Pneumonic Pasteurellosis of Cattle: Microbiology and Immunology

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SUMMARY

Pasteurellosis was investigated under natural conditions by comparing bacterial and viral nasal flora and levels of bacterial and viral antibody in sera and nasal secretions between animals sick with the disease and those that remained well. The animals were classified sick or well on the basis of the levels of body temperature and plasma fibrinogen. The most significant feature of the bacterial flora was the higher frequency of isolation and the numbers of Past. hemolytica in the nasal flora in the first two weeks after shipment. As indicated by the number of animals with serum antibody to PI-3 virus, infection with this virus was active in both sick and well animals, and serologically, the incidence of infection was higher in animals that remained well. Nasal antibody to PI-3 virus was slightly lower in incidence than serum antibody. Examination of untreated fatal cases and animals killed during the experiment suggests that in some animals there may be a relationship between the high numbers of a bacterial species in the nasal passage and infection by that organism in the lung.

INTRODUCTION

The financial losses to the cattle industry in the United States due to Pasteurellosis have been pointed out by Jensen (11) and it is probable that this disease is of proportional significance to the beef industry in Canada. The conflict evident in papers presented at a recent symposium (3) is indicative of the confusion surrounding the understanding of many aspects of this disease. Few workers have related their work, either in isolation of suspected etiological agents from field material or in attempted experimental reproduction, to the pathogenesis of the lesions found in the lungs. Furthermore, few attempts have been made to study in detail the composite changes in the flora of the respiratory tract and the immune status of animals as they develop the disease.

The work reported here is the first phase of a study on the pathogenesis of the disease (Pasteurellosis) and was an attempt to evaluate the significance of some microbiological agents and the immune status of the host toward these agents in the natural disease. Randomly selected cattle were obtained following their transportation from western Canada, and the differences in factors traditionally considered to be important in the disease were studied between those animals that became ill or died and those that remained well.

MATERIALS AND METHODS

Five groups of range calves of beef breeding (Table I), six to 10 months of age, were purchased on arrival by train from western Canada, a journey of about five days, and were housed in a partially enclosed cement block shed. One group was removed before another was brought in. They were allowed into a small corral twice each day for oats, hay and water and were driven into a chute for handling. Sampling started on the day of arrival and continued until the sale of the animals at the conclusion of the experiment about one month

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TABLE I. Experimental Design and Data Collected

Animals					
Group	Date		Number	Sick	Well
A	Oct.	'67	10	5	5
B	Nov.	'67	10	7	3
C	Jan.	'68	10	9	1
D	Feb.	'68	10	1	9
E	Mar.	'68	15	11	4
			55	33	22

Data Collected			
Parameter	Sample	Frequency	Recorded as
Nasal bacterial flora — Past. hemolytica — Past. multocida — Neisseria sp.	nasal swab	daily[a]	mean colony count
Bacterial antibody titre — Past. hemolytica	serum	weekly	recip. of dilution
Bacterial antibody titre — Past hemolytica	nasal washing	weekly	recip. of dilution
Viral nasal flora — PI_3 — I.B.R.	nasal swab	2 x per week	+ or −
Viral antibody titre — PI_3	serum	weekly	recip. of dilution
— I.B.R.			+ or −
Viral antibody titre — PI_3	nasal washing	weekly	recip. of dilution
Fibrinogen	plasma	daily[a]	mg%
Temperature		daily	°F.

[a]each day for first 14 days and 2 — 3 times weekly for the next 2 weeks

later. Antibiotics were not given at any time.

Each calf was designated as *sick* or *well* (Table I) on the basis of the following criteria. The calf was designated *sick* for the entire experimental period if the body temperature reached 104.5°F and was followed within 48 hours by three consecutive daily plasma fibrinogen levels greater than 800 mg% regardless of when these changes occurred during the experimental period. Calves which did not fulfill these criteria for levels of temperature and fibrinogen were designated *well*. Plasma and nasal swabs were collected daily and serum and nasal washings on a weekly basis (Table I). The procedures of swabbing, plating, identification and tabulation of bacterial species and the calculation of mean colony count (mcc) were the same as those outlined by Magwood (16) except that colony count class "a" included 0 to 2 instead of 1 to 2 colonies. The swabs from both nostrils were inoculated onto bovine blood agar plates.

Three calves in group E were killed 24 hours after being designated *sick* on the basis of the above criteria to examine the lungs and determine whether or not pneumonia was present. This served as a check on the validity of the criteria for grouping into *sick* and *well* classes.

The same swabs were used for isolation of virus on the designated days after the agar plates were streaked. Cultures of primary fetal bovine kidney (FBK) were prepared and maintained in a Tris buffered Hank's medium (15). Nasal swabs from both nostrils of each animal were placed together in 3.0 ml of diluent of a phosphate buffered saline and after mild agitation, 0.2 ml of the dilution was inoculated immediately into each of four primary FBK culture tubes. When cultures of cells were not available on the day of collection, the contents of the tubes were frozen and held at −20°C until used 24 to 72 hours later. The inoculated tubes were examined daily for seven to 10 days for characteristic cytopathic changes and on the 3rd, 7th and 10th days, the tubes were examined for hemadsorbing activity (13) using both guinea pig and calf red blood cells. Each successful isolation was identified by its cytopathic ef-

