Possible mechanisms of viral-bacterial interaction in swine

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Summary: This paper reviews the major viral-bacterial interactions that cause or exacerbate respiratory disease in swine. It discusses the possible microbiological mechanisms of those interactions, and offers options for effective prevention and treatment of viral-bacterial infections.

Respiratory disease is a frequent and economically significant problem in swine units. The potential loss due to decreased average daily gain and feed efficiency and the cost to prevent or treat pneumonia is substantial.[1,2] Respiratory disease caused by virus or bacteria alone can be exacerbated by a number of environmental and management factors. Numerous researchers[3–14] have also shown that respiratory disease can be exacerbated by interactions of viruses and bacteria in pigs (Table 1). These interactions have also been noted in humans,[15–17] mice,[18–20] cows,[21] and other animals.[22–24] Most studies of viral-bacterial interaction mechanisms were made in mouse models; there have been few such studies in swine. A thorough understanding of the mechanisms of viral-bacterial interactions is important to help prevent costly respiratory syndromes. This review will report the synergistic effects of viruses and bacteria in the development of respiratory disease in swine, will offer possible explanations for the cellular mechanisms that are responsible for these interactions, and will offer suggestions for the management of these viral-bacterial infections.

Possible mechanisms involved

The upper respiratory system defenses include anatomical barriers, mucociliary clearance mechanisms, and reflex mechanisms to remove foreign particles. In the upper and lower respiratory tract, humoral substances play the following roles:

- local secretory immunoglobulins opsonize bacteria and effectively prevent them from adhering and therefore from colonizing;

- transferrin has a bacteriostatic effect on some bacteria, which strongly depend on iron;

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- surfactant alters surface charges that facilitate killing of microorganisms through improved phagocytic activity;

- fibronectin alters bacterial attachment and enhances phagocytosis; and

- lysozyme has a bactericidal effect.[27]

A key immunologic defense cell is the porcine alveolar macrophage (PAM), located throughout the alveoli.[28] The specialized function of PAMs is phagocytosis and bacterial killing. Briefly, when bacteria attach to specific immunologic (Fc and complement) and nonspecific receptors on the macrophage cell membrane they trigger phagocytic ingestion (Figure 1 top). After ingestion, the macrophages internally isolate the bacteria in the phagosomes, which then fuse with the lysosomes — bodies with sequestered microbicidal enzymes — to kill the bacteria (Figure 1 bottom).

Other PAM functions include:

- release of chemotactic factors that attract more immune cells to enhance the immune response;

- release of cytokines. Cytokines play an important role in the lung defense mechanisms. For instance, during viral infections, the tissue immediately reacts to produce interferon, an important cytokine. Interferon limits the rate of virus replication and modulates host defense to virus and superinfecting bacteria; and

- antigen processing. PAMs construct molecules from pieces of the pathogen's proteins and from cellular proteins called major histocompatibility complex molecules and "present" them to the immune cells. This antigen processing is the key to the flexibility, specificity, and thoroughness of all immune responses.

Other cells such as lymphocytes, neutrophils, eosinophils, and basophils also contribute to the pulmonary bacterial defense functions.[15]

During the early studies of viral-bacterial interactions, Green, et al.,[29] postulated two possible mechanisms to explain bacterial superinfection after exposure to virus:

- viral replication inhibits the ciliary removal of bacteria by desquamating the bronchial epithelium; and

