Porcine Hemophilus parahemolyticus Pneumonia in Saskatchewan
I. Natural Occurrence and Findings

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ABSTRACT

An outbreak of fibrinous pleuropneumonia was observed in October 1971 in Saskatchewan on a farm of 900 feeder pigs. Morbidity and mortality were low. Pathologic-anatomic findings included fibrinous pleuritis, pulmonary vascular thrombosis and necrotizing fibrinous pneumonia. Hemophilus parahemolyticus was isolated from the lungs of affected animals. In addition pulmonary lesions were found which suggested an adenovirus infection. It was speculated that the viral infection possibly predisposed the pigs to the Hemophilus infection. The H. parahemolyticus isolate was sensitive to common antibiotics.

RÉSUMÉ

En octobre 1971, une éruption de pleuropneumonie fibrineuse a sévi en Saskatchewan, dans une porcherie où on gardait 900 porcs d'engraissement. La morbidité et la mortalité s'avérèrent peu élevées. Les changements anatomo-pathologiques se traduisirent par de la pleurésie fibrineuse, de la thrombose et de la nécrose des vaisseaux pulmonaires et de la pneumonie fibrineuse. Les auteurs isolèrent Hemophilus parahemolyticus des poumons des porcs malades. Ils décelèrent en plus des lésions pulmonaires suggestives d'une infection à adénovirus et présumèrent que cette infection virale aurait prédisposé les porcs à l'infection par H. parahemolyticus, lequel s'avéra sensible aux antibiotiques usuels.

INTRODUCTION

Bacteria of the genus *Hemophilus* and species *parainfluenzae, pleuropneumoniae* or *parahemolyticus* have been reported to cause pleuropneumonia in pigs (Table I). The first description, judging by photomicrographs, was given by Pattison *et al* (24) who described an "Hemophilus-like" isolate which was later identified as *H. parainfluenzae* (13). Shope (27) and Shope *et al* (28) reported an outbreak of porcine contagious pleuropneumonia in Argentine due to *Hemophilus pleuropneumoniae*. This isolate was found to be closely related to *H. parasuis* (30).

Additional outbreaks of this disease attributed to *H. parahemolyticus* were observed by Olander (23) in California and by Nicolet *et al* (15, 16, 17) and Häni *et al* (6, 7) in Switzerland. Nicolet *et al* (15, 16. 17) found their isolate and that of Shope (27) to be similar. Observations by Nielsen (19, 20), Häni *et al* (6, 7) and Bachmann (1) indicated that *H. parahemolyticus* pneumonia in pigs is rather widespread in Denmark and Switzerland. The Danish isolates were found to be identical to one of the Swiss strains (Serotype 2).

This paper describes an outbreak of *H. parahemolyticus* pleuropneumonia in Saskatchewan, Canada.

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TABLE I. Infections in Pigs Attributed to Hemophilus spp

Causative Agent	Name of Disease	Prevalent Lesion	References
H. suis H. parasuis	Glässer's Disease	Fibrinous polyserositis	11, 12, 23
H. parainfluenzae		Pleuritis and necrotizing pneumonia	13, 23
H. parainfluenzae		Pleuritis and pneumonia	22
H. parainfluenzae		Septicemia	29
H. parainfluenzae		Meningitis and pericarditis	25
H. parainfluenzae		Enzootic pneumonia-like lesions	14
H. pleuropneumoniae	Porcine contagious pleuropneumonia	Pleuritis and necrotizing pneumonia	27,28
H. parahemolyticus		Pleuritis and necrotizing pneumonia	23
Type 1 (Dutch isolate or syn. H. pleuropneumoniae)	Hemophilus-pleuropneumonia	Pleuritis and necrotizing pneumonia	16
Type 2 (Swiss, Danish or Swedish isolate)	Hemophilus-pleuropneumonia	Pleuritis and necrotizing pneumonia	16
Type 3 (U.K. or a Swiss isolate)	Hemophilus-pleuropneumonia	Periarticular and pulmonary abscesses	5, 6, 16
Type K 17 (North American isolate)	Hemophilus-pleuropneumonia	Pleuritis and necrotizing pneumonia	this paper

METHODS OF DIAGNOSIS

CLINICAL FINDINGS

In October 1971 a respiratory disease occurred in a group of 900 feeder pigs in Saskatchewan. The pigs were housed in a new barn with open pens. Ventilation was good and no dust problems had been previously experienced. Whereas the sows and older pigs did not show any signs, groups of three month old pigs developed anorexia, vomition and hemorrhage from the mouth and nostrils. Although coughing and dyspnea were not observed, the attending veterinarian suspected pneumonia. Clinical examination of the pigs revealed body temperatures around 39.9°C, pulse rates of 160 per minute and respiration rates of 50 per minute with noticeably increased vesicular sounds on auscultation of the lungs. Ten pigs were found dead without premonitory signs of disease. Treatment with NF 180 concentrate[1] and Tylan[2] was instituted immediately and four animals were submitted to the Department of Veterinary Pathology for necropsy.

