Experimental Model of Swine Pneumonic Pasteurellosis using Crude *Actinobacillus pleuropneumoniae* Cytotoxin and *Pasteurella multocida* given Endobronchially

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ABSTRACT

This study was designed to develop and characterize a swine pneumonic pasteurellosis model by concurrent introduction of *Pasteurella multocida* type A and *Actinobacillus pleuropneumoniae* crude cytotoxin. After a series of preliminary experiments, a combination of 4×10^9 *P. multocida* and 4,000 toxic units of *A. pleuropneumoniae* crude cytotoxin was determined to produce optimal results. A total of 48 pigs were divided into four groups of 12 pigs each. The control group received buffered saline only. Four pigs from each group were randomly selected for necropsy 3, 7 and 14 days postinoculation (PI). Inoculation of pigs with *P. multocida* and *A. pleuropneumoniae* cytotoxin (group 1) resulted in moderate to severe pneumonia. *Pasteurella multocida* was isolated from pneumonic lesions, grossly normal lung, and bronchial lymph nodes of all group 1 pigs throughout the 14 day experimental period. Pathological changes typical of field cases of swine pneumonic pasteurellosis were produced. Pigs inoculated with *P. multocida* alone (group 2) had pneumonic lesions and *P. multocida* was reisolated from lungs at three days PI. *Pasteurella multocida* was not isolated from these pigs at 7 and 14 days PI, except for one pig in which an abscess developed in the thorax. Pulmonary lesions induced by *A. pleuropneumoniae* crude cytotoxin alone (group 3) were transient and resolved by seven days PI. Group 1 pigs had significantly greater lung lesion volumes than group 2 and 3 pigs at 3, 7 and 14 days PI. Statistical analysis indicated a significant interactive effect of *P. multocida* and *A. pleuropneumoniae* cytotoxin on the development of lung lesion volumes at 7 and 14 days PI ($p < 0.05$). This suggests that there is an additive or synergistic interaction between *P. multocida* and cytotoxin that is best demonstrated experimentally at 7 to 14 days after challenge.

RÉSUMÉ

Le but de la présente étude était de développer et caractériser un modèle de pasteurellose pulmonaire chez le porc par inoculation simultanée de *Pasteurella multocida* type A et de la cytotoxine brute d'*Actinobacillus pleuropneumoniae*. Suite à des essais préliminaires, il a été déterminé qu'une combinaison de 4×10^9 *P. multocida* et de 4,000 unités toxiques de la cytotoxine brute d'*A. pleuropneumoniae* produisait les meilleurs résultats. Quatre groupes de 12 porcs chacun furent formés. Le groupe témoin n'a reçu que de la saline tamponnée. Quatre porcs de chacun des groupes ont été choisis au hasard et soumis à une nécropsie aux jours 3, 7 et 14 post-inoculation (PI). L'inoculation de porcs avec *P. multocida* et la cytotoxine d'*A. pleuropneumoniae* (groupe 1) entraîna une pneumonie modérée à sévère. *Pasteurella multocida* fut isolé des lésions de pneumonie, des poumons apparemment normaux macroscopiquement, et des ganglions bronchiques des porcs du groupe 1 tout au long de la période expérimentale de 14 jours. Des changements pathologiques typiques de cas naturels de pasteurellose pulmonaire porcine étaient produits. Les porcs inoculés avec *P. multocida* seulement (groupe 2) ont développé des lésions de pneumonie et *P. multocida* fut réisolé du poumon au jour 3 PI. *Pasteurella multocida* ne fut pas isolé de ces animaux aux jours 7 et 14 PI, à l'exception d'un animal chez qui un abcès se développa dans la cavité thoracique. Les lésions pulmonaires induites par la toxine brute d'*A. pleuropneumoniae* administrée seule (groupe 3) étaient transitoires et avaient disparu au jour 7 PI. L'étendue des lésions pulmonaires des porcs du groupe 1 était significativement plus grande que celle des porcs des groupes 2 et 3 aux jours 3, 7 et 14 PI. Les analyses statistiques démontrent une interaction significative entre les effets causés par *P. multocida* et la cytotoxine d'*A. pleuropneumoniae* dans le développement des lésions pulmonaires aux jours 7 et 14 PI ($p <$ 0,05). Ces résultats suggèrent qu'il y a un effet additif ou synergique entre *P. multocida* et la cytotoxine qui est mieux démontré de 7 à 14 jours après l'inoculation. (*Traduit par Dr Serge Messier*)