Table I

Enhanced effects of combined bacterial-viral infections in pigs

Virus	Bacteria	Combined effect in pigs	Recommendations	Ref.
Influenza	*Haemophilus suis*	Accentuated clinical signs of swine influenza	Influenza— vaccination for influenza *H. suis*— antibiotics for secondary bacteria	3
Adenovirus	*Mycoplasma hyopneumoniae*	Severe pneumonia	Adenovirus— no vaccine available *Mycoplasma*— vaccines, antibiotics, medicated early weaning (MEW)	4
Hog cholera [Vaccinal low passage]	*Salmonella cholerasuis*	Increased subclinical salmonellosis	Hog cholera—safer vaccines where allowable (high-passage attenuated, lapinized, inactivated); *Salmonella*—antibiotics, husbandry procedures, autogenous bacterins, modified live vaccine	25
Hog cholera	*Pasteurella multocida*	Increased pneumonia by PAMs (porcine alveolar macrophages); Likely impairment in PAMs phagosome-lysosome fusion; Defective bacterial killing	Hog cholera—as above; *P. multocida*—antibiotics, management practices, autogenous vaccine	6 and 26
Aujeszky's disease virus (ADV)	*Pasteurella multocida*	Severe pneumonia; increased *P. multocida* isolations; reduced average daily gain; altered PAM intracellular killing; depressed phagosome-lysosome fusion in PAMs related to viral strain virulence; depressed Fc-mediated phagocytosis related to viral strain virulence	ADV—test & removal, offspring segregation, depopulation & repopulation, vaccination *P. multocida*—as above	11
Aujeszky's disease virus	*Actinobacillus pleuropneumoniae*	Decreased PAM phagocytosis and increased bacterial intracellular survival related to *A. pleuropneumoniae* strain virulence	ADV—as above; *A. pleuropneumonia*—bacterins, antibiotics, management practices	12
Aujeszky's disease virus	*Streptococcus suis*	Increased polyarthritis, pericarditis, and meningitis	ADV—as above; *S. suis*— autogenous vaccines (use appropriate serotype[s] isolated from brain or meninges)	13
Porcine reproductive and respiratory syndrome virus (PRRSv)	*Streptococcus suis*	Exacerbation of central nervous signs and lameness	PRRSv—vaccine, management practices; *S. suis*—as above	14
Aujeszky's disease virus	Toxigenic *Pasteurella multocida*	Death in adult pigs	ADV—as above; *P. multocida*—as above	10

- alveolar exudate in the viral lesion provides nutrients for bacterial growth.

However, Jakab[30] discovered that virus-induced suppression does not seem to significantly affect the ability of the immune system to physically remove bacteria from the lungs. In addition, bactericidal activity occurs in both consolidated and nonconsolidated portions of the same virus-infected lung. This suggests that bactericidal activity may not be altered in virus-infected lungs, and that the presence of exudates and consolidation are not critical to viral-bacterial interactions. Therefore, the two original hypotheses do not satisfactorily explain bacterial superinfection after viral exposure.
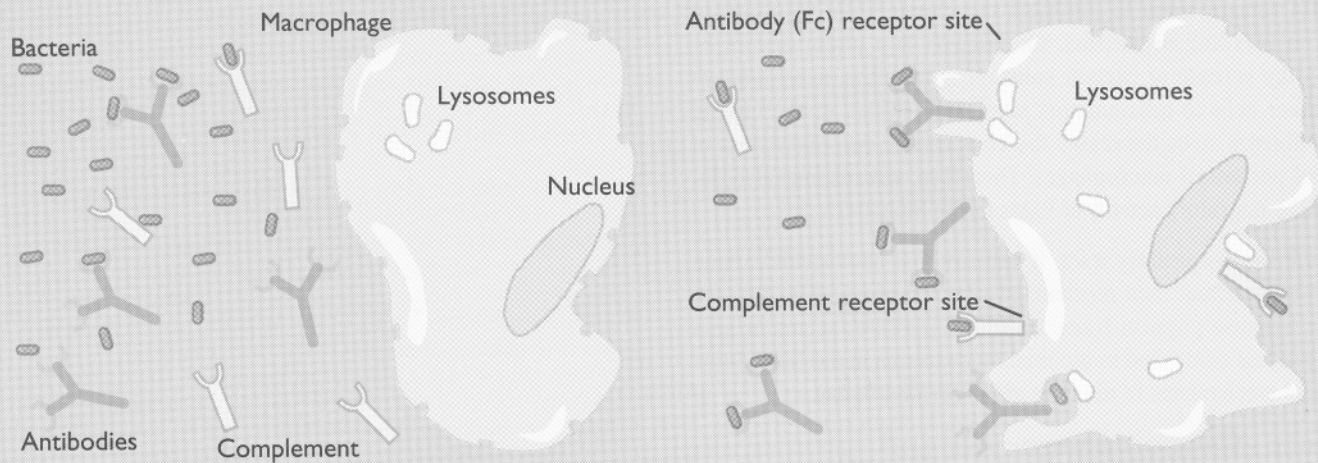
Antibodies (Fc) and complement bind to bacteria, then attach to receptor sites on a macrophage.
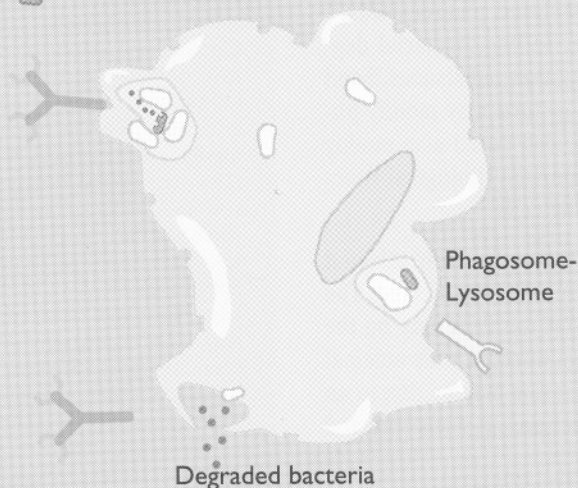
The bacteria are then engulfed and degraded by phagosome-lysosome complexes. The antibody or complement and degraded bacteria are released.