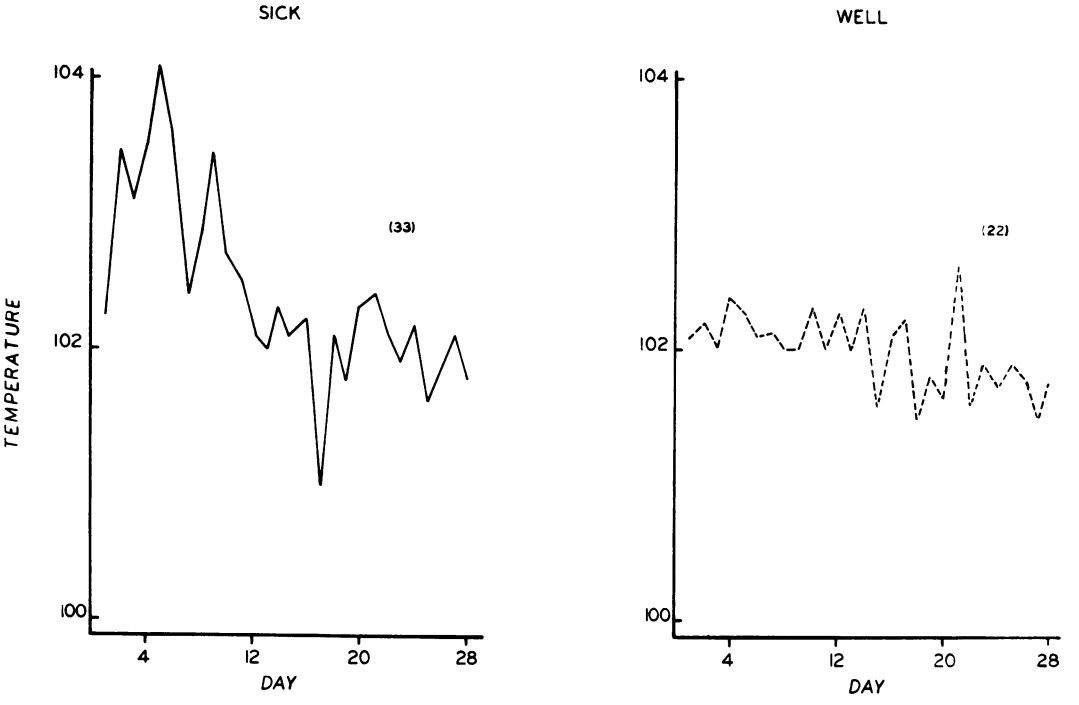

Fig. 1. The mean daily body temperature in degrees Fahrenheit of 33 sick animals is recorded on the left and 22 well animals on the right. The mean temperature for sick animals is below the required level (104.5°) since the day on which the maximum temperature occurred in individual animals varied.

fect, neutralization with specific antisera, hemagglutinating and hemadsorbing ability and chloroform sensitivity.

Nasal secretions were collected, lyophilized and standardized for protein as outlined by Duncan (7) except that two rather than three collections were pooled from one day.

The level of antibody to *Past. hemolytica* type I in serum and nasal secretions was determined by indirect hemagglutination (IHA) as outlined by Biberstein (2). Hemagglutination at a serum dilution of 1:2 or greater was recorded as positive. Antibody levels against Parainfluenza-3 (PI-3) virus in serum and nasal secretions were determined by the standard hemagglutination inhibition test (14) and these were carried out in disposable plastic trays[1]. Serum samples from groups D and E were tested for antibody to PI-3 virus by the microtitre technique[2] (23). Hemagglutination inhibition at a serum dilution of 1:10 or greater was recorded as positive. The presence or absence of serum antibody to Infectious Bovine Rhinotracheitis (IBR) virus was established by serum-virus neutralization tests using 100 $TCID_{50}$ of virus (26).

The plasma fibrinogen was assayed according to the method outlined by Henry (10).

Statistical analyses were carried out to evaluate differences between the *sick* (class "S") and the *well* (class "W") animals. Calculations for the analysis of variance of mcc and serum antibody were made according to the methods outlined in Steel and Torrie (24) for a two-way classification with unequal and disproportionate sub-class numbers with interaction. For both mcc and serum antibody, factor A was "health" (*sick* and *well*) and factor B was "time" (week 1, 2, 3 and 4 for mcc; day 1, 7, 14, 21 and 28 for serum antibody) and logarithmic transformations for the values (x) were made i.e. $\log_{10}(x+1)$. When "F" values for "time" were significant "Least Significant Differences" were calculated. Frequency data were compared by a simple proportion analysis and "t" test. *Differences stated in the results include only those which were significant at*

[1]Linbro Chemical Co., New Haven, Conn.
[2]Cooke Engineering Co., Alexandria, Va.

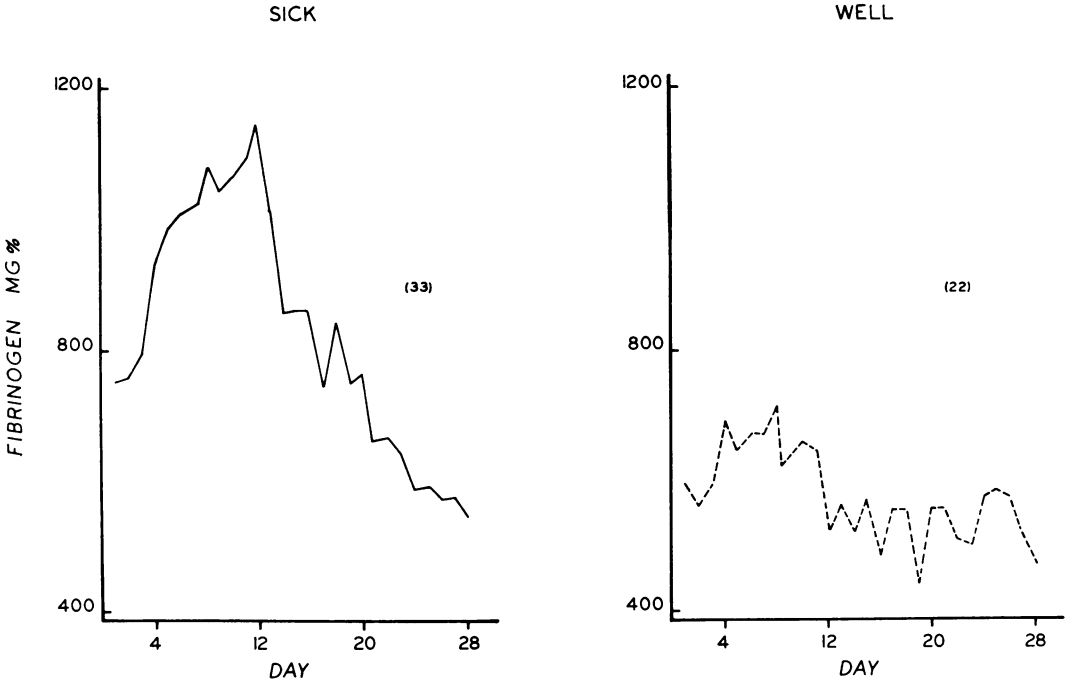

Fig. 2. The mean daily plasma fibrinogen levels of 33 sick animals is shown on the left and 22 well animals on the right.

the 0.05 level of probability.