Fig. 1. Fibrinous pleuritis and consolidation of apical and cardiac lobes in a pig with Hemophilus parahemolyticus **infection.**

[1]**150 gm furazolidone/lb; Norwich Pharmacal Co. Ltd., Paris, Ontario.**

[2]**Tylosin, Elanco, Division of Eli Lilly Co., Indianapolis, Indiana.**

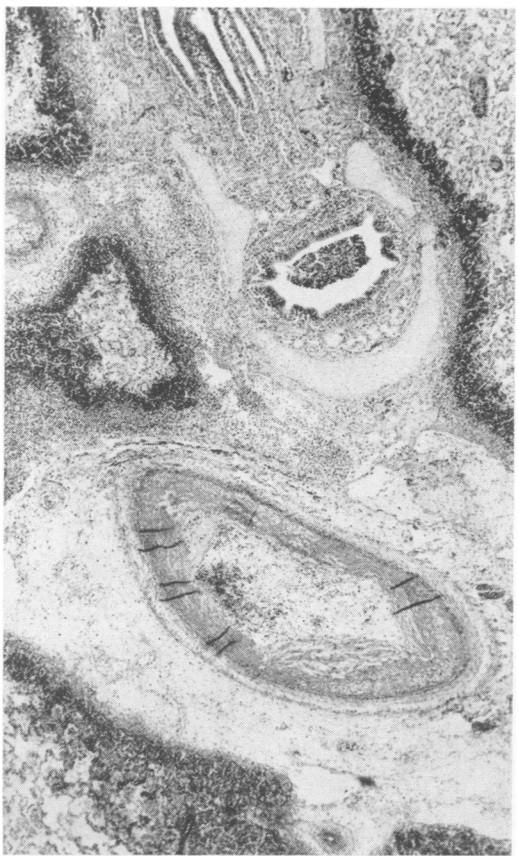

Fig. 2. Irregular necrosis, demarcated by a zone of cellular infiltration. Thrombosed vessels are shown. H & E. X40.

Gross Findings

All pigs were in fair to good bodily condition. There was a bluish discoloration of the skin of the abdomen and ears. Gross lesions were restricted to the respiratory system with the exception of one pig which had an increased amount of serosanguinous fluid in the pericardial sac. Tracheas contained fine, blood-tinged froth. In two cases the thoracic cavity was filled with dense sheets of fibrinous exudate while in the other animals the fibrinous coating was found only in localized areas overlying pulmonary lesions (Fig. 1). Firm dark red irregular shaped infarct-like areas were scattered through the lungs, involving entire lobes in some instances. Firm nodules could be palpated in the depths of the parenchyma of all lobes. Interlobular septa were edematous. On cross section of the lung the focal infarct-like pattern was more noticeable and areas of necrosis could be identified grossly.

Bronchial lymph nodes were moderately enlarged and firm. Other lesions such as endocarditis, arthritis or meningitis were not found.

A tentative diagnosis of fibrino-purulent pleuropneumonia was made and samples were submitted for bacteriological and histological examination.

Histopathological Findings

Samples for histopathological examination were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 6 μm and stained with hematoxylin and eosin (H & E) and phosphotungstic acid hematoxylin (PTAH) (4).

The most striking lesions were seen in the lung. At a low power magnification the sections had irregularly shaped areas outlined by basophilic bands composed of

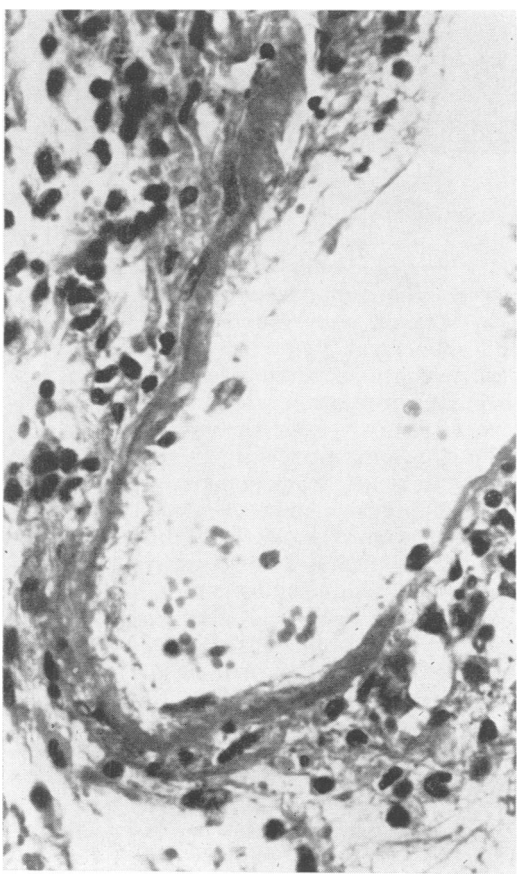

Fig. 3. Fibrinoid necrosis of pulmonary vessel wall. H & E. X40.

