Comparative Virulence of Porcine *Haemophilus* Bacteria

S. Rosendal, D.A. Boyd and K.A. Gilbride*

ABSTRACT

The virulence of strains of *Haemophilus pleuropneumoniae* serotype 1, 2, 3, 7 and strains of the "minor-group" and *Haemophilus parasuis* were compared by inoculating specific pathogen-free pigs into the lower airways with specified doses of bacteria. *Haemophilus pleuropneumoniae,* strain W, serotype 1, given in 1 x 10^8 colony-forming units, produced a lethal acute pleuropneumonia in four pigs. Nonlethal localized pulmonary necrosis was induced in four groups of two pigs given 1 x 10^7, 1 x 10^6, 1 x 10^5 and 1 x 10^4 respectively of the same strain. Two groups of four pigs developed chronic lesions when inoculated with 1 x 10^7 colony-forming units of *H. pleuropneumoniae,* strain Shope 4074, serotype 1 and 1 x 10^7 colony-forming units of *H. pleuropneumoniae,* strain WF83, serotype 7, respectively. Of 20 pigs given 1 x 10^8 colony-forming units of strain 1536, serotype 2, two died of acute pleuropneumonia and 18 had lesions of pulmonary necrosis or abscessation and pleuritis. A dose of 4 x 10^9 colony-forming units of strain BC181, serotype 3, induced pulmonary necrosis similar to the lesions in pigs given 10^7 colony-forming units or less of strain W, serotype 1, suggesting that the serotype 3 strain is less virulent. No clinical signs, but focal areas of pulmonary fibrosis and pleural adhesions were induced in four pigs inoculated with 4 x 10^9 colony-forming units of the "minor-group" strain 7ATS. Similarly, four pigs inoculated with "minor-group" strain 33PN did not show clinical signs, but had focal necrotic and fibrotic pulmonary lesions and pleural adhesions. The virulence of "minor-group" strains is therefore probably low. Strain E751 of *H. parasuis* induced septicemia and Glasser's disease without evidence of meningitis in two pigs. Except for spleen and pericardial fluid of one pig, none of the pigs exposed to *H. pleuropneumoniae* strains were positive for these bacteria outside the respiratory tract when cultured at necropsy.

Results of this study suggest that differences in virulence between serotype 1, the strains of serotype 3, and the "minor-group" are of potential importance in comparative studies on microbial virulence factors.

Key words: *Haemophilus,* pleuritis, pneumonia, swine, pathology, virulence.

RÉSUMÉ

Cette expérience consistait à comparer la virulence de souches des sérotypes #1, #2, #3 et #7 d'*Haemophilus pleuropneumoniae,* de même que celle de souches du groupe mineur, et d'une souche d'*Haemophilus parasuis,* en les inoculant dans les bronches de porcs exempts de germes pathogènes spécifiques. L'injection de 1 x 10^8 unités formatrices de colonies de la souche W du sérotype #1 d'*H. pleuropneumoniae* produisit une pleuro-pneumonie fatale, chez quatre porcs. Une nécrose pulmonaire focale, mais non mortelle, résulta de l'injection respective de 1 x 10^7, 1 x 10^6, 1 x 10^5 et 1 x 10^4 unités formatrices de colonies de cette souche, à quatre groupes de porcs qui en comptaient chacun deux. Deux groupes de quatre porcs dont l'un avait reçu 1 x 10^7 unités formatrices de colonies de la souche Shope 4074, i.e. du sérotype #1 d'*H. pleuropneumoniae,* et l'autre, la même dose de la souche WF 83, i.e. le sérotype #7 de cette bactérie, développèrent des lésions chroniques. Des 20 porcs auxquels on avait injecté 1 x 10^8 unités formatrices de colonies de la souche 1536, i.e. du sérotype #2 de cette bactérie, deux moururent d'une pleuro-pneumonie aiguë et 18 développèrent des lésions de nécrose pulmonaire qui s'accompagnaient d'abcès et de pleurésie. L'injection de 4 x 10^9 unités formatrices de colonies de la souche BC 181, i.e. du sérotype #3 de cette bactérie, provoqua une nécrose pulmonaire semblable à celle des porcs qui avaient reçu jusqu'à 1 x 10^7 unités formatrices de colonies du sérotype #1, indice d'une virulence moins grande de la part du sérotype #3. L'inoculation de 4 x 10^9 unités formatrices de colonies de la souche 7 ATS du groupe mineur à quatre porcs n'entraîna pas de signes cliniques, mais elle provoqua des foyers de fibrose pulmonaire et d'adhérences pleurales; l'inoculation d'une dose identique de la souche 33PN du même groupe produisit des résultats similaires et en plus de la nécrose pulmonaire focale, indice d'une virulence probablement moins grande de la part des souches du groupe mineur. La souche E751 d'*H. parasuis* produisit une septicémie et la maladie de Glasser, sans évidence de méningite, chez deux porcs. La culture de divers organes des porcs expérimentaux ne se solda jamais par l'isolement d'*H. pleuropneumoniae* ailleurs que dans leurs organes respiratoires, à l'exception de la rate et du liquide péricardique de l'un d'eux.

