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PATHOLOGY OF TRACHEA IN TURKEYS EXPOSED BY AEROSOL TO NEWCASTLE DISEASE VIRUS

Iowa State University PH.D. 1983

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Pathology of trachea in turkeys exposed by aerosol to Newcastle disease virus

by

Tahseen Ali Abdul-Aziz

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GENERAL INTRODUCTION

Newcastle disease is a highly contagious, viral disease affecting several avian species. The disease occurs in several forms ranging from mild inapparent infection to acute disease with high mortality. Different avian species vary in their susceptibility to Newcastle disease, and although turkeys are considered relatively resistant (8,25), the economic impact from this disease in turkeys may be severe. For this reason, Newcastle disease vaccine has become one of the most important elements in vaccination programs for turkey flocks.

The most common types of Newcastle disease vaccines used for vaccination of chickens and turkeys are live lentogenic virus vaccines. Lentogenic strains are capable of multiplying in the upper respiratory epithelium and of inducing an immune response (6). To economically vaccinate large poultry flocks and obtain a reliable immune response, aerosol vaccination is widely practiced.

Despite the efficiency of aerosol vaccination in inducing protective immunity, multiplication of the virus in respiratory epithelium may damage the respiratory mucosa and cause a severe postvaccinal respiratory reaction. Moreover, damage to the mucosa of upper respiratory tract may enable pathogenic

bacteria to reach the lower respiratory tract and establish secondary bacterial infections such as Escherichia coli septicemia (36). In chickens, E. coli septicemia is a well-known complicating factor of aerosol vaccination with lentogenic strains of Newcastle disease virus (50). In the last few years, we have seen an increased incidence of E. coli septicemia in turkeys following spray vaccination with live lentogenic Newcastle disease virus vaccines. Because turkeys are more resistant to Newcastle disease, it is generally recommended that more efficient immunizing strains be used, such as LaSota. Since the immunogenicity of a strain is directly related to its virulence (66), the more efficient immunizing strains are also those which induce the most severe respiratory reactions (6).

No information is available on the pathology of the trachea in turkeys exposed by aerosol to Newcastle disease virus. The increased incidence of E. coli septicemia in Iowa turkey flocks following spray vaccination with live lentogenic Newcastle disease virus vaccines provided the impetus for this investigation on the extent of tracheal damage caused by aerosol exposure to different strains of Newcastle disease virus.

The objectives of this research were: (1) to characterize tracheal lesions in turkeys exposed by aerosol to

lentogenic, mesogenic, and velogenic strains of Newcastle disease virus, (2) to determine Newcastle disease virus titers in tracheas of turkeys exposed to these three strains, and (3) to determine whether there is a relationship between the level of strain virulence and the degree of tracheal damage and virus titers in tracheas.

This dissertation is presented in the alternate format including 3 manuscripts which are accepted or submitted for publication in Avian Diseases. All manuscripts are presented in the format required by Avian Diseases. References cited in each manuscript are included with that manuscript and conform to journal format. The manuscripts are preceded by a general introduction and literature review. The general discussion and conclusions, literature cited, and acknowledgements follow the third manuscript. Literature cited refers to citations in the general introduction, literature review, and general discussion and is presented in the journal format consistent with that used in the manuscripts.

The Ph.D. candidate, Tahseen A. Abdul-Aziz, was the principal investigator for each of the studies and is the senior author of each manuscript.

LITERATURE REVIEW

Newcastle Disease Virus

Classification and morphology

Newcastle disease virus (NDV) is a paramyxovirus, a genus of the paramyxoviridae family. Viruses in this family contain unsegmented, linear, single-stranded ribonucleic acid (RNA) surrounded by protein to form a helical nucleocapsid which, in turn, is enclosed within a lipoprotein envelope (35,79). Mature virions are pleomorphic (90) and range in size from 120-300 nm (132) although values as high as 600 nm have been recorded (9). The outer envelope is covered with short spikes or projections of glycoprotein that are 8 nm long, 1-1.5 nm wide, and 8-10 nm apart (3,86, 132). These surface projections are of 2 kinds: one contains both the hemagglutinin and the neuraminidase and is responsible for the hemagglutination property of the virus; the other is called F protein and is responsible for the hemolysis and cell fusion properties of the virus (35).

Strains

The possibility that more than one strain of NDV existed was first suggested when great differences were

noted in the clinicopathological expression of Newcastle disease (ND) among flocks in various parts of the world. In general, all strains are morphologically, structurally, and serologically indistinguishable (86) and are grouped in a single serotype (115). On the basis of mean death time (MDT) of 10-day-old chick embryos inoculated into the allantoic sac with the minimum lethal dose, NDV is divided into velogenic, mesogenic, and lentogenic strains. Velogenic strains have an MDT of 40-60 hours, the mesogenic 60-90 hours, and the lentogenic 90-150 hours (55). These three strains also vary in their virulence for birds; the velogenic strains are the most virulent, the mesogenic moderately virulent, and the lentogenic the least virulent (54). Tissue tropisms are also shown by NDV strains; some strains are pneumotropic, while others are viscerotropic, and all strains are neutrotropic to some extent if time permits (71). Therefore, the word strain when applied to NDV is used to mean a stable, well-characterized line of virus and does not imply serologically distinguishable types (3).

Newcastle disease virus populations are exceedingly diverse within a single isolate (33,34,53,117); a virus of low virulence has been derived from an originally virulent isolate (107) and vice versa (74). This observation

indicates that a particular virus population is subject to a continual selection (107).

Effect on cell metabolism

Newcastle disease virus has been shown to inhibit the synthesis of cellular RNA (5,94,134,137), DNA (40,41,134), and protein (24,61,94,108,137). In general, as with other paramyxoviruses, inhibition of host cell protein synthesis by NDV is not dramatic (42) and occurs relatively late in the virus growth cycle (91). There seems to be a direct correlation between the virulence of NDV strain and the magnitude of inhibition of cell protein (94,108) and RNA (5) synthesis. However, this correlation is not absolute since some lentogenic and mesogenic strains have profound inhibitory effects (94). Other metabolic alterations which occur in NDV-infected cells are degradation of cellular RNA (67) and activation of cellular lysosomes (138).

Cytopathology

The primary cytopathic effects produced by NDV grown in tissue culture include cell death (plaque formation), cell fusion (formation of polykaryocytes), and alterations in permeability of plasma membranes (54). The morphology and size of plaques depend on the virulence of the strain

(33,109,116) and on the type of cell culture (116). Polykaryocytosis is the initial principal cytopathic effect produced by NDV (13,69,81,106,109). The ability to form polykaryocytes in chick embryo cell cultures and the sizes of polykaryocytes are directly related to the virulence of the infecting strain (13,100,106,109). It has been shown that the modification of the cell surface by NDV is responsible for fusion of cells (110) and that the rate of modification is related to the virulence of infecting strain (100, 106,110). Allison and Mallucci (7) found that the less cytopathic strain caused transient reversible changes in lysosomes, whereas the more cytopathic strain caused irreversible destruction of lysosomes with the release of enzymes contained in them and consequent cell damage. It has been suggested that NDV may damage plasma membranes resulting in the release of cellular enzymes (78).

Newcastle Disease

Newcastle disease is a virus-induced, worldwide, highly contagious disease affecting several avian species. In chickens, the disease occurs in one of several clinicopathologic forms, depending on the strain of the virus involved. The diversity in the clinicopathological expression of ND caused difficulty in recognizing it on its

first appearance in different parts of the world. As a result, ND had different local names, some of which have become synonyms. Some of these names are: pseudo-fowl-pest, pseudo-Vogelpest, avian pest, pseudo-poultry plaque, Madras fowl pest, avian distemper, and Ranikhet disease (21).

Newcastle disease has been studied more extensively in chickens than in other avian species; therefore, information in this review will pertain to chickens.

Historical aspect

What is now termed ND was first broken out as a new disease entity of poultry in the Dutch East Indies (Indonesia) near Batavia (now Djakarta) (83). Doyle (39), not aware of the outbreak in the Dutch East Indies, reported an acute disease that occurred in 1916 in a flock of chickens in the town of Newcastle-upon-Tyne in England, from which the disease derived its name. In his report, Doyle (39) demonstrated the filterability of the causative agent and announced it to be a virus serologically distinguishable from the virus of fowl plague. The disease was probably carried by ship from Batavia to the port of Newcastle-upon-Tyne (87). In England, the disease was summarily eradicated following its first outbreak in the same year; however, this was not the case in Batavia, from which the disease disseminated

throughout the world (56).

In the United States, ND first appeared in the early 1940s as a nervous disorder and respiratory disease and affected 1-12 week-old chickens in northern California (98). The respiratory disease was assumed to be infectious bronchitis and the nervous disorder was thought to be avian encephalomyelitis (98). During the year following the first report, consideration was given to the possibility that the nervous disorder was caused by invasion of the brain by the virus of infectious bronchitis (14); the possibility of dietary causes was also investigated (14). The malady remained unrecognized as ND and was called "respiratory-nervous disorder" (124,125) and later "pneumoencephalitis" (15). It was not until 1942 that Stover (124) reported that the nervous disorder and the respiratory disease were both manifestations of one disease caused by a filterable virus that was distinct from the virus of infectious bronchitis and from that of infectious laryngotracheitis; however, Stover did not realize that he was dealing with NDV. The nature of the virus remained obscure until 1944 when Beach (17) reported that the virus of "pneumoencephalitis" was antigenically identical with the virus of ND.

Host spectrum

Although ND is prevalent among chickens and turkeys, other domestic, captive, and wild birds are also susceptible (84). Reports on the isolation of NDV from different avian species are voluminous and beyond the scope of this review. A comprehensive review was provided by Lancaster (84).

Incubation period

After natural infection, the incubation period varies from 2 to 15 days or longer with an average of 5 to 6 days (54). Following experimental infection, the incubation period has been reported to vary from 1 to 25 days (85). With both natural and experimental infections, the incubation period diminishes gradually from hatching to maturity because of the loss of passive immunity transferred from immunized hens to offspring through the egg yolk (54).

Pathogenicity and pathogenesis

The terms pathogenicity and virulence are nearly synonymous and mean the capacity to produce disease (120). The pathogenicity of NDV has been discussed by Jungherr (71) and Hanson (54). Although the pathogenicity of NDV is determined largely by the strain, several factors may

modulate the behavior of a specific strain within the body; among these factors are: dose (75), route of administration (18,19,20), age of the chicken and certain environmental conditions (119). And while the pathogenicity of NDV of chickens is an outstanding marker, it must be assessed for individual strains in terms of transmissibility of the virus by various routes to birds of a specific age, the rate of viral multiplication in the visceral organs, passage through the blood-brain barrier, formation of antibody, and tissue tropism (71).

Under natural conditions, the virus enters the body through the respiratory tract and also through the digestive tract and starts to multiply in mucosal epithelium within 24 hours. The next event depends on the strain of the virus; multiplication of avirulent strains may be limited to the site of entry, whereas infection with virulent strains leads to viremia and secondary multiplication of virus in distant tissues (28,71). During viremia, both erythrocytes and leucocytes are infected (28). Following subcutaneous inoculation of a virulent strain, the virus titer increases gradually for 12 hours in visceral organs, followed by a regression period which lasts 12 to 24 hours. After this regression period, the titer increases in all visceral organs and in the brain (10). Hofstad (65) used strains

of different virulence and followed sequentially the virus titer in different tissues following subcutaneous or intranasal inoculation. He found a wide variation in the response of chickens to different strains and also to different routes of inoculation of the same virus strain.

Karzon and Bang (75) compared the pathogenesis of virulent and avirulent strains in 10-week-old chickens following intramuscular inoculation of approximately equivalent viral doses. Both strains multiplied in visceral organs at approximately the same rate, but in the brain, the virulent strain multiplied more rapidly and killed birds in a relatively low titer, while the avirulent strain reached a higher titer a few days later but did not even cause paralysis. Within 24 hours after intracerebral inoculation, the titer of the virulent strain was 3 times higher than the titer of avirulent strains.

