6	9.26×10^5	4	4	0	14(3)
L	Not determined	4.26×10^5	4	4	1	8(2)

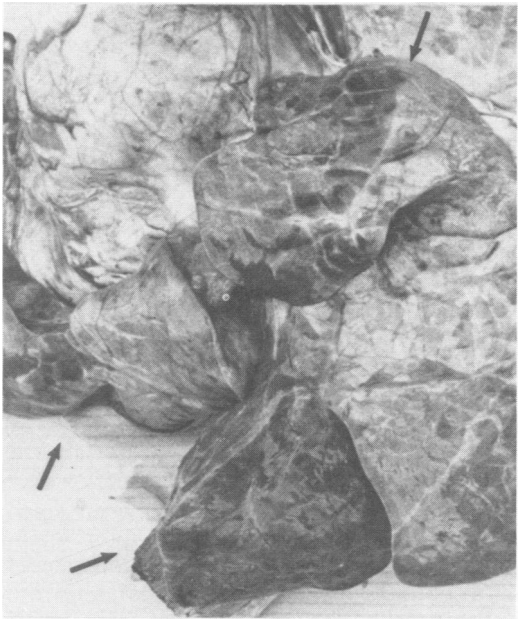

Fig. 3. Ventral aspect of lung. Pneumonia of distal part of apical (arrow, left), cardiac (arrow, bottom), and intermediate (arrow, top) lobes. Pneumonic tissue was congested and consolidated. Fibrinous adhesions were absent. Note sharp line of demarcation between pneumonic (left) and functional tissue of intermediate lobe (arrow, top). Lung tissue on the right of photo was not affected but has mottled appearance due to uneven distribution of blood.

gitis. With the exception of two calves from experiment J, all calves used in experiments J, K and L had petechial changes in the upper trachea which were an extension of similar laryngeal changes.

Lobar pneumonia was produced in five experiments (G, I, J, K and L) in a total of 42 lung lobes of 11 calves. This pneumonia was usually, but not always, associated with fibrinous pleural adhesions and the presence of straw-colored thoracic fluid. The volume of this fluid increased with the extent of pneumonic tissue. These changes were observed in only those experiments where bacterial exposure occurred at least four days after viral exposure (Table I). Production of any macroscopic pathological changes was not proportional to the concentration of infectious units of virus or bacterial counts in the aerosols (Table III). Similarly, the occurrence of lobar pneumonia was not proportional to the number of lesions affecting only lobules. These lobular lesions were indistinguishable from those in lungs of calves exposed to only BHV1 (16). Lobar pneumonia occurred in lungs which had very few such BHV1-like lobular lesions. Conversely, in experiments where lobar pneumonia occurred, some lungs without lobar pneumonia had numerous BHV1-like lobular lesions.

One calf died in each of experiments G, H, I, K and L. All these calves had lobar pneumonia with the exception of the calf from experiment H which died with numerous lobular lesions in its lungs and severe hemorrhagic diarrhea. The extent of pneumonia in the other four calves which died was 25 to 80% of the lung tissue, whereas for calves which did not die 5 to 40% of the lung tissue was pneumonic.

DISCUSSION

Throughout these experiments every effort was made to use calves of similar background. The only exception being calves in experiment E which were brought into the chamber during severe winter conditions. Variations in the management of the calves in the chamber were as follows: (a) time of habituation to the chamber, (b) time between virus and bacterial exposure, (c) length of experiment and (d) the controlled climatic conditions within the chamber. Additional stressors on all the calves were anxiety and fear due to weaning, transport to the chamber (⅓ km), introduction into the chamber, daily handling, sampling procedures and aerosal exposures. However, they did have the benefit of feed and water *ad lib* and the opportunity of rest between experimental procedures. The sale and transport of calves by the cattle industry is also associated with many stressors and much respiratory disease in calves. It is not known how the physiological effects of the stressors by industry compare with the experimental stressors noted above.

The effect of constant or daily fluctuations of climatic conditions on the incidence of respiratory disease is not elucidated by these studies. In some experiments with various daily climatic conditions lobar pneumonia was not produced. In a similar study using only virus (BHV1) aerosol, the number of macro- or microscopically recognizable lesions produced in the lungs

was not influenced by variations in the ambient temperature or humidity (16). Similarly, the period of habituation allowed calves of some experiments did not influence the susceptibility to this experimental respiratory disease.