An autopsy of the animals which died or were killed on experiment, tissue from each lobe of the lungs (whether lesions were visible or not) was cultured for bacteria and two lobes with lesions were cultured for virus. The methods for isolation and identification of agents were similar to those outlined for nasal swabs.

RESULTS

The daily mean body temperature of *sick* animals was higher than the *well* animals and peaked at about seven days (Fig. 1). The daily mean levels of plasma fibrinogen followed a similar pattern to the temperature rise and peaked at day 10 (Fig. 2). Some sick animals maintained temperatures in the range of 105-106°F for several days, whereas others rose to such levels for only one or two days. The manner in which an elevation in plasma fibrinogen followed the temperature rise in individual animals was remarkably consistent. However, there were instances in which a temperature rise to 105°F was not followed by an increase in plasma fibrinogen.

A comparison of the incidence of isolation from swabs (daily from each nostril), noses (daily from at least one nostril) and calves (at least once during the week), for *Past. hemolytica* and *Past. multocida* and *Neisseria* sp. in all calves indicated that *Neisseria* sp. were lower in incidence than the other two organisms (Fig. 3). A high percentage of all the calves carried the three organisms. The proximity of the "nose" and "swab" lines to each other (Fig. 3) is an indication of how often the organism occurred bilaterally in the nose. The frequency of isolation from noses in *sick* and *well* animals during each week indicated that *Past. hemolytica* was more frequently isolated from the class "S" than from class "W" in the first two weeks (Fig. 4).

The relative numbers of *Past. hemolytica, Past. multocida* and *Neisseria* sp. in the nasal flora in the *sick* and *well* animals were expressed as mean colony count (Fig. 5). The mean colony count of *Past. hemolytica* in the nasal flora varied among the five groups (Fig. 6). Groups C and E had proportionally more *sick* animals than the other three groups and in addition, the

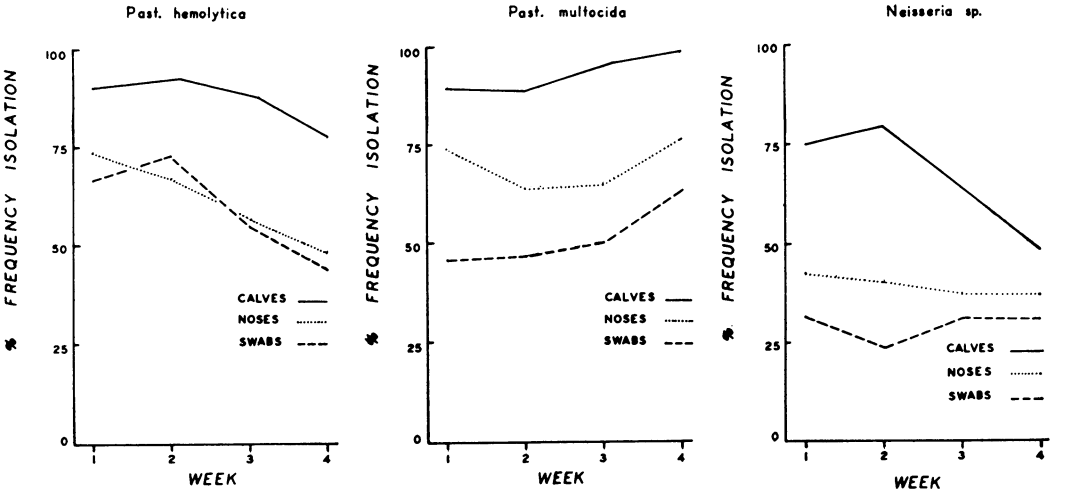

Fig. 3. The frequency of isolation for Past, hemolytica, Past, multocida **and** Neisseria, sp. **on an overall basis without regard to** sick **and** well **animals is illustrated. "Swabs" refers to the incidence in each nostril each day on all calves. "Noses" refers to the incidence each day in at least one nostril. "Calves" indicates the frequency on a weekly basis of the calves which had the particular organism isolated at least once during the week.**

