Evaluation of nasal culturing procedures and immunization as applied to the control of Bordetella bronchiseptica rhinitis in swine

Daniel Owen Farrington

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Evaluation of nasal culturing procedures and immunization as applied to the control of
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by

Daniel Owen Farrington

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INTRODUCTION

Infectious atrophic rhinitis is a serious economic disease of swine. It has been estimated that from 5 to 25 percent of all market swine have lesions of turbinate atrophy. *Bordetella bronchiseptica* infection is considered to be a major cause although not the only cause of atrophic rhinitis epizootics. Even though *Bordetella bronchiseptica* isolates from several species of animals have been demonstrated to infect swine, the usual mode of transmission is for certain baby pigs to become infected within the first week of life by exposure to chronically infected dams. These infected young pigs then serve to infect other young pigs by aerosol exposure to the organism and thus establish an aerosol epizootic within a group of closely housed, susceptible young pigs.

Naturally infected pigs 4 to 10 weeks of age have been shown by nasal swab culture to have the highest percentage of infected pigs as well as the greatest numbers of *B. bronchiseptica* organisms present. After the peak incidence of infection is reached, a gradual recovery occurs until in mature animals 10 to 15 percent remain as infected carriers. These carriers serve as a reservoir of infection for the next generation of susceptible young pigs, thus perpetuating the cycle of infection. This gradual clearance of the respiratory tract infection usually proceeds so that the nasal cavity is the last area to be freed of the infection with the ethmoid turbinates
reputedly the last area of the nasal cavity to be freed of *B. bronchiseptica*.

A procedure to monitor swine with *B. bronchiseptica* nasal infection has been developed. This procedure is based on the collection of nasal secretions on sterile swabs, with subsequent culturing of the material on a selective medium. Several years favorable experience with this detection system by research workers in this laboratory utilizing experimentally infected swine culminated in the initiation of a pilot, field control effort in October of 1970. This control program was an effort to eliminate Bordetella rhinitis from certain swine herds by detection and culling of infected breeding animals. The control program was based on the observation that *Bordetella bronchiseptica* can be recovered with reasonable accuracy from the nasal cavity of carrier swine by the culturing of nasal secretions and that the detection and elimination of such infected animals leaves a group of negative animals in the breeding herd that can be expected to produce Bordetella rhinitis free pigs. Two criteria for success of the nasal swab culturing procedure were utilized: (1) production of swine free from clinical atrophic rhinitis and (2) production of swine culture-negative for *B. bronchiseptica*. Due to possible reinfection from external sources, the second criteria was considered to be the most demanding.

The nasal swab culturing procedure is not applicable in all Bordetella rhinitis situations, is expensive, and if
successful produces a completely susceptible herd of swine. The necessity and desirability of an efficacious immunizing product are evident. It has been demonstrated that naturally recovered swine are extremely resistant to reinfection. This resistance has been shown to last for several months and is not considered to be associated with significant levels of circulating, agglutinating antibody. In several other chronic infections of the mucous membranes, circulating antibodies have relatively little significance or are only indirectly related to resistance to infection. Local immunity and mucoantibodies are of primary importance in such infections, particularly those which are confined to the mucous surfaces. In addition many pathogenic organisms gain access to the host via the mucosal surfaces of the respiratory, gastrointestinal and urogenital tracts and via the mammary gland. It has been suggested that adequate immunization against these pathogens must include immune protection at this portal of entry.

The present study was initiated to: (1) evaluate the nasal swab culture procedure as a method to control clinical Bordetella rhinitis epizootics, and (2) develop and evaluate \textit{B. bronchiseptica} immunizing agents for the control of Bordetella rhinitis.
REVIEW OF THE LITERATURE

This literature review covers three areas: 1. *Bordetella bronchiseptica* infection in swine and other animals. 2. Resistance of swine to *Bordetella bronchiseptica* infection, and 3. The secretory immune system with emphasis on swine secretory immunoglobulins, immunoglobulin A, and respiratory tract local mucosal resistance and immunity. Previous reviews by Winsser (1960), Duncan (1965), Ross (1965), Harris (1970) and Switzer (1970b) have dealt extensively with the history, pathology, bacteriology, animal pathogenicity, and infectivity of and resistance to *Bordetella bronchiseptica*. Since 1970 considerable additional information has been added to the literature concerning *B. bronchiseptica* and this review will primarily concentrate on these newly developed sources.

*Bordetella bronchiseptica* Infection
in Swine and Other Animals

*Bordetella rhinitis* is a chronic disease of swine caused by infection of the nasal cavity with *Bordetella bronchiseptica*. This bacterium has been isolated from a wide variety of healthy as well as diseased animals and its main habitat is the respiratory tract.

*Bordetella bronchiseptica* has been found to be widely distributed throughout the swine population (Phillips, 1943; Switzer, 1956; Ray, 1959; L'Ecuyer, Roberts and Switzer, 1961; Dunne, Kradel and Doty, 1961; Cross and Claflin, 1962; Ross,
Switzer and Mare, 1963b; Pearce and Roe, 1966; Pashov, Kurbala and Sereda, 1967; Harris, Ross and Switzer, 1969; Ogata, Koshimizu, Kang, Atobe, Yamamoto, Kino and Ikeda, 1970; Kang, Koshimizu and Ogata, 1971; Mermerski, 1973). Switzer (1956) reported that a bacterium subsequently identified as \textit{B. bronchiseptica} was capable of causing turbinate atrophy when inoculated intranasally as a pure culture into young pigs free from respiratory tract disease. This finding has been verified by many workers in several different laboratories (Cross and Claflin, 1962; Ross, Duncan and Switzer, 1963a; Duncan and Ramsey, 1965; Duncan, Ross, Switzer and Ramsey, 1966b; Pearce and Roe, 1966; Ross, Switzer and Duncan, 1967; Pashov, Kurbala and Sereda, 1967; Harris and Switzer, 1968; Agar, 1969; Shimizu, Nakagawa, Shibata and Suzuki, 1971; Maeda, Tokuhisa and Shimizu, 1971; Kemeny, 1972 and 1973; Fetter, Switzer and Capen, 1973). \textit{Bordetella bronchiseptica} is considered as an important cause of atrophic rhinitis epizootics in the United States (Harris and Switzer, 1972; Switzer and Farrington, 1972), Japan (Ogata et al., 1970; Hasebe, 1971; Shimizu et al., 1971), England (Done, 1972; Betts, 1972), and Bulgaria (Mermerski, 1973).

Akkermans, Ouwerkerk and Terpstra (1968) stated that analysis of smears from swine nasal cavities showed a close correlation between the presence of \textit{B. bronchiseptica} and the occurrence of "contagious sneezing" and turbinate atrophy. Schoss (1971) and Schoss, Dirks and Schimmelpfennig (1972) considers \textit{Pasteurella multocida} to be the primary causal agent of
atrophic rhinitis in Northwestern Germany. Transmission trials were carried out with 18 strains of *P. multocida* isolated from pigs with atrophic rhinitis. Turbinate atrophy was demonstrated in 26 of 94 test animals necropsied at the age of 3 months. This work is subject to further evaluation however, as no nasal swab cultures for the isolation and identification of *B. bronchiseptica* nasal infections were taken or negative controls utilized. Typical atrophic rhinitis was produced in 3 to 7 day old piglets after intranasal instillation of cultures of *P. multocida* and/or *B. bronchiseptica* by Pashov et al. (1967). Sterile filtrates of the cultures had no effect. MacKenzie (1969) does not regard atrophic rhinitis as an etiological entity since it has been associated with a variety of infectious agents, e.g. *B. bronchiseptica*, *P. multocida*, *Hemophilus suis*, *Corynebacterium pyogenes*, *Mycoplasma* species, inclusion body rhinitis, cytopathic viruses and trichomonads. He stated other factors such as heredity, vitamin deficiency (A, D, or C), nutritional hyperparathyroidism, altered Ca and P levels, chemical irritation, age of exposure, the use of chemotherapeutic agents, management and other environmental factors may influence the severity of the atrophic rhinitis in a given herd.

Agar (1969) inoculated gnotobiotic pigs fed a ration deficient in calcium (0.3% level) at 3 days of age with *B. bronchiseptica* and caused some degree of turbinate atrophy in 96 percent of them. The atrophy was evident within 7 days of inoculation and at varying ages up to 56 days. Osteodystrophia
fibrosa and pericytic osteolysis as a result of a dietary insufficiency of calcium did not contribute significantly to turbinate atrophy in the pigs in the age group 0 to 56 days. His results indicated that osteodystrophia fibrosa as a result of dietary insufficiency of calcium and turbinate atrophy induced by *B. bronchiseptica* occurred as two separate disease entities in this age group of gnotobiotic pigs.

Shimizu *et al.* (1971) in a work to determine if *B. bronchiseptica* is the causative agent of atrophic rhinitis in hysterectomy produced colostrum-deprived pigs caused typical turbinate atrophy in 100 percent of *B. bronchiseptica* inoculated pigs. They further noted the organisms decrease in incidence by 3 months of age and that agglutinating antibodies appeared as early as 37 days post-challenge. A *Bordetella bronchiseptica* culture that was isolated from infectious nasal washings was used by Kemeny (1972) to produce turbinate atrophy in 36 percent of intranasally inoculated pigs. The crude nasal washings caused turbinate atrophy in 81 percent and sedimented crude nasal washings caused turbinate atrophy in 100 percent of intranasally inoculated pigs.

Fetter, Switzer and Capen (1973) caused turbinate atrophy in 100 percent of respiratory disease-free pigs inoculated with swine virulent (B strain) *B. bronchiseptica* in the first days of life. Turbinate atrophy was evident by 2-weeks post-inoculation.

The extent of atrophic rhinitis in the swine population is
difficult to assess. Dunn (1969) reported that the inspection of the nasal cavities of more than 1,600 swine slaughtered from 1962 to 1969 indicated a prevalence of about 25 percent. Switzer (1970b) estimated that from 5 to 10 percent of slaughtered swine from the United States swine producing areas have turbinate atrophy and that the growth rate may be retarded about 5 percent in the affected pigs as compared with healthy ones. He stated the economic loss caused by the occurrence of atrophic rhinitis consists of retarded growth, reduced feed efficiency and predisposition to other respiratory tract diseases rather than a high mortality rate among the infected swine. Ross (1965) found an incidence of \textit{B. bronchiseptica} in purebred and commercial herds ranging between 38 and 54 percent while Harris \textit{et al.} (1969) found 25 percent of 102 Iowa swine herds culture-positive for this organism. In Japan, the first case of atrophic rhinitis was reported in 1966, but it is assumed that it may have existed in Japan for quite some time prior to this date (Hasebe, 1971). Hasebe (1971) considered that the disease may have spread to about 20 percent of the Japanese swine population and it was estimated that the disease retarded growth rate by about 10 percent. Kang \textit{et al.} (1971), in a field survey utilizing the agglutination test to determine the incidence of \textit{B. bronchiseptica} and turbinate atrophy in 400 4 to 8 week old swine from various districts of Japan, found antibodies against \textit{B. bronchiseptica} in 54.5 percent of the pigs. Comparison of 3 diagnostic methods, i.e.
serological (agglutination test), pathological (gross lesion examination) and bacteriological (B. bronchiseptica isolation) tests, revealed that the results were not always correlated.

Pearce and Roe (1967) in studying 3 specific pathogen-free (SPF) herds that contracted atrophic rhinitis, found an initial incidence of turbinate atrophy of 45 percent which declined to an average of 35 percent. Their findings indicated atrophic rhinitis did not cause a significant difference in the maturity time from birth to 200 pounds of weight.

Based on examination of the nasal cavities of 3,500 Landrace market weight swine, Behrens (1971), stated that the gross lesions (turbinate atrophy, septal distortion) associated with atrophic rhinitis were not pathognomonic. Sectioning of the nasal cavity at the level of the first premolar tooth was said to be of limited diagnostic value without a thorough understanding of the clinical and bacteriological picture. 

*Bordetella bronchiseptica* may cause severe damage to the nasal turbinate without causing significant damage to the dense bones of the wall of the nasal cavity (Duncan et al., 1966b). Therefore, inspection for twisted noses or nasal hemorrhage will enable diagnosis in only a small percentage of the actual cases of turbinate atrophy.

*Bordetella bronchiseptica* can also cause primary pneumonia as well as atrophic rhinitis in swine (L'Ecuyer et al., 1961; Duncan, Ramsey and Switzer, 1966a). No incidence figures are available to indicate the morbidity of *B. bronchiseptica*
pulmonary disease but Brown\textsuperscript{1} states \textit{B. bronchiseptica} is a consistant and relatively frequent bacterial pathogen which may be recovered in pure culture from pneumonic lesions of swine submitted to the Veterinary Diagnostic Laboratory, College of Veterinary Medicine, Iowa State University.

The pigs nasal cavity may become infected with \textit{B. bronchiseptica} as early as 48 hours after birth (Harris and Switzer, 1968). Switzer (1970a) states there are 3 general requirements necessary for \textit{B. bronchiseptica} nasal infection to cause severe turbinate damage. These are: 1. The infection must occur at an early age, usually during the first 3 weeks of life. Swine several months of age or older may become infected, but they do not develop lesions. 2. The infection must be with a virulent strain. 3. The infection must be of adequate duration lasting at least 3 to 5 weeks.

Once turbinate atrophy occurs the lesions may persist for long periods even though the infection may be cleared by therapy or by the pigs natural defense mechanisms (Harris and Switzer, 1969). Harris and Switzer (1969) state the recovery of infected swine usually proceeds so that the lung and tracheal infection is eliminated before the nasal infection is cleared. The ethmoid turbinates are reported to be the last area of the nasal cavity to be cleared from the infection (Ogata

\textsuperscript{1}Brown, L. N. 1973. Personal Communication. Veterinary Diagnostic Laboratory, Iowa State University, Ames.
et al., 1970; Kemeny, 1973). Switzer and Farrington (1972) presented data on the natural clearing rate of *B. bronchiseptica* nasal infections that indicated up to 90 percent of pigs 4 to 10 weeks of age may have infected nasal cavities. This infection gradually clears until at approximately 10 to 12 months of age only 10 to 15 percent of the animals remain as infected carriers. These pigs may serve as a reservoir of infection for their offspring, thus perpetuating the cycle of infection.

Kang, Koshimizu and Ogata (1971) stated that *B. bronchiseptica* can be demonstrated from the nasal cavity in the early (1 to 3 months after birth) or middle (4 to 5 months) stages of infection, but gradually decreased in the older animals. Turbinate atrophy was said to usually appear at 4 to 5 months of age and agglutinating antibodies against *B. bronchiseptica* were generally present at the end of the early stage or the beginning of the middle stage of infection.

Switzer and Farrington (1972) proposed that Bordetella rhinitis spreads by 3 general methods: via nasal contact between infected sows and their progeny; via aerosol infection of young pigs, especially in farrowing houses or nursery environments from other young pigs; and via exposure of susceptible swine to nonswine sources of infection. MacLean (1971) reported that infectious atrophic rhinitis was transmitted over a distance of 6 feet, but not over 16 feet and that the infective droplet size must therefore be between 10 and 80 microns.

Various isolates of *B. bronchiseptica* are not of equal
virulence for swine. Some nonswine strains have been demonstrated to cause typical atrophic rhinitis in swine, whereas other strains recovered from swine or other mammals may be of low virulence for pigs (Ross et al. 1967). One canine origin isolate was demonstrated to be regularly eliminated from the swine nasal cavity by the sixth to seventh week postinoculation. Ross et al. (1967) further state that differences in pathogenicity of B. bronchiseptica isolates may account for differences in the severity of rhinitis and turbinate atrophy in different field cases where this agent is present. Agents such as Pasteurella multocida, Hemophilus spp. and inclusion body rhinitis virus may also play a significant role in the field disease.

Swine experimentally infected with B. bronchiseptica were successfully freed of the infection by treatment with sulfa­methazine or sulfaethoxypyridazine (SEZ) at the level of 100 grams per ton of feed (Switzer, 1963). Since that time these sulfonamides have been incorporated with growth-promoting antibiotics and have enjoyed wide popularity as feed additives to swine rations. Harris et al. (1969) conducted a survey that indicated 80 percent of the B. bronchiseptica isolates recovered from infected herds were resistant to sulfonamides as determined by sensitivity disc assay. Woods et al. (1972) found a ration containing 100 grams chlortetracycline, 100 grams sulfamethazine, and 50 grams of procaine penicillin per ton of feed effective in controlling B. bronchiseptica in the
nasal tract of pigs. Suhara et al. (1970) noted that in pigs infected with Bordetella rhinitis, the addition of tylosin-sulfonamide feed additives reduced the severity of the nasal lesions and improved the weight gain, but did not eradicate B. bronchiseptica from the herd even when used in conjunction with pen disinfection and the parenteral and intranasal administration of antibiotics.

In the course of work with experimentally induced B. bronchiseptica infections in swine it became necessary to develop a procedure to monitor nasal infections. This procedure was based on the collection of nasal secretions on sterile swabs with subsequent culturing on a selective medium (Ross, 1963). Akkermans (1972) concluded that the bacteriological investigation of the nasal flora in pigs is a useful method with which an impression of the occurrence of B. bronchiseptica and Pasteurella multocida on infected farms can be obtained. After several years experience with the nasal swab culture detection system in infected swine, it was decided the method had sufficient merit to warrant its application to an initial experimental field control effort (Switzer, 1970a). A pilot field control effort was therefore initiated in October, 1970 with the production of Bordetella rhinitis free pigs from B. bronchiseptica negative sows as the goal of the nasal swab control procedure. Switzer and Spear (1973) showed that 34 B. bronchiseptica culture-negative sows from 4 known Bordetella rhinitis herds weaned 317 pigs, all of which were culture-
negative for *B. bronchiseptica* at 8 weeks of age. Switzer and Farrington (1972) found that the nasal swab culture control procedure was effective in elimination of clinical signs of atrophic rhinitis in 16 of 19 (85 percent) herds sampled. In 21 initially culture-positive herds, 14 (67 percent) produced pigs that were free of *B. bronchiseptica* at weaning age. Nasal swab cultures from 6,970 mature breeding animals revealed 542 (8 percent) culture-positive for *Bordetella bronchiseptica*.

MacKenzie (1969) states that in the field respiratory disease epizootics in swine are usually caused by multiple etiological factors. Pathological changes often have to be interpreted within the context of combined infections. Hereditary, environmental, nutritional or stress factors may be essential predisposing causes or other infective agents may act synergistically to produce intensified respiratory tract lesions.

Detailed histologic studies of atrophic rhinitis caused by *B. bronchiseptica* (Duncan and Ramsey, 1965; Duncan et al., 1966b) have revealed that the organism localizes on the epithelial surfaces of the respiratory tract. Under the influence of inflammation produced by *B. bronchiseptica* the normal porcine ciliated nasal epithelial cells became polyhedral in shape. The cilia were reduced in numbers and spaced further apart. Microscopic lesions consisted of hyperplasia and metaplasia of the epithelium, fibrosis and collagen formation in the lamina propria and resorption and replacement fibrosis in the bony core. The major part of the atrophy was accounted for by the
reduction in the size of the osseous core. Shimizu et al. (1971) reported that microscopic examination of experimentally produced turbinate atrophy caused by *B. bronchiseptica* revealed rarefaction of the osseous core and inflammatory changes of the nasal mucosa that were essentially the same as reported previously by Duncan et al. (1966b). Fluorescent antibody staining indicated *B. bronchiseptica* among the cilia of the trachea and turbinates, but not in the underlying tissues. Conversely, Fetter, Switzer and Capen (1973) observed in the bone cells of the nasal turbinates of pigs experimentally infected with *B. bronchiseptica*, bacterial organisms with ultrastructural features resembling those described for *B. bronchiseptica* in the immediate vicinity of degenerating bone cells and adjacent to mineralized bone matrix. Turbinate atrophy was clearly evident in the respiratory disease free pigs experimentally inoculated with *B. bronchiseptica* at two weeks post inoculation. The ventral scroll of the ventral turbinate was most often affected first and most severely.

Fetter and Capen (1971a and b) studied the ultrastructural lesions of bone cells in pigs with naturally occurring and experimentally produced atrophic rhinitis. The experimental atrophic rhinitis was produced with an inoculum of pooled material from the nasal turbinates of pigs with the naturally occurring disease. A variety of organisms were isolated from the inoculum including *B. bronchiseptica* and *Pasteurella multocida*. Fetter, Switzer and Capen (1973) evaluated ultrastructure
changes in the bone cells of the nasal turbinates of pigs experimentally infected with *B. bronchiseptica* by intranasal inoculation. These were compared to control pigs of the same age and to pigs with experimentally transmitted (atrophic turbinate suspension inoculated) and naturally occurring atrophic rhinitis. Severe degenerative changes were observed in the osteoblasts and osteocytes in the diseased pigs. These alterations progressed to atrophy and lysis of bone cells, reduction of osteoid formation and the degenerative cells were observed directly adjacent to the mineralized matrix of bone. Osteoclasts were similar in number and appearance to those in the control pigs. Fetter et al. (1973) concluded that the fundamental lesions in pigs with Bordetella rhinitis was reduced formation of the organic matrix by bone cells. Bone resorption in the vicinity of degenerating osteocytes and osteoblasts appeared to contribute to the overall loss of bone. They concluded that there was no significant difference in the ultrastructural lesions of bone cells in pigs with Bordetella rhinitis as compared to pigs with experimentally transmitted (atrophic turbinate suspension inoculated) or naturally occurring atrophic rhinitis.

The nasopharynx, in common with many other mucous membranes, is covered by a film of mucus kept in continuous motion by the cilia arising from its epithelium. Mucus transport by the ciliary escalator is a major defense mechanism for clearance of airborne materials from the respiratory tract. This
action is brought about by coordinated ciliary beat, which is translated into effective clearance only in the presence of an intact respiratory tract mucosa and a mucus film of suitable properties. Sade et al. (1970) showed that the systemic role of mucus is highly specific and in a depleted tissue, the cause of failure of the mucociliary function is a decrease in the quantity of mucus. Mucus was stated to be an essential intermediary in the transport system and a minimal amount must be available to allow the cilia to perform their function. Kilburn (1967) states that the cohesiveness of hydrated mucin permits the mucus sheet to be dragged along above the surface of the ciliated epithelium. Its major functions may be to control transudation and to protect underlying cilia and cells from penetration by particles, viruses and bacteria. The ciliary mucous transport system's protective ability is reversibly decreased by cold, dehydration, trauma, oxidant gases and the action of bacterial and viral infection which probably destroy the epithelium. The mucosa can also be altered by inanition, vitamin A deficiency and hypothyroidism. Kilburn (1967) further stated that air pollutants such as SO₂, H₂S, NH₃ at lesser concentrations approaching those in environmental pollution may affect mucus transport by changing mucus or ciliary beating. Once protection has been destroyed or reduced from whatever cause, bacteria which could not penetrate the intact mucus layer or find a hospitable environment may become concentrated and colonization occurs.
To avoid exclusion and removal, *Bordetella pertussis* must be able to anchor itself to some fixed structure on the nasopharyngeal surface (Holt, 1972). It was shown that in whooping cough in children *B. pertussis* has a marked tendency to attach itself to the cilia of the nasopharynx. Holt (1972), using fibroblast cell cultures in a model system for the early stages of infection in natural whooping cough, found that *B. bronchiseptica* (strain B8760) was strongly adherent to MRC-5, Hela, Hep 2 and monkey kidney cell (MKC) cell lines in all suspending fluids. Electron microscopic study of intraventricular infection of mice with *B. pertussis* has shown that the bacteria adhere to the microvilli between the ependymal cilia (Hopewell et al., 1972). Harris (1970) demonstrated *in vitro* that *B. bronchiseptica* organisms attach to nasal epithelial cells and indicated this accounts for its high infectivity for the nasal mucosa of pigs. He further stated the eventual ciliary destruction by this organism probably reduces the efficiency of the mucociliary system and allows for the establishment of secondary respiratory pathogens (unattaching organisms) such as *Pasteurella multocida*.

