Pathomorphological changes of oviduct in spontaneous cases of Newcastle disease in layer chicken

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ABSTRACT


Newcastle disease is a major constrain to commercial layer production in India due to high mortality and egg production loss. The disease was found to be one of the predominant cause affecting oviduct with an overall prevalence rate of 27.23% and commonly noticed in the age group of 21 to 50 weeks during the summer season. In the affected flocks drop in egg production, morbidity and mortality were in the range of 5 to 40, 2 to 35 and 0.5 to 15%, respectively. Grossly, in acute death, the visceral organs revealed marked congestion and petechial haemorrhages on the proventricular papillae. Oviduct was congested and the uterus contained partially or fully formed eggs. Cyanosis of comb, congested tracheal mucosa with catarrhal exudate, petechial haemorrhages on the proventricular papillae and caecal tonsils were noticed in chronic cases. The ovarian follicles were misshapened, congested, haemorrhagic and ruptured with yolk materials in the abdominal cavity. Mild to moderate reduction in the size of the oviduct, congestion of the serosal vessels and pale and dry less prominent mucosal folds were observed. Microscopically, in birds died of acute disease, the oviduct showed congestion, edema and haemorrhages, and necrotic changes in the surface epithelia and glandular epithelial cells resulting in desquamation and accumulation of cellular debris in the lumen. In chronic cases, along with the above changes, focal to scattered lymphocytic and plasmacytic infiltration in the interstitium was noticed throughout the oviduct, however more pronounced in the magnum and uterus region. In immunoperoxidase test, NDV antigen appeared as moderate to dark golden brown fine granular material in the cytoplasm of surface epithelium, tubular glands and adjoining to the damaged epithelial cells.

Keywords: Layer, Newcastle disease, oviduct, pathology

INTRODUCTION

Newcastle disease is a highly contagious and widespread viral disease of avian species causing severe economic losses in domestic poultry especially chicken. In India, Newcastle disease virus (NDV) infections are the major cause of economic losses in poultry farming and to control the disease extensive vaccinations (with both lentogenic and mesogenic strains) are being carried out. It is known that vaccination of poultry provides an excellent means to reduce clinical form of disease caused by virulent NDV, however egg production is frequently depressed and often associated with abnormal shell formation and albumen quality. Decrease in egg production is a functional manifestation of the structural damages caused by NDV replication in the reproductive tract. Despite the fact that NDV is known to induce pathology in the reproductive tract of infected poultry, few reports are available on the reproductive pathology associated with NDV under experimental infections. The objective of the present study is to describe the pathomorphological changes of oviduct in spontaneous cases of Newcastle disease among the vaccinated commercial layer chicken.

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MATERIALS AND METHODS

The study on the prevalence of Newcastle disease in commercial layer chicken was carried out for a period of three years (2005 to 2008). A total of 85 commercial layer flocks, above 20 weeks of age with flock strength of 3000 to 25,000 birds belonging to white leghorn breed located in Namakkal district, Tamil Nadu, India were investigated. All the flocks were vaccinated against Marek’s disease, Newcastle disease, infectious bronchitis, infectious bursal disease, fowl pox and infectious coryza according to a standard vaccination schedule. The flocks were inspected, records verified and the information regarding breed and strain of chicken, flock strength, age, method of rearing, vaccination schedule, source of feed and water, production performance including time of peak production, percentage of production, production drop and mortality were collected.

The dead birds were subjected to detailed postmortem examination as per approved procedure. Organs were examined thoroughly for gross pathological changes. Heart blood and oviduct swabs were collected for screening of bacterial agents. The samples were placed in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours and cultured aerobically in sheep blood agar, MacConkey agar and...
eosin methylene blue agar (EMBA) for isolation of bacteria. Bacterial isolates were identified on the basis of their morphology, growth characteristics, sugar fermentation and biochemical characteristics. Pooled tissues (trachea, lung, spleen, proventriculus, ceacal tonsils and oviduct) samples from each flock were finely minced and 20 per cent suspension was made in PBS containing Penicillin (2000 IU/ml) and Streptomycin (2 mg/ml). The samples were kept at room temperature for 1 h before centrifugation at 2000 rpm for 15 min. The supernatant was collected, filtered through millipore membrane filter treated with antibiotics and subjected to Haemagglutination (HA) test for detection of Newcastle disease virus (NDV), infectious bronchitis virus (IBV) and EDS-76 virus. A volume of 0.2 ml of supernatant was injected into the allantoic cavity of six numbers of nine days old embryonated SPF chicken eggs and embryos with typical lesions were tested for presence of NDV in the allantoic fluid by HA and haemagglutination inhibition (HI) tests. Those samples which were found negative for NDV at 3rd passage were further passaged twice before being discarded as negative.