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INTRODUCTION

Pasteurella multocida is a major cause of swine pneumonia (1–4). Predisposing conditions enhance the colonization of *P. multocida* in the lung leading to pneumonia. Hog cholera virus (5), pseudorabies virus (6), *Mycoplasma hyopneumoniae* (7,8) and *Actinobacillus pleuropneumoniae* (9) have been shown to impair host defense systems, thus allowing *P. multocida* to survive and proliferate in the porcine lung. Studies also have shown that the normal porcine lung can efficiently clear experimentally introduced *P. multocida* (6,7,10). *Pasteurella multocida* challenge results in a transient lesion that resolves quickly (11).

Efforts have been made to use *P. multocida* alone to establish a pneumonia model. Successful models have been reported by inoculation of specific-pathogen-free (SPF) pigs with field isolates of *P. multocida* type A (12,13), but this method has been unreliable (14). Bentley *et al* (15) successfully induced swine pneumonic pasteurellosis by using a serotype B strain of *P. multocida* originally isolated from an American bison. However, *P. multocida* type B pneumonic pasteurellosis rarely occurs in swine (16,17). Hall *et al* (14) established a swine pneumonia model by concurrent intratracheal inoculation of *P. multocida* type A and 8 mL/kg body weight of saline. The success of this model was dependent on whether pigs survived anesthesia and inoculation of such a large volume of fluid.

Experimental and field studies have demonstrated that *A. pleuropneumoniae* causes damage to the lung and enables *P. multocida* type A to colonize, causing severe pneumonia (9). Surveys of the incidence of swine pneumonia have shown that concurrent infection of *A. pleuropneumoniae* and *P. multocida* type A is not uncommon (18). The cytotoxin of *A. pleuropneumoniae* is both hemolytic to red blood cells (19) and toxic to swine pulmonary alveolar macrophages, peripheral blood monocytes, and neutrophils (20–23). Given alone, *A. pleuropneumoniae* culture supernatant causes moderate to severe lung lesions (24), probably primarily due to the cytotoxin produced by the organism. The objective of this study was to test whether the crude *A. pleuropneumoniae* cytotoxin could act as a priming factor to predispose swine to pneumonic pasteurellosis when concurrently challenged with *P. multocida*.

MATERIALS AND METHODS

ANIMALS AND HOUSING

A total of 72 crossbred five-week-old SPF pigs were used. Pigs originated from the University of Wisconsin swine herd, which was free from *P. multocida* and *A. pleuropneumoniae* infection according to continual abattoir surveys and bacteriological culture of nasal samples. Animal experimental protocol was approved by the University of Wisconsin animal care committee and conformed to the standards of the Canadian Council on Animal Care (25).

PASTEURELLA MULTOCIDA INOCULUM

A nontoxigenic strain of *P. multocida* type A (PSIL6, isolated from a pneumonic pig lung) was grown on blood agar for 16 hours and then suspended in phosphate buffered saline. The number of organisms was adjusted to the necessary concentration using a spectrophotometer at 540 nm and a previously established standard curve against plate count data.

ACTINOBACILLUS PLEUROPNEUMONIAE CYTOTOXIN

Preparation of *A. pleuropneumoniae* crude cytotoxin has been described previously (26). A preparation containing 400 toxic units per mL was used throughout the experiments. *Limulus* lysate assay (E-Toxate®, Sigma) was used to detect endotoxin in the preparation using *Escherichia coli* endotoxin as the reference standard.

DETERMINATION OF *A. PLEUROPNEUMONIAE* CYTOTOXIN CONCENTRATION

A previously described modified, quantitative MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay to measure the cytotoxicity of *A. pleuropneumoniae* cytotoxin to swine neutrophils was used. A toxic unit was defined as the cytotoxin dilution that caused 50% cytotoxicity of neutrophils under defined conditions (26).

INOCULATION TECHNIQUES

Pigs were anesthetized with 5.7 mg Telazol®/kg plus Rompun® at 2.2 mg/kg intramuscularly to achieve about one hour of anesthesia. Pigs were held in ventral recumbency and 4.0 mm ID, 18 cm long endotracheal tube was introduced into the trachea. A 16 gauge, 16 inch intravenous catheter (Deseret Medical, Inc., Utah) with wire stylet was inserted through the endotracheal tube until resistance was felt. After injection of 10 mL of inoculum into the lungs, the pigs were kept in ventral recumbency for one hour.

