Mycoplasma hyopneumoniae Increases the Susceptibility of Pigs to Experimental *Pasteurella multocida* Pneumonia

Abel Ciprián, Carlos Pijoan, Tonatiuh Cruz, José Camacho, Jorge Tórtora, Germán Colmenares, Rubén López-Revilla and Mireya de la Garza*

ABSTRACT

The interaction between *Mycoplasma hyopneumoniae* and *Pasteurella multocida* in experimental pneumonia was investigated in conventional pigs. The experimental animals were 49 days old when inoculated with *M. hyopneumoniae*; they were inoculated with *P. multocida* after 23 days, and killed 13 days later. In pigs inoculated only with *P. multocida*, clinical signs and lung lesions were not observed, and the agent was not recovered. Pigs inoculated with *M. hyopneumoniae* developed fever, moderate cough and dyspnea which tended to disappear, and small proliferative lung lesions from which *M. hyopneumoniae* was isolated. Pigs inoculated with both agents had higher fever, severe cough and dyspnea which tended to aggravate, and extensive exudative lung lesions from which organisms were isolated. All animals had similar growth rates, but the group infected with both agents consumed 60% more food. Therefore, *M. hyopneumoniae* causes mild pneumonia, whereas *P. multocida* is not pathogenic alone but aggravates the pneumonia initiated by *M. hyopneumoniae*.

RÉSUMÉ

Cette expérience consistait à étudier l'interaction entre *Mycoplasma hyopneumoniae* et *Pasteurella multocida*, lors d'une pneumonie expérimentale, chez des porcs conventionnels. Les sujets expérimentaux étaient âgés de 49 jours, lorsqu'on leur inocula *M. hyopneumoniae*; 23 jours après, on leur inocula *P. multocida* et, au bout de 13 jours, on les sacrifia. On n'observa pas de signes cliniques ou de lésions pulmonaires, chez ceux qui n'avaient reçu que *P. multocida*, et on ne recouvra pas la bactérie. Par ailleurs, les sujets qui avaient reçu *M. hyopneumoniae* développèrent de l'hyperthermie, une toux modérée et de la dyspnée qui s'amenuisèrent, ainsi que des lésions pulmonaires prolifératives discrètes desquelles on recouvra *M. hyopneumoniae*. Les porcs auxquels on avait injecté les deux microorganismes expérimentaux développèrent une hyperthermie marquée, une toux sévère et une dyspnée qui tendaient à s'aggraver, ainsi que des lésions pulmonaires exsudatives extensives desquelles on recouvra les deux microorganismes. Tous les porcs affichèrent un taux de croissance semblable, mais ceux qui avaient reçu les deux microorganismes consommèrent 60% plus d'aliments. Par conséquent, *M. hyopneumoniae* cause une pneumonie légère, tandis que *P. multocida* seule n'est pas pathogène; elle aggrave cependant la pneumoie déclenchée par *M. hyopneumoniae*.

INTRODUCTION

Enzootic pneumonia is a chronic pulmonary disease which develops in pigs of any age (1), and whose primary causative agent is *Mycoplasma hyopneumoniae* (2,3,4). In a study performed on slaughtered pigs, *M. hyopneumoniae* and *Pasteurella multocida* were the agents most frequently found in the lung lesions of enzootic pneumonia (5).

Morrison *et al* (6) recently reported that, in slaughtered pigs, the pneumonic lesions from which *M. hyopneumoniae* and *P. multocida* were simultaneously isolated were more severe than those from which only one of these agents was recovered. On the other hand, Smith *et al* (7) found that in gnotobiotic pigs experimentally inoculated with either *M. hyopneumoniae* or *P. multocida* type A, the lesions caused by each agent were mild, not extensive, and histologically identical. However, simultaneous inoculation with both agents produced extensive and severe lung lesions (7). These reports suggest the possibility of synergy between *M. hyopneumoniae* and *P. multocida*.

