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STRUCTURAL AND FUNCTIONAL EFFECTS OF BORDETELLA AVIUM INFECTION IN THE TURKEY RESPIRATORY TRACT

Iowa State University PH.D. 1987

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Structural and functional effects of *Bordetella avium* infection in the turkey respiratory tract

by

William George Van Alstine

A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of the
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GENERAL INTRODUCTION

The poultry industry in the United States accounts for approximately 11 billion dollars worth of poultry products each year and the turkey industry contributes 20 percent of the total product value.[1] Large flocks, multiple ages of turkeys on a farm, intensified rearing practices, and concentrated production in limited geographic areas have contributed to increased incidence of multiple respiratory infections. The total loss from disease and management related problems costs the poultry industry approximately 2 billion dollars each year.[1] Respiratory infections account for 50 percent of the losses due to mortality and morbidity.[1]

Bacterial pathogens, such as Bordetella avium and Escherichia coli, are important causes of turkey respiratory disease. Turkey coryza, caused by B avium, is a highly contagious upper respiratory disease of turkeys and chickens. It has been reported in most major turkey producing regions of the United States.[2] In the 1983 Iowa turkey health survey, turkey coryza and colibacillosis-coryza syndrome were estimated to be the most important causes of monetary loss from disease in Iowa market turkeys (W. J. Owings, Department of Poultry Science, Iowa State University, Ames, IA, 1983). Turkey coryza was classified as 1 of the top 10 priorities for turkey respiratory disease research in 1986.[1]

Turkey coryza is characterized clinically by excessive

oculonasal discharge, sneezing, tracheal collapse, and decreased rate of weight gain.[3-5] Although B avium is noninvasive, it persistently colonizes the ciliated epithelium of the nasal cavity and trachea leading to inflammation of the upper airways, loss of ciliated epithelial cells, and accumulation of nasal and tracheal mucus.[3,6] Morphological and functional alterations caused by B avium infection in the turkey lung have not been described.

Mechanisms responsible for damage to the ciliated epithelium are not known, but a bacterial toxin has been incriminated in several studies.[7,8] Heat-labile toxin, prepared from sonicated live B avium, is lethal for turkeys when injected parenterally, but the contribution of this toxin to the clinical signs and tracheal lesions of turkey coryza is unknown.[9]

Turkey coryza can be a clinically mild disease with low mortality, but infected turkeys may be predisposed to other respiratory pathogens.[4] Excessive mortality has been reported when B avium-infected turkeys are exposed to other respiratory pathogens.[10-12] Escherichia coli, the cause of avian colibacillosis, is the most common secondary bacterial pathogen isolated from B avium-infected turkeys.[2] Vaccination of B avium-infected turkeys with live Newcastle disease virus vaccine can result in high death losses due to colibacillosis.[12] Mechanisms by which B avium predisposes

turkeys to colibacillosis and other respiratory diseases are not known.

The objectives of this study were to i) evaluate the effects of B avium infection on the pulmonary clearance of E coli in turkeys, ii) determine if B avium influences the adherence and colonization of E coli in the turkey trachea, iii) describe the pulmonary lesions and changes in bronchus-associated lymphoid tissue in turkeys infected with B avium, and iv) characterize the morphological and metabolic alterations caused by B avium heat-labile toxin on turkey tracheal organ cultures.

This dissertation is presented in the alternate format and consists of four manuscripts submitted to refereed scientific journals. The format used is that of the American Journal of Veterinary Research. The review of the literature precedes the first manuscript. The first, second, and third manuscripts have been submitted for publication to the American Journal of Veterinary Research. The fourth manuscript has been accepted for publication in Avian Diseases. A general summary and discussion follow the last manuscript. A list of references appears at the end of each manuscript. Literature cited in the general introduction, literature review, and general summary and discussion appears at the end of the dissertation.

The Ph.D. candidate, William George Van Alstine, was the principal investigator for each study.

LITERATURE REVIEW

Turkey coryza - Turkey coryza, caused by Bordetella avium, is a highly contagious upper respiratory disease of turkeys characterized clinically by excessive oculonasal discharge, sneezing, coughing, dyspnea, tracheal collapse and decreased rate of weight gain.[3,4,11,13-16] Morbidity within B avium-infected flocks often reaches 100%, but mortality usually remains low unless turkeys are concurrently infected with a second respiratory pathogen.[4,17] Chickens and quail can be infected with B avium, but clinical signs and lesions are far less severe than in turkeys.[18-23]

Initial searches for the cause of this disease centered on isolating respiratory viruses. Respiratory adenoviruses were associated with the disease, but turkey coryza could not be consistently reproduced with these viruses.[24,25] In 1967, Filion et al. described 2 outbreaks of respiratory disease in Canadian turkeys with clinical signs similar to turkey coryza.[26] An organism similar to Bordetella bronchiseptica was isolated from the affected turkeys and the disease was experimentally reproduced in inoculated and contact turkeys.[26] In 1978, Hinz et al. reported outbreaks of upper respiratory disease in West Germany and isolated a Bordetella bronchiseptica-like bacillus from the respiratory tracts of affected turkeys.[17] This bacillus was used to reproduce the disease in turkeys, and the disease was termed

turkey bordetellosis.[17] In 1979, Simmons et al. isolated a gram-negative bacillus from naturally infected turkeys in North Carolina and concluded this organism was the primary etiologic agent of turkey coryza.[5] Based on physical and biochemical properties, Simmons et al. identified this organism as Alcaligenes faecalis and termed the disease alcaligenes rhinotracheitis.[27] Since 1979, a gram-negative, motile, aerobic, nonfermentative bacillus has been isolated from affected turkeys throughout the United States.[9,15,24,28] This organism is now classified as Bordetella avium based on morphological, physiological, biochemical, and serological studies, and analysis of bacterial DNA and RNA composition.[29] The disease is called turkey coryza.[30]

Gross lesions found in turkeys infected with B avium typically include excessive foamy ocular and nasal exudate, excessive thick tracheal mucus, tracheal collapse, mild submandibular swelling, and staining of periocular, cervical and wing feathers by brownish exudate.[3,6]

Like other Bordetella species, B avium has specific tissue tropism for ciliated respiratory epithelium.[3] Colonization of the nasal cavity and trachea by B avium causes mild catarrhal rhinitis and fibrinopurulent tracheitis within the first 10 days of infection.[3-6,16] From 14 to 21 days postinfection (PI), tracheal lesions include multifocal loss of ciliated cells, epithelial hyperplasia, depletion of

mucus, and diffuse infiltration of lymphocytes and macrophages into the mucosa.[3,6,16] From 21 to 40 days PI, the tracheal mucosa is composed of basophilic, low columnar to cuboidal, nonciliated cells; squamous metaplasia occurs sporadically.[3,6] Mucus glands become cystic and lined by cuboidal epithelium.[3] Tracheal rings may flatten dorsoventrally and become severely distorted.[3,6] By 53 days PI, tracheas return to normal except for residual distortion of tracheal rings and shortened luminal epithelium.[3,6] Bordetella avium can be isolated from infected tracheas for at least 53 days PI.[3] Histological evaluations of lung of B avium-infected turkeys have been included in several early studies of turkey coryza, but pulmonary lesions attributed to B avium infection were either not found or not reported.[4,31-33]

Attempts at treatment and prevention of turkey coryza have met with only limited success in commercial turkey flocks.[13,34,35] Bordetella avium can persist at least 6 months in litter, and can survive wide ranges of temperature (10 C - 40 C) and humidity (32% - 78%).[13,32,34] Because B avium is resistent to many environmental influences, antibiotic therapy and vaccination have been the focus of control measures. Antibiotics, administered orally, parenterally, or as an aerosol, cause only temporary remission of clinical disease.[16,35,36] Vaccination of turkey

breeder hens with B avium bacterins has increased survival rates, improved growth rates, and delayed the onset and severity of clinical disease in progeny of vaccinated hens.[13,37] Vaccination of young turkeys with a temperature-sensitive mutant, attenuated live cultures, or formalin-inactivated cultures of B avium produce incomplete or short-lived protection.[38-41] These procedures are usually not sufficient to eliminate the disease from a flock.[34]

Isolates of B avium have been divided into 2 groups based on colony morphology, biochemical profiles, and hemagglutination reactions.[42,43] Type I isolates, described by Jackwood et al.[42], are similar to group I isolates described by Rimler and Simmons.[43] Type I (group I) isolates are pathogenic for turkeys. These isolates agglutinate erythrocytes of turkeys, chickens, sheep, and guinea pigs, and form round, raised, colorless colonies on bovine blood agar and MacConkey agar. Type II isolates are nonpathogenic for turkeys and do not agglutinate erythrocytes. Colonies of type II isolates are larger than those of type I isolates. Type II isolates form round, raised, opaque, mucoid colonies on bovine blood agar and MacConkey agar.[42] Rimler and Simmons[43] reported that group II isolates have colony morphology similar to type II isolates and do not agglutinate erythrocytes; however, some group II isolates are pathogenic for turkeys. Type I and type II isolates can also be

distinguished by comparing the cellular fatty-acid content of the bacteria.[30]

Potential virulence factors of B avium include: hemagglutinins[44], plasmids for antibiotic resistance[45,46], lipopolysaccharide[7], histamine-sensitizing factors[47], heat-labile dermonecrotic toxin[9], heat-stable toxin[48], and surface pili.[49] However, the contribution of these factors in the pathogenesis of turkey coryza is unknown. Bordetella avium may bind via a surface ligand to a monosaccharide receptor on tracheal epithelial cells or to N-acetylneuraminic acid and D-galactose moieties of tracheal mucus, but the mechanism by which B avium adheres to tracheal epithelium is incompletely understood.[7,50]

Adherence of B avium causes ciliostasis and loss of ciliated cells in turkey tracheal organ cultures and degeneration and loss of ciliated cells in vivo.[7,8,51] Mechanisms responsible for the local effects of B avium infection on the trachea are unknown. Bordetella avium is noninvasive yet marked alteration of the tracheal mucosa results from bacterial colonization. Such local injury is suggestive evidence for bacterial toxins as mediators of local tracheal lesions[7], and adherence of the bacteria to tracheal epithelium appears to be a prerequisite for local cytopathic effects.[7] One study, utilizing turkey tracheal organ cultures, suggested that acute lethal injury to

ciliated cells appeared to be induced by B avium itself or toxic bacterial metabolites rather than the host's tissue response to the colonizing bacteria.[8]

Several physiological changes have been found in turkeys infected with B avium. McCorkle et al. reported that tissue levels of norepinephrine, dopamine, and serotonin were lower in B avium-infected turkeys compared to B avium-free controls after administration of alpha methylparatyrosine. Based on these findings, they suggested that postganglionic sympathetic neurons in poults may be involved in the pathogenesis of turkey coryza.[52] In another study, McCorkle et al. found increased levels of serum corticosterone in B avium-infected turkeys compared to normal turkeys.[53] Simmons et al. described a histamine-sensitizing factor similar to that reported for Bordetella pertussis and suggested this factor may be responsible in part for the excessive lacrimation and mucus production observed in B avium-infected turkeys.[47] Edens et al. found depressed colonic and foot pad temperatures in B avium-infected turkeys and suggested that endotoxin was responsible for this change.[54] In each instance, the authors suggested these effects may be due to the action of a toxin. However, no toxin which induces these effects has been isolated, and the relationship of heat-labile toxin or heat-stable toxin to the clinical disease or to these physiological effects is

unknown.[9,48] Whether these physiological changes are specific for B avium infection or represent a nonspecific response to any bacterial infection of the respiratory tract is unknown.

Based on field observations, B avium-infected turkeys may have impaired resistance to other infectious diseases.[2] Simmons et al. reported that B avium infection caused impaired function of the cellular immune system as evidenced by depressed in vitro lymphocyte stimulation by concanavalin A and disrupted thymic architecture.[55] However, later studies using delayed hypersensitivity[56], graft versus host reaction[57], leucocyte migration[58], and anaphylactic response[59] in B avium-infected turkeys demonstrated normal or enhanced cellular immune responses. No definitive impairment of the immune system has been demonstrated in B avium-infected turkeys.[2]

Uncomplicated infections of B avium cause a mild disease which, in most cases, results in low mortality.[4,13] However, serious losses may result when B avium-infected turkeys are simultaneously exposed to other respiratory pathogens such as adenovirus[10], Yucaipa virus[11,60], Newcastle disease virus[61], CELO virus[10], Mycoplasma gallisepticum[10], and Mycoplasma meleagridis.[10] Escherichia coli is the most common secondary bacterial invader, but other gram-negative bacteria such as Pasteurella multocida can concurrently infect B avium-

infected turkeys.[2] Vaccination of B avium-infected flocks with live Newcastle disease virus can result in high death losses due to colibacillosis.[12] The mechanisms by which B avium predisposes turkeys to other infectious agents have not been characterized.

Avian colibacillosis - Colibacillosis, caused by Escherichia coli, is a serious and potentially fatal disease of chickens and turkeys and is responsible for major economic losses in the poultry industry.[62] Infection with E coli can produce a variety of lesions in poultry including enteritis[63], salpingitis[64], airsacculitis[65], colisepticemia[66], osteomyelitis and synovitis[67], coligranuloma[68], and cloacal bursitis.[69] Three forms of respiratory disease have been described in naturally-occurring colibacillosis.[70] First, colisepticemia occurs in young birds and causes typical lesions of acute gram-negative bacterial septicemia affecting the respiratory tract, heart, liver, spleen, and lymphoid tissues. The second and most common form of respiratory disease is subacute fibrinopurulent polyserositis and air sacculitis in birds surviving acute septicemia. A third and sporadic form of E coli infection is chronic granulomatous pneumonitis, hepatitis, and enteritis in flocks some time after acute septicemia or subacute polyserositis.[70]

Attenuation of normal pulmonary defense mechanisms may

be important in the pathogenesis of avian colibacillosis. Epizootics of E coli infection are commonly associated with viral or mycoplasmal respiratory disease, vaccination, and environmental stress.[64,71-73] Bordetella avium infection is considered to be one of the most important predisposing factors involved in outbreaks of colibacillosis following Newcastle disease virus vaccination in turkeys.[74] Colibacillosis is believed to occur in turkeys by inhalation of poultry house dust contaminated with feces. Infection may extend from the lower respiratory tract into the bloodstream.[70,75] Pathogenic serotypes of E coli are commonly found in poultry house dust and litter.[76,77] Escherichia coli septicemia has been reproduced experimentally by exposing turkeys to virulent serotypes of E coli via air sac, intratracheal, aerosol, and oral routes.[64,69,78,79] The mechanism whereby E coli enters the bloodstream from the respiratory tract remains a mystery; however, E coli bacteremia is necessary for most, if not all, manifestations of colibacillosis in turkeys.[80]

Strains of E coli vary in their capacity to produce disease.[81] This variation may be due in part to differences in their capacity to adhere to the respiratory mucosa. Virulent strains of E coli are more capable of colonizing chicken tracheal epithelium compared with avirulent strains.[82] This colonization is considered an important

initial step in the pathogenesis of colibacillosis in the chicken.[82] Type 1 (F1) fimbriae can promote adherence of E coli to urinary tract and oral epithelium in mice and humans by binding to mannose residues on mucosal epithelial cell membranes.[83-85] However, the involvement of F1 fimbriae in the pathogenesis of disease in mammals and poultry is controversial.[86,87] Naveh et al. reported that E coli expressing type 1 fimbriae adhere to chicken tracheal epithelial cells; however, they did not prove that type 1 fimbriae were responsible for the adherence.[88] Higher morbidity and mortality were reported in chickens inoculated with E coli expressing type 1 fimbriae compared with chickens inoculated with nonfimbriated E coli.[88] Gyimah and Panigrahy reported partial protection in chickens challenged with pathogenic E coli after vaccination with a vaccine containing type 1 fimbriae prepared from E coli homologous to the challenge strain.[89] In this study, neither E coli adherence nor clearance in the respiratory tract were evaluated.

Most strains of E coli which are virulent for turkeys produce type 1 fimbriae when cultured in vitro.[81,90] In turkeys, type 1 fimbriae do not play a significant role in adherence of pathogenic E coli to intestinal epithelium, but may promote bacterial trapping or adherence to intestinal mucus.[91] Antibodies to type 1 fimbriae do not enhance clearance of fimbriated E coli from the bloodstream.[92] The

role of type 1 fimbriae in the adherence of E coli to the tracheal mucosa of turkeys is unknown.

Other mechanisms of virulence for E coli have been proposed. Virulent E coli resist the bactericidal effects of serum and resist phagocytosis by producing exopolysaccharide capsular material and external carbohydrate moieties of lipopolysaccharide. The roles of plasmid-mediated bacterial products such as toxins, adhesins, and siderophores as virulence factors have been reviewed.[62,69,80]

Anatomy of the avian respiratory tract - The respiratory system in turkeys begins at the nostrils and consists of the nasal cavities, oropharynx, trachea, bronchi, lungs, and air sacs. The trachea is composed of complete interlocking cartilage rings and is covered by pseudostratified ciliated columnar epithelium with numerous shallow mucous glands.[93-95] The distal end of the trachea connects to the syrinx just inside the thoracic inlet. Two extrapulmonary primary bronchi arise at the syrinx. These are relatively short and enter the lungs on the ventromedial aspect. Structurally, the extrapulmonary bronchi resemble the trachea.[93,94]

Instead of dicotymously branching airways terminating in alveoli as in mammalian lung, the avian lung is composed of a series of interconnecting bronchi.[94] The tubular system of the lung consists of 3 bronchial subdivisions: the intrapulmonary primary bronchus, various secondary bronchi,

and many parabronchi (tertiary bronchi). The cartilage rings of the extrapulmonary primary bronchi are replaced by a layer of smooth muscle in the intrapulmonary primary bronchi. Within the lung, the mucosa of the primary bronchi is composed of low columnar, ciliated, pseudostratified epithelium with goblet cells and occasional mucous glands. Primary bronchi pass through the lung with a slight S-bend while giving rise to several groups of secondary bronchi before entering the abdominal air sacs.[93-95] Each secondary bronchus gives rise to many parabronchi. Parabronchi, which communicate with several secondary bronchi, contain numerous invaginations or atria that lead to smaller round openings termed infundibula.[93,95] Extending from the infundibula are the air capillaries, anastomosing cylindrical tubes which function in gaseous exchange.[93] Air sacs are thin walled extensions of the respiratory tract connecting to the lung by the primary and secondary bronchi and the parabronchi.[93,95]

In the chicken, lymphoid nodules project into the airways at the bifurcations of the primary and secondary bronchi.[96] The epithelium over this bronchus-associated lymphoid tissue (BALT) is flattened, lacking in cilia and goblet cells, and is similar to lymphoepithelium of mammalian BALT.[96] Microfold (M) cells within the lymphoepithelium are believed to selectively transfer soluble and particulate antigens from the airway lumen into the internal lymphoid

environment.[96] Below the epithelium, BALT consists of a well organized reticulin network filled with lymphocytes and macrophages.[96] In the chicken, lymphocytes in BALT commonly express immunoglobulin A on the cell surface, but immunoglobulins are only rarely found within lymphocytes.[97] Although antigen does not appear to be necessary for the development of BALT, antigen is necessary for expanding the size and number of lymphoid aggregates.[97] Unlike mammalian BALT, germinal centers are commonly found in BALT of chickens.[96] Similar BALT has not been described in the turkey lung.