MODEL ANALYSIS

The optimal concentration of *A. pleuropneumoniae* cytotoxin (4,000 units) and dose of *P. multocida* type A (4×10^9) were determined by preliminary experiments. Pigs, weaned at four weeks of age, were transferred to the experimental station, and randomly allotted to four groups of 12 pigs each. At the age of five weeks, pigs in each group were inoculated endobronchially with the following inocula (10 mL total volume):

Group 1: 4×10^9 *P. multocida* plus 4,000 units of *A. pleuropneumoniae* cytotoxin.

Group 2: 4×10^9 *P. multocida* only.

Group 3: 4,000 units of *A. pleuropneumoniae* cytotoxin only.

Group 4: Phosphate buffered saline only.

Four pigs from each group were randomly selected for necropsy at 3, 7 and 14 days postinoculation (PI). Pigs were euthanized by anesthesia, as described above, followed by exsanguination.

PARAMETERS FOR THE MEASUREMENT OF PNEUMONIA

Lung lesion volume — At necropsy the length of both right and left lungs was measured, then each was cut into 1 cm slices. The pneumonic area in each parallel section was traced onto a transparent plastic sheet. The cross-sectional area of the lesion at each slice was measured using a semiautomatic digitizing tablet (Zeiss Zidas, Thornwood, New York). Total lung lesion volume was calculated by the following formula (27):

$$\text{Vol} = \bar{t} \times \Sigma A$$

where

TABLE I. Changes in lung lesion volume and bacterial quantitation over time in each treatment group

Group	Days post-challenge	Pigs with lung lesions	Lung lesion volume (cm^3)***	Log_{10} *P. multocida*/gm tissue***		
				Pneumonic lung	Normal lung	Bronchial lymph node
1	3	4/4	78.6 ± 32.9[a]	8.5 ± 0.5	5.1 ± 0.8	5.3 ± 0.7
1	7	4/4	53.7 ± 24.6[a]	7.3 ± 0.3	4.2 ± 1.0	4.6 ± 1.1
1	14	4/4	41.7 ± 14.3[a]	7.8 ± 0.5	4.7 ± 0.5	4.9 ± 0.6
2	3	4/4	17.5 ± 4.4[b]	4.5 ± 1.8	2.7 ± 1.0	1.8 ± 1.1
2	7	3/4	1.2 ± 0.6[b]	0	0	0
2	14	2/4	4.0 ± 3.9[b]	1.4 ± 1.4	1.2 ± 1.2	1.0 ± 1.0
3	3	4/4	4.8 ± 0.4[b]	0	0	0
3	7	3/4	1.3 ± 0.7[b]	0	0	0
3	14	1/4	0.3 ± 0.3[b]	0	0	0
4	3	0/4	0	0	0	0
4	7	0/4	0	0	0	0
4	14	0/4	0	0	0	0

* Pigs received the following inoculum (10 mL in volume) for each group:
Group 1: 4×10^9 *P. multocida* and 4,000 toxic units of *A. pleuropneumoniae* cytotoxin
Group 2: 4×10^9 *P. multocida* only
Group 3: 4,000 toxic units of *A. pleuropneumoniae* cytotoxin only
Group 4: Phosphate buffered saline only

** Data expressed as no. of pigs with lesion/no. of pigs inoculated

*** Data expressed as mean ± SEM. For bacterial count data 0 represents no bacteria isolated rather than 10^0

[a,b] Different superscripts at the same day postinoculation indicate a significant difference ($p < 0.05$) between means. The data expressed in this table are the original data but a transformed (log_e) data was used for statistical analysis

$\bar{t}$ = [length of lung − (first and last slice)]/(number of slices − 2)
A = the area of each measurement

Bacterial concentration per gram tissue — At necropsy, two samples (approximately one gram for each sample) from the pneumonic lungs and one sample each from grossly normal lung and bronchial lymph node were collected in sterile plastic bags and weighed. Ten mL of phosphate buffered saline was added to each bag and the tissues were homogenized for 5 min by a Stomacher (Tekmar® Co., Ohio) homogenizer. Each suspension was centrifuged at 100 × *g* for 10 min, and the supernatant collected. After serial tenfold dilutions, the total bacterial concentration in each tissue was determined using a pour-plate counting method.

