

AN ABSTRACT OF THE THESIS OF

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Title : RESPIRATORY PATHOGENESIS OF PASTEURELLA MULTOCIDA
IN TURKEYS

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Pasteurella multocida causes diseases in many animal species including fowl cholera, a septicemic disease of poultry and other birds. Pathogenesis of the disease has been studied by many investigators by the systemic administration of the organism in poultry. However, only a few studies have been done as to the respiratory pathogenesis of the organism. The objective of the study was to investigate the fate of *P. multocida* after the intratracheal administration in turkeys

The fate of four strains of *Pasteurella multocida* was studied after their intratracheal inoculation in young adult turkeys. Viable bacterial counts were made in respiratory tissues as well as in the liver, spleen and blood at 6 and 9 hrs after the inoculation of approximately 10^9 viable organisms of each strain. A virulent, encapsulated strain, P-1059, invaded systemically by 6 hrs postinoculation (PI)

and multiplied vigorously in all tissues and organs examined. A blue colony mutant of P-1059, T-325, which does not possess a thick layer of capsule, as well as CU vaccine strain, invaded the parenchymal organs, but did not show significant increase in viable counts at 9 hrs PI compared with at 6 hrs PI. Another vaccine strain, M-9, also invaded blood and internal organs by 6 hrs PI, however, its viable counts showed no significant change between 6 and 9 hrs PI, or in some tissues significant decrease at 9 hrs PI. The results indicate that all the four strains possess high capacity to invade respiratory tissues with varying capacity to persist in host tissues.

The lesions caused by two strains of *Pasteurella multocida* (P-1059 and M-9) were observed after their intratracheal inoculation in young adult turkeys. The lesions were observed in the respiratory organs at 0, 0.25, 0.5, 1, 2, 3, and 6 hrs after inoculation of approximately 10^9 viable organisms of each strain. Both virulent strain, P-1059 and non-virulent vaccine strain, M-9, have capacity to invade and multiply in the tissues examined.

Macroscopicly, the lesions in the lung and in the airsac were found as early as 1 hr PI, including the infected lung was foamy and the airsac became cloudy. They became more severe by 2 to 6 hrs PI. Microscopicly, heterophiles were present, occasionally, in the lung, trachea and airsac by 0 to 1 hr after inoculation. Then they became more severe by 2 to 6 hrs PI. By 6 hrs PI, there were diffuse

heterophiles infiltration in the trachea, lung, and airsac. The lung vascular was edema. The trachea ciliate and mucous gland was cystic or hyperplasia, and the airsac showed increased in thickness and cloudiness. These results of study indicate that the lesion caused by P-1059 and vaccine strain, M-9, were not significantly different.

RESPIRATORY PATHOGENESIS OF *PASTEURELLA MULTOCIDA*

IN TURKEYS

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Respiratory Pathogenesis of
Pasteurella multocida
in Turkeys

Chapter I

Introduction

(Literature review)

The Organism

Pasteurella multocida has been recognized as an important veterinary pathogen for over a century, and its pathogenic importance has been increasingly recognized in the last 50 years. The organism causes acute septicemia with high mortality including hemorrhagic septicemia in cattle and fowl cholera in poultry (Bain, 1963; Rhoades & Rimler, 1991), or non-septicemic diseases including shipping fever in cattle, pneumonia in sheep and goats, pneumonia and rhinitis in swine and chronic upper respiratory infection (snuffles) in rabbits (Carter and Bain, 1960). The organism can also occur as a commensal in the naso-pharyngeal region of apparently healthy animal of many species, and it can be either a primary or secondary pathogen in a variety of domestic or feral mammals and birds (Griefz et al., 1986). Infection in human can occur most frequently after being

bitten or scratched by infected animals such as cats or dogs (Yeff, 1983). The clinical picture includes a cellulitis, bronchitis, pneumonia, and pleural empyema (Griefz et al., 1986).

Pasteurella multocida is a Gram-negative, non-motile, non-sporogenous, coccobacillus, and the cell occurs singly, in pairs or occasionally as a chained filament (Krieg, 1984). Virulent strains of *P. multocida* are usually encapsulated and the capsule can be seen in tissues from infected animals and from laboratory cultures (Rimmler and Rhoades, 1989). The capsule contains carbohydrate and other materials and differs in size. They are frequently lost upon repeated passages *in vitro* (Heddleston, 1962)

Generally, description of colonies are made from 18-24 hrs cultures incubated aerobically at 35-37 C on enriched media, containing serum, blood or dextrose starch (Carter 1981; Rhoades and Rimmler, 1989). Under these conditions, colonies usually range in size from 1.0 to 3.0 mm in diameter. Two principal colony forms, mucoid and smooth, have been recognized with no hemolysis. It does not grow on MacConkey agar. Metabolically, the organism is catalase-positive, oxidase-positive, indole-positive, and urease-negative. On the triple sugar iron medium (TSI) it produces an acid slant and acid butt with no gas/H₂S (Heddleston, 1964; Carter, 1967; Oberhofer, 1981). The large colonies are mucoid and composed of cells with capsule

consisting in part of hyaluronic acid (Jasmin, 1945). The mucoid characteristic can range from a culture whose colonies are discrete, circular, convex or having slight mucous like consistency to a culture whose colonies are confluent. The latter colonies, sometimes referred to as watery mucoid colonies, possess flowing margin.

Observation of the colonial morphology on semitransparent media under obliquely transmitted light with a stereomicroscope gives useful characteristics of *P. multocida* that distinguish variants (Elberg 1950; Heddleston, 1964). Except for watery mucoid colonies which appear gray, colonies consisting of capsulated cells display a yellowish green, bluish-green or pearl-like iridescence (Heddleston, 1964; Rhoades & Rimler, 1991). Colonies that consist primarily of noncapsulated cells are not iridescent and appear blue, grayish-blue, or grey. Dissociation, which is manifested primarily by a change from an iridescent to a blue-grayish-blue colony form, is often associated with the loss of the bacterial capsule (Heddleston, 1964).

Antigen and Serology

Serological classification of *P. multocida* is complicated because of its antigenic complexity (Brogden and Packer, 1970). Various investigators have developed different serological methods, but no international classification system has yet been established (Brogden &

Packer, 1970; Heddleston, 1972). By convention, most workers use Carter's indirect hemagglutination method (Carter, 1955) for typing the capsular antigen and Heddleston's gel diffusion method (Heddleston et al., 1970) for typing the somatic antigen. Capsule types of *P. multocida* can be separated into five serological groups (A, B, D, E or F) by the indirect hemagglutination test. Sixteen somatic types (type 1 through 16) have currently been recognized by the immunodiffusion test with antiserum against purified LPSs (Rimler & Rhoades, 1987). Capsular types B and E strains of *P. multocida* are the frequent cause of hemorrhagic septicemia in cattle (Carter & Bain, 1960). Most strains associated with acute fowl cholera belong to capsular type A, but their somatic antigens are heterogeneous (Carter, 1967; Rimler and Rhodes, 1987).

To eliminate difficulties and time involved with the indirect hemagglutination test, Carter and Rudle (1975) developed a simple test in which serogroup A strains are recognized by depolymerization of the capsule after growth in proximity to hyaluronidase-producing strain of *Staphylococcus aureus*. The basis for the presumptive recognition of the serogroup D isolate is the characteristic floccular reaction with acriflavine (Carter and Subronto, 1979). However, Rimler and Rhoades, (1987) found that newly recognized serogroup F strains reacted similarly.

Characteristic mucoid colonies, due to the presence of

hyaluronic acid in the capsule, has been found in most capsule type A and a few type D strains (Carter, 1967). Most strains of *P. multocida* that produce colonies of large watery mucoid variety have been found to belong to serogroup A or D (Carter, 1967). Some colonies of capsular types A, D or F strains display pearl-like iridescence under oblique transmitted light. Subgroup B and E strains produce small capsule and form smooth colonies that usually have yellowish or bluish-green iridescence (Rhoades and Rimler, 1989). The presence of capsule is not an absolute criterion for virulence of *P. multocida* because capsulated or non-capsulated strains may be virulent. However, Penn and Nagy (1974) reported transformation of colonies from iridescence to noniridescence and concomitant loss of capsule are associated with reduction or loss of virulence in some strains (Heddleston et al., 1964).

The capsule antigen of *P. multocida* responsible for capsular types specificity is intimately associated with lipopolysaccharide (LPS), as well as with non-antigenic polysaccharide material. Both capsule specific antigen and LPS are adsorbed onto erythrocyte from crude cell extract (Rimler and Rhoades, 1991). However, the indirect hemagglutination test with serum containing antibody against each usually shows a reaction only with the capsule-specific antigen. Carter (1955) reported that LPS and capsule-specific antigen are considered to be identical in some

report, but the exact chemical composition of the surface antigens is unknown. Despite the importance of the capsule specific antigen, few studies have been aimed at determining its composition. After heating crude extract at 56 C for 30 min, the antigen is retained and can be adsorbed onto erythrocytes. Lipopolysaccharide can be extracted from the cell by using the hot phenol-water method of Wesphal and Jann (1965) and precipitated from the aqueous phase with ethanol. Knox and Bain (1960) characterized preparation of capsular polysaccharides isolated from supernatant fluid by ethanol precipitation after removing the pH 3.8 isoelectric precipitates. Protein was removed from final product by the trypsin treatment. Although the substance was antigenic and non-toxic, the best polysaccharide preparation are heterogeneous and formed two precipitin lines in an immunodiffusion test. It contained fructose, mannose and glucosamine. The polysaccharide containing fructose was produced by both an iridescent and noniridescent variant of strain. Penn and Nagy (1976) isolated capsule antigen from serogroup B and E strains. The nontoxic antigens were isolated by fractional precipitation from aqueous solution with polar solvents. The chemical composition of the antigens were not established. However, they were assumed to be acidic polysaccharides of high molecule weights, based upon gel filtration chromatography, precipitation with ethyl pyridium chloride, insensitivity to pronase and nonreaction

with protein stains (Rimler and rhoades, 1991)

Pasteurella multocida LPS have chemical and biological properties similar to those found in many species of Gram-negative bacteria. As an antigen LPS has been associated with protection of animals and is believed to be the chemical basis for specificity of somatic typing system. It has been studied as a purified form or in association with protein as complexes. Prosky (1938) was able to extract LPS-protein complexes from two or three strains of *P. multocida* using three chloroacetic acid. The complexes were characteristics of Boivin type antigen (endotoxin) being both immunogenic and toxic. Rabbit antiserum, made against the antigen, produced active immunity in mice against the endotoxin challenge. Similar complexes were extracted from fowl cholera strains (serotype 1 and 3) and a bison hemorrhagic septicemia strain (serotype 2) with formaldehyde saline solution (Heddleston et al., 1966; Rebers et al., 1966). After purification by ultracentrifugation, the complexes had endotoxin activity and induced serotype specific active and passive immune protection against the challenge with live organisms.

