Effects of intranasal inoculation of porcine reproductive and respiratory syndrome virus, *Bordetella bronchiseptica*, or a combination of both organisms in pigs

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Objective—To examine effects of co-infection with porcine reproductive and respiratory syndrome virus (PRRSV) and *Bordetella bronchiseptica* in pigs.

Animals—Forty 3-week-old pigs.

Procedure—30 pigs (10 pigs/group) were inoculated with PRRSV, *B bronchiseptica*, or both. Ten noninoculated pigs were control animals.

Results—Clinical signs, febrile response, and decreased weight gain were most severe in the group inoculated with both organisms. The PRRSV was isolated from all pigs in both groups inoculated with virus. All pigs in both groups that received PRRSV had gross and microscopic lesions consistent with interstitial pneumonia. *Bordetella bronchiseptica* was cultured from all pigs in both groups inoculated with that bacterium. Colonization of anatomic sites by *B bronchiseptica* was comparable between both groups. Pigs in the group that received only *B bronchiseptica* lacked gross or microscopic lung lesions, and *B bronchiseptica* was not isolated from lung tissue. In the group inoculated with *B bronchiseptica* and PRRSV, 3 of 5 pigs 10 days after inoculation and 5 of 5 pigs 21 days after inoculation had gross and microscopic lesions consistent with bacterial bronchopneumonia, and *B bronchiseptica* was isolated from the lungs of 7 of those 10 pigs.

Conclusions and Clinical Relevance—Clinical disease was exacerbated in co-infected pigs, including an increased febrile response, decreased weight gain, and *B bronchiseptica*-induced pneumonia. *Bordetella bronchiseptica* and PRRSV may circulate in a herd and cause subclinical infections. Therefore, co-infection with these organisms may cause clinical respiratory tract disease and leave pigs more susceptible to subsequent infection with opportunistic bacteria. (*Am J Vet Res* 2000;61:892–899)

Respiratory tract disease in pigs is one of the most important health concerns for swine producers. Pneumonia in swine is often a multifactorial disease caused by infection with multiple pathogens and involving environmental and management factors. **Porcine respiratory disease complex (PRDC)** is a term that has been used to describe this condition. Initially described as a problem affecting pigs 15 to 20 weeks old in the grower-finisher stage of production, PRDC also has been used to describe multifactorial pneumonia at other stages of production.[1] Numerous infectious organisms have been implicated, most commonly **porcine reproductive and respiratory syndrome virus (PRRSV)**, *Mycoplasma hyopneumoniae*, *Pasteurella multocida*, and swine influenza virus.[2-4] Other agents isolated less commonly include *Actinobacillus pleuropneumoniae*, *Streptococcus suis*, *Haemophilus parasuis*, and *Bordetella bronchiseptica*.[3,4]

Porcine respiratory disease complex can develop in herds despite the use of segregated early weaning. Segregated early weaning generally refers to systems in which pigs are weaned at a relatively young age (< 21 days old) and separated from the sows to prevent or decrease transmission of some pathogens from dam to baby pigs, including pathogens such as *M hyopneumoniae*, *P multocida*, *B bronchiseptica*, and *A pleuropneumoniae*.[5] Although use of such systems may result in a general decrease in the number of infected pigs, in large herds it may also result in a low number of infected pigs in which the infection can persist into the grower phase, which spreads the agents to naive pigs when maternal immunity wanes.[6] Certain secondary pathogens such as *S suis* and *H parasuis* colonize efficiently even with early weaning systems.[5]

Even though many potential bacterial pathogens colonize the nasal cavity or tonsils of pigs, normal defense mechanisms of the respiratory tract, such as the mucociliary apparatus, alveolar macrophages, and secretory IgA, prevent infection from spreading to the lungs and causing pneumonia. Primary pulmonary bacterial and viral pathogens have virulence factors that allow them to overcome natural defenses in the lungs. Once primary infections are established, secondary pathogens can subsequently proliferate and cause disease. Primary agents include PRRSV, *M hyopneumoniae*, *A pleuropneumoniae*, swine influenza virus, and *B bronchiseptica*.[3,4]

Porcine reproductive and respiratory syndrome virus is a widely disseminated pathogen of swine capable of causing reproductive and respiratory tract disease. There is evidence that PRRSV is associated with outbreaks of disease caused by other pathogens.[2,7-15] Furthermore, PRRSV can be involved in respiratory tract disease in neonatal pigs, pneumonia that develops in pigs shortly after weaning, and pneumonia in pigs in the grower-finish-

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er stage.[2,8,10,11,14,16,17] Differences in virulence among strains of PRRSV, environmental conditions, passive and active immunity, and infection with other organisms may affect the outcome of natural infection.[9,12,14,18-26] In 1996, severe epizootics of **porcine reproductive and respiratory syndrome (PRRS)**, described as atypical PRRS, were reported, which involved a high number of abortions, increases in sow mortality, and pneumonia in nursery pigs.[27] Experimentally, PRRSV isolates from these herds appear more virulent than previous PRRSV isolates, resulting in pneumonic lesions that are more severe and persist longer.[28]