In this paper we analyzed the effect that primary infection of conventional pigs with *M. hyopneumoniae* has on the establishment and severity of *P. multocida* secondary lung infection.

*Coordinación General de Investigación y Estudios de Posgrado, Facultad de Estudios Superiores Cuautitlán, Universidad Nacional Autonóma de México, Ap. Postal 222, Cuautitlan Izcalli Méx. 54750, México (Ciprián, Cruz, Camacho, Tortora), Department of Large Animal Clinical Sciences, University of Minnesota, St. Paul, Minnesota 55108 (Pijoan) and Departamento de Biología Celular, Centro de Investigación y de Estudios Avanzados del IPN, Ap, Postal 14-740, México D.F. 07000, México (Colmenares, López-Revilla, de la Garza).

This report represents a portion of the doctoral thesis project submitted by A. Ciprián to Facultad de Estudios Superiores Cuautitlán, UNAM.

This research was partially supported by Consejo del Sistema Nacional de Enseñanza Tecnólogica (COSNET grant 506/86).

Submitted December 21, 1987.

MATERIALS AND METHODS

SWINE

Nineteen conventional Yorkshire male pigs were obtained from three different litters of fifth parturition offspring and weaned after the fifth week of life. The piglets were fed with commercial pelleted antibiotic-free weaning ration (Purina, México).

EXPERIMENTAL DESIGN

Sixteen pigs were divided in four groups, each consisting of four animals. Each group was housed separately under conventional conditions. The animals had free access to drinking water and food. Group A were untreated controls; group B consisted of pigs inoculated only with *M. hyopneumoniae* on the first experimental day; group C contained pigs inoculated only with *P. multocida* on day 23; group D pigs were inoculated with *M. hyopneumoniae* on day 1 and with *P. multocida* on day 23.

MICROORGANISMS

Mycoplasma hyopneumoniae strain 194 was isolated from a field case of enzootic pneumonia and was kindly supplied by Dr. Richard Ross, Veterinary Medical Research Institute, College of Veterinary Medicine, Iowa State University, Ames, Iowa in a pneumonic lung suspension. *Pasteurella multocida* strain A52 (capsular type A) was isolated from a field case of pig pneumonia and obtained from Dr. Carlos Pijoan's collection.

CULTURE MEDIA

For mycoplasma cultures, Friis' broth (8) was supplemented with kanamycin sulfate (1 mg/mL), 20% (v/v) horse serum and 10% (v/v) fresh yeast extract. To prepare Friis' solid medium, 0.9% (w/v) Noble agar (Difco Laboratories, Detroit, Michigan) was added to the broth. *Pasteurella multocida* was cultured either in brain-heart infusion (BHI, Bioxon de México, Oaxaca, Oax, México) broth or agar supplemented with 5% (v/v) bovine blood.

PREPARATION AND STANDARDIZATION OF INOCULA

Pneumonic lung suspension containing M. hyopneumoniae — Three piglets were sedated with 2 mg azaperone (Janssen Pharmaceutica, Beerse, Belgium) per kg body weight, deeply anesthetized with 1.5 mg metomidate hydrochloride (Janssen Pharmaceutica, Beerse, Belgium) per kg body weight and inoculated intratracheally with 10 mL of a pneumonic lung suspension containing *M. hyopneumoniae* strain 194 that was free of other mycoplasma species as well as of *P. multocida, Bordetella* sp., *Actinobacillus pleuropneumoniae*, porcine *Haemophilus* sp., and respiratory viruses. The piglets were observed daily and killed when clinical signs of pneumonia appeared. The pneumonic lungs were collected aseptically and homogenized in a Ten-Broeck tissue grinder as reported by Piffer and Ross (1). Samples of homogenate were diluted serially in Friis' medium through 10^{-5} and incubated five to seven days in a 10% CO_2 atmosphere at 37°C; for mycoplasma titration, dilutions were cultured in fresh Friis' solid medium and incubated in a candle jar under the same conditions. An aliquot was taken to determine the concentration of viable organisms in color-changing units (CCU). The homogenate diluted to contain 10^4 CCU/mL was used to inoculate group B and D animals, using the procedure described above.