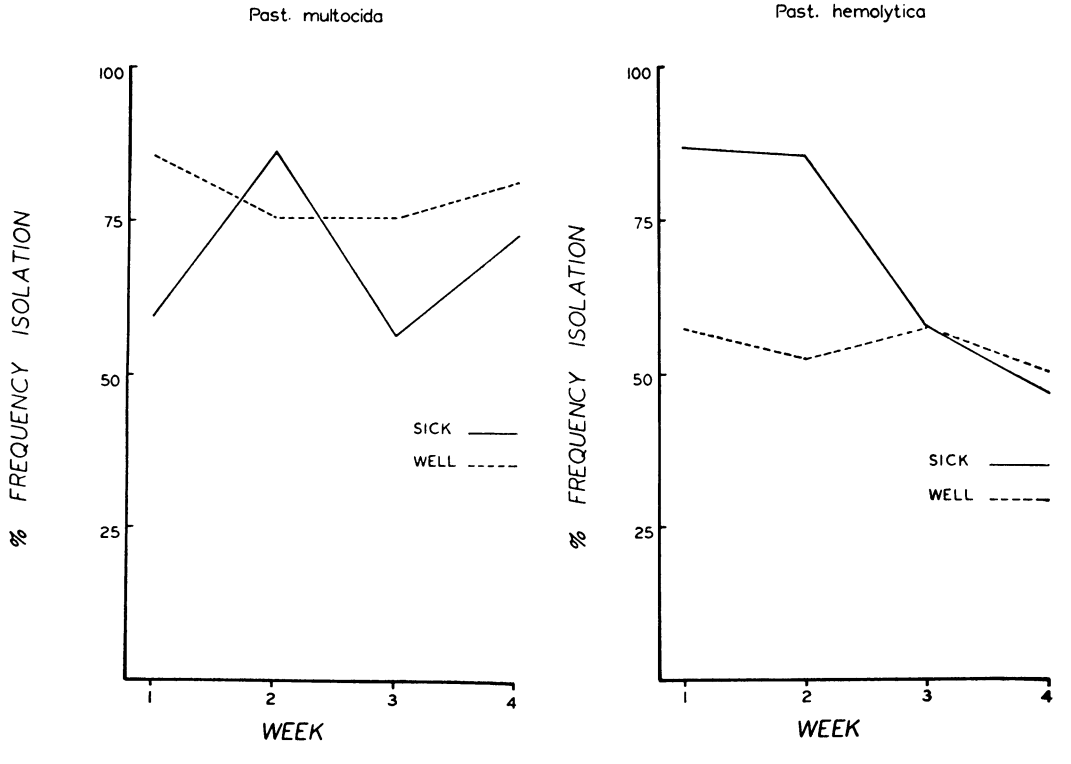

Fig. 4. The frequency of isolation of Past. hemolytica **is recorded on the right and** Past. multocida **on the left in** sick **and** well **animals. The difference between the two groups in the weeks one and two was statistically significant.**

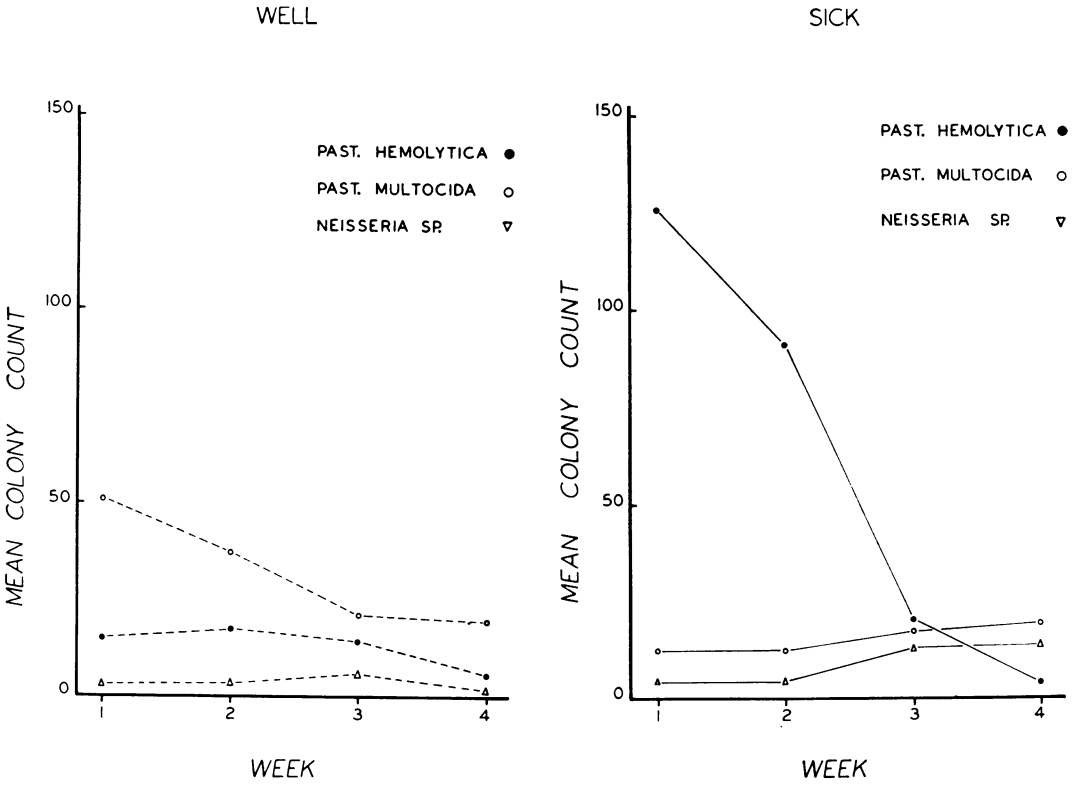

Fig. 5. The relative number of three species of bacteria on the nasal mucosa in sick and well animals is illustrated.

TABLE II. Analysis of Variance of Bacterial Mean Colony Counts of the Nasal Flora

Group	Factor	Degrees of Freedom	Past. hemolytica Mean Square	Past. hemolytica "F"	Past. multocida Mean Square	Past. multocida "F"
A	Time	2	0.745	0.8824	1.8899	4.5386[a]
	Health	1	0.053	0.0628	0.0540	0.1296
	Interaction	2	0.950	1.250	0.2799	0.6721
	Error	24	0.8843		0.4164	
B	Time	3	2.5618	8,2718[a]	0.1907	0.6569
	Health	1	0.0008	0	3.1198	10.7468[a]
	Interaction	3	0.3788	1.2231	0.4841	1.6675
	Error	29	0.3097		0.2903	
C	Time	3	1.0275	3.0256[a]	0.2617	1.4284
	Health	1	0.9867	2.9054	0.0252	0.1375
	Interaction	3	0.2932	0.8633	0.0166	0.0906
	Error	24	0.3396		0.1832	
D	Time	3	0.1026	0.3444	0.3552	0.3445
	Health	1	0.4367	1.4659	1.0611	1.0292
	Interaction	3	0.1302	0.4370	0.0530	0.0514
	Error	32	0.2979		1.0309	
E	Time	3	3.8665	14.1630[a]	0.1740	0.7699
	Health	1	2.2591	8.2750[a]	0.9754	4.3159[a]
	Interaction	3	0.8196	3.0021[a]	0.1047	0.4632
	Error	35	0.2730		0.2260	