Bordetella bronchiseptica also is widely disseminated in pig herds. *Bordetella bronchiseptica* can cause primary bronchopneumonia in neonatal pigs and may be a secondary pathogen in respiratory tract disease in older swine. It colonizes the nasal cavity causing mucosal inflammatory lesions with loss of cilia and atrophy of the turbinates. A sow is a major source of infection for her litter, and the infection can then spread when litters are mixed. Prevalence of *B bronchiseptica* infection peaks when pigs are approximately 12 weeks old, and a high percentage of pigs remain infected until 18 to 20 weeks of age.[29-31] Infection with *B bronchiseptica* can predispose pigs to disease with secondary pathogens such as *P multocida* and *S suis*.[32-34]

Because *B bronchiseptica* and PRRSV are common pathogens that can be found in pigs from birth through the grower-finisher stage, the purpose of the study reported here was to determine whether co-infection would result in a greater severity of disease than infection with either agent alone. Clinical signs were monitored, and pigs were euthanatized and necropsies performed 10 and 21 days after inoculation for detection of lesions and to enable us to collect samples for viral and bacterial isolation.

Materials and Methods

PRRSV and *B bronchiseptica* inocula—The PRRSV isolate used (strain NADC-21)[a] was isolated from the serum of a prematurely born, weak pig on a farm that had an acute onset of abortions and an increase in sow mortality, characteristics compatible with an outbreak of atypical PRRS. In a preliminary study in our laboratory, 10-week-old pigs experimentally infected with the isolate developed signs of respiratory tract disease and interstitial pneumonia. The virus was isolated on MARC-145 cells, passaged 2 times in that cell line, and frozen at –70 C.

Bordetella bronchiseptica strain KM22 is a virulent phase-I swine isolate from a herd with atrophic rhinitis. In a preliminary study in our laboratory, strain KM22 induced turbinate atrophy and pneumonia in 1-week-old cesarean-derived colostrum-deprived pigs. *Bordetella bronchiseptica* was cultured at 37 C for 40 hours on Bordet-Gengou agar supplemented with 10% sheep blood.

Experimental design—Forty baby pigs from a herd with high health status were used in the study. The herd was certified free of brucellosis and had negative results when tested for pseudorabies virus. Furthermore, the herd was monitored quarterly, using serologic tests, and periodic slaughter checks had not revealed evidence of swine dysentery, swine influenza, rotavirus, PRRSV, transmissible gastroenteritis, *Mycoplasma* organisms, *Salmonella* spp, mange, lice, or *B bronchiseptica*.

Pigs were weaned when they were 2 weeks old and transferred to isolation facilities at the National Animal Disease Center. Pigs had not been given medications or vaccinations prior to weaning. Tonsil and nasal swab specimens were obtained from all pigs prior to the start of the study, and *B bronchiseptica* or *P multocida* were not isolated. Pigs were randomly assigned to 4 groups (10 pigs/group). A 1-week period was allowed for acclimation, and pigs were then inoculated (day 0) as follows: group 1, *B bronchiseptica*; group 2, PRRSV; group 3, PRRSV and *B bronchiseptica*; and group 4, noninoculated (control group). For PRRSV, pigs were inoculated intranasally with 4 ml (2 ml/nostril) of cell-culture medium containing $10^{3.2}$ **median cell-culture infectious dose ($CCID_{50}$)**/ml. For *B bronchiseptica*, pigs were inoculated intranasally with 1 ml (0.5 ml/nostril) of bacterial suspension containing $10^{5.8}$ **colony-forming units (CFU)**/ml.

Clinical signs were recorded daily, including number of coughs and sneezes heard during a 15-minute period for each group. Rectal temperature was recorded daily through day 15 of the study, and body weight was recorded twice weekly. Five pigs from each group were euthanatized on day 10 after inoculation, and the remaining 5 pigs in each group were euthanatized on day 21 after inoculation. Necropsies were performed on all pigs.

Postmortem evaluation—During gross examination, an estimate of percentage of lung involvement was assigned on the basis of the percentage of each lung lobe affected and the percentage of total lung volume represented by each lobe. Percentage of total lung volume of each lobe was estimated as 5% for the left cranial, 6% for left middle, 29% for left caudal, 11% for right cranial, 10% for right middle, 34% for right caudal, and 5% for the intermediate.[35] The amount of interstitial pneumonia, characterized by multifocal, tan-to-red mottled areas with irregular borders, was estimated; we also estimated the amount of bronchopneumonia, characterized by tan-to-red consolidation with clearly demarcated borders.

Tissue sections were obtained from the nasal turbinates, trachea, and lungs for microscopic evaluation. All tissues were fixed in neutral-buffered 10% formalin for 24 hours and then placed in 90% ethanol. After being fixed, nasal turbinates were decalcified by placing them in EDTA decalcifying solution for an additional 24 hours. All sections were routinely processed and embedded in paraffin, sectioned, and stained with H&E.