Recent research has suggested a number of new explanations for the mechanisms of viral-bacterial interactions.

Increased bacterial adherence due to viral infection

Bacterial adherence to cells of the respiratory tract is an initial step in bacterial infection. Plotokowski, et al.[31] found that viral infection alters surface membrane receptors, which modifies the microenvironment. The modified environment allows bacteria to proliferate, a phenomenon called "opportunistic adherence."[32] Opportunistic adherence may explain the interactions among pseudorabies virus (PRV), porcine reproductive and respiratory syndrome virus (PRRSV) and *Bordetella bronchiseptica* with *Streptococcus suis*, which have been reported to enhance *Streptococcus suis* disease. All these predisposing agents cause damage to the nasal epithelium.

Destructive virus enzyme

Some viruses use specific enzymes (e.g., neuraminidase in swine influenza virus [SIV]) that may destroy some of the mucous gly-coproteins that normally prevent bacterial attachment and infection of epithelial cells.[27]

Reduction of mucociliary clearance

Viruses may diminish mucociliary clearance by reducing the production of bactericidal substances. This mechanism has been proposed as part of the interaction of hog cholera virus (HCV) and *Pasteurella multocida*. Iglesias, et al.,[7] studied the effect of a strain of HCV vaccine on cilia destruction of *P. multocida* by using tracheal explants collected from embryonic pigs. The tracheal explants were infected in vitro with a vaccine suspension of HCV and later *P. multocida* type D was added. Hog cholera virus affected the bactericidal activity of tracheal explants against *P. multocida*, reducing lysis of *P. multocida* to 58% at 24 hours after viral infection and to 44% during the following 24 hours.

The authors suggested that the damage caused by the virus may be responsible for bacterial colonization of the lung by altering epithelial cell bactericidal secretions.

Diminished chemotaxis

Certain viruses seem to diminish the chemotactic response of cells to invading organisms. Chemotaxis is a phenomenon by which the release of certain substances mobilize macrophages and other cells to inflammatory sites. Kleinerman, et al.,[33] found that influenza virus decreases the chemotactic responsiveness of normal peritoneal macrophages. Therefore, viruses may also depress the migratory activity of PAMs in the lung.

In the lower respiratory tract, it appears that bactericidal action is more important than mechanical removal of bacteria. In part, this is because physical translocation of particles seems to be a slow process compared to biocidal mechanisms in the lung. Some biocidal mechanisms in the lung are described below.