Sinha et al. (119) exposed 6- to 8-week-old chickens by aerosol to 6 different strains of low, moderate, and high virulence and compared tissue tropism and the virus titers in lung, brain, spleen, blood and breast muscles 4 days after exposure. All strains multiplied to a high titer in the lung with a direct correlation between the titer and the strain virulence. More virulent strains were consistently isolated from brain, lung, blood, and spleen;

the mesogenic strain was isolated from lung, blood, and spleen, but not from the brain; and the lentogenic strain was isolated from lung and spleen only. Again, there was a direct correlation between the rate of multiplication and the virulence of the strain. They concluded that viremia followed infection with all strains but it lasted longer in chickens infected with virulent strains.

It is not clear yet why different strains have widely varying effects on the host. Virulence is the manifestation of a complex host-virus relationship and if considered unilaterally in terms of virus properties, it can be assumed that strains differing in virulence differ in their ability to damage host cells rather than attain high titre (118). The blood-brain barrier which develops during the first few days of life may prevent the invasion of the brain by lentogenic strains of NDV. Velogenic strains are capable of breaking the barrier and are lethal even to adult chickens, while mesogenic and lentogenic strains are not able to pass the barrier (130). It had also been suggested earlier that virus particles of avirulent strains are released slowly from infected cells, and that this relatively slow release may give time for the formation of antibodies which confine the infection (71). But later, Reeve et al. (111) found that titers of released virus were not related to virus virulence.

It has been shown that NDV strain B1 replicates to a high titer in the brain of 1-day-old chicks following intracerebral inoculation (135). Immunofluorescent studies revealed that multiplication of this strain is confined to ependymal cells and choroid plexus (135), whereas virulent strains replicated in vascular endothelia, ependymal cells, choroid plexus, glial cells, and neurons (31,135). These observations and the finding that the virus surface glycoproteins for virulent strains are cleaved by cellular proteases which do not act on the same glycoproteins of avirulent strains (95) resulted in the development of a new approach to explain the neurovirulence for NDV. Wilczynski et al. (135) suggested that endothelial cells, glial cells, and neurons possessed proteases capable of selectively cleaving the appropriate glycoproteins of virulent virus. This cleavage is necessary for maturation of the virus.

Clinical syndromes

Clinical signs, as well as morbidity and mortality rates, are variable and dependent on virus strain and age of the bird. Depending on the virus strain, ND occurs in 4 main forms.

Doyle's form (Asiatic form) This is an acute, lethal infection of chickens of all ages originally described

by Doyle (39). It is caused by certain velogenic viscerotropic strains of NDV (VVNDV). The disease appears suddenly and spreads rapidly throughout the flock (85). Infected birds initially have dullness followed by depression, rapid respiration, weakness, and prostration. Profuse watery, greenish or bloody diarrhea is a common feature of this form (2). Edema of the periorbital tissues and of the throat (57,121) and corneal opacity (129) have also been reported. Birds surviving the initial acute stage of the disease often develop nervous signs of paralysis, clonic spasm, muscular tremor, torticollis, and opisthotonos (112). Duration of illness has been reported to vary from 1 to 10 days (85). Mortality is usually greater than 90 per cent (38).

Beach's form This is an acute, often fatal infection of all ages of chickens caused by certain velogenic, neurotropic strains. It was first described by Beach (15) who called it "pneumoencephalitis." In immature chickens, the disease is characterized by respiratory distress followed by nervous signs. The respiratory phase is manifested by cough, rales, and gasping. Within 1 to 2 days or later, nervous signs may appear (54); they include ataxia, partial or complete paralysis of legs or wings, and torticollis. In mature chickens and laying hens, the respiratory phase may predominate the course of the disease. Even respiratory signs may

be absent in laying hens and the disease is manifested by depression, loss of appetite, and decrease in egg production (15). Morbidity is 100 per cent. Mortality is 10 to 50 per cent but may be as high as 90 per cent in young birds (54).

Beaudette's form This is an acute respiratory and occasionally nervous infection of young chickens caused by mesogenic strains. It was first described by Beaudette and Black (22). The disease appears suddenly and spreads rapidly. Affected birds show respiratory signs of coughing and gasping. There is a decrease in appetite and dropping or cessation of egg production (23). Respiratory signs usually disappear within 2 to 3 weeks and may be followed by nervous signs (127). Involvement of the nervous system is variable (99) and is more common in young than older birds (1). A feature of this form is the change in egg quality; affected hens usually lay eggs with abnormal size, shape, shell, or interior quality (80,105). The mortality in this form varies from 5 to 50 per cent in adult flocks but may exceed 50 per cent in young chickens (85).

Hitchner's form This is a mild or inapparent respiratory infection caused by lentogenic strains. This form was originally recognized by Hitchner and Johnson (62). Respiratory signs when present are mild and may be detected only when birds are roosting (85). Nervous signs have not

been observed in North America (54). Mortality is usually negligible although it may reach 30 per cent, particularly when complicated by other infections. Affected birds may recover in 1 to 8 weeks, depending on the health status of the flock.

Lesions

There is considerable variation in lesions found in chickens with ND. Differences in tissue tropism and in virulence of infecting strains account largely for the variation in post-mortem findings. In addition, the age of the bird and the presence of intercurrent infection may affect the pathologic picture of the disease.

Gross lesions are not always dramatic in ND and may be absent in birds infected with lentogenic strains. However, severe lesions are found in birds infected with virulent, especially viscerotropic, strains.

Microscopically, hyperemia, edema, and hemorrhage are found in various organs (32,54). There are intravascular coagulation and lysis of erythrocytes (29,32). Lesions in small blood vessels consist of thrombosis, hyaline degeneration of the tunica media, and necrosis of the endothelial cells (54). Macrophage and perivascular plasma cell infiltration is another tissue reaction observed in many organs of chickens naturally infected with virulent NDV (68).

Respiratory system Grossly, serous, or catarrhal exudate may be present in the nasal passages, larynx, and trachea (54,112). Congestion and hemorrhage of the trachea have been observed in chickens infected naturally (46,76,97) and experimentally (4,77) with velogenic and mesogenic strains of NDV. Occasionally, there is cloudiness or yellowish discoloration of air sacs (16,72). The lung may be enlarged, edematous, congested, and foamy (32,112,122).

Microscopically, rhinitis and sinusitis have been described in chickens inoculated intranasally with a lentogenic strain (26). There was exudate in the nasal sinuses and focal areas of necrosis and desquamation in the mucosa. Ultrastructurally, irregularity of the cell surfaces and distortion of the microvilli are the predominant lesions. A microscopic lesion of acute, mild, local rhinitis was found in chickens inoculated intranasally with a mesogenic strain (12). Lesions were demonstrated 18 hours after inoculation and consisted of necrosis of mucous glands and mucosal epithelium followed by infiltration of polymorphonuclear leucocytes and lymphocytes. Cheville et al. (32) described focal destruction of epithelial cells in the nasal cavity of chickens inoculated intranasally with a velogenic strain.

Microscopic lesions have been described in trachea of chickens infected with lentogenic (20,51), mesogenic (96), and velogenic (32,72,77) strains. Gross (51) exposed 3-week-

old chickens to an aerosol of the lentogenic B1 strain and followed histologic changes up to the 21st day postexposure. Hyperemia, edema, mononuclear cell infiltration, and epithelial cell hyperplasia were observed beginning 2 days after exposure. Mononuclear cell infiltration increased toward the end of the study period. Beard and Easterday (20) described the histopathology of the trachea in chickens infected by aerosol with the lentogenic strain B1 and the velogenic strain GB. Both strains produced a remarkable reaction in the trachea consisting of hyperemia, loss of cilia, necrosis of the mucosa, squamous metaplasia of the mucosal epithelium, and dense mononuclear infiltration. Lesions reached a peak on the 3rd and 4th day postexposure and started to regress by the 5th or 6th days. Tracheal lesions in chickens inoculated intratracheally with mesogenic strains consist of loss of cilia, degeneration of epithelial cells, edema of the lamina propria and submucosa, and infiltration of heterophils and macrophages (96). Catarrhal tracheitis has been described in chickens inoculated intramuscularly with a velogenic strain (72). Velogenic viscerotropic NDV administered intranasally or intraocularly to chickens produces severe tracheitis (77). Early lesions are characterized by vascular degeneration, edema, and mild heterophil infiltration; these are followed by degeneration and desquamation of the epithelium, and degeneration and coagulative necrosis of

small blood vessels. Marked, focal destruction of the epithelium was the only lesion observed by Cheville et al. (32) in the trachea of chickens inoculated intranasally with VVNDV. Ultrastructural changes in trachea of chickens challenged with the virulent GB strain consist of inter- and intra-cellular edema, necrosis, and loss of cilia (29).

Jungherr et al. (73) described proliferative and exudative lesions in the lungs of chickens infected naturally and experimentally with virulent NDV. Interstitial pneumonia is marked by hyperplasia and hypertrophy of connective tissue septae and of pneumocytes lining the tertiary bronchi, atria, and air capillaries. The proliferative lesions may cause partial or complete consolidation of the lung (73,112). Peribronchial air capillaries and tertiary bronchi may contain serous exudate, desquamated cells, erythrocytes, and necrotic heterophils. Stevens et al. (122) noted hemorrhagic and fibrinous pneumonia in chickens experimentally infected with velogenic strains. Multifocal necrotizing pneumonia has also been observed by some researchers (73,112). Sometimes, the only changes seen are hyperemia and edema of the parabronchi, or catarrhal bronchitis (32,37,72). Ressang (112) reported interstitial edema as a frequent finding with occasional perivascular mononuclear cell infiltration.

Chickens challenged intratracheally with a mesogenic strain have an airsacculitis characterized by hyperplasia of

the mesothelial cells, and by macrophages, heterophils and plasma cell infiltration (113). Air sac lesions in chickens experimentally infected with a velogenic strain are edema, heterophil and mononuclear cell infiltration, and metaplasia and hyperplasia of mesothelial cells (72,73). In subacute and chronic cases, the lesion is primarily proliferative and is marked by proliferation of fibroblasts (73).

Central nervous system Gross lesions have not been reported in the central nervous system (CNS). Microscopic lesions in the CNS are a significant feature of NDV infection (54). Lesions are uniformly distributed in the spinal cord, medulla, midbrain, and cerebellum, but cerebral lesions are rare. Wilczynski et al. (135) stated that the cerebellum was most commonly involved. Sullivan (128) emphasized involvement of the vestibular, reticular, and cerebellar nuclei, and Purkinje cells as the cause of tremor and inability to maintain head posture. Auer (11) did an extensive study on the microscopic lesions in the hypothalamus, optic lobe, midbrain, hindbrain, and cerebellum with special emphasis on changes in the neurons of different nuclei; he found lesions in all parts examined. Vascular reactions in the CNS are characterized by hypertrophy and hyperplasia of endothelial cells (32,70,72,73,128) and by infiltration of the blood vessel walls with mononuclear cells (37), particularly

lymphocytes (60). Focal meningitis, characterized by lymphocytic infiltration and thickening of leptomeninges is seen in some cases of ND (72). Occasionally, heterophil infiltration occurs in the early phase of the infection, and is associated particularly with the necrosis of neurons (128). The gray matter of the cerebellum has been reported by some authors to be intensively infiltrated by lymphocytes, which in some areas form bands radiating from the inner layer to the surface of the cerebellum (11,75). Multifocal gliosis is a very common finding throughout the CNS (37,60,72,73,122). Glial foci have been reported to be more common in the cerebellar cortex than in other parts (73). Jungherr and Minard (72) described degenerative changes in neurons; these changes were characterized by disintegration of the cytoplasm, which progressed from severe vacuolation to irregular coarse fibrillation. Degenerating neurons were found in the large motor nuclei of the spinal cord but were not uncommon in the medulla and cerebellar nuclei. Other changes found in neurons are chromatolysis, hyalinization, and necrosis (128). Damaged neurons may incite satelletosis (60,112) and neuronophagia (11,112). Purkinje cells may show degenerative changes and shrinkage (11,112,122,128,135).