Snouts were transversely sectioned at the level of the first premolar tooth, and each of the 4 scrolls of the ventral turbinates was assigned a score, using the following scale: 0 = normal, 1 = less than half of turbinate gone, 2 = half or more of turbinate gone, 3 = turbinate is straight with only a small portion remaining, and 4 = total atrophy. The nasal septum also was scored, using the following scale: 0 = normal, 1 = slight deviation, and 2 = severe deviation. Total snout score (range, 0 to 18) was determined by adding the 4 turbinate scores and the score for the nasal septum.

Viral and bacterial isolation—Specimens were obtained from the nasal turbinates, trachea, and lungs of each pig. Specimens were weighed, and each specimen was ground, using a Ten Broeck grinder and PBS solution. Number of CFU of *B bronchiseptica* per gram of tissue was determined by plating serial 10-fold dilutions of homogenates on duplicate selective blood agar plates containing 20 µg of penicillin/ml, 10 µg of amphotericin B/ml, 10 µg of streptomycin/ml, and 10 µg of spectinomycin/ml. Samples of serum and pulmonary lavage fluid were obtained for virus isolation. Pulmonary lavage was performed by instilling four 10-ml aliquots of PBS solution into the right lung at the bifurcation of the trachea. The right lung was massaged gently, and PBS solution was collected by aspiration. Serum samples and lavage fluid were

stored frozen at –80 C. Isolation of PRRSV was performed by inoculating 100 µl of serum or lavage fluid onto a monolayer of MARC-145 cells in a 24-well plate. Cells were examined for cytopathic effects daily for 1 week. Isolation of PRRSV also was attempted on porcine blood monocytes grown on feeder layers of STO mouse embryonic fibroblasts.[36]

Serologic testing—Serum antibodies to PRRSV were detected, using an ELISA.[b] Serum antibodies against *B bronchiseptica* were detected by use of an agglutination test. Briefly, serial 2-fold dilutions of serum were mixed with an equal volume of 10^8 CFU of *B bronchiseptica*/ml suspended in PBS solution. Then, mixtures were incubated at 37 C for 1 hour and observed for agglutination.

Statistical analysis—Statistical analysis was conducted, using a Student *t*-test with significance defined at $P < 0.05$. Mean CFU of *B bronchiseptica* per gram of tissue isolated from the turbinates and trachea of pigs were compared on day 10 between the 2 groups inoculated with *B bronchiseptica*. A similar analysis was performed by using data from the 5 pigs of each group euthanized on day 21 of the study. Mean weight gain of pigs and mean rectal temperatures of pigs were compared.

Results

Clinical signs—Control pigs remained clinically normal throughout the study. In pigs inoculated with PRRSV alone, clinical signs included lethargy, anorexia, slight increase in respiratory rate, and infrequent sneezing, but coughing was not observed. In pigs inoculated with *B bronchiseptica* alone, clinical signs included infrequent sneezing and coughing. Pigs inoculated with both organisms had clinical signs that included lethargy, anorexia, increased respiratory rate, and frequent sneezing and coughing. In pigs inoculated with both organisms, sneezing and coughing were detected sooner and lasted longer (days 3 to 21) than in pigs infected with either agent alone (days 7 to 14). There were approximately 10 times as many sneezes heard in the group inoculated with both organisms, compared with the number for the groups inoculated with PRRSV alone or *B bronchiseptica* alone, and there were 4 times as many coughs heard in the group inoculated with both organisms, compared with the number for the group inoculated with *B bronchiseptica* alone.

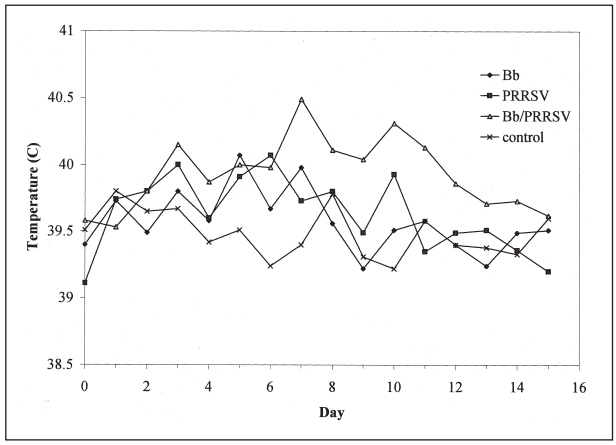

Figure 1—Mean rectal temperature of noninoculated control pigs and pigs inoculated (day 0) with *Bordetella bronchiseptica* (Bb), porcine reproductive and respiratory syndrome virus (PRRSV), or both organisms (Bb/PRRSV).