Les résultats de cette expérience permettent de penser que les différences entre la virulence des souches des sérotypes #1 et #3, ainsi que de ceux du groupe mineur, pourraient revêtir une certaine importance, lors d'études comparatives sur les facteurs de virulence microbienne.

Mots clés: *Haemophilus,* pleurésie, pneumonie, porcs, pathologie, virulence.

INTRODUCTION

Pleuropneumonia in pigs caused by

*Department of Veterinary Microbiology and Immunology, Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

Submitted April 2, 1984.

Haemophilus pleuropneumoniae is a serious problem in modern industrialized swine production in most parts of the world. Seven serotypes have been recognized; whether they are equally virulent is not clear. Recent studies comparing serotypes 1 through 6 indicate that serotype 3 is less virulent than the remaining types (1). In a survey of 240 slaughtered pigs we isolated several strains of the so-called "minor-group" (2) which is a group of porcine *Haemophilus* strains of uncertain taxonomical position (3). The significance of the "minor-group" in pneumonia of pigs needs evaluation. The purpose of this study was to compare the virulence of *H. pleuropneumoniae* serotype 1, 2, 3 and 7 and to evaluate the virulence of two field strains of the "minor-group". A strain of *H. parasuis* was included for comparison.

MATERIALS AND METHODS

ANIMALS

Eighty-seven specific pathogen-free (SPF) pigs of 20 to 25 kg body weight were obtained from the Arkell Swine Research Station Centre of the Ontario Ministry of Agriculture and Food. They were free of pleuropneumonia caused by *H. pleuropneumoniae* and were negative for complement fixing antibodies to *H. pleuropneumoniae* serotypes 1, 2, 5 and 7 (4). The pigs were housed in enclosed isolation units with each experimental group kept separately. They were fed nonmedicated pig grower ration and water.

EXPERIMENTAL DESIGN

Four experiments were carried out as designed and shown in Table I.

PREPARATION AND STANDARDIZATION OF INOCULUM

The *Haemophilus* strains used are listed in Table I. All *H. pleuropneumoniae* strains originated from field cases of pleuropneumonia. Strains Shope 4074, 1536 and WF83 are reference strains of serotype 1, 2 and 7 respectively (5). Strains 7ATS and 33PN were isolated from tonsil and nose of slaughtered pigs without evidence of pneumonia (2). *Haemophilus parasuis,* straing E751 was obtained from J. Stevens, Animal Diseases Research Institute, Agriculture Canada, Nepean, Ontario, Canada.

The strains were cultured on trypticase soy agar (TSA) with 5% calf blood and 0.1% NAD (Nicotinamide adenine dinucleotide, Eastman Kodak Co., Rochester, New York). A heavily inoculated plate culture incubated overnight at 37°C in ordinary atmosphere was suspended in 10 mL of phosphate buffered saline pH 7.4 (PBS). Except in experiment 4 this suspension was diluted with PBS to give an optical density (OD) reading of 0.2 at 625 nm using a spectrophotometer (Spectronic 20, Bausch and Lomb, Rochester, New York). Previous experiments had established that this density was equivalent to 1×10^8 colony-forming units (CFU) per mL. The OD 0.2 suspension was diluted in tenfold serial dilutions in PBS to provide inoculation material for the pigs in experiment 1. The pigs in groups F and G received a 1:100 dilution of the OD 0.2 suspension and the pigs in group I received a 1:10 dilution. No attempts were made to dilute or concentrate the standardized bacterial suspensions used for inoculation in experiment 4, except in the case of *H. parasuis,* E751 which was cultured in 200 mL of tryptone yeast extract broth (6) centrifuged and resuspended in 20 mL of PBS. The actual concentrations of bacteria in the inocula were determined by standard plate-counting techniques.

INOCULATION TECHNIQUE

The pigs were restrained in dorsal recumbency and a 13-gauge needle inserted into the trachea distal to the larynx. Plastic tubing of 1 mm diameter was introduced through the needle and into the lower airways. Ten mL of inoculum was injected through the tubing. Both needle and tubing were withdrawn immediately thereafter.