A sequential study on the brain lesions in NDV infected chickens has shown that early lesions, detectable 5 days after infection, reached their maximum intensity approximately

20 days after infection (135).

Alimentary tract Severe lesions are commonly seen throughout the alimentary tract of chickens infected with VVNDV. Hemorrhagic, necrotic, and ulcerative lesions may occur in the mouth, pharynx, and esophagus (93,112). Early in the course of the disease, these ulcers may have bleeding surfaces, but later they become covered by diphtheritic membranes (93). Prominent lesions are multifocal hemorrhages and ulcers in the proventriculus, especially at its junction with the esophagus. Focal and diffuse hemorrhage occurs occasionally in the gizzard musculature. A very common, prominent feature is the hemorrhagic, necrotic, and ulcerative lesions in the intestinal wall, especially in the posterior part of the duodenum and in the jejunum and ileum (54). These intestinal lesions are associated primarily with the Peyer's patches and lymphoid follicles, including cecal tonsils (73). Depending on the stage of the disease, lesions in the intestine may appear as blue-red or yellow-red hemorrhages, as necrotic foci, as bleeding ulcers, or as ulcers covered by diphtheritic membranes (93). The large hemorrhagic necrotic areas may appear somewhat like blebs (54). Hemorrhage in the rectum and cloaca is also a common finding in some outbreaks (97).

Microscopically, lesions in the alimentary tracts of chickens infected with VVNDV are hemorrhagic and necrotic,

rather than inflammatory (37). The hemorrhagic-necrotic foci usually occur in or near lymphoid aggregates (73). The center of these foci is completely necrotic while there is intense capillary congestion in the periphery. Epithelial cells over these foci are partially or completely denuded and are often covered by serous or fibrinous exudate. Lesions usually involve the lamina propria, muscularis mucosa, and submucosa, but occasionally may reach the muscular layer (73,112). Fibrinous necrotic clots sometimes bulge into the lumen of the gut (73,129). Microscopic lesions have also been described in the intestine of chickens infected with the velogenic neutrotropic-pneumotropic NDV (30). These lesions consist of edema, vascular degeneration, appearance of heterophils between the epithelial cells, and hyperplasia of the goblet cells.

Lymphoid organs The spleen is often enlarged in acute cases and in the early stage of the disease but becomes small or shrunken in subacute and chronic cases (76). Multiple pinpoint gray areas have also been observed in the spleen of chickens infected with VVNDV (129). The splenomegaly seen in the early stages of ND is caused by congestion, dilation of sinusoids, and swelling of endothelium (29). Lymphoid follicles do not have remarkable histologic changes in the early disease process (29), but as the disease progresses, lymphocytes in the lymphoid sheaths undergo degeneration

and necrosis (29,32,37). Later, these lymphoid follicles become depleted of lymphocytes (37,72) and may be displaced by degenerate macrophages (29). Areas of hemorrhage, hyalinization, and necrosis are frequently scattered throughout the spleen (72,112). Cheville et al. (32) reported extensive erthrocyte destruction and erythrophagocytosis. Varying numbers of hemosiderin-laden macrophages are distributed throughout the spleen (29,37,112).

Cheville and Beard (29) and Cheville et al. (32) described histologic changes in the bursa and thymus of chickens experimentally infected with velogenic strains. The bursa was edematous and hyperemic. Lymphoid follicles became depleted of lymphocytes, and in severely affected chickens, these follicles were heavily infiltrated with macrophages. Early necrosis appeared in individual follicles, but later all follicles became markedly necrotic. In the thymus, both cortex and medulla were depleted of lymphocytes. The medulla was altered by destruction of vascular and lymphoid elements, by hypertrophy of Hassal's corpuscles epithelium, and by heterophil infiltration.

Other tissues

Petechiae sometimes occur in the subcutaneous tissues, musculature, heart, and mesentery (52,76). Edema of the subcutaneous tissue of the head and neck is a feature of

infection with VVNDV (32,52,89,112).

There may be cloudiness of the pericardium and an increased amount of clear serous or yellow fluid in the pericardial sac (32,52,112).

Biswal and Morrill (23) described lesions in the reproductive tract of laying hens inoculated intranasally with NDV. Grossly, atresia of ovarian follicles was the predominant lesion. In addition, many follicles were degenerating and cystic, and the oviduct was edematous and shrunken. Some infected birds had egg yolk in their peritoneal cavity. Microscopically, there were oophoritis and salpingitis characterized by varying degrees of degeneration of ovarian follicles, infiltration of granulocytes and mononuclear cells, and formation of lymphoid nodules. Edema and necrosis has also been observed in some regions of the oviduct.

Newcastle Disease in Turkeys

Historically, turkeys have been observed dying during natural outbreaks of ND in chickens (21). In the United States, Hoffman (64) reported a respiratory-nervous disorder in turkeys in California, and was able to reproduce the disease in turkeys and chickens. After doing cross immunization studies, Hoffman (64) suggested a relationship between the disease in turkeys and the respiratory-nervous disorder in chickens described by Stover (124,125).

Turkeys have been reported to be more resistant to virulent NDV infection (8,25). Natural infection can be asymptomatic (45,48) or may be followed by a clinical disease (43,47,131); peracute cases with high mortality have also been reported (47). Fenstermacher et al. (43) indicated that symptoms were less pronounced in turkeys than in chickens. In young turkeys, respiratory signs of cough, gasping, and rales may be followed in 1 to 4 days by nervous signs of chronic spasm, muscle contraction, and unilateral or bilateral paralysis of wings or legs (101). In old turkeys the disease may be so mild as to go unrecognized (45,101). Drop in egg production, laying of misshapen and poor quality eggs, and decrease in fertility and hatchability may be the only clinical signs in laying hens (45).

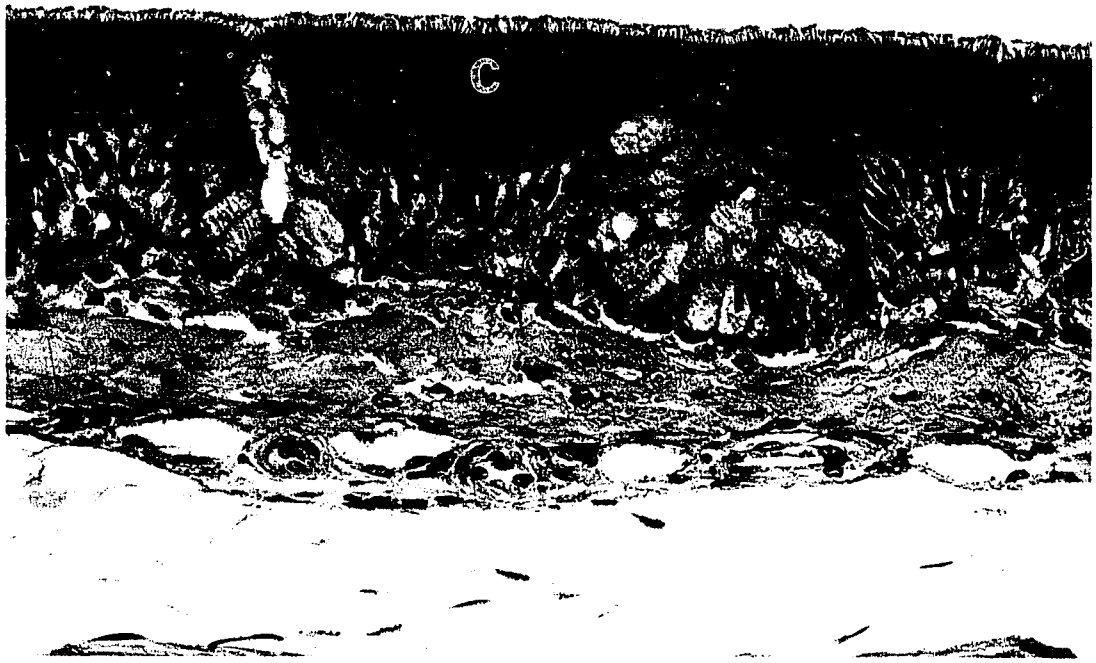
The pathology of natural (45,48,126) and experimental (8,123) NDV infections in turkeys is essentially similar to that described in chickens.

Histology of the Trachea in Fowls

The trachea is a flexible tube, made up of a large number of articulating rings of hyaline cartilage and lined by respiratory mucosa. The tracheal lumen is lined by pseudostratified ciliated columnar epithelium with goblet cells. Within the mucosa are many simple alveolar mucous

glands, which are unique to avian species (63) (Fig. 1). The ciliated cells have oval or elongated nuclei located near the epithelial surface. The nuclei of basal cells are round or irregular. Goblet cells extend from the basement membrane to the lumen; they are noncilated and their nuclei are similar in shape to those of ciliated cells (103). The cells of mucous glands are typical mucous secreting cells having large basal nuclei and foamy, pale cytoplasm. Mucous glands open either directly onto the mucosal surface or connect with it by a short neck, which is lined by ciliated cells (103). A thin lamina propria supports the mucosal epithelium and consists of dense, irregular connective tissue containing many blood vessels and lymphoid cells. Directly beneath the lamina propria is a submucosa consisting of collagen and elastic fibers. The submucosa blends with the perichondrial connective tissue and the whole tube is surrounded by a layer of adventitial connective tissue. There are three pairs of striated muscles associated with the trachea; however, in most sections of the trachea, the muscle bands are composed of only one pair, the muscularis sternotracheolaryngeous, which run down each side of the trachea (63).

Fig. 1 Normal trachea from a 4-week-old turkey. The tracheal lumen is lined by typical pseudo-stratified ciliated columnar epithelium with mucous glands. C, ciliated columnar cells; M, mucous glands; arrows, basal cells. Goblet cells are not shown in this section. H & E 630X.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PART I: PATHOLOGY OF THE TRACHEA IN TURKEYS EXPOSED BY AEROSOL TO LENTOGENIC STRAINS OF NEWCASTLE DISEASE VIRUS

PATHOLOGY OF THE TRACHEA IN TURKEYS EXPOSED BY AEROSOL TO LENTOGENIC STRAINS OF NEWCASTLE DISEASE VIRUS

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SUMMARY

Five groups of 4-week-old turkey poults were each exposed by aerosol to a different lentogenic strain of Newcastle disease virus. Four days postexposure, sections of tracheas were collected for histopathologic characterization and virus titration. The most prominent lesions were fibrinopurulent exudate in tracheal lumens, hyperplasia of epithelial cells, and infiltration by lymphocytes. All strains multiplied to high titers and produced similar microscopic lesions, but the number of poults with severe microscopic lesions varied among groups.

INTRODUCTION

Although most domestic and many wild avian species are susceptible to Newcastle disease virus (NDV) (15), turkeys have been reported to be relatively resistant to infection (2,4). Newcastle disease (ND) in turkeys may occur in subclinical (7,12) or clinical (6,10,14,20) forms; peracute cases with high mortality have also been reported (10).

Not only the virulent strains of NDV but also the lentogenic vaccinal strains can initiate infection in the respiratory tract (5). Although lesions have been described in the trachea of turkeys infected naturally (7) and experimentally (2,19) with velogenic strains of NDV, there is a lack of information on tracheal lesions in turkeys infected with lentogenic strains. In chickens, aerosol vaccination with live lentogenic strains B1 and LaSota often causes respiratory distress (5,11), and lesions have been described in tracheas of chickens experimentally infected with strain B1 (3,13).

This study was undertaken to characterize and compare lesions in tracheas of turkeys exposed by aerosol to different lentogenic strains of NDV.

MATERIALS AND METHODS

Two trials were conducted. The first trial was a preliminary study carried out to determine virus titers in tracheas. The second trial was done to determine virus titers and to characterize tracheal lesions histologically. Since the same procedure was followed in both trials, materials and methods will be described as a single experiment.

Poults

Day-old poults were obtained from a commercial hatchery (Cuddy Farms, Ellsworth, Iowa) and raised to 4 weeks of age. At 4 weeks of age, 36 poults were divided randomly into six equal groups, and each group was held throughout the experiment in a separate modified Horsfall-Bauer isolation unit in an isolation room. During the first 4 weeks and throughout the trial, the poults were fed turkey starter ration and tap water ad libitum.

