STUDIES ON PNEUMONIA OF CATTLE
I. EXPERIMENTAL INFECTION OF CALVES WITH PASTEURELLA HAEMOLYTICA

By G. R. CARTER*

Previous reports (2, 3) have shown that *Pasteurella haemolytica* was the predominant bacterium recovered from cases of shipping fever in cattle in Canada during the fall and winter 1953-1954 and 1954-1955. The trials described below were carried out in order to determine the pathogenic potentialities of *P. haemolytica.*

Stamp and associates (7) have produced *P. haemolytica* infections in sheep by the administration of cultures via different routes. Mild infections were initiated in sheep by Florent and Godbille (6). However, there do not appear to be reports on experimental infections of calves. In six trials Carter (2) was unable to produce a shipping fever-like infection in calves by the administration of morbid tissues from cases of shipping fever.

MATERIALS AND METHODS

Experimental Calves:

These were male calves of dairy breeding ranging in age from 1½ to 4 months. They were obtained from the Ontario Agricultural College herd usually within a week after birth. Some of the calves were placed immediately in isolation units while others, of necessity, were reared in a stable housing the Ontario Veterinary College herd. There had been no history of shipping fever in recent years in either of these herds. None of the experimental calves (listed in Table 1), from which blood samples were taken prior to exposure to *P. haemolytica,* possessed anti-bodies for this bacterium, as determined by the haemagglutination test.

Cultural Methods:

Blood agar and serum beef heart infusion agar plates were employed. The former consisted of tryptose agar with 7-8 percent sheep's blood added and the latter was a fresh beef heart infusion to which was added 20 percent horse serum. The liquid medium employed was a freshly prepared beef heart infusion broth.

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Haemagglutination Test:

The tests were carried out according to the method described previously (4, 5). The samples of serum were inactivated at 56°C. for one hour. The type O human red cells were treated with a 0.3 percent solution of the capsular substance of *P. haemolytica.*

Attempts to Produce Experimental Infections

Trial No. 1:

Calves 68 and 69 were each given 5.0 ml. of a young broth culture of *P. haemolytica* intratracheally and 10.0 ml. intranasally. Sufficient mucin* was added to the broth cultures to give a final concentration of 5 percent. The calves showed no evidence of disease.

Trial No. 2:

Calf 72 was given, intranasally, 20.0 ml. of broth washings from the nasal passages of a calf with symptoms of acute shipping fever. The calf was observed for longer than a month and there was no evidence of disease except occasional coughing.

On necropsy the apical lobes were partially consolidated and *P. multocida* was the only organism recovered.

Trial No. 3:

Calf 73 was given, intranasally, a serum agar plate culture that had been comminuted in 50.0 ml. of tryptose phosphate broth. This inoculum and subsequent ones prepared from agar plate cultures contained both the growth and the agar. The serum agar plate had been inoculated from a nasal swab from an acute case of shipping fever.

On the 20th day after exposure the calf showed a rise in temperature. The temperature readings were as follows:

Days After Exposure	A.M.	P.M.
20	105.3	105.0
21	104.6	103.2
22	104.5	104.2
23	103.2	103.0
24	103.4	Killed

On the 20th day the calf was markedly depressed and would take only a small amount of milk and no pellets. Stethoscopic examination revealed diffusely congested lungs.

* Gastric mucin, Nutritional Biochemicals Corporation.

At necropsy, the apical, cardiac and intermediate lobes of the lungs were found to be consolidated. *P. haemolytica* in large numbers was the only organism recovered from the affected lungs.

Calf 74 was exposed in the same manner as calf 73. The nasal swab was taken from another acute case of shipping fever. The calf remained normal and necropsy showed no evidence of pneumonia.

Trial No. 4:

P. haemolytica isolated from the lungs of calf 73 was used in this trial. Calf 70 was given, intranasally, 20.0 ml. of a preparation consisting of a primary blood agar plate culture of *P. haemolytica* comminuted in 25.0 ml. of broth. Five ml. of this preparation were given intratracheally. The calf remained normal. Necropsy revealed no evidence of pneumonia.

Calf 71 was treated the same as calf 70. The exposure had no apparent effect on its health.

Trial No. 5:

Calf 66 was given, intranasally, a primary blood agar plate culture of *P. haemolytica* comminuted in 50.0 ml. of broth. The blood agar plate had been inoculated with a nasal swab from an acute case of shipping fever. The culture appeared to be pure *P. haemolytica*. The following temperatures were recorded:

Days After Exposure	A.M.	P.M.
2	102.8	103.6
3	104.2	105.4
4	105.4	105.8
5	103.8	102.0
6	Killed	—

On the 4th day there was an abundant nasal discharge and persistent coughing. The calf lost condition rapidly.

On necropsy, the right lung was found to be normal but the left lung displayed areas of congestion in the apical and cardiac lobes. *P. haemolytica* was recovered from the nasal passages but not from the lung.