NECROPSY

All pigs, except those in experiment 3, were observed for 14 days after

TABLE I. Experimental Design of Experiments to Determine Virulence of Various Strains of *Haemophilus* Bacteria Isolated from Pigs

Experiment	Inoculum	Group	No. of Pigs	Inoculation Dose
1	*H. pleuropneumoniae,* strain W, serotype 1	A	4	1×10^8 CFU
		B	2	1×10^7
		C	2	1×10^6
		D	2	1×10^5
		E	2	1×10^4
2	*H. pleuropneumoniae,* strain Shope 4074, serotype 1	F	4	1×10^7
	H. pleuropneumoniae, strain WF83, serotype 7	G	4	1×10^7
	Phosphate-buffered saline	H	4	—
3	*H. pleuropneumoniae,* strain 1536, serotype 2	I	20	1×10^8
	Phosphate-buffered saline	J	20	—
4	*H. pleuropneumoniae,* strain Shope 4074, serotype 1	K	3	5×10^9
	H. pleuropneumoniae, strain BC181, serotype 3	L	4	4×10^9
	Haemophilus sp. "minor-group" strain 7ATS	M	4	4×10^9
	Haemophilus sp. "minor-group" strain 33PN	N	4	4×10^9
	H. parasuis strain E751	0	2	4×10^{10}
	Phosphate buffered saline	P	4	—

inoculation when those not dying were killed with euthanyl forte (M.T.C. Pharmaceuticals, Hamilton, Canada) injected intravenously. Four from each of group I and J (in Experiment 3) were killed four, five, seven, eight and nine days after inoculation, except day 8 and 9 when only three pigs from group I were killed, since two pigs from this group had died 24 hours after inoculation. All animals were examined for gross morphological changes and tissues were fixed in 10% buffered formalin, embedded in paraffin, sectioned (6 μ) and stained with hematoxylin and eosin for microscopy. Nasal cavity, lung tissue, pulmonary lymph nodes and spleen of all pigs were cultured on TSA blood agar plates with NAD. In some experiments, other tissues were cultured as well. The recovered *Haemophilus* bacteria were identified as described previously (5).

RESULTS

EXPERIMENT 1

The pigs in groups A and B, given 10^8 and 10^7 organisms respectively, developed signs of acute respiratory distress starting 10-15 minutes after inoculation. Some of these pigs vomited and later became recumbent. The pigs in groups C, D and E never showed signs and the two in group B recovered from the acute dyspnea. Three pigs in group A died within 48 hours and the fourth one died four days after inoculation.

NECROPSY

Group A — Large volumes of hemorrhagic froth were seen in the airways. The lungs were swollen, firm and dark and did not collapse. The interstitial septae were widened due to edema and hemorrhage. Abundant hemorrhagic fluid oozed from cut surfaces. The thoracic cavity was filled with serohemorrhagic fluid and fibrin clots. The surfaces of the visceral and parietal pleurae were covered with fibrin. The submandibular and cervical lymph nodes were swollen and hemorrhagic. The lung changes were unilateral in one case only. Pericarditis was present in another case.

Histologically the airways were filled with abundant erythrocytes, proteinaceous fluid and exfoliated epithelial cells. The alveolar structure was markedly disrupted and in some areas not recognizable due to severe hemorrhage, edema and exfoliation of alveolar lining cells. Neutrophils were seen in low numbers evenly distributed in the pneumonic lung. The pleura and interstitial tissue were dramatically thickened and had abundant fibrin. The lymphatic vessels were markedly distended and often occluded by fibrin. Severe vasculitis with thrombosis was present in blood vessels throughout inflamed lung tissue (Fig. 1). Vessel walls were necrotic, invaded by neutrophils and often surrounded by zones of lymphocytes and plasma cells. In the pig dying four days after inoculation abundant basophilic cells of spindle-shape were present in alveoli. Clumps of bacteria were observed in lung tissue of all pigs. Pulmonary and cervical lymph nodes were edematous and had afferent lymphatics distended by fibrin and cells. *Haemophilus pleuropneumoniae* was isolated in large numbers and pure culture from the lungs and pulmonary and cervical lymph nodes of all pigs dying of acute pleuropneumonia, but not from the spleen.

Groups B-E — The pigs given 10^7, 10^6, 10^5 and 10^4 had lung lesions except one given 10^4 organisms. The lesions were mostly confined to the diaphragmatic lobe of the right side and consisted of several encapsulated necrotic areas varying in size from 1 to 10 cm in diameter. Most necrotic areas were surrounded by zones of hyperemia and edema and were covered by fibrous adhesions to the overlying parietal pleura. There was evidence of sequestration of the necrotic tissue in two of the pigs. One of the pigs given 10^6 had diffuse pericarditis with abundant fibrin in the pericardial cavity. One pig inoculated with 10^5 organisms had approximately 40 mL of serosanguinous fluid in the pleural cavity. The pulmonary lymph nodes were enlarged in all the pigs with lung involvement. Histological examination of lung tissue revealed areas of coagulation necrosis surrounded by an inner zone of basophilic spindle-shaped cells and an outer zone of connective tissue which occasionally had lymphocytic aggregates. In the margin of necrotic zones vascular thrombosis was commonly observed. Organization with recanalization was seen occasionally. There was marked interstitial fibrosis and the pleura overlying pneumonic areas had a thick layer of fibrous connective tissue which occasionally was interrupted by more acute changes with edema, fibrin and hemorrhage.