Aerosolization with P. multocida suspensions — Pasteurella multocida strain A52 was cultured overnight in BHI broth at 37°C; the bacterial suspension was adjusted to an optical density of 0.70 at 660 nm in a Spectronic 20 spectrophotometer (Bausch and Lomb, Rochester, New York), equivalent to 1×10^8 colony-forming units (CFU) per mL. A fixed volume of standardized bacterial suspension was aerosolized at constant rate (22 mL per animal group in 30 min) to inoculate separately group C and group D animals, 23 days after the experiment began, in an aerosolization chamber containing three medical nebulizers (DeVilbiss, Somerset, Pennsylvania); the chamber was adapted from that designed by Lopez *et al* (9).

NECROPSY

Pigs were killed by electrocution followed by exsanguination, after sedation and deep anesthesia. Lungs were removed and areas of pneumonia were evaluated and sketched. The extent of pneumonic lesions was determined by planimetry in standardized dorsal and ventral pulmonary diagrams, considering the surface of both sides as 100%. Statistically significant differences between groups ($p < 0.05$) were determined with the *t*-test.

HISTOPATHOLOGY

Lung tissue samples were fixed in phosphate-buffered 10% formalin, embedded in paraffin, and stained with hematoxylin-eosin.

CULTURE TECHNIQUES

Mycoplasma hyopneumoniae — The whole trachea and lungs from each pig were collected. Sterile Friis' broth was poured through the trachea into the lungs, from which the medium was recovered. Samples of consolidated lung areas were homogenized. The medium recovered and the homogenates were cultured as described above. *Mycoplasma hyopneumoniae* was identified by its sterol dependency (10) and the biochemical and serological tests described by Goodwin *et al* (3).

Pasteurella multocida — Samples from pneumonic lesion homogenates were streaked on 5% bovine blood agar plates, which were incubated and examined 24 h and 48 h later. Suspect *P. multocida* colonies were picked and identified by the criteria of Cowan and Steel (11). The acriflavine (12) and the staphylococcal hyaluronidase (13) tests were used to determine their capsular types.

GROWTH PERFORMANCE OF PIGS

The amount of food was calculated according to the weight of animals. Pigs from 11 to 16 kg were given 1 kg/day, those from 16 to 28 kg received 1.5 kg/day, and pigs from 28 to 38 kg were given 2 kg/day. The average daily gain (ADG) and feed efficiency (FE) ratios were calculated for each experimental group. Variance analysis was applied to ADG and FE ratios of groups under comparison.