Calf 67 was exposed in the same manner as calf 66. The strain of *P. haemolytica* employed was isolated from a nasal swab from calf 66. The calf did not develop any apparent illness.

Calves 03 and 04 were exposed in the same manner as calf 66. Although neither calf became markedly ill, both showed occasional temperature increments with depression and inappetance. Calf 04 had afternoon temperatures as high as 104.6 and 105.2. However, both calves appeared quite healthy 10 days after exposure.

Trial No. 6:

Calves 01 and 02 were each given, intranasally, 20.0 ml. of a 24-hour chicken embryo culture of *P. haemolytica*. The eggs were inoculated in the same manner as described previously (1). Only the fluids of the infected 10-day embryos were given to the calves.

None of the calves showed evidence of an infection.

Trial No. 7:

Suspensions of morbid lung from an acute fatal case of shipping fever were administered intranasally to calves 07 and 08. No evidence of disease appeared.

Trial No. 8:

Calves 14 and 15 were each given, intranasally, 20 ml. of a 6-8 hour broth culture of *P. haemolytica* and 30 ml. of citrated blood intravenously. The blood was taken from a pool of blood equal parts of which were taken from three calves with acute shipping fever. The temperatures were:

Calf 14			Calf 15		
Days After Exposure	A.M.	P.M.	Days After Exposure	A.M.	P.M.
4	103.8	104.0	4	102.8	102.4
5	104.0	105.0	5	104.0	103.2
6	103.0	103.2	6	104.6	104.2
7	104.0	104.2	7	103.4	103.8
8	103.0	103.4	8	102.8	102.6
9	102.0	102.6	9	103.0	104.2
10	102.0	102.0	10	103.2	103.6
11	102.4	101.8	11	102.6	103.0
12	101.6	102.0	12	101.8	Died
13	Recovered	—			

During the course of the illness, *P. haemolytica* was recovered from the nasal cavities of both calves in almost pure culture.

Necropsy of calf 15 revealed an acute pneumonia with extensive consolidation. Fibrotic and suppurative processes suggested that the calf had probably suffered from a chronic pneumonia prior to exposure to *P. haemolytica*. *Corynebacterium pyogenes* and *P. haemolytica* were recovered from the lungs.

Calves 16 and 17 were exposed in the same manner as calves 14 and 15. Moderate temperature rises were registered for both calves but severe illness did not ensue.

Necropsy of calf 16 revealed limited pneumonic lesions involving chiefly the left apical lobe. *P. haemolytica* and *C. pyogenes* were recovered from the lungs.

Necropsy of calf 17 revealed limited pneumonic areas from which *C. pyogenes* was recovered but not *P. haemolytica*.

Trial No. 9:

Calves 13, 20 and 21 were each given, intranasally, 10 ml. of a young broth culture of *P. haemolytica* isolated from calf 14.

Other than displaying several rises in temperature with occasional coughing, calves 13 and 20 remained normal.

Calf 21 developed an acute pneumonia which resolved early. The following temperatures were recorded:

Days After Exposure	A.M.	P.M.
2	103.0	103.0
3	105.2	105.6
4	104.6	103.8
5	103.8	103.4
6	103.2	103.6
7	102.0	102.0
8	Recovered	

Serological Tests on Sera of Calves:

Blood samples were taken from some of the calves prior to exposure and after exposure to determine if there was an antibody response to *P. haemolytica.* The serological method employed was the previously described haemagglutination test (4, 5). The results of the tests are given in Table 1. Haemagglutinating titers were not observed in the sera taken from calves prior to exposure.

TABLE 1

Haemagglutination Titers for *P. haemolytica:* Sera Taken After Exposure

	Reciprocals of Dilutions				
Calf	20	40	80	160	180
13	+	+	+	+	−
14	−	−	−	−	−
16	+−	−	−	−	−
17	−	−	−	−	−
20	+−	−	−	−	−
21	+−	−	−	−	−
01	+	+	+−	−	−
02	+	+	+	+−	−
03	+	+	+	+−	−
05	+	+	+	+	+−
08	+	+	+	+	+−
04	+	+	+	+	+−
67	+	+	+	−	−
68	+	+	+	−	−
69	+	+	+−	+	−

DISCUSSION

One can conclude from the results of these trials that *P. haemolytica* is capable of causing mild to severe infections in calves. The most severe infections were seen in calves that would appear to have had inapparent chronic pneumonia prior to exposure to *P. haemolytica.*

This observation prompted an examination of a large number of lungs taken from calves slaughtered as normal at a local abattoir. It was noted that approximately 10 percent of the lungs displayed lesions of chronic pneumonia. This pneumonia has been successfully transmitted experimentally and has been shown to be caused by a filtrable agent. It will be the subject of the second communication in this series.