Materials for histopathology were collected from different parts of oviduct (infundibulum, magnum, isthmus and uterus) and fixed in 10 per cent neutral buffered formalin. After fixation, samples were processed by following the routine histological procedures, embedded in paraffin, sectioned at 5 µm thickness and stained with hematoxylin and eosin for histopathological examination. Tissue sections were subjected to immunoperoxidase test (IPT) for detection of NDV antigens.

RESULTS

In the present study, supernatant of the pooled organ samples (467) collected from 19 flocks revealed HA titre of 64 to 256 and it was neutralized in HI test by specific NDV antiserum. The first two egg passages of the samples produced neither any embryo mortality nor gross embryo lesions. At 3rd passage in embryonated eggs, the samples caused embryo mortality, varying degrees of haemorrhages throughout the body including haemorrhages at the occipital regions in 5-7 days post-inoculation. The virus was further confirmed in the allantoic fluid by HA test showing a titre value of 64 to 128 and specific haemagglutination inhibition by using NDV specific antiserum. The heart blood swabs from seven flocks revealed the presence of E. coli organisms, whereas the oviduct swab revealed no organisms of any etiological significance.

On postmortem examination, in birds died of acute disease, the condition of the carcasses were good and showed congestion of the pectoral muscles. Visceral organs revealed marked congestion and petechial haemorrhages on the epicardium, spleen and proventricular papillae. Ovarian follicles were congested and revealed follicular hierarchy. Oviduct was congested and the uterus showed partially or fully formed eggs. Birds which died two or more days after appearance symptoms, showed cyanosis of comb, marked congestion of the pectoral muscles, congested tracheal mucosa with catarhal exudates and cloudy airs. Petechial haemorrhages were noticed around the orifices of papillary glands of proventriculus. Intestinal mucosa showed congestion especially in the duodenal region with greenish watery content, haemorrhages and erosions on the caecal tonsils. Lungs and kidneys showed congestion. The ovarian follicles were misshapened, congested with variably haemorrhagic, ruptured (Fig.1) and yolk materials lied freely in the abdominal cavity. In few birds atresia of ovarian follicles were also noticed. Mild to moderate reduction in the size of the oviduct with severe congestion of the serosal blood vessels and the mucosal folds were less prominent and comparatively pale and dry.

Microscopically, in acutely died birds oviduct showed circulatory disturbances such as congestion, edema, haemorrhages and degenerative changes. While, in chronic cases, along with the above changes inflammatory changes were also noticed. Infundibulum showed loss of cilia and focal hyperplasia of epithelial cells. Mild edema, infiltration of lymphocytes and plasma cells in the lamina propria and muscular layer were noticed. Severe congestion of subepithelial and subserosal blood vessels was also observed. Magnum revealed loss of cilia, focal hyperplasia and necrosis of surface epithelial cells. Marked edema and haemorrhages in the submucosal stroma, distension of the tubular glands with pink intraluminal content were noticed in acutely died birds. In chronic cases, atrophy and depletion of granules in the glandular epithelial cells, multifocal infiltration of plasma cells and lymphocytes in the intertubular glandular space and necrosis were noticed (Fig. 2). Marked infiltration of heterophils and lymphocytes in the lamina propria and congestion of serosal blood vessels were observed. Isthmus showed focal hyperplasia, necrosis and desquamation of the mucosal epithelium. Congestion of vessels along with diffuse infiltration of mononuclear cells and a few heterophils were observed in the lamina propria. In few cases, cystic dilatation of tubular glands was also noticed. Uterus showed loss of cilia, necrosis and desquamation of the mucosal epithelial cells. Submucosal stroma revealed congestion and edema. Focal atrophic and necrotic changes were also observed.
in the tubular gland (Fig. 3). Focal to dispersed interstitial accumulation of lymphocytes and plasma cells were noticed. Vagina showed focal necrosis of surface epithelium and infiltration of heterophils in the stroma.

Newcastle disease virus antigens were detected as positive reaction of immuno peroxidase test in the form of moderate to dark golden brown fine granular material in the cytoplasm of surface epithelium, whereas, nuclei took the colour of counter stain i.e. blue in all the region of the oviduct except vagina. The positive reaction was also noticed in the tubular glands and adjoining to the damaged epithelial cells (Fig. 4).

