Dose Response Relationship of *Haemophilus pleuropneumoniae* Aerosols in Pigs

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ABSTRACT

The virulence of *Haemophilus pleuropneumoniae* was quantitated for ten and 12 week old pigs following aerosol exposure. The volume and concentration of culture aerosolized, the estimated numbers of organisms inhaled by the pigs and the mortality rates at 72 hours postexposure were computed and used to calculate the LD_{50}. There was correlation between the concentration of culture aerosolized, the amount of the estimated inhaled dose and the mortality rates. The ten week old pigs were apparently more susceptible to aerosols of *H. pleuropneumoniae* than the 12 week old pigs. The LD_{50} value or a multiple of it appears to be a reasonable basis for a standardized aerosol challenge of the immunity of pigs vaccinated with experimental or commercial *H. pleuropneumoniae* vaccines.

RÉSUMÉ

Cette expérience consistait à quantifier la virulence de *Haemophilus pleuropneumoniae*, pour des porcs âgés de dix à 12 semaines, à la suite d'une nébulisation de cette bactérie. Pour déterminer la DL_{50}, on calcula le volume et la concentration des cultures utilisées pour la production des aérosols, ainsi que le nombre approximatif de microbes inhalés par les porcs et le taux de mortalité enregistré 72 heures après la nébulisation. On constata une corrélation entre la concentration de la culture utilisée pour la nébulisation, la quantité approximative de microbes inhalés et le taux de mortalité. Les porcs âgés de dix semaines se révélèrent apparemment plus susceptibles aux aérosols de *H. pleuropneumoniae* que les porcs âgés de 12 semaines. La valeur de la DL_{50} ou un de ses multiples représenteraient une base raisonnable pour tester, à l'aide d'aérosols standardisés, l'immunité des porcs vaccinés avec des vaccins expérimentaux ou commerciaux, préparés avec *H. pleuropneumoniae*.

INTRODUCTION

An aerosol exposure system for induction of porcine *Haemophilus* pleuropneumonia (PHP) was shown to consistently result in *H. pleuropneumoniae* infections (1).

The purpose of this study was to quantitate the virulence of *H. pleuropneumoniae* aerosols in pigs for possible future use in the evaluation of *H. pleuropneumoniae* vaccines in the laboratory and subsequently in field trials.

MATERIALS AND METHODS

ANIMALS

All swine used in this study were specific pathogen free pigs[1] and were housed as previously described (2). The 20 pigs in experiment 1 were 11 to 12 weeks old and weighed 10-15 kg (average 12.5 kg) while the 20 pigs in experiment 2 were nine to ten weeks old and weighed 8-10 kg (average 8.5 kg).

All the pigs were bled before challenge and their sera subjected to the tube agglutination test with a 0.2% formalin killed culture of *H. pleuropneumoniae* (strain A79-9, serotype 1) as antigen (3).

BACTERIAL PREPARATION AND AEROSOL EXPOSURE OF PIGS

Cultures of *H. pleuropneumoniae* strain A79-9 were grown from stock egg yolk and prepared for aerosolization as before (1).

In experiment 1 five groups of four pigs selected at random were exposed (one group at a time) for ten minutes to *H. pleuropneumoniae* aerosols of a tenfold diluted culture in the improvised exposure chamber previously described (2). The estimated concentration of *H. pleuropneumoniae* per 1 mL culture before aerosolization was between 1×10^9-1×10^5 bacteria. The amount and the mean concentration of culture aerosolized per group were determined as before (1).

In experiment 2 five groups of four pigs were selected at random and exposed (one group at a time) for ten minutes to *H. pleuropneumoniae* aerosols of a tenfold diluted culture in a specially designed portable exposure chamber constructed of plexiglass sides and lid over a galvanized steel frame with dimensions of 107 cm wide × 137 cm long and 107 cm high.[2] The

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estimated concentration of culture before aerosolization ranged from 1×10^7 to 1×10^3 organisms/mL.