Duncan et al. (1966b) suggested that an organism capable of producing atrophic rhinitis such as *B. bronchiseptica* must release substances which diffuse into the tissue and elicit changes in the osseous core without causing a marked inflammatory reaction. They proposed the endotoxin or a similar substance of *B. bronchiseptica* as a potential initiating agent.
Harris, Harris and Green (1968) subsequently demonstrated that *B. bronchiseptica* boivin extracts had a profound effect upon the energy conserving and transducing properties of isolated beef heart mitochondria. The extract was tentatively described as endotoxin-like. Fetter and Capen (1971b) stated that bacterial endotoxins released during a localized inflammatory reaction in the Schneiderian membrane of diseased pigs could result in degenerative changes of bone cells similar to those reported in their investigation. Harris, Switzer and Harris (1971) experimenting further with the effects of *B. bronchisep-tica* boivin extracts on pig and beef heart mitochondria suggest that the boivin extract may contain the membrane-damaging component responsible for infectious rhinitis. They proposed that a diffusible product such as that suggested by Duncan et al. (1966b) may be contained in the boivin extract and that the presence of these toxins near the osseous core of the nasal turbinate prevents the uptake of Ca\(^{++}\) by the organic matrix of the turbinate by a mechanism similar to the effect of the extract upon mitochondria. The extract, however, was found to be heat labile and thus did not fit the classical characterization of endotoxins as heat stable lipopolysaccharides. No specific chemical constituent could be found as possessing activity against the mitochondrial energized processes. It was suggested that a possible mechanism for complete activity might be a complex composed of protein and lipopolysaccharide in a membranous configuration.
Bordetella bronchiseptica may be recovered from the respiratory tract of a wide variety of mammalian species (Winsser, 1960; Switzer et al., 1966; Switzer, 1970b). Of the various non-swine carriers of Bordetella bronchiseptica, the cat probably has the greatest potential for spread of the organism to susceptible young swine (Switzer and Farrington, 1972). Fisk and Soave (1973) in a study of cats for carriers of B. bronchiseptica found that 10 percent of newly purchased random source cats had the organism in their upper respiratory tract and that at the end of a 3 week confinement period, the proportion of carriers had increased to 48 percent. They considered cats a potential source of this infection for susceptible laboratory animal species as well as other cats. Snyder et al. (1973) isolate Bordetella bronchiseptica from the trachea and lungs of 10 cats that died from pneumonia.

In experimental B. bronchiseptica infection in guinea pigs, Nakagawa et al. (1971), found that infection rates were proportional to the number of organisms inoculated. Infection occurred in few of the animals receiving $10^2$ organisms, but occurred in the majority of the animals receiving $10^4$ organisms and in all of the animals inoculated with $10^6$ organisms. Subcultures on MacConkey's agar rapidly lowered the infectivity of the organisms for guinea pigs.

Mayer (1971) and Oldenburg et al. (1972) have reported on B. bronchiseptica infection in large rabbit farms. Bordetella bronchiseptica was found to cause bronchopneumonia
in both germfree and conventional rats by Burek et al. (1972)
and Farrington (1973) found *B. bronchiseptica* in 4 of 11 short-tailed shrews. Ioakimidis et al. (1970) reported a *B. bronchiseptica* epizootic on a large chinchilla ranch; Graves (1970) considered *B. bronchiseptica* as the cause of a pneumonia epizootic in a monkey colony containing 4 different species; Saxegaard et al. (1971) found *B. bronchiseptica* to be the etiological agent in a case of equine bronchopneumonia; and Hall et al. (1971) isolated *B. bronchiseptica* from the lung of a beached Pacific pilot whale. A case of cross-infection of *B. bronchiseptica* from infected pigs to the noninfected controls within an isolation unit was attributed to a heavy infestation of Pharaoh's ants by Beatson (1972)

Nakase (1957 a,b,c,d) made an extensive examination of the antigenic structure, phase variation, biological properties and serological relationships of *B. bronchiseptica*. He described a capsular thermolabile L antigen, flagellar thermolabile H antigen, surface slightly-thermostable S antigen and somatic thermostable 01, 05 and 07 antigens. *Bordetella bronchiseptica* was divided into 3 smooth phases and 1 rough phase and was said to be an extremely unstable organism with easy variation from phase I to phase IV by cultivation. Phase I organisms were considered to be pathogenic. Biological differences existed between phase I and III in regard to acid agglutination, hemagglutination, toxicity, virulence and protective potency to mice. Dog, guinea pig and human *B. bronchiseptica* isolates
showed antigenic variation.

Mason (1971) reported the growth of *B. bronchiseptica* cultures in a graded series of glycine concentration. Gradual inhibition of growth occurred in glycine concentration up to 1 percent while higher concentrations greatly inhibited growth and caused some autolysis. Sixteen hour cultures in 1 percent glycine were almost completely converted to aberrant forms and spheroplasts. Karaivanov *et al.* (1972) demonstrated the inhibitory effect of a *B. bronchiseptica* strain on growth of *Pasteurella multocida* organisms. The inhibitory factor was demonstrable only on solid media.

Terakado *et al.* (1973) demonstrated that R factors carrying sulfadimethoxine-streptomycin-aminobenzyl penicillin resistance was present in strains of *B. bronchiseptica* isolated from pigs. All strains could transfer their drug resistance as 1 unit to a sensitive strain of *Escherichia coli* as well as to *B. bronchiseptica* by mixed cultivation. This increased prevalence of R factors in bacteria of animal origin was said to be a serious problem for animal hygiene and public health.

Azechi *et al.* (1973) tested 61 strains of *B. bronchiseptica* isolated from swine for sensitivity to 52 antibacterial drugs by means of a plate dilution method. Thirty-nine isolates were from pigs showing clinical signs of atrophic rhinitis. Of the isolates tested 11.5 percent were resistant to sulfa drugs and showed a cross resistance to aminobenzyl penicillin and streptomycin. Wilkins and Helland (1973) found 17 isolates
of *B. bronchiseptica* from dogs with tracheobronchitis resistant to lincomycin, penicillin, streptomycin, nitrofurantoin and tylosin as determined by sensitivity disc testing. The activity of selected antibacterial agents against a swine isolate of *B. bronchiseptica* was evaluated in a 7-day-old embryonating hens egg system by Shirk and Kemp (1967). Single doses of the desired amount of drug were injected into the allantoic cavity prior to infection of the yolk sac with *B. bronchiseptica*. Survival data indicated the most effective drug was sulfaethoxypyridazine, followed by sulfathiazole and sulfaquinoxaline.

Blood chemistry changes in swine from 1 to 12 weeks of age infected with *Bordetella bronchiseptica* intranasally were examined by Baetz et al. (1974) to determine if they might be of diagnostic value. Lactic dehydrogenase (LDH) activity, lactic acid and total protein concentrations were found to be significantly higher in the infected piglets than in controls at a few time periods early in the infection. However, it was stated the minor changes in blood chemistries caused by *B. bronchiseptica* superimposed on the dynamic changes in blood chemistry taking place in the rapidly growing piglet make it impractical to use blood chemistry measurements for diagnostic purposes in this disease.
Resistance of Swine to 
Bordetella bronchiseptica Infection

Harris (1970) has extensively reviewed the resistance of various animals to Bordetella bronchiseptica and Bordetella pertussis; therefore, only those selected references which have relevance to Bordetella bronchiseptica resistance in swine will be included in this discussion.

Bordetella bronchiseptica and B. pertussis are antigenically related (Blair et al., 1970). Andersen (1953) demonstrated that B. bronchiseptica and B. pertussis have similar O-antigens, a similar hemorrhagic toxin and common as well as species-specific K-antigens. The mechanism of immunity following B. pertussis infection or immunization has not been clearly defined (Aftandelians and Connor, 1973) and no satisfactory purification of the protective antigen from B. pertussis has yet been accomplished (Sato and Nagase, 1967; Ross and Munoz, 1971).

Munoz (1963) listed the following antigens or substances to be present in B. pertussis: agglutinogen, heat-labile toxin, heat-stable toxin (endotoxin), hemagglutinin, histamine-sensitizing factor and protective antigen. The agglutinogen and hemagglutinin were thought to be cell surface capsular antigens while protective antigen, histamine-sensitizing factor and endotoxin were associated with the cell wall. Heat-labile toxin was considered to be of protoplasmic origin. Ross and Munoz (1971) considered it well established that agglutinogens,
endotoxin, heat-labile toxin and hemagglutinins were not involved in the active immunization of mice intracranially challenged with virulent *B. pertussis*. They further reported histamine-sensitizing factor and protective antigen were found in the same fraction and that their physical and chemical properties were similar if not identical.

Standfast (1967) agreed that most of the protective activity was found in the cell wall, but that it was still open to question whether the protective antigen was identical with the histamine-sensitizing factor. Milleck and Ocklitz (1972) reported that *B. pertussis* spheroplast cell walls gave better protection than the corresponding intact spheroplasts and purified K-agglutinogen was found to lack protective activity by Nakase and Kasuga (1971).

Ackers and Dolby (1972) investigated a circulating complement mediated bactericidal antibody appearing after an injection of vaccine to determine if it might be responsible for the protection of immunized mice. They concluded that the bactericidal antibody (bactericidin) was not necessary for the protection of mice. Complement-dependent bactericidal antibodies were detected in the sera of 24 persons with bacteriologically confirmed pertussis and the development of humoral bactericidal antibody during pertussis was considered to indicate a role in immunity although a mechanism was not demonstrated (Aftandelians and Connor, 1973). It was postulated that the protective antibody reacted with the invading
organism on the surface of the respiratory epithelial cells in
the case of intranasal challenge and prevented extension of
infection by limiting replication or adherence or by other
means.

Holt (1972) observed that the incubation of live B. pertus-
sis and B. bronchiseptica in the presence of certain cell
cultures resulted in adhesion of the bacteria onto the cell
surfaces. This adhesiveness was lost if the bacteria were
given prior treatment with immune antisera. A similar mechan-
ism was believed to operate on the surface of the respiratory
epithelium by the action of either locally produced (IgA) or
serum (IgG) antibody (Aftandelians and Connor, 1973). Holt
(1972) found it probable, in accord with Tomasi and Bienenstock
(1968) and Tomasi (1970), that B. pertussis reinfection, e.g.
after intranasal vaccination, is prevented by locally produced
IgA antibody which prevents bacterial adhesion to the
epithelium. This antiadhesive mechanism was said to be greatly
improved by the continuous flow of mucus over the epithelial
surface. Harris (1970) also demonstrated in vitro that swine
nasal epithelial cells attached to B. bronchiseptica organisms
and he postulated that such an inhibition of attachment to
nasal and tracheal epithelial cells may be a mechanism by which
respiratory tract resistance could be explained. Holt (1972)
further stated that the current practice of vaccinating infants
subcutaneously with B. pertussis vaccines may induce not a pro-
phylactic but a therapeutic immunity and that to confer true
prophylaxis against pertussis may require the addition of aerosol vaccination to the present immunization program.

Kang, Koshimizu and Ogata (1970) used live *B. bronchiseptica* grown in broth as an agglutinogen for use with immune serum in order to develop a serological test for the diagnosis of atrophic rhinitis in naturally infected pigs. A tube agglutination test was developed and great care was used to select the strain used in the agglutination reaction. The first appearance of agglutinating antibody in the naturally infected pigs was at about 20 weeks of age. Kang, Koshimuzu and Ogata (1971) subsequently used this agglutination test to carry out a field survey to determine the incidence of *B. bronchiseptica* and turbinate atrophy in 400 pigs 1 to 8 months of age from various districts of Japan. Lesions were found in 91.4 percent of the serologically positive pigs while pigs evidencing pathological lesions showed a 77 percent positive reaction in the agglutination test. They concluded that a serological test was available for the diagnosis of Bordetella rhinitis in swine. Ogata, Kodama and Koshimizu (1973) reported on the successful use of formalin-killed phase I *B. bronchiseptica* as antigens in a tube agglutination test for the diagnosis of *B. bronchiseptica* in swine.

Harris (1970) and Harris and Switzer (1972) reported the development of a particulate antigen settling test (PAST) for the detection of antibody against *B. bronchiseptica* infection in swine. Harris (1970) stated that nasal resistance to
B. bronchiseptica infection did not appear to be correlated with serum antibody as determined by the particulate antigen settling test.

Ross et al. (1967) reported that sera of experimentally infected pigs may contain low levels of agglutins 4 weeks after exposure while Shimizu et al. (1971) demonstrated an increase of agglutinins in sera from hysterectomy derived, colostrum deprived pigs after B. bronchiseptica challenge. Kemeny (1973) found that B. bronchiseptica multiplied rapidly on the mucosal surface of the respiratory tract of young pigs, that the infection induced the formation of agglutinins and that turbinate atrophy occurred in 65 percent of the experimentally exposed pigs. It was stated that the demonstration of a rise in agglutinating antibody titer in at least 2 properly timed serum samples may help confirm B. bronchiseptica infection in a swine herd where nasal swab culturing was unsuccessful.

Kemeny and Amtower (1973) used an agglutination test employing killed B. bronchiseptica to detect naturally-occurring antibody against this organism in the sera of 451 pigs 1 month to over 36 months of age. It was stated that nasal swab cultures did not reliably detect the carrier state of subclinically infected pigs and that detection of agglutinating antibody against B. bronchiseptica may be useful for diagnosing infection in swine herds.

Resistance to B. bronchiseptica infection in swine may be
due to the action of immunizing agents (acquired resistance) or to the pigs' own inherent immune mechanisms (natural resistance). Harris and Switzer (1969) reported that swine freed from virulent \textit{B. bronchiseptica} infection with sulfonamide treatment or by natural clearance of a low-virulence strain (canine origin D-1 strain) of \textit{B. bronchiseptica} were extremely resistant to reinfection. This natural resistance lasted for at least several months and was not found to be associated with the presence of demonstrable bactericidal substances or with significant levels of circulating agglutinating antibody (Harris and Switzer, 1969; Harris, 1970).

Tomasi and Bienenstock (1968) stated that in certain infections, circulating antibody has relatively little significance or is only indirectly related to resistance to infection. Local immunity and mucoantibodies may be of primary importance in the defense against some infections especially those which are noninvasive and confined to the mucous surfaces. Bienenstock and Perey (1972) noted that local presentation of antigen to the respiratory tract appears to preferentially induce local antibodies (IgA) which were better correlated with subsequent resistance to reinfection than were circulating antibodies stimulated by the parenteral administration of the same antigen.

Holt (1972) observed that it was probable that 2 immune systems were of importance in whooping cough: (1) A true prophylactic immunity in which locally produced IgA secretory
immunoglobulin prevented the adhesion of the organism to the mucous membrane; and (2) A therapeutic system, involving polymorphs and an opsonin which after the development of inflammation acted at the surface of the membrane and eliminated the infection.

Rabbits (Ferry and Hoskins, 1920), rats (Wickert et al., 1958), mice (Winsser, 1960) and guinea pigs (Nikkels and Mullink, 1971) have been protected against _B. bronchiseptica_ pneumonia by the use of _B. bronchiseptica_ vaccines. Yoda et al. (1972) demonstrated the development of resistance to reinfection in guinea pigs recovered from _B. bronchiseptica_ pneumonia. It was previously shown that no differences of susceptibility to intranasal infection by the organism existed among individuals and that the development of infection depended only upon the number of organisms inoculated (Nakagawa et al., 1971). The intranasally infected guinea pigs usually recovered within 20 weeks and most of the recovered animals were highly resistant for as long as 40 weeks or more after recovery from the natural infection. Graves and Shah (1972) stated that the development of a _B. bronchiseptica_ vaccine would be advantageous for the control of _B. bronchiseptica_ pneumonia epizootics in laboratory monkey colonies.

Kendrick et al. (1939) first reported on the successful immunization of humans against whooping cough by use of _B. pertussis_ vaccines. It is generally accepted that the degree of protection against whooping cough is not correlated with
the serum agglutinin titer associated with pertussis immunization (Medical Research Council, 1959). Holt (1972) used a \textit{B. pertussis} aerosol vaccine in an attempt to induce local immunity in a Taiwan monkey. Both humoral and locally produced secretory antibody were produced, but the results were in general unsatisfactory.

Daughtery (1941) reported that swine with atrophic rhinitis showed a clinical improvement when administered a canine mixed bacterin containing \textit{B. bronchiseptica}. Phillips (1944) and Ray (1959) stated that a bacterin containing \textit{B. bronchiseptica} prevented pneumonia caused by this organism in swine. Harris and Switzer (1969), however, made the first clear demonstration of the induction of resistance in swine to \textit{B. bronchiseptica} infection. Harris and Switzer (1972) using a sonicated vaccine with adjuvant, prepared by sonically disrupting \textit{B. bronchiseptica} strain D-1 and a commercially purchased pertussis vaccine demonstrated accelerated nasal clearance of \textit{B. bronchiseptica} nasal infections. They reported high levels of circulating, agglutinating antibodies were stimulated by these vaccines. They had previously demonstrated (Harris and Switzer, 1969) that a whole-cell vaccine (strain B) without adjuvant did not induce nasal resistance against virulent \textit{B. bronchiseptica} challenge. The accelerated recovery from infection was not manifested by a complete refractiveness against the challenge organisms since 40 to 60 days were required for the clearance of the challenge organisms.
Matsuura\(^1\) concluded that immunization with attenuated \textit{B. bronchiseptica} may be effective to prevent infection with \textit{B. bronchiseptica} to some extent and to significantly inhibit development of atrophic rhinitis in swine. \textit{Bordetella bronchiseptica} was detected in 49 percent of the vaccinated pigs and in 77 percent of the unvaccinated controls. Gross lesions of atrophic rhinitis were found in 7 percent of the vaccinated pigs and 59 percent of the unvaccinated pigs. Forty-five \(10^9\) organisms were administered subcutaneously in 2 doses, 2 weeks apart.

Kawahira\(^2\) parenterally immunized pigs with inactivated \textit{B. bronchiseptica} using a total dosage of \(225 \times 10^9\) organisms administered in 3 doses beginning the third day after birth. \textit{Bordetella bronchiseptica} was not cultured from the nasal cavity of these immunized pigs on or after 18 to 26 days post challenge while all control pigs yielded the organism continuously to 109 days post-challenge. It was concluded that \textit{B. bronchiseptica} immunization was effective, even in 3-day-old pigs, in preventing \textit{B. bronchiseptica} nasal infections.

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\(^{1}\text{Matsuura, S. 1972. The preventive effect of attenuated B. bronchiseptica on atrophic rhinitis in swine. Unpublished data. Western Regional Animal Hygiene Service Station, Ibaragi Prefecture, Japan.}\)

\(^{2}\text{Kawahira, M. 1972. An immunological study with attenuated B. bronchiseptica in swine. Unpublished data. Kitasato Biological Institute, Japan.}\)
Ohmura\textsuperscript{1}, however, parenterally immunized pigs of varying ages with 2 doses of an inactivated \textit{B. bronchiseptica} vaccine using a total dosage of $105 \times 10^9$ organisms and found the comparative ratios of \textit{B. bronchiseptica} isolation in the vaccinated pigs versus the unvaccinated pigs to be as follows: 14\% vs. 30\% 6 weeks post-vaccination, 20\% vs. 44\% 11 weeks after vaccination, 36\% vs. 63\% 15 weeks after inoculation, 62\% vs. 87\% at the time of slaughter, and a cumulative ratio of 77\% vs. 100\%. It was concluded that \textit{B. bronchiseptica} infection in swine was prevented to a limited extent by vaccination.

Koshimizu\textsuperscript{2} reported that immunization of 3-month-old naturally infected pigs with a \textit{B. bronchiseptica} bacterin with adjuvant did not significantly decrease the number of \textit{B. bronchiseptica} isolated from the nasal cavity as compared to the control group. Harris and Switzer (1972) reported a similar finding utilizing pertussis vaccine in a group of experimentally infected 4-week-old pigs.

Koshimizu\textsuperscript{2} in a trial utilizing sows immunized with a \textit{B. bronchiseptica} bacterin approximately 40 days prior to farrowing, reported that \textit{B. bronchiseptica} was established in all pigs farrowed by the control sows while in pigs farrowed from


the immunized sows infection was completely prevented even when they were challenged with *B. bronchiseptica* in a dose of $10^1$ to $10^5$ organisms per 0.2 milliliter of inoculum. Kemeny (1973) immunized gilts with a *B. bronchiseptica* bacterin with adjuvant on the 70th and 85th day of gestation and demonstrated the presence of colostral agglutinating antibodies in the newly farrowed piglets. Eighty percent of the colostrum-deprived control pigs and 40 percent of the immune colostrum-fed pigs developed turbinate atrophy subsequent to virulent *B. bronchiseptica* challenge. Harris (1970) immunized pregnant gilts with a *B. bronchiseptica* sonicate-vaccine in an attempt to passively immunize their offspring. The results indicated that colostral immunity did not induce resistance to intranasal infection or prevent the development of respiratory tract lesions. There was, however, a decrease in the number of tracheas and lungs positive for the organism in pigs from vaccinated sows (14 percent) as compared to pigs from unvaccinated sows (50 percent).

The Secretory Immune System

The newborn pig is born essentially agammaglobulinemic and receives immunoglobulins entirely from ingested colostrum (Aalund, 1972). Immune protection in the young pig is initially determined by antibody passively acquired from maternal colostrum by absorption from the small intestine (Porter, 1973). To date, 3 classes of porcine immunoglobulins have
been described and based on their physicochemical and antigenic characteristics may be considered to be analogous to the human immunoglobulins IgG, IgA and IgM (Porter and Allen, 1972; Kaltreider and Johnson, 1972). The IgG class of immunoglobulins is quantitatively the most important in the pig while IgM is quantitatively the least abundant accounting for approximately 5 percent of the total immunoglobulin in serum and colostrum (Porter and Allen, 1972). Immunoglobulin M is believed to play an important role against gram-negative bacteria and provides shortlived passive immunity in neonatal life.

Curtis and Bourne (1971) showed by immunochemical studies of post-colostral piglet serum that all 3 classes of immunoglobulin (IgG, IgA and IgM) are absorbed. In order to ensure that an entire litter of newborn pigs received colostral antibodies Aalund (1972) suggested that suckling be postponed until the entire litter was born. This would help prevent the situation where firstborn piglets deplete the amount of colostral immunoglobulins available for the pigs born toward the end of the farrowing. The immunoglobulin serum concentration of the neonatal pig reaches its maximum 24 hours after ingestion of colostrum and thereafter steadily decreases for 5 to 6 weeks (Aalund, 1972). Porter and Allen (1972) state that approximately 80 percent of the immunoglobulin content in porcine mammary secretions is IgG. They further observed that although IgA is absorbed from the colostrum by the newborn pig,
very little secretory immunoglobulin A (SIgA) antibody is absorbed and it was concluded that SIgA antibody throughout lactation had as its main function local defense of the alimentary tract.