HISTOPATHOLOGICAL EXAMINATION

After examining gross lung lesions, specimens for light microscopy were collected and fixed in buffered 10% formalin. The fixed specimens were embedded in paraffin, sectioned, and stained with hematoxylin and eosin.

STATISTICAL ANALYSES

Analysis of variance was employed to evaluate overall differences among groups at each time point for lung lesion volumes and the average daily weight gains (ADG). Multiple comparisons were done using Fisher's protected least significant difference test. The differences in lung lesion volumes of pigs killed at different time points in the same group were also examined using this method. Transformed (log_e) lung lesion volumes were used for the statistical analysis due to heterogeneous variances. When transforming these data, pigs with lung lesion volumes of zero were replaced by a number of 0.001. Planned comparisons were done to examine the interaction between *P. multocida* and *A. pleuropneumoniae* cytotoxin on lung lesion volume at the same time point.

RESULTS

Preliminary experiments had shown that pigs inoculated with a combination of 4,000 toxic units of crude *A. pleuropneumoniae* cytotoxin and 4×10^9 *P. multocida* type A consistently developed pneumonic lesions averaging 34.1 ± 11.8 cm^3 in size. Reducing the dose of *P. multocida* gave inconsistent pneumonia, and increasing it resulted in acute death.

CLINICAL SIGNS

Group 1 pigs received both *P. multocida* and cytotoxin. All pigs had severe depression, shivering, increased respiratory and heart rates, and loss of appetite by six hours PI. Pigs showed varied degrees of clinical signs at 24 hours PI. Most pigs showed some depression and labored breathing (some were open mouth in character) while other pigs returned to normal and were active. Two days PI, clinical signs had stabilized or diminished, however, a few pigs had more severe clinical signs and were reluctant to move. Lameness was observed in some pigs when forced to rise and walk. At this stage some pigs were cyanotic, especially on the skin of the ears and abdomen. Three days PI the general physical condition, including respiration and appetite, was improved for all pigs. Pigs with lameness had swollen joints that persisted for four to five days and then gradually improved. Coughing was infrequent for all pigs. There were no deaths during the 14 day experimental period.

Groups 2 pigs received *P. multocida* alone. These pigs had moderate clinical signs initially, as described for group 1 pigs, but quickly improved by 24 hours PI. Clinical signs had subsided for most pigs by two days PI, although two pigs showed occasional labored breathing.

Group 3 pigs received cytotoxin alone. These pigs had clinical signs similar to those of group 2 pigs that lasted only 12 to 24 hours PI. These pigs then returned to normal physical condition.