Galancs et al. (1969) made a preparation of LPS from ethanol-killed cells that had been washed and dried. Cells killed by formalin have been used, but the use of formalin has resulted in alteration of extractibility, toxicity and chemical composition of LPS from certain strains (Rebers

and Rimler 1984). Extraction is done by the phenol-water method of Westphal and Jan (1965) or phenol:chloroform:petroleum ether (PCP) method of Galanos et al. (1965). The presence or the absence of the capsule seems to have no influence on extractibility by either method. Lipopolysaccharides can be extracted from most strains by one of the methods but not both. With the other strains, either method can result in comparable yield. Because cells are not destroyed by the PCP method, they can be recovered, washed with diethyl ether and dried for use in the phenol-water method. All LPS contain lipid A, glucose, heptose, and 2-keto-3 deoxy octanate (KDO), and glucosamine (Erler et al., 1977). Rimler et al. (1984) reported that buoyant densities of *P. multocida* LPS determined by ultracentrifugation in cesium chloride gradient were about 14 g/ml; the LPS caused direct hemagglutination with chicken and turkey erythrocytes but not with horse and sheep erythrocytes (Rimler, 1984). Purified *P. multocida* LPS is antigenic and can react with avian and mammalian antisera made against the whole organism. An immunoprecipitation line of partial identity usually occurs with LPS and specific heat-stable antigen of the Heddleston type system (Rhoades and Rimler, 1989). Rimler (1984) found that LPS from some serotypes produce antibodies in chicken whereas others did not. Rebers (1980) found that antibodies made against purified LPS of avian strains protected chicken

against challenge with the homologous organism.

Fowl Cholera

Fowl cholera is an acute septicemic disease with high morbidity and mortality which may affect many types of birds throughout the world (Rhoades & Rimler, 1991). The disease has also been called avian cholera, avian pasteurellosis, or avian hemorrhagic septicemia. Strains of capsular serotype A of *P. multocida* are recognized as the cause of fowl cholera (Carter 1967; Rhoades and Rimler, 1987).

Fowl cholera is widely distributed geographically, occurring in most poultry producing countries of the world. The occurrence of the disease is frequent or sporadic depending on location and prevalence from year to year. Poultry, particularly the chicken, turkey, pheasant, duck, and goose, are commonly affected and most reports on disease occurrence and research are concerned with these hosts. However, the disease is also of major importance in some wild birds.

The degree of susceptibility varies among different age groups within a species. Turkeys and pheasants are more susceptible than chickens (Heddleston, 1962; Curtis et al., 1980). Generally speaking, older chickens and turkeys were more susceptible to fowl cholera than younger ones (Heddleston, 1962; Hangerford, 1968). The natural disease does not occur in chickens or turkeys of 5 weeks of age or

younger.

The transmission of the disease within a flock is considered to be primarily through direct or indirect contact, including exposure to water or feed contaminated by infected members of the flock. Generally, the manner in which a flock becomes infected is unknown. However, a number of potential sources of infection have been recognized. Chronically affected birds in poultry flocks may shed the organism for years, and serve as a source of infection for susceptible birds (Van and Olney, 1940; Hall et al, 1955). Wilds birds and mammals may often serve as carriers of the disease (Snipes et al., 1987).

Common clinical signs include depression, ruffled feathers, fever, anorexia, mucous discharge from the mouth, diarrhea and high temperature. In some cases, such signs are present only a few hours before death, so the first observed evidence of disease is often death. Near the time of death, birds often become cyanotic; the cyanosis is particularly evident in the comb and wattle of chicken and snoods and unfeathered skin of the head of turkeys. Fecal materials from acutely affected bird is generally watery and predominantly white in early stage of disease and later become predominantly green containing mucus. Rhoades and Rimler (1991) found that signs in chronically infected birds are usually associated with localized infection, involving such structures as wattles, sinuses, preorbital subcutaneous

tissues, leg or wing joints, sternal bursae and foot pads. Exudative conjunctivitis and pharyngitis are also observed.

Pathogenesis

Pathogenicity or virulence of *P. multocida* in relation to fowl cholera is complex and variable depending on the strain, host species and variation within the strain or host and on the condition of contact between the two. The ability of *P. multocida* to invade and reproduce in the host is related to a capsule that surrounds the organism.

Portal of entry of *P. multocida* in natural infection in poultry is probably the pharynx and/or upper respiratory tract (Hughes, 1930 and Arsove, 1965), but it may also enter through the conjunctiva or cutaneous wound. The rapid multiplication of bacteria within the bird may produce an acute fatal septicemia, and the cause of death is presumed to be endotoxic shock (Hunter and Wobeter, 1980).

Maheswaran et al., (1971) demonstrated that when only 5×10^4 organism of virulent *P. multocida* were endotracheally inoculated into turkeys, the bacteria emerged in blood and spleen as early as 6 hrs after inoculation. The organism appears to invade the mucosal epithelium soon after colonization and enter the blood stream. Pabs-Garnon and Soltys (1971) demonstrated that virulent *P. multocida*, when inoculated intravenously into turkeys, initially localized in the liver and spleen. The bacteria multiplied primarily

in the two organs and were abruptly released into the blood stream at the terminal stage; resulting in septicemia. The implication of this observation is that the bacteria may be trapped by the reticulo-endothelial phagocytes in the liver and spleen, and multiplied intracellularly in the phagocytes. Some histopathological evidence which support this view has recently been reported (Wallner-Pendleton and Matsumoto, 1988). The manner by which infection of the lungs occurred could not be ascertained by experimental inoculation (Rhoades & Rimler, 1990). Generally, infection did not spread from colonized pharyngeal tissue to trachea. The presence of a few organisms in the trachea supported the possibility that infection of the lungs may be originated from the air passage. Tsuji and Matsumoto (1989) demonstrated that the blood-borne *P. multocida* in both encapsulated and nonencapsulated form was rapidly removed from the blood stream into the liver and spleen regardless of the presence or absence of capsule. However, encapsulation seemed to be essential for survival of *P. multocida* after being entrapped in the liver or spleen.

Gross and Histopathological Lesions

Lesions of fowl cholera are not constant but vary in type and severity. The greatest variation is related to the course of the disease, whether acute or chronic. with the former, postmortem lesions are associated with vascular

disturbances. General hyperemia is most evident in veins of the abdominal viscera, and may be quite pronounced in small vessels of the duodenal mucosa. Petechial and ecchymotic hemorrhages are frequently found and may be widely distributed. Subepicardial and subserosal hemorrhages are common, as are hemorrhages in the lung, abdominal wall, and intestinal mucosa (Rhoades, 1964). Disseminated intravascular clotting causing fibrinous thrombosis has been observed in chickens and ducks that died from acute experimentally induced fowl cholera (Hunter and Wobeser, 1980; Park, 1982).

The liver of acutely affected birds may be swollen and usually contain multiple, small focal areas of coagulative necrosis and heterophilic infiltration. However, some of less virulent *Pasteurella multocida* do not produce necrotic foci in the liver (Rhoades & Rimler, 1991). Rhoades (1964) had shown heterophilic infiltration also occurred in lungs and certain other parenchymatous organs. The lung of affected birds may be partly or completely consolidated, dark red and covered by a yellow exudate. The consolidation is due to a massive exudation of fibrin and heterophiles affecting all parts of lungs, including the pleura. The lung of turkeys are affected more severely than those of chicken, with pneumonia being a common sequela.

Prantner et al. (1991) reported that in the lungs after 16 to 24 hrs inoculated with a highly virulent field

isolated of *P. multocida* serotype A:3,4 (86-1913) by an oculo-nasal-oral route, large bronchi were completely filled with bacteria, fibrin, and a few necrotic heterophils. The parabronchi were distended by a loose network of fibrin, degenerated heterophils, and large number of bacteria. Changes in pulmonary veins and small arteries included fibrinoid necrosis, subendothelial accumulation of heterophils (Prantner et al., 1991). Large amounts of viscid mucus were observed in the digestive tract particularly in the pharynx, crop, or intestine. Prominent splenic changes occurred at 16 to 24 hrs postinoculation and varied in severity among individual birds. There was degeneration of periartericular reticular cells; these cells progressed to by coalescing areas of coagulative necrosis containing solid sheets of extracellular bacteria and heterophils. Hungerford (1968) reported there was catarrhal exudates in mass passage and in the wind pipe; the air sacs may be partly filled with caseous material.

Chronic lesion is usually characterized by localized infection in contrast to the septicemic nature of the acute disease. They often occur in the respiratory tract and may involve any part including sinuses and pneumatic bones. Pneumonia is an especially common lesion in turkeys. Chronic localized infection can involve the middle ear and cranial bones and can result in torticollis (McCune and Olsen, 1974). Affected birds have a caseous exudate filling

many of air spaces around the middle air in the cranium. The exudate consists primarily of fibrin and heterophiles. In some areas, the cell may become necrotic and form an eosinophilic mass containing nuclear debris and surrounded by giant cell (Olson et al., 1966).

Immunity

Successful induction of active acquired immunity against fowl cholera has been observed by Pasteur in 1881. This attempt to immunize against the disease was accomplished using an attenuate bacterial strain. Later, attempts to immunize poultry generally involved the use of bacterin (inactivated whole bacteria) which were often combined with adjuvant. Bacterins continue to be widely used commercially and are generally efficacious. Heddleston (1970) demonstrated that immunity induced by the bacterin is limited within somatic types of strain. There is a good correlation between immunotype and somatic serotype determined by the procedure of Heddleston et al. (1972) which utilizes bacterial LPS as an antigen (Brodgen and Rebers, 1978). Because of the relationship between immunotype and somatic serotype, the LPS antigen is considered an important contributor to the immunity induced by bacterins (Rebers et al., 1980 ; Rimler and Philips, 1986). Immunity induced by bacterins, however, covers only limited antigenic scope and is short-lived. Live vaccine

strain M-9 and Minnesota (MN) strains are safe, generally effective and offer immunity against wider antigenic diversity than bacterins do, but the induced immunity lacks in duration and intensity (Matsumoto, 1989; Friedlander et al., 1991). Other live vaccines such as CU, PM#1, and #3, mostly belonging to serotype 3,4, have been used in various parts of the country. Immunity induced by a live vaccine is cross-protective against the challenge infection with the heterologous somatic serotype strains of *P. multocida* (Bierer et al., 1979). A serious disadvantage of this vaccine is that the vaccine strain of *P. multocida*, by itself, appears to cause significant mortality when it is administered to the bird under undefined stresses or immunosuppressive conditions.