[a]Significant at the .C5 level of probability

TABLE III. Analysis of Variance of Serum Antibody Titres

Group	Factor	Past. hemolytica I Degrees of Freedom	Past. hemolytica I Mean Square	Past. hemolytica I "F"	PI_3 Degrees of Freedom	PI_3 Mean Square	PI_3 "F"
A	Time	4	2.7364	8.2001[a]	3	3.6181	32.2468[a]
	Health	1	0.0037	0.0111	1	0.0016	0.0142
	Interaction	4	0.1424	0.4267	3	0.0021	0.0187
	Error	40	0.3337		31	0.1122	
B	Time	5	1.1350	7.4376[a]	4	1.5649	5.0644[a]
	Health	1	0.2844	1.8661	1	5.1250	16,5857[a]
	Interaction	5	0.1660	1.0892	4	0.1716	0.5553
	Error	45	0.1524		35	0.3090	
C	Time	5	1.0115	3.5491[a]	4	0.1722	0.3018
	Health	1	0[b]	0[b]	1	0.4025	0.7056
	Interaction	5	0.3803	1.3343	4	0.0256	0.0448
	Error	36	0.2850		30	0.5704	
D	Time	4	0.0416	0.11861	4	1.7401	5.1345[a]
	Health	1	0[b]	0[b]	1	0.0100	0.0295
	Interaction	4	0.0291	0.8297	4	0.3331	0.9828
	Error	39	0.3507		39	0.3389	
E	Time	8	1.0443	3.2431[a]	4	0.8646	3.1589[a]
	Health	1	0.1053	0.3270	1	4.6225	16.8889[a]
	Interaction	8	0.1629	0.5059	4	0.2699	0.9861
	Error	71	0.3220		43	0.2737	

[a]Significant at the .05 level of probability
[b]Value less than 10^{-6}

most severe clinical illness. With regard to mcc, the mean for all groups is not truly representative of any one group (Fig. 6).

In the analysis of variance of mean colony counts of the nasal flora (Table II) the number of *Past. hemolytica* isolated was greater in class "S" than in class "W" only in Group E. This occurred during week 1. The number of *Past. hemolytica* isolated in weeks 1 and 2 was greater than in weeks 3 and 4 in groups B, C and E. In two groups, (B and E) *Past. multocida* was isolated in larger numbers from class "W" than from class "S".

The mean titres of serum antibody to *Past. hemolytica* type I (Fig. 7) increased over the four week period in four groups (A, B, C and E), in both *sick* and *well* animals, the most marked change occurring between day 1 and day 7. The number of animals having serum antibody to *Past. hemolytica* type I was very high (Table IV). Only a few nasal washings contained measurable IHA antibody to *Past. hemolytica* (Table IV) and the levels were very low and inconsistent.

The incidence of isolation of PI-3 virus and IBR virus was very low (Table V).

The frequency of animals having serum antibody (Table IV) to PI-3 virus and IBR virus increased over the experimental period. Class "W" had a greater number of animals with antibody to PI-3 virus than did class "S". The presence of antibody to PI-3 virus was less frequently demonstrated in the nasal secretions than it was in the serum (Table IV).

The overall mean titres of serum antibody to PI-3 virus in the *sick* and *well* animals were similar in pattern (Fig. 8) and a difference between the two classes was not demonstrated statistically. In two groups (B and E), the serum antibody to PI-3 virus was greater in class "W" than in class "S" (Table III). In four groups, (A, B, D, E), the level of serum antibody to PI-3 virus increased over the 4 week period (Table III), the most marked change occurring between day 14 and day 21. The levels of nasal antibody to PI-3 varied greatly and did not form a pattern similar to the serum levels (Fig. 9).

The relationship between serum and nasal antibody to PI-3 virus over the period of observation varied considerably between animals. The following combinations occurred: (a) an early level of nasal antibody which decreased or disappeared with time as the level of serum antibody increased, (b) serum antibody was present

TABLE IV. Frequency of Positive Serum and Nasal Antibody Titres

Antibody	Day 1 Sick	Day 1 Well	Day 7 Sick	Day 7 Well	Day 14 Sick	Day 14 Well	Day 21 Sick	Day 21 Well	Day 28 Sick	Day 28 Well
Serum Past. hemolytica										
Number	30/32	20/20	25/27	22/22	24/25	22/22	23/23	22/22	18/18	17/17
Per cent	94	100	93	100	96	100	100	100	100	100
Serum PI_3										
Number	10/33	11/22	9/28	9/22	11/23	17/22	19/23	21/22	14/18	14/16
Per cent	30	50	32	41	48	77	83	95	78	88
Serum I.B.R.										
Number	2/31	2/21	1/26	0/16	2/22	3/22	2/22	3/20	5/18	4/16
Per cent	6	9.5	4	0	9	14	9	15	28	25
Nasal Past. hemolytica										
Number	3/32	2/22	4/25	3/22	6/22	2/21	9/23	5/22	5/17	1/17
Per cent	9	9	16	14	28	9.5	39	23	29	6
Nasal PI_3										
Number	20/32	13/22	9/26	10/19	6/19	10/20	8/23	13/21	6/17	13/15
Per cent	63	59	35	53	32	50	35	62	35	87

and nasal antibody absent throughout the experiment, (c) the levels of serum and nasal antibody remained close to the original values throughout the experiment, and (d) serum antibody was present and nasal antibody increased in later time periods. PI-3 virus was isolated from animals in which antibody to the virus was present in both serum and nasal secretions, from others in which only nasal antibody was present and from others only after nasal antibody was no longer detectable.

Ten calves died or were killed on experiment (Table VI). Calves 1 to 5 died with extensive bilateral fibrinous pneumonia. Calves 6 and 7 had advanced clinical signs of pneumonia when they were killed. Calves 8, 9 and 10, which were killed as a check on the validity of the *sick* criteria, had bilateral bronchopneumonia of moderate severity. The lesions in the lungs of calves 6 and 7 were intermediate in severity between those of calves 8, 9 and 10 and those which died naturally (calves 1 to 5).