The discovery in the early 1960's that a secretory immune system develops and functions independently of the system responsible for the formation of circulating antibody, with immunoglobulin A (IgA) as the predominant immunoglobulin of external secretions, is the basis for the concept of the secretory immunologic system (Tomasi, 1970; Waldman, 1970).

The role of the secretory immune system in the development of resistance to certain viral and bacterial infections is becoming increasingly clear and it has become apparent that in mammals the burden of defense against foreign antigens is shared by the systemic and local immune mechanisms (Plaut, 1972). Harris and Switzer (1969) postulated a role for the secretory immune system in their consideration of nasal resistance in swine against reinfection by B. bronchiseptica and Holt (1972) stated that in order to confer true immunity to whooping cough it may be necessary to use aerosol immunization in order to stimulate a secretory immune response. Extensive reviews by Tomasi and Bienenstock (1968), Heremans (1968), Tomasi (1970), Tomasi and DeCoteau (1970), Plaut (1972), Bienenstock and Perey (1972), Tomasi and Grey (1972) and Waldman (1973) cover the historical, biochemical, immunological and biological aspects of the secretory immune system.
Key observations establishing the existence of local immunity are: (1) that antiviral and antibacterial antibodies are present in the secretions of individuals recovered from active infection, (2) that the secreted antibodies are predominately IgA, (3) that protection against reinfection of the mucous membranes can best be stimulated by local application of antigen to the mucous surface, (4) that if the antigen is present in sufficient amounts a systemic immune response may also occur, (5) that local immunity may be of both antibody and cell mediated types, (6) that there is often no correlation between the levels of circulating antibody and local mucosal resistance, and (7) that the presence of secretory immunoglobulin A (SIgA) antibody correlates with protection against disease (Smith, 1969; Waldman, 1970; Bienenstock and Perey, 1972). Bienenstock and Perey (1972) further state that a local immune response can occur after administration of parenteral antigen if sufficient quantity of antigen is administered or if a live replicating antigen (viral) is used and that not all antigens given locally will produce a local IgA immune response. Heremans (1968) stated that 4 types of cells (antigen-processing cells, plasma cells, intraepithelial lymphocytes and epithelial glandular or mucosal cells) should be considered in understanding the local immune system.

In general, all secretions which are derived from mucosal surfaces can be considered part of the secretory immune system including tears, respiratory secretions, gastrointestinal
secretions, colostrum and secretions of the female and male genital tracts (Waldman, 1973). Waldman (1973) states there are several components which play a role in the protection of mucosal surfaces against infectious agents. These include secretory IgA antibody, antibodies of other immunoglobulin classes such as IgG, IgM, IgD and IgE, cellular immunity, the lysozyme-complement-immunoglobulin system and interferon.

Secretory immunoglobulin A is the predominant class of antibody in secretions bathing mucous membranes (Williams and Gibbons, 1972), but it cannot account for all aspects of local immunity (Smith, 1969). Waldman (1973) states IgG, IgM, and IgD are present in external secretions but the evidence indicates that the largest quantity of these immunoglobulins reach the mucosal surface by simple diffusion. There is reason to believe, however, that in vivo the complement fixing antibodies may play an important role in protection against bacterial infection. The fact that immunoglobulin E has a powerful biological effect in releasing vasoactive substances on mucosal surfaces and is present in every normal individual, predicts some role for it in mediating normal response to antigen exposure on mucosal surfaces (Smith, 1969). It has been shown that there are large numbers of IgE producing cells lining the respiratory and gastrointestinal tracts and IgE is present in larger amounts than would be present by simple diffusion (Waldman, 1973).

Respiratory mucous secretions contain a number of
immunologic resistance factors which include lymphocytes and macrophages as well as antibody (Bienenstock and Perey, 1972). Waldman (1973) states that macrophages and lymphocytes contribute to cell-mediated immunity and that cell-mediated immunity can be induced by the local application of antigen.

Bienenstock and Perey (1972) report that in man *Mycoplasma pneumoniae* infection, which is localized on the respiratory tract epithelium, does not apparently give rise to a predominately IgA antibody local response. It was shown that although the local antibody response was primarily IgM a major component of resistance in this infection was related to local lymphocyte-mediated immunity. It was considered by Waldman and Ganguly (1973) that cell-mediated immunity might play an important role in protection and immunity on mucosal surfaces.

Waldman (1973) reported a study of the lysozyme-complement-immunoglobulin system that may explain how IgA antibody can function in a bactericidal manner. The study indicated that IgA antibody to *Escherichia coli*, plus complement and lysozyme led to the death of *E. coli* while removal of any 1 of the 3 components did not result in the death of *E. coli*.

Interferon has been detected in external secretions following viral infection, however, its role in the protection of secretory surfaces is not clearly understood (Waldman, 1973). Vengris and Mare (1972) showed that swine can produce interferon in response to viral and synthetic inducers and stated that this mechanism probably plays a significant role in
antiviral defense in swine.

The most widely studied immunoglobulin on secretory surfaces is IgA. Waldman (1973) states that serum IgA is found predominately in a monomeric form with a molecular weight of approximately 170,000 and a sedimentation coefficient of approximately 7. The predominant form of IgA in external secretions is as a dimer with a molecular weight of approximately 380,000, a sedimentation coefficient of 11 and contains a glycoprotein called secretory component. Secretory IgA is made in two different cells. The dimerized IgA is synthesized in the subepithelial plasma cells and secretory component is apparently synthesized in the epithelial cells.

The exact biological function of secretory component is unknown but possible functions are transport and/or stabilization of the molecule (Waldman, 1973). Butler (1973) states that secretory component is attached covalently and extracellularly to an IgA dimer and that the evidence indicates that 1 function of secretory component is stabilization of the SIgA molecule resulting in resistance to enzymatic proteolysis. Secretory 11S IgA antibodies appear to have a high degree of resistance to proteolytic enzymes, whereas serum antibodies are easily destroyed and it can be concluded that only these locally produced antibodies may be expected to function consistently on mucosal surfaces (Freter, 1971). Porter (1973) considers that the secretory component also assists in the binding of the immunoglobulin to the mucus which coats the
epithelium.

Studies have demonstrated that about 80 percent of the IgA found in nasal secretions is of the 11S secretory type while 10 to 20 percent is of the 7S type and is antigenically identical to serum IgA (Tomasi and Bienenstock, 1968; Tomasi, 1972).

The mechanisms of the biological action of SIgA antibodies are being investigated at this time. Secretory IgA has been shown to have neutralizing activity against viruses on the mucosal surface and thus can prevent colonization of this site (Tomasi, 1972). It is not so clear, however, how SIgA antibodies exert a protective effect against bacterial infections. Plaut (1972) reports that immunoglobulin A has no clearly demonstrable ability to promote phagocytosis of bacteria by polymorphonuclear leukocytes (opsonization). Tomasi (1972), however, believed it likely that IgA antibodies do promote phagocytosis, especially by monocytes and that this mechanism would apply more to particles that have penetrated below the mucous membrane into the submucosal area. Neither serum IgA or SIgA fix complement, but SIgA antibody plus complement plus lysozyme was reported to kill E. coli (Waldman, 1973). Secretory IgA, therefore, is not generally considered to be bactericidal, to mediate the usual complement-dependent bacterial lysis, or to enhance phagocytosis.

Tomasi (1972) suggests that SIgA could act in concert with other nonimmunoglobulin agents produced at the mucosal surface and that there are indications of a new pathway by which
complement might be implicated in IgA-mediated antibacterial reactions.

It has been shown that SIgA binds to and agglutinates bacteria to which it is specifically directed and may interfere with the attachment of the microorganism to the mucosa, thereby impairing its ability to penetrate the mucosa and rendering it more susceptible to mechanical clearing by ciliary action (Plaut, 1972; Gibbons, 1973). Williams and Gibbons (1972) suggest that by merely binding to and agglutinating bacteria, SIgA may exert an immune protective function as this could influence bacterial adherence to and subsequent colonization of the mucosal surface. They demonstrated that human parotid SIgA did interfere with bacterial adherence to epithelial surfaces in vitro and thought that this function in itself would limit the colonization of microorganisms on mucosal surfaces and could be the major mechanism of immunity mediated by secretory immunoglobulins.

Fubara and Preter (1973) reported that purified antibody preparations, obtained from the lumen of the intestine of orally vaccinated germfree mice, contained very small amounts of SIgA but that no other serologically active immunoglobulins were found that could protect mice against experimental cholera infection. This is the first time that protection against a bacterial infection has been demonstrated with purified preparations of SIgA antibodies.

Reynolds and Thompson (1973a and b) report that in rabbits
immunized with *Pseudomonas aeruginosa*, the immunoglobulin class stimulated that had antibody activity in bronchial secretions was a function of the immunizing route. Parenteral injections resulted in only IgG antibody in the bronchial secretions, probably as a result of simple diffusion. Intranasal immunization, in contrast, produced agglutinins in the mucosal secretions of both IgA and IgG class. Immune IgA was shown to inhibit the growth of pseudomonas serotypes, but this effect was not mediated by complement and was thought to be related to the agglutinative effect of IgA.

Gibbons (1973 and 1974) observed that bacterial adherence to a mucosal surface was a parameter which should relate to the virulence of pathogenic organisms. Several correlations between the ability of pathogenic bacteria to adhere to mucosal surfaces and their virulence are reviewed by Gibbons (1973 and 1974). These are as follows: (1) pathogenic strains of *Mycoplasma* species have been found to adhere to tracheal epithelial cells, while nonpathogenic strains generally do not, (2) virulent strains of *Streptococcus pyogenes* possessing M protein firmly adhere to human oral and pharyngeal epithelial cells, whereas an avirulent strain lacking M protein adhered poorly, and (3) pathogenic strains of *Escherichia coli* possessing K88 antigen have been observed to adhere better to segments of small intestine than avirulent strains lacking this antigen. Ellen and Gibbons (1972) presented data indicating that the surface "fuzz" of *S. pyogenes* which is known to contain M
protein, functions in the attachment of the organism to epithelial surfaces.

Gibbons (1973) observed that when colonization by a pathogen progresses slowly and elicits a secretory antibody response comparable to that imposed against indigenous bacteria, the pathogen could exist in a transient balanced or "carrier state" in which its adherence and colonization would be similar to that of indigenous bacteria. Artenstein (1969) stated that while there is a lack of information on antibacterial activity in nasal secretions that for several species of bacteria there is well documented evidence of local immunity.

Live agents, either in the form of natural pathogens or live attenuated vaccines, elicit better immunity than do killed organisms (Waldman and Ganguly, 1973; Bellanti, 1969). Bellanti (1969) reported that both nasal secretion and serum antibody response followed induced experimental F. tularensis infection or parenteral immunization with a live, attenuated tularemia vaccine containing approximately $10^8$ viable cells. Waldman and Ganguly (1973) state however, the same results could theoretically be obtained by administering the inactivated organisms repeatedly and in larger doses. It is known that secretory antibody response can be elicited by parenterally administered vaccines probably by transportation of the antigen to the secretory lymphoid tissue, but such levels of local antibody are usually low and result in only partial protection (Waldman and Ganguly, 1973). They further noted that
optimal mucosal antibody response against microorganisms may be induced by either natural infection or by direct application of killed or live attenuated organisms to the mucosal surface.

Ogra and Karzon (1969) presented evidence to indicate that the output of SIgA antibody from mucosal surfaces is anatomically restricted to the site where antigen stimulation has occurred. Live, attenuated poliovirus was used to immunize children with double-barreled colostomies. Secretory IgA responses were confined to the part of the colon vaccinated and no other local response occurred, although there was a marked serological response. When killed poliovirus vaccine was administered the response was confined to the site vaccinated and there was no serological response.

Waldman et al. (1970) observed influenza antibody response following aerosol administration of inactivated virus. They noted that large particle aerosols (40 to 100 u) or nose drops were the most effective in the stimulation of nasal antibody while the highest serum antibody responses resulted from aerosolization of small particle (1.5 u) size. In several field trials with influenza vaccine, the aerosol route of immunization gave protection rates of 70 to 80 percent.

Tomasi and DeCoteau (1970) noted that F. tularensis administered intranasally immunized subjects so that they were resistant to subsequent aerosol challenge with live organisms, but that this resistance could be overcome even in the presence of significant titers of nasal antibody if higher doses of the
organism were administered. Waldman and Ganguly (1973) stated that both local and systemic immune mechanisms are related to protection against disease, but that mucosal colonization is more effectively inhibited by local immunity.

In humans, small-particle aerosol immunization utilizing inactivated influenza virus vaccine appears to be the most effective method of immunoprophylaxis (Waldman et al., 1973). Aerosol administration of parainfluenza type 2 vaccine to humans was shown to be a more effective stimulus of antibody in respiratory secretions than subcutaneous administration (Wigley et al., 1970). Live attenuated rubella virus (Ganguly et al., 1973) was shown to stimulate respiratory secretion antibody when given by aerosol. Protection after nasal challenge appeared to be best in those groups which had the highest nasal secretion antibody response after immunization. Wigley et al. (1969) demonstrated that soluble tetanus toxoid given as an aerosol led to an increase in serum antibody titers comparable to those resulting from subcutaneous injection and that sputum antibody titers significantly increased. The sputum antibody titers were due to the production of secretory antibody. Buscho et al. (1972) reported that intranasal vaccination of human volunteers with an inactivated rhinovirus type 13 (RV13) vaccine induced resistance to challenge that lasted 6 months. The local neutralizing antibody which developed was specific for RV13 and this antibody persisted for at least 330 days post vaccination. The suggestion was made that the local
respiratory tract immune response possesses some degree of immunologic memory. It was concluded that intranasal immunization (nosedrops) with an inactivated RV13 vaccine was more effective in inducing nasal immunity than parenteral administration of this vaccine. It has been shown in man that respiratory tract resistance to infection by rhinovirus, parainfluenza viruses, and influenza virus is more closely correlated with antibodies present in nasal secretions than with circulating antibodies and that these nasal secretion antibodies are more consistently produced following intranasal administration than by parenteral injection (Todd, 1973).

Barclay et al. (1973) reported that Bacillus Calmette-Guerin bacterin administered by aerosol to rhesus monkeys induced a much greater degree of protection against aerosol challenge with virulent *Mycobacterium tuberculosis* than the same bacterin administered by intracutaneous vaccination. Nash and Holle (1973) working with guinea pigs immunized with DNP-HGG administered either by direct injection into the lower respiratory tract or intravenously, made the following observations: that systemic immunity could be as effective as local immunity in terms of the appearance of sensitized cell populations in the respiratory tract; that circulating, sensitized lymphocytes could be induced to enter the respiratory tract following local administration of a nonrelated substance; and that under specified conditions, systemic immunity (cell mediated immunity) combined with local antigen administration, may
be the most efficient procedure for obtaining an effective local immunity.

Rouse (1971) states that in the horse as in man, nasal antibody seems to be intimately associated with protection against influenza. High levels of nasal antibody were produced by the use of parenteral injections containing sufficient antigen and Freund's adjuvant. It was presumed that if the antigenic stimulation is sufficient, then either the antigen circulates and stimulates the antibody producing cells in the respiratory tract, or the respiratory tract is colonized by antibody producing cells migrating from the vaccination site.

Collins and Carter (1972) reported that mice receiving living, attenuated *Salmonella enteritidis* intragastrically developed an effective antisolmonella immunity against subsequent reinfection.

Duncan and Thomson (1970a) hypothesized that under normal circumstances a host can maintain an attenuated infection of the upper respiratory tract, but that with a decrease or alteration of the specific immunity the organism can proliferate and lead to the development of the disease state. They demonstrated that *Pasteurella hemolytica* was capable of colonizing the nasal passages of cattle in the presence of specific nasal and serum indirect hemagglutinating antibody. Bull and McKee (1928) reported that in the rabbit specific upper respiratory tract antibody to pneumococcus was responsible for the nasal rejection of the same strain, but specific antibody to *Bacillus*
_bronchiseptica_ did not influence its nasal residence. Duncan and Thomson (1970b) reported that aerosol vaccination of calves with live _Pasteurella hemolytica_ resulted in a significant nasal antibody response while parenterally vaccinated calves with equivalent serum titers had no significant nasal antibody response. Acquired immunity was said to be more effective in controlling the manifestation of disease than in preventing the establishment of infection.

Todd _et al._ (1971) showed that an avirulent strain of infectious bovine rhinotracheitis (IBR) virus used as an intranasal vaccine was capable of eliciting a higher humoral antibody response than presently available intramuscular vaccines and that such an intranasal vaccine also stimulated the production of secretory antibodies. Gutekunst _et al._ (1969) reported that calves intranasally vaccinated with parainfluenza-3 (PI-3) virus vaccine were better protected against infection than were intramuscularly vaccinated animals. Preliminary studies indicated that an animal could be immunized against PI-3 infection by intranasal inoculation of vaccine in the presence of significant levels of maternal serum-neutralizing antibodies, since there is no significant spill-over of circulating antibodies into the respiratory system. Todd (1973) states that subcutaneous administration of formalin-inactivated PI-3 virus failed to elicit nasal secretory antibodies after a single injection, but did evoke secretory antibodies following a booster dose 2 to 3 weeks later. Nasal secretion
antibody was said to persist for a relatively short time (6 to 8 weeks) following the initial response, but can be expected to persist at higher levels for prolonged periods following restimulation.

Porter (1973) states immunohistological studies in the pig have defined a local secretory immune system mediated by an immunoglobulin analogous to human IgA. This immunoglobulin is synthesized in plasma cells situated in the tissues close to the epithelium of those organs which are in intimate contact with the external environment and thus is the predominant immunoglobulin in the secretions of the gastro-intestinal, respiratory, and genito-urinary tracts and of the mammary, lacrimal and salivary glands.

Porcine IgA has been isolated and characterized from serum and mammary secretions (Bourne, 1969a and b; Vaerman and Heremans, 1970) and IgA concentrations in porcine sera and mammary secretions have also been determined (Curtis and Bourne, 1971). Vaerman and Heremans (1970) reported that pig serum and milk IgA closely resembled its human counterpart, however, the major part of pig serum IgA seemed to consist of 10S dimer molecules as compared to the predominant human 7S monomer. Curtis and Bourne (1971) found IgG to be the main immunoglobulin of sow serum and colostrum while IgA was the major immunoglobulin of milk. The role of IgA in milk was thought to be two-fold: to protect the mammary gland and to provide adequate levels of antibody to the intestinal mucosa.
of young pigs until the intestinal secretory immunoglobulin system is fully developed.

Bourne and Curtis (1973) reported that milk IgA is almost entirely (90 percent) locally produced, but that considerable amounts of IgG and IgM (40 percent) are also formed in the mammary tissue. All colostral IgG and a high proportion of IgM was reported to be of serum derivation as was 40 percent of the IgA. It was suggested that colostrum was not a true secretion since 90 percent of its immunoglobulin content is of serum origin. Svendsen and Brown (1973) measured IgA levels in the sera of pigs from birth to maturity and in the colostrum of their dams. Immunoglobulin A synthesis in young colostrum-fed pigs reached a significant level by 6 weeks of age and adult levels were reached by 18-21 weeks of age. Significant differences in IgA concentrations in secretions from individual glands of the same sow and from different sows were demonstrated and it was postulated these differences may be part of the explanation for some of the variations between pigs in their susceptibility to enteric infections.

Studies of the ontogenesis of the intestinal secretory immune response have not yet been reported in the literature (Allen and Porter, 1973b), however, they observed that the local immune response may commence predominately with IgM synthesis. In both the human infant and the pig IgM appears in the serum during the first week of life while IgA is not detected until several days later. Allen and Porter...
(1973 a and b) demonstrated that the onset of active secretion of intestinal IgA did not occur before the second week of life. They stated that the major antigenic stimulus for the development of IgA synthesizing cells in the lamina propria appeared to be the enteric flora.

Saif et al. (1972) reported that in the milk and colostrum from sows naturally or experimentally infected with live transmissible gastroenteritis (TGE) virus, IgA is the predominant immunoglobulin class of TGE antibodies. In the serum of such animals TGE antibody appeared to be associated with both IgA and IgG classes, but with more of the antibody activity in the IgG fraction. In contrast, intramuscular vaccination of sows with live attenuated TGE virus produced predominately if not entirely IgG antibodies in the serum, colostrum and milk. The mechanism accounting for the predominance of IgA TGE antibodies in the milk and colostrum of naturally and experimentally infected swine is not known, however, it was suggested that plasma cells either of local origin or relocated from the intestinal tract may be the source of mammary gland IgA TGE antibodies. Bohl et al. (1972) stated the limited effectiveness of live, attenuated TGE vaccines, especially when administered by the intramuscular route is probably due to the production of IgG rather than IgA antibodies in mammary secretions. Immunoglobulin A TGE antibodies were shown to provide more adequate passive immunity than IgG TGE antibodies.

Harris (1970) demonstrated antibodies against
B. bronchiseptica in concentrated nasal washings collected from pigs cleared of B. bronchiseptica nasal infections and from pigs receiving a sonicated B. bronchiseptica vaccine both before and after nasal challenge with a virulent B. bronchiseptica strain.
MATERIALS AND METHODS

Source of *Bordetella bronchiseptica* Isolates

Three isolates of *B. bronchiseptica* were used: (1) Virulent strain B was isolated\(^1\) from a pig with turbinate atrophy and had been previously used to produce turbinate atrophy in experimental swine (Ross *et al.*, 1963b; Harris and Switzer, 1968). (2) Strain 55 was isolated\(^1\) in 1955 from the pneumonic lungs and normal nasal cavity of an experimental pig inoculated intranasally with crude pneumonic swine lung suspension. The initial inoculum was secured from a pig in a herd experiencing clinical atrophic rhinitis and pneumonia. This organism was seeded into rubber stoppered 100 milliliter vials of tryptose phosphate broth (TPB) in 1955 and incubated at 37°C. These vials were then committed to a duration of survival trial. The cultures were sampled several times during the ensuing years and found to be viable. In January of 1972, after 17 years without serial passage, contents of a vial were cultured on 5 percent horse blood agar. Two extremely rough bacterial colonies developed and 1 colony was picked into TPB. This organism was subsequently identified as a highly modified *B. bronchiseptica* and designated as strain 55. (3) An isolate of *B. bronchiseptica* designated low-virulence D-1 strain was

\(^1\)Dr. W. P. Switzer, Veterinary Medical Research Institute, Iowa State University, recovered this isolate.
isolated\textsuperscript{1} from a puppy infected with canine distemper. Strain D-1 had been shown in a previous study to be incapable of causing turbinate atrophy in swine (Ross \textit{et al.}, 1967).