Direct effect on phagocytic and postphagocytic PAM functions

Some viruses suppress or alter the following functions: Fc-membrane receptor-attaching activity, Fc-mediated phagocytosis,[34] phagosome-lysosome fusion, intracellular killing, bacterial degradation due to low levels of enzymes or other substances,[34] and macrophage metabolic processes which in turn may modify phagocytosis.[35] Alterations of the phagocytic cell could also modify cytokine secretions, thereby altering important biological functions and disrupting communication among cells.

Pijoan, et al.,[26] reported that although HCV selectively affected PAM killing functions, phagocytic activities were not affected. The results of their experiments suggested that the PAM may be the target cell for immune suppression in pigs infected with vaccinal strains of HCV. The authors postulated that this may be due to an impairment of phagosome-lysosome functions in virus-infected PAMs.

Fuentes, et al.,[8] studied phagocytosis and killing of *P. multocida* using PRV-infected and noninfected PAMs. *Pasteurella multocida* survived intracellular digestion in greater numbers in PRV-infected macrophages. Infection with virulent PRV resulted in a 4–5 logrithmic increase in intracellular viable bacteria as early as 1 hour postviral infection. As with the HCV model,[26] PRV selectively affects killing but not phagocytosis.

Iglesias, et al.,[11] studied the effect of various strains (field and attenuated) of PRV on PAMs. Viability, phagocytosis, phagosome-lysosome fusion, phagocytosis of opsonized particles, and superoxide release (an assay to measure metabolic activity) were the macrophage activities measured. Viability of infected cells when compared to non-infected cells was less for field isolates of PRV than for the attenuated strains. Phagocytosis was not affected by PRV except for one virulent field strain. However, phagosome-lysosome fusion was depressed by PRV infection. Fc-mediated phagocytosis (using opsonized particles) was negatively influenced by PRV infection except with one attenuated strain when compared to non-infected PAMs. As virus concentration increased, Fc-mediated phagocytosis decreased. All viral strains depressed metabolic activity, indicating decreased bactericidal function, and this reduction depended upon the quantity of virus particles.

The authors concluded that even with low numbers of virus particles, PRV infection impairs PAM function and that the inability to proliferate and induce macrophage impairment may be related to strain virulence.

Immature phagocytes

The alveolar macrophage is the replication site of such swine viruses as PRV and PRRSV, and it is has been hypothesized that after viral infection, mature macrophages are destroyed and probably replaced by immature phagocytes. These cells are not fully capable of bactericidal activity; consequently bacteria can proliferate.

Decrease of surfactant levels

In SIV infections in swine, the function of the alveolar type-2 pneumocyte is impaired. These cells synthesize and secrete surfactant, which plays an important role in phagocytosis of microorganisms. Reduction of surfactant may therefore be an additional mechanism of virus-induced macrophage dysfunction.

Increased viral replication/lethality

Hall, et al.,[10] studied the effects of exposure to PRV as well as exposure to *P. multocida* type D toxin in mice, swine, and nasal turbinate cell cultures. They noticed elevated mortality in mice when nonlethal doses of toxin were given along with nonlethal doses of PRV. Clinical disease and death in adult pigs was observed after an intradermal injection of toxin and intranasal exposure to PRV. Nasal turbinate cell cultures incubated with toxin and PRV had increased viral protein synthesis, DNA synthesis, and increased recovery of virus particles. These findings showed that the toxin from *P. multocida* type D enhances PRV replication and lethality in cell cultures and animal models. Like other toxins, *P. multocida* toxin may use membrane receptors for protein hormones or growth factors as sites for binding and entry into susceptible cells, leading to changes in cellular metabolism which PRV may then use to produce more infectious virus.

Immune response

Indirect effects of the virus on the host have also been considered. In experiments of viral-induced phagocytic dysfunction, the bactericidal defect is associated with declining viral titers and increasing antiviral immunity, suggesting decreased bactericidal function due to host response as well as virus-induced effects on macrophages.[15]

Discussion

Synergistic effects of viruses and bacteria in swine have been demonstrated both in vivo and in vitro. Commonly, an opportunistic bacteria superinfects after a primary viral infection. Multiple mechanisms appear to be involved in virally-induced suppression of pulmonary antibacterial defenses. However, impairment in postphagocytic digestion may be the most important mechanism in viral-bacterial interactions in swine. The conditions for viral enhancement of bacterial superinfections have to be appropriate and several important factors such as strain virulence, viral dose, and health status of the pigs must be taken into account.