Haemophilus pleuropneumoniae

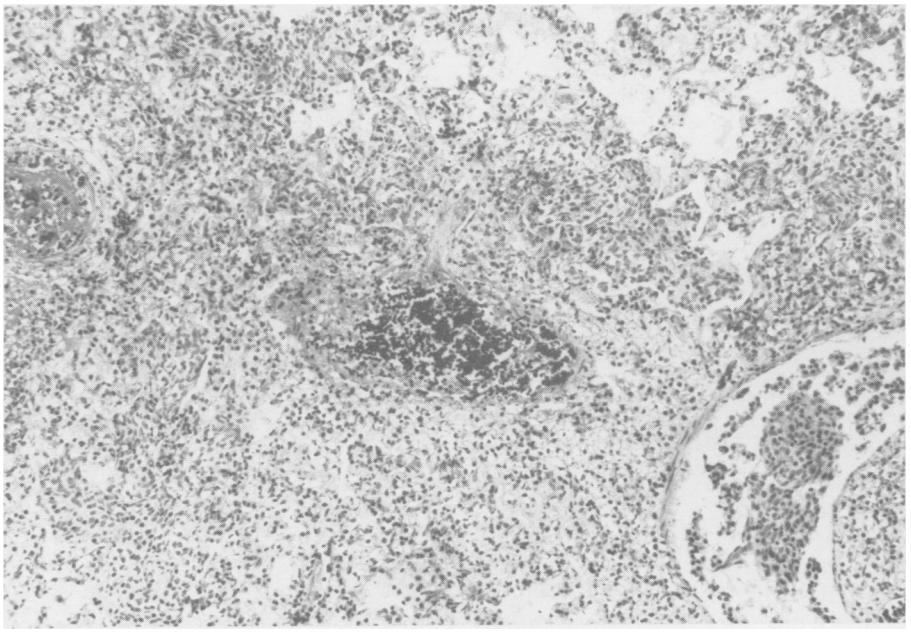

Fig 1. Acute exudative pneumonia in pig inoculated endobronchially with 10^8 CFU of *H. pleuropneumoniae*, serotype 1, strain W. There is a severe necrotizing vasculitis with thrombus formation. Alveoli are filled with proteinaceous fluid and mononuclear cells. H & E X100.

was isolated only from lesions in case of the pigs with chronic pneumonia.

EXPERIMENT 2

Twenty-four hours after inoculation the eight infected pigs (Group F and G) were lying down, anorectic and dyspneic. All pigs recovered clinically and resumed eating by the fourth day. The control pigs (Group H) appeared normal throughout the experiment.

All pigs in groups F and G had necrotic pulmonary lesions ranging in size from 2 to 14 cm in diameter and localized in the right diaphragmatic lobe. Three animals had additional lesions in the left lung. The lesions were either sequestra or well encapsulated abscesses. Fibrous connective tissue was invariably present over the necrotic lung tissue forming adhesions to the thoracic wall. One animal in group G had diffuse fibrous pleuritis on both sides. Four animals had fibrous pericarditis. There was hyperplasia of the pulmonary lymph nodes in all infected pigs. The histological changes were essentially similar to those seen in groups B-E of experiment 1. None of the control pigs (Group H) had pulmonary and pleural lesions at necropsy.

Haemophilus pleuropneumoniae, serotype 1, was recovered consistently from pulmonary lesions, trachea and bronchi of pigs in group F and from the nose of one; but on no occasion from the spleen or pulmonary lymph nodes. *Haemophilus pleuropneumoniae,* serotype 7 was recovered from the lungs of all four pigs in group G from the trachea of three, pulmonary lymph nodes of two and the nose of one pig. The spleen was consistently negative.

EXPERIMENT 3

Ten pigs in group I developed clinical signs of respiratory distress and became anorectic. Two of these died within 30 hours, whereas the remaining recovered clinically. Group J pigs remained clinically normal.

The two pigs dying acutely had extensive fibrinohemorrhagic pleuropneumonia and pericarditis similar to the pigs in group A. The remaining pigs had pulmonary sequestra or abscesses varying in size from 3 to 10 cm in diameter usually located in the dorsal part of the right diaphragmatic lobe. There was a fibrinohemorrhagic pleuritis localized to the areas of lung change. Histologically the changes in the two pigs with acute pleuropneumonia were similar to group A in experiment 1 and the changes in the 18 remaining pigs were similar to those described for groups B-E in experiment 1 with the exception that the connective tissue reaction around necrosis and on the pleura became more pronounced in the pigs killed late (day 9 after inoculation).

Haemophilus pleuropneumoniae serotype 2 was recovered from the nose of two, the lungs of 20, pulmonary lymph node of four pigs; but not from the spleen. There were no morphological changes in the lungs and pleura of the group J pigs, and lungs, pulmonary lymph nodes and spleen were negative for bacteria. Nasal cavity had bacteria interpreted as belonging to the normal flora.