Group 4 pigs were placebo controls. Except for some pigs with mild

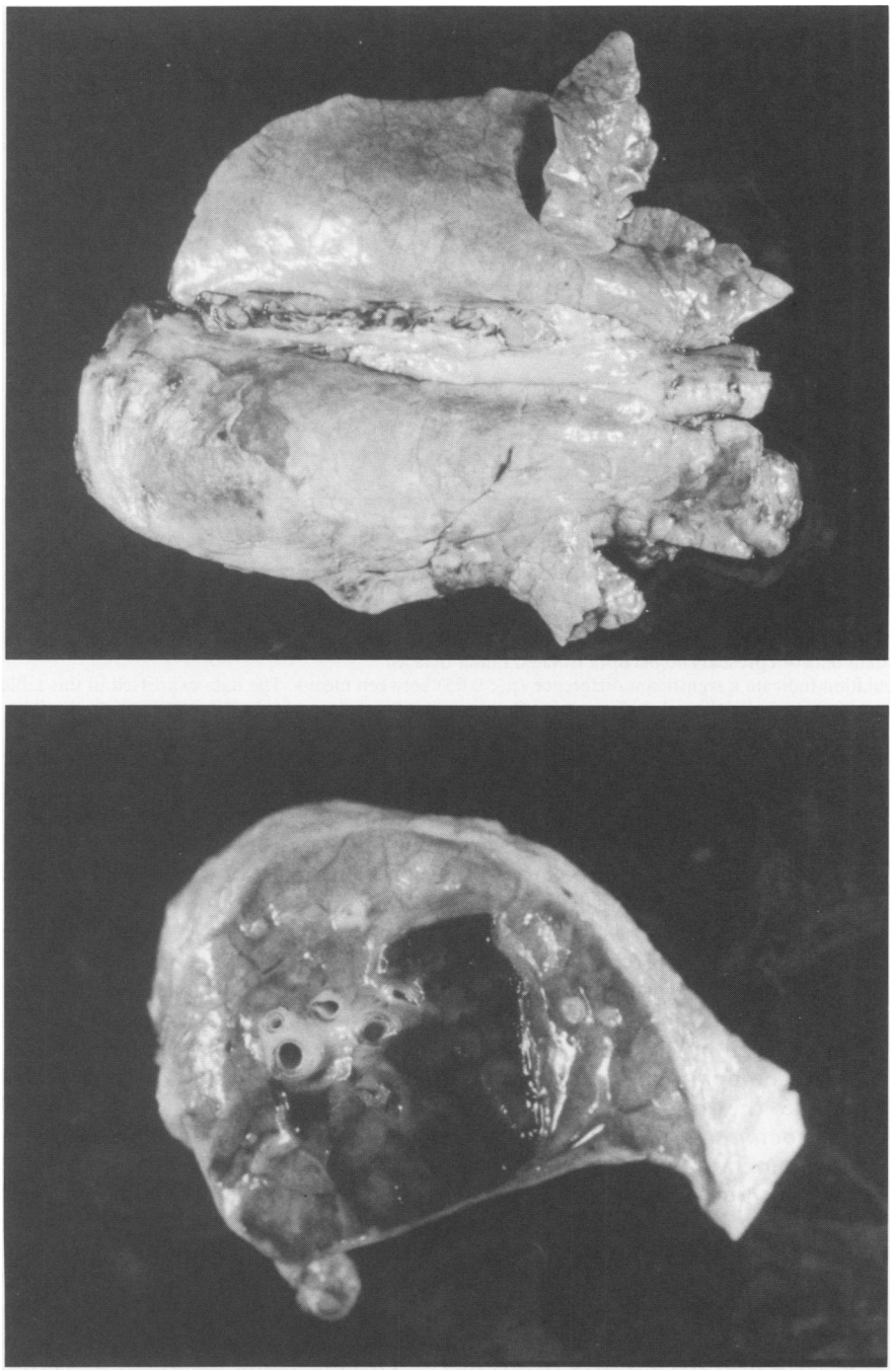

Fig. 1. Lung from a pig challenged with 4×10^9 *P. multocida* type A and 4,000 toxic units of *A. pleuropneumonia* crude cytotoxin and killed three days PI. A) Consolidated lesion with dark-red appearance involving more than one-third of the diaphragmatic lobe of the right lung; the pleura of the affected area was thickened and covered by sheets of fibrin. B) Cross-section of the lung shows the hemorrhagic and necrotic lesion typically produced in the model.

to moderate depression that lasted for four to six hours, no other clinical signs were observed.

LUNG LESION VOLUME

Of the 29 lungs with gross lesions, for 22 the lesions were located in the right diaphragmatic lobe, four were in the left diaphragmatic lobe, two in the intermediate lobe, and one in the right cardiac lobe. All but one had localized lung lesions.

Lung lesions of group 1 pigs were significantly greater in volume than for pigs of group 2 or 3 pigs at 3, 7 and 14 days PI (Table I). Further statistical analysis showed there was interactive effect between *P. multocida* and *A. pleuropneumoniae* cytotoxin on lung lesion volume development at 7 and 14 days PI ($p < 0.05$), but not at three days PI ($0.05 < p < 0.1$). Significant reduction in lung lesion volumes over time was observed for group 3 pigs. Although gradual reduction in lung lesion was noticed 7 and 14 days PI, no significant changes were observed for group 1 ($p = 0.6$) and group 2 ($p = 0.085$) pigs.

MACROSCOPIC LESIONS

In group 1 pigs pulmonary lesions of pigs killed three days PI were severe with an average lung lesion volume of 78.6 cm^3 (range 27.6 to 174.5 cm^3). Affected areas were reddish or dark-red, firm, and covered with sheets of fibrin (Fig. 1A). Cut surfaces revealed multiple hemorrhagic and necrotic foci. They were often confluent and formed locally extensive lesions (Fig. 1B). Lesions outside these local foci were predominantly purulent bronchopneumonia, often involving the entire lung.