The mechanism by which active acquired immunity prevents development of fowl cholera is not well understood. Both humoral and cell mediated immunity are considered important contributors. Since immunity can be passively transferred to susceptible birds using either serum or serum-globulin from immunized birds (Rebers et al., 1975; Rimler, 1987), humoral factors play a major role. However, Tsuji and Matsumoto (1990) indicated that a macrophage-stimulating factor enhances bactericidal activity against *P. multocida*

Chemotherapy

Anti-bacterial chemotherapy has been used extensively in treatment of fowl cholera with varying success, depending to large extent on the promptness of treatment and the kind of drugs used. Sensitivity testing is often advantageous if it is done in a prompt manner, since strains of *P. multocida* vary in susceptibility to chemotherapeutic agents and resistance to treatment may develop, especially during prolonged use of a drug. The therapeutic agents are effective in improving clinical disease of fowl cholera, especially by decreasing mortality. However, none of the drugs with any means of application is useful for the complete recovery of affected birds. Some reports indicated that *P. multocida* multiplies in phagocytes of liver and spleen and also can localize in joints and cerebrospinal spaces as well as on the surface of the respiratory tract. The ineffectiveness of the drugs may be explained by the intracellular localization of the organism (Matsumoto, 1989). Sulphonamides have been employed both experimentally and in natural outbreak. The main disadvantage of sulphonamides are their bacteriostatic instead of bactericidal action, inability to cure localized abscesses and toxic effect on birds. Kiser (1948) reported 63-85 percent reduction in mortality from experimentally produced fowl cholera compared to the untreated control when using sulfamethazine and sodium sulfamethazine. Favorable results

were obtained with 0.5-1.0 percent of drug in food, or 0.1 percent in drinking water.

Administeration of penicillin, streptomycin, or tetracyclin via an intramuscular route, all showed therapeutic effects (Bierer, 1962). Little (1948) reported that the use of chlortetracyclin reduced losses in chickens about 80 % when given at the rate of 40 mg/ kg body weight intramuscularly a half hour after parenteral inoculation of the organism.

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Chapter II

The invasive and Persisting Capacity of
Four Strainsof *Pasteurella Multocida*
After The Intratracheal Inoculation into Turkeys

Summary

The four strains of *Pasteurella multocida*, P-1059, T-325, CU, and M-9 were used in this study. They are intratracheally (IT) inoculated into turkeys, the number of viable bacteria in the trachea, lung, liver, spleen and the blood were enumerated during periods of 6 and 9 hrs postinoculation (PI). The four strains of organism were present in the organ and the tissues observed. In the liver, spleen and blood, the virulent P-1059 strain showed significant increases within 6 or 9 hrs PI. Strain CU showed significant increase only in the spleen. Two other strains did not show significant increase in any organs; strain M-9, on the other hand, showed significant decrease in the trachea. These results indicated that P-1059, and three other strains showed their ability to invade the tissues or organs when they are intratracheally (IT) inoculated.

Introduction

Fowl cholera is a bacterial disease of fowl and other birds caused by *Pasteurella multocida* (Rhoades and Rimler, 1991). Economically, it causes significant worldwide poultry losses, including death loss, condemnation loss, and vaccination and medication cost (Carpenter et al., 1988). A variety of vaccines are available, but have shown only a limited value in preventing the disease. Inactivated vaccines (bacterins) induce only type-specific immunity for limited duration and must be applied individually (Heddleston, 1972). Live vaccine strains are generally effective when given in the drinking water (Bierer and Derieux, 1972), or the wing web application (Marshall, 1981). However, various field observations have suggested that the live vaccines may cause substantial mortality as well as persistent infection in vaccinates, which may serve as a source of further infection (Schlink and Olson, 1987b).

Pathogenesis of fowl cholera is poorly understood. The invasion of the organism occurs primarily through the upper respiratory tract mucosa (Rhoades and Rimler, 1991) or through the lower respiratory tract (Ficken and Barnes, 1989). The organism in the blood is rapidly cleared and localized in the liver and spleen. The virulent organism rapidly multiplies in these organs and is abruptly released again into the blood shortly before death of the host (

Pabs-Garnon and Soltys, 1971; Tsuji and Matsumoto, 1989). Some attention has been focused on the initial adhesion and invasion of the organism. Maheswaran (1973) demonstrated that when a low number of a virulent strain were endotracheally inoculated into turkeys, the bacteria emerged in the blood and spleen as early as 6 hrs after inoculation. Intraairsac inoculation of CU vaccine strain resulted in the detection of the organism in the blood at 3 hrs postinoculation (Ficken and Barnes, 1989). Rhoades and Rimler (1990) inoculated a virulent or CU vaccine strain into the upper respiratory tract of turkeys and determined the presence of the organism in various tissues by the swab culture method. They observed the systemic invasion of the virulent organism at 12 hrs but not 6 hrs after inoculation. On the other hand, the CU vaccine strain showed a significantly lower invasion rate than the virulent strain. Matsumoto et al. (1991) found that the intratracheal inoculation of a virulent strain in the order of 10^8 to 10^9 organisms showed the organism multiplied *in situ* to gradually spread downward to lower respiratory tract in the first few hours. By 6 hrs, the organisms invaded the blood circulation to multiply vigorously in the liver and the spleen in the majority of the inoculated turkeys.

The purpose of this study was to investigate the respiratory pathogenesis of four strains of *P. multocida* in turkeys. The four strains were an encapsulated P-1059, non-

encapsulated T-325 strain derived from P-1059, and two vaccine strains, CU and M-9

Material and Methods

Experimental Turkeys. Medium white turkeys were maintained as a closed flock at the Department of Poultry Science, Oregon State University (Hales et al., 1989). Progenies of the flock were maintained in a brooder unit up to seven weeks of age, and moved to a concrete animal isolation unit with wood shavings on the floor.

Bacteria: *Pasteurella multocida*, strain p-1059, was originally obtained from Dr. K. R. Rhoades, National Animal Disease Center, Ames, Iowa. Strain T-325 was a spontaneous mutant lacking capsule derived from P-1059 strain (Tsuji and Matsumoto, 1989). Strain CU was obtained from Kee Vet Laboratories, Anniston, Alabama. Strain M-9 was obtained from Dr. M. Jensen, Brigham Young University, Provo, Utah. All strains were propagated on dextrose starch agar (DSA; Difco, Detroit, MI), harvested in brain heart infusion broth (BHI; Difco) and stored at - 70 C. Characteristics of the form strains are listed in Table 1.

Inoculation: *Pasteurella multocida*, strain P-1059, T-325, CU, and M-9 were recovered from frozen cultures to DSA and incubated at 41 C (P-1059 and T-325) or 35 C (CU and M-9) overnight. Bacteria were spread out for confluent growth on warmed DSA plates (100 x 15 mm) and incubated for 4 hrs at the appropriate temperature. The growth was harvested with 5 ml of BHI broth/plate and enumerated for viable counts by plating out serial dilutions on DSA. The culture

harvest of each strain diluted to 1:10 in BHI was used as inoculum.

Table II.1. Characteristics of the strains of *P. multocida* used in the study

Strain	Encapsulation	Colony	Optimal Growth Temperature
P-1059	++ ^a	iridescent	41°C
T-325	<u>+</u> ^b	blue	41°C
CU	++	iridescent	35°C
M-9	<u>+</u>	blue/gray	35°C

^a) a substantial amount of capsule present; bacteria cannot be aggregated by antiserum

^b) no capsule was visible by capsular stains; bacteria were agglutinable by antiserum

Intratracheal inoculation: The inoculum was transferred into a 5 ml syringe, and polyethylene tubing (1.19 mm in inner diameter) in 5.1 cm length was attached on 18 ga. needle. The tubing was carefully inserted through the laryngeal cleft, and 2.5 cm (1 inch) below the cleft, the inoculum in 1 ml was delivered slowly drop wise. After inoculation, the head was held with the mouth open for 1 minute to prevent immediate expulsion of inoculum.

Sampling procedures: Blood was drawn in 5 ml from the cutaneous ulnar vein into a syringe containing heparin (final concentration; 40 units/ml). The turkeys were killed by electrical shock. A sterile cotton swab was inserted through the platine cleft, rotated a few times, and streaked onto sheep blood/MacConkey agar. The skin of the neck was pulled back and the trachea was exposed and a ring of 1 cm width was cut 2 cm below the laryngeal cleft for the upper tracheal sample. At about 2 cm above the bifurcation, a ring of 1 cm width was cut for the lower tracheal sample. For the rest of isolation, the body was placed in the metal tray at a 30 degree angle with the head upward. The rib was cut and the sternum were reflected to expose the thoracic and abdominal cavities. Swab samples were taken from thoracic and abdominal airsacs and directly plated onto DSA plates. Blood vessels were clamped off using hemostats, and the heart, liver, and digestive tract were lifted out. A portion of the liver and the entire spleen were removed. The right and the left lungs

were removed. All the tissue samples were placed in sterile plastic bags and immediately placed on ice.

Sample processing: Blood and tissue samples were immediately processed as follows; the blood was transferred to a sterile tube and mixed well. Serial 1:10 dilutions were made in 0.05 M phosphate buffered saline (0.85%) pH 7.2 (PBS) by transferring 0.2 ml of blood to 1.8 ml of sterile PBS. The appropriate dilution in 0.1 was spread out on DSA plates and incubated. Broth culture was made by adding 1.0 ml of blood to 100 ml of sterile BHI broth and incubating at 41 or 35 °C. Liver, spleen, and right/left lungs were weighed. Sterile PBS in 9 ml was added for every 1 g of tissue to make the original 1:10 dilution. The sample was then processed for 30 sec. in a homogenizer (Stomacher, Model STO-80, Tekmar, Cincinnati, Ohio). Serial 1:10 dilutions were made and samples plated out by the same method as for blood. Broth cultures for tissues were made by adding 1.0 ml of the 1:10 dilution to 100 ml of BHI broth and incubated at 41 or 35 °C. Upper trachea and lower trachea were weighed and the amount of PBS to add for the 1:10 dilution was determined in the same way as for other organ samples. Trachea samples were then cut into small pieces using sterile scissors, and placed into sterile mortars. A small amount of sterile sand and predetermined amounts of sterile PBS was added followed by grinding with pestles. The sample was then poured into the test tube and placed on ice.

Serial 1:10 dilutions were made and plated out by the same method as for blood and the other organ samples. Colony counts were made on all plates on the following day. If they were still negative at 48 hrs the colony counts were recorded as 0. If all the plates for a sample were negative, the broth culture was checked for signs of bacterial growth. Subcultures of the positive broth cultures were made in blood/MacConkey agar for identification of *P. multocida*. The broth culture was judged negative when no bacterial growth was observed after 48 hrs of incubation. When colonies of apparent pure culture with mucoid appearance and strong iridescence were observed, they were identified as *P. multocida*. When mixed cultures or atypical colonies were observed, the following criteria was used to identify *P. multocida*; characteristic staining and morphology with gram stain, negative growth on MacConkey agar, positive catalase test, weakly positive oxidase test, acid slant/acid butt with no gas or H₂S in the triple sugar iron medium, and positive reaction for indole.

Experiment 1: Ten 8-week-old turkeys were inoculated intratracheally (IT) with 1.3×10^9 CFU of P-1059. Five of them were terminated at 6 or 9 hrs postinoculation, and their tissues were examined for bacterial counts. Two uninoculated control birds were also processed.

Experiment 2: Ten 9-week-old turkeys were IT inoculated with 4.0×10^9 CFU of *P. multocida* strain T-325

per bird. Other procedures were done in a same manner as in Experiment 1.

Experiment 3: Ten 10-week-old turkeys were IT inoculated with 1.3×10^9 CFU of strain CU per bird. Other procedures were done in a same manner as in Experiment 1.