EXPERIMENT 4

Group K — Approximately ten minutes after inoculation the three pigs vomited and were breathing rapidly. They preferred to be in sternal recumbency and gradually became dyspneic. All animals were dead within 16 hours after inoculation. Blood was oozing from the nostrils and two of the pigs had cyanotic skin discoloration on extreme parts of the body. Hemorrhagic froth was in the airways and extensive fibrinohemorrhagic pneumonia was bilateral in two pigs and unilateral in one. There was serosanguinous pleurisy in all pigs. Edema and fibrin deposition extended into the pericardium in two pigs. The histological changes were similar to group A in experiment 1. *Haemophilus pleuropneumoniae* serotype 1 was isolated in large numbers and pure culture from thoracic organs and cervical lymph nodes of all pigs. In one case these bacteria were isolated from the spleen in low numbers.

Group L — The animals appeared slightly depressed after inoculation and did not thrive as well as the control pigs in group P. At necropsy, three pigs had pulmonary necrosis undergoing sequestration in the dorsal area of the right lung in the cardiac lobe region. These lesions varied in size from 2 to 6 cm in diameter. There was fibrosing pleuritis causing pleural adhesions over the necrosis. The bronchial lymph nodes were enlarged in these three pigs. One of the pigs with pulmonary necrosis also had fibrinopurulent polyarthritis and peritoneal lymph nodes appeared edematous and hyperaemic. One of the four pigs had an abscess (7 cm in diameter) in the soft tissues at the thoracic inlet, but no other lesions. Histologically the changes in the lungs were similar to the description for group B-E in experiment 1. There was marked villous proliferation of joint capsules in the animal with polyarthritis. The synovial lining showed mild hyperplasia and occasionally foci of necrosis (Fig. 2). *Haemophilus pleuropneumoniae* serotype 3 was isolated in large numbers and pure culture from the pulmonary lesions. In one pig it was isolated from the trachea and pericardial fluid as well. The animal with polyarthritis was negative for bacteria (including *Haemophilus*) or mycoplasmas in the joints, but had *Haemophilus pleuropneumoniae* in the pulmonary lesions. *Staphylococcus aureus* grew in large numbers from the abscess at the thoracic inlet of one pig.

Group M — The animals in this group did not show clinical signs of pneumonia. At necropsy one pig had a 1-2 cm cyst-like cavity surrounded by a 2-3 mm fibrotic capsule in the dorsal area of the right cardiac lobe. Few fibrous strands over the lesion formed adhesions to the parietal pleura. Another pig had a wedge-shaped firm nodule approximately 1 x 1 cm with fibrous pleuritis in the same part of the lung. The third pig in this group had diffuse pleural adhesions among the ventral face of the right lung, diaphragm and pericardium. Gross changes were not seen in the fourth pig in this group. Histologically the focal pneumonia in two pigs was characterized by marked fibrosis with occasional aggregates of lymphocytes in the interstitial tissue and adjacent pleura. One pig had increased thickness of the pleura due to fibrosis and the remaining animal had no visible lesions. No bacteria (including *Haemophilus*) from any pig in this group were isolated from tissues without a normal flora.

Group N — The pigs in this group did

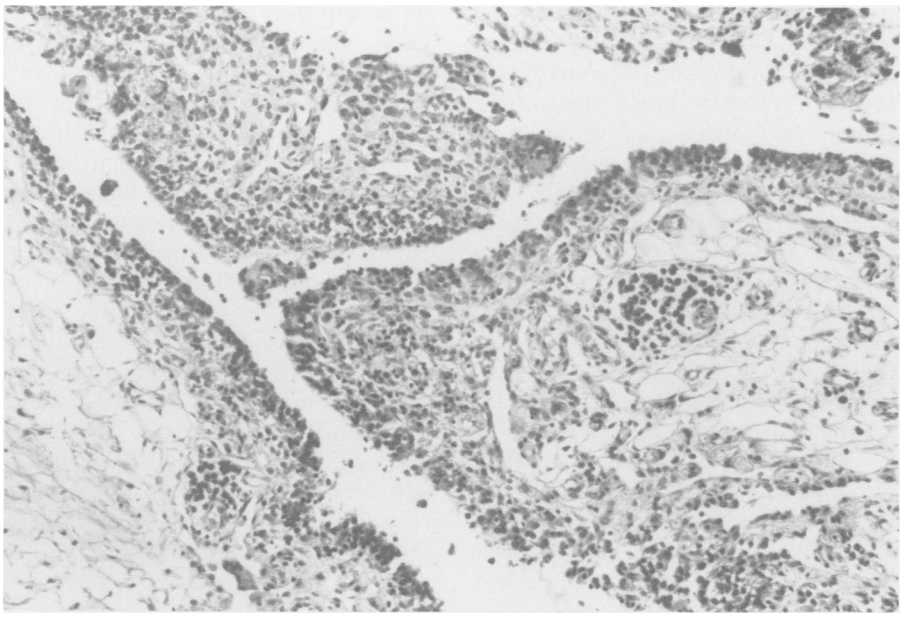

Fig 2. Arthritis in pig inoculated endobronchially with 4 x 10^9 CFU of *H. pleuropneumoniae* serotype 3, strain BC181. There is necrosis of the synovial membrane, edema and perivascular accumulation of lymphocytes. H & E. X100.