**Culture Media**

\textit{Bordetella bronchiseptica} was isolated from swab specimens by inoculation onto MacConkey's agar with crystal violet\textsuperscript{2} modified by the addition of 1 percent dextrose (Ross, 1963). The MacConkey's agar was further modified to retard overgrowth of the cultures by bacterial and fungal contamination. The modifications were as follows: (1) Furaltadone\textsuperscript{3} (NF-260) was added to the medium at a concentration of 0.02 mg./ml. The Furaltadone (0.01 gram) was autoclaved separately in 250 ml. of distilled water and added to 250 ml. of double strength MacConkey's agar with crystal violet and 2 percent dextrose. \textit{Bordetella bronchiseptica} isolates were found to be resistant to Furaltadone at the 0.02 mg./ml. level while many common contaminating organisms contributing to overgrown plates were significantly inhibited. (2) After autoclave sterilization and cooling to 45°C, 50 units of Mycostatin\textsuperscript{4} was added to each milliliter of the medium. Fungal contamination of nasal

\textsuperscript{1}Dr. R. F. Ross, Veterinary Medical Research Institute, Iowa State University, recovered this isolate.

\textsuperscript{2}Difco Laboratories, Incorporated, Detroit, Michigan.

\textsuperscript{3}Norwich Pharmacal Company, Norwich, New York.

\textsuperscript{4}E. R. Squibb \& Sons, Incorporated, New York, New York.
swab cultures was completely prevented by this procedure without interfering with the isolation of *B. bronchiseptica*.

Horse blood agar composed of 5 percent defibrinated horse blood in tryptose blood agar base medium\(^1\) was used to determine bacterial titers expressed as colony forming units, purity checks at all stages of vaccine production and serial passage through embryonating 6 to 8 day old hen eggs and for hemagglutination (HA) studies.

Mueller-Hinton agar medium\(^1\) was used for sulfamethazine sensitivity testing and tryptose blood agar base in Roux flasks was used for vaccine production. Fluid cultures of *Bordetella bronchiseptica* were propagated in tryptose phosphate broth\(^1\) (TPB). Media for the biochemical identification of *B. bronchiseptica* were obtained from a commercial source.\(^1\)

**Identification of *Bordetella bronchiseptica***

Forty-eight hour cultures of *B. bronchiseptica* on modified MacConkey's agar incubated at 37°C appeared as grayish-tan colonies with a decolorized alkaline zone surrounding the colony. Further identification was based on an alkaline reaction in dextrose broth and lactose broth, hydrolysis of urea within 4 hours, utilization of citrate, alkalinization of litmus milk in 48 to 72 hours and a positive hemagglutination reaction.

\(^1\)Difco Laboratories, Incorporated, Detroit, Michigan.
Preparation of Buffered Solutions

**Phosphate buffered saline (PBS):**

The PBS was composed of the following:

- \( \text{Na}_2\text{HPO}_4 \) (0.1 M) \( 85.7 \) ml.
- \( \text{KH}_2\text{PO}_4 \) (0.1 M) \( 14.3 \) ml.
- Saline (0.85 percent NaCl) \( 900.0 \) ml.

The above solution was pH 7.4.

**Veronal buffered diluent (VBD):**

The VBD was composed of the following:

- Stock solution 100.00 ml.
- Distilled H\(_2\)O 400.00 ml.
- Gelatin (0.25 percent) 16.00 ml.

The VBD was pH 7.4 and was stored at 4-8°C. It was used within 24 hours of preparation.

**Bordetella bronchiseptica Inocula**

Cultures of *B. bronchiseptica* used in transmission experiments were stored lyophilized in low passage. Strain B inocula for intranasal challenge was prepared by injecting 0.1 ml. of
24 hour TPB cultures into the yolk sac of 6 to 8 day old embryonating hens eggs. The embryos were incubated at 37°C and candled daily. Embryos dying after 24 hours incubation were incubated an additional 24 hours. Yolk sac fluids were harvested aseptically, pooled, checked for purity on 5 percent horse blood agar and stored frozen at -20°C. The pooled egg yolk fluid inocula containing B strain B. bronchiseptica had approximately 9 x 10^6 colony forming units per milliliter.

Strain 55 B. bronchiseptica was checked for ability to regain swine virulence by serial passage in embryonating hens eggs. One-tenth ml. of fourth passage 24 hour TPB culture was injected into the yolk sac of 6 to 8 day old embryonating hens eggs. The embryos were incubated at 37°C and candled daily. The yolk sac fluids were aseptically harvested from the dead embryos, pooled, purity checked on 5 percent horse blood agar and used to inoculate the next group of 6 to 8 day old embryonating hens eggs for 11 serial passages. The eleventh passage was stored at -20°C until used. The pooled egg yolk fluid had approximately 1 x 10^7 colony forming units per milliliter.

Intranasal Inoculation of Pigs

Pigs were inoculated intranasally by inserting the tip of a syringe into the external naris and discharging 0.5 ml. of the challenge inoculum into each nostril. The injection of the egg yolk inoculum into the nasal cavity was timed with the
Preparation of Vaccines

Strain D-1 sonicate, whole-cell, formalinized bacterin

Two to 5 milliliters of a 24 hour tryptose phosphate broth (TPB) culture of seventh, eighth or ninth passage D-1 strain B. bronchiseptica was inoculated onto tryptose blood agar base (without blood) in Roux flasks and incubated at 37°C for 24 hours without inversion of the flasks. The growth was harvested at 24 hours by washing with 20 milliliters of physiological saline solution (PSS) per Roux flask. Glass beads were used to loosen the growth from the agar. The harvested growth was sonicated in 50 to 100 milliliter volumes in a Raytheon model DF101 sonic oscillator\(^1\) for 2.5 minutes. The titer of the bacterial suspension was determined by culturing 10-fold dilutions onto 5 percent horse blood agar both prior and subsequent to the sonication procedure. A 1 log drop in the bacterial titer usually occurred post sonication. The sonicated culture was inactivated with a final concentration of 1:1000 formalin at 4°C for 48 hours. The 4°C temperature was selected because the hemagglutination (HA) titers of the bacterins were maximized by inactivation at refrigerator temperatures. Strain D-1 sonicate bacterin was generally prepared prior to each experiment and the approximate number of organisms per

\(^1\)Raytheon Company, Norwalk, Connecticut.
milliliter of bacterin after sonication varied from $8 \times 10^8$ to $2 \times 10^9$.

Adjuvanted parenteral bacterins were prepared as follows:
The parenteral bacterins were emulsified with equal parts of adjuvant by repeated passage through an 18-gauge needle attached to a 20 milliliter syringe.

**Strain D-1, whole-cell, formalinized bacterin**

Two milliliters of a 24 hour TPB culture of seventh passage D-1 strain *B. bronchiseptica* was inoculated into Roux flasks containing tryptose blood agar base (without blood) and incubated for 48 hours at 37°C without inversion of the flask. The growth was harvested at 48 hours with 20 ml. of PSS with the aid of glass beads. The titer expressed as colony forming units was determined on 5 percent horse blood agar. The culture was formalinized with a final concentration of 1:1000 formalin, inactivated for 24 hours at room temperature and then refrigerated at 4°C. The bacterial concentration was approximately $1 \times 10^{10}$ organisms per milliliter.

**Strain 55, whole-cell, formalinized bacterin**

A whole-cell bacterin using fourth passage 55 strain *B. bronchiseptica* was prepared utilizing the same procedure as used for the D-1 whole-cell bacterin. The bacterial concentration was approximately $2 \times 10^{10}$ organisms per milliliter.
Partially purified Bordetella bronchiseptica hemagglutinin extract

Bordetella bronchiseptica hemagglutinin was extracted by a modification of the technique employed by Masry (1952). The extraction procedure was as follows: (1) Five milliliters of seventh passage D-1 strain B. bronchiseptica 24 hour TPB culture containing approximately $2 \times 10^7$ organisms/ml. was inoculated onto tryptose blood agar base (without blood) in 6 Roux flasks and incubated at $37^\circ C$ for 24 hours with the agar down. (2) The growth was harvested after 24 hours incubation with 15 ml. of 1 M sodium acetate per flask. Glass beads were used to loosen the growth from the agar. The harvest was pooled and incubated at $37^\circ C$ for 48 hours. (3) The sodium acetate-bacterial suspension was centrifuged at $4^\circ C$ for 1 hour at 10,000 Xg. The supernatant was retained. The pH was adjusted to 6.7 to 6.9 with 1 N sodium hydroxide, and cooled to $-3^\circ C$. (4) Methanol cooled to $-20^\circ C$ was used in the extraction process. The supernatant was placed in a 250 ml. Erlenmeyer flask in a salt water ice bath kept at approximately $-5^\circ C$. Methanol at $-20^\circ C$ was added to the supernatant with constant stirring at a rate of 2 ml./minute until methanol was 40 percent of the total volume. (5) The resultant solution was kept at $-20^\circ C$ for 24 hours and then centrifuged at 10,000 Xg for 1 hour at $-10^\circ C$ in a Beckman L2-65B ultracentrifuge. The centrifugate was

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1Beckman Instruments, Inc., Spinco Division, Palo Alto, California.
harvested with 5 milliliters of PBS, checked for hemagglutinin content, and stored at -20°C.

**Live, low-virulence 55 strain Bordetella bronchiseptica intranasal vaccine**

Three milliliters of sixth passage 55 strain *B. bronchiseptica* 24 hour TPB culture was inoculated into cotton-stoppered flasks containing 50 ml. of TPB and incubated at 37°C for 24 or 48 hours. The live seventh passage TPB cultures of 55 strain were used for intranasal vaccination. Twenty-four hour cultures were administered for the first dose and 48 hour cultures were used for the second and subsequent doses. The 24 hour 55 strain seventh passage TPB culture contained approximately $1 \times 10^6$ organisms/ml, and the 48 hour TPB culture approximately $1 \times 10^7$ organisms/ml. The vaccines were administered on the day of their preparation.

A dosage of 0.5 ml. per nostril of the 24 and 48 hour TPB cultures in the form of nosedrops was administered to each intranasally immunized pig. The tip of a 5 milliliter syringe was inserted into each nostril and used to instill the TPB culture into the nasal cavity. Administration of the culture was timed with the inspiration of the pig.

**Strain 55 Bordetella bronchiseptica TPB culture sterile filtrate intranasal vaccine**

Three milliliters of sixth passage 55 strain *B. bronchiseptica* 24 hour TPB culture was inoculated into 50 ml. of TPB and incubated at 37°C for 24 hours. The 55 strain TPB culture
was passed through a 0.45 micrometer Millipore filter. The filtrate was sterility checked on 5 percent horse blood agar and stored at 4°C. The TPB culture contained approximately 1 x 10^6 organisms/ml. prior to filtration. The 55 strain sterile filtrate was administered in the same manner as the live 55 strain intranasal vaccine.

Source and Care of Experimental Swine

Experimental swine were obtained from 4 sources: (1) From a *Bordetella bronchiseptica* rhinitis infected Iowa State University production herd, Ames, Iowa, (2) from a *Bordetella bronchiseptica* rhinitis free commercial herd, Ames, Iowa, (3) from a *Bordetella bronchiseptica* rhinitis free purebred SPF herd, Clear Lake, Iowa, and (4) from the respiratory-disease-free herd maintained by the Veterinary Medical Research Institute, Ames, Iowa.

The Veterinary Medical Research Institute respiratory-disease-free herd was originated in 1951 from surgically derived stock and maintained as an isolated herd since that time. The ration fed to the herd and all experimental swine did not contain antibiotics, sulfonamides or arsenicals. The feed contained a calcium:phosphorus ration of 1.9:1 based on calculation of phytophosphorus as 30% available. Newborn pigs were injected intramuscularly with 2 milliliters of

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1 Millipore Filter Corporation, Bedford, Massachusetts.
Dexiron 100\(^1\) at 7 days of age. The pigs had access to a preweaning meal starter ration (19 percent protein) beginning at 1 week of age and were weaned at approximately 5 weeks of age. A grower ration (17 percent protein) was fed in the herd from the fifth week of age. Experimental pigs weaned from the supply herd were fed the grower ration while on experiment.

Pregnant dams brought to the Veterinary Medical Research Institute for experimental purposes were housed in individual farrowing units and fed a gestation-lactation ration (12 percent protein). Newborn pigs were injected intramuscularly with 2 milliliters of Dexiron 100 at 3 to 5 days of age. The pigs were fed the preweaning meal starter ration until weaning age (5 weeks) and then were placed on grower ration for the duration of the experiments.

Animal Experiments

Experimental pigs were housed in individual isolation units or at the Veterinary Medical Research Institute hilltop swine rearing unit.

Collection of Specimens

Nasal secretions were collected in vivo on sterile cotton-tipped plastic stemmed applicator sticks\(^2\) by inserting a separate sterile swab into each nostril and gently twisting. The

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\(^1\)Norden Laboratories, Lincoln, Nebraska.

\(^2\)Tomac, American Hospital Supply, Evanston, Illinois.
external nares were cleaned prior to nasal sampling with a cot-
ton pledget saturated with 70 percent ethyl alcohol and then
squeezed dry. The swabs were inserted into the nares at a slight
angle with the cotton tip directed toward the nasal septum and
twisted with gentle pressure until the area of the second cheek
tooth was reached. In very young pigs the cotton tip of the
nasal swab was all that was inserted into the nasal cavity.
Samples of nasal secretions from the ethmoid turbinates for
bacterial culture were collected at necropsy by sectioning the
head longitudinally and carefully removing the medial septum.
A sterile swab was then used to obtain secretions from the pos-
terior portion of the nasal cavity. The secretions gathered on
the paired nasal swabs were streaked as soon as possible on the
selective modified MacConkey's medium. The nasal swabs were
thoroughly rubbed over the surface of the culture medium with a
twisting motion in 3 different directions.

Necropsy Procedures

Experiments were terminated by either animals being killed
by electrocution and exsanguination at the Veterinary Medical
Research Institute with immediate necropsy or by the animals
being sent to commercial swine slaughter plants where gross
examinations were made after processing.

The procedure for the determination of gross turbinate
atrophy was as follows: A cross-section of the nasal cavity
was made at the level of the first premolar tooth. Gross
distortion or atrophy of the nasal turbinates and nasal septal defects were characterized depending on severity as mild, moderate or severe.

Determination of Resistance to Sulfamethazine in *Bordetella bronchiseptica*

Sulfamethazine sensitivity of *B. bronchiseptica* isolates was determined by utilizing Mueller-Hinton agar medium and sulfamethazine sensitivity discs containing approximately 0.15, 0.30, 0.60 or 0.90 milligrams of sulfamethazine\(^1\) per 1/4 inch disc. The sulfamethazine discs were prepared by saturating blank discs\(^2\) in solutions containing 5, 10, 20 and 30 milligrams of sulfamethazine per milliliter. Each disc was expected to retain about 0.03 ml. of the solution prior to drying. The sulfamethazine saturated discs were dried overnight in a 37°C incubator. A culture was considered sensitive if *B. bronchiseptica* was inhibited by the disc saturated in the 5 or 10 mg./ml. solution. Sensitivity to the discs saturated in the 20 and 30 mg./ml. solutions was recorded as S20 or S30. Isolates not inhibited by the discs saturated in the 30 mg./ml. solution were considered resistant.

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\(^1\)Sulfamethazine, Vet Products Corporation, Kansas City, Missouri.

\(^2\)Schleicher & Schuell, Inc., Keene, New Hampshire.
Preparation of *B. bronchiseptica* for Electron Microscopy

*Bordetella bronchiseptica* 55 and B strain thin sections were prepared in the following manner: Forty-eight hour TPB cultures were pelleted by centrifugation and fixed for 45 minutes in 1.5 percent cold glutaraldehyde in Milloning's phosphate buffer at pH 7.2. They were post-fixed in buffered 1 percent osmium tetroxide for 1 hour. The pellets were embedded in Epon 812 epoxy resin\(^1\) and the capsules were incubated according to the following schedule: overnight at 35°C, 24 hours at 45°C and 24 hours at 60°C. After polymerization, sections were cut and stained in uranyl acetate and lead citrate. The grids were examined\(^2\) with a Hitachi HS-8 electron microscope.

For negative contrast staining, the following procedure was utilized:\(^3\) Strain 55 and B strain *B. bronchiseptica* was grown for 24 and 48 hours on 5 percent horse blood agar or in TPB. The preparations were negatively stained with neutralized phosphotungstic acid. A drop of lightly centrifuged TPB culture centrifugate or a cotton-tipped swab with organisms from the agar cultures was added to a spot plate well containing 3 to 4 drops of 4 percent phosphotungstic acid, 15 to 20

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\(^1\)Polysciences Inc., Rydal, Pennsylvania.

\(^2\)C. L. Annis, Veterinary Medical Research Institute, Ames, Iowa.

\(^3\)A. E. Ritchie, National Animal Disease Laboratory, Ames, Iowa.
drops of water and 1 to 2 drops of a freshly prepared approximately 0.1 percent bovine serum albumin solution. After mixing, the preparations were sprayed on carbon-coated colloidion-filmed grids with a Vaponefrin all-glass nebulizer. The grids were immediately examined in a Phillips EM-200 electron microscope.

Particulate Antigen Settling Test

The particulate antigen settling test (PAST) was utilized as described by Harris (1970) and Harris and Switzer (1972).

Preparation of PAST antigen

Strain D-1 E. bronchiseptica was grown in TPB for 16 hours at 37°C. A 1 milliliter aliquot was then inoculated into 550 ml. of TPB in a 1000 ml. Erlenmeyer flask and incubated for 20 to 24 hours at 37°C. Formalin was added to the flask at a 1:1000 concentration and incubated at 37°C for another 20 to 24 hours. Blood agar cultures were made to verify that the organisms were inactivated by this treatment. The cells were harvested by centrifugation at 4-8°C at 15,000 Xg for 1 hour, resuspended in 0.85 percent NaCl solution containing 1:1000 formalin and stored at 4°C.

The test was conducted in plastic microtitration trays

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1 Vaponefrin Company, New York, New York.
2 A. E. Ritchie, National Animal Disease Laboratory, Ames, Iowa.
3 Linbro Chemical Company, New Haven, Connecticut.
with U-shaped wells. Diluent was 5 percent NaCl solution. Diluent was added to all wells in 0.05 ml, amounts with a dropper and 0.05 ml of serum was added to the first well. Serial two-fold dilutions were made with a diluter which carried 0.05 ml of fluid from 1 well to the next.

The appropriate antigen dilution (0.05 ml,) was added to each well. The plate was covered with a plastic sealer, shaken for 2 minutes on a mechanical shaker and incubated at 37°C for 2 hours. The trays were kept at 4°C for 48 to 72 hours. After incubation the results were read with the aid of a mirror.

The *B. bronchiseptica* antigen suspension was diluted in 0.85 percent NaCl solution containing 1:1000 formalin to a concentration that registered 120 to 130 nephlos units as determined by a Coleman Nepho-colorimeter set to a standard of 79. This was determined to be the optimum working antigen concentration.

**Bordetella bronchiseptica** Hemagglutination

A characteristic of *B. bronchiseptica* is its ability to agglutinate red blood cells. This characteristic is not known to be shared with other closely related organisms except

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1 Linbro Chemical Company, New Haven, Connecticut.
4 Coleman Instruments Corporation, Maywood, Illinois.
members of the genus *Bordetella* and can be used as an additional diagnostic tool in the identification of *B. bronchiseptica*.

A *B. bronchiseptica* colony was suspended in a drop of PSS on a glass slide or a brucellosis test box. The colonies were picked from 24 hour cultures as aged cultures tend to lose their ability to agglutinate red blood cells. A drop of approximately 3 percent washed sheep red blood cells (SRBC) was added to the bacterial suspension and mixed. A drop of SRBC was added to a drop of PSS as a control. The slide was rotated to insure adequate mixing. Hemagglutination occurred within 1 to 8 minutes.

**Determination of B. bronchiseptica hemagglutination (HA) titers**

Three milliliters of sixth passage 55 strain, ninth passage D-1 strain, and eighth passage B strain *B. bronchiseptica* 24 hour TPB cultures were inoculated onto 5 percent horse blood agar and tryptose blood agar base in Roux flasks and into 25 milliliters of TPB. Blood agar cultures were incubated 24, 48 and 72 hours, tryptose blood agar base cultures were incubated 24 and 48 hours and TPB cultures were incubated 24 hours at 37°C. The 24, 48 and 72 hour blood agar cultures and 24 and 48 hour tryptose blood agar base cultures were harvested with 20 milliliters of PSS. The bacterial suspensions in PSS and the TPB cultures were pelleted by centrifugation. The supernatant was discarded and the bacteria were resuspended to a 2 percent concentration in PSS.
The hemagglutination test was done in plastic trays with V-shaped wells. Diluent for the 2 percent bacterial suspensions was Veronal buffered diluent (VBD) with gelatin. Diluent was added to all wells in 0.05 ml. amounts with a dropper and 0.05 ml. of the 2 percent bacterial suspensions was added to the first well. Dilutions were made with a diluter which carried 0.05 ml. of fluid from 1 well to the next to obtain two-fold dilutions. Washed sheep red blood cells at a concentration of 0.7 percent in VBD were added to all wells in 0.025 ml. amounts with a dropper. The trays were covered with a plastic sealer, shaken gently on a mechanical shaker for 2 minutes and incubated overnight at 4°C. The HA tests were set up immediately after the cultures were harvested and the 2 percent bacterial suspensions produced. The vaccine HA titers were determined utilizing the same procedure.
RESULTS
Swine Experiments

Bordetella rhinitis control by detection and elimination of infected swine.

Experiment I Nasal secretions were collected on sterile swabs and cultured on a selective modified MacConkey's agar medium for the isolation and identification of Bordetella bronchiseptica. Culture-positive breeding animals were culled. Switzer and Spear (1973) have demonstrated that pregnant sows negative for B. bronchiseptica nasal infection will usually wean litters negative for this organism. A pilot field control attempt was initiated in October, 1970 (Switzer, 1970a). The details of the detection and control method that evolved were as follows: (1) Nasal secretions were collected by means of nasal swabs from 4-to-10-week-old pigs in a herd experiencing atrophic rhinitis and cultured to determine whether B. bronchiseptica was present in the herd. A random sample of 25 pigs was considered adequate to determine the status of the herd. (2) If B. bronchiseptica was isolated from the pigs the following criteria were evaluated before a control attempt was initiated: (a) the herd must be of sufficient genetic merit to justify the expense and effort of the control procedure; (b) the herd should have adequate facilities to enable the necessary isolation, cleanup, disinfection and fumigation; and (c) the producer should have a high degree of disease control awareness. (3) The entire breeding herd was subjected to nasal
swabbing. (4) All culture-positive animals were eliminated from the breeding herd as soon as they were detected. (5) Three negative nasal swab cultures were required for each breeding animal retained in the herd as a single nasal swab culture is estimated to be 75 to 90 percent accurate (Switzer and Farrington, 1972). (6) Animals that yield 3 or more cultures overgrown with contaminating organisms were eliminated providing the nasal swab sampling technique was satisfactory. (7) All bred sows and gilts were sampled an additional time within 2 weeks prior to farrowing and only culture-negative animals entered the farrowing house. (8) The farrowing house(s), nursery(s), and growing-finishing buildings were emptied, cleaned, disinfected, fumigated and kept empty for 6 weeks if possible. (9) After all the above steps were satisfactorily completed, nasal swab samples from 1 (or more) 4-to-10-week-old pig per litter from subsequent farrowings were utilized to monitor the success of the control effort. (10) All additions to the herd were from a herd free of Bordetella rhinitis or must pass 3 negative nasal swab cultures.