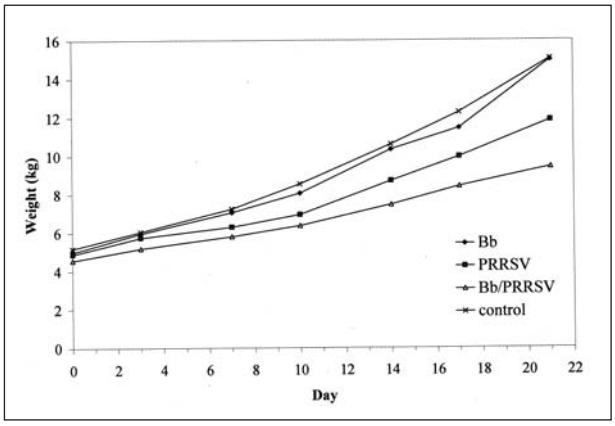

Figure 2—Mean weight of noninoculated control pigs and pigs inoculated (day 0) with *B bronchiseptica* (Bb), porcine reproductive and respiratory syndrome virus (PRRSV), or both organisms (Bb/PRRSV).

Febrile response—The febrile response was of longer duration and higher magnitude for the group of pigs inoculated with *B bronchiseptica* and PRRSV than for any other group of pigs (**Fig 1**). The duration of

Table 1—Lesions in the lungs and nasal turbinates grossly visible 10 and 21 days after inoculation in control pigs and pigs inoculated with *Bordetella bronchiseptica* (Bb), porcine reproductive and respiratory syndrome virus (PRRSV), or both organisms (Bb/PRRSV)

		Group			
Day*	**Type of lesion**	**Bb**	**PRRSV**	**Bb/PRRSV**	**Control**
10	Interstitial†	0	36.8 ± 25.5	38.6 ± 27.0	0
	Consolidation†	0	0.8 ± 1.2	7.15 ± 7.6	0
	Turbinate score	1.6	0.2	0.2	0
21	Interstitial†	0	41.3 ± 32.4	33.8 ± 18.8	0
	Consolidation†	0	0	14.8 ± 4.6	0
	Turbinate score	5.8	1.8	5.8	0.7

*Day after inoculation on which necropsy was performed; day of inoculation = Day 0. †Values reported are mean ± SD.

Interstitial = Mean percentage of lungs involved in pigs with pneumonia characterized by multifocal, tan-to-red mottled areas with irregular borders. Consolidation = Mean percentage of lungs involved in pigs with pneumonia characterized by tan-to-red consolidation with clearly demarcated borders. Turbinate score = Mean snout score for each group (0 to 2, normal; 3 to 6, mild atrophy; 7 to 10, moderate atrophy; 11 to 18, severe atrophy).

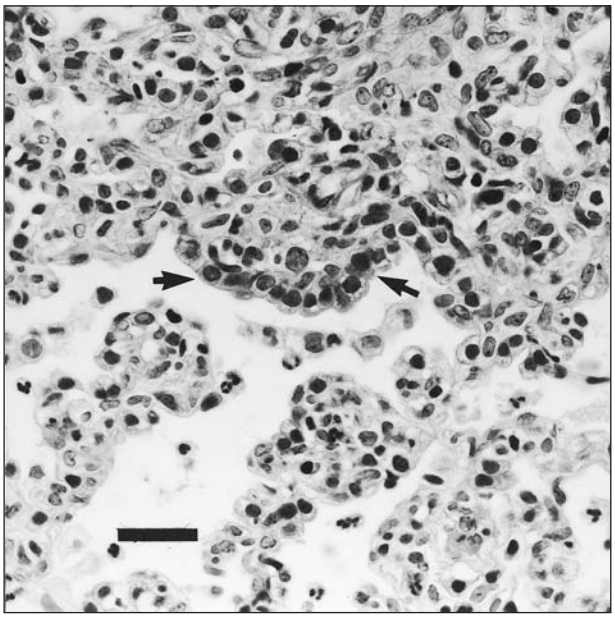

Figure 3—Photomicrograph of a section of lung tissue obtained from a pig 10 days after inoculation with *B bronchiseptica* and PRRSV. Notice the interstitial pneumonia characterized by septal infiltration with lymphocytes, plasma cells, and macrophages; alveolar lumens containing macrophages, neutrophils, and necrotic debris; and type-2 pneumocyte hypertrophy and hyperplasia (between arrows). H&E stain. Bar = 35 μm.