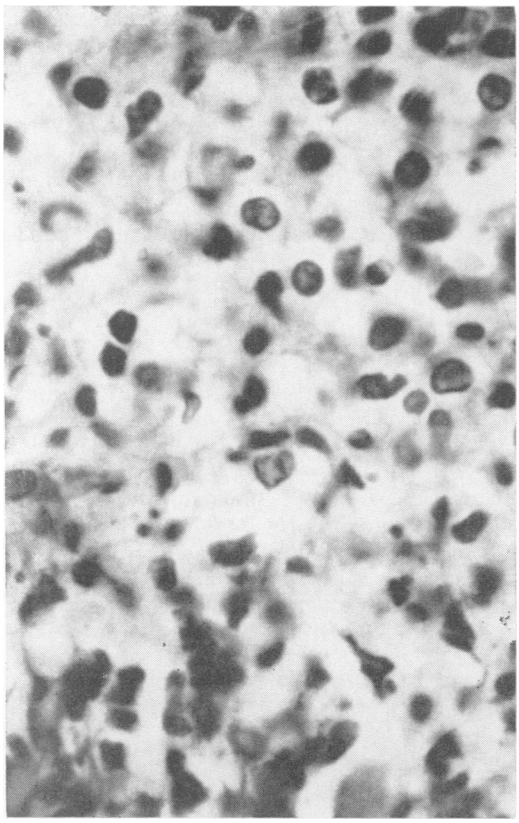

Fig. 4. Cellular reaction at the line of demarcation. Note the absence of polymorphonuclear granulocytes. H & E. X1000.

densely packed cells surrounding either necrotic pulmonary tissue or lymphatics. Fibrin thrombi were found in the interstitial and subpleural lymph vessels and a fibrinous exudate covered the pleura. Acute thrombus formation was present in the lumen of many vessels throughout the lungs (Fig. 2). Some vessels in the affected areas had thickened homogenous walls which stained intensely pink (Fig. 3). At higher magnification the dark staining cellular elements resembled degenerating macrophages and lymphocytes rather than granulocytes (Fig. 4). The alveoli were filled with fibrin and desquamated alveolar cells. Bronchi and bronchioles, particularly in areas free of fibrinous exudate, contained a small amount of cellular debris. Hyperemia and microhemorrhage were seen throughout the lungs. Alveolar and interstitial edema were widespread.

The pulmonary interstitial tissue in unaffected areas had slightly increased cellularity. High power magnification revealed that numerous mesenchymal cells as well as alveolar macrophages had undergone nuclear changes. The nuclear membrane stained darker than normal and basophilic inclusions were found in the nucleus (Fig. 5). These inclusion bodies resembled those described in adenovirus infections (8, 26).

Vascular thrombosis was also observed in the bronchial lymph nodes, in larger vessels of the kidney and in smaller vessels of the gastric mucosa. The PTAH-stain failed to demonstrate thrombi in glomeruli nor was there evidence of disseminated intravascular coagulation in other organs examined such as spleen, liver, myocardium, and adrenal glands.

Bacteriological Findings

Initial routine bacteriological culture of porcine lung tissue yielded only scant colonies of *Escherichia coli* and a non-hemoly-

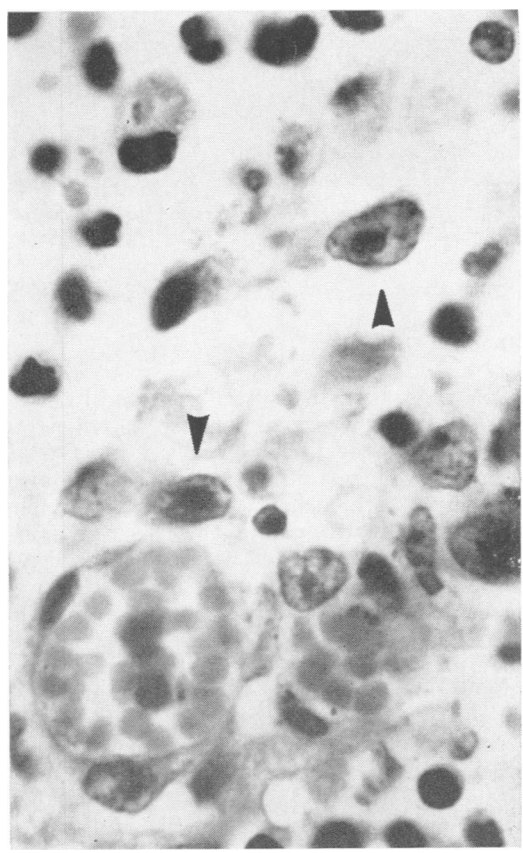

Fig. 5. Hyperchromasia of nuclear wall and basophilic intranuclear inclusions in two cells (arrowed) which suggest adenovirus infection. H & E. X1000.

tic *Streptococcus* sp. neither of which was considered to be significant.