The rate of isolation of *B. bronchiseptica* from nasal swab specimens from swine breeding animals submitted to the Veterinary Medical Research Institute, Iowa State University from October, 1970 to June, 1972, is presented in Table 1.
Table 1. Rate of isolation of *Bordetella bronchiseptica* from swine nasal swab specimens from October, 1970 to June, 1972.

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive (No.)</th>
<th>Negative (No.)</th>
<th>Usable (No.)</th>
<th>Overgrown (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>First</td>
<td>409</td>
<td>2,895</td>
<td>3,304</td>
<td>---</td>
</tr>
<tr>
<td>Second</td>
<td>93</td>
<td>2,103</td>
<td>2,196</td>
<td>---</td>
</tr>
<tr>
<td>Third</td>
<td>40</td>
<td>1,430</td>
<td>1,470</td>
<td>---</td>
</tr>
<tr>
<td>Total</td>
<td>542</td>
<td>6,428</td>
<td>6,970</td>
<td>1,052</td>
</tr>
</tbody>
</table>

Inspection of isolates of *B. bronchiseptica* from infected pigs does not allow an evaluation of their virulence. Therefore, recovery of *B. bronchiseptica* cannot be considered as a certain indication of an actual atrophic rhinitis problem in an infected herd.

Because of the large number of swine producers and veterinarians who expressed interest in the nasal swab culture control procedure, many veterinarians established this technique in their practice laboratories. Several training sessions were held to facilitate this. In an attempt to evaluate progress during the first 18 months of the control effort, a questionnaire was mailed to the 66 veterinarians who had sent nasal swab samples to the Veterinary Medical Research Institute or who had established their own laboratories for the culturing of nasal swab specimens. Replies were received from 54 veterinarians. The results are summarized in Figure 1. No attempt was made to select veterinarians for participation in
the testing program.

Dear Dr._____________________

For over one year, the Bordetella bronchiseptica nasal swabbing program has been available as an experimental pilot project. We are now conducting an evaluation of field results in order to better define the potential of this program. We need your observations regarding this testing procedure.

1. How many herds have you used this procedure on? 110 How many herds were positive for B. bronchiseptica? 80 How many had clinical rhinitis? 65 Total number of breeding animals involved? 8,995.

2. Was there clinical improvement in those herds in which the bordetella carriers were removed from the breeding herd? Yes 23 No 6.

3. Was your client's reaction to the test favorable? Yes 34 No 9.

4. Is swab collection too time consuming? Yes 3 No 41.

5. Do you have to wait too long for laboratory results? Yes 2 No 39.

6. Are too many breeding animals removed from the herd? Yes 3 No 36.

7. Have you had complaints as to collection costs? Yes 8 No 37.

8. Has the program produced the desired results? Yes 13 No 8 Partially 21.

9. Is sulfonamide therapy effective in clinical B. bronchiseptica situations in which you have used it? Yes 11 No 7 Partially 28.

---

Figure 1. Summary of questionnaire response from 54 private practitioners participating in a Bordetella rhinitis control project.

From October, 1970 to December, 1973, approximately 30,182 nasal swab samples were submitted to the Veterinary Medical Research Institute, Iowa State University for the isolation and identification of B. bronchiseptica. These samples were
collected from 152 herds of which 107 were determined to be infected with *B. bronchiseptica*. Sulfamethazine sensitivity was determined for *B. bronchiseptica* isolates from 76 herds. Forty of the 76 *B. bronchiseptica* isolates (53 percent) were resistant at the 30mg./ml. level indicating a high degree of sulfamethazine resistance. Fourteen isolates (18 percent) were sensitive at the 20 or 30mg./ml. level, but resistant at the 5 and 10mg./ml. level indicating partial resistance to sulfamethazine. Twenty-two isolates (29 percent) were sensitive to sulfamethazine at the 5, 10, 20 and 30mg./ml. levels indicating that they were sensitive to the drug. The data obtained from the 152 herds submitting swine nasal swabs to the Veterinary Medical Research Institute, Iowa State University is presented in Table 2.

Table 2. Rate of *Bordetella bronchiseptica* isolation from swine nasal swab specimens, October, 1970, to December, 1973. Composite of all samples.

<table>
<thead>
<tr>
<th>Total Cultures</th>
<th>Positive (No.)</th>
<th>Negative (No.)</th>
<th>Usable (No.)</th>
<th>Overgrown (No.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
<td>(%)</td>
</tr>
<tr>
<td>3,006</td>
<td>11.6</td>
<td>22,812</td>
<td>85.5</td>
<td>4,364</td>
</tr>
<tr>
<td>11.6</td>
<td>88.4</td>
<td>85.5</td>
<td>14.5</td>
<td></td>
</tr>
</tbody>
</table>

Twenty-five herds utilizing the control procedure were followed in adequate detail to allow evaluation of the efficiency of the procedure. Two criteria for success were used. The first was the production of swine free from clinical
atrophic rhinitis and the second was the production of swine free from *B. bronchiseptica*. These 25 herds comprised approximately 2,400 breeding animals and ranged in size from 20 to 300 breeding animals. Twenty-four herds were culture-positive for *B. bronchiseptica*. Seventeen of these 24 herds were experiencing clinical atrophic rhinitis at the initiation of the control effort. The culture-negative herd was established with sows that were culture-negative for *B. bronchiseptica* at the time of entry into the herd and the swine producer was interested in whether the negative status of the herd could be maintained through subsequent farrowings.

Of the 17 herds with clinical atrophic rhinitis prior to the *B. bronchiseptica* control attempt, 13 (77 percent) subsequently produced pigs that did not have clinical evidence of atrophic rhinitis at weaning age. Nasal sampling of weaned pigs was completed in 12 of the 13 herds that eliminated clinical atrophic rhinitis. Of these 12 herds, 10 (83 percent) produced pigs that were culture-negative for *B. bronchiseptica* at 4-to-10-weeks of age.

Seven herds were initially culture-positive for *B. bronchiseptica*, but were not experiencing clinical atrophic rhinitis. Five of these 7 herds (71 percent) produced pigs that were culture-negative for *B. bronchiseptica* at 4-to-10-weeks of age.

Of the 23 initial culture-positive herds that sampled weaning age pigs produced after the control effort, 15 (65 percent) produced pigs that were culture-negative for
B. bronchiseptica at weaning age. The herd founded with B. bronchiseptica free breeding stock was found to be free of clinical evidence of atrophic rhinitis and failed to yield B. bronchiseptica from nasal swab specimens from weaning age pigs for a 2 year period.

Individual herd data of those 25 herds participating in the initial control effort is presented in Table 3.

Natural clearance of a sulfamethazine resistant Bordetella bronchiseptica nasal infection.

Experiment II Thirty naturally infected Bordetella rhinitis pigs averaging 12 weeks of age were secured from a production herd.1 These pigs had received a ration containing Aureo S-P 2502 at the recommended level from approximately 3 to 7 or 8 weeks of age. The pigs had mild to moderate clinical evidence of atrophic rhinitis at the time of procurement. They were placed in an isolated confinement area and fed a complete ration free of antibiotics or sulfonamides. The initial B. bronchiseptica infection was determined to be sulfamethazine resistant. The nasal swab culturing method was utilized for the isolation of B. bronchiseptica from the infected pigs.

The natural clearance of the B. bronchiseptica nasal infection and reestablishment of sulfamethazine sensitivity in the

1Swine Teaching Herd, Department of Animal Science, Iowa State University.
2American Cyanamid Company, Princeton, New Jersey.
Table 3. Bordetella rhinitis nasal swab culture control program herd data

<table>
<thead>
<tr>
<th>Herd</th>
<th>Approximate Number in Breeding Herd</th>
<th>B. bronchiseptica Positive/negative</th>
<th>Clinical Evidence of Atrophic Rhinitis</th>
<th>Turbinate Atrophy Observed at Slaughter or Necropsy</th>
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<tr>
<td>1</td>
<td>80</td>
<td>Positive</td>
<td>Severe</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>300</td>
<td>Positive</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>166</td>
<td>Positive</td>
<td>Severe</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>Positive</td>
<td>Severe</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>125</td>
<td>Positive</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>Positive</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>Positive</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>8</td>
<td>200</td>
<td>Positive</td>
<td>Severe</td>
<td>Yes</td>
</tr>
<tr>
<td>9</td>
<td>80</td>
<td>Positive</td>
<td>Mild</td>
<td>Yes</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>Positive</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>11</td>
<td>122</td>
<td>Positive</td>
<td>Mild</td>
<td>No</td>
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<tr>
<td>12</td>
<td>31</td>
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<td>No</td>
</tr>
<tr>
<td>13</td>
<td>92</td>
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<td>No</td>
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<td>14</td>
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<td>None</td>
<td>No</td>
</tr>
<tr>
<td>15</td>
<td>25</td>
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</tr>
<tr>
<td>16</td>
<td>Primary Lab Pigs Only 41</td>
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<td>No</td>
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<tr>
<td>17</td>
<td>220</td>
<td>Positive</td>
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<tr>
<td>18</td>
<td>60</td>
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<td>Severe</td>
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</tr>
<tr>
<td>19</td>
<td>39</td>
<td>Positive</td>
<td>Moderate</td>
<td>Yes</td>
</tr>
<tr>
<td>20</td>
<td>180</td>
<td>Positive</td>
<td>None</td>
<td>No</td>
</tr>
<tr>
<td>21</td>
<td>60</td>
<td>Positive</td>
<td>Moderate</td>
<td>No</td>
</tr>
<tr>
<td>22</td>
<td>200</td>
<td>Positive</td>
<td>Severe</td>
<td>Yes</td>
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<tr>
<td>23</td>
<td>150</td>
<td>Positive</td>
<td>Mild</td>
<td>No</td>
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<tr>
<td>24</td>
<td>200</td>
<td>Positive</td>
<td>Mild</td>
<td>—</td>
</tr>
<tr>
<td>25</td>
<td>60</td>
<td>Negative</td>
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</table>

*a Based on observations by herd owners and program veterinarians.

b Not determined.
<table>
<thead>
<tr>
<th>B. bronchiseptica Sulfamethazine Sensitivity</th>
<th>Elimination of Clinical signs of Atrophic Rhinitis</th>
<th>Production of 4 to 10 week old Pigs free of B. bronchiseptica</th>
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</thead>
<tbody>
<tr>
<td>Sensitive</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Resistant</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Resistant</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Partial</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitive</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Resistant</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Resistant</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Sensitive</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Resistant</td>
<td>Yes</td>
<td>Yes</td>
</tr>
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<td>Resistant</td>
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<td>Resistant</td>
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<td>Yes</td>
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<tr>
<td>Resistant</td>
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<td>Yes</td>
</tr>
<tr>
<td>Sensitive</td>
<td>Not applicable</td>
<td>No</td>
</tr>
<tr>
<td>Resistant</td>
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<tr>
<td>Sensitive</td>
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<td>Resistant</td>
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<tr>
<td>Sensitive</td>
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<td>No</td>
</tr>
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<tr>
<td>Partial</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Resistant</td>
<td>Not applicable</td>
<td>Yes</td>
</tr>
</tbody>
</table>

---

*a* Elimination of Clinical signs of Atrophic Rhinitis

---

*b* Not applicable
population of infecting organisms is presented in Figure 2. The individual pig data is presented in Table 4. At least 1 colony from each nasal culture of *B. bronchiseptica* isolate was tested for sulfamethazine sensitivity. Pigs negative for *B. bronchiseptica* on 2 consecutive samplings were considered as recovered from the infection. Six pigs became negative for *B. bronchiseptica* before the organism regained sulfamethazine sensitivity.

**Parenteral immunization of young pigs to produce resistance to *Bordetella bronchiseptica* nasal infections**

**Experiment III** This experiment tested the ability of a commercial canine origin *B. bronchiseptica* bacterin\(^1\) and laboratory prepared immunizing agents to produce resistance to *B. bronchiseptica* nasal infections in a group of 4-to-8-week-old pigs. A partially purified *B. bronchiseptica* hemagglutinin extract was also administered to determine its ability to stimulate respiratory tract resistance. The laboratory bacterins were prepared in a manner calculated to preserve maximum hemagglutinin (HA) titers. Vaccine HA titers are indicated in Table 5.

Thirty-two pigs, 4-to-8-weeks of age, were obtained from the Veterinary Medical Research Institute normal herd and from a *Bordetella rhinitis* free commercial herd. All pigs were determined to be negative for *B. bronchiseptica* nasal infection.

---

\(^1\)Pitman-Moore, Inc., Washington Crossing, New Jersey, Bacterin Number 19-521.
Figure 2. Natural clearance of *Bordetella bronchiseptica* nasal infection and reestablishment of sulfamethazine sensitivity in a group of thirty pigs
Percent Sulfamethazine Sensitive B. bronchiseptica Cultures

Percent Positive B. bronchiseptica Cultures

a Average Number of B. bronchiseptica Colonies recovered per sample.

b Number of pigs positive for B. bronchiseptica over number of pigs sampled.
Table 4. Clearance of a natural *Bordetella bronchiseptica* nasal infection and reestablishment of sulfamethazine sensitivity

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Pig Number</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D25-3</td>
<td>+R&lt;sup&gt;a&lt;/sup&gt;,&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+R</td>
<td>-&lt;sup&gt;c&lt;/sup&gt;</td>
<td>+S&lt;sup&gt;d&lt;/sup&gt;</td>
<td>+S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D24-6</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D22-2</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+R</td>
<td>OG&lt;sup&gt;e&lt;/sup&gt;</td>
<td>+S</td>
<td>-</td>
</tr>
<tr>
<td>D-20-3</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+S</td>
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<td>D-24-3</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S</td>
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<td>+S20</td>
<td>+S</td>
</tr>
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<td>D-21-10</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H28-4</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+S</td>
<td>-</td>
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<td>-</td>
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<td>H-29-3</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+R</td>
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<td>-</td>
</tr>
<tr>
<td>X21-5</td>
<td>+R</td>
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<td>+R</td>
<td>+S30</td>
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<td>-</td>
</tr>
<tr>
<td>Y24-7</td>
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<td>+R</td>
<td>-</td>
<td>+R</td>
<td>+S</td>
<td>+R</td>
<td>+S</td>
</tr>
<tr>
<td>X24-2</td>
<td>+R</td>
<td>+R</td>
<td>+S20</td>
<td>+R</td>
<td>+S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X26-8</td>
<td>-</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H-26-7</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td></td>
</tr>
<tr>
<td>X22-5</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td></td>
</tr>
<tr>
<td>D26-5</td>
<td>+R</td>
<td>+R</td>
<td>+S20</td>
<td>+S</td>
<td>+S</td>
<td>+S</td>
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</tr>
<tr>
<td>D23-2</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+S30</td>
<td>+S</td>
<td>+S</td>
<td>+S</td>
</tr>
<tr>
<td>X27-4</td>
<td>OG</td>
<td>+R</td>
<td>+S30</td>
<td>+R</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Y23-11</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S</td>
<td>+S20</td>
<td>+R</td>
<td>-</td>
</tr>
<tr>
<td>D21-6</td>
<td>-</td>
<td>+R</td>
<td>+R</td>
<td>+S20</td>
<td>+R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H27-2</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S</td>
<td>-</td>
<td>+S</td>
<td>-</td>
</tr>
<tr>
<td>Y25-8</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>D19-6</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S</td>
<td>+S</td>
<td>+S20</td>
<td>-</td>
</tr>
<tr>
<td>P24-7</td>
<td>+R</td>
<td>+R</td>
<td>+R</td>
<td>+S20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Y26-3</td>
<td>+R</td>
<td>-</td>
<td>+R</td>
<td>+S20</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>X30-5</td>
<td>+R</td>
<td>+R</td>
<td>-</td>
<td>+S</td>
<td>-</td>
<td>+S20</td>
<td>+S</td>
</tr>
<tr>
<td>P22-3</td>
<td>+R</td>
<td>+R</td>
<td>+S20</td>
<td>-</td>
<td>+S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H31-3</td>
<td>+R</td>
<td>OG</td>
<td>+R</td>
<td>-</td>
<td>+S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>H52-2</td>
<td>+R</td>
<td>+R</td>
<td>-</td>
<td>+S</td>
<td>+S</td>
<td>+S</td>
<td>-</td>
</tr>
<tr>
<td>H32-8</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+S20</td>
<td>+S</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CW2-7</td>
<td>+R</td>
<td>+R</td>
<td>+S30</td>
<td>+R</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Positive for *B. bronchiseptica*.

<sup>b</sup>R = Resistant to 30 mg. level of Sulfamethazine.

<sup>c</sup>- = Negative for *B. bronchiseptica*.

<sup>d</sup>S = Sensitive to 10 mg. level of Sulfamethazine.

<sup>e</sup>OG = Overgrown sample not usable.
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5/3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5/17</td>
<td>+S</td>
<td>-</td>
<td>+S20</td>
<td>OG</td>
<td>+S30</td>
</tr>
<tr>
<td>6/1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6/8</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6/15</td>
<td>+S20</td>
<td>+S20</td>
<td>+S20</td>
<td>OG</td>
<td>+S</td>
</tr>
<tr>
<td>6/25</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7/9</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7/21</td>
<td>+S30</td>
<td>+S</td>
<td>+S20</td>
<td>OG</td>
<td>+S</td>
</tr>
<tr>
<td>8/6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8/20</td>
<td>+S20</td>
<td>+S20</td>
<td>+S20</td>
<td>+S</td>
<td>+S</td>
</tr>
</tbody>
</table>

+S Died
by nasal swab culturing. The pigs were placed in individual pens by litter and randomly selected for immunization or as non-immunized controls. Four pigs died from stress during the trial.

The pigs were vaccinated subcutaneously in the fold of the flank. Five pigs received D-1 strain sonicate, whole-cell, formalinized bacterin with adjuvant, 5 received D-1 strain whole-cell, formalinized bacterin with adjuvant, and 4 pigs received 55 strain whole-cell, formalinized bacterin with adjuvant. The D-1 and 55 strain bacterins were mixed in equal 1 milliliter volumes with Freund's incomplete adjuvant immediately prior to administration. Four pigs received *B. bronchiseptica* partially purified hemagglutinin extract without adjuvant (1 ml. dosage), 7 received Pitman-Moore 19-521 bacterin without adjuvant (2 ml. dosage) and 7 pigs served as unvaccinated controls. Two doses of the immunizing agents were administered at a 1 week interval and the pigs were challenged with virulent B strain *B. bronchiseptica* 18 days post-immunization. A booster dose of the immunizing material was administered 7 weeks post-B strain challenge and 9 weeks post-immunization. Average PAST serum titers by vaccine group are presented in Table 5. A summary of the performance of the vaccine groups during the experiment in regards to resistance to the *B. bronchiseptica* nasal challenge and the average *B. bronchiseptica* colony count is shown in Table 6.

Eighteen snouts were examined for gross evidence of turbinate atrophy at the termination of the experiment. Gross
Table 5. Average PAST serum titers by vaccine group

<table>
<thead>
<tr>
<th>Product</th>
<th>3-28-72 Prevac</th>
<th>4-14-72 1st Dose</th>
<th>4-28-72 Post-2nd Dose</th>
<th>6-16-72 Pre-3rd Dose</th>
<th>7-6-72 2nd Dose</th>
<th>8-21-72 Termination</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1 Sonicate With Adjuvant (64)</td>
<td>2</td>
<td>&gt;4096</td>
<td>3686</td>
<td>&gt;4096</td>
<td>&gt;4096</td>
<td>&gt;4096</td>
</tr>
<tr>
<td>D-1 Whole-Cell With Adjuvant (128)</td>
<td>4</td>
<td>3379</td>
<td>&gt;4096</td>
<td>&gt;4096</td>
<td>&gt;4096</td>
<td>&gt;4096</td>
</tr>
<tr>
<td>55 Whole-Cell With Adjuvant (64)</td>
<td>3</td>
<td>10</td>
<td>289</td>
<td>320</td>
<td>3088</td>
<td>1707</td>
</tr>
<tr>
<td>Partially Purified B. bronchiseptica</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemagglutinin Extract (128)</td>
<td>1</td>
<td>2560</td>
<td>1536</td>
<td>271</td>
<td>1616</td>
<td>176</td>
</tr>
<tr>
<td>Pitman-Moore 19-521 (0)</td>
<td>2</td>
<td>651</td>
<td>585</td>
<td>539</td>
<td>3547</td>
<td>860</td>
</tr>
<tr>
<td>Nonvaccinated Controls</td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>59</td>
<td>53</td>
<td>32</td>
</tr>
</tbody>
</table>

Vaccine hemagglutination titer.
Table 6. Resistance of parenterally immunized pigs to *B. bronchiseptica* challenge

<table>
<thead>
<tr>
<th>Product</th>
<th>3-28-72</th>
<th>5-9-72</th>
<th>5-18-72</th>
</tr>
</thead>
<tbody>
<tr>
<td>D-1 Sonicate With Adjuvant</td>
<td>0/5^b</td>
<td>2/4</td>
<td>4/4</td>
</tr>
<tr>
<td>D-1 Whole-Cell With Adjuvant</td>
<td>0/5</td>
<td>3/5</td>
<td>4/5</td>
</tr>
<tr>
<td>55 Whole-Cell With Adjuvant</td>
<td>0/4</td>
<td>2/4</td>
<td>3/4</td>
</tr>
<tr>
<td>Partially Purified <em>B. bronchiseptica</em> Hemagglutinin Extract</td>
<td>0/4</td>
<td>3/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Pitman-Moore</td>
<td>0/7</td>
<td>3/7</td>
<td>5/6</td>
</tr>
<tr>
<td>19-521</td>
<td>0/7</td>
<td>5/7</td>
<td>5/7</td>
</tr>
<tr>
<td>Non-vaccinated Controls</td>
<td>0/7</td>
<td>5/7</td>
<td>5/7</td>
</tr>
</tbody>
</table>

^aThird dose vaccine administered 6-19-72.

^bNumber of pigs positive for *B. bronchiseptica* over number sampled.

^cNumber of pigs with turbinate atrophy at necropsy over number of pigs examined.

