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REVIEW ARTICLE

OCCURRENCE OF INFECTIOUS LARYNGOTRACHEITIS IN POULTRY POPULATION OF MIZORAM, INDIA

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ABSTRACT

A study was undertaken to survey the prevalence of viral diseases of poultry in Mizoram during March 2013 to February 2014. Out of 476 poultry carcasses examined, 208 (43.69%) cases were diagnosed as viral diseases. Only 10 cases of infectious laryngotracheitis (2.10%) could be diagnosed basing on the clinical history, gross and histopathology, which were found to affect the birds older than 3 weeks with higher incidences (40.00%) in 3-6 and 6-9 weeks followed by 9-12 weeks of age (20.00%) with morbidity and mortality rates of 20-40% and 2-5% respectively. All the incidences were found in winter season only, not in summer and rainy seasons. The characteristic signs recorded during the study included conjunctivitis, ocular discharge, mouth breathing, gasping, respiratory rales and coughing of blood, while some birds showed great respiratory distress by extending their neck with prolong difficult inspiration through wide open beak, expectoration of bloody mucus and high mortality while some exhibited mucoid tracheitis, sinusitis, unthriftiness and low mortality. The significant gross lesions were mucoid tracheitis, laryngitis and severe hemorrhages in the trachea which was filled with mucus mixed with blood leading to obstruction. Most striking microscopic changes were severe congestion and hemorrhages of the tracheal mucosa which showed the presence of several syncytial cells with intranuclear inclusion bodies in the epithelium, while sections revealed complete desquamation of tracheal mucosa with infiltration of lymphocytes and plasma cells.

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INTRODUCTION

Infectious Laryngotracheitis (ILT) is an economically important and highly contagious viral respiratory disease of chicken caused by *Gallid herpes virus 1* (Hughes *et al*., 1991). Domestic fowl is the only natural primary host of ILT virus. It has been shown that ILTV can also infect pheasants, pheasant-bantam crosses and peafowl (Crawshaw and Boycott, 1982). All ages of chickens are affected but chickens older than 3 weeks are most susceptible to ILTV (Bagust *et al*., 1986). The main route of transmission is windborne spread, contaminated clothing, dead birds and other inanimate objects (Hidalgo, 2003). Sources of ILTV are clinically affected chickens, latent infected carriers, contaminated dust, litter, beetles, drinking water and fomites (Ou *et al*., 2012). Severe forms of ILT cause high morbidity of 90%-100% and mortality of 5%-70% (Beach, 1926; Hinshaw, 1931). Mild form of the disease may cause morbidity as low as 5% and very low mortality 0.1%-2% (Sellers *et al*., 2004).

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Characteristic clinical signs included conjunctivitis, ocular discharge, mouth breathing, gasping, respiratory rales, coughing of blood, increased morbidity and mortality; dried blood may be present around the nostril and lower beak and severe drop in egg production (Barhoom and Dalab, 2012). Severe forms of ILT causes severe respiratory distress birds extend their neck with prolong difficult inspiration through wide open beak, expectoration of bloody mucus and high mortality and the mild forms of infection are characterized by mucoid tracheitis, sinusitis, unthriftiness and low mortality (Srinivasan *et al*., 2012). Along with respiratory signs some birds may become cyanotic before death (Preis *et al*., 2013). The most commonly seen gross lesions are mucoid tracheitis, laryngitis and severe hemorrhages in the trachea. The tracheal lumen may be filled with mucus mixed with blood, exudates, caseous materials and sometimes existence of blood casts along the entire length of the larynx and trachea. Inflammation may extend down the bronchi into the lungs and air sacs (Barhoom and Dalab, 2012; Aziz, 2010). Lesions are most commonly noticed in the larynx and upper trachea and less frequently in lower trachea. ILT diagnosis requires laboratory assistance as other respiratory pathogen of poultry can cause similar clinical signs and lesions. Only in case of severe acute disease with

high mortality and expectoration of blood, ILT can be reliably diagnosed on basis of clinical signs. Otherwise, diagnosis of ILT should be based on one or more confirmatory laboratory diagnostic procedures including detection of intranuclear inclusion bodies, virus isolation and detection of ILTV antigen in tracheal tissues or respiratory mucus. Detection of ILTV specific DNA can be done by PCR (Hinshaw, 1931; Hughes and Jones, 1988; Tripathy and Hanson, 1989). Indirect immunoperoxidase (IP) can be used to detect ILT virus antigen in frozen tissue sections (Guy *et al*., 1992).