Experiment 4. Ten 12-week-old turkeys were IT inoculated with 4.7×10^9 CFU of *P. multocida* strain M-9 per bird. Other procedures were done in a same manner as in Experiment 1.

Statistical analysis: The colony counts were transformed into \log_{10} values, and the mean and standard deviation were calculated in each group. Bacterial concentration for the two interval times (6 and 9 hrs) were compared using the Student's *t*-test.

Results

Experiment 1. Turkeys were IT inoculated with 1.3×10^9 CFU of P-1059 and tissues were examined for bacterial counts at 6 hrs and 9 hrs postinoculation (Fig II.1). No *Pasteurella* was isolated from any tissue of the two uninoculated control turkeys. All the birds showed evidence of *Pasteurella* infection at 6 hrs PI; the organism was isolated in high numbers in respiratory tissues and in moderate numbers in the liver, spleen and blood. At 9 hrs PI all birds showed high numbers of the organism in all tissue samples. Between 6 and 9 hrs PI, there was a highly significant ($p < 0.01$) increase in the number of the organisms isolated from the liver, spleen and blood (Table II.2). The data indicate that P-1059 strain invaded systemically before 6 hrs PI followed by the rapid multiplication in the liver and spleen. Gross and histopathological lesions are described in the accompanied chapter.

Experiment 2. Turkeys were IT inoculated with non-encapsulated T-325 strain and examined at 6 and 9 hrs PI (Fig II.2). The two uninoculated controls birds showed negative isolation results with any tissues. At 6 hrs PI, the bacteria were detected in high numbers in the respiratory tissues and also in the systemic organs. However, the number of organisms detected in the liver, spleen and blood were smaller than those observed with P-1059 strain. Three out of the five birds showed negative

results with blood samples. At 9 hours PI, T-325 strain did not show the vigorous multiplication in the systemic organs as seen with P-1059 strain. In fact, the number of organism in blood and liver, did show no significant difference ($p > 0.05$) between 6 and 9 hrs sampling time (Table II.2). One turkey showed less than 10 CFU/g in the blood at 9 hrs PI.

Experiment 3. Strain CU was IT inoculated and tissues were examined at 6 and 9 hrs PI (Fig II.3). No *Pasteurella* was isolated from any tissues of the two control turkeys. At 6 hrs PI the organism was abundant in respiratory tissues similar to the observation with P-1059 or T-325 strain. As with the case of T-325, no significant ($p > 0.05$) increase of the organism was seen in the blood or liver. In the spleen, however, a significant ($p < 0.05$) increase in the bacterial number was observed.

Experiment 4. Turkeys were IT inoculated with M-9 strain and tissues were examined at 6 and 9 hrs PI (Fig II.4). No organism was isolated from any tissue of the two uninoculated control birds. The organism was isolated in moderate numbers in the respiratory tissues at 6 hrs PI. Unlike the three other strains, significantly fewer ($p < 0.05$) organisms were detected at 9 hrs than 6 hrs PI in the upper and lower trachea, the right lung and blood

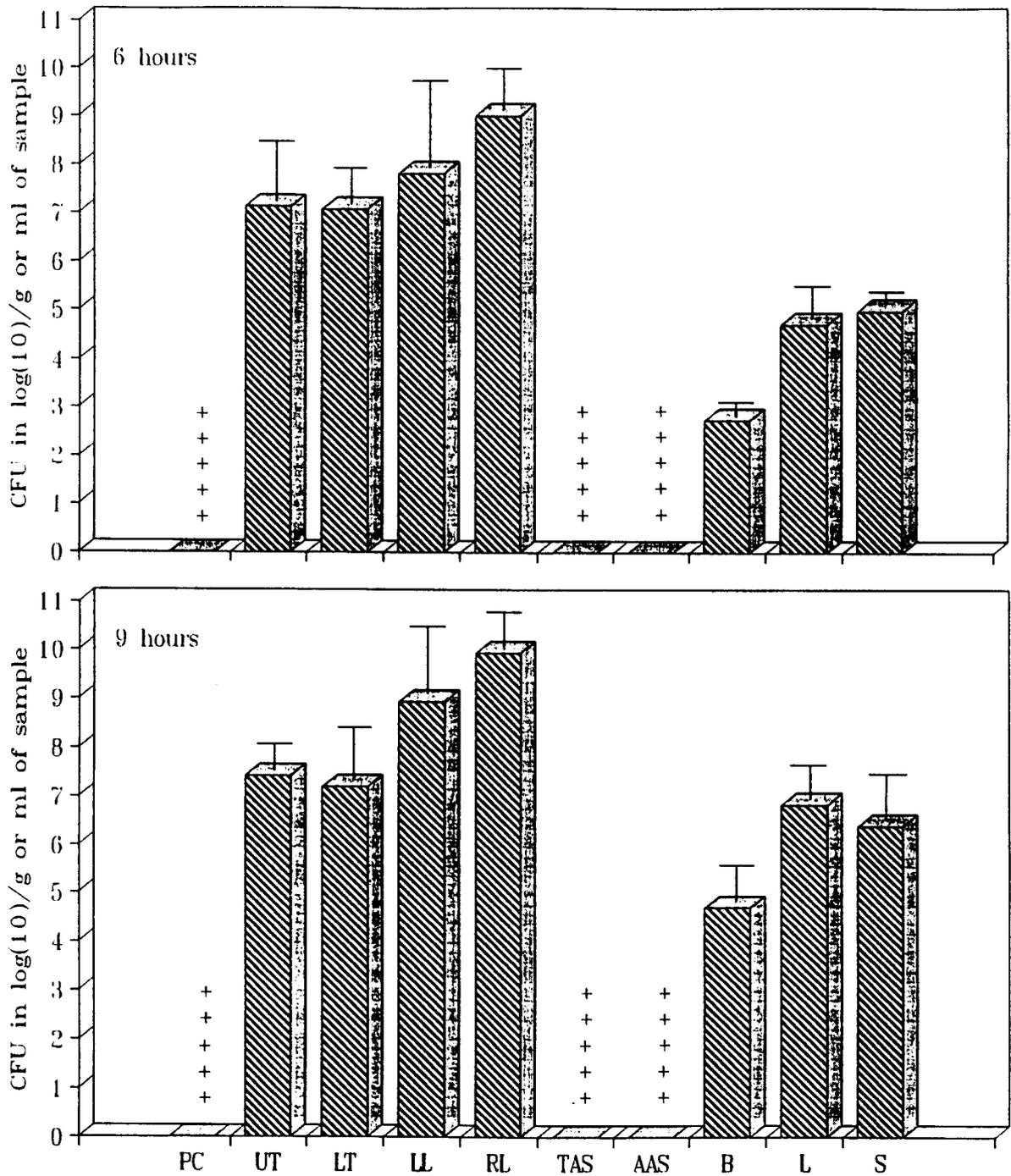


Fig. II.1. Bacterial recovery (mean \pm standard deviation) from the palatine cleft (PC), upper trachea (UP), left lung (LL), right lung (RL), thoracis airsac (TAS), abdominal airsac (AAS), liver (L), and spleen (S) of inoculation turkeys at 6 or 9 hrs after intratracheal (IT) inoculation with encapsulated P-1059 strain. (+) or (-) for PC, TAS, and AAS shows positive or negative respectively, isolation from a swab sample.

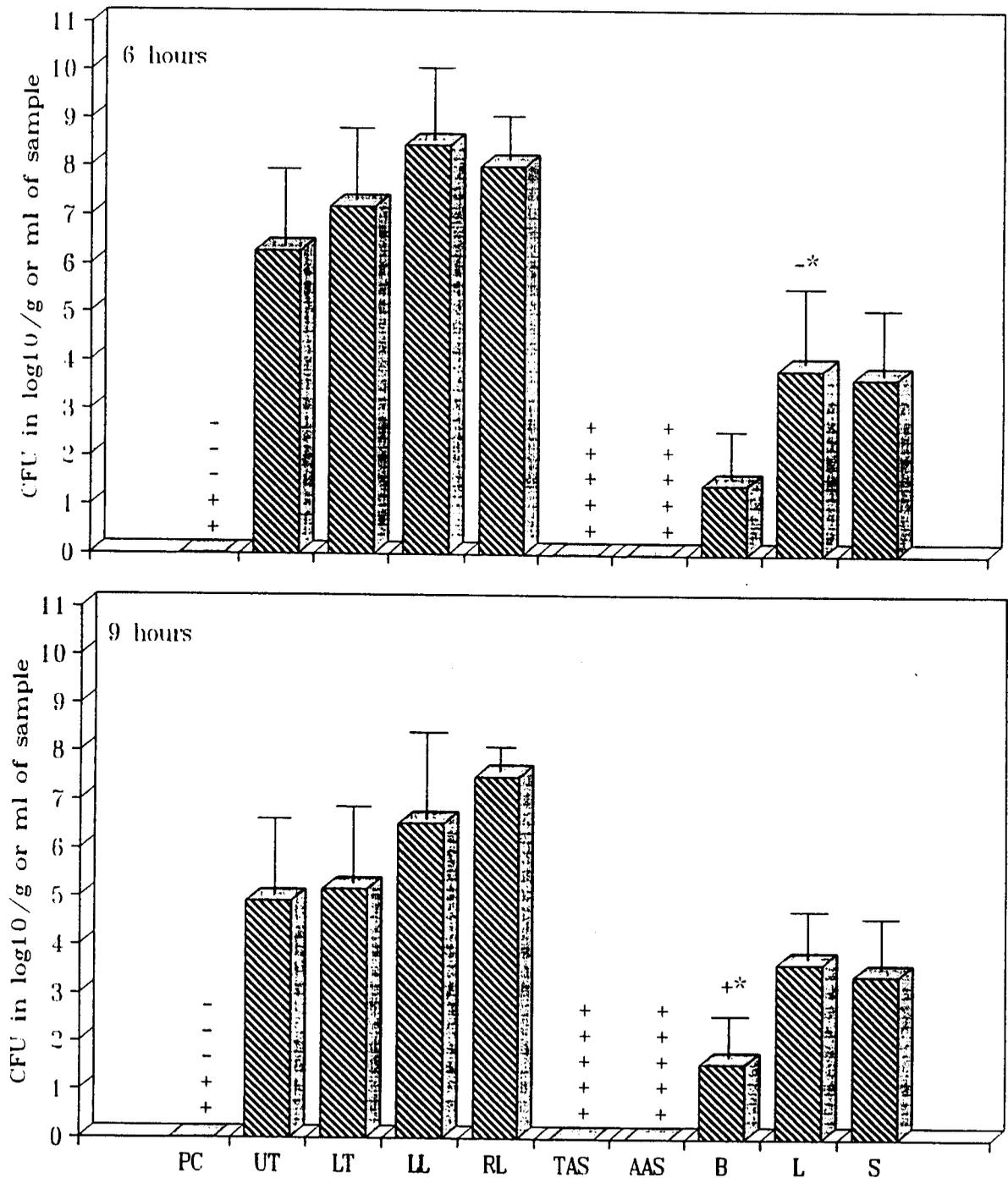


Fig. II.2. Bacterial recovery (mean \pm standard deviation) from various samples of inoculated turkeys at 6 or 9 hrs after IT inoculation of nonencapsulated T-325 strain. Abbreviation for tissues are explained in Fig II.1. (+) or (-) for PC, TAS, and AAS shows positive or negative, respectively, isolation from a swab sample. (+) or (-) indicates the result of broth culture when plate count was less than 10 CFU/g.