Viral-bacterial interactions do not always begin with a viral infection and do not always involve secondary bacteria. Bacteria can also act as predisposing factors for viral infection, as is the case of *P. multocida* (toxin), which has been shown to intensify PRV infection. Primary bacterial pathogens like *Salmonella cholerasuis* and *Actinobacillus pleuropneumoniae* also interact with viruses leading to enhanced disease.

Viruses are not the only agents that predispose the host to bacterial superinfections. In some experimental models, secondary bacterial infections are predisposed by other bacteria, not a virus. Such are the cases of *B. bronchiseptica-P. multocida*[36] and *B. bronchiseptica-S. suis*[37] as well as the classic *Mycoplasma hyopneumoniae-P. multocida*[38] lung interaction.

Viral and bacterial virulence factors are important in developing disease. It has been demonstrated with the PRRSV-*S. suis* model that a strain of *S. suis* lacking a protein associated with virulence would not reproduce clinical signs of *S. suis* disease even with PRRSV preinfection.[14]

Viral dose also seems to be important in developing bacterial superinfection.[9,11] Higher doses of PRV resulted in greater reduction of macrophage functions.

Although viral-bacterial interactions appear to be important, the interactions are not the only cause of bacterial superinfection. For example, outbreaks of *S. suis* meningitis — frequently considered a secondary agent — have been described in SPF pigs where no known mycoplasma or viral agents were present. Thus, other mechanisms must also be involved.

In addition, the pig's immune status is important. From the viral agents that interact with bacteria mentioned throughout this paper — adenovirus, enterovirus, HCV, PRV, PRRSV — a degree of herd immunity may be established. Even though bacterial superinfection has been demonstrated in gnotobiotic pigs after exposure with adenovirus, most conventional herds have been exposed to this virus, reducing the likelihood of the viral enhancement occurring under field conditions. Similar situations occur with enterovirus infections. Therefore, herds that have high-health status and are susceptible to those viral infections must be watched carefully because some of the secondary bacteria mentioned previously are ubiquitous.

Results of experiments performed to study the role of immunity in viral-bacterial interactions in lungs have indicated that:

- the degree of virus-induced suppression of pulmonary antibacterial defenses is associated with the virus's virulence;

- specific viral immunity reduces viral infection and, consequently, complications due to secondary bacteria;

- antiviral immunity does not protect against an heterologous virus, so host susceptibility to secondary bacteria is increased;

- the efficacy of antibacterial immunity in preventing bacterial superinfection appears to depend on the microorganism;[39] and

- immunization and passive transfer against bacterial microorganisms do not always prevent bacterial superinfection in virus-infected lungs.

Interactions of virus and bacteria are important in developing respiratory and other swine diseases, making control of these diseases difficult. Strategies must control and prevent the primary agents (most commonly viral) rather than simply treating the secondary agents that cause clinical disease.

Implications

- Morbidity and mortality will be increased when a combined infection of virus and bacteria is present compared to an infection with either agent alone.

- Multiple mechanisms are involved in virus-induced suppression of pulmonary antibacterial diseases.

- Many bacterial infections are difficult to initiate without the presence of viral infection (e.g., *S. suis* and *P. multocida* and *H. suis*).

- Once pneumonia is initiated, the host itself may contribute to the immunopathology and severity of the disease.

- It may be necessary to immunize against both viral and bacterial pathogens.

- Therapy against bacterial agents should include both management and antibacterials.

- Time the administration of vaccines and antibiotics to ensure their efficacy. Treating with antibiotics after extensive damage has been done and bacterial colonies have formed is of little value.

- If you use antibiotics, use a sensitivity test to make sure they are effective against the agent.

References

1. Morrison RB, Pijoan C, Leman AD. Association between enzootic pneumonia and performance. *Pig News Info*. 1986;1(7):23.