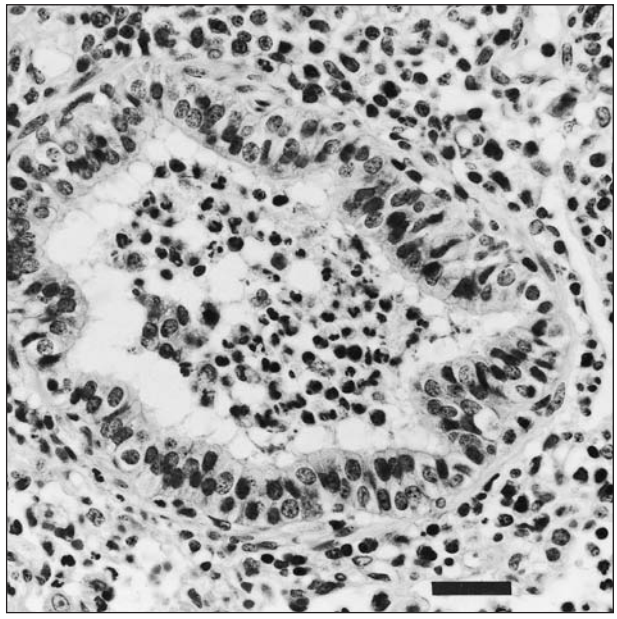

Figure 4—Photomicrograph of a section of lung tissue obtained from a pig 10 days after inoculation with *B bronchiseptica* and PRRSV. Notice the bronchopneumonia characterized by infiltrates of neutrophils with a few macrophages in the airway. H&E stain. Bar = 35 μm.

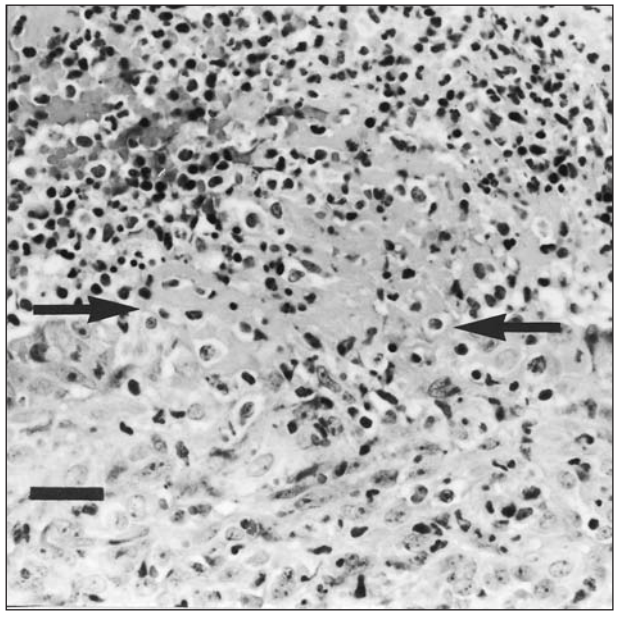

Figure 5—Photomicrograph of a section of turbinate from a pig 21 days after inoculation with *B bronchiseptica* and PRRSV. Notice the flattening of the epithelium with developing ulcers (between arrows) covered by an exudate of fibrin and neutrophils. Also notice the submucosal infiltrates of lymphocytes, plasma cells, and neutrophils. H&E stain. Bar = 88 μm.

febrile response, measured as the mean number of days pigs in a group had a rectal temperature of ≥ 40 C, was 8.2 days for pigs inoculated with both organisms in contrast to 2.6 and 4.2 days for pigs inoculated with *B bronchiseptica* alone or with PRRSV alone, respectively. The magnitude of febrile response, measured as the overall mean rectal temperature for the first 15 days of the study, was significantly ($P = 0.005$) higher for the group of pigs inoculated with both organisms than for any other group of pigs.

Weight gain after inoculation—Pigs inoculated with *B bronchiseptica* alone gained weight at a rate similar to that for control pigs (**Fig 2**). Mean weight gains for the control group and *B bronchiseptica* group were 9.8 and 10.0 kg, respectively, during the 21-day recording period. Pigs inoculated with PRRSV alone and pigs inoculated with PRRSV and *B bronchiseptica* had significantly lower mean weight gains (7.0 kg [$P = 0.004$] and 4.9 kg [$P < 0.001$], respectively) than that for control pigs. Pigs inoculated with both organisms also had significantly lower weight gains than that for pigs infected with *B bronchiseptica* alone ($P < 0.001$) or PRRSV alone ($P = 0.004$).

Gross postmortem examination—Gross lesions were not observed in any of the control pigs at 10 or 21 days after inoculation (**Table 1**). Ten days after inoculation, pigs inoculated with *B bronchiseptica* alone had slight hyperemia of the mucosa of the nasal passages with mucopurulent nasal discharge, and 1 pig in that group had mild atrophy of the turbinates (turbinate score of 4). Gross lung lesions were not observed in any of the 5 pigs in that group. The 5 remaining pigs euthanatized 21 days after inoculation with *B bronchiseptica* had findings similar to those for the pigs euthanatized 10 days after inoculation, except there was mild to moderate atrophy of the turbinates in all 5 pigs on day 21 (turbinate score for pigs ranged from 4 to 7).