^dAverage *B. bronchiseptica* colony count.
<table>
<thead>
<tr>
<th>Date</th>
<th>4 Weeks Post-Challenge</th>
<th>8 Weeks Post-Challenge</th>
<th>10 Weeks Post-Challenge</th>
<th>14 Weeks Post-Challenge</th>
<th>Gross Evidence of Turbinate Atrophy at Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-31-72</td>
<td>4/4</td>
<td>3/4</td>
<td>1/4</td>
<td>2/4</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>(7)</td>
<td>(13)</td>
<td>(.25)</td>
<td>(10)</td>
<td></td>
</tr>
<tr>
<td>7-5-72</td>
<td>5/5</td>
<td>4/5</td>
<td>2/5</td>
<td>3/4</td>
<td>0/4</td>
</tr>
<tr>
<td></td>
<td>(75)</td>
<td>(133)</td>
<td>(20)</td>
<td>(2)</td>
<td></td>
</tr>
<tr>
<td>8-3-72</td>
<td>4/4</td>
<td>3/4</td>
<td>1/4</td>
<td>0/3</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>(63)</td>
<td>(13)</td>
<td>(4)</td>
<td>(0)</td>
<td></td>
</tr>
<tr>
<td>8-21-72</td>
<td>4/4</td>
<td>3/4</td>
<td>2/4</td>
<td>2/4</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>(115)</td>
<td>(100)</td>
<td>(225)</td>
<td>(57)</td>
<td></td>
</tr>
<tr>
<td>8-23-72</td>
<td>6/7</td>
<td>6/7</td>
<td>6/7</td>
<td>4/6</td>
<td>1/4</td>
</tr>
<tr>
<td></td>
<td>(170)</td>
<td>(227)</td>
<td>(180)</td>
<td>(30)</td>
<td></td>
</tr>
<tr>
<td>10-21-72</td>
<td>5/7</td>
<td>7/7</td>
<td>6/7</td>
<td>6/7</td>
<td>2/3</td>
</tr>
<tr>
<td></td>
<td>(230)</td>
<td>(104)</td>
<td>(102)</td>
<td>(105)</td>
<td></td>
</tr>
</tbody>
</table>
turbinate atrophy was not observed in the pigs receiving laboratory produced *B. bronchiseptica* bacterins with adjuvant. Two of 3 control pigs, 1 of 4 Pitman-Moore 19-521 immunized pigs and 1 of 3 *B. bronchiseptica* partially purified hemagglutinin extract immunized pigs evidenced moderate to severe turbinate atrophy.

At 10 weeks post-challenge, 4 out of 13 pigs vaccinated with laboratory experimental whole-cell bacterins were positive for *B. bronchiseptica* while 12 out of 14 pigs vaccinated with either Pitman-Moore 19-521 bacterin or unvaccinated controls were positive. At 14 weeks, the values for the two groups were 5 for 11 and 10 for 13 respectively. The average colony counts were lower in the pigs immunized with the laboratory experimental bacterins with adjuvant throughout the experiment.

All pigs receiving bacterins containing Freund's incomplete adjuvant injected subcutaneously in the fold of the flank developed palpable, persistent enlargements at the site of injection. Bacterins administered without adjuvant or containing AB-65 adjuvant did not produce a comparable enlargement at the site of injection.

**Experiment IV**  *Bordetella bronchiseptica* strain D-l sonicate, whole-cell, formalinized bacterin with adjuvant containing approximately $2 \times 10^9$ *B. bronchiseptica* organisms per milliliter after sonication was administered to 3 and 6 week old pigs. A second group of 6-week-old pigs was immunized with the same bacterin without adjuvant. The bacterin contained a HA titer of 128 at the time of administration.
Thirty-three pigs, 3-to-6-weeks of age, were obtained from the Veterinary Medical Research Institute normal herd. The pigs were randomly assigned within the 3 or 6 week age groups to individual pens by vaccine groups. Two pigs died from stress during the experiment.

The pigs were vaccinated subcutaneously in the fold of the flank. Five 3-week-old pigs and 7 6-week-old pigs received D-1 strain, sonicate, whole-cell, formalinized bacterin (2 ml.) with adjuvant. The bacterin was mixed in equal volumes with Freund's incomplete adjuvant immediately prior to injection. Eight 6-week-old pigs received D-1 strain, sonicate, whole-cell, formalinized bacterin without adjuvant (2 ml. dosage) and 5 3-week-old and 8 6-week-old pigs served as nonvaccinated controls. Two doses of the vaccine were administered at a 10 day interval and the pigs were challenged with virulent B strain B. bronchiseptica 14 days post-vaccination. Average PAST serum titers by age and vaccine groups are presented in Table 7. Resistance of the parenterally immunized pigs to B. bronchiseptica nasal challenge is summarized in Table 8.

Thirty-one snouts were examined for gross evidence of turbinate atrophy at necropsy. Two 6-week-old control pigs and 1 6-week-old D-1 sonicate without adjuvant immunized pigs had evidence of mild turbinate atrophy. Gross turbinate atrophy was not present in the 3 and 6-week-old pigs receiving the adjuvanted bacterin.

At 2 weeks post-challenge, 4 out of 12 pigs vaccinated
Table 7. Average PAST serum titers by vaccine group

<table>
<thead>
<tr>
<th>Age of Pigs</th>
<th>Vaccinated/Product</th>
<th>10-13-72</th>
<th>11-2-72</th>
<th>11-16-72</th>
<th>3-8-73</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Week Old Pigs: Nonvaccinated Controls</td>
<td></td>
<td>0.8</td>
<td>No Test</td>
<td>33</td>
<td>12</td>
</tr>
<tr>
<td>3 Week Old Pigs: D-1 Sonicate With Adjuvant</td>
<td></td>
<td>1.6</td>
<td>640</td>
<td>3076</td>
<td>269</td>
</tr>
<tr>
<td>6 Week Old Pigs: D-1 Sonicate With Adjuvant</td>
<td></td>
<td>0.5</td>
<td>&gt;4096</td>
<td>3072</td>
<td>4096</td>
</tr>
<tr>
<td>6 Week Old Pigs: D-1 Sonicate Without Adjuvant</td>
<td></td>
<td>0.8</td>
<td>2816</td>
<td>2286</td>
<td>1381</td>
</tr>
<tr>
<td>6 Week Old Pigs: Nonvaccinated Controls</td>
<td></td>
<td>3</td>
<td>No Test</td>
<td>4.5</td>
<td>22</td>
</tr>
</tbody>
</table>

1st Vaccination 10-24-72 2nd Vaccination 11-2-72 B. bronchiseptica Nasal Challenge 11-16-72

Vaccine hemagglutination titer 128.
Table 8. Resistance of parenterally immunized pigs to *B. bronchiseptica* challenge

<table>
<thead>
<tr>
<th>Age of Pigs Vaccinated/Product</th>
<th>10-13-72 Pretrial Nasal Culture</th>
<th>12-1-72 2 Weeks Post-Challenge</th>
<th>12-19-72 4 Weeks Post-Challenge</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Week Old Pigs: Nonvaccinated Controls</td>
<td>0/5(^a)</td>
<td>5/5</td>
<td>4/5</td>
</tr>
<tr>
<td>3 Week Old Pigs: D-1 Sonicate With Adjuvant</td>
<td>0/5</td>
<td>2/5</td>
<td>2/5</td>
</tr>
<tr>
<td>6 Week Old Pigs: D-1 Sonicate With Adjuvant</td>
<td>0/7</td>
<td>2/7</td>
<td>4/7</td>
</tr>
<tr>
<td>6 Week Old Pigs: D-1 Sonicate Without Adjuvant</td>
<td>0/8</td>
<td>5/8</td>
<td>4/8</td>
</tr>
<tr>
<td>6 Week Old Pigs: Nonvaccinated Controls</td>
<td>0/7</td>
<td>8/8</td>
<td>8/8</td>
</tr>
</tbody>
</table>

\(^a\) Number of pigs positive for *B. bronchiseptica* over number sampled.

\(^b\) Number of pigs with turbinate atrophy at necropsy over number of pigs examined.
<table>
<thead>
<tr>
<th>1-19-73</th>
<th>2-23-73</th>
<th>3-8-73</th>
<th>Gross Evidence of Turbinate Atrophy at Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 Weeks Post-Challenge</td>
<td>12 Weeks Post-Challenge</td>
<td>15 Weeks Post-Challenge</td>
<td></td>
</tr>
<tr>
<td>2/4</td>
<td>2/4</td>
<td>1/4</td>
<td>0/4(^b)</td>
</tr>
<tr>
<td>3/4</td>
<td>3/5</td>
<td>2/4</td>
<td>0/5</td>
</tr>
<tr>
<td>4/7</td>
<td>4/7</td>
<td>3/7</td>
<td>0/7</td>
</tr>
<tr>
<td>3/7</td>
<td>3/7</td>
<td>2/7</td>
<td>1/7</td>
</tr>
<tr>
<td>6/8</td>
<td>4/8</td>
<td>3/8</td>
<td>2/8</td>
</tr>
</tbody>
</table>
with D-1 sonicate bacterin with adjuvant were culture-positive for *B. bronchiseptica* while 13 out of 13 controls were positive. This resistance waned until at 12 weeks post-challenge the figures for the 2 groups were 5 of 12 and 6 of 12 respectively. At 16 weeks post-immunization the average PAST serum titers were >4096 in the 6-week-old pigs and 269 in the 3-week-old pigs receiving D-1 sonicate bacterin with adjuvant. The 6-week-old pigs receiving D-1 sonicate without adjuvant averaged 1381 at this time.

**Experiment V** Seven 5-to-6-week-old pigs were obtained from the Veterinary Medical Research Institute normal herd and were inoculated intranasally with virulent B strain *B. bronchiseptica*. Nasal secretions of all 7 pigs were culture-positive for *B. bronchiseptica* 2 weeks post-challenge. Strain D-1 sonicate, whole-cell, formalinized bacterin containing approximately \(2 \times 10^9\) organisms per milliliter was administered subcutaneously 3 weeks post-challenge to 4 of the pigs. The first dose (2 ml.) was given without adjuvant and the second dose (2ml.) given 7 days later contained an equal volume of Freund's incomplete adjuvant. At 3 weeks post-vaccination 4 of 4 vaccinates and 2 of 3 controls were culture-positive for *B. bronchiseptica*. At 15 weeks post-vaccination 1 of 4 vaccinates and 2 of 3 controls were culture-positive for *B. bronchiseptica*. The average PAST serum titer of vaccinated pigs was 2688 2 weeks post-vaccination and 2112 15 weeks post-vaccination. Mild gross turbinate atrophy was observed at necropsy in 1 vaccinate while 3
vaccinated swine had normal turbinates. Two control swine had normal turbinates and 1 had mild turbinate atrophy.

**Experiment VI** Seven 6-week-old pigs were obtained from the Veterinary Medical Research Institute normal herd. Strain D-1 sonicate, whole-cell, formalinized bacterin with AB-65 adjuvant containing approximately $1 \times 10^8$ organisms per milliliter was subcutaneously administered to the 7 pigs. No control swine were utilized in this experiment. Equal volumes of AB-65 adjuvant and D-1 sonicate bacterin were mixed and injected in 2 ml. dosages.

The pigs were immunized with 2 doses of the immunizing agent 30 days apart with a virulent B strain *B. bronchiseptica* nasal challenge midpoint between vaccinations.

Nasal secretions of 6 of 7 pigs were culture-positive for *B. bronchiseptica* 2 weeks post-challenge and 0 of 7 were culture-positive by 9 to 12 weeks post-challenge. The average PAST serum titer of the pigs was 2962 3 weeks post-vaccination and 1351 at 16 weeks post-vaccination. Two of 7 pigs had slight malformation of the ventral scroll of the ventral turbinates while 5 had normal nasal cavities when examined at necropsy.

Intranasal vaccination of young pigs to produce resistance to *Bordetella bronchiseptica* nasal infections

**Experiment VII** The ability of live, low-virulence 55

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1 Armour-Baldwin Laboratories, Omaha, Nebraska.
strain B. bronchiseptica intranasal vaccination to produce nasal resistance to subsequent challenge with swine virulent B strain B. bronchiseptica was tested. The nasal persistence of 55 strain infection was also evaluated.

Twelve pigs, 4-to-8-weeks of age, were obtained from the Veterinary Medical Research Institute normal herd and a Bordetella rhinitis free commercial herd. All pigs were culture-negative for B. bronchiseptica. The pigs were selected into 2 groups and housed in individual isolation units.

Six pigs received 3 doses over a 4 day period of live, low-virulence 55 strain intranasal vaccine at 7 weeks of age and 7 pigs served as nonvaccinated controls. All pigs were challenged with virulent B strain B. bronchiseptica 3 weeks post-intranasal vaccination.

Strain 55 was not detected by the nasal swab culture method in the nasal secretions of the vaccinated pigs 2 weeks post-intranasal vaccination.

A summary of the incidence of B. bronchiseptica in the nasal secretions of the B strain challenged pigs is presented in Table 9. At 3 weeks post-B strain challenge 0 of 6 vaccinateds and 5 of 7 control pigs were culture-positive for B. bronchiseptica. At 4 weeks post-B strain challenge the nasal secretions of 3 of 6 vaccinated pigs were culture-positive for B. bronchiseptica with an average colony count of 6 organisms per culture plate. The 8 week termination figures were 0 of 6 and 7 of 7 respectively. The average termination PAST serum
Table 9. Incidence of *Bordetella bronchiseptica* in the nasal secretions of swine after intranasal installation of live, low-virulence 55 strain *B. bronchiseptica* vaccine and subsequent challenge with virulent B strain *B. bronchiseptica*

<table>
<thead>
<tr>
<th>Swine/Product</th>
<th>Number of Swine</th>
<th>Pretrial</th>
<th>3</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>Gross evidence of turbinate atrophy at necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 to 8-week-old Pigs, live, low-Virulence 55 Strain Intranasal Vaccination</td>
<td>6</td>
<td>0/6</td>
<td>0/6</td>
<td>3/6</td>
<td>0/6</td>
<td>0/6</td>
<td>0/6*</td>
</tr>
<tr>
<td>4 to 8-week-old Pigs, Non-Vaccinated Controls</td>
<td>7</td>
<td>0/7</td>
<td>5/7</td>
<td>6/7</td>
<td>6/7</td>
<td>7/7</td>
<td>2/3</td>
</tr>
</tbody>
</table>

*Number of pigs showing gross turbinate atrophy over number necropsied.*
titer was 39 for the vaccinated pigs and 53 for the nonvaccinated controls. At necropsy, gross examination revealed all intranasally immunized pigs had normal nasal turbinates while 2 of 3 controls examined had moderate to severe turbinate atrophy.

Experiment VIII The ability of live, low-virulence strain 55 B. bronchiseptica intranasal vaccination to produce nasal resistance to subsequent challenge with B strain swine virulent B. Bronchiseptica was reevaluated. In addition, sterile filtrates from 55 strain B. bronchiseptica 24 hour TPB cultures were instilled intranasally to determine their protective ability. The nasal persistence of 55 strain was also evaluated.

Fifteen pigs, 3-to-4-weeks of age, were obtained from the Veterinary Medical Research Institute normal herd. The pigs were randomly selected into 3 groups and placed in individual isolation units.

Six pigs received 3 doses over a 4 day period of live, low-virulence 55 strain B. bronchiseptica intranasal vaccine, 5 pigs received 3 doses over a 4 day period of 55 strain TPB culture sterile filtrate and 4 pigs served as unvaccinated controls. All pigs were challenged with swine virulent B strain 2 weeks post-intranasal immunization. The 55 strain B. bronchiseptica was not detected on nasal swab culture 6 days post-vaccination. The pigs were 4 to 5 weeks of age at the time of 55 strain vaccination and 6 to 7 weeks of age at the time of B strain challenge.
A summary of the incidence of *B. bronchiseptica* in the nasal secretions of the B strain challenged pigs during the first 12 weeks of the experiment is presented in Table 10. At 2 weeks post-B strain challenge 0 of 4 live, 55 strain vaccinates, 5 of 6 55 strain sterile TPB culture filtrate vaccinates and 4 of 4 control pigs were culture-positive for *B. bronchiseptica*. The 12 week figures were 0 of 5, 1 of 6 and 1 of 4.

At 8 weeks and 17 weeks post-first B strain challenge, the live 55 strain intranasally vaccinated pigs were rechallenged with B strain. *Bordetella bronchiseptica* was not detected in the nasal secretions of live, low-virulence 55 strain intranasally vaccinated pigs for the entire 19 week sampling period.

The average termination PAST serum titer was 38 for the live 55 strain vaccinates, 8 for the 55 strain TPB sterile filtrate vaccinates and 13 for the nonvaccinated controls. At necropsy gross examination revealed all intranasally vaccinated pigs had normal turbinate structures while 3 of 4 controls had mild turbinate atrophy.

**Effects of colostral antibodies and intranasal immunization on resistance to Bordetella bronchiseptica nasal infection and turbinate atrophy.**

**Experiment IX** Three pregnant sows were obtained from a Bordetella rhinitis negative commercial herd. The sows were culture-negative for *B. bronchiseptica* and were housed in individual isolation units.

Two of the sows (numbers 1 and 2) were immunized with
Table 10. Incidence of *Bordetella bronchiseptica* in the nasal secretions of swine after intranasal installation of live, low-virulence 55 strain *B. bronchiseptica* vaccine or 55 strain TPB culture sterile filtrate and subsequent challenge with virulent B strain *B. bronchiseptica*

<table>
<thead>
<tr>
<th>Swine/Product</th>
<th>Number of Swine</th>
<th>Pretrial</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>12</th>
<th>Gross Evidence of Turbinate Atrophy at Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-week-old pigs. Live, low-virulence 55 strain Intranasal Vaccination</td>
<td>5</td>
<td>0/5</td>
<td>0/4(^a)</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/5</td>
<td>0/4(^b)</td>
</tr>
<tr>
<td>5-week-old pigs. 55 strain TPB Culture sterile Filtrate intranasal Vaccination</td>
<td>6</td>
<td>0/6</td>
<td>5/6</td>
<td>5/6</td>
<td>1/6</td>
<td>1/6</td>
<td>1/6</td>
<td>0/6</td>
</tr>
<tr>
<td>4 to 5-week-old pigs. Non-Vaccinated Controls</td>
<td>4</td>
<td>0/4</td>
<td>4/4</td>
<td>3/4</td>
<td>3/4</td>
<td>2/4</td>
<td>1/4</td>
<td>3/4</td>
</tr>
</tbody>
</table>

\(^a\)One sample was not interpretable due to overgrowth with contaminating organisms.

\(^b\)Number of pigs with turbinate atrophy at necropsy over number of pigs examined.
strain D-1 sonicate, whole-cell bacterin with adjuvant containing approximately $8 \times 10^8$ organisms per milliliter. The sows received 2 subcutaneous doses of the bacterin 7 days apart, 24 days (in the case of number 1) and 32 days (in the case of number 2) prior to farrowing. The bacterin was incorporated with Freund's incomplete adjuvant for the first dose and AB-65 adjuvant for the second dose. One sow (number 3), served as a non-immunized control. Sows 1 and 2 developed PAST serum titers of $>4096$ 20 days post-immunization. This was 4 days prefarrowing for sow 1 and 12 days prefarrowing for sow 2. At 52 days post-immunization and 28 days post-farrowing sow 1 had a PAST serum titer of $>4096$. Litters farrowed by sows 1 and 2 had PAST serum titers that averaged 1283 and 156 respectively at 3 weeks of age. The litter farrowed from sow 3 had an average PAST serum titer of 11 at 6 weeks of age.

The pigs from sow 2 were accidentally killed at 22 days of age due to a malfunction of the isolation building. Litters from sows 1 and 3 were weaned at 5 weeks of age.

Pigs farrowed by sows 1 and 3 were administered 3 doses of live, low-virulence 55 strain B. bronchiseptica intranasal vaccine during the first 5 days of life. The pigs were challenged with swine virulent B strain B. bronchiseptica 12 to 14 days after the last intranasal immunization. Results of nasal swab culturing and necropsy observation of these challenged pigs is presented in Table 11.

One of 10 intranasally immunized pigs nursing sow 1 was
Table 11. Resistance to *Bordetella bronchiseptica* nasal infection in live, low-virulence 55 strain *B. bronchiseptica* intranasally vaccinated newborn pigs nursing immunized and nonimmunized sows

<table>
<thead>
<tr>
<th>Immunization State of sows/ Number of pigs</th>
<th>Age of pigs at B strain Challenge</th>
<th>Number of swine culture-positive for <em>B. bronchiseptica</em> over number sampled at various times after B strain challenge</th>
<th>Gross Evidence of turbinate Atrophy at Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immunized</td>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>(Sow 1) 11 pigs</td>
<td>19 days</td>
<td>1/9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0/10</td>
</tr>
<tr>
<td>Nonimmunized</td>
<td></td>
<td>2 weeks</td>
<td>4 weeks</td>
</tr>
<tr>
<td>(Sow 3) 9 pigs</td>
<td>16 days</td>
<td>2/7</td>
<td>3/7</td>
</tr>
</tbody>
</table>

<sup>a</sup>Pig number 479 was culture-positive at the 2 week and 6 week sampling period with an average colony count of 5.

<sup>b</sup>The experiment involving the litter from sow 1 was terminated after the negative 12 week nasal swab culturing and the pigs assigned to other research.

<sup>c</sup>Number of pigs showing gross turbinate atrophy over number necropsied.
culture-positive subsequent to B strain challenge. Intranasally immunized pigs nursing sow 3 (nonimmune colostral control) were all culture-positive for *B. bronchiseptica* at some time during the experiment. At necropsy pigs from sow 3 had an average PAST serum titer of 9, *B. bronchiseptica* was found in the ethmoid turbinates of 4 of 7 pigs and all turbinates were grossly normal.

**Experiment X**  Six bred gilts were obtained from a *Bordetella* rhinitis free purebred SPF herd. The gilts were culture-negative for *B. bronchiseptica* and were housed in individual isolation units.

Three gilts (numbers 1, 2, and 3) were immunized with 2 3 ml. doses of strain D-1 sonicate, whole-cell bacterin with AB-65 adjuvant. The doses of bacterin were administered 12 days apart approximately 4 to 6 weeks prior to farrowing, the bacterin contained approximately $1 \times 10^9$ organisms per milliliter and was administered subcutaneously with equal volumes of adjuvant. Three gilts (numbers 4, 5, and 6) served as nonimmunized controls.

The immunized gilts developed high titers of agglutinating antibody as indicated in Table 12. The PAST serum titers observed in the pigs nursing these gilts is also presented in Table 12.