At 6 hrs PI, 4 turkeys showed negative isolation with the blood, 1 with liver, and 1 with spleen. At 9 hrs PI, 3 out of 5 turkeys showed negative results with blood, and 1 out of 5 with spleen.

Table II.2. Summary of the statistical analysis using two sample comparison test based on $H_0: \mu_{6hrs} = \mu_{9hrs}$ for each strain

Tissue	<i>P. multocida</i> strain			
	P-1059	CU	T-325	M-3
Upper Trachea	-	-	-	**
Lower Trachea	-	-	-	**
Left Lung	-	-	-	-
Right Lung	-	-	-	*
Blood	**	-	-	**
Liver	**	-	-	-
Spleen	**	*	-	-

**) significant difference at 1% level

*) significant difference at 5% level

-) non significant difference at 5% level

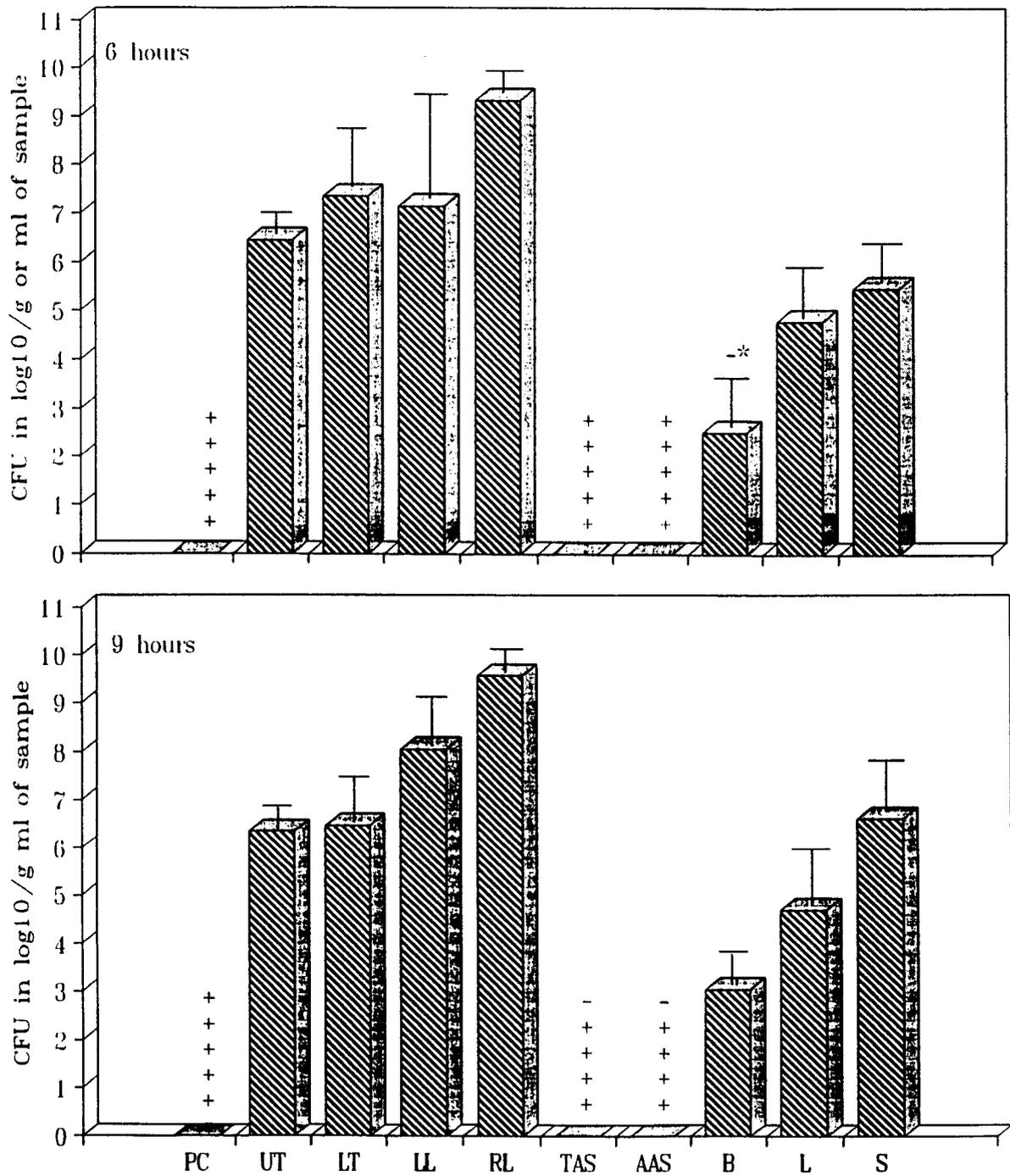


Fig. II.3. Bacterial recovery (mean \pm standard deviation) from various samples of inoculated turkeys at 6 or 9 hrs after IT inoculation of CU strain. Abbreviation for tissues are explained in Fig II.1. (+) or (-) for PC, TAS, and AAS shows positive or negative, respectively, isolation from a swab sample. (+) or (-) indicates the result of broth culture when the plate count was less than 10 CFU/g

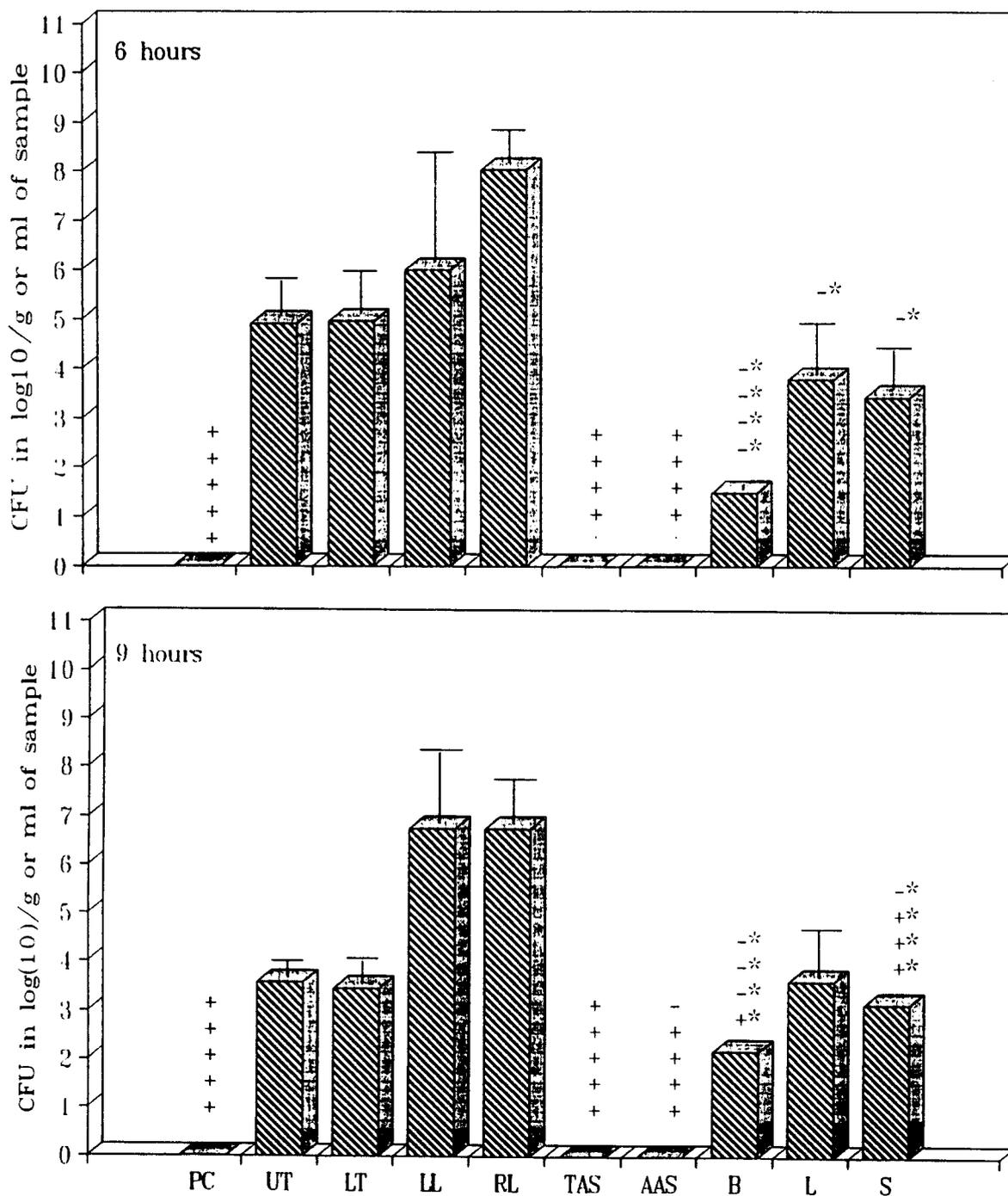


Fig. II.4. Bacterial recovery (mean \pm standard deviation) from varicous samples of inoculated turkeys at 6 or 9 hrs after IT inoculation of M-9 strain. Abbreviation for tissues are explained in Fig. II.1. (+) or (-) for PC, TAS and AAS shows positive or negative, respectively, isolation from a swab samples. (+) or (-) indicates the result of broth culture when plate count was less than 10 CFU

Discussion

In the present study, high numbers (10^9 CFU) of viable *P. multocida* were deposited on the surface of tracheal mucosa. Our preliminary study indicated that even a virulent P-1059 strain caused systemic infection at a low rate when it was IT inoculated in lower than 10^8 CFU. Processing each tissue for accurate CFU counts technically limited us to examine 12 bird in each experiment. The combination of these two factors were the reason for the use of high inoculum amount to pursue the main objective; evaluating invasiveness of the four strains after IT inoculation.

Pasteurella multocida is suggested to invade the upper respiratory mucosa to reach the blood stream (Rhoades and Rimler, 1991). When virulent P-1059 strain was swabbed at the palatine cleft, however, no systemic infection was detected at 6 hrs postinoculation (Rhoades and Rimler, 1990). Intratracheal inoculation of P-1059 strain resulted in the detection in the liver as early as 3 hrs postinoculation (Maheswaran, 1973). A live vaccine strain, CU, was detected in the blood at 3 hrs after the inoculation of the organism into the airsac (Ficken and Barnes, 1989). When P-1059 strain was IT inoculated into turkeys, it multiplied *in situ*, spreading gradually downwards along the airway in a majority of the animals, while, in some animals, the organism invaded the blood and

systemic organs in less than one hour (Matsumoto et al., 1991). In the present study, P-1059 and three other strains showed their high rate of invasiveness when they were IT inoculated in high numbers, suggesting that there is not a significant difference among the four strains in their capacity to cause systemic invasion from the respiratory tract in the turkeys. The four strains, however, showed differences in their capacity to multiply in various organs.