Viruses

The five lentogenic strains of NDV used in this study were cloned LaSota (Sterwin Laboratory, Inc., Millsboro, Delaware), uncloned LaSota (Salsbury Laboratories, Charles City, Iowa), B1, 2024, and ET. The last three strains were

obtained from M. S. Hofstad, Veterinary Medical Research Institute, Ames, Iowa. Strains 2024 and ET were originally isolated from turkeys with severe respiratory disease. Both cloned and uncloned LaSota strains were rehydrated with sterile distilled water from commercial lyophilized vaccines. Strains B1, 2024, and ET were in the form of allantoic fluid harvested from infected chicken embryos.

Before the experiment was initiated, all strains were propagated in embryonated chicken eggs. The 50% embryo lethal dose (ELD_{50}) was determined for each strain (Table 1) by the method of Reed and Muench (18).

Table 1. Virus titer of the NDV-infected allantoic fluid

Strain	Virus titer (log 10 ELD_{50}/ml)
Uncloned LaSota	9.4
Cloned LaSota	9.5
B1	10.0
ET	9.7
2024	9.6

Experimental Procedure

At 4 weeks of age, five groups were each exposed to a different lentogenic strain of NDV; the sixth group served as

a control. Each virus was administered as an aerosol through a port on top of the isolation unit. A Peralta vaporizer (Peralta Hospital, Oakland, California) connected to a pump was used to generate the aerosol using 8 lb/in^2 of pressure. The virus was administered as a 10^{-2} dilution of allantoic fluid in sterile distilled water. Three groups were exposed on each of two successive days. The aerosol was generated for 10 min, during which the vaporizer delivered 0.98-1.02 ml of the virus suspension. During aerosolization and 30 min after, the ventilating system for the units was turned off to allow poults to breathe the aerosol. Poults in the control group were exposed similarly to an aerosol of sterile distilled water.

Necropsy

Four days postexposure, poults in all groups were examined for clinical signs and killed. They were first anesthetized by injecting intravenously 0.3-0.4 ml of a 5% solution of thiamylal sodium (Surital, Parke-Davis, Morris Planes, New Jersey) and then exsanguinated by cutting the right jugular vein with a scalpel. The ventral part of the body was then skinned to expose the trachea. Beginning 2 cm below the larynx, a 4-cm section of trachea was removed, examined grossly, and placed in a sterile test tube for virus

titration. The remaining section of upper trachea and a 2-cm section of lower trachea (about 1 cm above the thoracic inlet) were removed, fixed in Bouin's fixative for 5 hours, washed in several changes of 70% ethanol for 2-3 hours, and stored in 70% ethanol until processed. Tissue sections were prepared by conventional paraffin-embedding procedures and stained with hematoxylin and eosin (H & E).

Virus Assay of Tracheas

Tracheas were frozen at -75 C until they could be processed for virus titration. At that time, they were placed in a porcelain mortar, ground with sterile alundum, and diluted in tryptose phosphate broth (TPB) containing approximately 7,500 I.U. penicillin and 7.5 mg streptomycin/ml to make a 1:5 dilution. The suspension was centrifuged 5 min at 1,520 X g, and the supernatant was used to make seven serial 10-fold dilutions (10^{-1}-10^{-7}) with TPB containing approximately 150 I.U. penicillin and 1.5 mg streptomycin/ml. For each dilution, five 10-day-old chicken embryos were inoculated via the allantoic sac with 0.1 ml. The ELD_{50} of each tracheal suspension was determined by the method of Reed and Muench (18).

RESULTS

Clinical Findings

Most poults remained clinically normal. A few appeared restless and frequently shook the head and extended the neck. Mild inspiratory dyspnea with tracheal rattling sounds during inspiration were detected in one poult from the uncloned LaSota strain group and one poult from the ET strain group.

Virus Titers

Table 2 summarizes the virus titers of tracheas in both trials. Newcastle disease virus was not isolated from the uncloned LaSota group in the first trial or from the B1 or cloned LaSota group in the second trial. In both trials, all other strains achieved high titers, and with very few exceptions there was only a slight difference in virus titers of tracheas from individual poults within groups.

Gross Lesions

Gross tracheal lesions were detected in 46% of the infected poults (Table 2). In those that had gross lesions, the mucosal surface was hyperemic and covered with white, tenacious exudate. In some poults, a plug of this exudate

was lodged at different levels of the trachea and nearly occluded the tracheal lumen.

Microscopic Lesions

Tracheas from control poults were histologically normal. The microscopic lesions were similar in all infected poults regardless of the NDV strain used. In groups exposed to cloned LaSota and B1 strains, the severity and extent of lesions varied. The main difference among groups was the number of poults that had severe lesions (Table 2). With few exceptions, lesions were similar in type, extent, and severity in the upper and lower parts of the trachea.

A large amount of luminal exudate consisting of fibrin and heterophils was present in a few tracheas (Fig. 1). In some cases, only threads of fibrin and a few heterophils covered the mucosal surface. The most remarkable lesion in the surface epithelium was the replacement of normal pseudostratified ciliated columnar epithelium by four to eight layers of hyperplastic undifferentiated cells. Epithelial hyperplasia was associated with thickening and distortion of the tracheal mucosa. These cells had basophilic cytoplasm and round, hyperchromatic nuclei with one or two prominent nucleoli. Mitotic figures were frequently seen in undifferentiated cells (Fig. 2). In most cases, ciliated columnar

epithelium was not detected in areas of hyperplasia, but occasionally the hyperplastic basal cells were covered with a single layer of squamous or low cuboidal cells having short, indistinct cilia. In a few cases, vacuolation was sometimes seen to various degrees in the mucosal epithelium; in severe cases, only cell boundaries and nuclei could be identified in the vacuolated areas (Fig. 3). In less severely affected tracheas, the only changes in the epithelium were intercellular edema and marked vacuolation of mucous gland cells.

After epithelial hyperplasia, the next most prominent tracheal lesion was infiltration of the mucosa and/or submucosa with lymphocytes, alone or with epithelial cell hyperplasia. Lymphocytic infiltration varied in intensity and occurred in either a diffuse or focal pattern. Focal, dense lymphoid nodules were a feature in some tracheas; these lymphoid nodules frequently produced a dome-shaped elevation in the mucosa that bulged into the lumen (Fig. 4). The mucosal epithelium overlying the lymphoid nodules lost its normal pseudostratified appearance and was composed of a single layer of low cuboidal or squamous nonciliated cells. In areas heavily infiltrated with lymphocytes, mucous glands were absent or indistinct. In tracheas with both hyperplastic and infiltrative lesions, the hyperplastic epithelium was

partly masked by the dense lymphocytic infiltration; however, individual hyperplastic cells still could be identified between lymphocytes. Among tracheas with marked microscopic lesions, 60% had epithelial cell hyperplasia, 15% had lymphocytic infiltration, and 25% had a combined lesion, regardless of NDV strain.

Mild to moderate edema of the submucosa was seen occasionally, and numerous lymphocytes, macrophages, and plasma cells were scattered throughout the edematous areas. Blood vessels in the submucosa were congested, and many were filled with lymphocytes and fewer heterophils. Heterophils were sometimes present throughout the mucosa and submucosa.

Table 2. Comparative data on five lentogenic strains of NDV as indicated by virus titer of the tracheas, clinical signs, gross lesions, and microscopic lesions

Strain	Virus titer log 10 ELD_{50}/ml of tracheal suspension		Clinical signs	Gross lesions
	Trial 1	Trial 2		
Uncloned LaSota	0	6.3[b]	2[c]	3
Cloned LaSota	5.8	0	0	1
B1	5.3	0	0	2
ET	5.9	6.0	3	5
2024	5.7	5.2	2	3

[a]Marked hyperplasia and/or intense lymphocytic infiltration.

[b]Mean of six tracheas.

[c]Number of poults out of six.

Severe[a] microscopic lesions	Type of microscopic lesions		
	Epithelial hyperplasia	Lymphocytic infiltration	Combined lesion
6	4	1	1
1	1	-	-
4	2	1	1
6	4	-	2
6	3	1	2

Fig. 1 Trachea infected with ET strain. The tracheal lumen contains a plug of fibrinopurulent exudate. There is thickening and distortion of the mucosa. The submucosa is slightly edematous and infiltrated with lymphocytes, macrophages, and plasma cells. H & E 335X.

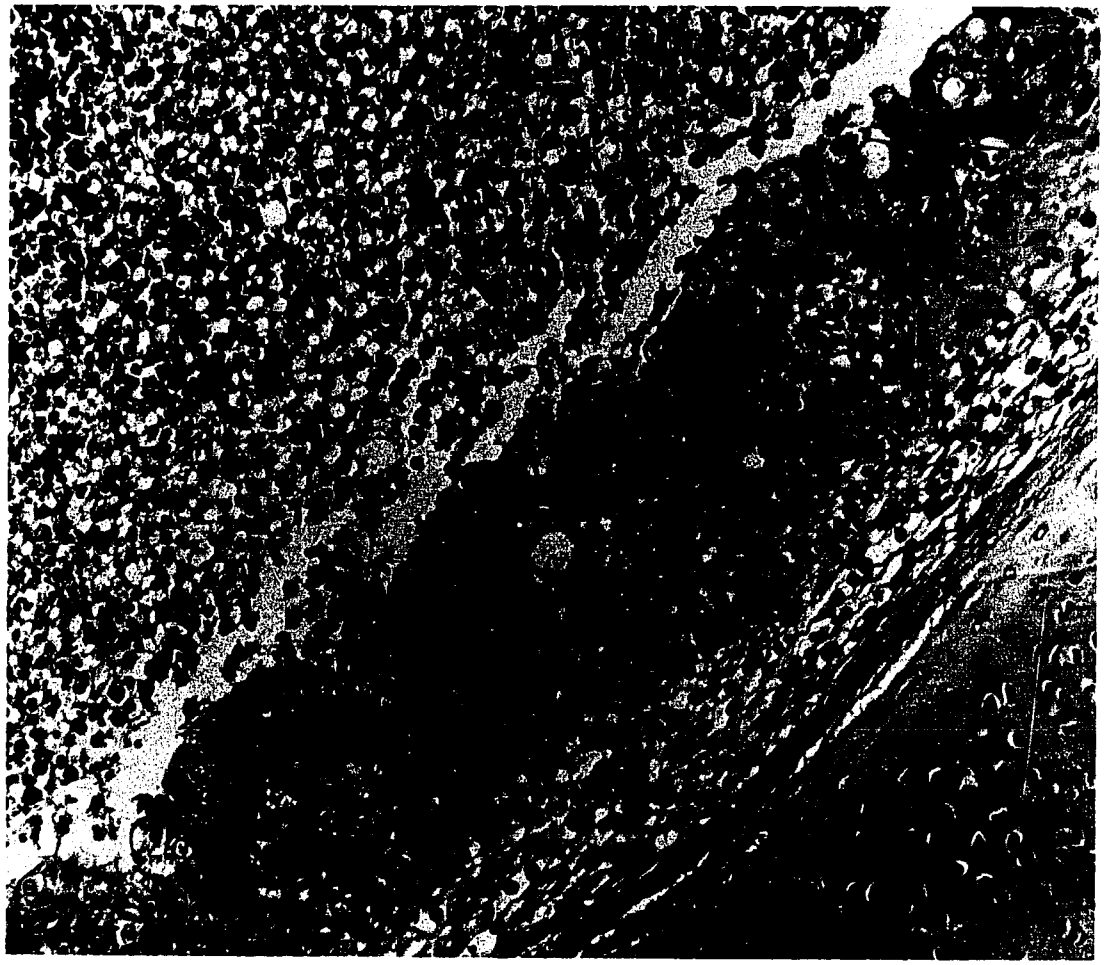


Fig. 2 Trachea infected with uncloned LaSota strain. The mucosa is markedly thickened and consists of several layers of undifferentiated hyperplastic cells. Two cells are undergoing mitosis (arrows). The tracheal lumen contains fibrin. H & E 800X.