PAPER I. EFFECTS OF *BORDETELLA AVIUM* INFECTION ON THE PULMONARY CLEARANCE OF *ESCHERICHIA COLI* IN TURKEYS

Effects of *Bordetella avium* infection on the pulmonary clearance of *Escherichia coli* in turkeys

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SUMMARY

One-day-old turkeys were inoculated intranasally with Bordetella avium. Noninoculated hatchmates were housed separately. At 2 and 4 weeks of age, B avium-infected (BA+) and B avium-free (BA-) turkeys were exposed to an aerosol of virulent Escherichia coli. The remaining turkeys were used as controls not exposed to E coli. Turkeys were necropsied on postaerosolization days 0 (immediately after aersolization), 1, 3, 5, and 7. Lung and tracheal samples were collected from each turkey for bacterial quantitation and histopathologic examination. A 1-ml blood sample was collected for detection of bacteremia. Numbers of E coli in lung from both 2 and 4-week-old turkeys were not significantly different between BA+ and BA- groups using pooled data over time, however numbers of E coli isolated from trachea were significantly greater in BA+ turkeys compared with BA- turkeys at most sample times. Pulmonary abcesses and E coli bacteremia were much more frequent in 2-week-old turkeys than in 4-week-old turkeys, but no differences were found between BA+ and BA- turkeys. Air sacculitis was more common and more severe in BA+ turkeys at both ages. Hyperplastic bronchus-associated lymphoid tissue was more common in BA+ turkeys than in BA- turkeys and appeared to be the first site of heterophil infiltration following E coli aerosolization.

INTRODUCTION

Turkey coryza, alcaligenes rhinotracheitis, and avian bordetellosis are terms used to describe a specific upper respiratory disease of young turkeys and chickens caused by Bordetella avium (Alcaligenes faecalis).[1-4] This highly contagious disease is characterized by bacterial colonization of the nasal and tracheal mucosae, rhinitis, conjunctivitis with frothy exudate, tracheitis, tracheal collapse, and decreased weight gain.[1,3] Although turkey coryza is usually associated with low mortality, affected turkeys may be predisposed to E coli infection resulting in increased mortality and condemnations.[1]

The high incidence of E coli infection in B avium-infected turkeys and a report of simultaneous seroconversion to both Newcastle disease virus (NDV) and B avium suggest a potential interaction between these respiratory pathogens.[5] In flocks where B avium is endemic, increased mortality due to E coli is seen following Newcastle disease virus vaccination.[6] Other pathogens which have been isolated from B avium-infected flocks include adenovirus, CELO virus, and Mycoplasma meleagridis.[7]

The purpose of this study was to determine the effects of B avium infection (turkey coryza) on pulmonary and tracheal clearance of pathogenic E coli.

MATERIALS AND METHODS

Experimental design - From a group (n=72) of 1-day-old turkeys (Nicholas strain) of both sexes, 1 group of 36 turkeys was inoculated intranasally with B avium; the remaining 36 turkeys were raised as B avium-free controls. At 2 weeks of age, 15 B avium-infected (BA+) turkeys and 15 B avium-free (BA-) turkeys were exposed to an aerosol of pathogenic E coli. Three BA+ turkeys and 3 BA- turkeys were randomly selected for necropsy from each treatment group 1 day prior to aerosolization and at postaerosolization day (PAD) 0, 1, 3, 5, and 7. Three turkeys from each group were necropsied and cultured prior to aerosolization to confirm that BA- turkeys were free of B avium and that turkeys were free of E coli. Turkeys necropsied at PAD 0 were killed and sampled within 30 to 60 minutes after aerosolization. A blood sample was collected for detection of bacteremia, and gross pathologic changes were recorded. Lung and trachea were collected from each turkey for bacterial quantitation and histopathologic examination. The above procedures were repeated using 18 BA+ and 18 BA- turkeys when the remaining 36 turkeys were 4 weeks old.

Bacteria and culture conditions - Turkeys were inoculated with B avium strain 838 as previously described.[3] Briefly, 0.05 ml of inoculum was placed on one nostril for inhalation into the oronasal cavity. The inoculum contained

1.2×10^7 colony-forming units (CFU) of B avium per ml in phosphate buffered saline solution (PBSS).

The E coli strain, EC1 (078:K80:H9:F1), is pathogenic for turkeys and has been used in a previous experiment.[8] A stock culture maintained at -60 C was thawed and used to inoculate 3 tissue culture flasks containing 60 ml of brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI), and a sample of the stock culture was streaked onto solid medium to verify a pure culture. The flasks were incubated aerobically at 37 C with agitation for 24 hours. Cultures were centrifuged at 3000 x g and the bacterial pellet was resuspended in PBSS to the original volume. The bacterial suspension was maintained at 5 C and used for aerosolization within 1 hour of preparation. For aerosolization of 2-week-old and 4-week-old turkeys, the E coli suspension contained 2.6×10^9 CFU/ml.

Aerosolization of E coli - BA+ and BA- turkeys were commingled in an aerosol chamber (0.35 m^3) with air exchange of 4 liters per minute. The E coli suspension was administered through an ultrasonic nebulizer (Model 65, DeVilbis Co., Somerset, PA) with power adjustment and air flow at maximum settings. The nebulizer delivered 125 ml of E coli suspension over a period of 30 minutes. At the end of aerosol exposure, the chamber was exposed to ultraviolet light for 10 minutes before turkeys were removed.

Necropsy procedure - At each necropsy time, 3 BA+ and 3 BA- turkeys were selected at random. A blood sample (1 ml) was aseptically collected from the wing vein, and the turkeys were anesthetized with pentobarbital sodium (5-10mg) given intravenously. The femoral vessels were severed and the turkeys were maintained with the cranial end elevated to facilitate exsanguination. Turkeys were immersed in a disinfectant solution for 1 minute before exposure of the body cavities. Gross lesions in the thoracic and abdominal viscera were noted. A 1 cm section of the midcervical trachea was removed aseptically, opened longitudinally, and placed in 9 ml cold (5 C) PBSS for bacterial quantitation. The left lung was removed aseptically and placed in 45 ml cold PBSS for homogenization and bacterial quantitation. The lower trachea, right lung and air sacs were perfused with 10% neutral buffered formalin and fixed in situ for 30 minutes before being removed from the turkey.

Bacterial quantitation - Blood samples were placed in 3 ml of BHI broth and incubated at 37 C for 24 hours. Tubes with turbidity were subcultured onto solid media for bacterial identification. Tracheal sections, each in 9 ml PBSS, were mixed on a vortex-mixer (Vortex-Genie, Fisher Scientific, Pittsburgh, PA) at maximum speed for 1 minute. A 1-ml sample of PBSS from each tracheal segment was serially diluted 10-fold 3 times, and 50 ul of each dilution were

placed as a band on MacConkey medium. Lungs were homogenized for 1 minute with a motorized tissue grinder (Model 23, The Virtis Co., Gardiner, NY) operated at 70% power. Lung homogenates were serially diluted 10-fold 3 times and 50 ul of each dilution was placed on MacConkey medium. Additionally, 0.5 ml of the lowest dilution of lung and tracheal tissues was inoculated onto MacConkey medium. All plates were incubated 36 hours at 37 C and colonies were counted for determining CFU/cm of trachea and CFU/lung. Each colony morphotype was tested by slide agglutination with antisera specific for B avium or E coli EC1.

Microtiter agglutination test - Serum was harvested from 6 of 36 2-week-old and 6 of 36 4-week-old turkeys prior to E coli aerosolization. Sera were tested for antibody to E coli by microtiter agglutination as previously described,[9] except that live E coli EC1 cells in 2% NaCl were used as the antigen. The sera were also tested for antibody to avian mycoplasmas (bacterial agglutination) and NDV (microtiter hemagglutination-inhibition test) (Dr. M. S. Hofstad, VMRI, Iowa State University, Ames, IA).

Histopathology - Lung and tracheal tissues were fixed 48 hours in 10% neutral-buffered formalin, paraffin embedded, sectioned at 3 um and stained with hematoxylin and eosin by routine methods. For each turkey, 2 sections of trachea and 2 sections of lung were examined.

Statistical analysis - Numerical data means from the 30 turkeys exposed to E coli in each experiment were evaluated for significant differences by the F test (Statistical Analysis System, ANOVA procedure).

RESULTS

Bacterial numbers in tissue - Numbers of E coli in lungs from 2-week-old and 4-week-old turkeys were not significantly different ($P > 0.3$) when BA+ and BA- groups were compared using data pooled over time (Figs 1 & 2). An initial decrease in E coli numbers was followed by stable or increased numbers at PAD 3 in 2-week-old turkeys and PAD 5 in 4-week-old turkeys.

The number of E coli isolated from trachea was significantly greater ($P < 0.05$) in BA+ turkeys compared with BA- turkeys at most sample times. In 2-week-old turkeys, numbers of E coli in trachea generally increased with time. At PAD 3 to 7, E coli numbers were significantly greater ($P < 0.05$) in BA+ turkeys compared with BA- turkeys (Fig 3). In 4-week-old turkeys, numbers of E coli were significantly greater ($P < 0.05$) in tracheas of BA+ turkeys compared with BA- turkeys at most sample times (Fig 4). Escherichia coli was not detected in tracheas of BA- turkeys after PAD 5. Numbers of E coli were generally greater in the tracheas of 4-week-old BA+ turkeys compared with 2-week-old BA+ turkeys.

Detection of bacteremia after E coli aerosolization was much more frequent in 2-week-old turkeys than in 4-week-old turkeys (Table 1). All positive blood cultures produced a single colony morphotype on MacConkey medium and colonies from each culture agglutinated with antisera specific for

E coli EC1.

Bordetella avium was isolated from all turkeys inoculated with B avium. Numbers of B avium ranged from 10^5 - 10^7 CFU/cm trachea. Colonies isolated from each turkey agglutinated with antiserum specific for B avium.

Lungs of both 2 and 4-week-old BA+ turkeys contained 10^3 - 10^6 CFU of B avium per lung. At PAD 7, B avium was isolated (10^5 CFU/cm) from trachea, but not from lung, in 2, 2-week-old turkeys that had not been inoculated with B avium. Bordetella avium was not isolated from 4-week-old BA- control turkeys.

Lesions - Gross lesions were restricted to the respiratory tract and were similar in 2 and 4-week-old turkeys. All BA+ turkeys had mucopurulent oculonasal exudate and increased tracheal mucus. Mild to severe dorso-ventral flattening of the trachea was present in 15 of 30 BA+ turkeys. BA- turkeys had no gross lesions of rhinitis or tracheitis.

Histological changes in the tracheas of BA+ turkeys were similar to those previously described[3] and included mild suppurative tracheitis with loss of ciliated epithelial cells, depletion of mucus, bacterial colonization, and diffuse infiltration of mononuclear cells in the lamina propria. Nonciliated epithelial cells were hyperplastic in the tracheas of 4-week-old turkeys. Tracheas of BA- turkeys

had no lesions B avium infection.

Tracheal lesions following E coli aerosolization were similar in all BA+ turkeys and consisted of low numbers of heterophils in the lamina propria at PAD 1 through PAD 7. Tracheas of BA- turkeys had a mild infiltration of heterophils in the lamina propria at PAD 1, but not at later times.

The predominant lung lesion B avium-infected turkeys was massive peribronchial lymphocytic hyperplasia (Fig 5) around the intrapulmonary primary bronchus. This lesion persisted throughout the experiment in 27 of 30 BA+ turkeys; only 9 of 30 BA- turkeys had similar lesions (Table 1).

Lung lesions following E coli aerosolization were present at all PAD sample times. At PAD 0, locally-extensive heterophil infiltrates, within and around peribronchial lymphoid nodules (Figs 6, 7), were accompanied by mild diffuse heterophil infiltration throughout the lung. By PAD 1 most of the inflammatory response centered around primary bronchi. The peribronchial connective tissue contained serofibrinous exudate and massive infiltrates of heterophils with fewer macrophages. Bacteria were rarely found within the lesions. Similar, but less severe changes were present in adjacent secondary bronchi. Microabscesses, more common in 2-week-old turkeys than in 4-week-old turkeys, were found around primary bronchi or within secondary bronchi (Table 1).

From PAD 3 to 7, the acute suppurative inflammatory response gradually subsided and macrophages and lymphocytes became the predominant inflammatory cells.

Forty-nine of the 60 turkeys exposed to the E coli aerosol developed airsacculitis (Table 2). Mild lesions, common in BA- turkeys, involved only the thoracic and/or abdominal air sacs. Mildly affected air sacs were slightly cloudy and contained occasional 1-3 mm white to yellow nodules composed of aggregations of macrophages and lymphocytes. Epithelial surfaces were separated by serofibrinous fluid and small numbers of heterophils and macrophages diffusely infiltrated the interstitium.

Severe air sac lesions were more common in BA+ than in BA- turkeys and more common in 2-week-old than in 4-week-old turkeys (Table 2). Severe air sac lesions usually involved all air sacs and occasionally the pericardium. The air sacs were opaque to white and contained multiple 5-7 mm raised yellow caseous nodules. Microscopically these nodules were pyogranulomas and lymphoid nodules in the connective tissue of the air sacs. Although bacteria were present in some pyogranulomas, no bacteria were isolated from these lesions.

Serology - All sera were negative for antibody to E coli EC1, Mycoplasma meleagridis, M. gallisepticum, M. synoviae, and Newcastle disease virus.

TABLE 1 - Incidence of pulmonary lesions and *E coli* bacteremia

Turkeys	Peribronchial lymphoid hyperplasia	Pulmonary abscesses	*E coli* bacteremia
2-week-old BA+	12/15	9/15	9/15
2-week-old BA-	5/15	8/15	8/15
4-week-old BA+	15/15	5/15	2/15
4-week-old BA-	4/15	4/15	1/15

BA+ = *B avium*-infected; BA- = *B avium*-free

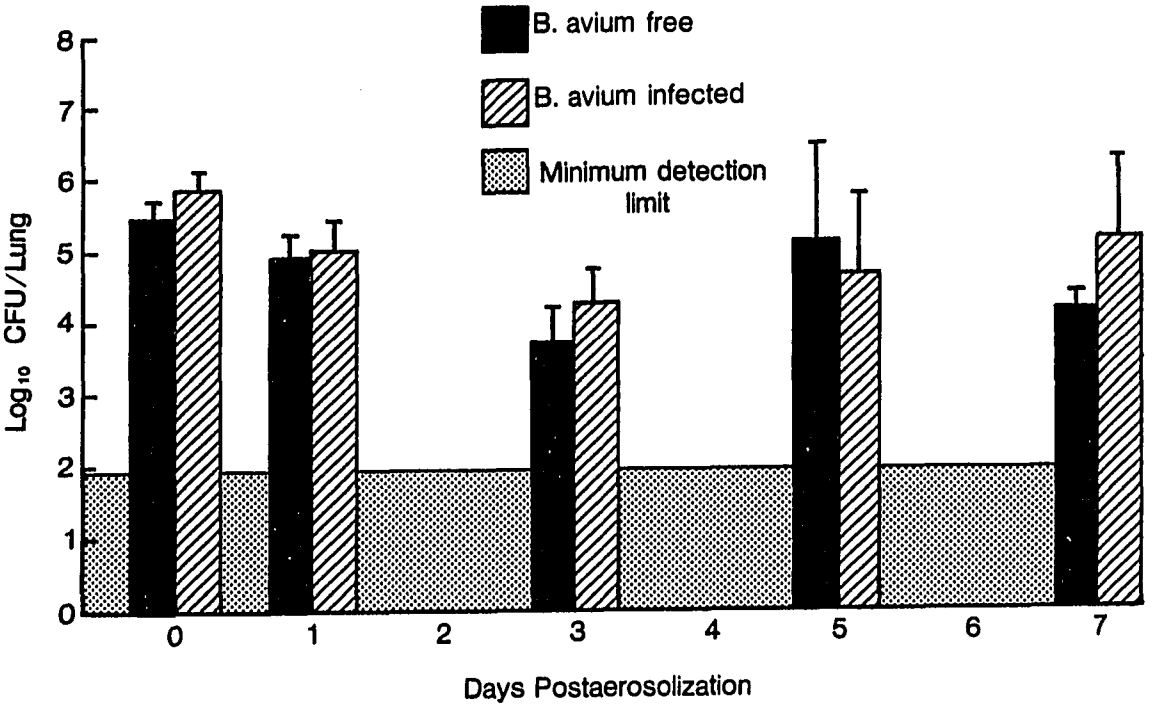
TABLE 2 - Incidence of air sac lesions

Turkeys	Mild	Severe
2-week-old BA+	5/15	9/15
2-week-old BA-	9/15	3/15
4-week-old BA+	10/15	5/15
4-week-old BA-	8/15	0/15

BA+ = *B avium*-infected; BA- = *B avium*-free

Fig 1 - Recovery of viable *E coli* from lungs of 2-week-old turkeys. Data expressed as the mean (n=3) + standard error of the mean

Fig 2 - Recovery of viable *E coli* from lungs of 4-week-old turkeys. Data expressed as the mean (n=3) + standard error of the mean


B. avium free
B. avium infected
Minimum detection limit
Log10 CFU/Lung
0
1
2
3
4
5
6
7
8
0
1
2
3
4
5
6
7
Days Postaerosolization