Points to be noted in the data concerning these 10 calves (Table VI) include the dominance of *Past. hemolytica* in cultures from the lung; the lack of viral isolations from the lung from even the very early lesions (calves 8, 9, 10); the level of serum antibody to *Past. hemolytica* I and PI-3 virus was low or absent, and that *Past. multocida* was isolated from the lungs of calves in which the ratio of the number of nasal *Past. hemolytica* to the number of *Past. multocida* was low. In addition, nasal antibody to PI-3 virus was present in all ten calves and ranged from 1:4 in calf 4 to 1:128 in calf 7.

A possible indication of the relative importance of PI-3 virus and *Past. hemolytica* in pneumonic Pasteurellosis is illustrated by a comparison of levels of serum antibody to these organisms during the period of observation in a group with 9 out of 10 *sick* animals (C) and one with 9 out of 10 *well* animals (D) (Table I). The level of serum antibody to PI-3 virus on the five days sampled (lower part of Table VII) did not differ greatly between the two groups (C and D) whereas there was a marked difference between the groups in the level of serum antibody to *Past. hemolytica* on these days. In group D (Table II) there was a significant increase in serum antibody to *Past. hemolytica* type I but not to PI-3 virus over the four week period whereas the reverse was true in group D.

DISCUSSION

The approach used for this investigation differs from that of the previous work of others in that a few animals were examined in greater detail as they became ill with Pasteurellosis under natural conditions. Previous workers concentrated on serological data and the isolation of viruses with less emphasis on bacteria, since only a few individuals have recognized the significance

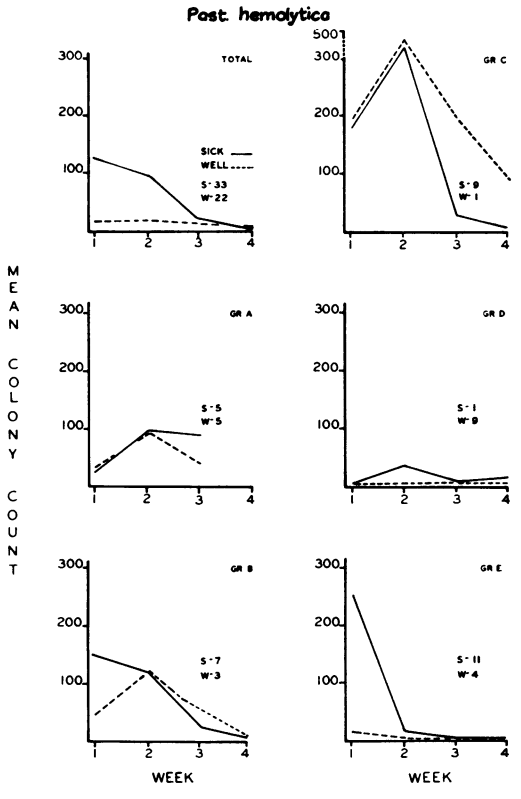

Fig. 6. This figure demonstrates the variation in mcc of Past. hemolytica in all the animals and in the five groups. Differences in what might be termed sick groups (C,E) can be compared to a well group (D).

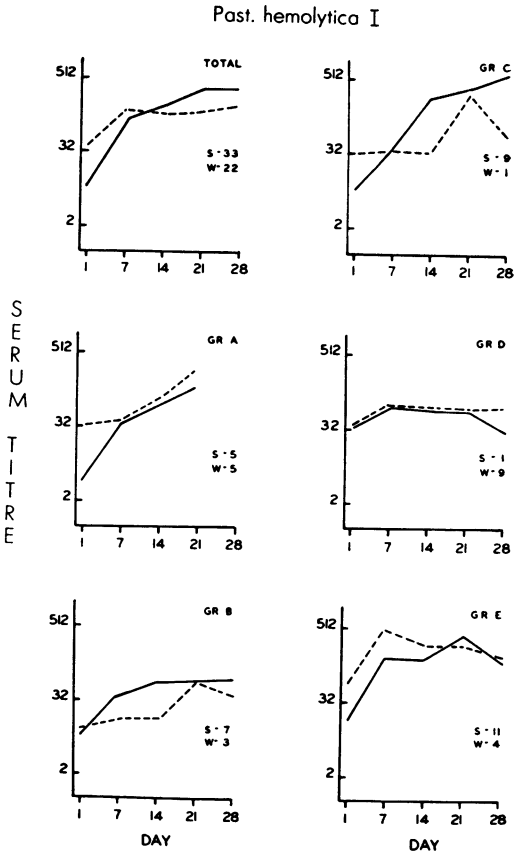

Fig. 7. The mean serum antibody titre to Past. hemolytica type I in sick and well animals can be compared in predominantly sick and well groups and on an overall basis. The solid line represents the sick animals and the dotted line the well.

of *Past. hemolytica* in this diseases (5, 6, 21). The methods developed by Magwood (16) and Duncan (7) have allowed investigation of parameters not previously studied in the naturally occurring disease.

The use of plasma fibrinogen in conjunction with body temperature allows detection of illness at a time when clinical assessment of the acute disease would be difficult or impossible. In man, plasma fibrinogen is elevated in patients with lobar pneumonia and does not rise only as a result of an increase in body temperature (8). McSherry (18) suggests that plasma fibrinogen may become a useful prognostic index in many diseases of cattle. Three factors indicate that our criteria for dividing the animals into *sick* and *well* classes were probably valid but not specific indicators of pneumonia (Pasteurellosis): (a) three animals killed as a check on pneumonic lesions immediately after fulfilling the criteria for inclusion in the *sick* class had early lesions of pneumonia, (b) prolonged high levels of plasma fibrinogen existed in animals with clinical signs of pneumonia, and (c) levels of plasma fibrinogen were elevated in most but not all of the animals in which a rise in body temperature occurred.