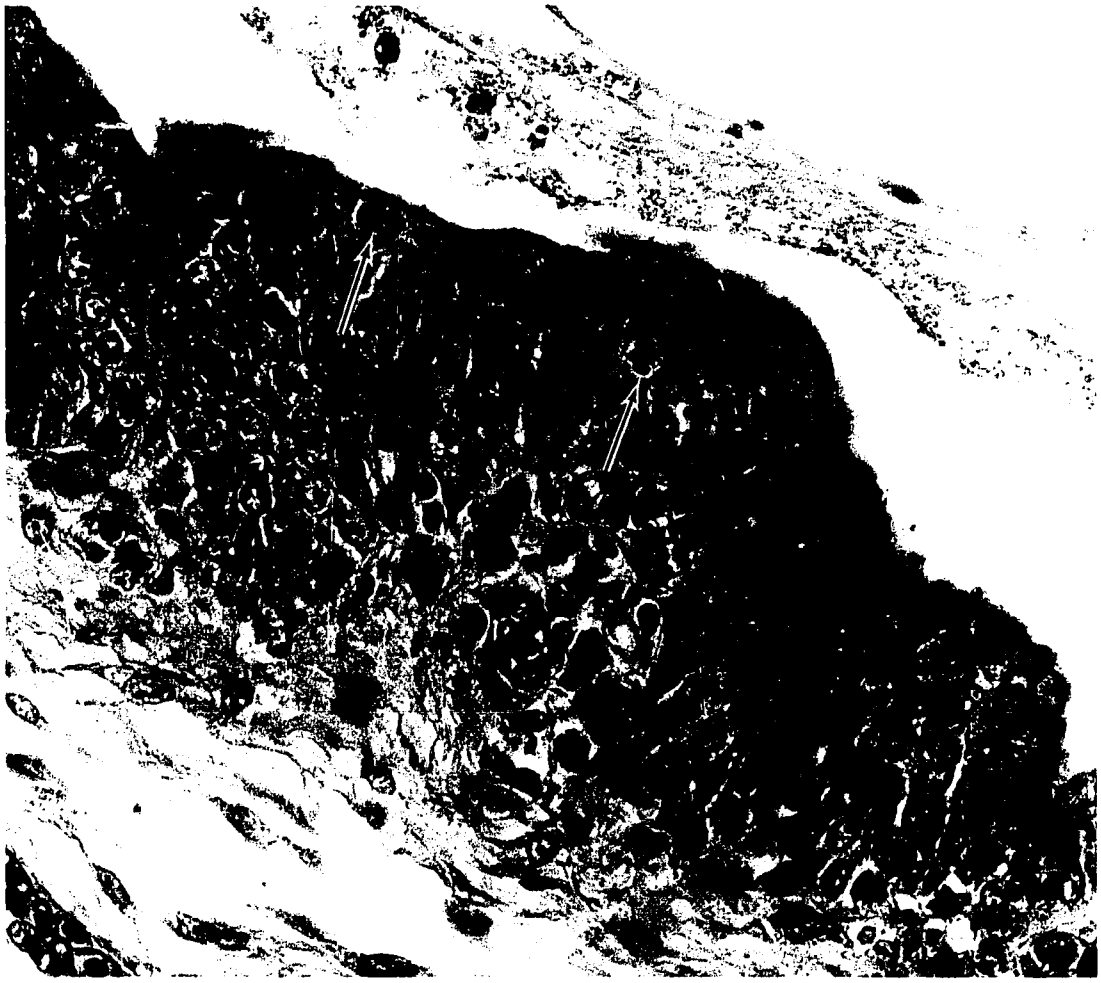


Fig. 3 Trachea infected with strain 2024. Severe vacuolation of mucosal epithelium. Few heterophils on mucosal surface and in mucosa. H & E 855X.

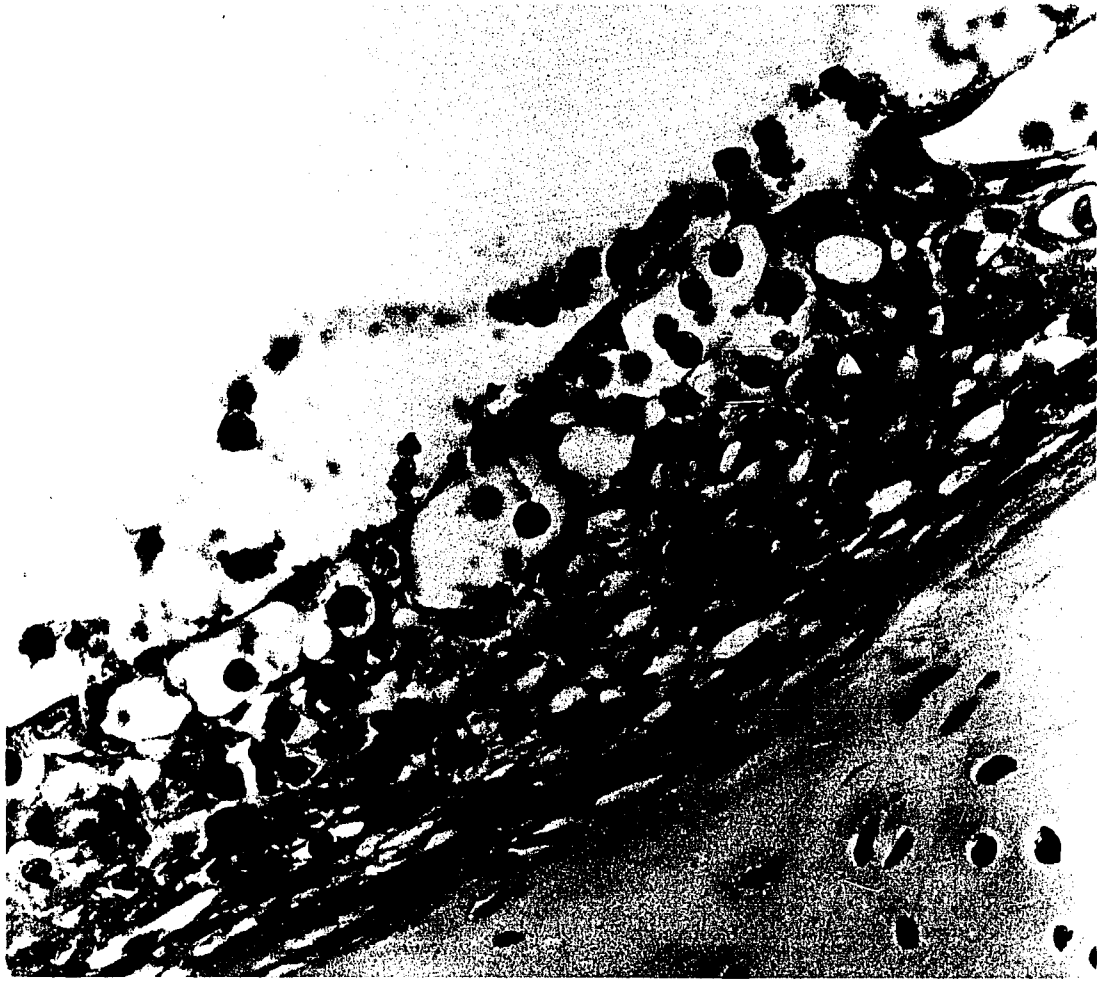
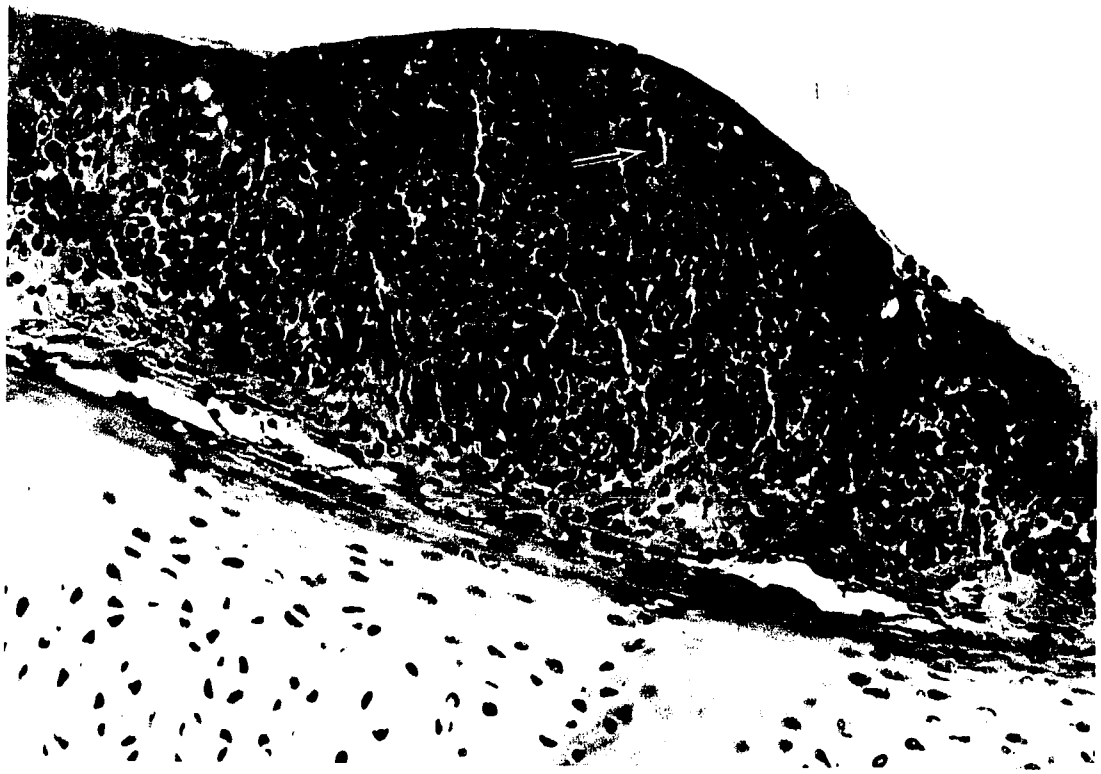


Fig. 4 Trachea infected with strain 2024. The mucosa is expanded by a proliferative nodule of lymphocytes (dark nuclei. Note hyperplastic epithelial cells (nuclei with prominent nucleoli) between lymphocytes. One cell is in mitosis (arrow). The luminal epithelium is flattened and non-ciliated. H & E 355X.



DISCUSSION

The results indicate that lentogenic strains of NDV multiply to high titer in the trachea following aerosol exposure and that viral replication is associated with marked damage to the tracheal mucosa. The failure to isolate NDV from the uncloned LaSota group in the first trial and from the B1 and cloned LaSota groups in the second trial may be due to technical errors during processing of the tissue. Some tracheas from which virus was not isolated had severe lesions; a virus titer of zero seems unlikely.

Regardless of the strain used, lesions can be classified into two types: epithelial cell hyperplasia and extensive lymphocytic infiltration. It is difficult to explain why some tracheas responded to infection by hyperplasia while others responded by intense lymphocytic infiltration, considering that there was no correlation between virus titer and the type of lesion. Garside (9) examined tracheas from chickens that died from ND and stated that hyperplasia was the main lesion in chickens that were more resistant to infection.

Hyperplasia of the tracheal epithelial cells has been described in chickens infected by aerosol with the B1 strain (13). In chickens, hyperplasia of tracheal epithelium is not a specific response to NDV infection, since it occurs also

in infectious bronchitis (17) and infectious laryngotracheitis (16). The intense lymphocytic reaction is also a prominent lesion in chickens exposed to an aerosol of B1 strain (3,13). The lymphonodular reaction has also been noted in tracheas of chickens infected with infectious bronchitis virus (17) and adenovirus (8).

Surprisingly, no attempt has been made to describe lesions in tracheas of chickens exposed to lentogenic NDV strains other than B1. The tracheal lesions in turkeys exposed to the B1 strain were similar but more severe than those described in chickens. Moreover, the large plugs of exudate seen in tracheas of two poults from the B1 group have not been reported in chickens. Differences in the dose and technique used for exposure may account for this variation.