Pigs nursing gilts 1, 2, 5 and 6 were intranasally vaccinated with live, low-virulence 55 strain *B. bronchiseptica* according to the schedule presented in Tables 13 and 14. Pigs
Table 12. PAST serum titers in experimental gilts and their offspring

<table>
<thead>
<tr>
<th>Gilt Number</th>
<th>Immunization Status</th>
<th>Gilt Prefarrowing PAST</th>
<th>Litter Post-farrowing Average PAST/age</th>
<th>Litter Termination Average PAST/age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td>1024 4 days prefarrowing (22 days post-immunization)</td>
<td>50 3-5 weeks (8 pigs)</td>
<td>33 14-16 weeks (9 pigs)</td>
</tr>
<tr>
<td>2</td>
<td>Immunization with 2 doses of D-1 sonicate Bacterin with Adjuvant</td>
<td>&gt;4096 1 day prefarrowing (30 days post-immunization)</td>
<td>19 4 weeks (6 pigs)</td>
<td>13 16 weeks (8 pigs)</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>&gt;4096 10 days prefarrowing (30 days post-immunization)</td>
<td>176 5 days 5 weeks (4 pigs)</td>
<td>116 16 weeks (4 pigs)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>128 7 weeks (3 pigs)</td>
<td>20 weeks (3 pigs)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Nonimmunized</td>
<td>256 4 weeks (7 pigs)</td>
<td>35 20 weeks (7 pigs)</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>128 &lt;1 4 weeks (10 pigs)</td>
<td>17 14-16 weeks (10 pigs)</td>
<td></td>
</tr>
</tbody>
</table>
Table 13. Resistance to *Bordetella bronchiseptica* nasal infection in live, low-virulence 55 strain *B. bronchiseptica* intranasally vaccinated pigs nursing immunized dams

<table>
<thead>
<tr>
<th>Gilt number/ Age of pigs at Intranasal Vaccination/ Number of pigs</th>
<th>Age of pigs at B strain Challenge</th>
<th>Number of swine culture-positive for <em>B. bronchiseptica</em> over number sampled at various times after B strain challenge</th>
<th>Gross Evidence of turbinate Atrophy at Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gilt 1 2, 3, 35 days, 4 pigs</td>
<td>45 Days</td>
<td>2/4 4/4 3/4 2/4</td>
<td>Not Sampled</td>
</tr>
<tr>
<td>Gilt 1 2, 3, 21 days, 5 pigs</td>
<td>30 Days</td>
<td>2/5 2/5 1/5 5/5</td>
<td>3/4</td>
</tr>
<tr>
<td>Gilt 2 2, 3 days, 8 pigs</td>
<td>21 Days</td>
<td>0/8 1/7 2/8 3/8</td>
<td>3/6</td>
</tr>
</tbody>
</table>

*Number of pigs showing gross turbinate atrophy over number necropsied.*

*Five pigs showed slight malformation of the ventral scroll of the ventral turbinate.*

*One pig showed slight malformation of the ventral scroll of the ventral turbinate.*
Table 14. Resistance to *Bordetella bronchiseptica* nasal infection in live, low-virulence 55 strain 
*B. bronchiseptica* intranasally vaccinated pigs nursing nonimmunized dams

<table>
<thead>
<tr>
<th>Gilt number/ Age of pigs at Intranasal Vaccination/ Number of pigs</th>
<th>Age of pigs at B strain Challenge</th>
<th>Gross Evidence of turbinate Atrophy at Necropsy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of swine culture-positive for <em>B. bronchiseptica</em> over number sampled at various times after B strain challenge</td>
<td></td>
</tr>
<tr>
<td>Gilt 6 2, 3, 35 days, 5 pigs</td>
<td>45 Days</td>
<td>0/5 1/5 0/5 0/5 Not Sampled 1/5^a</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3/4 3/4 1/5 2/5 1/5 0/5^b</td>
</tr>
<tr>
<td>Gilt 5 2, 3 Days, 7 pigs</td>
<td>21 Days</td>
<td>0/7 1/7 1/7 2/7 1/7 0/7^c</td>
</tr>
<tr>
<td>Gilt 4 Pigs not Immunized, 3 pigs</td>
<td>21 Days</td>
<td>3/3 3/3 2/3 3/3 3/3 2/3</td>
</tr>
</tbody>
</table>

^aNumber of pigs showing gross turbinate atrophy over number necropsied.

^bOne pig showed slight malformation of the ventral scroll of the ventral turbinate.

^cThree pigs showed slight malformation of the ventral scroll of the ventral turbinate.
farrowed by gilts 3 and 4 were not intranasally immunized. All pigs were challenged with swine virulent B strain *B. bronchiseptica* according to the schedule presented in Tables 13 and 14. The recovery of *B. bronchiseptica* from nasal swabs collected from these pigs and the production of turbinate atrophy in groups of the pigs is also presented in Tables 13 and 14. The pigs were weaned at 21 days of age and moved to individual isolation units or to the Veterinary Medical Research Institute hilltop swine rearing unit. Some unplanned mixing of the different groups took place at the hilltop unit.

**Inoculation of three-week-old pigs with eleventh serial embryonating hens egg passage of 55 strain *Bordetella bronchiseptica***.

**Experiment XI** The eleventh serial passage of 55 strain *B. bronchiseptica* in 6-to-8-day-old embryonated hens eggs was used as challenge inoculum in susceptible swine to determine if an increase in swine virulence had occurred. Embryo mortality caused by 11 back passages of 55 strain *B. bronchiseptica* is summarized in Table 15. Eleventh passage 55 strain *B. bronchiseptica* egg yolk inoculum was administered in 2 doses 2 days apart to 4 3-week-old pigs. The pigs were obtained from the Veterinary Medical Research Institute normal herd and housed in an individual isolation unit. Strain 55 was not detected in the nasal secretions of the 4 pigs 1 or 2 weeks post-challenge.
Table 15. Serial passage of 55 strain *B. bronchiseptica* in 6 to 8 day old chicken embryos

<table>
<thead>
<tr>
<th>Passage Number</th>
<th>Number of dead embryos/total embryonated hens eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hours</td>
</tr>
<tr>
<td>1</td>
<td>0/10</td>
</tr>
<tr>
<td>2</td>
<td>2(^a)/10</td>
</tr>
<tr>
<td>3</td>
<td>0/10</td>
</tr>
<tr>
<td>4</td>
<td>0/10</td>
</tr>
<tr>
<td>5</td>
<td>1/10</td>
</tr>
<tr>
<td>6</td>
<td>0/10</td>
</tr>
<tr>
<td>7</td>
<td>2/10</td>
</tr>
<tr>
<td>8</td>
<td>0/10</td>
</tr>
<tr>
<td>9(^b)</td>
<td>1/18</td>
</tr>
<tr>
<td>10</td>
<td>0/18</td>
</tr>
<tr>
<td>11</td>
<td>0/10</td>
</tr>
</tbody>
</table>

\(^a\)The early embryonic mortality was ascribed to inoculation trauma.

\(^b\)A staphylococcus contaminant occurred in the ninth passage. The eighth embryonating hens egg passage was plated onto blood agar. An individual colony of 55 strain *B. bronchiseptica* was picked into three milliliters of TPB, incubated for 24 hours and used to inoculate the 9th passage.

\(^c\)The embryos were moribund but not dead.
The pigs were subsequently challenged with B strain \textit{B. bronchiseptica} 3 weeks post-eleventh passage 55 strain challenge. Three weeks post-B strain challenge nasal secretions of 3 of 4 pigs were culture-positive for \textit{B. bronchiseptica}. At 5 weeks post-B strain challenge 0 of 4 pigs were culture-positive for \textit{B. bronchiseptica}. At necropsy all 4 pigs had grossly normal turbinates. The ethmoid turbinates were culture-negative for \textit{B. bronchiseptica} at the time of necropsy.

**Morphology of 55 and B Strain Compared with Previous Characterizations of \textit{Bordetella bronchiseptica}**

\textit{Bordetella bronchiseptica} is a gram-negative, short coccobacillar rod motile by means of peritrichous flagella (Breed \textit{et al.}, 1957). Richter and Kress (1967) found considerable variation in the size of \textit{B. bronchiseptica} grown on solid media. Cells measured from 3.0 by 0.5, 1.0 by 0.5 to 0.5 by 0.4 micrometers respectively. They found that the fine structure of \textit{B. bronchiseptica} closely resembled that of other gram-negative bacteria. The organism was said to possess: (1) a cell wall of 5 or more layers, (2) a trilaminar cell membrane, (3) abundant ribosomes embedded in the cytoplasmic matrix, (4) nucleoidal material consisting of dense bodies and fibrils, and (5) peritrichous flagella composed of interlacing strands.

Since 55 strain \textit{B. bronchiseptica} has been demonstrated to have some potential as a live intranasal vaccine, the electron microscope was used to further characterize this organism.
Strain B *B. bronchiseptica* (a swine virulent strain) was utilized as a control organism.

An average sized (0.4 x 0.7 micrometer), single coccobacillary 55 strain cell is pictured in Figure 3. This bacterium shows the convoluted surface typical of gram-negative bacteria. Richter and Kress (1967) described the surface of *B. bronchiseptica* as wrinkled, with lobules up to several hundred angstroms in width that are separated by furrows about 100 to 200 angstroms in width.

Filamentous organisms were regularly observed in negatively stained 55 strain TPB cultures. A representative 55 strain filamentous form showing the typical convoluted surface is shown in Figure 4.

Cell division with asymmetry in the size of the daughter cells was commonly observed in 55 and B strain cultures. Asymmetrical division showing daughter 55 strain cells with the membranous tubular connection still intact is pictured in Figure 5.

A longitudinal cross-section of B strain is shown in Figure 6. Retraction of the bacterial cell membrane and cytoplasm from the cell wall is evident. Richter and Kress (1967) state that retraction of the cell wall from the cell membrane may not always be an artifact, but may indicate impending disintegration of the cell wall. The nuclear zones in this electron micrograph are quite distinct. The nucleoplasm is whorled and the cell wall is furrowed.
Figure 3. Negatively stained 55 strain *Bordetella bronchiseptica*. Forty-eight hour blood agar culture. Note the convoluted surface. Cellular dimensions approximately 0.4 x 0.72 micrometers. X98,000. Bar equals 0.1 micrometer.

Figure 4. Negatively stained 55 strain *Bordetella bronchiseptica*. Twenty-four hour tryptose phosphate broth culture. Representative filamentous form. Many organisms were longer or shorter in the same culture. X17,000. Bar equals 1.0 micrometer.
Figure 5. Negatively stained 55 strain Bordetella bronchiseptica. Forty-eight hour blood agar culture. Asymmetrical division with membranous tubular connection (arrow). X85,000. Bar equals 0.1 micrometer.

Figure 6. Longitudinal cross-section of B strain Bordetella bronchiseptica. Forty-eight hour tryptose phosphate broth culture. Uranyl acetate and lead citrate stain. Note the furrowed cell wall. Appearance of the nucleoplasm is whorled and rarefied (N). Retraction (shrinkage) of the bacterial cell membrane and cytoplasm from the cell wall is evident (arrow). X90,000. Bar equals 0.1 micrometer.
A membranous lamellar structure\(^1\) oriented perpendicular to the cytoplasmic membrane is shown in Figures 7 and 8. Due to its central location and close association with the bacterial nucleoplasm the structure may be of mesosomal origin. Richter and Kress (1967) questioned whether previously described intracytoplasmic membranes were not actually mesosomes.

In cross section the cell wall has a multilaminar structure. Three osmophilic layers alternate with less opaque layers (Richter and Kress, 1967). Morse and Morse (1970) stated that the cell wall of *Bordetella pertussis*, in common with other gram-negative cells, is composed of 5 layers. The outer 3 layers were said to have the appearance of a unit membrane. The outer unit membrane is separated from a deeper, dense, mucoprotein layer by a less dense layer. A thin section of 55 strain illustrating the multilaminar nature of the cell wall is presented in Figure 9. Three dense layers separated by 2 less dense layers are seen. Richter and Kress (1967) determined that in perpendicular cross sections of fixed, embedded *B. bronchiseptica*, cell wall thickness was 100 to 200 angstroms.

Uniformly distributed fibrillar appendages\(^1\) were observed on negatively stained 55 and B strain cells from 24 hour blood agar cultures (Figures 10 and 11). These appendages were

\(^{1}\)A. E. Ritchie, National Animal Disease Laboratory, Ames, Iowa.
Figure 7. Longitudinal cross-section of B strain *Bordetella bronchiseptica*. Forty-eight hour tryptose phosphate broth culture. Uranyl acetate and lead citrate stain. Note membranous lamellar structure oriented perpendicular to the cytoplasmic membrane (arrow) and polar granular inclusion (I). Cellular dimensions approximately 0.4 x 1.0 micrometers. X120,000. Bar equals 0.1 micrometer.

Figure 8. Perpendicular cross-section of B strain *Bordetella bronchiseptica*. Forty-eight hour tryptose phosphate broth culture. Uranyl acetate and lead citrate stain. Note membranous lamellar structure (arrow) and apparent retraction of the bacterial cell membrane and cytoplasm from the cell wall. X210,000. Bar equals 0.1 micrometer.

Figure 9. Perpendicular cross-section of 55 strain *Bordetella bronchiseptica*. Forty-eight hour tryptose phosphate broth culture. Uranyl acetate and lead citrate stain. Note multilaminar structure of cell wall (arrow). Cell wall thickness approximately 160 angstroms. X176,000.
estimated to be less than 3 nanometers in diameter.\(^1\) Morse and Morse (1970) described a filamentous particle found in the supernatant fluids of phase I *Bordetella pertussis*. These filaments had a diameter of approximately 2 nanometers and ranged in length from 40 to 70 nanometers. They also observed these filaments in cultures of *B. bronchiseptica* and *Bordetella parapertussis*. The filaments were seen attached to the bacterial cell during the early stages of growth and numerous free filaments were found in the culture medium during the latter part of log phase growth. Morse and Morse (1970) concluded that the filaments were not readily comparable with any previously described bacterial structure.

The fibrillar appendages present on the 55 strain cell surface were considerably fewer in number than on the B strain cells. Figure 12 shows a portion of the cell wall of a B strain cell with the fibrillar appendages apparently emanating from the cell surface.

Labaw and Mosley (1955) reported that the flagella of *B. bronchiseptica* had the external contour of a counter-clockwise or left-handed triple helix. The average periodicity along the length of the flagella was measured as 190 angstroms with an average flagellar diameter of 139 angstroms. They estimated that the average length of *B. bronchiseptica* flagella was 17

\(^1\)A. E. Ritchie, National Animal Disease Laboratory, Ames, Iowa
Figure 10. Negatively stained B strain *Bordetella bronchiseptica*. Twenty-four hour blood agar culture. Note uniformly distributed fibrillar appendages (arrow). Cellular dimensions approximately 0.6 x 0.9 micrometers with fibrillar appendages less than 3.0 nanometers in diameter. X114,000. Bar equals 0.1 micrometer.

Figure 11. Negatively stained 55 strain *Bordetella bronchiseptica*. Twenty-four hour blood agar culture. Fibrillar appendages (arrow) present but considerably fewer in number than in Figure 11. Cellular dimensions approximately 0.6 x 1.0 micrometers. X88,000. Bar equals 0.1 micrometer.
micrometers. Richter and Kress (1967), however, found the flagellum to appear as braids with interlacing strands. They were unable to determine the exact number of strands, but were able to observe profiles of 5 to 6 strands per flagellum. The thickness was determined to be generally between 180 and 200 angstroms. A portion of a 55 strain flagellum is pictured in Figure 13. A 55 strain flagellated cell showing the hooked portion of a flagellum as it exits from the cell surface is shown in Figure 14.

A cellular surface protrusion of unknown significance is shown in Figures 15 and 16. This structure was frequently encountered in a negatively stained 55 strain 48 hour TPB culture. It was not observed in a similar B strain culture. The dimensions of the protrusion are approximately 0.25 x 0.25 micrometer. It is not evident by negative staining techniques whether the structure is of cytoplasmic, cell membrane or cell surface origin.

**Biological Comparison of 55, D-1 and B Strain Bordetella bronchiseptica**

Three strains of *B. bronchiseptica* were compared in biological and hemagglutination tests. All 3 strains (55, D-1 and B) are small gram-negative rods. The comparisons between 55, D-1 and B strain *B. bronchiseptica* are presented in Table 16.

Strain 55 differed from D-1 and B strains in a number of significant ways. These were as follows: (1) Strain 55 colonies on modified MacConkey's agar medium without Furaltaladone
Figure 12. Negatively stained B strain *Bordetella bronchiseptica*. Enlargement of portion of Figure 11 showing fibrillar appendages (arrow). X195,000. Bar equals 0.1 micrometer.

Figure 13. Negatively stained 55 strain *Bordetella bronchiseptica*. Twenty-four hour blood agar culture. Part of flagellum of approximately 135 angstrom diameter. A multistranded appearance is suggested. X230,000.

Figure 14. Negatively stained 55 strain *Bordetella bronchiseptica*. Twenty-four hour blood agar culture. Flagellated cell showing the hooked portion at the point of a flagellum's exit from the cell surface (arrow). X104,000. Bar equals 0.1 micrometer.
Figure 15. Negatively stained 55 strain *Bordetella bronchiseptica* from 48 hour culture in tryptose phosphate broth. Circular surface protrusion of unknown significance frequently encountered in this culture. X130,000. Bar equals 0.1 micrometer.

Figure 16. Negatively stained 55 strain *Bordetella bronchiseptica* from 48 hour culture in tryptose phosphate broth. Enlargement of circular surface protrusion present in another cell in the same culture as Figure 16. Dimensions of structure approximately 0.25 x 0.25 micrometer. X190,000. Bar equals 0.1 micrometer.
incubated at 37°C had a "lacy" margin. Strains D-1 and B colony margins were undulate. The colony diameter at 48 hours was approximately 1 ml. with colonies up to several millimeters in diameter present after prolonged incubation. All 3 strains formed colonies on 5 percent horse blood agar that were circular, smooth, opaque, and homogeneous with an entire edge. The colony size and morphology of the *B. bronchiseptica* strains varied greatly depending on the concentration of organisms on the culture plate, hours (days) of incubation at 37°C or room temperature and the relative humidity under which the culture plates were maintained. Aged colonies on modified MacConkey's agar without Furaltadone were characterized by central collapse of the colony and pronounced undulating rays from the cell margin giving the colony a "wagon wheel" shape. (2) Strains D-1 and B were motile while 55 strain was often nonmotile when examined by the hanging drop method. (3) Strain 55 was sensitive to Furaltadone at the 0.02 mg./ml. concentration in the modified MacConkey's medium while D-1 and B strain were not. (4) Strain 55 gave a slow and weak positive reaction (<24 hours) on Simmon's citrate agar slants while D-1 and B strains were strongly positive at 18 to 24 hours. (5) Strain 55 had the highest hemagglutinating titer among the 3 strains. Additionally, 55 strain, and D-1 strain as reported by Ross *et al.* (1967) have been shown to be incapable of causing turbinate atrophy in swine, while B strain (Ross *et al.*, 1963a) has been used to produce turbinate atrophy in experimental swine.
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DISCUSSION

In the past, the general approach to the control of infectious atrophic rhinitis has been to reduce the exposure of young pigs to possible infectious agents and to institute measures to increase the resistance of the pig. With the recent documentation that Bordetella bronchiseptica is the principal cause of infectious atrophic rhinitis epizootics in major swine producing areas it becomes logical to focus control efforts on this specific organism. Bordetella rhinitis control by the detection and elimination of infected swine was shown in the present work to be a useful procedure in many herds for reducing the incidence of B. bronchiseptica nasal infections and the elimination of clinical atrophic rhinitis.

Table 1 indicates that on the first nasal swab culture 12 percent of 3,304 breeding animals were culture-positive. The efficiency of B. bronchiseptica nasal infection detection in swine breeding stock by the nasal swab culturing procedure used in the present study was determined by compiling results obtained in 3 sequential samplings with the removal of culture-positive animals after each sampling. These results are presented in Table 1. It is apparent that 113 culture-positive animals were detected on the second and third tests after being called culture-negative on the previous tests. When this number (113) of culture-positive animals is adjusted to reflect the decreased sample size, it becomes 201. This
indicates that a single nasal swab culture was capable of detecting approximately 67 percent of the total number of infected animals detected by 3 samples. Of the 113 culture-positive animals detected on the second and third test, 40 escaped detection on the second test. If adjustment is made again for the decreased sample size then the second sampling had an efficiency of 62 percent. Assuming a 62 percent efficiency for the third sampling allows the determination that 3 nasal swab cultures have a detection accuracy of 94.7 percent.

Animals removed on the second and third nasal swab culture generally had lower culture plate *B. bronchiseptica* colony counts. *Bordetella bronchiseptica* may be present in such low numbers in these animals that it was beyond the detection ability of our sampling technique.

It was recommended that all sows and gilts be sampled an additional time within 2 weeks prior to farrowing. By assuming a 62 percent efficiency for the fourth sampling a 98.2 percent detection accuracy can be predicted for 4 nasal swab cultures.

A summary of the questionnaire response from 54 veterinary practitioners participating in the Bordetella rhinitis control project indicated that there was clinical improvement in 79 percent of those herds experiencing atrophic rhinitis epizootics to which the procedure was applied. This figure is considered to be highly significant considering the varying conditions under which the procedure was applied in the various herds.
Approximately 30,182 nasal swab samples from 152 herds were cultured in this laboratory in the course of the pilot field control project. One hundred and seven of the herds were culture-positive. A total of 76 of these isolates was tested for sulfamethazine resistance with 53 percent showing complete sulfamethazine resistance and 18 percent showing partial resistance. The rate of *B. bronchiseptica* isolation from all nasal swab specimens was found to be 11.6 percent.

The nasal swab culturing procedure in 25 herds cultured at this laboratory was shown to be 77 percent efficient in eliminating clinical atrophic rhinitis and 65 percent efficient in producing pigs that were culture-negative for *B. bronchiseptica* at weaning time.

It was not possible in the present study to obtain detailed evaluations of the economic performance of swine herds before and after application of the nasal swab culture procedure, however, the owners of most of the severely affected herds reported that they were able to reestablish their swine production on a profitable basis.

These results indicate that the nasal swabbing procedure is an effective tool for the control of clinical *Bordetella rhinitis* in suitable swine herds. It can be used in certain herds to eradicate the organism entirely from the swine herd by continual nasal swab culturing pressure and strict herd health management practices. Possible reinfection from uncontrollable external sources makes eradication of *B. bronchiseptica*
difficult to achieve and maintain for long periods of time. Nasal swab culturing serves an additional function as a diagnostic tool for the detection of *B. bronchiseptica* nasal infections in herds with and without clinical atrophic rhinitis.

In Experiment II, it was found that 50 percent of the *B. bronchiseptica* isolates from the initially sulfamethazine resistant infection had returned to complete or partial sensitivity within 2 months after removal of sulfamethazine medication. After 5 months without medication 100 percent of the infected pigs yielded isolates showing some degree of sulfamethazine sensitivity.

The natural clearing rate observed in Experiment II was supplemented with field infection rate incidence observations by Switzer and Farrington (1972) to formulate an expected natural clearing rate of *B. bronchiseptica* from the nasal cavity of swine. Their predicted natural clearing rate is reproduced in Figure 17.

The ability of swine to resist nasal infection by *B. bronchiseptica* with subsequent development of atrophic rhinitis probably involves several mechanisms. An age resistance to the development of turbinate atrophy occurs (Switzer, 1970b). Pigs infected after 3 to 5 weeks of life are expected to develop less severe lesions than those infected in the first 3 weeks of life. This is probably associated with the reduction in rate of tissue growth. Pigs infected after 8 to 10 weeks of age may develop inapparent infections and become carriers.
Figure 17. Expected natural clearing rate of *Bordetella bronchiseptica* from the nasal cavity of swine. (Reproduced from Switzer, W. P. and D. O. Farrington. 1972. *J.A.V.M.A.* 161(11):1327.)
PERCENT NASAL CAVITIES
POSITIVE FOR BORDETELLA BRONCHISEPTICA

AGE IN MONTHS

0 10 20 30 40 50 60 70 80 90 100
Harris and Switzer (1969) produced nasal and tracheal resistance against *B. bronchiseptica* reinfection in swine. They noted that swine freed from a virulent *B. bronchiseptica* infection by sulfonamide treatment or after natural clearing of low-virulence D-1 strain infection were extremely resistant to subsequent nasal challenge. Swine which had recovered from infection with D-1 strain were resistant to reinfection for up to 90 days post-nasal clearance. This resistance was not associated with high serum agglutinating antibody titers. Resistance was also not stimulated by intramuscular injection of a formalinized, whole-cell B strain *B. bronchiseptica* bacterin without adjuvant even though the bacterin induced high levels of circulating agglutinating antibody. Harris (1970) demonstrated that almost all of a virulent challenge inoculum disappears from the nasal cavities of D-1 strain cleared resistant swine in 2 to 6 hours after challenge while viable organisms were readily recovered from the nasal cavities of susceptible control animals.