The statistical analysis shown in Table 2 indicates there is differences among strains in their capacity to multiply in various organs. Between 6 and 9 hrs of isolation, P-1059 strain showed significant increase in bacterial numbers in the blood, liver, and spleen. Strain CU showed a significant increase only in the spleen. The other two strains did not show a significant increase in any organs. Strain M-9, on the other hand, showed significant decrease between the two isolation attempts in the upper and lower trachea, right lung and the blood. Two factors should be considered for the cause of these differences. Strain P-1059 and CU are encapsulated, while T-315 and M-9 are not or poorly encapsulated; P-1059 or T-325 grow optimally at 41 C, while the two vaccine strains are temperature-sensitive mutants, growing optimally at 35 C. Differences in their growth rate in the turkey combined with some unknown "protective" effect of the capsule from the host defense mechanism may explain the observed differences in the fate

of the organism *in vivo*.

Pathogenesis and immunity in fowl cholera are poorly understood. The organism invades from the respiratory tract, enters the bloodstream, and localizes in the liver and spleen (Pabs-Garnon and Soltys, 1971; Tsuji and Matsumoto, 1989). Virulent organisms multiply rapidly in these organs, while those belonging to low virulent strains are killed at various rates (Tsuji and Matsumoto, 1989). In immune turkeys, a virulent strain is killed in the liver (Tsuji and Matsumoto, 1990). The mechanism by which the organism is killed in the liver is not known. The results of the present study generally support these pathogenic processes. In addition, the results indicate that both virulent and current vaccine strains all possess high capacity to invade from the respiratory tract into the bloodstream.

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Chapter III

The Lesions Caused by *Pasteurella Multocida*
Strain P-1059 and M-9
After Intratracheally Inoculated into
Turkeys

Summary

Lesions caused by *Pasteurella multocida* strain P-1059 and M-9 were investigated in turkeys. The birds were intratracheally (IT) inoculated with a virulent strain, P-1059 or M-9 vaccine strain, and the lesions were observed in the lung, trachea and airsac during period of 0, 0.25, 0.5, 1, 2, 3, and 6 hrs PI. No serious lesions were found in the early infection (0.25-1 hr). At later stages, the trachea showed numerous heterophil infiltrations and hyperplastic mucous glands. In the lung, there were intense infiltration of heterophils and moderate to severe vessel edema. The airsac showed increased thickness and cloudiness due to infiltration of heterophils and edema. No major differences were noted in the microscopic lesions between birds infected with P-1059 and those infected with M-9 strain. These result suggest that both P-1059 and M-9 vaccine strain have an ability to infect the respiratory organs.

Introduction

Pasteurella multocida is a highly invasive bacterium which causes fowl cholera in turkeys, chicken, and other avian species (Rhoades and Rimler, 1991). Death often results from septicemia, but in some birds, chronic disseminated pasteurellosis can occur (Lucan, 1961). Pneumonia and airsacculitis are often found in turkeys with fowl cholera; however, morphological description of *P. multocida*-induced acute experiment pneumonia and airsacculitis in the turkeys are lacking (Arya, 1971).

There are conflicting reports on whether or not *P. multocida* caused persistent bacteremia and whether the bacterial replication occurs intracellular or extracellular (Pab-Garnon and Scltys, 1971; Snipes et al., 1987; Tsuji and Matsumoto, 1989). The site of bacterial replication and the associated tissue lesions have not been adequately described. The significance of phagocytosis and bacterial killing by either heterophiles or macrophage also is not known. Rhoades (1964) reported that when *P. multocida* strain X-73 inoculated via the nasal cleft, the lung of infected birds revealed moderate to general infiltration of interstitial tissue with heterophiles and intravascular of this cell. Prantner et al. (1990) observed a fibrinopurulent bronchopneumonia followed by severe pulmonary necrosis, pleuritis and vasculitis when a field isolate was inoculated via oculo-nasal-oral route. Ficken

and Barnes (1990) inoculated CU strain of *P. multocida* into the caudal thoracic airsac and found that the airsac reacted rapidly and intensively with exudation and accumulation of heterophils. Kenneth et al. (1990) reported that *P. multocida* strain M-9, CU and 86-1913 were capable of generating chemotactic factor when exposed to pooled turkey serum *in vitro*.

In natural field infection of turkeys, the respiratory tract is probably the initial site of infection. The present study was designed to investigate the invasive and persisting ability of two strains of *P. multocida*, P-1059 and M-9, in various tissues after their intratracheal inoculation.

Materials and Methods

Experimental Turkeys: Medium white turkeys were raised in a closed flock at the Department of Poultry Science, Oregon State University (Hales, 1989) until seven weeks of age. Poultry were raised under conventional management in a room partitioned by wooden walls with curtain windows. No vaccines were administered and no disease problem was noted during the growing period. At 7 weeks of age, they were transferred to concrete animal isolation units with wood shaving litter on the floor.

Bacteria: *P. multocida* strain P-1059 and M-9 were originally obtained as described in material and method section in the previous chapter. Both strains were propagated on dextrose starch agar (DSA), harvested in brain heart infusion broth (BHI) and stored at -70 C.

Inoculation: *P. multocida* strains were recovered from frozen cultures to DSA and incubated at 41 C for 5 hrs (P-1059), or at 35 C for 8 hrs (M-9). Confluent growth was harvested with 5 ml of BHI broth/plate and enumerated for viable counts by plating out serial dilutions on DSA. The culture harvest of each strain diluted 1:10 in BHI was used as inoculum.

Turkey inoculation: An intratracheal inoculation (IT) technique described in the previous chapter was used for administration of both P-1059 and M-9 strain. One milliliter per bird of diluted culture was deposited slowly

25 cm below the laryngeal cleft via polyethylene tubing. After the inoculation the head of the turkey was held for 20 second with the mouth open to prevent immediate expulsion of the inoculum.

Experimental design: Thirty-five 14-week-old turkeys were randomly assigned to three groups as follows:

Experiment 5: Seven control birds were inoculated IT with 1 ml broth heart infusion (BHI) per bird.

Experiment 6: Fourteen experimental turkeys were IT inoculated containing $1,2 \times 10^9$ CFU of P-1059 per bird.

Experiment 7: Fourteen experimental turkeys were inoculated similarly with 2.1×10^9 CFU of M-9 strain per bird.

At 0, 0.25, 0.5, 1, 2, 3, and 6 hrs postinoculation, one turkey from control bird and 2 turkeys from each challenge group were euthanatized for histopathological evaluation.

Histopathology: The turkeys were killed by electrical shock. At necropsy, the skin of neck was pulled back and the trachea was exposed. Three tracheal samples were taken; 2.5 cm below the laryngeal cleft (upper trachea); 2.5 cm above the bifurcation (lower trachea); and the mid point between upper and lower trachea. The abdominal cavity of each bird was opened and airsac and left and right lung were taken. During the sample processing, macroscopic lesions were examined. Samples were taken from the right and left lung,

airsac and upper, middle and lower trachea at 0, 0.25, 0.5, 1, 2, 3, and 6 hrs PI. These samples were placed in 10% neutral buffered formalin solution. The tissues were processed, stained with hematoxylin and eosin according to standard procedures, and examined by light microscopy.

Section Evaluation: The right and left lung were evaluated for heterophil infiltration, edema, vascular changes and lymphocyte infiltration. The airsac was evaluated for heterophil infiltration, lymphocyte infiltration, and thickness. The trachea was evaluated for heterophil infiltration, changes in ciliated epithelium, and changes in mucous glands. The severity of the lesions were indicated as follows: (-) = no lesion; (+) = rare; (++) = mild; (+++) = severe; (++++) = very severe.

Statistical analysis: The degree of lesions were analyzed by using the Categorical Data Analysis.

Results

Experiment 5. In birds inoculated with the BHI broth, generally no change was observed in tissue samples (Fig III.1 and 2). But occasionally heterophils were present in intra or extra vascular of respiratory tissue samples with varying intensity.

Experiment 6. After a variety time of inoculation, all the birds showed both macroscopic and microscopic lesions. Macroscopically, the lesion was observed as early as 1 hr after inoculation. Affected small areas of the lung were foamy and had yellowish purulent exudates, and commonly pus was squeezed out. The airsacs typically showed cloudy spots. By 2 to 6 hrs PI, one fourth to one third area of either right or left lung showed fibrinous pneumonia with accumulation of pus. Distal parts along the chest wall were most frequently affected. The airsacs increased in thickness and cloudiness.

Microscopically, in the early infection, (0.25 to 1 hr), lesions were found only slight number infiltration of heterophils in the lung. The airsacs showed mild edema. In more severely affected lungs (2 to 6 hrs PI), there was infiltration and accumulation of a large number of heterophiles (Fig III.3). Lymphocytes showed nodular and diffuse accumulation in the large focal area, and edema occurred around vessels (Fig III.4).

The airsac, examined at 6 hrs PI, was increased in

thickness and cloudiness. There was severe infiltration of heterophiles in the vessels, intra- and extravascular. Mesothelial/epithelial showed hyperplasia and edema, and space was filled with fibrin.

Tracheal changes occurred at 3 to 6 hrs PI and varied in severity between individual birds. These changes include hyperplasia of the ciliated epithelium and heterophil infiltration. Mucous gland was cystic and hyperplastic (Fig III.5). Moderate changes consisted of mild diffuse infiltration of heterophils in intravascular or perivascular areas of trachea in the mucosa and submucosa .