It is difficult to compare the severity of lesions among NDV strains used in the present study. Scoring these strains on the basis of number of poults with severe lesions is unreliable. However, it is safe to conclude that all strains can cause severe injury to the trachea. Although the LaSota strain is known to cause more severe postvaccinal respiratory disease than the B1 strain following spray vaccination (1), in the present experiment tracheal lesions produced by the B1 strain were as severe as those produced by the LaSota strain. Whether similar tracheal lesions occur following

spray vaccination of turkey flocks with live lentogenic NDV vaccines is unknown.

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PART II: PROGRESSION OF TRACHEAL LESIONS IN TURKEYS EXPOSED BY AEROSOL TO LASOTA STRAIN OF NEWCASTLE DISEASE VIRUS

PROGRESSION OF TRACHEAL LESIONS IN TURKEYS EXPOSED BY AEROSOL TO LASOTA STRAIN OF NEWCASTLE DISEASE VIRUS

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SUMMARY

Five-week-old turkeys were exposed by aerosol to the LaSota strain of Newcastle disease virus. Poults were killed on days 2, 4, 6, 8, 10, 12, and 14 postexposure, and tracheas were processed for virus quantitation and histologic examination. Newcastle disease virus was recovered at a high titer from all tracheas collected 2, 4, and 6 days postexposure. The initial tracheal lesion observed on day 2 was swelling of ciliated columnar and mucous gland cells. Some of the affected cells contained intracytoplasmic inclusions. Cell swelling and degeneration were followed by epithelial cell proliferation, fibrinopurulent exudation, and lymphocytic infiltration. Epithelial cell proliferation was most severe on days 4 and 6, when tracheas were lined with several layers of immature cells. Lymphoid nodules appeared on day 6 and persisted up to day 14. From day 8 and on, there was regression of the proliferative lesion accompanied by differentiation of the immature epithelium. By day 14, the tracheal mucosa regained its normal histologic appearance.

INTRODUCTION

The LaSota strain of Newcastle disease virus (NDV) is a lentogenic strain used for vaccinating chickens (8) and turkeys (11,31,32) against Newcastle disease (ND). For large poultry populations, the vaccine is applied via the drinking water or in the form of an aerosol (2). Although application of the vaccine by aerosol induces a greater immunity than application in drinking water (2,12), aerosol vaccination is sometimes associated with various degrees of postvaccinal respiratory reactions (8,13). When turkeys were exposed to an aerosol of different lentogenic strains of NDV, including LaSota, high virus titers and severe microscopic lesions were detected in tracheas 4 days postexposure (1).

The purpose of this study was to follow sequentially the histological changes in the tracheas of turkeys exposed by aerosol to the LaSota strain and to relate the lesions with virus titers.

MATERIALS AND METHODS

Poults

Day-old poults were obtained from a commercial hatchery (Cuddy Farms, Ellsworth, Iowa) and raised to 5 weeks of age. At 5 weeks of age, 44 poults that were serologically negative for ND hemagglutination-inhibition antibodies were divided randomly into six groups. Each of the first five groups (seven poults each) were kept in a separate modified Horsfall-Bauer isolation unit in an isolation room. The sixth group (nine poults) was kept in a double-decker cage in another isolation room. During the first 5 weeks and throughout the experiment, poults were fed turkey starter ration and tap water ad libitum.

Virus

The LaSota strain (B1 type) of NDV was rehydrated with sterile distilled water from a commercial lyophilized vaccine (Salsbury Laboratories, Charles City, Iowa). Before the experiment was initiated, the virus was propagated in enbryonating chicken eggs. The allantoic fluid, harvested from inoculated chicken embryos, contained $10^{9.6}$ mean embryo lethal doses (ELD_{50})/ml as determined by the method of Reed and Muench (28).

Experimental Procedure

At 5 weeks of age, poults in each of the first five groups were exposed to the LaSota strain of NDV; the sixth group served as a control. The virus was administered as an aerosol through a port on top of each isolation unit. A peralta vaporizer (Peralta Hospital, Oakland, California) connected to a pump was used to generate the aerosol using 8 lb/in^2 of pressure. The virus was administered as a 10^{-2} dilution of allantoic fluid in sterile distilled water. The aerosol was generated for 10 min, during which time the vaporizer delivered 1.32 - 1.36 ml of the virus suspension. During aerosolization and 30 min after, the ventilating system for the units was turned off to allow poults to breath the aerosol. The control group was exposed similarly to an aerosol of distilled water.

Necropsy

One poult from each exposed group was killed on days 2, 4, 6, 8, 10, 12, and 14 postexposure (PE); three poults from the control group were killed on days 4, 8, and 12 PE. Poults were anesthetized by an intravenous injection of 0.3-0.5 ml of a 5% solution of thiamylal sodium (Surital, Parke-Davis, Morris Planes, New Jersey) and then were exsanguinated by cutting the right jugular vein. The ventral part of the body was skinned to expose the trachea. Beginning 2 cm below the

larynx, a 4-cm section of the trachea was removed, examined grossly, and placed in a sterile vial for virus titration. The remaining section of upper trachea and a 2-cm section of lower trachea (about 2 cm above the thoracic inlet) were removed and fixed in Bouin's solution for 5 hours, washed in several changes of 70% ethanol for 2-3 hours, and stored in 70% ethanol until processed for histopathologic examination. Histologic sections were prepared by conventional paraffin-embedding techniques and stained with hematoxylin and eosin (H & E).

Virus Assay of Tracheas

The method for quantitating NDV in tracheas has been described (1).

RESULTS

Gross Lesions

Gross tracheal lesions were detected on days 4, 6, and 8 PE. The mucosal surface was covered with white, tenacious exudate. In four poults killed on the 4th day and three poults killed on the 6th day, a plug of this exudate was lodged in tracheal lumina.

Microscopic Lesions

Two days PE, microscopic lesions were observed in two of five poults, and one trachea was affected more severely than the other. The mucosa was thickened owing to swelling of ciliated columnar cells and mucous gland cells. Swollen cells had a pale, coarsely granular cytoplasm with distinct cellular outlines (Fig. 1). In some cells, cellular degeneration was characterized by partial or complete dissolution of the cytoplasm. A few ciliated cells and mucous gland cells had one to three round, eosinophilic, intracytoplasmic inclusions (Fig. 1). These inclusions were 1-3 µm in diameter and sometimes were surrounded by a narrow, clear halo. Numerous heterophils were scattered throughout the mucosa.

All poults killed on day 4 had severe tracheal lesions. Tracheal lumina contained abundant exudate consisting of fibrin and heterophils (Fig. 2). A prominent and consistent

finding was epithelial cell hyperplasia accompanied by thickening and distortion of the mucosa (Fig. 2). Normal pseudostratified columnar epithelium was replaced by four to ten layers of immature cells that were sometimes covered by ciliated epithelium. Generally, immature cells were closely packed and had indistinct cellular outlines, basophilic cytoplasm, and hyperchromatic vesicular nuclei with one or two prominent nucleoli. Many immature cells contained mitotic figures. Vacuoles containing remnants of necrotic cells frequently were seen in the mucosa. Blood vessels in the mucosa were congested and contained marginated heterophils. Focal areas of the mucosa were vacuolated and infiltrated by heterophils (Fig. 3). Occasionally, epithelial cell hyperplasia was accompanied by intense lymphocytic infiltration. In three of the five tracheas, the submucosa was edematous and infiltrated by macrophages, plasma cells, and heterophils.

Tracheal lesions in poults killed 6 days PE were characterized by hyperplasia of epithelial cells and mucous gland cells. Mucous glands were lined with three to five layers of immature cells and had small or obliterated lumina (Fig. 4). Ciliated cells were absent and the mucosa was composed of immature cells only. In two tracheas, the mucosa was heavily infiltrated by lymphocytes, which in some areas formed nodules that bulged into the tracheal lumen (Fig. 5).

In addition to lymphocytes, lymphoid nodules sometimes contained a few lymphoblasts. These lymphoid nodules were covered by a single layer of flattened, nonciliated epithelium.

On day 8 PE, epithelial cell hyperplasia was less extensive, and tracheas generally were lined with three to four layers of immature cells (Fig. 6). Mitotic figures rarely were observed. Differentiation of the immature cells to mature epithelium was obvious on days 10 and 12 PE, and ciliated columnar cells, mucous glands, and a few goblet cells appeared in the mucosa (Fig. 7). By day 14, all tracheas were lined with normal pseudostratified ciliated columnar epithelium. Intact mucous glands occurred throughout the mucosa, but goblet cells were decreased in number. Prominent, circumscribed lymphoid nodules covered by ciliated cuboidal to columnar epithelium were seen in some tracheas examined 10, 12, and 14 days PE (Fig. 7). Lymphoblasts were the predominant cells in these lymphoid nodules.

Virus Titers

Newcastle disease virus was recovered from tracheas of all poults killed on days 2, 4, and 6 (Table 1). The highest titer was detected on day 4. Virus was recovered from only one trachea on day 8. Tracheas from all poults killed on days 10, 12, and 14 were negative for virus.

Table 1. Titer of the LaSota strain of NDV in tracheas following aerosol exposure

Days postexposure	Virus titer (log_{10} ELD_{50}/ml)
2	5.5(5)[a]
4	6.5(5)
6	5.5(5)
8	3.5(1)
10	0
12	0
14	0

[a]Number of poults from which NDV was isolated.

Fig. 1 Trachea 2 days PE. Epithelial cells are swollen, and cytoplasm has a granular appearance. Some cells contain intracytoplasmic inclusions (arrows). H & E 650X.

Fig. 2 Trachea 4 days PE. The trachea is lined with several layers of immature cells. The mucosal surface is distorted, and the tracheal lumen contains fibrinopurulent exudate. The submucosa is slightly edematous and is infiltrated by macrophages, plasma cells, and lymphocytes. H & E 430X.

Fig. 3 Trachea 4 days PE. Mucosal epithelium is vacuolated and infiltrated by numerous heterophils. Blood vessels in the mucosa are prominent and contain marginated heterophils. H & E 265X.

Fig. 4 Trachea 6 days PE. There is hyperplasia of mucous gland cells causing narrowing or obliteration of glandular lumina. Arrows, mucous gland lumina. H & E 430X.

Fig. 5 Trachea 6 days PE. The mucosa is expanded by a lymphoid nodule. H & E 415X.

Fig. 6 Trachea 8 days PE. The tracheal lumen is lined with three to four layers of immature cells. H & E 715X.

Fig. 7 Trachea 12 days PE. Ciliated columnar epithelium, goblet cells, and mucous glands appear in the mucosa. There is a well-demarcated, subepithelial lymphoid nodule. H & E 415X.

DISCUSSION

The present study shows that after turkeys are exposed by aerosol to the LaSota strain of NDV, the virus multiplies to a high titer in the trachea and was followed by damage to the tracheal mucosa. Although early replication of the virus was associated with minimal histologic changes, thereafter damage to the mucosa was extensive. Disappearance of NDV from the trachea was followed rapidly by remodeling and normalization of the mucosa.

In a previous study, NDV was recovered from the trachea of chickens as early as 1 day and up to 5 days after aerosol exposure to the B1 or GB strain of NDV (6). Why viruses cease to multiply in target organs after a period of productive infection is still controversial. Possible nonimmunological mechanisms that terminate viral multiplication are production of interferon, local hypoxia and acidity induced by inflammation, and the febrile response (4). Destruction of virus receptor sites on the cell surface or loss of permissive cells also may be important factors that terminate virus infection.

Little information is available about the site of NDV multiplication in the tracheal epithelium. Fluorescent-antibody examination in chickens has shown NDV antigen in the ciliated epithelium (6,18) as well as in mucous gland cells (18) of the trachea. In this experiment, we also

observed early microscopic changes in the ciliated cells and mucous gland cells that were suggestive of early viral infection. However, recovery of a high virus titer from tracheas lined with nonciliated immature cells, particularly on day 6 PE, suggests that immature cells can also support virus multiplication after loss of mature ciliated epithelium and mucous cells.