Ten days after inoculation, pigs inoculated with PRRSV alone had mucopurulent nasal discharge without evidence of atrophy of the turbinates (Table 1). All 5 pigs in that group had generalized lymphadenopathy

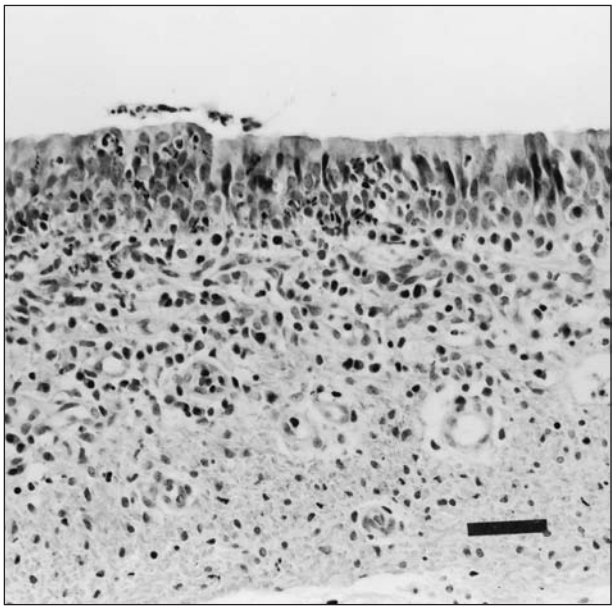

Figure 6—Photomicrograph of a section of trachea obtained from a pig 10 days after inoculation with *B bronchiseptica* and PRRSV. Notice the submucosal infiltrates of lymphocytes, plasma cells, and neutrophils and intraepithelial infiltration with neutrophils. H&E stain. Bar = 55 μm.

Table 2—Isolation of *B bronchiseptica* from turbinate, trachea, and lung specimens obtained 10 and 21 days after inoculation

		Group*			
Day	Tissue	Bb	PRRSV	Bb/PRRSV	Control
10	Turbinate	5 ($10^{7.5}$)	0	5 ($10^{6.6}$)	0
	Trachea	5 ($10^{7.0}$)	0	5 ($10^{6.5}$)	0
	Lung	0	0	3 ($10^{6.2}$)	0
21	Turbinate	5 ($10^{6.8}$)	0	5 ($10^{6.3}$)	0
	Trachea	5 ($10^{6.1}$)	0	5 ($10^{5.1}$)	0
	Lung	0	0	4 ($10^{6.0}$)	0

*There were 5 pigs/group. Values reported are No. of pigs from which *B bronchiseptica* was isolated, with mean titer per gram of tissue indicated in parentheses.

and lesions consistent with interstitial pneumonia, characterized by multifocal, tan-to-red mottled areas with irregular borders that appeared to be of greatest severity in the cranioventral portions of the lungs. Two pigs had small areas (≤ 2.5%) of consolidation characterized by tan-to-red consolidation with clearly demarcated borders. Twenty-one days after inoculation with PRRSV, the nasal turbinates of the 5 remaining pigs appeared normal with little evidence of nasal discharge. All pigs had pronounced generalized lymphadenopathy, and there were lesions consistent with interstitial pneumonia, but we did not detect areas of consolidation in the lungs.

Ten days after inoculation, pigs inoculated with both organisms had mucopurulent nasal discharge without turbinate atrophy (Table 1). All 5 pigs had slight generalized lymphadenopathy and lesions consistent with interstitial pneumonia, which appeared similar in severity to that observed in pigs inoculated with PRRSV alone. Lungs of 3 pigs had areas of consolidation characterized by tan-to-red consolidation with clearly demarcated borders and a cranioventral distribution with percentage of lungs affected ranging from 6 to 17%. By 21 days after inoculation, the remaining 5 pigs inoculated with both organisms had profuse mucopurulent nasal discharge and mild to moderate turbinate atrophy (turbinate score for pigs ranged from 3 to 8). All 5 pigs had generalized lymphadenopathy and lesions consistent with interstitial pneumonia. Lungs of all 5 pigs had areas of consolidation characterized by tan-to-red consolidation with clearly demarcated borders and a cranioventral distribution with percentage of lungs affected ranging from 9 to 20%.

Histologic examination—Tissue specimens were examined to detect pathologic changes.

Lungs—Pigs in the control group and the group inoculated with *B bronchiseptica* alone did not have appreciable microscopic lesions of the lungs at 10 or 21 days after inoculation. All pigs in both groups inoculated with PRRSV had multifocal interstitial pneumonia of similar severity 10 and 21 days after inoculation (**Fig 3**). Alveolar septa contained infiltrates of lymphocytes, plasma cells, and macrophages. Alveolar lumina contained necrotic cellular debris and neutrophils. Hypertrophy and hyperplasia of type-2 pneumocytes were observed; a syncytial cell formation also was observed. Hyperplasia of bronchus-associated lymphoid tissue and lymphocytic perivascular cuffing were typical 21 days after inoculation. Ten days after inoculation, 2 pigs inoculated with PRRSV alone and 3 pigs inoculated with both organisms had lesions of suppurative bronchopneumonia with infiltrates consisting primarily of neutrophils with a few macrophages in the airways (**Fig 4**). Twenty-one days after inoculation, 4 pigs inoculated with both organisms had lesions of suppurative bronchopneumonia, and 1 pig in that group had multifocal alveolar hemorrhages.