The overall frequency of isolation of *Past. hemolytica* reported here is higher than that reported previously by Magwood *et al* (17) in normal cattle and by Hamdy (9) in "shipping fever". According to Magwood (17) the number of these organisms in the nasal passage was the outstanding difference in the nasal flora of shipped animals compared to normal animals. In our work the frequency of isolation of *Past. multocida* was generally similar to that of *Past. hemolytica* but there was a marked difference in the two bacterial species with regard to the numbers of organisms cultured. The high frequency of isolation, and large numbers of organisms present in the nasal passages and the lungs indicates the

TABLE V. Frequency of Isolation of Virus from Nasal Swabs

Virus		Week 1		2		3		4	
		Sick	Well	Sick	Well	Sick	Well	Sick	Well
PI-3	— number[a]	2/32	1/22	0/26	1/22	1/23	0/22	0/18	1/17
	— per cent	6	5	0	5	4	0	0	6
IBR	— number	1/32	0/22	2/25	1/22	4/23	2/22	0/18	0/17
	— per cent	3	0	8	5	17	9	0	0

[a]Numerator is number of isolations and denominator is number of attempts

TABLE VI. Bacterial and Viral Recovery from the Respiratory Tract and the Immune Status of Calves which Died or were Killed on Experiment[a]

Calf	Day of Death	Nasal Mean Colony Count[b] P. hem.	P. mult.	Neis.	Nasal Isolation PI-3	IBR	Serum Titre[c] PI-3	P. hem.	Nasal Titre PI-3	Isolation from Lung[d] PI-3	P. hem.	P. mult.	Neis
1	7	450	3	7	+	0	0	4	8	0	+++	0	0
D							0	32	–				
I 2	2	1800	0	1800	0	–	–	0	–	0	+++	0	0
E 3	6	90	5	2	0	0	0	4	8	0	+++	0	0
							0		0				
D 4	2	560	235	0	0	0	0	4	4	0	+++	++	+
5	7	1465	0	0	0	0	0	16	32	0	+++	0	0
							0	0	–				
6	8	560	5	315	0	0	10	0	8	0	+++	0	0
K							10	16	16				
I 7	3	380	380	0	0	0	0	16	128	0	+++	++	++
L 8	9	10	70	0	0	0	0	64	16	0	0	+	0
L							0	128	32				
9	4	175	10	0	0	0	0	16	64	0	+	0	0
E													
10	4	200	0	2	0	0	0	4	32	0	+	0	0
D													

[a] + — positive; 0 — negative; – — not sampled; P. hem. — **Past. hemolytica**; P. mult. — **Past. multocida**; Neis. — **Neisseria** sp.

[b] number includes all samples until death

[c] — titre refers to day 1 and if a second is listed, day 7

[d] 0 — netagive; + — 1 to 30 colonies per plate; ++ — 30 to 300 colonies per plate; +++ — over 300 colonies per plate

importance of *Past. hemolytica* in this disease as had been pointed out by Collier (6) and Carter (5).

Magwood *et al* (17) were the first to consider not only the frequency of isolation of bacteria in the nasal flora of cattle but the numbers of organisms present. Magwood (16) developed the system of assessing the numbers of bacteria on nasal swabs which was also used in this work. Such a system allows statistical evaluation of the results. However, in expressing daily mean colony counts on a weekly basis, the mean for the week does not necessarily indicate either the daily fluctuations occurring during the week or the number of positive samples.

The cattle were divided into a *sick* class and *well* class for the entire experimental period but few values such as mean colony count were statistically significant between the *sick* and *well* classes over the entire period. Some values were significant over shorter periods as exemplified by the levels of nasal *Past. hemolytica* in the early time periods (Fig. 5 and Table II).

Our results suggest that the high level of bacteria (*Past. hemolytica* in particular) in the nasal passages eventually results in descent to the lungs and subsequent pneumonia (Table VI); however, there is no clear evidence to substantiate this hypothesis. In man, there is evidence that as the frequency of nasal isolations of bacteria, such as streptococci, rises, the incidence of pneumonia from which streptococci may be isolated increases, but the actual numbers of bacteria were not assessed (1).

Rice *et al* (22) used the complement fixation (CF) test for antibody to *Past. hemolytica* (serotype not distinguished) in the sera of cattle and concluded that increasing

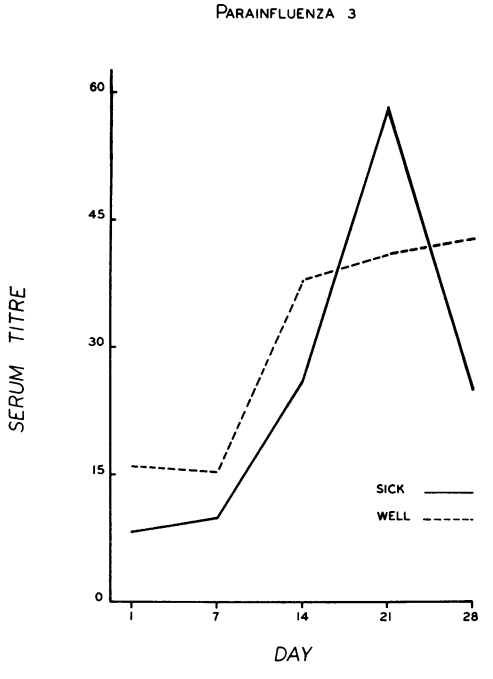

Fig. 8. The mean serum antibody titre to PI-3 virus in sick and well animals is shown. On an overall basis there was no significant difference between the two. In general the well animals had higher titres to PI-3 virus and Past. hemolytica than the sick at the start of the period of observation.