Initial cell swelling observed in the ciliated cells and mucous cells was interpreted as intracellular edema possibly caused by disruption in the integrity of cell membranes. Very similar changes have been described in tracheas of chickens examined 3 days after intratracheal inoculation with a viscerotropic velogenic strain of NDV (18). Newcastle disease virus has been shown to alter the permeability of plasma membranes of cells infected in vitro (20). It was postulated that insertion of new virus antigens in the membranes of infected cells is responsible for altering the functional properties of cell membranes. Increasing the permeability of lysosomal membranes may lead to the release of lysosomal enzymes and consequent cell damage (30).

Intracytoplasmic inclusions are either areas of viral factories or nonviral inclusions of cellular origin resulting from cell injury. As with other paramyxoviruses, transcription and replication of viral RNA and assembly of virions take place in the cytoplasm (16). Garside (10) observed

cytoplasmic inclusions in tracheal epithelium of two chickens that died of ND, and Katoh (18) described similar inclusions in the tracheal and conjunctival epithelium of chickens inoculated intratracheally or intraocularly with a viscerotropic velogenic strain of NDV. In his review of the histopathology of avian respiratory infections, Garside (10) mentioned that Butler (7) found cytoplasmic inclusions in tracheal organ cultures infected with NDV. Cytoplasmic inclusions have also been reported in tracheal epithelium of rats (3) and calves (29) infected by aerosol with the paramyxoviruses Sendai and parainfluenza type 3, respectively.

We considered epithelial cell hyperplasia a nonspecific mechanism of mucosal repair. The immature cells that replaced normal epithelium are most likely basal cells that proliferate in response to damage and loss of mature ciliated epithelium. Basal cells in the trachea retain the ability to proliferate and differentiate into ciliated columnar cells and mucous cells (24). Epithelial cell proliferation also occurs in the trachea of chickens with infectious bronchitis (27) and infectious laryngotracheitis (26). "Regenerative hyperplasia" has been described in mechanically injured tracheal mucosa in rats (21) and chemically injured tracheal mucosa in golden hamsters (25).

Lymphoid nodules have been described in tracheas of chickens exposed by aerosol to the B1 strain of NDV (6,15).

These lymphoid nodules are not a pathognomonic lesion for NDV infection since they also occur in the tracheas of chickens infected with infectious bronchitis (27), infectious laryngotracheitis (26), and adenovirus (9). Jones and Hunt (17) stated that the principal function of lymphocytes is to mediate immunologic responses and that their presence in tissues indicates such a response. The presence of lymphoblasts in the lymphocytic infiltrate indicates that there is local antigenic stimulation and suggests that these lymphoid cells may serve an immunologic function. It has been shown that chickens vaccinated with the lentogenic strain B1 of NDV by aerosol rather than by the intramuscular route do not develop respiratory tract infection following aerosol challenge with the velogenic strain GB (5). It was speculated that this resistance might be due to production of local antibodies by lymphoid cells in the tracheal mucosa following aerosol vaccination with the B1 strain. This speculation was confirmed by finding immunoglobulin in lymphoid cells, mucous gland cells, and surface mucus in the trachea of chickens aerosol-vaccinated with NDV (15). Subsequently, both IgG and IgA have been identified in the tracheobronchial secretion of chickens (19,22,23), with IgA being the predominant immunoglobulin (22).

Although aerosol vaccination of turkeys with the LaSota strain induces protective immunity against ND (11,32), we

have seen an increased incidence of Escherichia coli infection in turkey flocks following spray vaccination with this strain. Tracheal damage demonstrated in this experiment may explain in part the vulnerability of vaccinated turkeys to E. coli infection. Presumably, damage to the tracheal mucosa impairs the ability of the trachea to clear inhaled poultry house dust, which may contain large numbers of E. coli, particularly in littered-floor confinement (14). However, whether the same degree of tracheal damage occurs in commercial turkey flocks vaccinated by aerosol with the LaSota strain of NDV has not been determined.

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PART III: PROGRESSION OF TRACHEAL LESIONS IN TURKEYS EXPOSED BY AEROSOL TO ROAKIN AND GB STRAINS OF NEWCASTLE DISEASE VIRUS

PROGRESSION OF TRACHEAL LESIONS IN TURKEYS EXPOSED BY AEROSOL TO ROAKIN AND GB STRAINS OF NEWCASTLE DISEASE VIRUS

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SUMMARY

Five-week-old turkeys were exposed by aerosol to either the Roakin or GB strain of Newcastle disease virus. Poults were killed on days 2, 4, 6, 8, 11, and 14 post-exposure and tracheas were processed for virus quantitation and histologic examination. Newcastle disease virus was recovered from all tracheas collected 2, 4, and 6 days. Two days postexposure swelling of epithelial cells occurred in tracheas infected with the Roakin strain, whereas there were loss of ciliated cells and epithelial cell hyperplasia in tracheas infected with the GB strain. On day 4, there was epithelial cell hyperplasia with both strains, and this was followed on days 6 and 8 by differentiation of the immature epithelium. By days 11 and 14, the tracheal mucosa regained its normal histologic appearance.

INTRODUCTION

Pathology of Newcastle disease has been studied more extensively in chickens than in turkeys. Tracheal lesions have been described in chickens experimentally infected with mesogenic (7) and velogenic (2,3) strains of Newcastle disease virus (NDV). Severe damage to the tracheal mucosa occurs in turkeys infected by aerosol with lentogenic strains of NDV (1). There has been no report describing tracheal lesions in turkeys infected by aerosol with mesogenic or velogenic strains of NDV.

The objective of this study was to characterize tracheal lesions in turkeys exposed by aerosol to the mesogenic strain Roakin and the velogenic strain GB of NDV. Newcastle disease virus titers in tracheas were determined for each strain. Tracheal lesions and virus titers are compared with those reported in poults exposed by aerosol to the LaSota strain of NDV (1).

MATERIALS AND METHODS

Poults

Day-old poults were obtained from a commercial hatchery (Cuddy Farms, Ellsworth, Iowa) and raised to 5 weeks of age. At 5 weeks of age, 56 poults that were serologically negative for ND hemagglutination-inhibition antibodies were divided randomly into nine groups. Each of the first eight groups (six poults each) was kept separately in a modified Horsfall-Bauer isolation unit in two isolation rooms (four units in each room). The ninth group (eight poults) was kept in a double-decker cage in a separate isolation room. Poults were fed turkey starter ration and tap water ad libitum.

Viruses

The two NDV strains used were the mesogenic strain, Roakin and the velogenic strain, GB. Both strains were obtained from M. S. Hofstad, Veterinary Medical Research Institute, Ames, Iowa. Before the experiment was initiated, both strains were propagated in 10-day-old chicken embryos. Embryo lethal doses (ELD_{50}) of the allantoic fluid, harvested from inoculated chicken embryos, were determined by the method of Reed and Muench (8). The allantoic fluid contained $10^{9.6}$ mean ELD_{50}/ml for the GB strain and $10^{9.7}$ mean ELD_{50}/ml for the Roakin strain.

Experimental Procedure

At 5-weeks of age, poults in the first four groups were exposed by aerosol to the Roakin strain, whereas poults in the second four groups were exposed by aerosol to the GB strain. Poults in the ninth group served as controls and were exposed to an aerosol of sterile distilled water. The aerosolization procedure has been described (1). Poults in all groups were observed daily for clinical signs.

Necropsy

One poult from each infected group was killed on days 2, 4, 6, 8, 11, and 14 postexposure (PE). Four poults from the control group were killed on days 6 and 11 PE. Poults were euthanatized by intravenous injection of 1-1.5 cc of 5% solution of thiamylal sodium (Surital, Parke-Davis, Morris Planes, New Jersey). The ventral part of the body was skinned and the trachea was exposed. Sections from tracheas were removed for virus titration and histopathologic examination (1). Handling of tissues, preparation of histologic sections, and virus assay of tracheas have been described (1). Sections from tracheas infected with the GB strain and collected on day 2 PE were stained with periodic acid-Schiff stain.

RESULTS

Clinical Signs

Clinical signs were only observed in poults exposed to the GB strain. Three days PE, poults were depressed and 7 of 24 were coughing. Coughing was prominent in all poults at 4 days PE, but disappeared by day 6. On PE day 8, nervous signs of head tremor were seen in 2 poults. Another two poults developed leg paralysis on PE day 10.

Gross Lesions

On days 2, 4, and 6 PE, similar gross lesions were detected in all infected tracheas. The mucosal surface was congested and covered with white tenacious exudate, which in some poults formed a plug that lodged in the tracheal lumen. In addition, in poults exposed to the GB strain and killed on days 4 and 6 PE, there was marked congestion of the external surface of trachea and the fascia around it.

Microscopic Lesions

At 2 days PE, severe lesions occurred in tracheas infected with the GB strain. There was loss of nearly all ciliated epithelium associated with epithelial cell hyperplasia. The mucosa consisted of one to three layers of

immature epithelial cells covered by a thick layer of fibrin, mucus, and necrotic heterophils (Fig. 1). In 2 tracheas, there were indistinct mucous glands; the periodic acid-Schiff stain showed that cells of these glands were completely depleted of mucus, but the glandular lumina contained a small amount of mucus. Some immature cells had abundant basophilic cytoplasm, whereas others were markedly swollen and had pale, eosinophilic, coarsely granular cytoplasm. Many swollen cells contained one to three round, eosinophilic, intracytoplasmic inclusions that were 1-3 μm in diameter and frequently surrounded by a narrow, clear space (Fig. 2). There were numerous heterophils and a few mitotic figures throughout the mucosa. Two tracheas exposed to the Roakin strain had degenerative lesions characterized by swelling of ciliated cells and mucous gland cells. A few cells contained intracytoplasmic inclusions similar to those seen in tracheas infected with the GB strain (Fig. 2).

On day 4 PE, there was epithelial cell hyperplasia in all tracheas, regardless of the strain. Tracheas were lined with three to six layers of immature cells that had deeply basophilic cytoplasm and hyperchromatic, vesicular nuclei with one or two prominent nucleoli (Fig. 3). Cells in different stages of mitosis were frequently seen in the mucosa. In 2 tracheas, there was focally intense lymphocytic infiltration of the mucosa. Tracheal lumina contained

fibrinopurulent exudate.

By days 6 and 8 PE, there was diminished hyperplasia accompanied by differentiation of the immature epithelium to ciliated columnar cells, goblet cells, and mucous gland cells. In most areas, the epithelium was composed of ciliated columnar cells overlying one to three layers of basal cells (Fig. 4). However, the cilia of some columnar cells were short and indistinct. An intense, diffuse, lymphoplasmacytic infiltrate occurred in mucosas on day 6. In poults exposed to the GB strain and killed on day 6 PE, the perichondrium was infiltrated with lymphoid cells, particularly around blood vessels and in some nerves.

At 11 and 14 days PE, all tracheas were lined by normal pseudostratified ciliated columnar epithelium with well-developed mucous glands and goblet cells.

Virus Titers

Newcastle disease virus was recovered from tracheas of all exposed poults killed on days 2, 4, and 6 PE (Table 1). With both strains, virus titers were highest on day 2 and lowest on day 6. Tracheas from all poults killed on days 8, 11, and 14 were negative for virus isolation.

Table 1. Titers of Roakin and GB strains of NDV in tracheas following aerosol exposure

Days postexposure	Virus titer (log_{10} ELD_{50}/ml)	
	Roakin	GB
2	5.6	6.0
4	4.8	4.7
6	2.5	2.4
8	0	0
11	0	0
14	0	0

Fig. 1 Trachea 2 days PE to the GB strain. The mucosa is composed of two to three layers of swollen immature epithelial cells. One cell is markedly swollen and in mitosis (arrowhead). There is an intracytoplasmic inclusion (arrow). The mucosal surface is covered by a layer of necrotic heterophils. H & E 640X.