The results of the haemagglutination tests on cattle with experimental infections confirm the conclusion that *P. haemolytica* produces infections in cattle. Antibodies for *P. haemolytica* have been demonstrated in the cattle of three herds which sustained severe outbreaks of shipping fever attributable to *P. haemolytica.*

SUMMARY

Nineteen calves were exposed to *P. haemolytica* in various ways. The usual procedure was to inoculate calves intranasally with young primary cultures of *P. haemolytica.* By this means, eight clinical infections were initiated. *P. haemolytica* was recovered at necropsy from calves showing clinical signs of pneumonia.

Bacteriological and pathological observations of the lungs of calves with *P. haemolytica* infections suggested that some of the experimental calves had been affected with chronic pneumonia prior to exposure. It is postulated that the inapparent chronic pneumonias contributed to severe *P. haemolytica* infections in some of the calves.

Blood samples were taken from the experimental calves before and after exposure to *P. haemolytica.* Antibodies for *P. haemolytica* were demonstrated after exposure but not before.

RESUME

L'auteur rapporte le cas de 19 veaux exposés à *P. haemolytica* de diverses façons. L'inoculation expérimentale des veaux fut habituellement faite par voie intranasale en utilisant des cultures primaires jeunes de *P. haemolytica.* Par cette méthode l'auteur a réussi à produire huit infections cliniques et il a retrouvé *P. haemolytica* chez tous les animaux présentant des symptômes de pneumonie.

Le résultat de l'examen bactériologique et anatomo-pathologique des poumons de ces animaux suggère que quelques-uns des veaux souffraient déjà de pneumonie chronique au moment de leur infection expérimentale. L'auteur croit que la pneumonie chronique inapparente est responsable de la gravité des symptômes présentés par certains des veaux.

Les anticorps de *P. haemolytica* ont été mis en évidence dans le sang des veaux prélevé après l'inoculation expérimentale mais non avant.

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AIRBORNE TRANSMISSION OF GASTROENTERITIS IN BABY PIGS

Transmissable gastroenteritis is a highly infectious disease of the intestinal tract of baby pigs. The possibility of airborne transmission for short distance, 2 to 3½ feet, has been demonstrated experimentally at the time of the initial appearance of vomitus and diarrhoea; the incubation period in 4 to 6 day old Hampshire pigs so exposed, ranged from 18 to 24 hours (Reber, R. F., Amer J. Vet. Research, 17:194, 1956).

MUCOSAL DISEASE IN DEER

During the spring and summer of 1955, several young mule and white-tailed deer were found sick or dead in various parts of North Dakota. Catarrhal, ulcerative and or haemorrhagic inflammation of the gastrointestinal tract were observed at autopsy. Richards, Schepper, Eveleth and Shumard (Vet. Med., 51:358, 1956) have reported on successful transmission of the condition from deer to deer and from deer to antelope, using infected deer spleen or blood. Deer inoculated with spleen material from cattle that died of mucosal disease developed identical symptoms and pathology. The stringy mucoid nasal discharge and lachrymation with erosion around the eyes, which are the outstanding symptoms of mucosal disease in cattle, were seldom seen in deer. The absence of oral lesions in the deer may result from their tougher oral mucosa.

CANINE HISTOPLASMOSIS IN ONTARIO

In a recent issue of the Canadian Medical Association Journal (74:734-735, 1956), Fish, Schroder and Fischer have described a case of canine histoplasmosis which they believe to be the first reported in Ontario and possibly in Canada. The animal which died shortly after admission, was much emaciated and had a history of chronic cough and laboured breathing. At necrospy, the entire parietal pleura was covered with small nodules; larger nodules were seen on the mediastinum and lung surface. The thoracic lymph nodes were much enlarged. Focal necrosis with very profound reticulo-endothelial cell proliferation was noted in liver, spleen, and lymph nodes. Many of the macrophages were filled with spherical basophilic staining bodies with a colourless halo. These bodies, morphologically typical of *Histoplasma capsulatum*, were also seen free in the tissue spaces. The organisms were isolated on horse-blood agar and Sabouraud agar plates. White mice inoculated intraperitoneally with these cultures when destroyed three months later, showed numerous *H. capsulatum* organisms in liver, and spleen.

ANTIBIOTICS IN THE TREATMENT OF VIRUS PNEUMONIA IN PIGS

A dosage of 10 to 20 mg. per kg. of bodyweight of the antibiotics tetracycline or exytetracycline has been found by Lannek and Bornfors (Vet. Rec. 68: 53, 1956) to completely or nearly completely inhibit the development of virus pneumonia in pigs. In two experiments 40 pigs were inoculated intranasally and later exposed to pigs with spontaneous pneumonia; the third experiment was a field trial with 60 pigs.

VITAMIN B SUPPLEMENT FOR HORSES

A distinct improvement in the appearance and condition of debilitated horses was observed after 8 weeks feeding of a high energy diet supplemented by vitamin B_{12} (Clifford, Henderson, and Wilkins, Vet. Rec. 68: 41, 1956).