Out of 1715 birds from 85 farms with oviduct abnormalities, 467 birds from 19 farms with the prevalence rate of 27.23 per cent were identified as ND on the basis of postmortem lesions and laboratory test. Affected flocks showed ruffled feathers, depression, reduced feed and water intake, cyanosis of comb and sudden drop in egg production ranged from 5 to 40 per cent accompanied with variation in the size of the eggs, soft or thin shelled eggs. Few birds also revealed paralytic symptoms. Morbidity and mortality in the NDV affected flocks were ranged from 2 to 35 and 0.5 to 15 per cent, respectively. Age wise analysis on the occurrence of NDV in 467 layer chickens showed highest infection at 31-40 (28.9 %) and 41-50 (23.3%) weeks followed by 21-30 (16.3%), 51-60 (14.6%), 61-70 (9.6%) and 71 -80 (7.3%) weeks. Season wise analysis on the occurrence of NDV showed highest incidence rate during summer (52.9%) in comparison to southwest (27.2%), northeast (9.4%) monsoon and winter season (7.3%).
DISCUSSION

The reproductive efforts of birds can be influenced by disease process either by acting directly and altering the ability of the lining cells to perform their specialized function or generally compromising the health of the bird. Notable among those that affect the cells of the oviduct are infectious bronchitis, Newcastle disease and egg drop syndrome. Newcastle disease is an endemic disease in India and cause changes in the quantity and quality of egg production by the affected layers. Decrease in the egg production is a functional manifestation of oviduct. Hence this paper describes the pathomorphological changes in oviduct of Newcastle disease affected layer chicken.

Among the 1715 birds showing oviduct abnormalities 467 found positive for NDV in postmortem examination and laboratory test. The virus isolates were identified by micro HA test and specific inhibitions of the HA by known specific ND antisera. Similar methods to identify the virus isolates were also employed by earlier workers. The HA titres of the isolates varied between 64 to 128 and HA activity of the isolates were completely inhibited by reference ND antisera, which proved the presence of the virus.

In acute death, visceral organs revealed marked congestion and petechial haemorrhages on the epicardium, spleen and proventricular papillae. Oviduct was congested and the uterus contained partially or fully formed eggs. In chronic cases, cyanosis of comb, marked congestion of the pectoral muscles, congested tracheal mucosa with catarrhal exudate and cloudy air sacs were noticed. Petechial haemorrhages were noticed on the papillary glands of proventriculus and caecal tonsils. The ovarian follicles were misshapen, congested, haemorrhagic, ruptured and yolk materials lied freely in the abdominal cavity. Mild to moderate reduction in the size of the oviduct, severe congestion of the serosal vessels and pale and dry less prominent mucosal folds were observed. More or less similar lesions observed in our study had also been reported by other workers.

Microscopically, the oviduct of an affected bird may contain variable changes in acutely died birds oviduct showed circulatory disturbances such as congestion, edema and haemorrhages and degenerative and necrotic changes in the surface and glandular epithelial cells and subsequent desquamation and accumulation of cellular debris in the lumen of the oviduct. In chronic cases along with the above changes focal to scattered lymphocytic and plasmacytic infiltration in the interstitium was also noticed. These changes were observed throughout the oviduct, however lesions were most pronounced in the magnus and uterus region of the oviduct. Variation in the lesion manifestation in the present study may be due to virulence of infecting NDV and host factors.

The NDV antigen was found to be localized in the cytosol of epithelial cells as golden brown fine granular material in IPT. The positive reaction was also noticed in the tubular glands. These observations are positively correlated with Rao et al. and Raghul et al. observed that the localization of NDV antigen in the cytosol was due to viral replication in the cytoplasm of the cells. The presence of viral antigen adjoining to the epithelium and lamina propria might be due to spill over effect of damaged epithelial cells.

In the present study, Newcastle disease was found to be one of the predominant affections of oviduct. Among the 1715 birds showing oviduct abnormalities 467 (27.23 per cent) found positive for NDV. This observation is similar with findings of Mukhopadhyay et al. who observed 29.71 percent of oviduct problems due to ND infection in Namakkal area. In this study, occurrence of ND in vaccinated flocks might be due to the emergence of virulent strain, poor quality of vaccine due to the poor manufacturing standards, faulty application and inadequate storage facilities. The overall incidence of NDV is more common during the summer followed by south west monsoon season. The summer season in Namakkal poultry belt is characterized by rise in atmospheric temperature to 42 ºC, dryness and other harsh weather conditions and believed to lower the immune status of the birds making it susceptible to NDV in commercial birds that have ordinary or lower herd immune status of the birds making it susceptible to NDV. In this study, the peak production occurs between the age of 25th to 50th week, during this period the birds are subjected to high production stress associated with increased plasma corticosterone concentration and a number of modification to metabolic, physiological and immunological functions. The consequence are heavy economic losses due to reduced growth, production and immunity leading to disease outbreak and mortality. Moreover, the loss of immunoglobulins in laying birds through the egg yolk may leads to rapid decrease in the protective antibodies and predispose the bird to risk of contracting diseases. Though antibody assay was not done at peak hen-day production of lay in this study, we believe that there was significant loss through egg production and this in combination with hormonal stress have contributed in lowering the birds’ immunity.
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REFERENCES