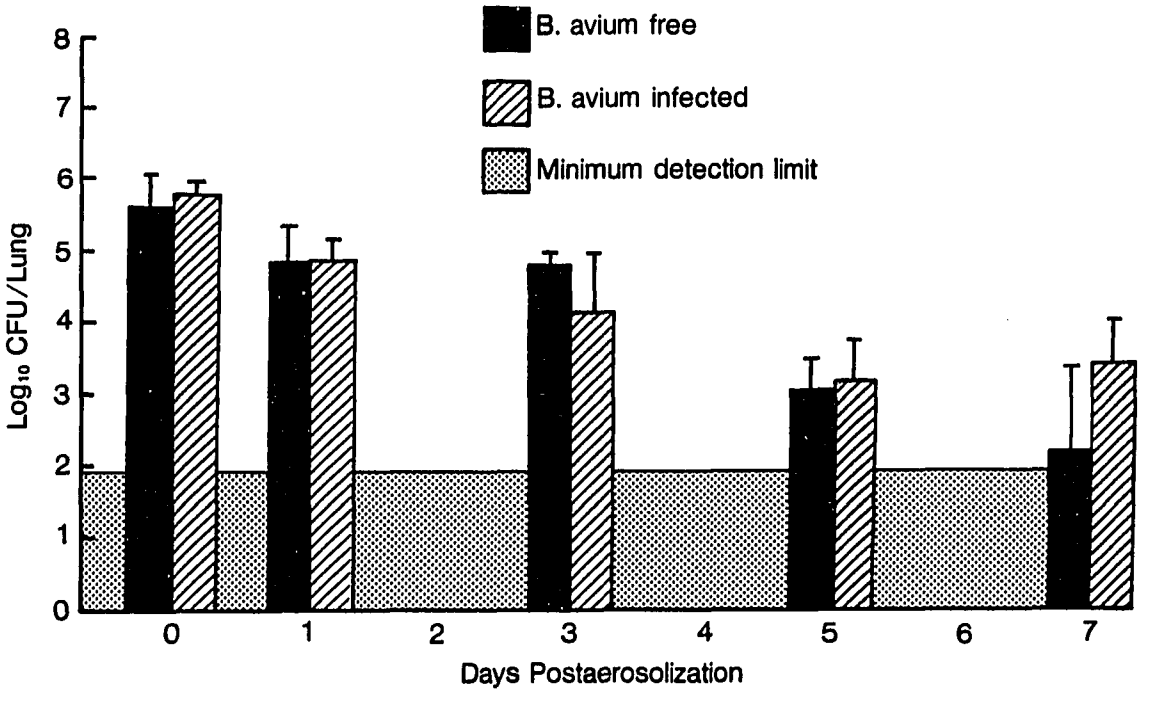
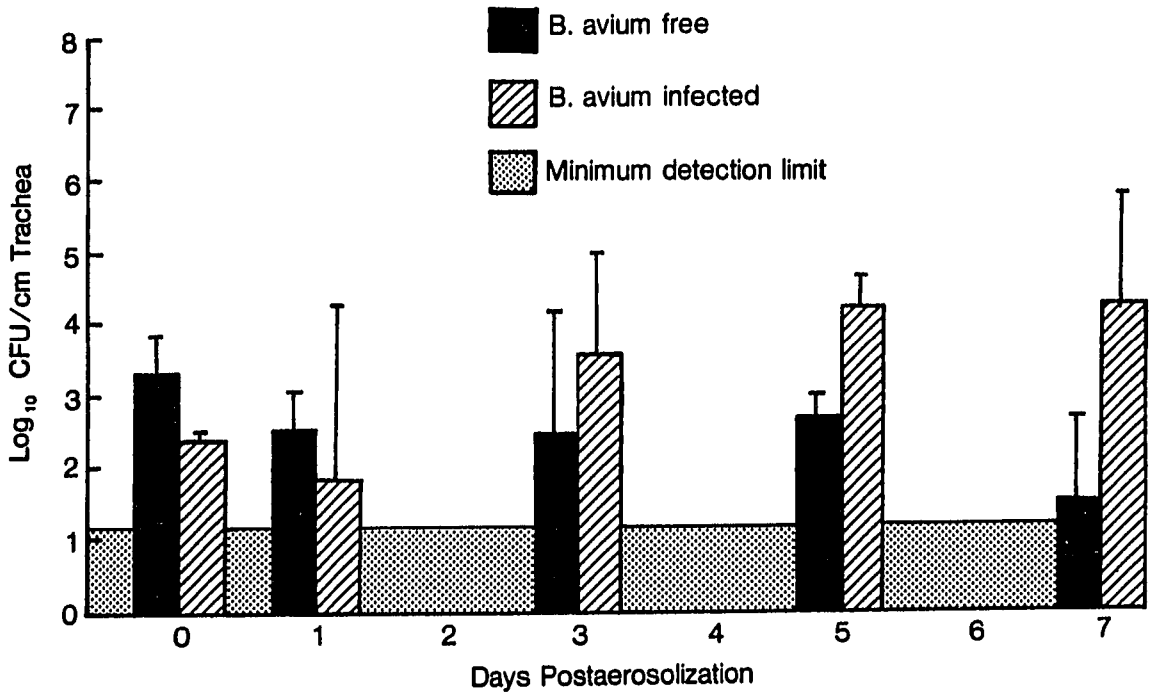

B. avium free
B. avium infected
Minimum detection limit
Log10 CFU/Lung
0
1
2
3
4
5
6
7
8
0
1
2
3
4
5
6
7
Days Postaerosolization

Fig 3 - Recovery of viable E coli from tracheas of 2-week-old turkeys. Data expressed as the mean (n=3) + standard error of the mean

Fig 4 - Recovery of viable E coli from tracheas of 4-week-old turkeys. Data expressed as the mean (n=3) + standard error of the mean. Escherichia coli was not isolated from tracheas of B avium-free turkeys at 7 days postaerosolization


B. avium free
B. avium infected
Minimum detection limit
Log10 CFU/cm Trachea
0
1
2
3
4
5
6
7
8
Days Postaerosolization

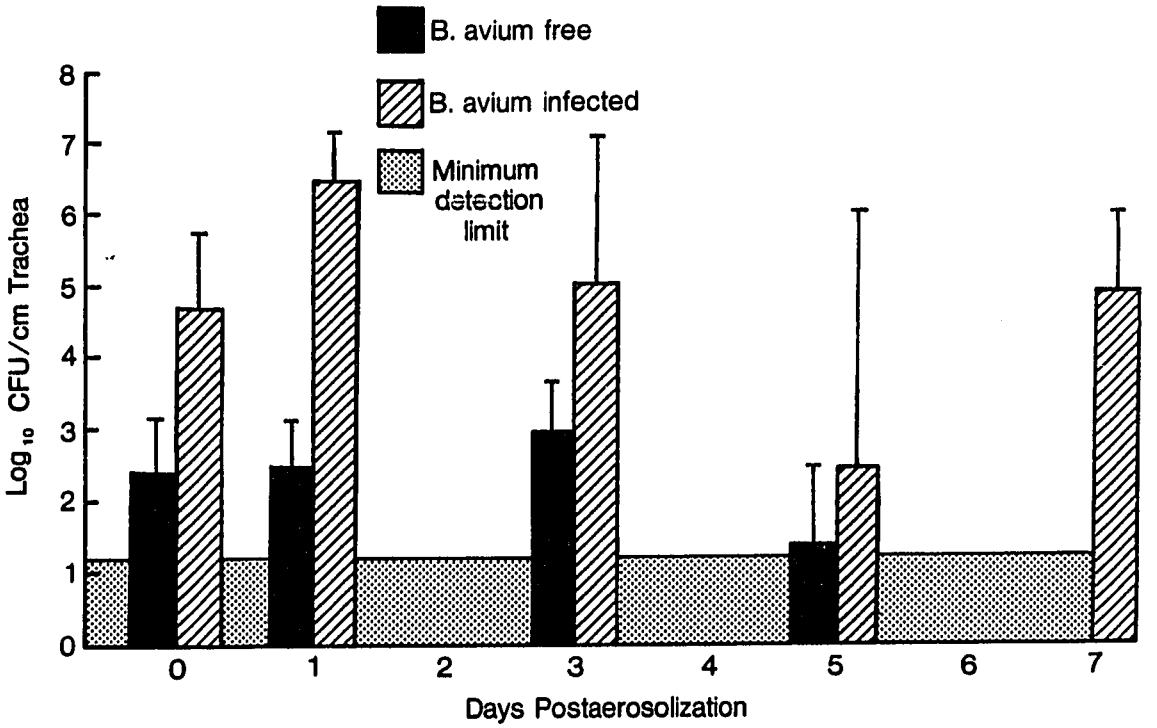
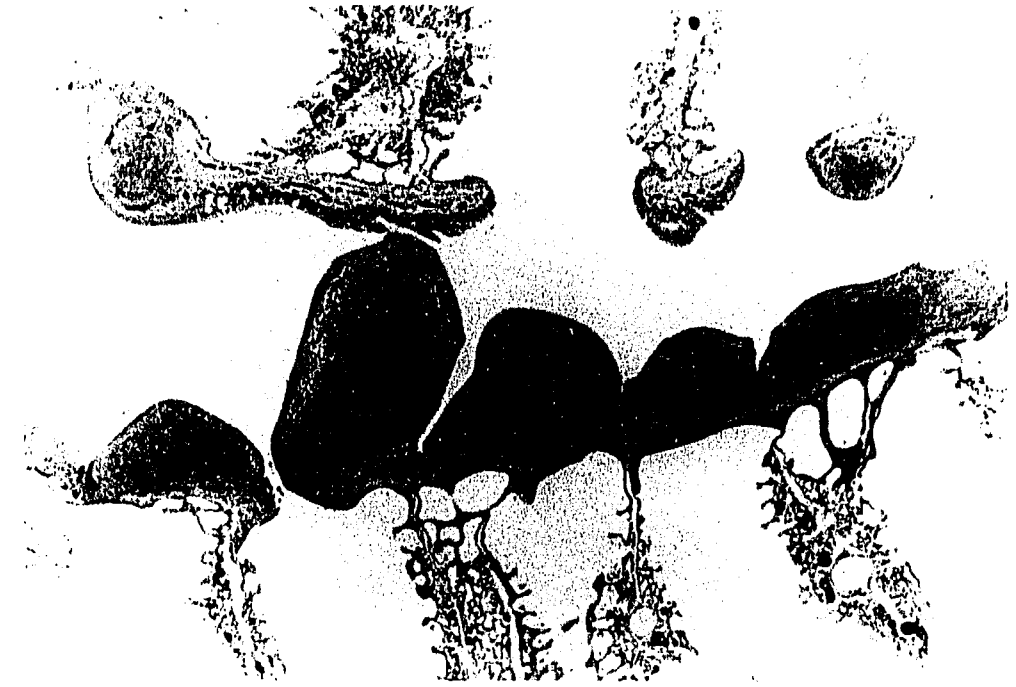

B. avium free
B. avium infected
Minimum detection limit
Log10 CFU/cm Trachea
0
1
2
3
4
5
6
7
8
Days Postaerosolization

Fig 5 - Peribronchial lymphocytic hyperplasia in the primary bronchus of B avium-infected turkey. H&E stain; X 70

Fig 6 - Heterophils (inset) infiltrating a peribronchial lymphoid nodule following E coli aerosolization at PAD 0. H&E stain; X 450



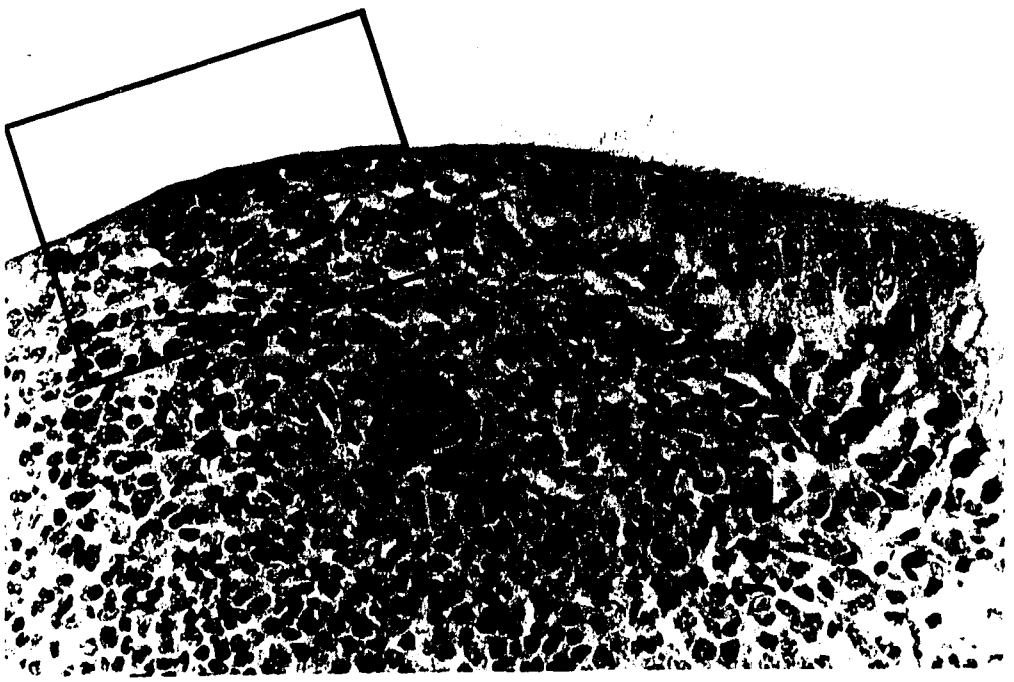


Fig 7 - (Inset of Fig 6) - Heterophils (arrows) in a peribronchial lymphoid nodule following *E coli* aerosolization (PAD 0). H&E stain; X 1100

DISCUSSION

Bordetella avium infection had no effect on numbers of *E coli* in lung, but was associated with increased numbers of *E coli* in trachea, particularly in the 4-week-old turkeys. The two principal mechanisms for pulmonary clearance of inhaled bacteria are: mechanical removal by the mucociliary escalator of the upper respiratory tract and removal by pulmonary macrophages. Although the importance of mucociliary clearance is unknown in turkeys, reduced tracheal clearance may exist when ciliated epithelial cells are damaged.[10,11] In this study, *B avium* caused severe damage to ciliated epithelial cells of the trachea, yet pulmonary clearance of *E coli* was similar in BA+ and BA- turkeys. Our results suggest that mucociliary clearance is of minor importance in removal of *E coli* from the lungs of turkeys.

Greater *E coli* numbers in the trachea of BA+ turkeys in this study could be due to colonization of the trachea by *E coli* or to increased mucociliary transit time of *E coli* being cleared from the lung. Gram-negative bacteria may opportunistically adhere to tracheal mucosa damaged by infectious or mechanical agents.[12-14] Bordetella-damaged trachea may be a primary site for *E coli* adherence and colonization.

Large numbers of *E coli* on the *B avium* injured tracheal mucosa may play a key role in the pathogenesis of air sac

lesions. Bordetella avium and E coli together produced severe airsacculitis without the presence of other respiratory pathogens. Escherichia coli dislodged from the trachea may act as a constant source of bacteria seeding the lower respiratory tract resulting in the more severe airsacculitis commonly found in B avium-infected turkeys. Under field conditions, E coli contaminated poultry house dust may act as the initial aerosol exposure of pathogenic bacteria to the poult.[15]

Pathogenic E coli in the respiratory tract may enter the bloodstream of turkeys when the respiratory lining epithelium is sufficiently damaged.[11,16] This concept is supported in the present study because 14 of 17, 2-week-old turkeys with E coli bacteremia had gross or histological evidence of pulmonary abscessation beginning PAD 1. The reason for increased resistance to bacteremia in 4-week-old turkeys is unclear. Turkeys may have age-related maturation of their local (mucosal) and systemic immune responses.[17] Factors involved in bacterial clearance and killing such as macrophage and heterophil function, complement, and immunoglobulins may be less developed in 2-week-old turkeys than in 4-week old turkeys.

The acute inflammatory response to E coli aerosol was focused on the pulmonary bronchi and peribronchial lymphoid tissue. The large peribronchial lymphoid nodules in BA+

turkeys represent a response to chronic antigenic stimulation in the bronchi. The early accumulation of heterophils among peribronchial lymphoid tissue suggests this may be a site of bacterial uptake and antigen processing. The bronchus-associated lymphoid tissue (BALT) may be an initial site of antigen uptake and processing following bacterial challenge by the aerosol route. Low cuboidal nonciliated cells covering the lymphoid nodules may be similar to lymphoepithelial M cells which function in antigen uptake and processing in mammals.[18] Further research will be needed to characterize the nature and importance of BALT in the turkey and its role in respiratory immunity.

REFERENCES

1. Saif YM, Moorhead PD, Dearth RN, Jackwood DJ. Observations on Alcaligenes faecalis infection in turkeys. Avian Dis 1980; 24:665-684.

2. Simmons DG, Dees C, Rose LP. Toxin production by Bordetella avium (Alcaligenes faecalis), in Proceedings of the 66th Annual Meeting of the Conference of Research Workers in Animal Disease 1985; 30.

3. Arp LH, Cheville NF. Tracheal lesions in young turkeys infected with Bordetella avium. Am J Vet Res 1984; 45:2196-2200.

4. Simmons DG, Rose LP, Gray JG. Some physical, biochemical, and pathologic properties of Alcaligenes faecalis, the bacterium causing rhinotracheitis (coryza) in turkey poults. Avian Dis 1980; 24:82-90.

5. Meinecke CF, Duncan RL, Skeeles JK. Circulating Newcastle disease antibodies and mortality related to turkey coryza and vaccine breaks. Poult Sci 1981; 60:1696.

6. Ficken MD. Antibiotic aerosolization for treatment of alcaligenes rhinotracheitis. Avian Dis 1983; 27:545-548.

7. Boycott BR, Wyman HR, Wong FC. Alcaligenes faecalis rhinotracheitis in Manitoba turkeys. Avian Dis 1984; 28:1110-1114.

8. Arp LH. Pathology of spleen and liver in turkeys inoculated with Escherichia coli. Avian Pathol 1982; 11:263-279.

9. Arp LH, McDonald SM. Influence of temperature on the growth of Bordetella avium in turkeys and in vitro. Avian Dis 1985; 29:1066-1077.

10. Van Alstine WG, Hofstad MS. Antibiotic aerosolization: The effect on experimentally induced alcaligenes rhinotracheitis in turkeys. Avian Dis 1985; 29:159-176.

11. Nagaraja KV, Emery DA, Jordan KA, Sivanandan V, Newman JA, Pomeroy BS. Effect of ammonia on the quantitative clearance of Escherichia coli from lungs, air sacs, and livers of turkeys aerosol vaccinated against Escherichia coli. Am J Vet Res 1984; 45:392-395.

12. Marcus H, Baker NR. Quantitation of adherence of mucoid and nonmucoid Pseudomonas aeruginosa to hamster tracheal epithelium. Infect Immun 1985; 47:723-729.

13. Ramphal R, Pyle M. Adherence of mucoid and nonmucoid Pseudomonas aeruginosa to acid-injured tracheal epithelium. Infect Immun 1983; 41:345-351.

14. Ramphal R, Small PM, Shands JW, Fischlschweiger W, Small PA. Adherence of Pseudomonas aeruginosa to tracheal cells injured by influenza infection or by endotracheal intubation. Infect Immun 1980; 27:614-619.

15. Harry EG. The survival of Escherichia coli in the dust of poultry houses. Vet Rec 1964; 76:466-470.

16. Cheville NF, Arp LH. Comparative pathologic findings of Escherichia coli infection in birds. J Am Vet Med Assoc 1978; 173:584-587.

17. Hofstad MS, Jeska EL. Immune response of poults following intranasal inoculation with Artvax™ vaccine and a formalin-inactivated Bordetella avium bacterin. Avian Dis 1985; 29:746-754.