Seven days PI the pigs had lung lesion volumes ranging from 7.7 to 123.3 cm^3 (mean = 53.7 cm^3). The intensely necrotic foci became abscesses with heavy encapsulation. Outside the abscesses was interstitial pneumonia and fibrosis 7 and 14 days PI. Fibrin covering the visceral pleura gradually became fibrous tissue and adhesions developed to both the visceral and parietal pleura. Average lung lesion volume was 41.7 cm^3 (range = 6.9 to 76.7 cm^3) for pigs killed 14 days PI.

Group 2 pigs three days PI had localized, consolidated lung lesions that were dark-red to reddish-gray in color and oblong or circular in shape. The affected areas were characterized by purulent bronchopneumonia, but outside these areas was interstitial pneumonia. Mean lung lesion volume was 17.5 cm^3 (range = 12.3 to 30.6 cm^3). As lesion developed progressed, chronic interstitial pneumonia became the predominant lesion in pigs killed 7 and 14 days PI. Mean lung lesion volumes were 1.2 cm^3 (range = 0 to 2.8 cm^3) and 4.0 cm^3 (range = 0 to 15.7 cm^3), for group 2 pigs killed 7 and 14 days PI, respectively.

Group 3 pigs three days PI developed a localized necrotizing fibrinohemorrhagic pneumonia. Lung lesion volumes were consistent between pigs ranging from 4.2 to 5.2 cm^3 (mean = 4.8 cm^3). Lung lesions were significantly reduced in size seven days PI and were characterized by a typical local interstitial pneumonia.

No significant changes were seen in the lungs of placebo inoculated control pigs throughout the experimental period.

TABLE II. Comparison of average daily gain (ADG) among treatment groups

Group*	ADG** (grams) on different days postinoculation	
	7 days	14 days
1	60 ± 45[a]	106 ± 27[a]
2	142 ± 11[a]	217 ± 20[b]
3	179 ± 13[a]	177 ± 19[b]
4	145 ± 29[a]	217 ± 35[b]

* Pigs received the following inoculum (10 mL in volume) for each group:
Group 1: 4×10^9 *P. multocida* and 4,000 toxic units of *A. pleuropneumoniae* cytotoxin
Group 2: 4×10^9 *P. multocida* only
Group 3: 4,000 toxic units of *A. pleuropneumoniae* cytotoxin only
Group 4: Phosphate buffered saline only

** Data expressed as mean ± SEM

[a,b] There was a significant difference ($p < 0.05$) between means with different superscripts in the same column; using Fisher's least significant difference method

MICROBIOLOGICAL FINDINGS

Pasteurella multocida was reisolated from lungs and bronchial lymph nodes of all group 1 pigs (Table I). In addition, three days PI, *P. multocida* was reisolated from the blood of two pigs and from the spleen, liver, and joints of all group 1 pigs. *Pasteurella multocida* persisted in the spleen, liver, and joints of some pigs throughout the experimental period, although there was a gradual reduction in isolation rates (data not shown).

For group 2 pigs, *P. multocida* was reisolated from lungs of three of four pigs necropsied three days PI (Table I). However, *P. multocida* was not recovered from the pigs by seven days PI, except for one pig that had *P. multocida* reisolated from its lung 14 days PI due to an abscess in the thorax. *Pasteurella multocida* persisted in the spleens and livers of group 2 pigs for three days, after which no *P. multocida* were reisolated from the blood, spleen, liver, or joints.

No *P. multocida* was isolated from group 3 and 4 pigs.

AVERAGE DAILY WEIGHT GAIN

No significant difference in ADG between treatment groups was found at seven days PI. However, there was a significant reduction in ADG between group 1 pigs and all other groups of pigs at 14 days PI ($p < 0.05$) (Table II).

DISCUSSION

Endobronchial inoculation of pigs with a combination of 4×10^9 *P. multocida* and 4,000 toxic units of *A. pleuropneumonia* crude cytotoxin consistently induced swine pneumonic pasteurellosis. Using this method, moderate to severe pulmonary lesions persisting for up to 14 days after challenge were observed. *Pasteurella multocida* was reisolated from pneumonic lungs, grossly normal portions of lung, and bronchial lymph nodes throughout the experimental period. Pathological changes included: septicemia, purulent bronchopneumonia, lung abscesses, purulent pleuritis, pericarditis, and septic arthritis similar to those seen in field cases of swine pneumonic pasteurellosis.