RESULTS

TEMPERATURE

Group A pigs had normal temperatures during the experiment, with a

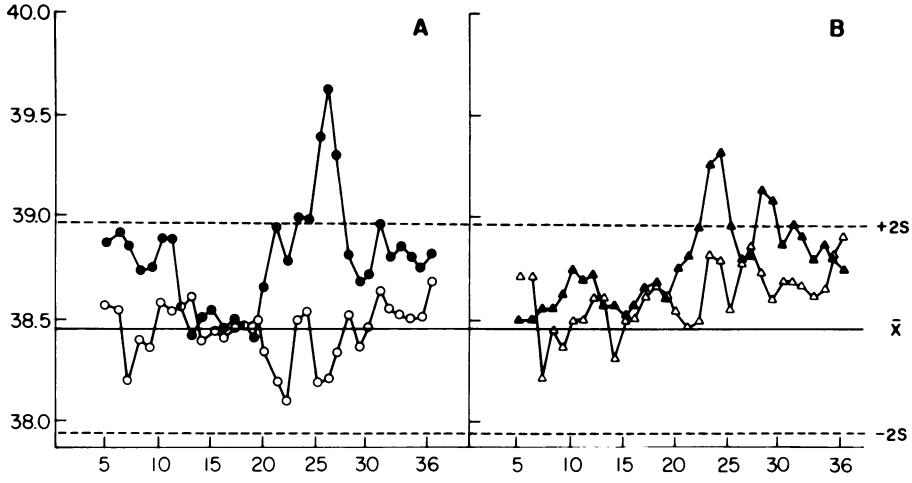

Fig. 1. Average temperature of experimental pig groups. (A) Temperature of untreated pigs (group A, o), and of group B (●) pigs inoculated on the first experimental day with *M. hyopneumoniae* strain 194; note the unimodal hyperthermia observed in this group. (B) Temperature of group C (Δ) pigs inoculated on day 23 with an aerosolized suspension of *P. multocida*, and of group D (▲) pigs that were inoculated sequentially: on day 1 with *M. hyopneumoniae* and on day 23 with *P. multocida*. Note the bimodal hyperthermia observed in group D. The continuous horizontal line represents the normal pig temperature (38.4°C); distontinuous lines are placed two standard deviations above (+2S) and below (-2S) the normal.

mean ± SD of 38.4 ± 0.25°C (see Fig. 1). Pigs in group B had hyperthermia beginning on day 19 and reaching its maximum (39.6°C) on day 26; later, the temperature was only slightly higher than normal (Fig. 1A). Group C pigs had normal temperatures during the experiment (Fig. 1B). Group D had bimodal hyperthermia with its first rise beginning on day 19 and reaching its maximum (39.3°C) on day 24; the second peak began on day 27 and reached its maximum (39.2°C) on day 28; after the second peak, the temperature in this group was maintained slightly above normal until the end of the experiment (Fig. 1B).

RESPIRATORY SIGNS

Respiration was normal in groups A and C. Pigs in groups B and D developed nonproductive cough which evolved from mild to severe, as well as dyspnea at rest (Fig. 2). In group B, mild cough started on day 18; on day 22 it had increased to become moderate in three out of four pigs; on days 23 to 27 all pigs had moderate cough. From day 28, cough severity slowly decreased to become mild again at the end of the experiment (Fig. 2A). On the other hand, on day 23 all pigs in group D had moderate cough, that became severe on day 32 in all pigs, and continued unchanged until the end of the experiment (Fig. 2B). Dyspnea on effort predominated over dyspnea at rest in group B (Fig. 2C) whereas dyspnea at rest predominated over dyspnea on effort in group D (Fig. 2D).

GROSS LUNG PATHOLOGY

The distribution, extent, and appearance of the macroscopic lung lesions are summarized in Table I. No macroscopic lesions were found in groups A and C, except for the right cardiac lobe of an animal of group A, which had a small reddish consolidated zone (covering 3.3% of the lung surface) with pleural adhesions, and from which *Mycoplasma hyorhinis* was isolated. Group B pigs had reddish-gray consolidated lesions ranging in extent from 4 to 17% of their lung surface, with pleural adhesions in one case. Group D animals had reddish or reddish-gray consolidated areas extending from 22 to 26% of the lung surface, with pleural adhesions in three cases; group D lesions were more extensive ($p < 0.05$) than those of the other two treated groups.

HISTOPATHOLOGY

Figures 3A and 3C exemplify the absence of histological changes in the lungs of groups A and C. Consolidated lung areas of group B pigs had alveolar collapse, alveolar septum enlargement, lymphocytic peribronchial infiltration (2-3 cells per septum), and a slight increase in alveolar macrophages (Fig. 3B). Group D had enlarged septa, a large increase in the number of polymorphs in alveoli and bronchi, alveolar and interstitial hemorrhages, marked alveolar macrophage proliferation, and perivascular and alveolar lymphocytic infiltration with fibrin deposition (Fig. 3D).