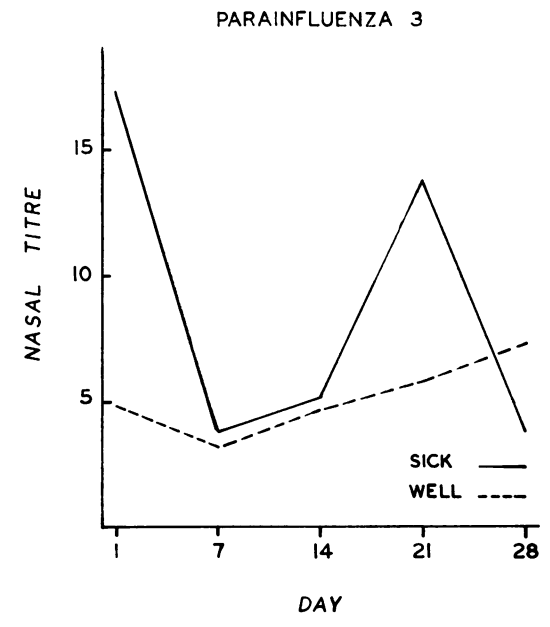

Fig. 9. The nasal antibody titres to PI-3 virus in the sick and well animals are illustrated.

levels of CF antibody in shipped animals indicated infection with *Past. hemolytica*. The IHA test was used on our animals because it indicated exposure to the organism (7) and because Cameron's work (4) suggested that it also indicated protection, at least in mice. In addition it was the only established technique which considered the serotype of the organism. The fact that significant differences in serum antibody to *Past. hemolytica* between the *sick* class and the *well* class were not present in all groups could have been due to several factors. The effect of considering all animals in the *sick* class together, rather than considering degrees of clinical illness, and time of onset of illness might tend to decrease the differences between *sick* and *well* classes within a group when analysed. The antibody measured indicated exposure but may not have actually measured protection.

There is considerable evidence that shipped cattle have an active PI-3 virus infection during or shortly after their change in location (3). Our work adds further support and demonstrates an active PI-3 virus infection on serological evidence (Fig. 8 and Table III). It was also evident that serum antibody to PI-3 virus was present in a greater number of *well* animals than *sick* animals (Table IV). This finding together with the lack of a significant antibody response to PI-3 virus in group C (Table III) would not support the contention that this virus has an obvious pathogenic role in the disease. In spite of the existence of PI-3 virus infection in shipped animals and the use of vaccines against it,

TABLE VII. Comparison of the Serum Antibody Titres to Past. Hemolytica and Parainfluenza-3 Virus in Groups C (9 sick) and D (9 well)

	Day	Group C (9 sick)	D (9 well)
Past. hemolytica	1	8[a]	16
	7	21	30
	14	130	23
	21	230	24
	28	346	30
Parainfluenza-3	1	14	3
	7	17	5
	14	36	23
	21	50	44
	28	27	12

[a]Mean of reciprocal of dilutions

a cause and effect relationship between this virus and the pneumonic lesions of Pasteurellosis has not yet been demonstrated; Sweat (25) concluded that the value of vaccines of PI-3 virus in protection against "shipping fever" has yet to be determined.

There is pathological evidence of the virulence of this virus in the lungs of calves with enzootic pneumonia (20), but there is no histological evidence of such lesions in the fibrinous pneumonia that is so characteristic of fatal cases of Pasteurellosis (12) and the two pneumonias are morphologically very different (12). There is a tendency to assume that all pneumonias are initially of viral etiology and that bacteria are secondary but a recent detailed investigation in man indicates that this assumption may not be true (19).

As outlined in the literature review by Duncan (7) nasal antibody to some virus infections in man is known to protect against respiratory infection and may be more protective than serum antibody. Our work has demonstrated antiviral (PI-3) nasal antibody in cattle for the first time and found it to occur more or less in a similar incidence to serum antibody. However, as indicated by the levels and time of occurrence of the nasal antibody in our animals, the relationship of the induction of serum antibody to nasal antibody, the specificity, the persistence and the protective effect of nasal antibody to PI-3 virus remains to be determined.

Results in man have been more encouraging with regard to protection against infection with anti-viral nasal antibody than anti-bacterial antibody (7). Duncan (7) demonstrated rises in nasal antibody in cattle after aerosol exposure to *Past. hemolytica* but did not demonstrate a protective effect or that such antibody could control the *Past. hemolytica* in the nasal flora. Our results have not demonstrated a pattern of development of nasal antibody in animals with prolonged high levels of *Past. hemolytica* in the nasal passages. At the present time, we can not account for the differences between our results and Duncan's.

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ADDENDUM

As the samples from the animals included in this paper were collected for the first time on arrival in Guelph, at least one week had elapsed from the time the animals were taken from the range. The status of the nasal flora and antibody levels were not known at the time the animals were removed from their normal environment. Therefore two groups of 10 calves each, F (November, 1968) and G (January, 1969) were purchased directly from the range and sampled during a three day period in Saskatoon prior to shipment by rail to Guelph. One sample of serum and one of nasal washings were collected on day 2. Nasal swabs were collected on days 1 and 3. The swabs were streaked onto bovine blood agar, incubated in Saskatoon and shipped via air express to be read in Guelph. The samples were collected and the plates streaked in the same manner as in the other groups. The swabs were shipped frozen for virus isolation. On arrival in Guelph the calves were sampled in a similar manner to groups A to E.

Five animals in group F were classified as *sick* and five as *well*. In group G, all ten were classified *well*. Statistical analysis was not conducted, but the results were graphed and compared to the figures in this paper. The frequency of isolation and mean colony counts of *Past. hemolytica* were markedly similar to Figs. 3, 4 and 5 during the observation period in Guelph. Group G was similar in pattern to group D in the level of serum antibody and mean colony count of *Past. hemolytica* as shown in Figs. 6 and 7. Group F had a rising level of serum antibody to *Past. hemolytica* in both *sick* and *well* animals and the peak occurred two weeks after arrival in Guelph. PI-3 virus was not isolated from any nasal swabs; the mean serum antibody levels to PI-3 virus were below 1:20 and did not rise during the period of observation in both groups. In addition, an increase did not occur in levels of nasal antibody to PI-3 in *sick* or *well* animals.

The significant features of groups F and G were the low frequency of isolation (group F=0%; group G=20%) and low numbers of *Past. hemolytica* in the nasal flora prior to shipment from Saskatoon.

The additional data was made available by the co-operation of Dr. O. M. Radostits, Dr. L. Keith and Dr. C. H. Bigland of the Western College of Veterinary Medicine in Saskatoon. Dr. Radostits purchased the animals and collected the samples.