MATERIALS AND METHODS

Collection of data

The epidemiological data pertaining to viral diseases in poultry from March 2013 to February 2014 were collected from both organized and unorganized poultry farms of Mizoram. Detailed information such as total birds in a flock, number of birds affected, number of birds died, age of the affected birds, month of occurrence of the disease, history of previous outbreaks of viral diseases and vaccination status were obtained from the affected flocks.

Collection of samples

Dead/moribund birds were collected for proper necropsy. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, etc.) showing typical lesions were collected for histopathological examination and laboratory analysis.

Epidemiological studies

Both organized and unorganized poultry farms of Mizoram were visited regularly and the morbidity, mortality, age of affection of various diseases were recorded. To assess the age-wise variations in the incidence of the diseases, the birds were grouped as 1-3, 3-6, 6-9, 9-12 and above 12 weeks old. To study the seasonal variations, the whole year was divided into conventional three seasons, namely summer (March-June), Rainy (July-October) and winter (November-February).

Recording of clinical signs

In case of mortality/outbreak of diseases in the poultry population, the clinical signs exhibited by the individual bird during illness were recorded in detail in a prescribed according to the description of the respective poultry farm's owner or attendant. In addition, sometimes some sick/moribund birds were kept under careful observation with feed and water *ad libitum* till death to record the detailed clinical signs along with other abnormalities.

Gross pathological examination

Detailed post-mortem examination of all the dead birds was performed. At necropsy, gross tissue changes were observed and recorded carefully. Representative tissue samples (heart, liver, spleen, lungs, kidneys, bursa of Fabricius, trachea, proventriculus, caecal tonsil, brain, feather follicles, etc.) showing lesions were carefully collected in ice and in 10% formaldehyde solution. Viable tissue samples were collected aseptically in sterile polypropylene zipper bags and stored in -80ºC for further analysis.

Histopathological examination

Formalin fixed tissues (2-3 mm thick) were taken, washed overnight in running tap water and then dehydrated in ascending grades of alcohol starting from 50%, 70%, 90% and absolute alcohol I, alcohol II, alcohol III and finally cleared in xylene. These dehydrated tissue pieces were then embedded in molten paraffin. Sections were cut at 4-5 µm thick with semi-automatic rotary microtome(MRS 3500, Histoline Laboratories) and stained with Mayer's hematoxylin and eosin (Bancroft and Stevens, 1980). The stained slides were examined under a trinocular research microscope (Olympus) and the magnified images of the tissue structures were captured for further study.

Diagnosis

The diagnosis of the disease was made mainly basing on the clinical signs and characteristic gross, as well as microscopic tissue alterations.

RESULTS

Epidemiology

During the period of March 2013 to February 2014, a total of 476 poultry carcasses were examined. Out of these, 208 (43.69%) cases were diagnosed as viral diseases. Only 10 cases of infectious laryngotracheitis (2.10%) could be diagnosed basing on the clinical history, gross and histopathology, which were found to affect the birds older than 3 weeks with higher incidences (40.00%) in 3-6 and 6-9 weeks followed by 9-12 weeks of age (20.00%) with morbidity and mortality rates of 20-40% and 2-5% respectively. All the incidences were found in winter season only, not in summer and rainy seasons.

Clinical findings

The clinical signs recorded in the ILT affected birds included conjunctivitis, ocular discharge, mouth breathing, gasping, respiratory rales and coughing of blood. In some cases, birds showed great respiratory distress by extending their neck with prolong difficult inspiration through wide open beak (Fig. 1), expectoration of bloody mucus and high mortality while some exhibited mucoid tracheitis, sinusitis, unthriftiness and low mortality. In addition to the respiratory signs, some of the birds became cyanotic before death.

Fig.1. ILT affected bird showing mouth breathing through wide open beak

Gross pathological findings

The gross changes were most commonly observed in the larynx and upper trachea and less frequently in lower trachea which included mucoid tracheitis, laryngitis and severe hemorrhages in the trachea (Fig. 2). The tracheal lumens were filled with mucus mixed with blood leading to obstruction (Fig. 3). In few cases, there were mucoid exudates blocking the lumen of cranial part of the trachea (Fig. 4). In addition, conjunctivitis with intense hyperaemia, oedema and sinuses with caseous exudates were present. Lungs of some affected birds exhibited reddening and white or yellow exudates on the surface.

Fig. 2. Photograph showing severe congestion and hemorrhagic trachea

Fig. 3. Photograph showing obstruction of tracheal lumen with blood clots

Fig. 4. Photograph showing congestion and mucoid exudates in the trachea

Histopathological findings

Trachea mucosa showed severe congestion and hemorrhages. In most of the cases, several syncytial cells with intranuclear inclusion bodies were present in the mucosa epithelium (Fig. 5 & 6). In some cases, there was complete desquamation of tracheal mucosa (Fig. 7). The mucosa and sub-mucosa were infiltrated by lymphocytes and plasma cells. Varying amounts of exudates composed of fibrin, heterophils, inflammatory mononuclear cells, sloughed epithelial cells; syncytial cells and red blood cells were present on the mucosal surface and in the lumen.