not show clinical signs of pneumonia. At necropsy one of them had a 3 x 3 cm pulmonary necrosis with a 2-3 mm fibrotic capsule in the dorsal part of the right lung over the cardiac lobe. Fibrous adhesions were present over this lesion. Another pig had a similar but smaller (1 x 1 cm) area of necrosis. The third pig in this group had an abscess (approx 4 x 4 cm) with overlying fibrous pleuritis in the same part of the lung. The fourth animal had a 1 x 1 cm fibrotic nodule in the ventral margin of the right diaphragmatic lobe and diffuse pleural adhesions among the ventral face of the right lung, diaphragm and pericardium. Histologically there was coagulation necrosis surrounded by connective tissue with occasional lymphocytic aggregates in the pneumonic tissue of three pigs. One pig had focal interstitial fibrosis and all pigs had thickened pleura due to fibrosis. One of the animals with pulmonary necrosis had *Haemophilus* bacteria, biochemically identical with strain 33PN, in the lung lesion. The same organism was isolated from the abscess together with *Corynebacterium pyogenes* and *Staph. aureus*. The two remaining pigs were negative for bacteria.

Group O — The pigs became anorectic after 24 hours and later recumbent. One died after 48 hours and the other one was moribund and euthanized. The extreme parts of the body of one pig were cyanotic. There was hemorrhage and edema in the subcutis and muscles. A brown area of consolidation of approx 2 x 5 cm in size was present in the ventral margin of the right diaphragmatic lung lobe. Blood vessels in the epicardium were congested. There was moderate fibrinous exudate in the peritoneum, especially in the tunica vaginalis of the testis. All joints had fibrin casts and the synovial membranes were edematous and congested. The periarticular tissue was very edematous. Body lymph nodes were enlarged due to hemorrhage and edema. The other pig had an area of consolidation (5 x 5 cm) in the right diaphragmatic lobe. This area was covered with fibrinous adhesions to the parietal pleura. Serous fluid in moderate amounts was present in the pericardial, pleural and peritoneal cavities. There was polyarthritis similar to the first pig. Histologically the pulmonary changes in both animals were characterized by collapse of alveoli with proliferation and hypertrophy of alveolar lining cells. Thère were few neutrophils in the alveoli. Fibrinous necrosis of synovium was seen in all joints examined histologically. One of the pigs had marked thrombosis of synovial blood vessels (Fig. 3). Lymph nodes from peritoneum and thoracic cavity were edematous and appeared depleted of lymphocytes resulting in fading of the follicular structure. Lymphatic sinuses were occluded by neutrophils. There was no evidence of meningitis in either pig. *Haemophilus parasuis* was isolated from lung, pleura, spleen, lymph nodes, peritoneal fluid, tunica vaginalis, blood and synovial fluid of both pigs, whereas the cerebrospinal fluid was negative.

Group P — None of the control pigs showed signs of disease or had morphological changes at necropsy and all tissues were negative for bacteria.

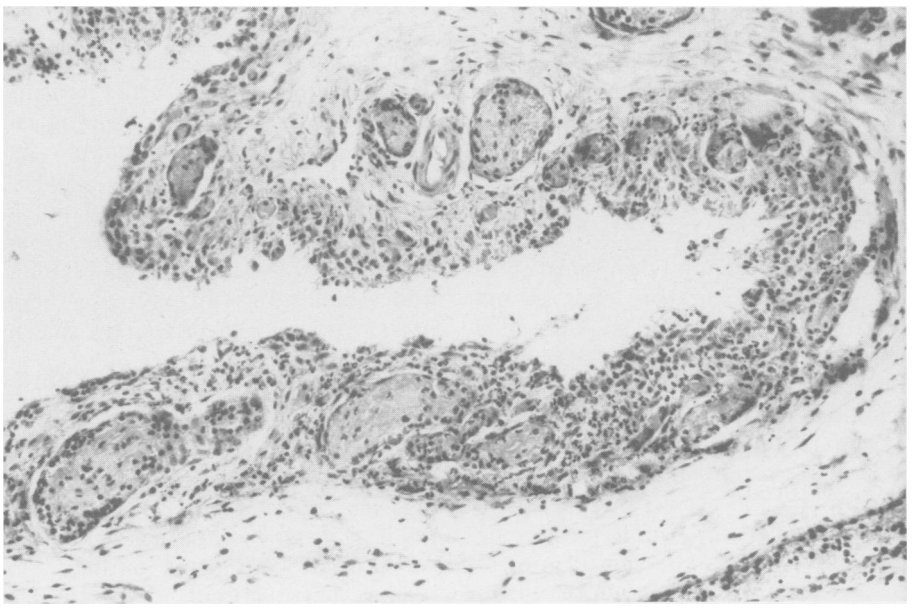

Fig 3. Arthritis in pig inoculated endobronchially with 4 x 10^{10} CFU of *H. parasuis* strain E751. There is edema, necrosis and thrombosis of venules. H & E. X100.