In both experiments the aerosol chamber was dismantled after aerosolization of each group of pigs and washed thoroughly with water. Each group of pigs was kept and observed in the isolation unit for up to six days.

ESTIMATION OF NUMBERS OF *H. PLEUROPNEUMONIAE* INHALED

An estimation was made of the concentration of viable *H. pleuropneumoniae* in the aerosols in the exposure chamber. The estimated total number of organisms aerosolized in each group of four pigs in experiment 1 was the product of the volume of culture (mL) aerosolized and the average concentration of culture aerosolized. In experiment 2 the number of organisms aerosolized per group was obtained by multiplying the volume of culture aerosolized by the concentration of culture immediately before aerosolization. The estimated concentration of *H. pleuropneumoniae* per litre of air was obtained by dividing the estimated total number of organisms by the chamber volume which was 1396.7 and 1568.5 liters for experiments 1 and 2, respectively. An estimation of the average number of organisms inhaled per specific group of pigs was derived from a formula that gave the respiratory volume per minute based on body weights (4).

DETERMINATION OF LD_{50}

Accumulated mortality rates per group at 72 hours and the estimated average dose of *H. pleuropneumoniae* inhaled were used to calculate the LD_{50} (5) for the two experiments.

POSTMORTEM EXAMINATIONS

The lungs, liver and spleen of dead pigs were examined pathomorphologically and by cultivation (1). The pigs that survived for six days postexposure were euthanized, blood collected and tissues sampled as previously described (1).

SEROLOGICAL EXAMINATION OF SERA

Sera obtained from the pigs before aerosol challenge and those killed at six days postexposure were tested for agglutinins to *H. pleuropneumoniae* (3).

RESULTS

The mortality data on aerosol exposure of pigs in experiments 1 and 2 are presented in Tables I and II. The LD_{50}, based on concentration of culture aerosolized, was calculated to be 1.9×10^5 organisms/mL in the first experiment and 7.0×10^3 organisms/mL in the second experiment.

The calculated average respiratory volumes per pig in ten minutes were 24.8 and 18.6 respectively, for experiments 1 and 2. The LD_{50} calculated in terms of the estimated inhaled dose per group of pigs was 2.9×10^4 viable organisms in experiment 1 and 2.1×10^3 in the second experiment (Table III).

GROSS POSTMORTEM FINDINGS

All the pigs that died had a hemorrhagic fibrinous pneumonia identical to that described previously (1) and *H. pleuropneumoniae* was isolated from all such lungs and tracheobronchial lymph nodes. The organism was also recovered from four and 17 out of a total of 20 samples, each of liver and spleen respectively. Pigs that survived for six days postexposure either had no lesions of PHP or the lesions present were less severe and more focal than in the acute disease.

TABLE I. Experiment 1 — Relationship Between Challenge Dose of Aerosolized *H. pleuropneumoniae* Culture, Time to Death and Mortality Rates in Pigs of 12.5 kg Average Weight

Group No.	Culture aerosolized (mL)[a]	Concentration/mL (Est.conc./liter)[b]	Time (hrs) to death and no. of pigs < 15	15-24	24-36	36-72	No. dead/no. in group (%)
1	29	1.86×10^4 (4×10^2)				1	1/4 (25)
2	31	3.0×10^5 (7×10^3)			2	1	3/4 (75)
3	19	3.78×10^6 (5×10^4)		1	1	1	3/4 (75)
4	22	2.4×10^7 (4×10^5)		3	1		4/4 (100)
5	33	5.4×10^8 (1.3×10^7)	2	1	1		4/4 (100)

[a]Overall mean culture aerosolized = 26.8 mL
[b]Average estimated *H. pleuropneumoniae*/liter of aerosols
Pig group $LD_{50} = 1.9 \times 10^5$ organisms/mL