Nasal turbinates—Pigs in the control group had mild lesions of the nasal turbinates, including submucosal infiltrates of neutrophils, lymphocytes, and plasma cells, and infiltration of the epithelium with neutrophils. Control pigs necropsied 21 after inoculation also had multifocal necrotic areas in the mucosa and submucosa. Pigs in the other 3 groups had rhinitis of greater severity with variable amounts of mucopurulent intraluminal exudate (**Fig 5**). Submucosal infiltrates of lymphocytes, plasma cells, and neutrophils were seen in addition to suppurative inflammation of submucosal glands, submucosal edema, and intraepithelial aggregates of neutrophils. Epithelial changes included loss of cilia, attenuated epithelium, squamous metaplasia, mucosal hyperplasia, erosions, and ulcers. Pigs in the 3 inoculated groups necropsied 21 days after inoculation also had multifocal necrotic areas in the epithelium and submucosa. The lesions were of greater severity in pigs euthanatized 21 days after inoculation, compared with lesions for pigs euthanatized 10 days after inoculation, and were most severe in pigs inoculated with both organisms.

Trachea—Lesions were not seen in tracheas of control pigs. Lesions seen in the tracheas of pigs in the

other 3 groups included attenuated epithelium with loss of cilia, squamous metaplasia, and erosions; submucosal edema with infiltrates of lymphocytes, plasma cells, and neutrophils; intraepithelial infiltration of lymphocytes and neutrophils; and accumulation of intraluminal mucopurulent exudate (**Fig 6**). Lesions were of greater severity in pigs euthanatized 21 days after inoculation, compared with those in pigs euthanatized 10 days after inoculation, and were most severe in the group inoculated with both organisms.

Bacterial isolation—*Bordetella bronchiseptica* was not isolated from any tissues at either time in the control pigs or pigs inoculated with PRRSV alone (**Table 2**). *Bordetella bronchiseptica* was cultured from the nasal turbinates and trachea of all pigs in both groups inoculated with the bacterium. Although the amount of colonization of these 2 anatomic sites was slightly higher at both times for the group inoculated with *B bronchiseptica* alone, the values were not significantly ($P = 0.14$ and $P = 0.44$, respectively) different. Importantly, *B bronchiseptica* was isolated only from the lungs of pigs inoculated with both organisms. *Bordetella bronchiseptica* was isolated from 3 of 5 pigs euthanatized 10 days after inoculation and 4 of 5 pigs euthanatized 21 days after inoculation.

Virus isolation—The PRRSV was not isolated from sera obtained from any pigs prior to inoculation. Similarly, PRRSV was not isolated from the sera or pulmonary lavage fluid obtained during necropsy of control pigs or pigs inoculated with *B bronchiseptica* alone. Virus was isolated from sera, pulmonary lavage fluid, or both obtained during necropsy of all pigs in both groups inoculated with PRRSV.

Serologic tests—Geometric mean agglutination titer against *B bronchiseptica* for the pigs prior to start of the study was 1:16. The group inoculated with *B bronchiseptica* alone had slight increases of 1.5- and 2-fold in geometric mean agglutination titer 10 and 21 days after inoculation, respectively. The other 3 groups had approximately the same geometric mean titer 10 days after inoculation and had a 1.5-fold decrease in geometric mean titer 21 days after inoculation. Sera from all pigs had negative results when tested for PRRSV antibody by use of the PRRSV ELISA prior to start of the study, and sera from pigs in the control group and the group inoculated with *B bronchiseptica* alone remained seronegative at the time pigs were euthanatized. Sera from 4 of 5 pigs inoculated with PRRSV alone and 5 of 5 pigs inoculated with both organisms had positive results when tested for PRRSV antibodies 10 days after inoculation, and sera from all 10 pigs in these 2 groups had positive results when tested for PRRSV antibody 21 days after inoculation. There was not a significant difference in mean titer of antibody against PRRSV between these 2 groups at either time.

Discussion

Analysis of results for the study reported here revealed that infection with PRRSV can predispose pigs to pulmonary infection with *B bronchiseptica*. *Bordetella bronchiseptica* was not isolated from the lungs, and lesions were not seen in the lungs, of any of the pigs inoculated with bacteria alone. *Bordetella bronchiseptica* was isolated from the lungs of the pigs inoculated with both organisms, and they had lesions of bronchopneumonia. *Bordetella bronchiseptica* can be the primary cause of pneumonia in neonatal pigs or can be a secondary invader in older pigs. In the study reported here, inoculation with *B bronchiseptica* alone did not result in colonization or lesions in the lungs. Clinical course, including respiratory tract disease, febrile response, and decreased weight gain, was more pronounced in pigs inoculated with both organisms than in pigs inoculated with either organism alone. Although lesions of PRRSV-induced interstitial pneumonia did not appear more severe in the group inoculated with both organisms, it is not known whether they would have persisted longer than those in the group infected with PRRSV alone, because lesions were still evident in both groups 21 days after inoculation. Prolongation of PRRSV-induced pneumonia has been reported for pigs infected with PRRSV and *M hyopneumoniae*.[26]