DISCUSSION

The etiological significance of *H. pleuropneumoniae* in acute and chronic pleuropneumonia has been proven in both forms (1). Aerogenous exposure is the preferred technique in order to reproduce pleuropneumonia resembling field cases. Nasal instillation or aerosol exposure of pigs with bacterial suspensions is usually successful; but difficult to standardize. To reproduce the chronic form with these methods it may be necessary to treat with penicillin or other antibiotics when the animals develop clinical signs of acute respiratory distress (1). In this study a specified dose of bacteria was deposited directly into the lower airways and in experiment 1 using a recent isolate of *H. pleuroneumoniae* serotype 1, 10^8 bacteria led to acute lethal pleuropneumonia. With doses between 10^7 and 10^4 the animals developed the chronic nonlethal form of pleuropneumonia. It is likely that with the large dose the pulmonary defence system becomes overwhelmed and breaks down with the result that bacteria can multiply and spread throughout the lung (7). This may be accentuated by a higher amount of inherent toxic material which may cause a marked edema and thus a good growth environment (8). With lower doses only local changes develop, perhaps because the defence system is only challenged locally and the immediate edema reaction is moderate or mild. Supporting this hypothesis is the fact that bacteria can be isolated from all parts of the lungs in acute pleuropneumonia but only from diseased parts in the chronic pleuropneumonia. The toxicity of large doses of *H. pleuropneumoniae* serotype 1 was also evident from the signs of respiratory distress and vomiting which was seen within 10-15 minutes after inoculation in the pigs in group A and B of experiment 1 and the pigs in group K of experiment 4. These clinical signs were also seen when pigs were exposed to sonicated bacteria and may be due to the endotoxin in the bacterial wall (8). The macroscopic and microscopic changes observed in the pigs with experimental acute or chronic pleuropneumonia are consistent with descriptions reported by others (1,9,10,11,12,13,14). However, we wish to emphasize an earlier observation (15) that vasculitis and thrombosis is a striking feature of the histological changes in both forms. If the bacteria or their products have a direct effect on the vasculature destroying its integrity then this can explain the marked edema, exudation and hemorrhage seen in the acute form. Thrombosis leading to necrosis or infarction would explain the necrotic changes seen in the chronic form.

With a dose predicted, on the basis of experiment 1, to induce chronic pleuropneumonia, four pigs in experiment 2 inoculated with the type strain (Shope 4074, serotype 1) and four pigs inoculated with the reference strain of serotype 7 (WF83) did in fact develop the typical chronic lesions. The minimal dose of WF83 which will induce acute pleuropneumonia is not known, but from the present experiment it seems that serotype 7 possess virulence similar to serotype 1. It is interesting to note that strain Shope 4074, which was isolated in 1964 and presumably highly passaged in the laboratory, seems to be as virulent as the recently isolated and low passaged strain W (9). Only two out of 20 pigs inoculated with 10^8 CFU of strain 1536, serotype 2, developed acute pleuropneumonia and died. The remainder, however, did have localized lesions of necrosis. This might suggest that strain 1536, serotype 2 is slightly less virulent than the serotype 1 strains.

Canadian strains of serotype 3 have been isolated in British Columbia so far (15). Strain BC181 was less virulent than strain Shope 4074. Even with 4×10^9 CFU only lesions consistent with chronic pleuropneumonia could be induced. It is possible that the inoculum in case of the one pig which had an abscess at the thoracic inlet and no pneumonia was actually deposited outside the airway resulting in local tissue damage, secondary *Staph. aureus* infection and abscess formation. Whether or not the polyarthritis seen in one pig inoculated with BC181 was induced by the organism is unclear since no bacteria or mycoplasmas could be isolated from the joints.

The lesions induced by the "minor-group" strains 7ATS and 33PN were mild and did not affect the pigs clinically. The fibrotic nodules and the focal necrotic lesions most likely developed at the inoculum deposition site. It is possible that these focal lesions represent a local effect of the endotoxin in the suspension and that similar lesions would develop with any Gram-negative bacterium inoculated under similar circumstances.

The diffuse pleuritis seen in one pig given strain 7ATS and one pig given strain 33PN is interesting and suggests that these organisms may be involved in diffuse pleuritis in pigs under natural conditions.