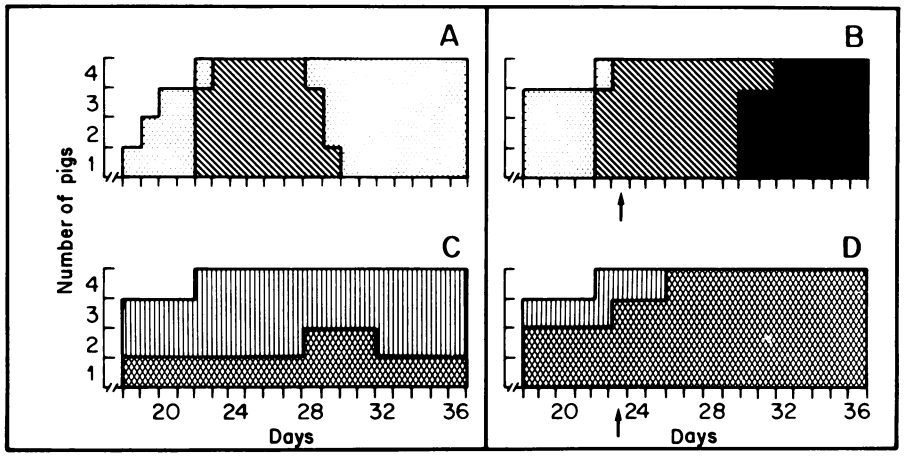

Fig. 2. Evolution of respiratory signs in pigs inoculated either with *M. hyopneumoniae* only, or with *M. hyopneumoniae* and *P. multocida*. On the left side of the figure (panels A and C) are shown the clinical signs of group B pigs (inoculated only with *M. hyopneumoniae*); on the right side of the figure (panels B and D) are shown the clinical signs of group D pigs (inoculated on the first day with *M. hyopneumoniae* and on day 23 (arrows) with *P. multocida*). (A) and (B) show the temporal course and severity of cough, whereas (C) and (D) show the temporal course and severity of dyspnea.

Cough: mild ▒, moderate ▓, severe ■. Dyspnea: on effort ▥, at rest ▩.

TABLE I. Location and Appearance of Macroscopic Lung Lesions[a]

	Consolidated Lobes							Appearance of Lesions		
	Apical		Cardiac		Diaphragmatic[b]					
Group	R[c]	L[c]	R	L	R	L	Accessory	Red	Reddish-gray	Pleural Adhesions
A	0	0	1[d]	0	0	0	0	1	0	1
B	4	4	4	4	3	2	3	1	3	1
C	0	0	0	0	0	0	0	0	0	0
D	4	4	4	4	3	3	3	2	2	3

[a]Data indicate the number of animals affected in each group of four
[b]Restricted to cephalic portions
[c]R = Right; L = Left
[d]*M. hyorhinis* was isolated from this lesion

RECOVERY OF MICROORGANISMS

None of the agents under study was isolated from group A or C animals. *Mycoplasma hyopneumoniae* was recovered from the endotracheal washings from all pigs in groups B and D; *M. hyopneumoniae* was also isolated from the pneumonic lesion homogenates in three pigs from each of groups B and D. *Pasteurella multocida* was isolated only in three pigs of group D (Table II). *Mycoplasma hyorhinis* was isolated from one group A pig.

GROWTH PERFORMANCE

Pigs from groups A and C had an ADG of 0.36 ± 0.07 kg; the FE ratio was 2.68 ± 0.62 for group A and 2.42 ± 0.62 for group C. Group B pigs had an ADG of 0.34 ± 0.07 kg and their FE ratio was 3.55 ± 0.62. Group D pigs had an ADG of 0.29 ± 0.07 kg and their FE ratio was 4.55 ± 0.44.