Experiment 7. Pathological changes induced by the IT inoculation of M-9 were essentially similar to those of P-1059 strain. In fact, both at early and later stage of infection. There was no significant difference in the reactions observed with the two strains (Table III.1 vs III.2 and Fig III.6)

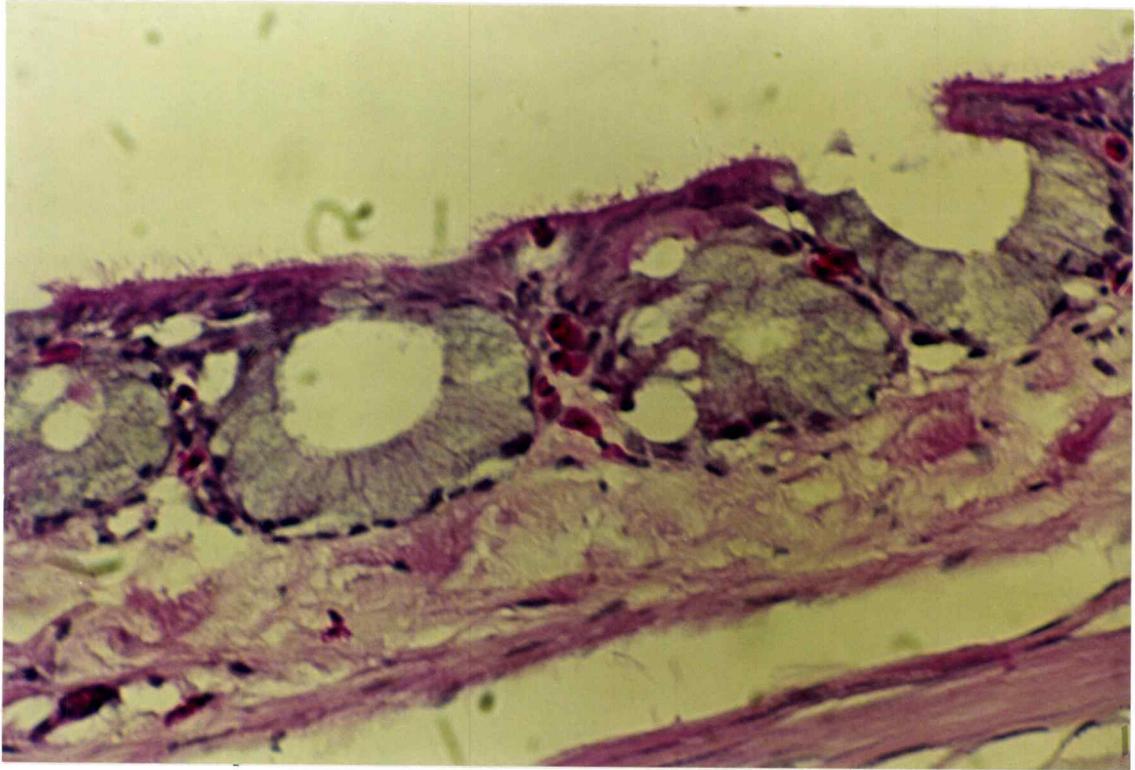


Fig III.1. Trachea of a controlled bird, showing normal ciliated epithelium and mucus gland. HE 100X

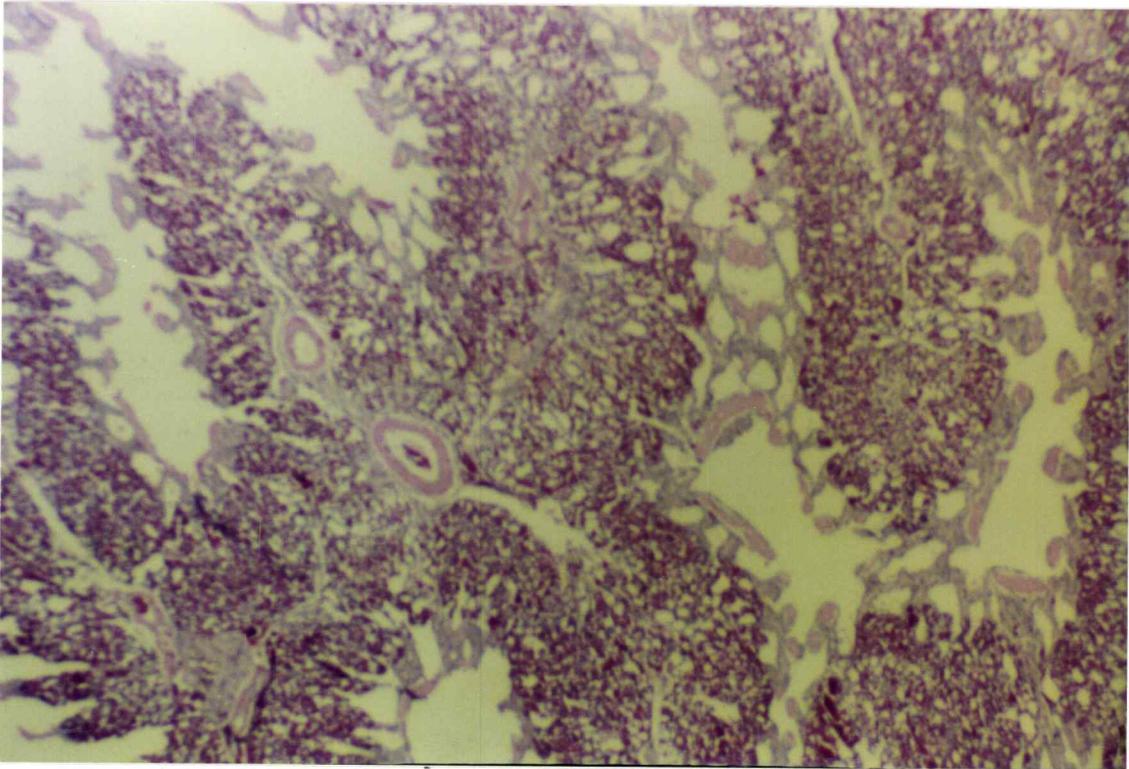


Fig III.2. Lung of a controlled bird, showing the vessels. HE 100X

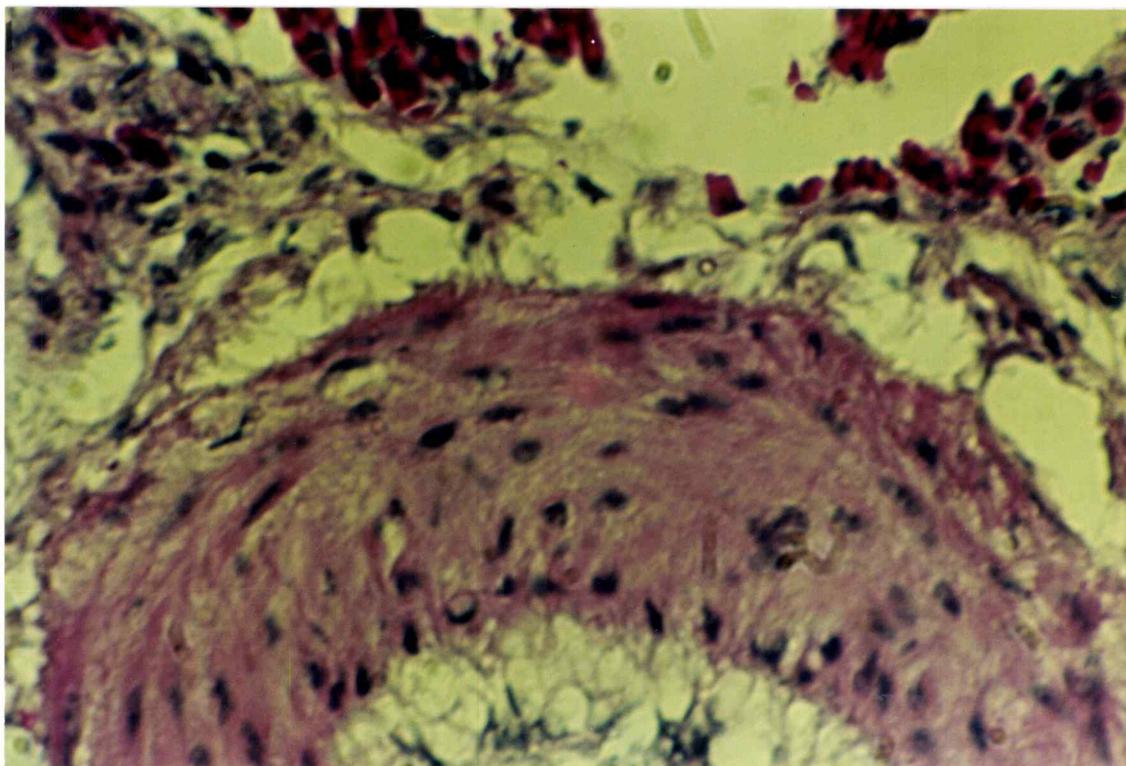


Fig III.3. Lung of a bird infected with P-1059 strain, 3 hrs postinoculation (PI), showing edema and heterophil infiltration in pulmonary vessel. HE 250X

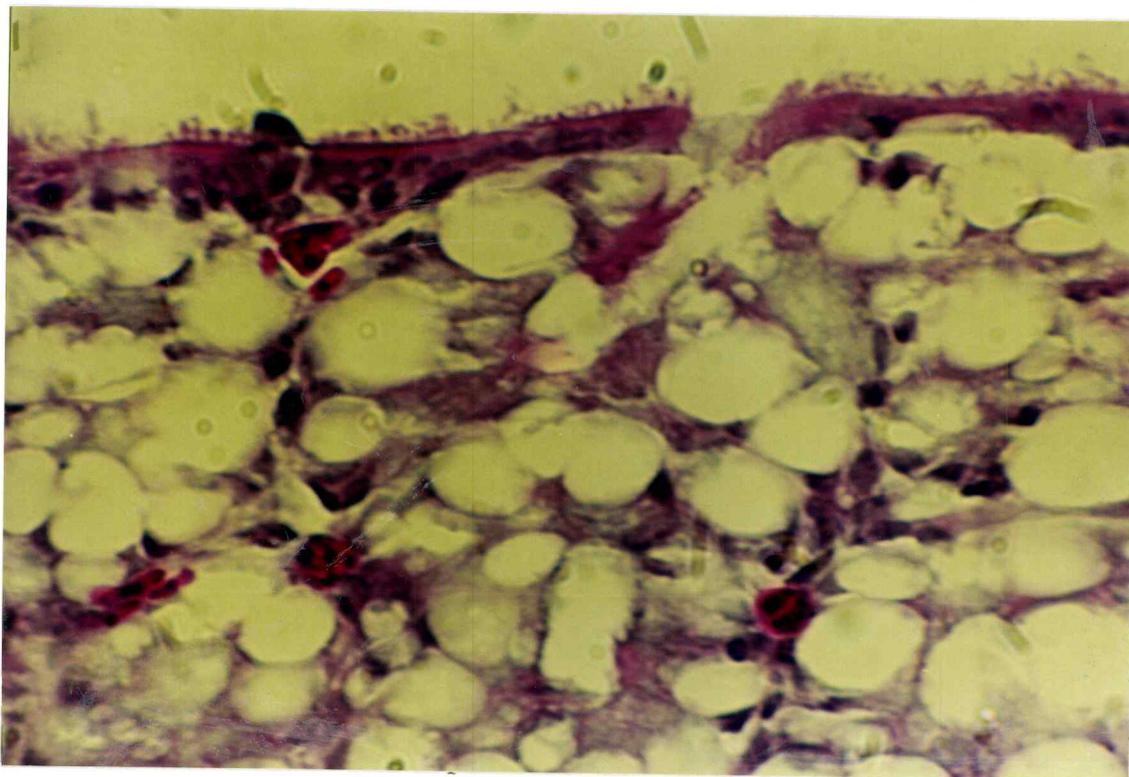


Fig III.4. Trachea of a bird infected with P-1059 strain, 6 hrs PI, showing cystic mucous gland. HE 400X