Accelerated nasal clearance of *B. bronchiseptica* was induced in 2-to-8-week-old swine by the subcutaneous administration of a sonically disrupted and filtered D-1 strain vaccine and by a commercial pertussis vaccine\(^1\) (Harris and Switzer, 1972). The bacterins were incorporated in equal amounts of Freund's incomplete adjuvant and injected in 2 ml. dosages 6

\(^1\)Eli Lilly and Company, Indianapolis, Indiana.
to 8 days apart. Twenty-seven of 30 vaccinated pigs were free from nasal infection approximately 60 days post-challenge while 10 of 14 control pigs were still infected. They stated that the vaccines somehow stimulated recovery of the swine nasal cavity from *B. bronchiseptica* infection, but that this accelerated recovery differed from the complete refractiveness against the challenge organisms shown by low-virulence strain D-1 cleared resistant swine.

Yoda *et al.* (1972) suggested that an immune state developed in guinea pigs infected with *B. bronchiseptica*. It was noted that infected guinea pigs usually recover within 20 weeks. Most guinea pigs were found to be in a highly resistant state after recovery from the natural infection and that this acquired resistance to nasal and tracheal infection lasted as long as 40 weeks or more. Few of the recovered guinea pigs demonstrated a measurable agglutinin titer. Nikkels and Mullink (1971) controlled *B. bronchiseptica* pneumonia outbreaks in an SPF guinea pig breeding colony by the use of an autogenous bacterin. They stated that a single dose of vaccine mixed with Freund's incomplete adjuvant not only controls losses due to pneumonia but may even eradicate the organism.

Ross *et al.* (1967) demonstrated that D-1 strain was regularly eliminated from the swine nasal cavity by 6 to 7 weeks post-inoculation and was incapable of producing turbinate atrophy. Harris (1970) compared the phagocytosis of D-1 and B strain *B. bronchiseptica* by swine polymorphonuclear (PMN)
leucocytes. Strain D-1 was ingested by the leucocytes at a significantly higher rate than B strain. He stated the PMN leucocytes present in the nasal cavity of pigs may remove D-1 strain infections more effectively than B strain infections and that this mechanism may be responsible for the more rapid natural clearance of D-1 strain. It was considered possible that the low-virulence strains were being phagocytized more rapidly than the virulent strains and that this reduction of the number of organisms present prevented the occurrence of turbinate atrophy. An increased rate of phagocytosis of *B. bronchiseptica* on the mucous surface may account for the increased clearance rate of nasal infection observed in pigs injected with a suitable *B. bronchiseptica* bacterin.

The mechanism of the local resistance of the nasal cavity of swine cleared from *B. bronchiseptica* infection is apparently different than the previous defense system. *Bordetella bronchiseptica* has been shown to attach to swine nasal epithelial cells (Harris, 1970). Holt (1972) reported that *B. bronchiseptica* adhered readily to live cultures of human fibroblasts. Gibbons (1973) considered bacterial adherence the first prerequisite for the successful colonization of a mucosal surface and that attachment ability was a critical determinant of the virulence of a particular organism. One of the early tissue damages in the area of a *B. bronchiseptica* microcolony on ciliated epithelium is the disruption of the cilia (Duncan and Ramsey, 1965). This affords a *B. bronchiseptica* microcolony an additional
defense against removal. Harris (1970) postulated that substances may be present in the respiratory tract secretions of pigs that may inhibit the attachment of *B. bronchiseptica* to nasal and tracheal epithelium. Such an inhibition of attachment provides a mechanism that explains the resistance of the ciliated mucosa of the nasal cavity to *B. bronchiseptica* infection. This inhibition could be due to a blockage of the receptor sites on either the bacteria or the epithelial cells. Williams and Gibbons (1972), based on the information that SIgA mediates a local immune system, suggested that the mechanism of SIgA activity is the inhibition of bacterial adherence to mucous surfaces. The possible blockage of bacterial attachment sites by SIgA and other factors present in the nasal secretions of pigs with subsequent bacterial disposal by removal on the mucociliary escalator, places the ciliated mucosal surface in perspective as the first line of immunological defense against *B. bronchiseptica* respiratory infections.

Parenteral immunization of 4-to-8-week-old swine with whole-cell bacterins was examined in Experiment III. At 12 weeks post-challenge 4 out of 13 (30 percent) experimental pigs receiving D-1 sonicate, whole-cell, D-1 whole-cell and 55 strain whole-cell bacterins with adjuvant were culture-positive for *B. bronchiseptica* while 6 out of 7 (86 percent) controls were culture-positive. This indicated that these whole-cell bacterins stimulated some degree of accelerated nasal clearance. The failure of the remaining bacterin to produce resistance to
infection or to significantly hasten nasal clearance may be related to production strain, lack of hemagglutinin content and/or the absence of an adjuvant.

Experiment IV further evaluated the potential of D-1 sonicate, whole-cell bacterin with adjuvant as a parenteral immunizing agent in 3 and 6 week old pigs. At 2 weeks post-challenge 4 out of 11 (36 percent) vaccinated pigs were culture-positive while 13 out of 13 (100 percent) of the controls were culture-positive. This resistance waned in the vaccinated pigs until at 8 weeks post-challenge 7 out of 11 (64 percent) were culture-positive. This waning of resistance is not consistent with the results observed in Experiment III. The 3-week-old immunized pigs failed to develop significant serum PAST titers. The 6-week-old pigs receiving the nonadjuvanted bacterin also failed to maintain a high serum PAST titer for the duration of the experiment. This failure to obtain consistent high PAST serum titers may account for the loss of resistance observed in this trial.

In Experiment V an attempt was made to clear virulent B strain infection from the nasal cavity of swine by the administration of D-1 sonicate, whole-cell bacterin without (1st dose) and with (2nd dose) adjuvant. This attempt was not successful. The infected immunized pigs cleared at about the same rate as the control pigs. This is consistent with the results of Harris and Switzer (1972).

The pigs in Experiment VI were challenged with B strain
B. bronchiseptica at a 2 week midpoint between doses of D-1 sonicate bacterin with AB-65 adjuvant. Accelerated nasal clearance of B. bronchiseptica was induced as all pigs had cleared the nasal infection by 9-12 weeks. The average PAST serum titer at the termination of the trial was 1201. All 7 pigs had grossly normal turbinates at necropsy.

In experiments III, IV and VI certain of the parenteral bacterins have shown some degree of accelerated nasal clearance of B. bronchiseptica nasal infections and have deterred gross turbinate atrophy. All pigs (28 of 28) immunized with D-1 sonicate, whole-cell bacterin with adjuvant prior to nasal challenge had grossly normal turbinates while 27 percent (4 of 15) of the nonimmunized controls had gross turbinate atrophy.

Holt (1972) stated the current practice of vaccinating infants subcutaneously with Bordetella pertussis vaccines may induce a therapeutic immunity and not a prophylactic immunity; that is, such vaccination may not protect against infection, but does protect against deeper tissue injury. This also appears to be the case with the D-1 sonicate, whole-cell bacterin with adjuvant failing to protect pigs against infection, but providing protection against deeper tissue damage (turbinate atrophy).

Intranasal immunization of 4-to-8-week-old pigs was evaluated in Experiment VII. Live, low-virulence 55 strain B. bronchiseptica TPB culture was instilled intranasally in pigs with subsequent B strain challenge. Strain 55 intranasal vaccination
prevented the establishment of a swine virulent B strain *B. bronchiseptica* nasal infection with no evidence of a persistent 55 strain infection. Intranasal immunization was reevaluated in Experiment VIII using 3-to-4-week-old pigs. Live, low-virulence strain 55 TPB culture intranasal vaccination again prevented the establishment of a swine virulent B strain *B. bronchiseptica* nasal infection. Sterile filtrates from 55 strain *B. bronchiseptica* 24 hour TPB cultures did not prevent the initial establishment of a *B. bronchiseptica* nasal infection, but by 6 weeks post-challenge 5 of 6 pigs were cleared from the infection. Live, low-virulence 55 strain intranasally vaccinated pigs were challenged twice more at 8 and 17 weeks post-intranasal immunization with no evidence of a *B. bronchiseptica* nasal infection. These results establish that live, low-virulence 55 strain intranasal vaccination of pigs 3 weeks of age or older prevented the establishment of virulent B strain *B. bronchiseptica* nasal infection following experimental challenge.

Necropsy examination of the pigs from Experiments VII and VIII revealed that all (10 of 10) of the live, low-virulence 55 strain intranasally immunized pigs had normal nasal cavities while 71 percent (5 of 7) of the control pigs had mild to severe gross turbinate atrophy. All of the pigs receiving the 55 strain TPB culture sterile filtrate had grossly normal turbinates. Strain 55 *B. bronchiseptica* was not detected in the nasal cavity of intranasally immunized swine as early as 6 days post-intranasal instillation of the vaccine. The
resistance developed against *B. bronchiseptica* nasal infections following intranasal immunization was not associated with significant levels of circulating agglutinating antibody.

Murray (1973) states that evidence now exists that there is a distinct immunological system at the mucous surfaces of most species and that this system plays a major protective role in host resistance. The major secretory and protective antibody found on the mucous surfaces is IIgA. The site and nature of the local immune response is influenced by a number of factors including the route of administration of antigen, the quantity and persistence of antigen at the mucosal surface, the method of presentation of the antigen, and the type of antigen used, e.g., live or dead. Live antigen was said to have a major advantage as live organisms may multiply and persist for long periods thereby producing higher and longer lasting levels of local antibody. Murray (1973) further states that immunization of local mucous surfaces has many disadvantages such as problems in adequate delivery of the antigen. Ogra and Karzon (1969) demonstrated that the output of SIgA antibody from mucosal surfaces is anatomically restricted to the site where antigen stimulation has occurred and that there is no good evidence of immunological memory at mucous surfaces as demonstrated by a secondary response. Nash and Holle (1973) did observe some evidence of a local memory effect. Murray (1973) recommended that for the optimum immunization of local mucous surfaces maximum dissemination of antigen to all parts of the system should be
accomplished and that immunological memory should be stimulated by systemic means. Live antigens, especially viruses, can fulfill both criteria following application at the mucous surface, but inactivated antigens may require a parenteral route of immunization as well in order to ensure the development of an immunological memory.

In experiments IX and X the potential of immunizing baby pigs with live 55 strain intranasal vaccine was explored. In Experiment IX the 55 strain intranasal vaccine appeared to protect pigs nursing a nonimmunized sow from turbinate atrophy even though a B. bronchiseptica nasal infection became established. Intranasally vaccinated pigs nursing an immunized sow withstood establishment of a B. bronchiseptica nasal infection better than the pigs not receiving colostral antibody.

The results of experiment X indicate the following:

1. The use of AB-65 adjuvant in the bacterin produced a high titer in only 2 of the 3 immunized gilts.
2. The PAST serum titers in pigs nursing these immunized gilts was lower than that obtained in the pigs nursing the immunized sows in Experiment IX.
3. The administration of live, low-virulence 55 strain intranasal vaccine at 2 to 3 days of age with or without a booster dose at 21 or 35 days resulted in a reduction of nasal infection following B strain challenge. Forty-three percent of the nasal swab cultures obtained from the intranasally immunized pigs nursing immunized gilts and 21 percent of those obtained from intranasally immunized pigs nursing nonimmunized
gilts were culture-positive. The figures were 83 percent and 89 percent respectively for the nonimmunized control pigs. Five of 6 intranasally vaccinated groups manifested resistance to *B. bronchiseptica* nasal infection through 14 weeks post-challenge. The resistance of pigs nursing gilt 1 (immunized) and intranasally vaccinated at 2, 3 and 21 days diminished between the 10th and 14th week samplings. (4) Nonintranasally vaccinated pigs nursing gilt 3 (immunized) were infected at about the same rate as nonintranasally vaccinated pigs nursing gilt 4 (nonimmunized) but the 2 groups of pigs developed a different degree of turbinate atrophy. One of 4 pigs nursing gilt 3 had moderate gross ventral turbinate atrophy at necropsy while 2 of 3 pigs nursing gilt 4 had severe (almost complete absence of nasal turbinates) turbinate atrophy. (5) Thirty-two of 33 (97 percent) intranasally immunized pigs nursing either immunized or nonimmunized gilts were protected against turbinate atrophy, while 3 of 7 (43 percent) of the pigs not intranasally immunized but nursing either immunized or nonimmunized gilts developed gross turbinate atrophy. (6) Pigs nursing gilt 6 (nonimmunized) that were intranasally immunized at 2, 3 and 35 days and challenged with B strain at 45 days was a successful third demonstration that intranasal immunization of 4-to-7-week-old pigs farrowed by nonimmunized dams (Experiments VII and VIII) were resistant to the establishment of *B. bronchiseptica* nasal infection for up to 12 weeks post-intranasal immunization. (7) No signs of clinical atrophic rhinitis were observed at any
time in any of the 55 strain B. bronchiseptica intranasally vaccinated groups of pigs. Clinical signs were observed in two of the seven nonintranasally vaccinated control pigs.

A summation of the results obtained from intranasally vaccinated pigs in Experiments IX and X indicates that pigs nursing immunized dams had 30 percent (35 of 115) positive cultures, while pigs nursing nonimmunized dams had 34 percent (36 of 107) positive cultures. It appears that the immune state of the dam did not affect either the incidence of infection or the development of turbinate atrophy in intranasally immunized pigs. However, immunization of the dam did reduce the incidence and severity of turbinate atrophy if the pigs were not intranasally immunized. In Experiments IX and X intranasally immunized baby pigs nursing immunized dams had a zero percent (0 of 16) incidence of gross turbinate atrophy while intranasally immunized baby pigs nursing nonimmunized dams had a 4 percent (1 of 26) incidence of gross turbinate atrophy. The nonintranasally immunized controls in Experiment X had an incidence of 25 percent (1 of 4) and 67 percent (2 of 3) respectively.

Live, low-virulence 55 strain intranasal vaccination appears to have protected pigs from turbinate atrophy caused by B. bronchiseptica even though some of the pigs did become infected. This is indicated by the fact that pigs of all ages in Experiments VII, VIII, IX and X that were intranasally vaccinated with live, low-virulence 55 strain TPB culture had an incidence of gross turbinate atrophy of less than 2 percent.
(1 of 52) while the controls from these experiments had an incidence of 57 percent (8 of 14).

Smith (1972) reported that the resistance of newborn pigs to infectious atrophic rhinitis can be raised by breeding from older sows, restricting the intake of gilts into the breeding herd, mixing replacement gilts with older pigs before and during pregnancy, selecting strains with inherent resistance and by immunization of the dams. Passively acquired maternal antibodies were pictured as being an important factor in the overall resistance of a newborn pig to atrophic rhinitis.

Kemeny (1973) found that pigs reared without colostrum developed turbinate atrophy more frequently than pigs fed either immune or nonimmune colostrum. He noted that parenteral immunization induced much higher agglutination titers in the serum of dams than did colonization of the respiratory tract with live \textit{B. bronchiseptica} organisms. Parenterally vaccinated gilts also had a much higher titer of antibody in their colostrum after parturition.

There is presently no evidence to indicate the persistence in or transfer between swine of 55 strain \textit{B. bronchiseptica}. In the absence of demonstrable swine to swine transfer for the determination of potential increased virulence of 55 strain the organism was back passaged through 11 passages in 6-to-8-day old embryonating hens eggs and introduced into the nasal cavity of susceptible 3-week-old pigs in Experiment XI. Strain 55 \textit{B. bronchiseptica} killed 100 percent of the embryos by 72 hours
in the first passage. Swine virulent B strain *B. bronchiseptica* regularly kills 100 percent of 6-to-8-day-old chicken embryos by 24 hours post-yolk sac inoculation. An increase in the virulence (earlier mortality) of 55 strain for chicken embryos was not evidenced through 11 serial passages.

Strain 55 eleventh passage egg yolk inoculum was not detected in the nasal secretions of the intranasally inoculated pigs at 1 or 2 weeks post-inoculation. Swine receiving the 55 strain eleventh egg passage inoculum were not completely refractory to subsequent B strain *B. bronchiseptica* nasal challenge but cleared the nasal infection by 5 weeks post-challenge. This may indicate that the serial embryonated hens egg passage reduced the antigenicity of the 55 strain *B. bronchiseptica*.

Electron microscopic examination of 55 and B strain *Bordetella bronchiseptica* revealed that morphologically they closely resembled another strain of *B. bronchiseptica* (R-21) described by Richter and Kress (1967). Strain 55 and B strain individual coccobacillary cells varied in size from 0.4 by 0.72 to 0.6 x 1.0 micrometers. The nuclear zones of *B. bronchiseptica* were quite distinct and consist of whorls or of a network of fibrils and dense bodies. The convoluted surface was typical of gram-negative bacteria, the cell wall was composed of 5 layers, the flagella had a multistranded appearance and nucleoidal material consisted of fibrils and dense bodies.

Strain 55 and B strain organisms were morphologically similar. Fibrillar appendages were more numerous on the B
strain organisms. Individual 55 strain cells may have large numbers of these appendages but on the average they were quantitatively fewer in 55 strain 24 hour blood agar cultures. Morse and Morse (1970) in discussing the biological properties of filamentous particles found in the supernatant fluids of phase I Bordetella pertussis cultures stated that induction of lymphocytosis and histamine-sensitizing activity was associated only with those gradient fractions in which these filaments were found. The degree of lymphocytosis and histamine-sensitizing activity paralleled the concentration of the filaments in the preparation. *Bordetella bronchiseptica* does not have lymphocytosis promoting activity (Morse and Morse, 1970).

The cellular protrusions of unknown significance encountered in a negatively stained 48 hour TPS culture of 55 strain were not observed in similar preparations of B strain. This may be a morphologically unique structure in strain 55. The exact origin of the circular protrusion was not ascertained.

The colony morphology of 55 strain on modified MacConkey's agar medium without Furaltaadone was modified as compared to D-1 and B strains. However, due to the wide variation in colony morphology and rapid phase variations observed with *B. bronchiseptica* isolates, depending on the cultural environment, colony morphology is not a satisfactory criteria for strain differentiation.

Motility was absent in some 55 strain cultures while D-1 and B strain organisms were uniformly motile. Strain 55 was
sensitive to the 0.02 mg./ml. Furaltaladone concentration in modifi
cified MacConkey's agar medium. This is significant as it allows
discrimination to be made between 55 strain and most other
known *B. bronchiseptica* isolates.

Strain 55 gives a weak and slow positive reaction in
Simmon's citrate agar. This may be a matter of cell concentra-
tions in the inoculum and/or growth inhibition.

Strain 55 had the highest hemagglutination titer among the
three strains. Harris (1970) determined that 48 hour blood
agar cultures of D-1 and B strain *B. bronchiseptica* had HA
titers of 128 and 64 respectively. He found a decrease in HA
titer in both D-1 and B strains on continued passage on artifi-
cial medium. The hemagglutinating ability of *B. bronchiseptica*
isolates does not appear to be a characteristic associated with
virulence for swine as both 55 and D-1 strains are more potent
hemagglutinating organisms than swine virulent B strain. The
hemagglutination titers were reduced by increased time of incu-
bation on the blood agar and tryptose blood agar base (without
blood) media.
SUMMARY

*Bordetella bronchiseptica* rhinitis control in swine breeding herds by culturing of 3 nasal swabs from each animal with subsequent elimination of culture-positive animals was evaluated. A total of 30,182 nasal swab samples from 152 swine herds were cultured. *Bordetella bronchiseptica* nasal infection detection by 3 nasal swab cultures was determined to be 94.7 percent accurate. Response from 54 veterinary practitioners participating in the *Bordetella* rhinitis control project indicated there was a reduction in clinical atrophic rhinitis in 79 percent of those herds utilizing the procedure. Thirteen of 17 (77 percent) herds cultured by this laboratory eliminated clinical atrophic rhinitis and 15 of 23 (65 percent) produced pigs that were culture-negative for *B. bronchiseptica* at weaning age. *Bordetella bronchiseptica* isolates from 76 herds were tested for sulfamethazine resistance. Fifty-three percent were resistant, 18 percent had partial resistance and 29 percent were sulfamethazine sensitive.

A group of pigs infected with a *B. bronchiseptica* isolate that was sulfamethazine resistant, yielded *B. bronchiseptica* with some degree of sulfamethazine sensitivity after the pigs had been removed from the drug for 5 months.

Four of 13 (30 percent) 4-to-8-week-old pigs parenterally immunized with *B. bronchiseptica* whole-cell bacterins with adjuvant were culture-positive for *B. bronchiseptica* 12 weeks
post-challenge while 6 of 7 (86 percent) controls were culture-positive. At 2 weeks post-challenge 4 of 11 (36 percent) 3-to-6-week-old pigs parenterally immunized with sonicated, whole-cell bacterin with adjuvant were culture-positive for *B. bronchiseptica* while 13 of 13 (100 percent) of the controls were culture-positive. Resistance waned in this group of pigs. At 8 weeks post-challenge 7 of 11 (64 percent) were culture-positive. In 4 trials, all pigs (28 of 28) immunized with sonicated, whole-cell bacterin with adjuvant prior to nasal challenge had grossly normal turbinates while 27 percent (4 of 15) of the nonimmunized controls had gross turbinate atrophy.

Live, low-virulence 55 strain intranasal vaccination of pigs 3 weeks or older prevented the establishment of virulent *B. bronchiseptica* nasal infection following experimental challenge with no evidence of a persistent 55 strain nasal infection. Gross necropsy examination of the intranasally vaccinated pigs revealed that all (10 of 10) of the pigs had normal nasal cavities while 71 percent (5 of 7) of the nonintranasally vaccinated control pigs had mild to severe turbinate atrophy.

Intranasally vaccinated baby pigs that nursed immunized dams had 30 percent (35 of 115) positive cultures while 34 percent (36 of 107) of the pigs nursing nonimmunized dams had positive cultures. Some of these pigs were subjected to constant exposure to the infected control pigs. Intranasally vaccinated baby pigs nursing immunized dams had a zero incidence (0 of 16) of gross turbinate atrophy at necropsy while intranasally vaccinated baby pigs nursing nonimmunized dams had a
4 percent (1 of 26) incidence. The nonintranasally immunized controls had an incidence of 25 percent (1 of 4) and 67 percent (2 of 3) respectively. It appears that the immune state of the dam did not affect either the incidence of infection or the development of turbinate atrophy in intranasally immunized pigs. However, immunization of the dam did reduce the incidence and severity of turbinate atrophy if the pigs were not intranasally immunized.

Live, low-virulence 55 strain intranasally vaccinated pigs appear to have been protected against turbinate atrophy caused by virulent \textit{B. bronchiseptica} even though some of the pigs did become infected. Pigs of all ages that were intranasally vaccinated with live, low-virulence 55 strain TPB culture had an incidence of gross turbinate atrophy of less than 2 percent (1 of 52) while the control pigs had an incidence of 57 percent (8 of 14). The 55 strain \textit{B. bronchiseptica} isolate was not detected on nasal swab culture 6 days post-vaccination.

Electron microscopic examination of the 55 strain immunizing and the B strain virulent challenge \textit{B. bronchiseptica} revealed that they both closely resembled previously described \textit{B. bronchiseptica}. 
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