18. McDermott MR, Befus D, Bienenstock J. The structural basis for immunity in the respiratory tract. Int Rev Exp Pathol 1982; 23:47-99.

PAPER II. INFLUENCE OF *BORDETELLA AVIUM* INFECTION ON ADHERENCE AND COLONIZATION OF *ESCHERICHIA COLI* IN THE TURKEY TRACHEA

Influence of *Bordetella avium* infection on adherence and colonization of *Escherichia coli* in the turkey trachea

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SUMMARY

Four-week-old Bordetella avium-infected (BA+) and B avium-free (BA-) turkeys were inoculated with Escherichia coli expressing F1 fimbriae or with E coli not expressing F1 fimbriae. The tracheal adherence of E coli at 1 postinoculation hour (1 PIH) and tracheal colonization of E coli at 6 PIH were determined using an in vivo bacterial adherence assay. Significantly greater numbers of E coli were isolated at both 1 PIH and 6 PIH from the tracheas of BA+ turkeys compared with BA- turkeys. The expression of F1 fimbriae on E coli had no significant effect on numbers of E coli in the trachea. The greater number of E coli in tracheas of BA+ turkeys was probably due to passive retention of bacteria in tracheal exudates. Neither specific adherence nor colonization could be demonstrated for the association of fimbriated or nonfimbriated E coli with the B avium-damaged trachea.

INTRODUCTION

Bordetella avium is the cause of turkey coryza, a highly contagious upper respiratory tract disease of young turkeys.[1-3] The disease is characterized by oculonasal discharge, sneezing, tracheal collapse, and decreased rate of weight gain.[3-5] Bordetella avium is a noninvasive, nonfermentative, gram-negative bacterium that persistently colonizes the ciliated epithelium of the respiratory tract leading to inflammation of the upper airways, loss of ciliated epithelial cells, and accumulation of nasal and tracheal mucus.[4,6] Although turkey coryza can be a clinically mild disease by itself, infected turkeys may be predisposed to other respiratory pathogens.[5] High mortality rates have been reported in B avium-infected turkeys exposed to other respiratory pathogens.[7-9]

Attenuation of the tracheal mucosa by B avium infection may enhance adherence and colonization by gram-negative respiratory pathogens such as E coli.[10,11] Bacterial adherence to normal or altered host epithelium is a prerequisite to colonization of mucosal surfaces and subsequent infection of deeper tissues.[12,13] The normal respiratory tract efficiently removes most bacteria from the airways; however, mechanical, chemical, or viral injury to the airway epithelium may predispose the airways to bacterial infection.[13,14] In turkeys, mycoplasmal and viral

respiratory infections predispose to colibacillosis.[15-17] Turkeys experimentally infected with B avium develop severe fibrinopurulent airsacculitis after aerosol exposure to E coli, whereas similar aerosol exposure of normal turkeys results in only mild, transient airsacculitis.[10]

In chickens, virulent strains of E coli apparently colonize the tracheal epithelium as a preliminary step in the development of colibacillosis.[18] Type 1 (F1) fimbriae are nonflagellar, filamentous surface appendages produced by E coli and other bacteria[19,20], which may enhance the attachment of E coli to ciliated tracheal epithelium in the chicken.[21] Although F1 fimbriae may enhance adherence of E coli to a variety of cells[22-26], the role of F1 fimbriae in the pathogenesis of disease is unclear.[19,27,28] The role of F1 fimbriae in the association of E coli with the B avium-damaged trachea is unknown.

The purpose of the present study was to determine if pre-existing B avium infection affects the adherence and colonization of fimbriated (F1+) and nonfimbriated (F1-) E coli in the turkey trachea.

MATERIALS AND METHODS

Experimental design - Turkeys inoculated at 6 days of age with B avium were housed separately from an equal number of noninoculated hatchmates. Adherence of E coli was determined in 5 B avium-infected (BA+) and 5 B avium-free (BA-) turkeys using an F1+ E coli inoculum; another 5 BA+ and 5 BA- turkeys were treated in a like manner using an F1- E coli inoculum. Adherence of E coli was determined 1 hour after E coli inoculation in isolated tracheal segments as described.[29]

To evaluate E coli colonization, BA+ and BA- turkeys were inoculated with either F1+ or F1- E coli as described above except the number of E coli associated with the tracheal mucosa was determined 6 hours after inoculation with E coli.

Eight turkeys (4 BA+ and 4 BA-) were treated intratracheally with phosphate-buffered saline solution (PBSS, pH 7.2) to serve as noninoculated controls.

Turkeys - Forty-eight one-day-old turkeys (Nicholas strain) were obtained commercially (Midwest Turkey Hatchery Inc., Dike, IA). They were maintained for 10 days in heated brooders and, for the remainder of the study, in metal cages with slatted floors. Turkey starter and water were provided ad libitum. Serum samples, collected 1 day before E coli inoculation, were assayed for agglutinating antibody to

somatic and fimbrial antigens of E coli EC1 by microtiter agglutination.[4] Turkeys were used in the study at 4 weeks of age.

Bacteria and culture conditions - Turkeys were inoculated with B avium strain 838 at 6 days of age as previously described.[4] Briefly, 0.05 ml of inoculum was placed on one nostril for inhalation into the oronasal cavity. The inoculum contained 2.4×10^6 colony-forming units (CFU)/ml in PBSS.

The E coli strain, EC1 (078:K80:H9:F1), is pathogenic for turkeys and has been used in previous studies.[19,30] A stock culture of E coli EC1 was stored in glycerine and brain-heart infusion broth (BHI) (Difco Laboratories, Detroit, MI) 1:1 at -60 C. For preparation of E coli inocula, bacteria were grown aerobically on a tryptose agar slant for 24 hours at 37 C. From this slant, E coli was subcultured (static and aerobically) in BHI broth, 2 times at 24 hour intervals, either at 37 C to enhance the expression of F1 fimbriae or at 18 C to suppress the expression of F1 fimbriae. The bacterial cultures were centrifuged 20 minutes at 3000 X g, and the supernatant was discarded. The bacterial pellet was suspended with PBSS, maintained at 5 C, and used within 1 hour of preparation. A sample of each E coli inoculum was examined for expression of fimbriae by negative-stain electron microscopy[31], mannose-sensitive

agglutination of guinea pig erythrocytes[32], and slide agglutination in antiserum to F1 fimbriae.[19] For slide agglutination, 1 drop of each E coli inoculum in BHI broth was placed on a clean glass slide and mixed at room temperature for 2 minutes with antiserum to F1 fimbriae. Visible white clumps of bacteria indicated a positive agglutination. The number of CFU/ml was determined for each inoculum immediately before use by standard plate counting methods.

Bacterial adherence assay - This assay is a modification of a previously described in vivo tracheal adherence assay.[29] Feed and water were withheld from turkeys 1 hour before they were anesthetized with 0.25 ml of a 4:1 mixture of ketamine hydrochloride (100 mg/ml) (Vetalar, Parke-Davis, Morris Plains, NJ) and acepromazine maleate (10 mg/ml) (Acepromazine, Fort Dodge Laboratories, Fort Dodge, IA) administered IV. Turkeys were placed on a rack to support the head and extend the neck. The midcervical area was sprayed with 70% ethanol and a 1 cm section of skin was removed to expose the trachea. Tracheal adventitia was opened, and the trachea was separated from subjacent tissues. A Satinsky cardiovascular forceps (V. Mueller, Chicago, IL) was applied to the trachea to occlude the lumen. A catheter (3.5 Fr. Tom Cat Catheter-open, Monoject, St. Louis, MO) was passed through the glottis to the base of the isolated

craniad tracheal segment, and after the trachea was completely filled with the bacterial inoculum, the inoculum was aspirated from the trachea and the Satinsky forceps was removed. Each turkey was laid on its side to recover from anesthesia. At 1 or 6 postinoculaton hours (PIH), turkeys were euthanatized, and a 1-cm section of trachea was obtained from each turkey as previously described.[29] The E coli CFU/cm trachea was determined by standard plate counting methods as described.[33]

To compensate for slight differences in the concentration of E coli inocula, an adjusted CFU/cm trachea was calculated as follows:

$$\log_{10}\left[\frac{1 \times 10^{9}\ \text{CFU/ml}}{\text{CFU/ml inoculum}} \times \text{observed CFU/cm trachea}\right]$$

The adjusted CFU/cm of trachea was used for statistical analysis.

Statistics - Numerical data were analyzed using analysis of variance procedures. Statistical differences between treatment means were determined by F tests and least significant differences ($P = 0.05$).

RESULTS

Bacterial numbers in trachea - The presence of B avium had a pronounced effect on E coli numbers in the trachea. Bordetella avium-infected turkeys had significantly greater ($P < 0.01$) numbers of E coli in tracheal segments compared with BA- turkeys at both 1 and 6 PIH.

Numbers of E coli in tracheal segments were not significantly different ($P > 0.5$) when data from BA+ turkeys killed at 1 PIH were compared with data from BA+ turkeys killed at 6 PIH (Fig 1). However, E coli numbers in tracheas were significantly less ($P < 0.05$) in BA- turkeys killed at 6 PIH compared with BA- turkeys killed at 1 PIH (Fig 1).

The presence of F1 fimbriae had no effect on E coli numbers in the trachea. Numbers of F1+ and F1- E coli were not significantly different in the tracheas of either BA+ or BA- turkeys at 1 PIH ($P > 0.4$) and 6 PIH ($P > 0.12$) (Table 1).

All BA+ turkeys exhibited clinical signs of severe rhinotracheitis evidenced by frothy nasal exudate, excessive tracheal mucus, mild tracheal flattening, and congestion of the tracheal vasculature. Bordetella avium was isolated (10^4-10^7 CFU/cm trachea) from all BA+ turkeys, but was never isolated from BA- turkeys. All BA- turkeys remained clinically normal. Escherichia coli was not isolated from BA+ or BA- turkeys inoculated with PBSS.

Escherichia coli inocula - The F1+ inocula, but not the F1- inocula, caused agglutination of guinea pig erythrocytes within 30 seconds. Hemagglutination was prevented when the F1+ inoculum was suspended in PBSS containing 0.5% D-mannose. The F1+ inocula, but not the F1- inocula, agglutinated when mixed on a slide with antiserum to F1 fimbriae. In F1+ inocula, but not the F1- inocula, most E coli contained surface filaments with size and morphologic features typical of F1 fimbriae.[34]

Serology - All sera were negative for antibody to somatic and fimbrial antigens of E coli EC1.

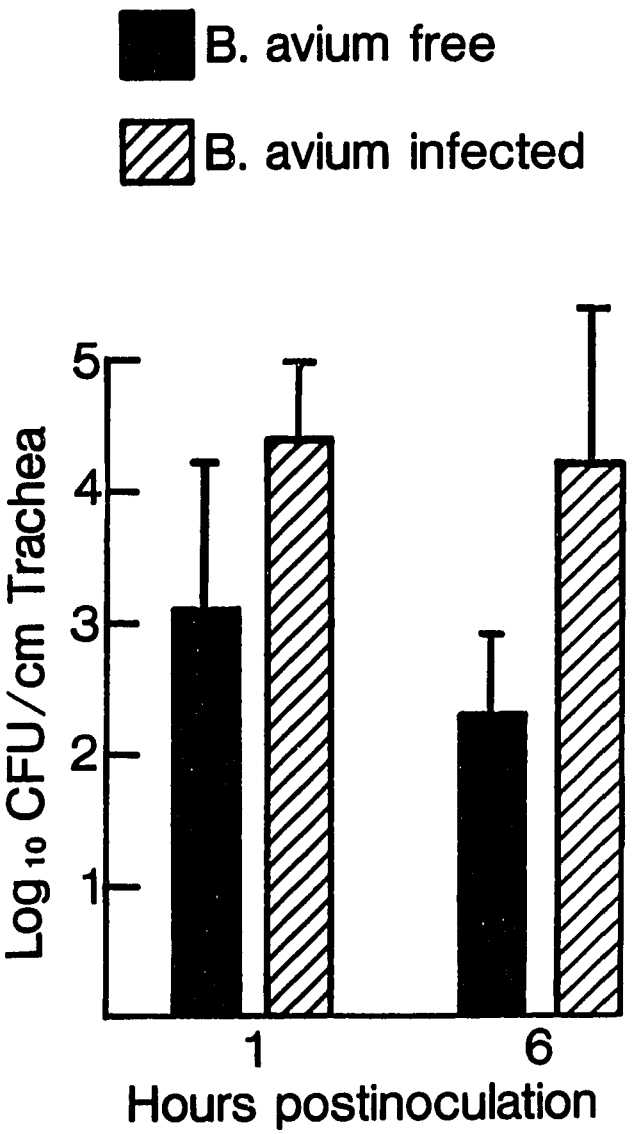
TABLE 1 - Numbers of *E coli* (Log_{10}CFU/cm) recovered from turkey tracheas following *E coli* inoculation

	BA+ turkeys		BA- turkeys	
PIH	F1+	F1-	F1+	F1-
1	4.34±0.28	4.66±0.26	3.61±0.53	2.65±0.46
6	4.54±0.37	3.88±0.74	2.65±0.28	1.85±0.20

Data (n=5/group) are expressed as means ± standard error of the mean.

BA+ = *B avium*-infected; BA- = *B avium*-free; PIH = post-inoculation hours; F1+ = fimbriated *E coli*; F1- = non-fimbriated *E coli* inoculum.

Fig 1 - Recovery of viable *E coli* from tracheas of *B avium*-infected and *B avium*-free turkeys. Data expressed as the mean (n=10/group) + standard error of the mean


B. avium free
B. avium infected
Log10 CFU/cm Trachea
5
4
3
2
1
1
6
Hours postinoculation

DISCUSSION

In turkeys inoculated intratracheally with E coli, greater numbers of E coli remained associated with B avium-damaged tracheas compared with normal tracheas. In mammals, mechanical, chemical, and viral damage to the tracheal epithelium is known to facilitate adherence of gram-negative bacteria.[14,35] These opportunistic bacteria, including E coli, can specifically adhere to epithelial cell receptors or to mucus of the tracheal mucosa.[36-38] The excessive, tenacious mucus, which is consistently present in B avium-damaged tracheas[4,6], may be the site for increased E coli adherence. However, altered receptor profiles in the B avium-damaged tracheal epithelium could potentially promote E coli adherence.

In the present study, it was not possible to determine whether increased association of E coli with the B avium-damaged trachea was due to specific adherence to epithelium or mucus, or whether the bacteria were just passively retained in tracheal exudates. Severe damage to ciliated epithelium by B avium probably inhibits clearance of the tracheal exudates and any bacteria associated with it.[39] Perhaps rinsing the trachea to remove excessive mucus and exudate would be useful in determining the site of E coli association.

Bacterial colonization following adherence is an

important feature in the pathogenesis of bacterial infections of mucosal surfaces.[40] Colonization represents a balance between clearance and killing of bacteria and multiplication of adherent bacteria. Bordetella avium has a specific tropism for tracheal epithelium, and colonization of the tracheal mucosa can be demonstrated within 6 PIH.[29] In the present study, we attempted to demonstrate E coli colonization based on an increase in E coli numbers between 1 PIH and 6 PIH. Six hours incubation was considered sufficient time for E coli to colonize the tracheal mucosa based on colonization studies using B avium.[29] In BA- turkeys, a 10-fold decrease in E coli numbers between 1 and 6 PIH indicated significant bacterial clearance from the trachea. In contrast, numbers of E coli in the BA+ tracheas were unchanged between 1 and 6 PIH; therefore, E coli colonization of the BA+ trachea could not be clearly demonstrated. The present study provided no evidence of a specific E coli adherence mechanism for the normal or B avium-damaged tracheal mucosa as suggested for the chicken trachea.[21]

The presence of F1 fimbriae did not affect the numbers of E coli in tracheas of BA+ or BA- turkeys. Although F1 fimbriae promote the colonization of several strains of E coli to urinary tract and oral epithelium of humans and mice[24-26], there is no evidence to suggest F1 fimbriae

mediate adherence of E coli in the turkey. F1 fimbriae are not important for adherence of pathogenic E coli to turkey intestinal epithelium, and antibodies to F1 fimbriae do not enhance clearance of F1+ E coli from the bloodstream of turkeys.[19,27] In chickens, F1 fimbriae are thought to facilitate the adherence of E coli to tracheal epithelial cells[21], and vaccines containing F1 fimbriae of E coli provide partial protection in chickens experimentally challenged with virulent E coli.[41] A role for E coli F1 fimbriae in the pathogenesis of colisepticemia of turkeys has yet to be established.

REFERENCES

1. Filion PR, Cloutier S, Vrancken ER, Bernier G. Infection respiratoire du dindonneau causee par un microbe apparente au Bordetella bronchiseptica. Can J Comp Med Vet Sci 1967; 31:129-134.

2. Hinz K-H, Glünder G, Lüders H. Acute respiratory disease in turkey poults caused by Bordetella bronchiseptica-like bacteria. Vet Rec 1978; 103:262-263.

3. Simmons DG, Gray JG, Rose LP. Isolation of an etiologic agent of acute respiratory disease (rhinotracheitis) of turkey poults. Avian Dis 1979; 23:194-203.

4. Arp LH, Cheville NF. Tracheal lesions in young turkeys infected with Bordetella avium. Am J Vet Res 1984; 45:2196-2200.

5. Saif YM, Moorhead PD, Dearth RN, Jackwood DJ. Observations on Alcaligenes faecalis infection in turkeys. Avian Dis 1980; 24:665-684.

6. Gray JG, Roberts JF, Dillman RC, Simmons DG. Pathogenesis of change in the upper respiratory tracts of turkeys experimentally infected with an Alcaligenes faecalis isolate. Infect Immun 1983; 42:350-355.

7. Boycott BR, Wyman HR, Wong FC. Alcaligenes faecalis rhinotracheitis in Manitoba turkeys. Avian Dis 1984; 28:1110-1114.

8. Heller ED, Weisman Y, Aharonovovitch A. Experimental studies on turkey coryza. Avian Pathol 1984; 13:137-143.

9. Ficken MD. Antibiotic aerosolization for treatment of alcaligenes rhinotracheitis. Avian Dis 1983; 27:545-548.

10. Van Alstine WG, Arp LH. Effects of Bordetella avium infection on the pulmonary clearance of Escherichia coli in turkeys. Am J Vet Res 1986; in press.

11. Ficken MD, Edwards JF, Lay JC. Clearance of bacteria in turkeys with Bordetella avium-induced tracheitis. Avian Dis 1986; 30:352-357.

12. Gibbons RJ. Adherence of bacteria to host tissue. In: Schlessinger, D, ed. Microbiology - 1977. Washington DC: American Society of Microbiology 1977; 395-406.