The exposure of calves in experiments A and B to only aerosols of *P. haemolytica* failed to produce any respiratory disease. This confirmed the results of reported similar experiments (17).

When the calves were exposed to the bacterial aerosol at least four days after the viral aerosol, pneumonia was produced under the conditions of these experiments. Evidently, this period of time is required by the virus infection to produce some impairment of pulmonary bactericidal activity (7, 12) or to inhibit the phagocytic activity of alveolar macrophages (6) or to effectively impair the clearance function of damaged respiratory epithelium (16). It is noteworthy that the virus used for experiments with macroscopic respiratory disease had undergone two more propagations than the virus of experiments B, C, D, E, F and H which were without respiratory disease. The significance of these two propagations to the results is not known.

The importance of the interval between infections with parainfluenza-3 and *P. haemolytica* to the production of respiratory disease in cattle has been suspected by Heddleston *et al* (10), Hetrick *et al* (11) and Baldwin *et al* (1). From experiments with intratracheal injections with BHV1 followed three days later by an aerosol of *P. haemolytica*, Collier *et al* (3) concluded that infection with BHV1 followed by *P. haemolytica* did not produce a "profound potentiating effect" of either organism. Similarly, in six experiments reported herein, *P. haemolytica* failed to potentiate the effects of BHV1 if given within three days of the virus. However, a considerable potentiation was observed in five other experiments of this study when the time interval between infections was more than three days. This observation is supported by reports of Collier (2) in which four calves infected intratracheally with BHV1 and 30 days later with an aerosol of *P. haemolytica* produced clinical disease and bronchopneumonia of a type described in this study.

The production of respiratory disease in experiments G, I, J, K and L may be regarded as a truly mixed infection and also dependent on factors other than the two infectious agents. Evidently the respiratory tract was only susceptible to infection by the bacterial aerosol when exposure occurred at least four days after virus exposure. This suggests that for the purpose of these experiments the marginal quantitative exposure of critical sites of the respiratory tract was most appropriate. Overwhelming exposure with bacteria may not have revealed differences in susceptibility of calves between experiments. Dao Trong Dat and Schimmel (4) reported the uniform production of extensive pneumonia in 11 of 13 calves two to ten weeks of age by injecting the relatively large volumes of 10 and 20 ml of a culture of *P haemolytica* into the noses and tracheas respectively.

The interval of four days between aerosols required for the production of experimental respiratory disease in this model is noteworthy in relation to studies on the importance of stressors on antibody levels in calves following active and passive immunization (9). It was found that humoral antibody production was impeded by repeated or lasting stressor effect (ACTH injections) and that the immunological reactions of the calves to antigen injections immediately after transport into the rearing unit were stronger than those to antigen application three days after transfer (9). With this observation in mind, the immune response of calves of this model should be investigated to elucidate the association of stressors and respiratory disease of cattle which is based on epidemiological considerations.

Based on results by Jericho *et al* (14) it is concluded that the viability of bacteria in aerosols for these experiments was not significantly influenced by the temperatures and humidities of the aerosols. These workers also reported that the addition of FCS was beneficial for viability of bacteria. Experiments A and B were done without FCS and thus yielded very few organisms in the fluids into which the aerosols were sampled.

Some or all of the noses and tracheas were colonized by *P. haemolytica* in all experiments of this study, regardless of the concentration of bacteria in the aerosols. The results of virological studies and the effect of virus exposure were in agreement with results of similar experiments without bacterial aerosols (16).

Death of calves was not always directly

related to the extent of lobar pneumonia. Although all calves with extensive lesions died, one with 40% of its lung tissue affected survived and one with only 25% involvement died. In experiment L, one animal, which died two days after exposure to the bacterial aerosol, had pneumonic lesions involving 80% of its lung tissue.

Pathological events were not uniform in affected respiratory tracts. Three out of 11 calves with lobar pneumonia had macroscopically recognizable inflammation in lower and upper trachea, larynx and nose. In seven calves with lobar pneumonia, macroscopically recognizable changes were not observed in the trachea. They had, however, rhinitis and/or tonsillitis. These findings suggest that the pathological sequence of events in the upper and lower respiratory tracts may be independent of each other, but nonetheless, similar immunopathological events may occur at the respective sites.

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