This experimental model of swine pneumonic pasteurellosis differs from those previously described in two respects. First, an endobronchial inoculation procedure was used. This mode of inoculation was chosen because: 1) to induce a consistent primary lung lesion, application of *A. pleuropneumoniae* cytotoxin to a local area was necessary; 2) alternative modes (aerosol, intranasal, or intratracheal inoculation) of inoculation of *P. multocida* results in a quick clearance of the organisms by the host and the development of transient lung lesions (6,7,10); and 3) it causes a localized, grossly evident lung lesion that could be easily measured volumetrically (27,28). Second, unlike most other models (6–9), a nonviable priming agent was used. Without the continued influence of a viable primary pathogen, we could examine the sequential development of swine pneumonic pasteurellosis induced predominantly by *P. multocida*. In addition, this model of swine pneumonic pasteurellosis should allow studies on the effects of drugs or vaccines specifically directed against *P. multocida* infection without interference by other microbial pathogens.

Of 29 lungs with gross lesions, 28 had localized lesions and 22 (76%) were located in the right diaphragmatic lobe. This lobe preference is due to the asymmetry of right and left lungs causing deflection of the trachea to the right of the median plane. Also, the bronchus of the right diaphragmatic lobe is a continuation of the right bronchus in a craniocaudal direction, parallel to the long axis of the trunk (29). Since the pigs were held in ventral recumbency during inoculation, insertion of the catheter through the trachea most frequently caused it to be directed into the right diaphragmatic lobe of lung.

Combined challenges of pigs with *P. multocida* and *A. pleuropneumoniae* cytotoxin caused more severe lung lesions than did inoculation of either factor alone (Table I). The mean of lung lesion volumes from group 1 pigs was higher than the sum of group 2 and 3 pigs at 3, 7 and 14 days PI. The interaction effect of *P. multocida* and *A. pleuropneumoniae* cytotoxin on lung lesion volume was significant at 7 and 14 days. However, due to the large variances and small sample sizes, it was not significant at three days PI. This suggests that an additive or synergistic interaction between these two factors exists and an experimental duration of 7 to 14 days is more appropriate. The interaction between *P. multocida* and other primary pathogens such as *M. hyopneumoniae* (7,8) and hog cholera virus (5) in the development of swine pneumonic pasteurellosis has been reported.

Lung damage caused by *A. pleuropneumoniae* crude cytotoxin was temporary and frequently resolved by seven days PI. The cytotoxin preparation contained less than two units of endotoxin (26) and likely contributed little to the observed pathology. Lung

lesions induced by *P. multocida* alone were reduced by 7 to 14 days PI. There was no significant change in lung lesion volume over a 14 day experimental period in group 1 pigs ($p = 0.6$). Delayed resolution of lung lesions in group 1 pigs was probably due to the larger lung lesions induced initially and the persistent insult of *P. multocida*. Judging from the gradual reduction in lung lesion volumes and *P. multocida* isolation rates from tissues (Table I), steady resolution of lung lesions could be expected in group 1 pigs over time.

Depressions in ADG and feed efficiency caused by swine pneumonia were reported in a field survey (30,31). Conflicting results on weight gain and feed efficiency have been found in other studies of experimentally induced swine pneumonic pasteurellosis (7,14). Experiment length was probably an important factor contributing to the different conclusions. In the present study, due to the limited sample size and the large variances in group 1 pigs, no significant differences ($0.05 < p < 0.10$) in ADG among groups were observed at seven days PI. However, a significant reduction in ADG was found with group 1 pigs as compared to other groups after 14 days ($p < 0.05$). For group 1 pigs, clinical signs gradually subsided and the intake of feed increased (data not shown). It is possible that the ADG of these pigs might return to normal given sufficient time, unless relapse of disease occurred.

Variation between pigs in susceptibility to *P. multocida* infection, the experiment length and the dose of *P. multocida* can affect the outcome of experimental swine pneumonic pasteurellosis. In spite of these variables, a successful swine pneumonic pasteurellosis model was established.

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