DISCUSSION

Smith *et al* (7) showed that gnotobiotic pigs infected with *M. hyopneumoniae* developed more severe pneumonic lesions when they were inoculated simultaneously with *P. multocida* type A. Morrison *et al* (6) recently demonstrated that pig lungs in which *M. hyopneumoniae* was identified by immunofluorescence, and from which *P. multocida* also was isolated, had more extensive lesions than lungs in which only one of these agents was identified or recovered. These reports show that both agents are found in severe cases of chronic pneumonia, but do not prove the pathogenic interaction of *M. hyopneumoniae* and *P. multocida*. The data presented here appear to demonstrate conclusively that *M. hyopneumoniae* predisposes to the opportunistic infection of *P. multocida* in conventional pigs.

Pasteurella multocida did not produce fever when inoculated alone, whereas *M. hyopneumoniae* produced fever in group B animals from day 19 to 26 postinoculation (Fig. 1A), as Livingstone *et al* (17) had previously reported. Similar data have been reported for gnotobiotic pigs inoculated simultaneously with *M. hyopneumoniae* and *P. multocida* (7). Based on these reports and our own observations, we decided to inoculate *P. multocida* on day 23 to group D pigs, whose fever was more intense and lasting (Fig. 1B) than that produced in pigs of group B by *M. hyopneumoniae* alone (Fig. 1A).

Pigs inoculated only with *M. hyopneumoniae* showed minimal dyspnea and tended to recover (Figs. 2A and 2C), as reported by Whittlestone (14). On the other hand, pigs sequentially inoculated with both agents developed severe cough and dyspnea (Figs. 2B and 2D), as reported for the bronchopneumonia caused by secondary infectious agents (6). It appears that *P. multocida* behaves as an opportunistic secondary infectious agent in enzootic pneumonia.

The control group, and the group of pigs inoculated only with *P. multocida* strain A52 had no macroscopic lung lesions, suggesting that when inoculated alone this species is not pathogenic. Pneumonic areas in the *M. hyopneumoniae*-inoculated group were less extensive than those found in pigs inoculated with both *M. hyopneumoniae* and *P. multocida*. These results suggest that *M. hyopneumoniae* infection facilitates the pathogenicity of *P. multocida*. In pigs inoculated with both agents, the pneumonic areas were more extensive and covered 22 to 26% of the lung surface; by Goodwin's method (15), such lesions would have had the maximum severity score (55 points).

The macroscopic lesions that we found in conventional pigs inoculated with *P. multocide* were different from those reported by Smith *et al* (7) in gnotobiotic pigs. This difference may be due to the gnotobiotic pigs being more susceptible to infection than our conventional pigs.

TABLE II. Recovery of *M. hyopneumoniae* and *P. multocida* from Postmortem Lungs

		Recovery of *M. hyopneumoniae*				Recovery of *P. multocida* in Lung Homogenates	
		Lung Homogenates		Endotracheal Washings			
Group[a]	Treatment	Number of Positive Pigs	Mean Titer of Positives[b]	Number of Positive Pigs	Mean Titer of Positives[b]	Number of Positive Pigs	Mean Titer of Positives[b]
A	None	0	0	0	0	0	0
B	*M. hyopneumoniae*[c] alone	4	10^2	2	$10^{2.5}$	0	0
C	*P. multocida*[d] alone	0	0	0	0	0	0
D	*M. hyopneumoniae*[c] and *P. multocida*[d]	4	10^2	4	10^2	3	10^1

[a]Each group contained four pigs
[b]Titer represents number of color-changing units (*M. hyopneumoniae*) or colonies (*P. multocida*) per g of lung tissue
[c]Pigs inoculated endotracheally with 10^4 color-changing units/mL (10 mL/pig) on day 1
[d]Pigs aerosolized for 30 min with 10^8 cells/mL (22 mL/group of 4 animals) on day 23