13. Ramphal R, Small PM, Shands JW, Fischlschweiger W, Small PA. Adherence of Pseudomonas aeruginosa to tracheal cells injured by influenza infection or by endotracheal intubation. Infect Immun 1980; 27:614-619.

14. Ramphal R, Pyle M. Adherence of mucoid and nonmucoid Pseudomonas aeruginosa to acid-injured tracheal epithelium. Infect Immun 1983; 41:345-351.

15. Cheville NF, Arp LH. Comparative pathologic findings of Escherichia coli infection in birds. J Am Vet Med Assoc 1978; 173:584-587.

16. Moorhead PD, Saif YM. Mycoplasma meleagridis and Escherichia coli infections in germfree and specific-pathogen-free turkey poults: Pathologic manifestations. Am J Vet Res 1970; 31:1645-1653.

17. Springer WT, Luskus C, Pourciau SS. Infectious bronchitis and mixed infections of Mycoplasma synoviae and Escherichia coli in gnotobiotic chickens. Infect Immun 1974; 10:578-589.

18. Dho M, Lafont JP. Escherichia coli colonization of the trachea in poultry: Comparison of virulent and avirulent strains in gnotoxenic chickens. Avian Dis 1982; 26:787-797.

19. Arp LH. Effect of antibodies to type 1 fimbriae on clearance of fimbriated Escherichia coli from the bloodstream of turkeys. Am J Vet Res 1985; 46:2644-2647.

20. Ottow JCG. Ecology, physiology, and genetics of fimbriae and pili. Annu Rev Microbiol 1975; 29:79-108.

21. Naveh MW, Zusman T, Skutelsky E, Ron EZ. Adherence pili in avian strains of Escherichia coli: Effect on pathogenicity. Avian Dis 1984; 28:651-661.

22. Silverblatt FJ, Dreyer JS, Schauer S. Effect of pili on susceptibility of Escherichia coli to phagocytosis. Infect Immun 1979; 24:218-223.

23. Svanborg Edén C, Bjursten L-M, Hull R, Hull S, Magnusson K-E, Moldovano Z, Leffler H. Influence of adhesins on the interaction of Escherichia coli with human phagocytes. Infect Immun 1984; 44:672-680.

24. Clegg H, Guerina N, Langermann S, Kessler TW, Guerina V, Goldman D. Pilus-mediated adherence of Escherichia coli K1 to human oral epithelial cells. Infect Immun 1984; 45:299-301.

25. Iwahi T, Abe Y, Nakao M, Imada A, Tsuchiya K. Role of type 1 fimbriae in the pathogenesis of ascending urinary tract infection induced by Escherichia coli in mice. Infect Immun 1983; 39:1307-1315.

26. Svanborg Edén C, Hansson HA. Escherichia coli pili as possible mediators of attachment to human urinary tract epithelial cells. Infect Immun 1978; 21:229-237.

27. Dominick MA, Schmerr MJF, Jensen AE. Expression of type 1 pili by Escherichia coli strains of high and low virulence in the intestinal tract of gnotobiotic turkeys. Am J Vet Res 1985; 46:270-275.

28. To SC-M, Moon HW, Runnels PL. Type 1 pili (F1) of porcine enterotoxigenic Escherichia coli: Vaccine trial and tests for production in the small intestine during disease. Infect Immun 1984; 43:1-5.

29. Arp LH, Brooks EE. An in vivo model for the study of Bordetella avium adherence to tracheal mucosa in turkeys. Am J Vet Res 1986; 47:2614-2617.

30. Arp LH. Pathology of spleen and liver in turkeys inoculated with Escherichia coli. Avian Pathol 1982; 11:263-279.

31. Arp LH, Jensen AE. Piliation, hemagglutination, motility, and generation time of Escherichia coli that are virulent or avirulent for turkeys. Avian Dis 1980; 24:153-161.

32. Salit IE, Gotschlich EC. Hemagglutination by purified type 1 Escherichia coli pili. J Exp Med 1977; 146:1169-1181.

33. Arp LH, McDonald SM. Influence of temperature on the growth of Bordetella avium in turkeys and in vitro. Avian Dis 1985; 29:1066-1077.

34. Jones GW, Isaacson RE. Proteinaceous bacterial adhesins and their receptors. CRC Crit Rev Microbiol 1983; 10:229-260.

35. Nagaraja KV, Emery DA, Jordan KA, Sivanandan V, Newman JA, Pomeroy BS. Effect of ammonia on the quantitative clearance of Escherichia coli from lungs, air sacs, and livers of turkeys aerosol vaccinated against Escherichia coli. Am J Vet Res 1984; 45:392-395.

36. McSweegan E, Walker RI. Identification and characterization of two Campylobacter jejuni adhesins for cellular and mucous substances. Infect Immun 1986; 53:141-148.

37. Ramphal R, Pyle M. Evidence for mucins and sialic acid as receptors for Pseudomanas aeruginosa in the lower respiratory tract. Infect Immun 1983; 41:339-344.

38. Cohen PS, Arruda JC, Williams TJ, Laux DC. Adhesion of a human fecal Escherichia coli strain to mouse colonic mucus. Infect Immun 1985; 48:139-145.

39. Ficken MD, Edwards JF, Lay JC, Tveter DE. Tracheal mucus transport rate in normal turkeys and in turkeys infected with Bordetella avium (Alcaligenes faecalis). Avian Dis 1986; 30:154-159.

40. Freter R, Jones GW. Models for studying the role of bacterial attachment in virulence and pathogenesis. Rev Infect Dis 1983; 5:S647-S658.

41. Gyimah JE, Panigrahy B. Immunogenicity of Escherichia coli (Serotype 01) pili vaccine in chickens. Avian Dis 1985; 29:1078-1083.

PAPER III. HISTOPATHOLOGY OF LUNG AND BRONCHUS-ASSOCIATED LYMPHOID TISSUE IN YOUNG TURKEYS INFECTED WITH *BORDETELLA AVIUM*

Histopathology of lung and bronchus-associated lymphoid tissue in young turkeys infected with Bordetella avium

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SUMMARY

One-day-old turkeys were inoculated intranasally with Bordetella avium. Noninoculated hatchmates were housed separately. At 1, 2, 3, 4, and 5 weeks postinoculation (PI), B avium-infected (BA+) and B avium-free (BA-) turkeys were necropsied; tracheas, intrapulmonary primary bronchi, and lung adjacent to primary bronchi were cultured. Lung was collected for histopathological examination. Lungs, perfused with acetic acid, were collected to determine the size, number, and distribution of lymphoid nodules associated with primary bronchi. Bordetella avium was isolated from trachea and primary bronchi of all BA+ turkeys, but was never isolated from lung parenchyma. Acute purulent bronchitis was associated with colonization of the primary bronchi by B avium from 1 to 3 weeks PI. Macrophages and lymphocytes persisted in the peribronchial connective tissue for 5 weeks PI. Bronchus-associated lymphoid tissue (BALT) consisted of discrete lymphoid nodules protruding into the lumen of primary bronchi. Lymphoid nodules, morphologically similar in BA+ and BA- turkeys, were composed of nonciliated, cuboidal epithelium covering a zone of loosely arranged lymphocytes and macrophages and a deeper, sharply demarcated lymphoid follicle. Compared with BALT of BA- turkeys, lymphoid nodules of BALT in BA+ turkeys were more numerous and widely distributed along primary bronchi. In both BA-

and BA+ turkeys, the mean diameter of lymphoid nodules doubled between 1 and 5 weeks of age.

INTRODUCTION

Bordetella avium is the cause of turkey coryza, a highly contagious upper respiratory disease of young turkeys.[1-3] The clinical disease is characterized by excessive oculonasal discharge, sneezing, dyspnea, tracheal collapse, and decreased weight gain.[1,2] Histological lesions in the nasal cavity and trachea consist of fibrinopurulent rhinitis and tracheitis associated with bacterial colonization of the ciliated epithelium. Loss of ciliated cells, epithelial hyperplasia, depletion of mucus, and diffuse infiltration of mononuclear inflammatory cells into subepithelial tissues are consistent features of B avium infection in turkeys.[1,4]

The significance of B avium as a pulmonary pathogen in turkeys is unknown. When lung tissue was cultured in previous studies of turkey coryza, B avium was rarely isolated.[2,5,6] In these experiments, lung was apparently cultured without specifically attempting to culture the primary bronchus. However, B avium is consistently isolated when lung homogenates from experimentally infected turkeys are cultured.[7] Therefore, bacterial colonization may be restricted to a small portion of lung such as the ciliated epithelium of large airways.

Histological examination of lungs from B avium-infected (BA+) turkeys was included in several early studies of turkey coryza, but lesions were not reported.[2,6,8,9] In contrast, a

recent study described massive hyperplasia of the bronchus-associated lymphoid tissue (BALT) in lungs of turkeys infected with B avium.[7] The purposes of the present study were to determine the distribution of B avium in lung and to describe the changes in lung and BALT of turkeys experimentally infected with B avium.

MATERIALS AND METHODS

Experimental design - Turkeys (n=30) were inoculated intranasally with B avium at 1 day of age; a second group of turkeys (n=25) was raised as B avium-free (BA-) controls. At 1, 2, 3, 4, and 5 weeks PI, 6 BA+ and 5 BA- turkeys were randomly selected for necropsy. Using 3 BA+ and 2 BA- turkeys, trachea, intrapulmonary primary bronchus, and lung adjacent to the primary bronchus from each turkey were cultured for B avium, and lung was collected for histopathology. Lungs collected at each necropsy time from a second group of turkeys (3 BA+ and 3 BA-) were fixed in acetic acid and examined using a dissecting microscope (Bausch and Lomb, Rochester, NY) to determine the size, number, and distribution of lymphoid nodules associated with primary bronchi.

Turkeys - One-day-old turkeys (Nicholas strain) were obtained commercially (Cuddy Farms, Ellsworth, IA). A group of 30 turkeys was inoculated with B avium at 1 day of age as previously described.[1] Briefly, 0.05 ml of inoculum was placed on one nostril for inhalation into the oronasal cavity. The inoculum contained 2.6×10^8 colony-forming units of B avium per ml phosphate-buffered saline solution. The remaining 25 turkeys were housed separately and served as noninoculated controls. All turkeys were maintained for the first 10 days in heated brooders and, for the remainder of

the experiment, in metal cages with slatted floors. Turkey starter and water were provided ad libitum. Serum from 3 BA+ and 3 BA- turkeys was collected at each necropsy time and tested for antibody to avian mycoplasmas (bacterial agglutination) and Newcastle disease virus (microtiter hemagluttination-inhibition test) (Dr. L. D. Koehnk, Jewell, IA).

Necropsy procedures - Turkeys were anesthetized with pentobarbital sodium (5-10 mg) given IV. The femoral vessels were severed, and turkeys were maintained with the craniad part of the body elevated to facilitate exsanguination. The body cavities were opened, and the left lung was removed aseptically. Left primary bronchi were opened longitudinally and cultured at the middle with a disposable swab (Calgiswab, American Can Co., Glenwood, IL). The lung parenchyma 1-cm lateral to the primary bronchus was flamed, opened longitudinally with a sterile knife, and cultured with a swab. Skin over the midcervical trachea was removed. The trachea was opened with a sterile knife and cultured with a swab. Lower trachea and right lung from each turkey were perfused with 10% neutral-buffered formalin and fixed in situ for 30 minutes before being removed from the turkey.

In turkeys used to determine the size, number, and distribution of lymphoid nodules associated with the primary bronchi, birds were anesthetized, exsanguinated, and tracheas

cultured as above. The lower trachea and both lungs were perfused with cold (5 C) 2.5% acetic acid and fixed in situ for 30 minutes before being removed.[10] The primary bronchus of each lung was opened longitudinally, and the lungs were placed in 2.5% acetic acid for 24 hours before examination under a dissecting microscope. Lymphoid nodules were counted, and the diameter of at least 7 nodules in each primary bronchus was measured using a calibrated eyepiece in the dissecting microscope.

Bacterial isolation - Swabs from trachea, bronchi, and lung parenchyma were streaked onto blood agar plates. All plates were incubated aerobically at 37 C for 36 hours. Representative small, isolated, translucent colonies were tested by slide agglutination with antiserum specific for B avium.

Histopathology - Formalin-perfused lungs were fixed 48 hours in 10% neutral-buffered formalin, paraffin embedded, sectioned at 3 um and stained with hematoxylin and eosin (H&E) by routine methods. Additional sections were stained with Masson's trichrome for collagen and periodic acid Schiff-Alcian blue (PAS-AB) for mucosubstances. From each turkey, a cross section of the entire lung was taken at the junction of the extrapulmonary and intrapulmonary primary bronchi. Also, a longitudinal section of the lung was taken through the intrapulmonary primary bronchus.

RESULTS

Clinical signs, gross lesions, and serology - Clinical signs of turkey coryza were present in all BA+ turkeys by 1 week PI and persisted throughout the study. Signs included sneezing, excessive oculonasal discharge, and staining of the periocular and cervical feathers with brownish exudate. Gross lesions in the nasal cavity and trachea included frothy nasal exudate, excessive thick tracheal mucus, mild to severe dorso-ventral flattening of the trachea, and hyperemia of the tracheal mucosa. Clinical signs and gross lesions of turkey coryza were not present in BA- control turkeys. All sera were negative for antibody to Mycoplasma meleagridis, Mycoplasma gallisepticum, and Newcastle disease virus.

Bacterial isolation - Bordetella avium was isolated from trachea and intrapulmonary primary bronchus of all BA+ turkeys at all necropsy times; however, B avium was never isolated from lung parenchyma adjacent to primary bronchi. All positive cultures produced a single colony morphotype on blood agar and colonies from each culture agglutinated with antiserum specific for B avium. Bordetella avium was not isolated from BA- control turkeys.

Histopathologic lesions in lung and BALT - Pulmonary lesions in BA+ turkeys were limited to primary bronchi. Diffuse peribronchial infiltration of heterophils was common in turkeys (5 of 6) at 1 and 2 weeks PI. Less common was

locally extensive infiltrations of heterophils within and around BALT (Fig 1). Primary bronchi contained exudates of mucus and cellular debris. From 3 to 5 weeks PI, the acute inflammatory response subsided, but lymphocytes and macrophages remained scattered throughout the peribronchial connective tissue. Lymphoid nodules were more numerous and often contained more germinal centers in BA+ turkeys compared to BA- turkeys. Occasionally, multinucleated giant cells were present within BALT. At 5 weeks PI, one lymphoid follicle contained a small granuloma.

Ciliated, columnar epithelium of the primary bronchi remained intact and without degenerative changes throughout this study. Similarly, mucosubstances stained with PAS-AB were continually present in goblet cells at all levels of primary bronchi. Increased peribronchial connective tissue was not apparent in sections stained with Masson's trichrome. Rarely, single bacteria were found adhered to the cilia of bronchial epithelium on H & E stained sections of lung. No lesions were present in BA- control turkeys.

Morphology of BALT - Greater numbers of lymphoid nodules were present in BA+ turkeys compared with BA- turkeys (Table 1). Numbers of lymphoid nodules were similar between right and left lungs. Lymphoid nodules ranged from 0.07 mm to 1.33 mm in diameter and were slightly smaller in BA+ turkeys compared to BA- turkeys (Table 2). The average

diameter of lymphoid nodules approximately doubled between 1 and 5 weeks of age in both BA+ and BA- turkeys.

In BA+ and BA- turkeys, BALT appeared as discreet, white, round to oval nodules on a clear to slightly opaque background of bronchial mucosa after fixation with acetic acid (Figs 2 and 3). Lymphoid nodules were concentrated at junctions of primary and secondary bronchi in BA- turkeys, but were more randomly distributed along the primary bronchus in BA+ turkeys. In BA+ and BA- turkeys, each lymphoid nodule contained a lymphoid follicle located immediately below the epithelium of the bronchus (Figs 4 and 5). The ciliated columnar bronchial epithelium changed to nonciliated, low-cuboidal cells over centers of BALT (Fig 6). The low-cuboidal cells lacked mucous granules. Collagen fibers and bundles of smooth muscle, which were regularly present under the surrounding columnar epithelium, were absent under the low-cuboidal epithelium covering lymphoid follicles of BALT.

Lymphoid follicles were well demarcated and loosely encapsulated with connective tissue on the lateral and antiluminal borders. Immediately under the epithelium, lymphoid follicles were composed of a zone of loosely arranged lymphocytes and large cells with foamy cytoplasm, presumably macrophages (Figs 4 and 5). Deeper in the follicle, lymphocytes were more densely arranged in sheets except around blood vessels where they were concentrically

aligned with vessels. Blood vessels were located at the periphery of follicles on lateral and antiluminal borders. Germinal centers, composed of lymphocytes and surrounded by a delicate collagen capsule, were occasionally present in lymphoid follicles. In BA+, but not in BA- turkeys, small to moderate numbers of heterophils were present in lymphoid follicles.

TABLE 1 - Mean number of lymphoid nodules associated with the primary bronchi of B avium-infected (BA+) turkeys and B avium-free (BA-) turkeys

		BA+			BA-		
Weeks	PI[2]	Right[1] Lung	Left[1] Lung	Mean[3]	Right[1] Lung	Left[1] Lung	Mean[3]
1		26	25.3	25.7	20.3	18.3	19.3
2		68	62.6	65.3	35.3	44.3	39.8
3		67.6	56	61.8	36.3	41.6	38.9
4		116.6	115	115.8	69.6	70.3	70
5		153	130	141.5	69.6	67.6	68.6

[1]n = 3/group.

[2]PI = postinoculation.

[3]n = 6/group.

TABLE 2 - Diameter (in mm) of lymphoid nodules associated with primary bronchi of turkeys

	BA+[1]		BA-[2]	
Weeks PI[3]	Range	Mean[4]	Range	Mean[4]
1	0.13-0.47	0.29	0.07-0.40	0.29
2	0.07-0.73	0.39	0.07-0.73	0.44
3	0.13-1.07	0.36	0.13-1.20	0.65
4	0.07-1.07	0.43	0.13-1.27	0.60
5	0.07-1.20	0.56	0.13-1.33	0.62

[1] BA+ = B avium-infected.

[2] BA- = B avium-free.

[3] PI = postinoculation.

[4] n = 6/group.