TABLE II. Experiment 2 — Relationship Between Challenge Dose of Aerosolized *H. pleuropneumoniae* Culture, Time to Death and Mortality Rates in Pigs of 8.5 kg Average Weight

Group No.	Culture aerosolized (mL)[a]	Concentration/mL (Est.conc./liter)[b]	Time (hrs) to death No. of pigs 15-24	24-36	36-72	No. dead/no. in group (%)
1	24	7.0×10^3 (1.12×10^2)		1	1	2/4 (50)
2	26	8.4×10^4 (1.4×10^3)	1	3		4/4 (100)
3	28	5.9×10^5 (1.1×10^4)	4			4/4 (100)
4	29	4.7×10^6 (9×10^4)	4			4/4 (100)
5	32	3.8×10^7 (8.1×10^5)	4			4/4 (100)

[a]Overall mean culture aerosolized = 27.8 mL
[b]Estimated *H. pleuropneumoniae* concentration per liter of aerosols
Pig group $LD_{50} = 7.0 \times 10^3$ organisms/mL

TABLE III. Estimated Inhaled Dose of *Haemophilus pleuropneumoniae* and Mortality Rates in Pigs in Experiments 1 and 2

Experiment No.	Group no. and estimated inhaled doses in ten minutes (% Mortality)				
	1	2	3	4	5
1	9.9×10^3 (25)	1.7×10^5 (75)	1.24×10^6 (75)	9.9×10^6 (100)	3.2×10^8 (100)
2	2.1×10^3 (50)	2.6×10^4 (100)	2.0×10^5 (100)	1.7×10^6 (100)	1.5×10^7 (100)

RESULTS OF AGGLUTINATION TESTS

The agglutination titers on pigs that survived are shown in Table IV. There was correlation between presence of PHP and an early but specific serological response. Sera from pigs before aerosol challenge had titers $< 1/5$.

DISCUSSION

The virulence of *H. pleuropneumoniae* aerosols was determined for two age groups of pigs. In each age group there was correlation between the concentration of culture aerosolized, the estimated concentration of organisms in the aerosol chamber, the incubation period of the disease and mortality rates. Although, the difference in age of the pigs in the two experiments was small, the younger pigs appeared to be more susceptible to *H. pleuropneumoniae* infections than the older ones (Table I and II). This seems to agree with previous observations (6) on the age dependent capability of pigs to clear nonpathogenic *Escherichia coli* from their lungs. These authors postulated that the reduced pulmonary bactericidal capacity of the young pig could be a factor in the pathogenesis of pulmonary infections.

The estimated doses of *H. pleuropneumoniae* inhaled represent an approximation of the actual amounts since the calculated porcine respiratory volumes were based on data obtained from laboratory animals and man (4). Corresponding values for the pig have apparently not yet been determined. Although actual totals for aerosols inhaled and the proportion retained by the pigs could not be determined, the results demonstrated a consistent relationship between the concentration of culture aerosolized on the one hand and times of death and the mortality rates on the other. Also, early development of a serological response correlated with the presence of *H. pleuropneumoniae* and PHP lesions. Under natural and experimental conditions, it has been previously shown that seropositive animals may be carriers of *H. pleuropneumoniae* (7,8).

Because the response of pigs exposed to different concentrations of culture aerosolized in this experimental system appeared to be predictable and reproducible, the system should be adaptable to controlled studies on the epidemiology and pathogenesis of PHP as well as on the efficacy of *H. pleuropneumoniae* vaccines or chemotherapeutic agents in the prevention or control of the disease.

TABLE IV. Experiment 2 — Pleuropneumonia and Agglutination Titers in Pigs Surviving Six Days After Challenge

Group No.	Pig. No.	Agglutination titer[a]	Pathological lesions
1	728	40	+[b]
	730	40-80	+
	701	<5	-[c]
2	738	5	-
3	766	80	+

[a]Reciprocal of highest dilution (two replicates) showing agglutination
[b]+ = Presence of PHP lesions
[c]- = No PHP lesions

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