2. Pijoan C. 1992. Pneumonic Pasteurellosis. In *Diseases of Swine*, editors Leman, Straw, Mengeling, D'Allaire and Taylor, Iowa State University Press, 7th edition, 552–559.

3. Shope R. Swine influenza. *J Exp Med*. 1931;54:373–385.

4. Kasza L, Hodges RT, Betts AO, Trexler PC. Pneumonia in gnotobiotic pigs produced by simultaneous inoculation of a swine adenovirus and *Mycoplasma hyopneumoniae*. *Vet Rec*. 1969;84:262–267.

5. Smith IM, Betts AO, Watt RG, Hayward HS. Experimental infections with *Pasteurella septica* (serogroup A) and adeno- or enterovirus in gnotobiotic piglets. *J Comp Path*. 1973;83:1–12.

6. Pijoan C, Ochoa G. Interaction between a hog cholera vaccine strain and *Pasteurella multocida* in the production of porcine pneumonia. *J Comp Path*. 1978;88:p167–170.

7. Iglesias G, Pijoan C. Effect of swine fever live vaccine on the mucociliary apparatus of swine and its interaction with *Pasteurella multocida*. *Rev Lat Microbiol*. 1980;22:52.

8. Fuentes M, Pijoan C. Phagocytosis and killing of Pasteurella multocida by pigs' alveolar macrophages after infection with pseudorabies virus. *Vet Immunol and Immunopathol*. 1986;13:165–172.

9. Fuentes M, Pijoan C. Pneumonia in pigs induced by intranasal challenge exposure with pseudorabies virus and *Pasteurella multocida*. *Am J Res*. 1987;48(10):1446–1448.

10. Hall MR, Williams PP, Rimler RB. A toxin from *Pasteurella multocida* serogroup D enhances swine herpesvirus 1 replication/lethality in vitro and in vivo. *Curr Micro*. 1987;15:277–281.

11. Iglesias G, Pijoan C, Molitor T. Interactions of pseudorabies virus with swine alveolar macrophages: Effects of virus infection on cell functions. *J Leukoc Biol*. 1989;45:410–415.

12. Ramirez MJ. The effect of different strains of pseudorabies virus on phagocytosis and killing of different serotypes of *Actinobacillus pleuropneumoniae* by porcine alveolar macrophages. Master's degree thesis 1990. College of Vet Med University of Minnesota.

13. Iglesias G, Trujano M, Xu J. Inoculation of pigs with *Streptococcus suis* type 2 alone or in combination with pseudorabies virus. *Am J Vet Res*. 1992;53(3):364–367.

14. Galina L, Pijoan C, Sitjar M, Christianson WT, Rossow K, Collins J.E. Interaction between *Streptococcus suis* serotype 2 and porcine reproductive and respiratory syndrome virus in SPF piglets. *Vet Rec*. 1994;134:60–64.

15. Jakab G. Mechanisms of virus-induced bacterial superinfections of the lung. *Clin Chest Med*. 1981;2(1):59–66.

16. Schwarzmann SW, Alder JL, Sullivan R, Marine WM. Bacterial pneumonia during the Hong Kong influenza epidemic of 1968-1969. *Arch Intern Med*. 1971;127:1037–1041.

17. Watson JM, Meredith SK, Whitmore-Overtone E, Bannister B, Darbyshire JH. Tuberculosis and HIV: Estimates of the overlap in England and Wales. *Thorax*. 1993;48(3):199–203.

18. Degre M, Glasgow LA. Synergistic effect in viral bacteria infection. Combined infections of the respiratory tract in mice with parainfluenza virus and *Haemophilus influenza*. *J Infect Dis*. 1969;118:449–462.

19. Jakab G. Suppression of pulmonary antibacterial activity following sendai virus infection in mice: Dependence on virus dose. *Arch Virol*. 1975;48:385–390.

20. Jakab G, Warr G, Sannes P. Alveolar macrophage ingestion and phagosome-lysosome fusion defect associated with virus pneumonia. *Infect Immun*. 1980;27(3)960–968.