In primary pneumonia caused by infection with *Bordetella* spp, severity of pneumonic lesions peaks between 10 and 14 days after infection when red, consolidated areas are noticeable in the lungs. By 21 days after infection, these lesions become more tan and contracted. Early histologic lesions are characterized by alveolar hemorrhage and neutrophilic infiltrates in alveoli and bronchioles. This is followed by epithelialization of alveoli and fibrosis.[32,37,38] The acute appearance of lesions seen in this study 10 and 21 days after inoculation supports the theory that *B bronchiseptica* was acting as a secondary invader in the lungs after initial damage or immunosuppression by PRRSV. It is interesting that alveolar hemorrhage was seen in only 1 pig inoculated with *B bronchiseptica*, which may mean the pathogenesis of disease was altered in pigs inoculated with both organisms. At 10 days after inoculation, there were small lesions of consolidation, which were characterized microscopically by bronchopneumonia in 2 pigs inoculated with PRRSV alone. Bacterial isolation was not successful even on media without antibiotics and with or without a streak of *Staphylococcus aureus*. Sections of lung from these pigs were sent to an independent diagnostic laboratory and found negative for *M hyopneumoniae* by immunohistochemical tests. Therefore, it is not known whether the lesions were caused by secondary bacteria other than *B bronchiseptica* or were the result of PRRSV infection alone.

Evidence as to whether PRRSV predisposes pigs to secondary infections is equivocal. Secondary bacterial infections are seen in field situations on farms where pigs are also infected with PRRSV, and PRRSV is often isolated from pigs with PRDC.[2,7-11,13,14] Experimentally, however, the association has been difficult to prove. In co-infection studies with PRRSV and other agents, results have ranged from no interaction to increased incidence and severity of disease, sometimes with conflicting results for the same infectious agents. In 1 study,[12] PRRSV predisposed pigs to infection with *S*

suis, whereas in another study,[39] it did not. Dual infections with PRRSV and porcine respiratory coronavirus or swine influenza virus caused disease of greater severity than that seen for infection with single viruses.[15] Other studies with dual infections of PRRSV and *H parasuis*, *S choleraesuis*, *P multocida*, *A pleuropneumoniae*, *M hyopneumoniae*, and transmissible gastroenteritis virus have been more equivocal.[26,39-44]

One reason for conflicting findings may be the use of isolates of infectious agents that differ in their ability to cause disease. Various isolates of PRRSV vary in their ability to cause disease in the respiratory and reproductive tracts.[20-22] The PRRSV isolate used in the study reported here was isolated from a herd that had some characteristics of an atypical PRRS outbreak; thus, this strain may have been more virulent than strains used in other co-infection studies with PRRSV. Other possible reasons for variation in outcome include time and sequence of exposure to the infectious agents, differing health status of pigs in various studies attributable to immune status, genetic predisposition for susceptibility or resistance to disease, age of pigs used in the study, other infectious agents the pigs may harbor, and differing environmental conditions.

Because PRRSV replicates in alveolar macrophages, 1 method by which it may predispose to secondary infections is alteration of alveolar macrophage function, including the ability to clear infectious agents and aid in the induction of inflammatory and immune responses.[25] Porcine reproductive and respiratory syndrome virus also replicates in pulmonary intravascular macrophages, which may leave the host more susceptible to septicemic infections. Decreased uptake of copper particles by pulmonary intravascular macrophages in pigs infected with PRRSV was reported in 1 study.[45] Macrophages in other tissues may be infected, and this may affect defense mechanisms in these areas as well. Mechanical damage of the respiratory tract may leave a pig more susceptible to secondary infection. Lesions of rhinitis, including alteration of the epithelial surface and loss of cilia, have been reported with PRRSV,[20,21] and similar lesions were seen in the turbinates and trachea of pigs infected with PRRSV in the study reported here.

Bordetella bronchiseptica can predispose to secondary infection with *P multocida* in atrophic rhinitis and *S suis* infections.[29-31] Possible mechanisms for predisposing to secondary infections include piracy of adhesins produced by *B bronchiseptica,*[46] turbinate atrophy and damage to the ciliated epithelium caused by toxins produced by the bacteria, and destruction or alteration of alveolar macrophages. *Bordetella bronchiseptica* is found in a high percentage of swine herds; thus, simultaneous infection with PRRSV and *B bronchiseptica* may result in severe inhibition of resistance to respiratory tract pathogens.

[a]PRRSV inoculum was supplied by Dr Kelly Lager, Virus and Prion Diseases of Livestock Research Unit, National Animal Disease Center, Agricultural Research Service, USDA, Ames, Iowa.

[b]HerdChek, Porcine Reproductive and Respiratory Syndrome Virus Antibody Test Kit, IDEXX Laboratories, Westbrook, Mass.

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