Fig 1 - Heterophils (arrows) within and around BALT in *B avium*-infected turkey at 2 weeks PI.
H&E stain; x 450

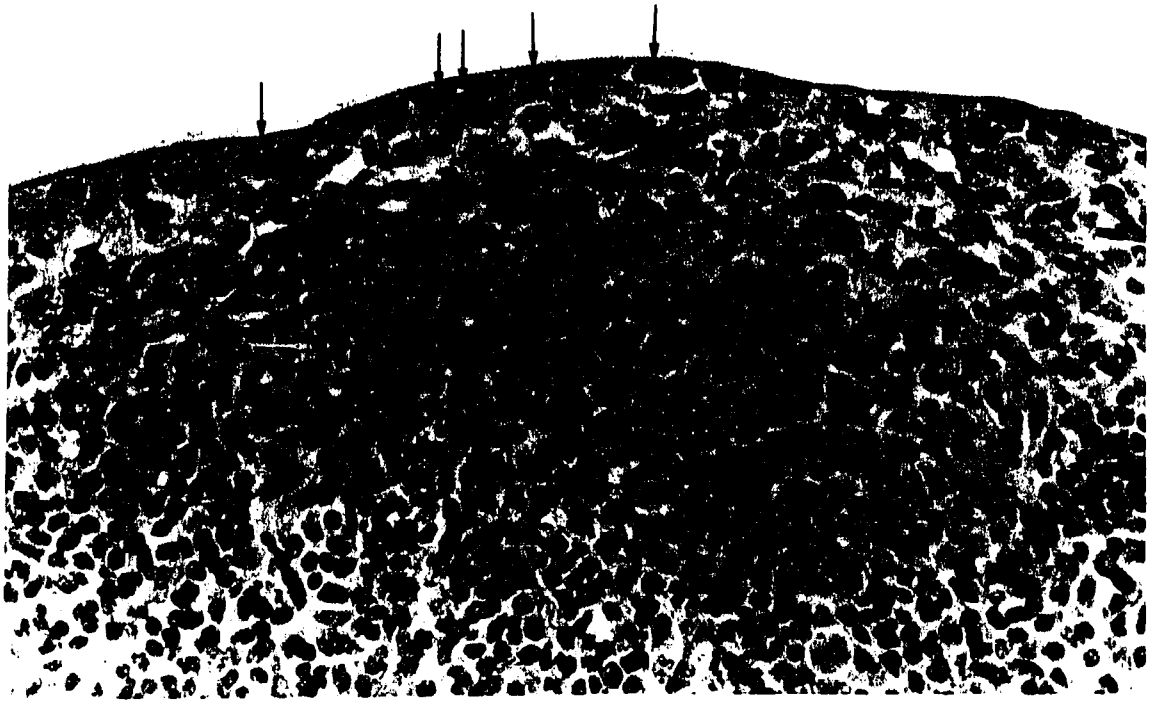
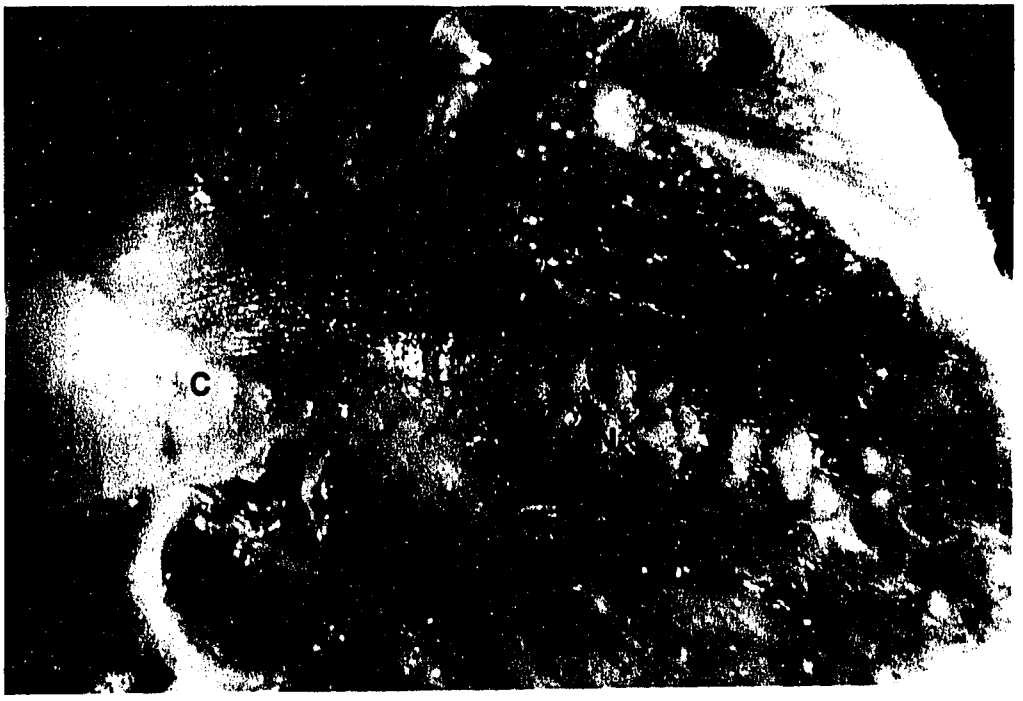


Fig 2 - Lymphoid nodules (N) in primary bronchus of B avium-infected turkey at 4 weeks PI. C = cartilage rings. L = lung parenchyma. x 15

Fig 3 - Lymphoid nodules (N) around openings of secondary bronchi (arrows). x 35


C


N

Fig 4 - Lymphoid nodule with lymphoid follicle in B avium-infected turkey at 4 weeks PI. Loose zone of macrophages and lymphocytes between black arrowheads. Blood vessels (white arrows). H&E stain; x 180

Fig 5 - Lymphoid nodule with lymphoid follicle in B avium-infected turkey at 4 weeks PI. Loose zone of macrophages and lymphocytes between black arrowheads. H&E stain; x 180

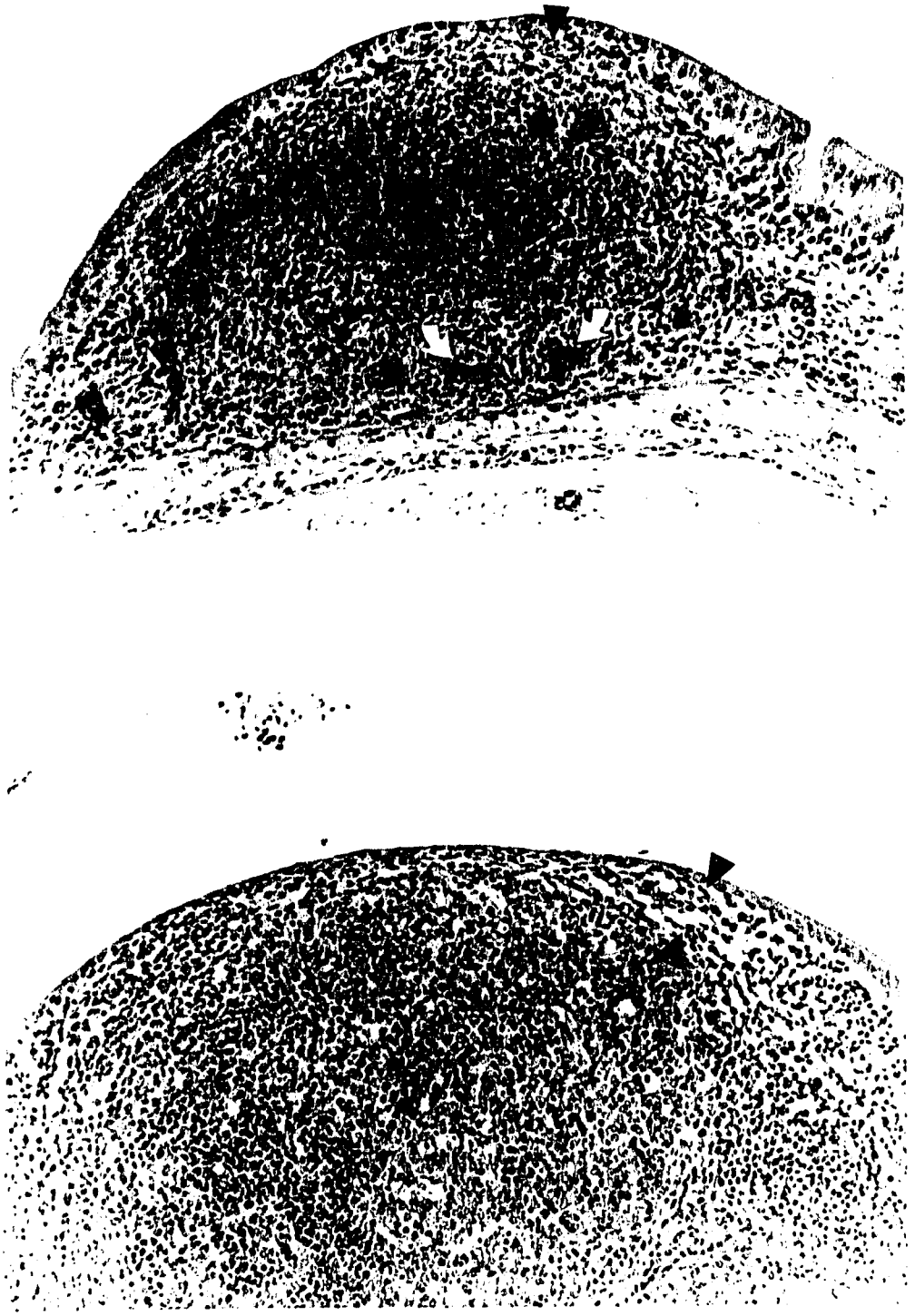


Fig 6 - Transition from ciliated, columnar epithelium (C) to nonciliated, cuboidal epithelium overlying loosely arranged macrophages and lymphocytes. H&E stain; x 1100

DISCUSSION

Bordetella avium has a striking tropism for the ciliated respiratory epithelium extending from the nasal cavity to the lung.[1,4,11] In the lung, ciliated epithelium is limited to the primary bronchi.[12] As in the trachea, B avium can be consistently isolated from the primary bronchi of turkeys with coryza, but is never isolated from the lung parenchyma adjacent to primary bronchi. This limitation of B avium to primary bronchi helps to explain the inconsistent isolation of B avium from lungs of BA+ turkeys in previous studies.[2,5,6]

Inflammation of the bronchial mucosa was interpreted to be a stereotypic response to persistent injury.[1] Pulmonary lesions associated with B avium infection were limited to primary bronchi. Mild diffuse purulent bronchitis subsided and changed to a predominantly mononuclear inflammatory cell response by 3 weeks PI. Similar progression of inflammatory change occurs in the tracheal and nasal mucosae of BA+ turkeys.[1,4] Loss of ciliated epithelial cells, epithelial cell hyperplasia, depletion of mucus, and microcolonization of ciliated epithelial cells are consistent tracheal lesions in BA+ turkeys[1], but these lesions are absent in intrapulmonary bronchi. Differences in lesions between lung and bronchi could be due to greater colonization of the trachea by B avium as compared to the lung.[7]

BALT in turkeys occurs as prominent, well demarcated lymphoid nodules projecting into the lumen of primary bronchi. Similar BALT has been described in chickens.[13,14] In BA- turkeys, lymphoid nodules were clustered around openings of secondary bronchi which are important sites for inertial impaction of inhaled particles. The more random spacial development of BALT in BA+ turkeys may result from the diffuse and persistent colonization of B avium throughout primary bronchi.

Greater numbers of lymphoid nodules were present in BA+ turkeys compared to BA- turkeys at each necropsy time, although BALT was present in all turkeys. Antigen may not be required for the development of BALT, but may be necessary for expansion and proliferation of this tissue.[13] Proliferation of BALT is not specific for B avium, but is a nonspecific response to persistent antigenic stimulation in bronchi. In both BA- and BA+ turkeys, the mean diameter of lymphoid nodules doubled in size between 1 and 5 weeks of age. A similar age related development of BALT occurs in other species.[13]

The low cuboidal epithelium covering each lymphoid nodule is morphologically similar to the lymphoepithelium of BALT and Peyer's patches of chickens and mammals.[15] This lymphoepithelium lacks cilia and mucus-producing cells. Microfold (M) cells, present in the lymphoepithelium of many

species, have the capacity to transport soluble and particulate antigens from the bronchial lumen to the interior of BALT.[15,16] Acute inflammation of BALT in the present study and in turkeys exposed to an Escherichia coli aerosol suggests that BALT in turkeys may be a preferential site of antigen uptake and processing.[7] Further studies will be needed to characterize the role of BALT in mucosal immunity of turkeys.

REFERENCES

1. Arp LH, Cheville NF. Tracheal lesions in young turkeys infected with Bordetella avium. Am J Vet Res 1984; 45:2196-2200.

2. Saif YM, Moorhead PD, Dearth RN, Jackwood DJ. Observations on Alcaligenes faecalis infection in turkeys. Avian Dis 1980; 24:665-684.

3. Simmons DG, Rose LP, Gray JG. Some physical, biochemic, and pathologic properties of Alcaligenes faecalis, the bacterium causing rhinotracheitis (coryza) in turkey poults. Avian Dis 1980; 24:82-90.

4. Gray JG, Roberts JF, Dillman RC, Simmons DG. Pathogenesis of change in the upper respiratory tracts of turkeys experimentally infected with an Alcaligenes faecalis isolate. Infect Immun 1983; 42:350-355.

5. Skeeles JK, Swafford WS, Wages DP, Hellwig HM, Slavik MF, Beasley JN, Houghten GE, Blore PJ, Crawford D. Studies on the use of a long-acting oxytetracycline in turkeys: Efficacy against experimental infections with Alcaligenes faecalis and Pasteurella multocida. Avian Dis 1983; 27:1126-1130.

6. Jackwood DJ, Saif YM, Moorhead PD, Dearth RN. Infectious bursal disease virus and Alcaligenes faecalis infections in turkeys. Avian Dis 1982; 26:365-374.

7. Van Alstine WG, Arp LH. Effects of Bordetella avium infection on the pulmonary clearance of Escherichia coli in turkeys. Am J Vet Res 1986; in press.

8. Saif YM, Moorhead PD, Whitmoyer RE. Scanning electron microscopy of tracheas from turkey poults infected with Alcaligenes faecalis. Avian Dis 1981; 25:730-735.

9. Slavik MF, Skeeles JK, Beasley JN, Harris GC, Roblee P, Hellwig D. Effect of humidity on infection of turkeys with Alcaligenes faecalis. Avian Dis 1981; 25:936-942.

10. Bienenstock J, Clancy RL, Perey DYE. Bronchus-associated lymphoid tissue (BALT): its relationship to mucosal immunity. In: Kirkpatrick et al., eds. Immunologic and infectious reactions in the lung. Lung biology in health and disease. New York, NY: Dekker, 1976; 1:29-58.

11. Van Alstine WG, Hofstad MS. Antibiotic aerosolization: The effect on experimentally induced alcaligenes rhinotracheitis in turkeys. Avian Dis 1985; 29:159-176.

12. Cover MS. Gross and microscopic anatomy of the respiratory system of the turkey. II. The larynx, trachea, syrinx, bronchi, and lungs. Am J Vet Res 1953; 14:230-238.

13. Bienenstock J. Bronchus-associated lymphoid tissue. In: Bienenstock J, ed. Immunology of the lung. New York, NY: McGraw-Hill, 1984; 96-118.

14. McDermott MR, Befus D, Bienenstock J. The structural basis for immunity in the respiratory tract. Int Rev Exp Pathol 1982; 23:47-99.

15. Wolf JL, Bye WA. The membranous epithelial (M) cell and the mucosal immune system. Ann Rev Med 1984; 35:95-112.

16. Bienenstock J. Bronchus-associated lymphoid tissue. Int Arch Allergy Appl Immun 1985; 76:62-69.

PAPER IV. EFFECTS OF *BORDETELLA AVIUM* TOXIN ON TURKEY TRACHEAL ORGAN CULTURES AS MEASURED WITH A TETRAZOLIUM-REDUCTION ASSAY

Effects of Bordetella avium toxin on turkey tracheal organ cultures as measured with a tetrazolium-reduction assay

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SUMMARY

Turkey tracheal organ cultures (TOCs) were exposed to 1 of the following Bordetella avium fractions or controls: live B avium, formalin killed B avium, B avium sonicate, heat-inactivated sonicate, culture supernatant, heat-inactivated culture supernatant, phosphate buffered saline solution, and brain heart infusion broth. After the TOCs were incubated for 2 hours with the bacterial fractions, the cellular metabolism of each TOC was evaluated using a tetrazolium chloride reduction assay and cellular morphology was determined by light microscopy. Additionally, bacterial fractions and controls were injected into turkeys to test lethality. Although the bacterial sonicate, containing heat-labile toxin, was lethal for turkeys, neither the sonicate nor any other B avium fraction significantly affected the metabolism or morphology of turkey TOCs.

INTRODUCTION

Bordetella avium is the cause of turkey coryza, an upper respiratory disease of turkeys and chickens.[1-3] The disease is characterized by rhinitis, conjunctivitis, oculonasal discharge, tracheal collapse, and decreased weight gain.[3] Colonization is restricted to ciliated respiratory epithelium of the nasal, tracheal, and bronchial mucosae.[4] Although *B avium* is noninvasive, it causes extensive damage to the colonized mucosae.[4] In 1 study, visual estimates of ciliary activity in turkey tracheal organ cultures (TOCs) were used as a subjective measurement of ciliary activity, and results suggested that intact live *B avium* is cytotoxic to tracheal epithelium.[5] Systemic effects reported with *B avium* infection include histamine sensitization, alterations in tissue monoamines, and alterations in immune functions.[6-8] Mechanisms responsible for the local and systemic effects of *B avium* infection have not been clearly elucidated. A toxin prepared from sonicated live *B avium* is lethal for turkeys when injected parenterally, but the contribution of this toxin to the clinical signs and lesions of turkey coryza is unknown.[9]

Tetrazolium reduction is a chemical reaction used to quantitate a variety of metabolic activities in viable cells.[10,11] The reduction of tetrazolium chloride provides an objective biochemical measurement of cellular metabolism

in organ cultures.[11] Tetrazolium acts as an artificial electron acceptor for pyridine nucleotide-linked enzymes, including several tissue dehydrogenases.[10,12] Colorless solutions of tetrazolium salts are reduced to insoluble red formazans at the site of enzymatic oxidation in metabolically active cells.[10] Tetrazolium reduction has been used to detect the viability of ferret tracheal explants[13], dehydrogenase activity in skin grafts[14], cellular activity in myocardial cells following ischemia[15], neutrophil function[16], and bacterial growth and motility.[17,18] In hamsters, tetrazolium reduction has been used to quantitate effects of Mycoplasma pneumoniae on metabolism of tracheal organ cultures.[11]

In the present study, TOCs from young turkeys were exposed to B avium and a variety of potentially toxic B avium culture products. A tetrazolium reduction assay was used as an objective measure of B avium-induced metabolic derangements in turkey TOCs.

MATERIALS AND METHODS

Experimental design - Tracheal organ cultures produced from 5-week-old turkeys were prepared daily and incubated for 2 hours with 1 of 8 B avium fractions or controls. The TOCs were removed from the fractions, rinsed, and incubated with tetrazolium chloride. After 2 hours, the TOCs were removed, rinsed, blotted, and extracted with acetone. The optical density of the formazan-containing acetone and the dry weight of each TOC were determined. The optical density per mg trachea was calculated daily using 5 TOCs for each bacterial fraction and control. This procedure was repeated on 5 consecutive days. On the last day of the experiment, 3 ml of each bacterial fraction and control was injected intravenously into 3, 5-week-old turkeys to determine lethality.