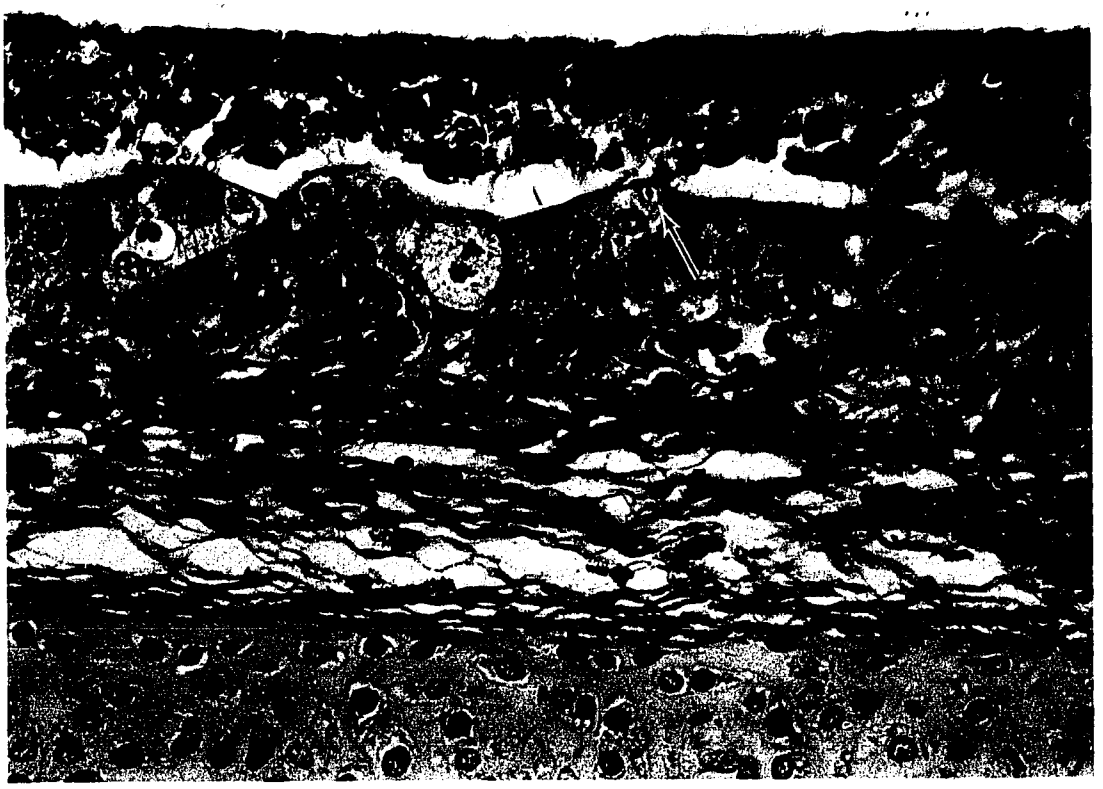
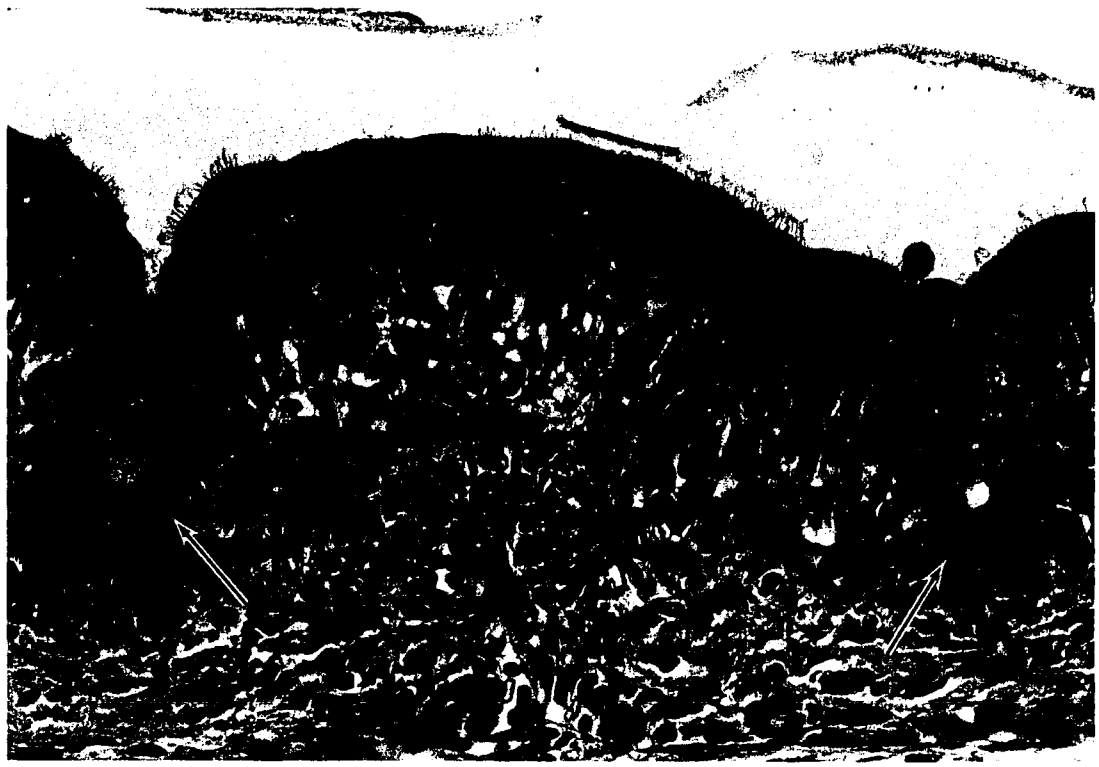


Fig. 2 Trachea 2 days PE to the Roakin strain. Some ciliated cells contain round intracytoplasmic inclusions (arrows) surrounded by a clear space. H & E 870X.

Fig. 3 Trachea 4 days PE to the Roakin strain. There is epithelial cell hyperplasia, and the mucosa consists of four layers of immature epithelial cells. Two cells are in mitosis (arrows). The tracheal lumen contains fibrin and a few heterophils. H & E 630X.

Fig. 4 Trachea 6 days PE to the GB strain. Ciliated columnar cells and mucous glands (arrows) appear in the mucosa. The cilia of some columnar cells are short and indistinct. H & E 565X.



DISCUSSION

Tracheal lesions described in this study are generally similar to those reported in poults exposed by aerosol to the LaSota strain of NDV (1). However, loss of ciliated cells occurred earlier in tracheas infected with the GB strain than in those infected with the Roakin or LaSota strain. This was evident by finding epithelial cell hyperplasia in tracheas infected with the GB strain as early as 2 days PE but not until the fourth day in those infected with the LaSota or Roakin strain. We considered epithelial cell hyperplasia to be a nonspecific response to damage of the mature cells.

Beard and Easterday (2) detected high virus titers in tracheas of chickens exposed by aerosol to GB strain of NDV. They found that virus titers peaked on day 3 and dropped to zero on day 6 PE. In this experiment, tracheal virus titers were low on day 6 and dropped to zero on day 8.

An interesting finding in this study is the more rapid regression of virus titers in tracheas infected with the Roakin or GB strain compared with those infected with the LaSota strain. All three strains attained high titers 2 days PE; however, the virus titer on days 4 and 6 were lower in tracheas infected with Roakin or GB strain than in those infected with the LaSota strain. Although velogenic and

mesogenic strains of NDV are better interferon inducers than lentogenic strains (4,5,6), NDV has been shown to be relatively insensitive to interferon (6). For this reason, it is difficult to explain the rapid decline of virus titers of mesogenic and velogenic strains. Other factors related to cell-virus interactions may limit the multiplication of mesogenic and velogenic strains. Rapid regression of virus titers in tracheas infected with the Roakin or GB strain was associated with early remodeling of the tracheal mucosa.

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GENERAL DISCUSSION AND CONCLUSIONS

Infection with NDV is initiated by multiplication of the virus at the site of entry (28,71). This experiment and others (18,20,51,96,77) indicate that NDV multiplies in the trachea following respiratory infection. Severe damage to the tracheal mucosa has been reported in chickens following aerosol exposure to lentogenic (20,51) and velogenic (51) strains of NDV. Intratracheal and intranasal inoculations of chickens with mesogenic (96) and velogenic (77) strains also produce severe tracheal lesions. Fluorescent antibody studies have demonstrated NDV antigen in the ciliated cells (6,18) and mucous gland cells (18). In this study, early microscopic lesions and cytoplasmic inclusions were also seen in these cells. It appears, however, that after destruction of ciliated cells and mucous gland cells, the virus also replicates in the immature epithelium before being eliminated from the trachea.

Epithelial cell hyperplasia is not a specific lesion, but rather a general response triggered by damage to the differentiated epithelium. Hyperplastic cells are most likely basal cells that later will differentiate into ciliated cells, goblet cells, and mucous gland cells. Therefore, epithelial cell hyperplasia (basal cell hyperplasia) is a nonspecific response indicating the onset of repair in the tracheal

mucosa. In chickens, epithelial hyperplasia of the tracheal mucosa is seen in many respiratory tract infections including infectious bronchitis (104), infectious laryngotracheitis (102), and respiratory cryptosporidiosis (44). Lymphoid nodules are also induced by a variety of infectious agents and are not specific for a particular infection (44).

All strains achieved high virus titers in tracheas on the second day following aerosol exposure. But whether there was a difference in the rate of virus multiplication between the time of exposure and the second day has not been determined. There have been several attempts to correlate virulence with the rate of virus replication (88,111). Most evidence indicates that both low and high virulence strains produce similar titers of free virus (88,111).

The sequelae of any nonpersistent viral infection are either death of the host or elimination of the virus from the body. Newcastle disease virus strains of low virulence appear to persist in the host longer than high virulence strains (133). The rapid clearing of highly virulent strains from the body has been attributed to the ability of these strains to induce a greater humoral immune response than low virulence strains (133). However, anti-NDV circulating antibodies in chickens have little if any effect on the infection of the trachea with NDV (18). It is not clear why tracheal virus titers declined more rapidly with the GB or Roakin

strain than with the LaSota strain. The possible role of interferon has been discussed in the last manuscript. Whether GB and Roakin strains are better inducers of IgA than the LaSota strain is unknown.

In mammals, respiratory mucosa from nasal cavity to small bronchioles is covered by the mucociliary blanket, which is a combination of ciliated epithelium, mucus, and mucus-secreting cells (mucociliary escalator) (92). The mucous coat is composed of two layers; a highly viscus superficial layer, and a serous layer, in which cilia beat (114). This mucociliary escalator contributes an important mechanism for the clearance of particulate materials from the respiratory tract. Inhaled particles are either entrapped in the mucous layer or reach the alveoli. The site of deposition in the respiratory tract is determined by size of particles (136); particles 10 μm or larger impinge on nasal mucosa, whereas those 2 to 10 μm impinge on the tracheal mucosa. Small particles 0.5 to 3 μm are likely to reach the lung. The wave-like propulsive action of the cilia moves entrapped particles through the serous layer toward the pharynx where they can be swallowed. Particles that reach the alveoli are phagocytized by alveolar macrophages. Loaded macrophages are moved from the alveolar surface to the mucociliary escalator and removed quickly from the lung (49).

The anatomic features of the avian respiratory tract are quite unlike those in mammals, but a pseudostratified ciliated epithelium similar to those of mammals lines the main conducting airways (nasal cavity, trachea, and primary bronchi) (63). Although no information is available on the clearance mechanism of the respiratory tract in avian species, at least the main respiratory airways may have a similar clearance mechanism as in mammals.

Poultry house dust has been shown to be a reservoir for Escherichia coli (27,58,59). Koon et al. (82) characterized the type of dust in chicken cages and found that dust particles ranged in size from one to 450 μm. Devitalization of the tracheal mucosa impairs its functional capacity to clear the inhaled particles. This may explain in part the increased incidence of E. coli septicemia in turkeys and chickens following aerosol vaccination with live lentogenic NDV. Damage to the tracheal mucosa by NDV destroys the mucociliary escalator with subsequent failure to remove E. coli-containing dust particles from the lower respiratory tract. Bacterial populations may then increase in the lower airways and lung. Defective clearance may be one of several factors that enable E. coli to colonize the lower respiratory tract and, in some cases, to invade the blood stream and cause septicemia.

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