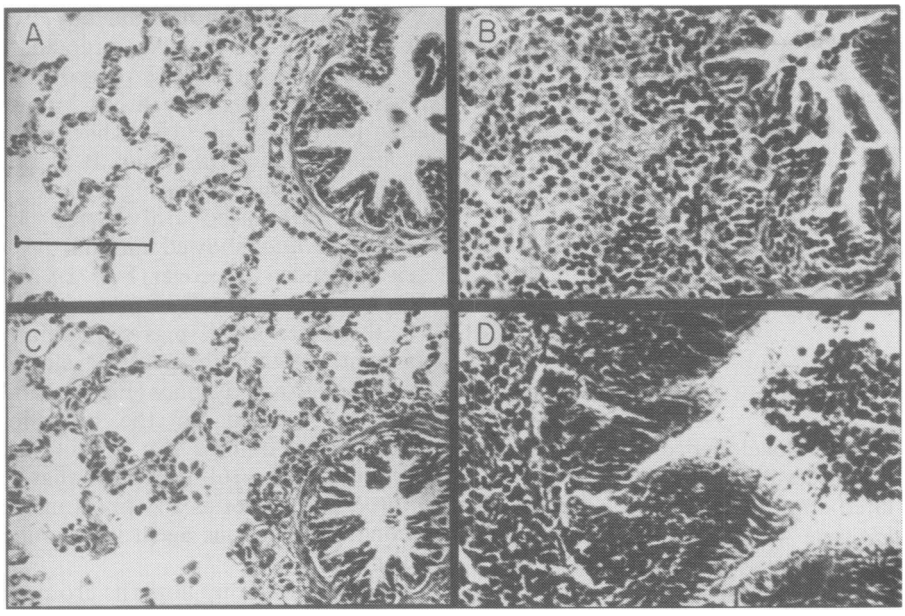

Fig. 3. Typical microscopic aspect of lung sections from each animal group. (A) Normal alveoli and bronchi in untreated pigs. (B) Proliferative lesions caused by *M. hyopneumoniae*. (C) Alveoli and bronchi without pathological changes in pigs inoculated with *P. multocida* alone. (D) Exudative lesions caused by the sequential inoculation of both infectious agents. Hematoxylin-eosin stain. Bar = 100 μm.

Lesions caused by *M. hyopneumoniae* were proliferative (Fig. 3B), whereas those caused by both infectious agents were exudative (Fig. 3D), like the lesions commonly found in secondary infections (6). This finding again shows that *P. multocida* behaves as a secondary infectious agent.

Mycoplasma hyopneumoniae was recovered from all the inoculated pigs, in agreement with reports in which it was isolated from experimentally induced active pneumonia (16) where it is known to have a long persistence in the lungs (14,17,18).

Pasteurella multocida was recovered from three of the pigs inoculated also with *M. hyopneumoniae* (group D) but not from pigs inoculated only with the first agent (group C), a finding indicating that pigs inoculated first with *M. hyopneumoniae* were not always able to eliminate *P. multocida*, in contrast with pigs inoculated only with *P. multocida*, which did eliminate this bacterial species from their lungs. An immunosuppressive event caused by the infection with a primary agent like *M. hyopneumoniae* may be needed for lung colonization by *P. multocida*, as has already been found in pigs inoculted with attenuated hog cholera virus, which enables subsequent lung colonization and invasion by *P. multocida* (19).

Growth performance was similar ($p > 0.05$) in all our experimental groups. Food consumption by pigs inoculated with *P. multocida* was not different from that of the control group. Pigs inoculated with *M. hyopneumoniae*, however, ate 32% more food. The infection with both agents caused a 59% increase in food consumption. These results suggest that food consumption increases due to the disease provoked by the interaction of *M. hyopneumoniae* and *P. multocida*, thus raising the costs of production.

We inoculated *M. hyopneumoniae* and *P. multocida* sequentially in order to simulate farm conditions. Our findings strongly suggest that there is a synergistic or additive pathogenic interaction between *M. hyopnaeumoniae* and *P. multocida*. We also showed that previous infection with *M. hyopneumoniae* favors the opportunistic colonization and lung damage caused by *P. multocida*, whose pathogenic mechanisms could now be analyzed in this experimental model.