Tracheal organ cultures - One-day-old broad breasted white turkeys (Nicholas-strain) were obtained commercially (Midwest Turkey Hatchery, Inc., Dike, Iowa) and raised to 5 weeks of age. Turkey starter and water were provided ad libitum. For 5 consecutive days of the experiment, 2 turkeys were killed by anesthetic overdose with pentobarbitol sodium, and tracheas were aseptically removed. Tracheas were immediately placed in a petri dish containing Tyrode's balanced salt solution (TBSS) (Sigma Chemical Corp, St. Louis, MO) pH 7.4 supplemented with 1.2% sodium succinate

(Sigma). Fascia and muscles were carefully removed from the external surface of tracheas. The tracheas were cut into 2-mm wide rings and incubated at 37 C. Ciliary activity was confirmed by examining each ring under an inverted phase-contrast microscope. On each of the 5 days, TOCs from 2 turkeys were pooled and used within 1 hour of preparation.

Bacterial fractions and controls - Bordetella avium strain P-4148 (obtained from Dr. R. B. Rimler, National Animal Disease Center, Ames, IA) was used, because this strain is capable of producing turkey coryza and a heat-labile lethal toxin.[9]

Live bacteria - Bordetella avium strain P-4148 was grown in brain heart infusion broth (BHI) (Difco Laboratories, Detroit, MI) for 18 hours at 37 C, aerobically, with agitation. The bacterial suspension was centrifuged (3000 X g, 20 minutes) and the supernatant was discarded. The bacteria were resuspended in phosphate-buffered saline solution (PBSS) to a concentration of 10^9 colony forming units (CFU)/ml. This inoculum was prepared fresh for each day of the experiment.

Killed bacteria - Bordetella avium strain P-4148 was cultured and centrifuged as above. The supernatant was decanted and saved. The bacteria were resuspended in PBSS to a final concentration of 10^9 CFU/ml and formalin was added to a final concentration of 0.02%. The formalinized bacteria

were mixed, incubated at 37 C for 1 hour, refrigerated (5 C) overnight, and then centrifuged (3000 X g, 20 minutes). The supernatant was discarded and the bacteria were resuspended in an equal volume of PBSS. This fraction was maintained at 5 C until needed. Sterility was confirmed by placing a sample of this fraction on blood agar plates.

Sonicate - A sterile filtered sonicate of B avium strain P-4148 was prepared by Dr. R. B. Rimler as previously described.[9] Protein content, 312 ug/ml, was determined by a modified Lowry method.[9] The sonicate was maintained at -60 C until needed.

Heat-inactivated sonicate - This fraction was prepared by heating the sonicate prepared as above to 56 C for 30 minutes.

Culture supernatant - BHI supernatant saved from preparation of the killed bacterial fraction was filtered through a 0.45 um filter followed by a 0.22 um filter (Millipore Corp., Bedford, MA). The filtered supernatant was cultured to confirm sterility. This fraction was maintained at -60 C until needed.

Heat-inactivated culture supernatant - For each day, culture supernatant, prepared as above, was thawed and heated to 56 C for 30 minutes.

Controls - Phosphate-buffered saline solution pH 7.2 and BHI were used.

Tetrazolium-reduction assay - On each day of the experiment, 48 TOCs were equally divided among 8, 16 x 100 mm glass test tubes (Vacutainer, Becton-Dickenson Company, Rutherford, NJ) containing 8 ml TBSS supplemented with 1.2% sodium succinate. To each tube, 3 ml of 1 of the 8 fractions were added. Rubber stoppers were replaced on the filled tubes and air bubbles were removed with a needle and syringe. A ninth tube was prepared by placing 6 ethanol fixed (killed) TOCs with 11 ml TBSS supplemented with 1.2% sodium succinate to serve as a negative control. All tubes were placed on a rocking platform (Model 4651, Ames Company, Elkhart, IN) and incubated for 2 hours at 37 C. From each tube, 5 TOCs were removed, rinsed four times in TBSS and placed in another test tube containing 11 ml of a solution of 0.02% 2,3,5 triphenyltetrazolium chloride (Sigma) in TBSS supplemented with 1.2% sodium succinate. All tubes were placed on a rocking platform and incubated for 2 hours at 37 C. After the incubation, TOCs were removed from the tubes, rinsed 4 times in distilled water, and blotted. Formazan was extracted from each TOC with 3 ml acetone for 10 minutes. The optical density of the acetone-formazan suspension was measured spectrophotometrically (Spectronic 20, Bausch and Lomb, Rochester, N.Y.) at 490 nm. Dry weight of each TOC was measured and the optical density per mg was calculated for each TOC. On each experimental day, 5 values were generated

for each of the 8 fractions and the ethanol fixed TOCs.

Histopathology - After 2 hours incubation with the fractions in TBSS, 1 TOC from each fraction on days 3, 4, and 5 of the experiment was fixed in 10% neutral buffered formalin. After fixation, the tracheas were routinely processed and embedded in paraffin, sectioned at 3 um, and stained with hematoxylin and eosin.

Statistical analysis - Differences in numerical data among treatment groups were analyzed for significance by the F test and least significant differences (Statistical Analysis System, Analysis of Variance) using a randomized block design.

RESULTS

Following incubation with tetrazolium, red pigment was visible on the tracheal mucosa of all TOCs except those fixed in ethanol. No statistically significant differences in tetrazolium reduction were found between the PBSS control and PBSS-containing bacterial fractions including live B avium, killed B avium, sonicate, and heat-inactivated sonicate ($P > 0.2$) (Table 1). No statistically significant differences were found between the BHI control and BHI-containing fractions including culture supernatant and heat-inactivated culture supernatant ($P > 0.2$) (Table 1). A significant difference ($P < 0.05$) was found between the PBSS control and the BHI control. The ethanol-fixed TOCs did not reduce tetrazolium to formazan; the optical density per mg trachea was zero.

Of all B avium fractions, only the sonicate was lethal for turkeys when injected intravenously. All 3 turkeys injected intravenously with the sonicate were depressed within 1 hour and dead within 3 hours following injection. Turkeys injected with all other fractions appeared healthy during 4 days of observation.

Histologically, no differences were seen among TOCs incubated in the different fractions. In all fractions, the epithelium appeared intact. Occasional bacteria lined the cilia in both the live and killed B avium-containing

fractions. Changes in all TOCs attributed to incubation in artificial media were mild diffuse vacuolation of mucosal epithelial cells, clumped chromatin in mitotically active basal cells, and pyknosis of leukocyte nuclei.

TABLE 1. Optical density (at 490 nm) per mg trachea for TOCs exposed to B avium fractions and controls

Fraction[1]	Day 1	2	3	4	5	Mean±SE[2]	Lethal[3]	
PBSS	37.3[4]	54.1	42.9	35.7	41.1	42.2±7.2	a	-
LBA	56.8	58.9	36.9	21.5	16.8	38.2±19.4	a,b	-
KBA	47.3	45.2	45.3	23.8	41.1	40.5±9.6	a,b	-
SON	43.1	39.6	42.7	26.3	41.1	38.6±7.0	a,b	+
H-SON	44.7	40.9	39.8	28.5	17.2	34.2±11.3	a,b	-
SUPN	33.7	33.4	38.3	26.7	33.7	33.1±4.2	a,b	-
H-SUPN	37.9	47.5	43.2	28.5	38.8	39.2±7.1	a,b	-
BHI	35.7	34.3	32.4	28.8	20.2	30.3±6.2	b	-

[1]PBSS = phosphate-buffered saline solution; LBA = live B avium; KBA = formalin-killed B avium; SON = sonicate; H-SON = heat-inactivated sonicate; SUPN = culture supernatant; H-SUPN = heat-inactivated supernatant; BHI = brain heart infusion broth.

[2]Mean ± standard error of the mean for each fraction. Means with same small letters do not differ significantly ($P < 0.05$). Optical density was multiplied by 10^3.

[3]Lethality following injection of each fraction into 3 turkeys.

[4]Each number represents a mean of five TOCs.

DISCUSSION

The tetrazolium reduction assay failed to detect metabolic differences among TOCs exposed to the various bacterial fractions and their comparable controls. Several authors have suggested that toxins of B avium may be responsible for the local and systemic effects of B avium infection.[5,19,20] Cytotoxicity, measured by visual estimates of ciliostasis, "blebing" of the cell surface, and sloughing of ciliated epithelium in TOCs, was seen 10 minutes after addition of live B avium to 12 hour or older TOCs.[5] The mild cytoplasmic vacuolization seen histologically in our 3 hour TOCs suggests that cellular metabolism and morphology may be altered in artificial medium.

The differences between PBSS control and BHI control TOCs were expected. BHI has a detrimental effect on the tracheal mucosa and on B avium adherence to tracheal mucosa (unpublished data).

Tracheal organ cultures taken directly from the lethal sonicate had no detectable morphological or biochemical evidence of cytotoxicity to suggest a direct effect of this toxin on tracheal epithelium. Similar to Bordetella affecting mammals, B avium contains endotoxin and both heat-labile and heat-stable toxins.[7,9,19] Endotoxin may have been present in the toxic sonicate used in this study. The role these toxins have in the production of tracheal cytopathology

is unknown.

Tracheal organ cultures are isolated from the immunological and hormonal influences of the living animal. An interaction between bacterial toxins and immunological mechanisms in the living animal may be necessary for cytotoxicity.[19,21] Because these interactions would not be possible in the in vitro system used in this study, an in vivo model may be useful for studying these mechanisms.

REFERENCES

1. Hinz K-H, Glünder G, Lüders H. Acute respiratory disease in turkey poults caused by Bordetella bronchiseptica-like bacteria. Vet Record 1978; 103:262-263.

2. Simmons DG, Gray JG, Rose LP. Isolation of an etiologic agent of acute respiratory disease (rhinotracheitis) of turkey poults. Avian Dis 1979; 23:194-203.

3. Saif YM, Moorhead PD, Dearth RN, Jackwood DJ. Observations on Alcaligenes faecalis infection in turkeys. Avian Dis 1980; 24:665-684.

4. Arp LH, Cheville NF. Tracheal lesions in young turkeys infected with Bordetella avium. Am J Vet Res 1984; 45:2196-2200.

5. Gray JG, Roberts JF, Dillman RC, Simmons DG. Cytotoxic activity of pathogenic Alcaligenes faecalis in turkey tracheal organ cultures. Am J Vet Res 1981; 42:2184-2186.

6. Simmons DG, Rose LP, McCorkle FM, Luginbuhl GH. Histamine-sensitizing factor of Alcaligenes faecalis. Avian Dis 1983; 27:171-177.

7. McCorkle FM, Edens FW, Simmons DG. Alcaligenes faecalis infection alters tissue monoamines in turkey poults, in Proceedings of the 65th Annual Meeting of the Conference of Research Workers in Animal Diseases 1984; 45.

8. Simmons DG, Gore AR, Hodgin EC. Altered immune function in turkey poults infected with Alcaligenes faecalis, the etiologic agent of turkey rhinotracheitis (coryza). Avian Dis 1980; 24:702-714.

9. Rimler RB. Turkey coryza: toxin production of Bordetella avium. Avian Dis 1985; 29:1043-1047.

10. Conn H. J., Lillie RD. H J Conn's Biological Stains. Baltimore: Williams and Wilkins, 1969; 154-155.

11. Gabridge MG, Polisky RB. Quantitative reduction of 2,3,5-triphenyltetrazolium chloride by hamster trachea organ cultures: Effects of Mycoplasma pneumoniae cells and membranes. Infect Immun 1976; 13:84-91.

12. Cantarow A, Schepartz B. Biochemistry. Philadelphia: WB Saunders Co, 1967; 364.

13. Toms GL, Rosztoczy I, Smith H. The localization of influenza virus: Minimal infectious dose determinations and single cycle kinetic studies on organ cultures of respiratory and other ferret tissues. Br J Exp Pathol 1974; 55:116-129.

14. Hershey FB, Cruikshank CDN, Mullins LI. The quantitative reduction of 2,3,5-triphenyltetrazolium chloride by skin in vitro. J Histochem Cytochem 1958; 6:191-196.

15. Robbins SL, Cotran RS, Kumar V. Pathologic Basis of Disease. 3rd ed. Philadelphia: WB Saunders Co, 1984; 559.

16. Ryu H, Kaeberle ML, Roth JA, Griffith RW. Effect of type A Pasteurella multocida fractions on bovine polymorphonuclear leukocyte functions. Infect Immun 1984; 43:66-71.

17. Kelly AT, Fulton M.. Use of triphenyltetrazolium in motility test medium. Am J Clin Pathol 1953; 23:512.

18. Gershman M. Use of tetrazolium salt for an easily discernible KCN reaction. Can J Microbiol 1961; 7:286.

19. Marshall DR, Simmons DG, Gray JG. Evidence for adherence-dependent cytotoxicity of Alcaligenes faecalis in turkey tracheal organ cultures. Avian Dis 1984; 28:1007-1015.

20. Simmons DG, Dees C, Rose LP. Toxin production by Bordetella avium (Alcaligenes faecalis), in Proceedings of the 66th Annual Meeting of the Conference of Research Workers in Animal Disease 1985; 30.

21. Etievant M, Chevance LG, Lesovrd M, Ohayon H. Complement activation and cytoimmunological alterations of the respiratory mucosa. Ann Immunol 1980; 131:13-42.

GENERAL SUMMARY AND DISCUSSION

This series of experiments was designed to evaluate the structural and functional effects of B avium infection in the turkey respiratory tract. Study 1 was designed to determine whether B avium infection predisposes turkeys to E coli airsacculitis and to evaluate the effect of B avium infection on pulmonary clearance of E coli. Study 2 was designed to determine if B avium infection affects tracheal adherence and colonization of E coli. Study 3 was designed to determine the distribution of B avium in lung and to describe changes in lung and bronchus-associated lymphoid tissue of turkeys experimentally infected with B avium. Study 4 was designed to determine if B avium heat-labile toxin would induce metabolic or morphological alterations in turkey tracheal organ cultures.

In these studies, turkeys experimentally infected with B avium developed severe fibrinopurulent airsacculitis after aerosol exposure to E coli, whereas similar aerosol exposure of normal turkeys resulted in only mild, transient airsacculitis. Association of E coli with the trachea may be an important step in the pathogenesis of airsacculitis in turkeys. Numbers of E coli in tracheas of B avium-infected turkeys were significantly greater than numbers of E coli in tracheas of B avium-free turkeys following aerosol exposure to E coli. Possibly, E coli dislodged from the trachea act

as a constant source of bacteria seeding the lower respiratory tract resulting in more severe airsacculitis in B avium-infected turkeys. Increased numbers of E coli in tracheas of B avium-infected turkeys were probably due to passive retention in tracheal exudate rather than specific adherence or colonization of E coli to tracheal epithelium.

No significant differences in pulmonary clearance of aerosolized E coli were found between B avium-infected and B avium-free turkeys. Although the importance of mucociliary clearance of inhaled bacteria is unknown in turkeys, these results suggest that mucociliary clearance may be of minor importance in removal of E coli from the lungs of turkeys. In turkeys exposed to aerosolized E coli, pulmonary abcesses and E coli bacteremia were much more common in 2-week-old turkeys compared to 4-week-old turkeys, but no differences were found between B avium-infected and B avium-free turkeys. Further studies will be needed to understand the development of this age-related resistance in turkeys.

Bordetella avium was found to colonize primary bronchi and persist in the lung of turkeys for at least 5 weeks postinoculation (PI). Pulmonary lesions in B avium-infected turkeys were limited to the primary bronchi and consisted of acute purulent bronchitis changing to a mononuclear response by 3 weeks PI. A similar progression of inflammatory lesions has been observed in tracheas of B avium-infected turkeys.[3]

However, severe tracheal lesions such as loss of ciliated epithelial cells, epithelial cell hyperplasia, depletion of mucus, and microcolonization of ciliated epithelial cells did not occur in the lung. Differences in lesions between primary bronchi and trachea are probably due to smaller numbers of colonizing B avium in bronchi compared colonization of trachea.

Compared to B avium-free turkeys, B avium-infected turkeys had greater numbers of lymphoid nodules in primary bronchi. This increase in the number of lymphoid nodules represents a nonspecific proliferation of bronchus-associated lymphoid tissue (BALT) in response to persistent B avium colonization. Average diameter of lymphoid nodules approximately doubled in both B avium-infected and B avium-free turkeys between 1 and 5 weeks of age. A similar age-dependent increase of BALT occurs in other species.[96]

Bronchus-associated lymphoid tissue in B avium-infected and B avium-free turkeys appeared as prominent, sharply demarcated lymphoid nodules projecting into the lumen of primary bronchi. Lymphoid nodules, histologically similar in B avium-infected and B avium-free turkeys, were composed of nonciliated, cuboidal epithelium overlying a single lymphoid follicle. Adjacent to the epithelium, the lymphoid follicle was composed of a zone of loosely arranged lymphocytes and macrophages. Deeper in the follicle, lymphocytes were more

densely arranged in sheets or concentrically around blood vessels. Some lymphoid follicles contained germinal centers. In turkeys, BALT may be an important site of antigen uptake and processing as evidenced by inflammatory responses centering on lymphoid nodules in turkeys exposed to an aerosol of E coli. Further studies will be needed to characterize the role of BALT in mucosal immunity of turkeys.

Sonicates of B avium strain P-4148 contain a heat-labile toxin which is lethal for turkeys.[9] Neither B avium sonicate containing heat-labile toxin, nor any other B avium bacterial fraction significantly altered the metabolism or morphology of turkey tracheal organ cultures. Therefore, heat-labile toxin is probably not directly responsible for local cytopathology in tracheal mucosa of B avium-infected turkeys.

From these studies, we conclude that B avium can colonize the primary bronchi of turkeys. This colonization results in inflammation focused on the primary bronchi and in proliferation of bronchus-associated lymphoid tissue. Secondly, E coli aerosolization in B avium-infected turkeys can result in severe fibrinopurulent airsacculitis. Although B avium infection had no effect on clearance of E coli from lung, turkeys retained a significant E coli population in their tracheas. Escherichia coli associated with the tracheal mucosa may serve as a continual source of infection to the airsacs. Thirdly, neither specific adherence nor

colonization could be demonstrated for the association of E coli with the normal or B avium-damaged trachea. Finally, heat-labile toxin of B avium does not alter cellular metabolism or epithelial morphology in turkey tracheal organ cultures.

Although these studies have contributed to our understanding of the structural and functional effects of B avium infection in the turkey respiratory tract, important questions remain. Why is E coli airsacculitis more severe in B avium-infected turkeys? Is mucociliary clearance from the lower respiratory tract impaired by B avium infection? What role does mucosal immunity have in resistance to B avium infection and is mucosal immunity age dependent? What role does BALT have in mucosal immunity? How does colonization of the respiratory tract by B avium lead to mucosal injury? And, do the toxins of B avium reported in the literature contribute to the pathogenesis of turkey coryza?