Histopathological evidence of *Hemophilus*-like infective process prompted a reinvestigation of tissue frozen at the time of necropsy.

Pulmonary tissues were smeared on 5% sheep blood agar plates on which a cross-streak of *Staphylococcus aureus* (SA) had been made to provide a source of diphosphopyridine-nucleotide (DPN or V factor). Cultures were incubated at 35°C in an atmosphere of approximately 10% carbon dioxide. Examination after 24 hours revealed a heavy growth of small, translucent, mucilaginous, hemolytic colonies which diminished in size distant from the SA and failed to grow more than 1.5 cm from the streak. Subsequent studies showed that this *Hemophilus*-like isolate did not require hemin (X factor) but it did require V factor.

Subcultures flourished on trypticase soy agar on which V factor impregnated discs were placed but the bacteria died rapidly after 48 hours.

Inoculation in appropriate biochemical reagents into which V factor had been added produced reactions which resembled closely those reported by Nielsen (20) to be characteristic for *H. parahemolyticus*. Identity of the first and subsequent isolates was confirmed as *H. parahemolyticus* strain K17 by Drs. Nicolet and Nielsen. The Saskatchewan isolate was sensitive to 12 common antibiotics and five common chemotherapeutic agents when tested by the *in vitro* disc method.

Lung tissue which had been frozen was later cultured in a medium described by L'Ecuyer (10) for the isolation of porcine mycoplasmas. No mycoplasmas were detected.

DISCUSSION

Based on gross, histological and bacteriological findings in this case a diagnosis of fibrinous, necrotizing pleuropneumonia due to *Hemophilus parahemolyticus* was made. As this is the first reported outbreak of the disease in Saskatchewan[3] to date it is impossible to comment on the incidence of the disease in this region. However, morbidity and mortality figures in the outbreak described in this paper were comparable with the average figures for Denmark where a morbidity of 8.5%-40% and a mortality of 0.4-24% have been reported (19).

The Saskatchewan isolate was serologically distinct from European types 1, 2 and 3 and it was isolated first in 1963 by Biberstein (2) in California from a lamb with arthritis.

Predisposing factors may be necessary to precipitate a severe outbreak and in this case there was evidence that an adenovirus infection was present. Porcine adenovirus infections are quite common but this virus alone rarely causes a clinically recognizable pneumonia (3, 5, 8, 26). Evidence was not found of a mycoplasma infection. Antibiotic treatment was instituted early in the course of the disease outbreak described in this paper. Since the agent was sensitive to common antibiotics it seems probable that some animals treated in the early stages of the disease recovered without residual signs of illness. Unfortunately, no follow-up of the surviving animals was made and it is not known what percentage of animals had old, encapsulated foci of necrosis at the time of slaughter.

The most intriguing aspect of this case is the type of lesions which were produced. The inflammatory exudate induced by *H. parahemolyticus* was quite different from those described for *Pasteurella* spp. or *Hemophilus suis* infections (9). Polymorphonuclear granulocytes were not prominent, whereas lymphocytes and macrophages were present in large numbers. Vascular damage, as evidenced by numerous thrombi in the vessels of the lungs and lymph nodes was reminiscent of endotoxin action. It has been speculated that generalized vascular damage and renal cortical necrosis might be the result of a reaction to bacterial endotoxin (6, 7, 21). In our cases, however, neither disseminated intravascular coagulation nor renal cortical necrosis was observed.

Hemophilus parahemolyticus is associated with necrotizing fibrinous pleuropneumonia whereas *H. parainfluenzae* is reported to cause a systemic disease (Table I). However, protoplasts of *H. parainfluenzae* can produce lesions which resemble those of enzootic pneumonia (14) and porcine pneumonic lungs when appropriately cultured

[3]Since this paper was written, two subsequent outbreaks have occurred.

have yielded various *Hemophilus* spp. relatively commonly (11). It is not unreasonable to assume that hemophilus pneumonia may be more commonplace than is generally supposed. A complement fixation test can detect infected pigs. Using an immunofluorescent technique the infective agent has been demonstrated in the tonsils of pigs which were serologically positive (1).

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