ACKNOWLEDGMENTS

The authors thank Virginia Lara, Susana Mendoza, and Pilar Yañez for skillful technical assistance, and Dr. Manuel V. Ortega and Dr. Irma Tejada for their useful comments.

REFERENCES

1. **PIFFER IA, ROSS RF.** Effect of age on susceptibility of pigs to *Mycoplasma hyopneumoniae* pneumonia. Am J Vet Res 1984; 45: 478-481.
2. **MARE CJ, SWITZER WP.** New species: *Mycoplasma hyopneumoniae*. A causative agent of virus pig pneumonia. Vet Med 1965; 60: 841-846.
3. **GOODWIN RFW, POMEROY AP, WHITTLESTONE P.** Characterization of *Mycoplasma suipneumoniae:* a mycoplasma causing enzootic pneumonia of pigs. J Hyg 1967; 65: 85-97.
4. **HODGES RT, BETTS AO, JENNINGS AR.** Production of pneumonia in gnotobiotic pigs with pure cultures of *Mycoplasma hyopneumoniae*. Vet Rec 1969; 84: 268-273.
5. **SMITH JE.** Analysis of autopsy data on pig respiratory disease by multivariate methods. Br Vet J 1977; 133: 281-291.
6. **MORRISON RB, PIJOAN C, HILLEY HD, RAPP V.** Microorganisms associated with pneumonia in slaughter weight swine. Can J Comp Med 1985; 49: 129-137.
7. **SMITH IM, HODGES RT, BETTS AO, HAYWARD AHS.** Experimental infections of gnotobiotic piglets with *Pasteurella septica* (sero-group A) alone or with *Mycoplasma hyopneumoniae*. J Comp Pathol 1973; 83: 307-321.
8. **FRIIS NF.** A selective medium for *Mycoplasma suipneumoniae*. Acta Vet Scand 1971; 12: 454-456.
9. **LOPEZ A, THOMSON RG, SAVAN M.** The pulmonary clearance of *Pasteurella haemolytica* in calves infected with bovine parainfluenza-3 virus. Can J Comp Med 1976; 40: 385-391.
10. **FRIIS NF.** The SPP and digitonin test applied to porcine mycoplasmas. Acta Vet Scand 1975; 16: 474-476.
11. **COWAN ST.** Cowan and Steel's Manual for the Identification of Medical Bacteria, 2nd ed. Cambridge: Cambridge University Press, 1974.
12. **CARTER GR, SUBRONTO P.** Identification of type D strains of *Pasteurella multocida* with acriflavine. Am J Vet Res 1973; 34: 293-294.
13. **CARTER GR, RUNDELL SN.** Identification of type A strains of *Pasteurella multocida* using a staphylococcal hyaluronidase. Vet Rec 1975; 93: 393.
14. **WHITTLESTONE P.** Porcine mycoplasmas. In: Tully JG, Whitcomb RF, eds. The Mycoplasmas, Vol. 2. New York: Academic Press, 1979: 133-176.
15. **GOODWIN RFW.** Respiratory diseases of pigs. Vet Rec 1971; 89:77-81.
16. **GOODWIN RFW.** Isolation of *Mycoplasma hyopneumoniae* from the nasal cavities and lungs of pigs affected with enzootic pneumonia or exposed to this infection. Res Vet Sci 1972; 13: 262-267.
17. **LIVINGSTONE CW, STAIR EL, UNDERDAHL NR, MEBUS CA.** Pathogenesis of mycoplasmal pneumonia in swine. Am J Vet Res 1972; 33: 2249-2258.
18. **UNDERDAHL NR, KENNEDY GA, RAMOS AS Jr.** Duration of *Mycoplasma hyopneumoniae* infection in gnotobiotic pigs. Can Vet J 1980; 21: 258-261.
19. **PIJOAN C, OCHOA G.** Interaction between a hog cholera vaccine strain and *Pasteurella multocida* in the production of porcine pneumonia. J. Comp Pathol 1978; 88: 167-170.