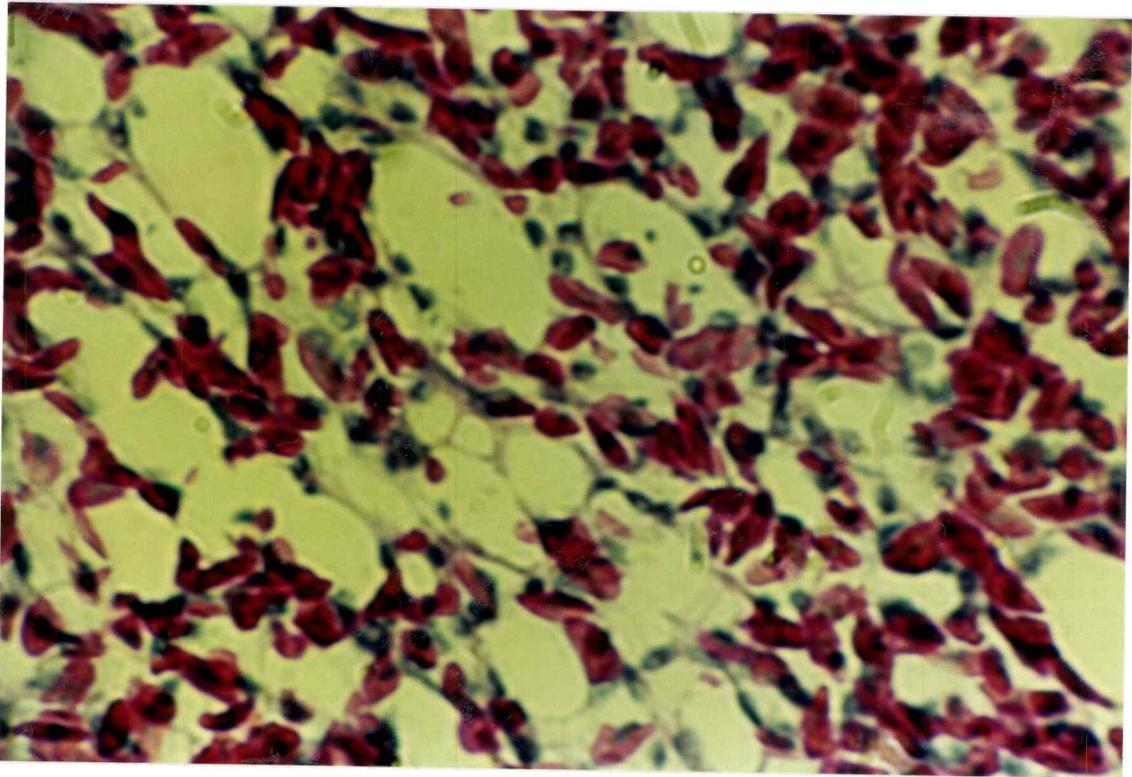


Fig III.5. Lung of a bird infected with P-1059 strain, 6 hrs PI, showing edema and heterophils infiltration in pulmonary vessels. HE 400X

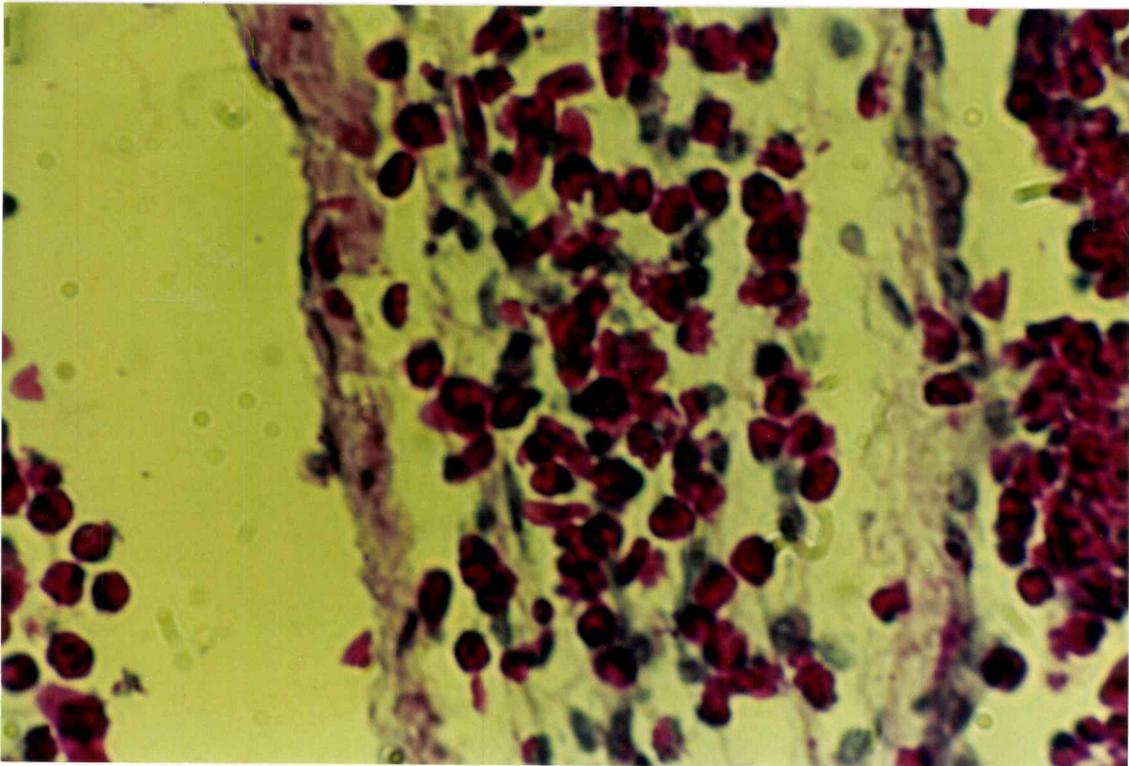


Fig III.6. Aairsac of a bird infected with M-9 strain, 6 hrs PI, showing numerous heterophil infiltrations. HE 400X

Table III.1. Summary of the lesions in the tissues after intratracheally inoculation with P-1059

Tissues & Lesions	Time (hrs)						
	0	0.25	0.5	1	2	3	6
Lung							
Het. inflt. ¹	+	-	+	+	++	+++	++++
Lymph. inflt. ²	-	-	+	+	+	++	++
Vessels ³	-	+	+	+	+	+	+
Edema ⁴	-	-	+	+	+	+	+
Trachea							
Het. inflt.	-	-	-	+	++	++	++
Cil. epith. ^{5,a,b}	-	+	-	+	++	++	++
Mucous glands ⁶	-	+	+	+	+	+	+
Airsac							
Het. inflt.	-	+	+	++	+++	+++	++++
Lymph. inflt.	-	+	+	+	+	+	+
Thickening	-	-	+	+	++	++	++

-) = negative. +) = rare. ++) = mild. +++ = severe.
++++) = very severe.

¹ Heterophile infiltration

² Lymphocyte infiltration

³ Heterophil infiltration

⁴ Vessels

⁵ Ciliate epithelium,

^aHeterophile infiltration and

^bIntact/Hyperplasia

⁶ cystic/hyperplasia

Table III.2. Summary of the lesions in the tissues after intratracheally inoculation with M-9

Tissues & Lesions	Time (hrs)						
	0	0.25	0.5	1	2	3	6
Lung							
Het. inflt. ¹	+	+	+	+	++	+++	++++
Lymph. inflt. ²	-	+	+	+	+	++	++
Vessels ³	-	-	-	-	+	+	+
Edema ⁴	-	-	+	+	+	+	+
Trachea							
Het. inflt.	-	+	+	+	++	++	++
Cil. epith. ^{5, a, b}	-	+	+	+	++	++	++
Mucous glands ⁶	-	+	+	+	+	+	+
Airsac							
Het. inflt.	-	+	+	++	+++	+++	++++
Lymph. inflt.	-	+	+	+	+	+	+
Thickening	-	-	+	++	++	++	++

-) = negative. +) = rare. ++) = mild. +++)) = severe.
 +++)) = very severe.

¹ Heterophile infiltration

² Lymphocyte infiltration

³ Heterophil infiltration

⁴ Vessels

⁵ Ciliate epithelium,

^aHeterophile infiltration and

^bIntact/Hyperplasia

⁶ cystic/hyperplasia

Discussion

Fowl cholera is caused by strains of *Pasteurella multocida* that belong to capsular type A and various somatic types. The organisms isolated from turkeys predominantly belong to type 3 or type 3,4 (Rhoades and Rimler, 1991). However, it is generally accepted that serotype specificity has no correlation with virulence of organism. Thus, among strains belonging to somatic type of 3,4, there are very virulent strains and those of low virulence; the latter include M-9 vaccine strain (Prantner et al., 1990). In the Chapter II, we examined the invasiveness and persistence of virulent or vaccine strain of *P. multocida* after their intratracheal inoculation into turkeys and found that significant difference among strains was detected in their persistence in various tissues but not their invasiveness. In the present investigation, we compared two strains, a virulent P-1059 and M-9 vaccine strain, in their pathological responses after their IT inoculation into turkeys

In the early course of infection (0.25 to 1 hr), trachea samples showed cystic in mucous gland followed by infiltration of heterophils in the epithelial layer. In the lung, edema of vessels and varying degrees of heterophil and lymphocyte infiltration were seen. Airsacs were thickened and infiltrated by heterophils. At later stages, the lesions were essentially similar in nature, but severe in

intensity and extended over larger areas.

The statistical analysis indicates there is no significant difference in these pathological lesions between birds infected with P-1059 strain and those infected with M-9 strain (Table II.1 and II.2). Prantner et al. (1990) also compared pathological changes in turkeys inoculated with a virulent strain with those inoculated with a nonvirulent strain. After oculo-nasal exposure to these organism, they observed significant differences in lesion scores only at some stage of infection. However, there were no qualitative differences in the pathological changes caused by virulent or vaccine strains. The results in the present study essentially confirm their results.

Some control birds that had received BHI broth showed mild degrees of heterophil infiltration in respiratory tissues. Although the experimental turkeys were raised under isolated condition without any vaccination, they were not reared under filtered air or with sterilized feed. Therefore, some subclinical infection of the respiratory tract may have occurred. Since the infiltration of heterophils is one of the major histopathological changes for *P. multocida* infection, pre-existed lesions obscure the early lesions induced by the organism. However, at later stages of infection, both strains induced such massive scale of heterophil infiltration, the presence of a minor degree of inflammation before infections does not have a major impact

on interpreting pathological lesions.

Edema and heterophil infiltration are major changes in the respiratory tissues after the inoculation of *P. multocida*. The organism produces endotoxin which may be diffused into tissues, causing edema and necrosis (Rhoades, 1964). The heterophil infiltration may be caused by a chemotactic factor described by Kenneth et al. (1990). Since both P-1059 and M-9 strain produced essentially identical histological changes, it can be concluded that both strains produce these pathogenic factors in a similar manner. However, as indicated in the previous chapter, M-9 strain does not persist in tissues as well as P-1059 does. The reason for the poor persistence is unknown other than the fact that M-9 strain does not multiply well at 41 C. Many investigators have indicated the M-9 strain has significantly lower virulence than P-1059 strain does (Snipe et al., 1987). However, the present results suggest that both strains induce very similar pathological changes in turkeys when fairly large doses of the organism are introduced into trachea.

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