21. Lopez A, Thomson R, Savan M. The pulmonary clearance of *Pasteurella hemolytica* in calves infected with bovine parainfluenza-3 virus. *Can J Com Med*. 1976;40:385–391.

22. Tashiro M, Ciborowsky P, Reinacher M, Pulverer HD, Klenk HD, Rott R. Synergistic role of staphylococcal proteases in the induction of influenza virus pathogenicity. *Virology*. 1986;157:421–430.

23. Akaike T, Molla A,o M, Araki S, Maeda H. Molecular mechanism of complex infection by bacteria and virus analyzed by a model using serratial protease and influenza virus in mice. *J Virol*. 1989;63(5):2252–2259.

24. Scheiblauer H, Reinacher M, Tashiro M, Rott R. Interactions between bacteria and influenza A virus in the development of influenza pneumonia. *J Inf Dis*. 1992;166:783–791.

25. Sierra ML, Rosas CP, Correa G, Jacobo R, Rodriguez S. Colera Porcino. In *Diseases of Pigs*, editors Necoechea and Pijoan, Mexico, DF. 1986:72.

26. Pijoan C, Campos M, Ochoa G. Effect of a hog cholera vaccine strain on the bactericidal activity of porcine alveolar macrophages. *Rev Lat Micro*. 1980:69–71.

27. Babiuk L, Lawman MJP, Ohmann HB. Viral-bacterial synergistic interaction in respiratory disease. *PRV Vir Res*. 1988;35:219–243.

28. Van Furth R. Cellular biology of pulmonary macrophages. *Int Archs Allergy Appl Immunol*. 1985:76 suppl 1:21–27.

29. Green GM, Jakab GL, Low RB, Davis GS. Defense mechanisms of the respiratory membrane. *Am Rev Respir Dis*. 1977;115:479–514.

30. Jakab G, Green G. Pulmonary defense mechanisms in consolidated and nonconsolidated regions of lungs infected with sendai virus. *J Infect Dis*. 1974;129(3):263–270.

31. Plotkowski MC, Puchelle E, Bech G, Jacquot J, Hannoun C. Adherence of type I *Streptococcus pneumoniae* to tracheal epithelium of mice infected with influenza A/PR8 virus. *Am Rev Respir Dis*. 1986;134:1040–1044.

32. Ramphal R, Samal PM, Shands JW, Fischlschweiger W, Small PA . Adherence of *Pseudomona aeruginosa* to tracheal cells injured by influenza infection or by endotracheal intubation. *Infect Immun*. 1980;27(2):614–619.

33. Kleinerman ES, Daniels CA, Polisson RP, Snyderman R. Effect of virus infection on the inflammatory response. *Am J Pathol*. 1976;85:373–382.

34. Warr G, Jakab GJ. Alterations in lung macrophage antimicrobial activity associated with viral pneumonia. *Infect Immun*. 1979;26(2):492–497.

35. Warr GA, Jakab GJ, Hearst JE. Alterations in lung macrophage immune receptor(s) activity associated with viral pneumonia. *J Reticuloendothel Soc*. 1979;26(4):357–366.

36. De Jong, MF. Progressive atrophic rhinitis. *Diseases of Swine* 7th edition;Leman AD, Straw BE, Mengeling WL, D'Allaire S, Taylor DJ:414–435.

37. Vecht U, Arends JP, Van der Molen EJ, Leengoed LAMG. Differences in virulence between two strains of *Streptococcus suis* type 2 after experimentally induced infection of newborn germfree pigs. *Am J Vet Res*. 1989;50:1037–1043.

38. Ciprian A, Pijoan C, Cruz T, Camacho J, Tortora J, Colmenares G, Lopez-Revilla R, de la Garza M. *Mycoplasma hyopneumoniae* increases the susceptibility of pigs to experimental *Pasteurella multocida* pneumonia. *Can J Vet Res*. 1988;53:434–438.

39. Jakab G. Viral-bacterial interactions in pulmonary infection. *PRV Vet Sci and Med*. 1982;26:155–171.