The inoculation strain was recovered from the lung lesion of only one of these pigs, suggesting that *Haemophilus* bacteria of the "minor-group" are low virulent and easily cleared from invaded tissue. The virulence of *H. parasuis*, strain E751, was confirmed. Even though the inoculum was deposited in the lower airways the pigs developed systemic infections resulting in polyserositis and polyarthritis. This differs markedly from infections with *H. pleuropneumoniae*. Neither of the two pigs developed meningitis or cerebral infection which contrasts to most standard descriptions of Glasser's disease (13).

The infection with *H. pleuropneumoniae* is confined to the respiratory system. Only in one case was *H. pleuropneumoniae* isolated from the spleen, confirming that septicemia only occasionally follows pulmonary infection in pigs of this age group. The pericarditis observed in several pigs infected with *H. pleuropneumoniae* may be regarded as a local extension of the infection.

In conclusion, the difference in virulence between *H. pleuropneumoniae* of serotypes 1, 2 and 7 is probably low. Serotype 3 seems less virulent than 1, although the strain did induce chronic lesions of a severity which affected the growth rate.

Strains of the so-called "minor-group" are probably insignificant in the field, although their role in pleuritis should be further evaluated. If these strains carry protective antigens shared by *H. pleuropneumoniae* they may be of potential use in live vaccines.

ACKNOWLEDGMENTS

Grants from the Ontario Ministry of

Agriculture and Food and the Ontario Pork Producers Marketing Board supported this research.

REFERENCES

1. **NIELSEN R.** *Haemophilus pleuropneumoniae* infection in pigs. Copenhagen: Carl Fr. Mortensen A S, 1982.
2. **GILBRIDE KA, ROSENDAL S.** Evaluation of a selective medium for isolation of *Haemophilus pleuropneumoniae*. Can J Comp Med 1983; 47: 445-450.
3. **KILIAN M, NICOLET J, BIBERSTEIN EL.** Biochemical and serological characterization of *Haemophilus pleuropneumoniae* (Matthews and Pattison 1961) Shope 1964 and proposal of a neotype strain. Int J Syst Bacteriol 1978; 28: 20-26.
4. **LOMBIN LH, ROSENDAL S, MITCHELL WR.** Evaluation of the complement fixation test for the diagnosis of pleuropneumonia of swine caused by *Haemophilus pleuropneumoniae*. Can J Comp Med 1982; 46: 109-114.
5. **ROSENDAL S, BOYD DA.** *Haemophilus pleuropneumoniae* serotyping. J Clin Microbiol 1982; 16: 840-843.
6. **O'REILLY T, ROSENDAL S. NIVEN DF.** Porcine haemophili and actinobacilli: characterization by means of API test strips and possible taxonomic implications. Can J Microbiol 1984; 30: 1229-1238.
7. **BENDIXEN PH, SHEWEN P, ROSENDAL S, WILKIE BN.** Toxicity of *Haemophilus pleuropneumoniae* for porcine lung marcophages, peripheral blood monocytes and testicular cells. Infect Immun 1981; 33: 673-676.
8. **ROSENDAL S, MITCHELL WR, WEBER M, WILSON MR, ZAMAN MR.** *Haemophilus pleuropneumoniae*. Lung lesions induced by sonicated bacteria and sterile culture supernatant. Proc Int Pig Vet Soc Congress 1980; 5: 221.
9. **SHOPE RE.** Porcine contagious pleuropneumonia. I. Experimental transmission, etiology and pathology. J Exp Med 1964; 119: 357-368.
10. **NIELSEN R.** Pleuropneumoni hos svin founkaldt of Haemophilus parahemolyticus. II. Undersogelser over den isolerede bakteries identitet og pathogenitet. Nord Vet Med 1970; 22: 246-255.
11. **HANI H, KONIG H, NICOLET J, SCHOLL E.** Zur Haemophilus-pleuropneumonie beim Schwein. V. Pathomorphologie. Schweiz Arch Tierheilkd 1973; 115: 191-203.
12. **SCHIEFER B, GREENFIELD J.** Porcine *Haemophilus parahaemolyticus* pneumonia in Saskatchewan II. Bacteriological and experimental studies. Can J Comp Med 1974; 38: 105-110.
13. **NICOLET J, SCHOLL E.** Haemophilus infections. In: Leman AD et al, eds. Diseases of swine. Ames: Iowa State University Press, 1981: 368-376.
14. **SEBUNYA TNK, SAUNDERS JR, OSBORNE AD.** A model aerosol exposure system for induction of porcine *Haemophilus* pleuropneumoniae. Can J Comp Med 1983; 47: 48-53.
15. **HANI H, KONIG H, NICOLET J. SCHOLL E.** Zur Haemophilus-pleuropneumonie beim Schwein. VI. Pathogenese. Schweiz Arch Tierheilkd 1973; 115: 205-212.