REFERENCES

1. National Turkey Federation Turkey Health Committee and American Association Of Avian Pathologists Poultry Health Advisory Committee. Report on turkey health research priorities. Kennett Square, PA:Author, 1986.

2. Simmons DG. Turkey coryza. In: Hofstad MS, Barnes HJ, Calnek BW, et al., eds. Diseases of Poultry. 8th ed. Ames, IA: Iowa State University Press, 1984; 251-256.

3. Arp LH, Cheville NF. Tracheal lesions in young turkeys infected with Bordetella avium. Am J Vet Res 1984; 45:2196-2200.

4. Saif YM, Moorhead PD, Dearth RN, Jackwood DJ. Observations on Alcaligenes faecalis infection in turkeys. Avian Dis 1980; 24:665-684.

5. Simmons DG, Gray JG, Rose LP. Isolation of an etiologic agent of acute respiratory disease (rhinotracheitis) of turkey poults. Avian Dis 1979; 23:194-203.

6. Gray JG, Roberts JF, Dillman RC, Simmons DG. Pathogenesis of change in the upper respiratory tracts of turkeys experimentally infected with an Alcaligenes faecalis isolate. Infect Immun 1983; 42:350-355.

7. Marshall DR, Simmons DG, Gray JG. Evidence for adherence-dependent cytotoxicity of Alcaligenes faecalis in turkey tracheal organ cultures. Avian Dis 1984; 28:1007-1015.

8. Gray JG, Roberts JF, Dillman RC, Simmons DG. Cytotoxic activity of pathogenic Alcaligenes faecalis in turkey tracheal organ cultures. Am J Vet Res 1981; 42:2184-2186.

9. Rimler RB. Turkey coryza: Toxin production of Bordetella avium. Avian Dis 1985; 29:1043-1047.

10. Boycott BR, Wyman HR, Wong FC. Alcaligenes faecalis rhinotracheitis in Manitoba turkeys. Avian Dis 1984; 28:1110-1114.

11. Heller ED, Weisman Y, Aharonovovitch A. Experimental studies on turkey coryza. Avian Pathol 1984; 13:137-143.

12. Ficken MD. Antibiotic aerosolization for treatment of alcaligenes rhinotracheitis. Avian Dis 1983; 27:545-548.

13. Barnes HJ, Hofstad MS. Susceptibility of turkey poults from vaccinated and unvaccinated hens to alcaligenes rhinotracheitis (turkey coryza). Avian Dis 1983; 27:378-392.

14. Page RK, Fletcher OJ, Lukert PD, Rimler R. Rhinotracheitis in turkey poults. Avian Dis 1978; 22:529-534.

15. Panigrahy B, Grumbles LC, Terry RJ, Millar DL, Hall CF. Bacterial coryza in turkeys in Texas. Poult Sci 1981; 60:107-113.

16. Van Alstine WG, Hofstad MS. Antibiotic aerosolization: The effect on experimentally induced alcaligenes rhinotracheitis in turkeys. Avian Dis 1985; 29:159-176.

17. Hinz K-H, Glünder G, Lüders H. Acute respiratory disease in turkey poults caused by Bordetella bronchiseptica-like bacteria. Vet Rec 1978; 103:262-263.

18. Berkhoff HA, Barnes HJ, Ambrus SI, Kopp MD, Riddle GD, Kradel MD. Prevalence of Alcaligenes faecalis in North Carolina broiler flocks and its relationship to respiratory disease. Avian Dis 1984; 28:912-920.

19. Berkhoff HA, McCorkle FM, Jr, Brown TT. Pathogenicity of various isolates of Alcaligenes faecalis for broilers. Avian Dis 1983; 27:707-713.

20. Simmons DG, Davis DE, Rose LP, Gray JG, Luginbuhl GH. Alcaligenes faecalis-associated respiratory disease of chickens. Avian Dis 1981; 25:610-613.

21. Montgomery RD, Kleven SH, Villegas P. Observations on the pathogenicity of Alcaligenes faecalis in chickens. Avian Dis 1983; 27:751-761.

22. Marshall DR, Simmons DG, Gray JG. An Alcaligenes faecalis isolate from turkeys: Pathogenicity in selected avian and mammalian species. Am J Vet Res 1985; 46:1181-1184.

23. Simmons DG, Gray JG. Transmission of acute respiratory disease (rhinotracheitis) of turkeys. Avian Dis 1979; 23:132-138.

24. Hofstad MS, Ritchie AE, Hill HT. Turkey coryza: A new disease of young poults, in Proceedings of the 26th North Central Poultry Disease Conference 1975; 58-59.

25. Simmons DG, Miller SE, Gray JG, Blalock HG, Colwell WM. Isolation and identification of a turkey respiratory adenovirus. Avian Dis 1976; 20:65-74.

26. Filion PR, Cloutier S, Vrancken ER, Bernier G. Infection respiratoire du dindonneau causee par un microbe apparente au Bordetella bronchiseptica. Can J Comp Med Vet Sci 1967; 31:129-134.

27. Simmons DG, Rose LP, Gray JG. Some physical, biochemic, and pathologic properties of Alcaligenes faecalis, the bacterium causing rhinotracheitis (coryza) in turkey poults. Avian Dis 1980; 24:82-90.

28. Kelly BJ, Ghazikhanian GY, Mayeda B. Clinical outbreak of Bordetella avium infection in two turkey breeder flocks. Avian Dis 1986; 30:234-237.

29. Kersters K, Hinz K-H, Hertle A, Segers P, Lievens A, Siegmann O, De Ley J. Bordetella avium sp. nov. isolated from the respiratory tracts of turkeys and other birds. Int J Syst Bacteriol 1984; 34:56-70.

30. Jackwood MW, Sasser M, Saif YM. Contribution to the taxonomy of the turkey coryza agent: Cellular fatty acid analysis of the bacterium. Avian Dis 1986; 30:172-178.

31. Saif YM, Moorhead PD, Whitmoyer RE. Scanning electron microscopy of tracheas from turkey poults infected with Alcaligenes faecalis. Avian Dis 1981; 25:730-735.

32. Slavik MF, Skeeles JK, Beasley JN, Harris GC, Roblee P, Hellwig D. Effect of humidity on infection of turkeys with Alcaligenes faecalis. Avian Dis 1981; 25:936-942.

33. Jackwood DJ, Saif YM, Moorhead PD, Dearth RN. Infectious bursal disease virus and Alcaligenes faecalis infections in turkeys. Avian Dis 1982; 26:365-374.

34. Cimiotti W, Glünder G, Hinz K-H. Survival of the bacterial turkey coryza agent. Vet Rec 1982; 110:304-306.

35. Glünder G, Hinz K-H, Lüders H, Stiburek B. Zur therapeutischen Wirksamkeit von Tetracyclin-HCl and Sulfaquinoxalin/Trimethoprim bei der Puten-Bordetellose. Zentralbl Veterinärmed Reihe B 1979; 26:591-602.

36. Skeeles JK, Swafford WS, Wages DP, Hellwig HM, Slavik MF, Beasley JN, Houghten GE, Blore PJ, Crawford D. Studies on the use of a long-acting oxytetracycline in turkeys: Efficacy against experimental infections with Alcaligenes faecalis and Pasteurella multocida. Avian Dis 1983; 27:1126-1130.

37. Hinz K-H, Korthas G, Lüders H, Stiburek B, Glünder G, Brozeit HE, Redmann T. Passive immunisation of turkey poults against turkey coryza (Bordetellosis) by vaccination of parent breeders. Avian Pathol 1981; 10:441-447.

38. Burke DS, Jensen MM. Field vaccination trials against turkey coryza using a temperature-sensitive mutant of Alcaligenes faecalis. Avian Dis 1981; 25:96-103.

39. Burke DS, Jensen MM. Immunization against turkey coryza by colonization with mutants of Alcaligenes faecalis. Avian Dis 1980; 24:726-733.

40. Jensen MM, Marshall MS. Control of turkey alcaligenes rhinotracheitis in Utah with a live vaccine. Avian Dis 1981; 25:1053-1057.

41. Hofstad MS, Jeska EL. Immune response of poults following intranasal inoculation with Artvax™ vaccine and a formalin-inactivated Bordetella avium bacterin. Avian Dis 1985; 29:746-754.

42. Jackwood MW, Saif YM, Moorhead PD, Dearth RN. Further characterization of the agent causing coryza in turkeys. Avian Dis 1985; 29:690-705.

43. Rimler RB, Simmons DG. Differentiation among bacteria isolated from turkeys with coryza (rhinotracheitis). Avian Dis 1983; 27:491-500.

44. Simmons DG, Rose LP, Brogden KA, Rimler RB. Partial characterization of the hemagglutinin of Alcaligenes faecalis. Avian Dis 1984; 28:700-709.

45. Luginbuhl GH, Rader JM, Simmons DG. Evidence for plasmid DNA in Alcaligenes faecalis. Am J Vet Res 1984; 45:1679-1681.

46. Simmons DG, Rose LP, Fuller FJ, Maurer LC, Luginbuhl GH. Turkey coryza: Lack of correlation between plasmids and pathogenicity of Bordetella avium. Avian Dis 1986; 30(3):593-597.

47. Simmons DG, Rose LP, McCorkle FM, Luginbuhl GH. Histamine-sensitizing factor of Alcaligenes faecalis. Avian Dis 1983; 27:171-177.

48. Simmons DG, Dees C, Rose LP. Toxin production by Bordetella avium (Alcaligenes faecalis), in Proceedings of the 66th Annual Meeting of the Conference of Research Workers in Animal Disease 1985; 30.

49. Jackwood MW, Saif YM. Pili (Fimbriae) identification and expression on Bordetella avium, in Proceedings of the 37th North Central Avian Disease Conference 1986; 22.

50. Finer RR. Alcaligenes rhinotracheitis of the turkey poult: Bacterial adherence and virulence. PhD Dissertation Texas A & M University, College Station, TX 1984.

51. Gray JG, Roberts JF, Simmons DG. In vitro cytotoxicity of an Alcaligenes faecalis and its relationship in in vivo tracheal pathologic changes in turkeys. Avian Dis 1983; 27:1142-1150.

52. McCorkle FM, Edens FW, Simmons DG. Alcaligenes faecalis infection alters tissue monoamines in turkey poults, in Proceedings of the 65th Annual Meeting of the Conference of Research Workers in Animal Disease 1984; 45.

53. McCorkle FM, Edens FW, Simmons DG. Alcaligenes faecalis infection in turkeys: effects on serum corticosterone and serum chemistry. Avain Dis 1985; 29:80-89.

54. Edens FW, McCorkle FM, Simmons DG. Body temperature response of turkey poults infected with Alcaligenes faecalis. Avian Pathol 1984; 13:787-795.

55. Simmons DG, Gore AR, Hodgin EC. Altered immune function in turkey poults infected with Alcaligenes faecalis, the etiologic agent of turkey rhinotracheitis (coryza). Avian Dis 1980; 24:702-714.

56. McCorkle FM, Simmons DG, Luginbuhl GH. Delayed hypersensitivity response in Alcaligenes faecalis- infected turkey poults. Avian Dis 1982; 26:782-786.

57. McCorkle FM, Simmons DG, Luginbuhl GH. Graft-vs-host response in Alcaligenes faecalis-infected turkey poults. Am J Vet Res 1983; 44:1141-1142.

58. McCorkle FM, Simmons DG. In vitro cellular migration of leukocytes from turkey poults infected with Alcaligenes faecalis. Avian Dis 1984; 28:853-857.

59. McCorkle FM, Simmons DG. Immediate hypersensitivity (anaphylaxis) response in uninfected and Alcaligenes faecalis-infected turkey poults. Avian Dis 1984; 28:579-585.

60. Yegana YY, Weisman Y, Herz A, Schapira R, Hoo I. The effect of combined and separate infection by Alcaligenes faecalis and paramyxovirus (Yucaipa) on the surface morphology of the trachea in turkey poults. Avian Pathol 1985; 14:543-551.

61. Meinecke CF, Duncan RL, Skeeles JK. Circulating Newcastle disease antibodies and mortality related to turkey coryza and vaccine breaks. Poult Sci 1981; 60:1696.

62. Gross WB. Miscellaneous bacterial diseases. In: Hofstad MS, Barnes HJ, Calnek BW, et al., eds. Diseases of Poultry. 8th ed. Ames, IA: Iowa State University Press, 1984; 270-278 .

63. Palmer CC, Baker HR. Studies on infectious enteritis of poultry caused by Bacterium coli communis. J Am Vet Med Assoc 1923; 63:85.

64. Fabricant J, Levine PP. Experimental production of complicated chronic respiratory disease infection ("air sac" disease). Avian Dis 1962; 6:13-23.

65. Gross WB. Escherichia coli as a complicating factor in chronic respiratory disease of chickens and infectious sinusitis of turkeys. Poult Sci 1956; 35:765-771.

66. Glantz PJ, Narotsky S, Bubash G. Escherichia coli serotypes isolated from salpingitis and chronic respiratory disease of poultry. Avian Dis 1962; 6:322-328.

67. Nairn ME. Bacterial osteomyelitis and synovitis of the turkey. Avian Dis 1973; 17:504-517.

68. Trylich C, Howell J, MacDonald DW. Avian coligranuloma: Case histories. Can Vet J 1977; 18:38-40.

69. Dominick MA, Cheville NF. Pathology of cloacal bursae of gnotobiotic turkeys orally inoculated with Escherichia coli. Vet Pathol 1985; 22:262-271.

70. Cheville NF, Arp LH. Comparative pathologic findings of Escherichia coli infection in birds. J Am Vet Med Assoc 1978; 173:584-587.

71. Gross WB. The role of Escherichia coli in the cause of chronic respiratory disease and certain other respiratory diseases. Am J Vet Res 1958; 19:448-452.

72. Saif YM, Moorhead PD, Bohl EH. Mycoplasma meleagridis and Escherichia coli infections in germfree and specific-pathogen-free turkey poults: Production of complicated airsacculitis. Am J Vet Res 1970; 31:1637-1643.

73. Springer WT, Luskus C, Pourciau SS. Infectious bronchitis and mixed infections of Mycoplasma synoviae and Escherichia coli in gnotobiotic chickens. Infect Immun 1974; 10:578-589.

74. Barnes HJ. Newcastle disease vaccination of market turkeys, in Proceedings of the Virginia turkey days 1983; 44-48.

75. Nagaraja KV, Emery DA, Jordan KA, Sivanandan V, Newman JA, Pomeroy BS. Effect of ammonia on the quantitative clearance of Escherichia coli from lungs, air sacs, and livers of turkeys aerosol vaccinated against Escherichia coli. Am J Vet Res 1984; 45:392-395.

76. Harry EG. The survival of Escherichia coli in the dust of poultry houses. Vet Rec 1964; 76:466-470.

77. Harry EG, Hemsley LA. The relationship between environmental contamination with septicaemia strains of Escherichia coli and their incidence of chickens. Vet Rec 1965; 77:241-245.

78. Piercy DWT, West B. Experimental Escherichia coli infection in broiler chickens: Course of the disease induced by inoculation via the air sac route. J Comp Path 1976; 86:203-210.

79. Arp LH, Graham CLG, Cheville NF. Comparison of clearance rates of virulent and avirulent Escherichia coli in turkeys after aerosol exposure. Avian Dis 1979; 23:386-391.

80. Arp LH. The response of liver and spleen to Escherichia coli bacteremia in turkeys. PhD Dissertation. Iowa State University, Ames, IA 1981.

81. Arp LH, Jensen AE. Piliation, hemagglutination, motility, and generation time of Escherichia coli that are virulent or avirulent for turkeys. Avian Dis 1980; 24:153-161.

82. Dho M, Lafont JP. Escherichia coli colonization of the trachea in poultry: Comparison of virulent and avirulent strains in gnotoxenic chickens. Avian Dis 1982; 26:787-797.

83. Clegg H, Guerina N, Langermann S, Kessler TW, Guerina V, Goldman D. Pilus-mediated adherence of Escherichia coli K1 to human oral epithelial cells. Infect Immun 1984; 45:299-301.

84. Iwahi T, Abe Y, Nakao M, Imada A, Tsuchiya K. Role of type 1 fimbriae in the pathogenesis of ascending urinary tract infection induced by Escherichia coli in mice. Infect Immun 1983; 39:1307-1315.

85. Svanborg Edėn C, Hansson HA. Escherichia coli pili as possible mediators of attachment to human urinary tract epithelial cells. Infect Immun 1978; 21:229-237.

86. To SC-M, Moon HW, Runnels PL. Type 1 pili (F1) of porcine enterotoxigenic Escherichia coli: Vaccine trial and tests for production in the small intestine during disease. Infect Immun 1984; 43:1-5.

87. Jayappa HG, Goodnow RA, Geary SJ. Role of Escherichia coli type 1 pilus on colonization of porcine ileum and its protective nature as a vaccine antigen in controlling colibacillosis. Infect Immun 1985; 48:350-354.

88. Naveh MW, Zusman T, Skutelsky E, Ron EZ. Adherence pili in avian strains of Escherichia coli: Effect on pathogenicity. Avian Dis 1984; 28:651-661.

89. Gyimah JE, Panigrahy B. Immunogenicity of Escherichia coli (Serotype 01) pili vaccine in chickens. Avian Dis 1985; 29:1078-1083.

90. Nagaraja KV, Emery DA, Newman JA, Pomeroy BS. Identification and isolation of somatic pili from pathogenic Escherichia coli of turkeys. Am J Vet Res 1983; 44:284-287.

91. Dominick MA, Schmerr MJF, Jensen AE. Expression of type 1 pili by Escherichia coli strains of high and low virulence in the intestinal tract of gnotobiotic turkeys. Am J Vet Res 1985; 46:270-275.

92. Arp LH. Effect of antibodies to type 1 fimbriae on clearance of fimbriated Escherichia coli from the bloodstream of turkeys. Am J Vet Res 1985; 46:2644-2647.

93. Fedde MR. Respiration. In: Sturkie PD, ed. Avian Physiology. 4th ed. New York: Springer-Verlag, 1986; 191-197.

94. Hodges RD. The histology of the fowl. New York, NY: Academic Press, 1974; 113-146.

95. Banks WJ. Histology and comparative organology: A text-atlas. Baltimore, MD: Williams and Wilkins, Co., 1974; 212-216.

96. Bienenstock J. Bronchus-associated lymphoid tissue. In: Bienenstock J, ed. Immunology of the lung. New York, NY: McGraw-Hill, 1984; 96-118.

97. Bienenstock J, Befus D. Gut and bronchus associated lymphoid tissue. Am J Anat 1984; 170:437-445.

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