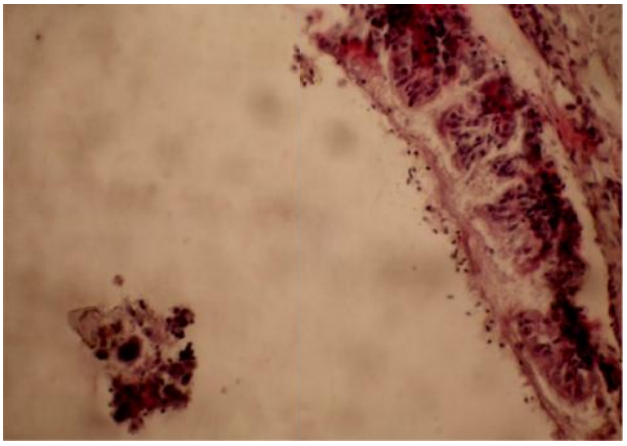

Fig. 5. Tracheal section showing congestion, hemorrhages and syncytial cells with intranuclear inclusion bodies (H&E x400)

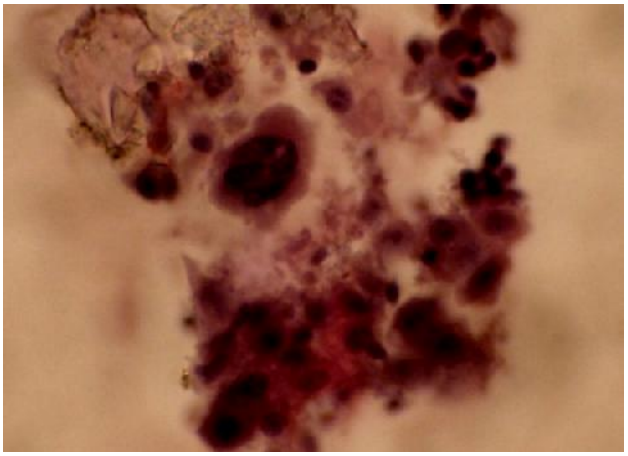

Fig. 6. Syncytial cells with intra-nuclear inclusion bodies (arrow) (H&E x100)

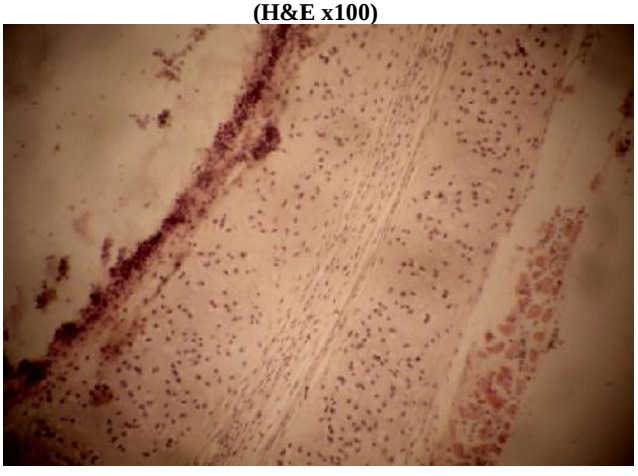

Fig. 7. Tracheal section showing complete desquamation of the mucosa (H&E x200)

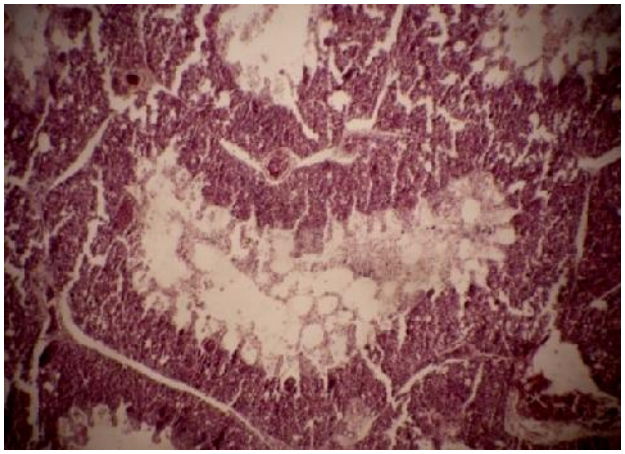

Fig. 8. Lung section showing congestion, haemorrhages and serous exudation in the parabronchial lumen (H&E x200)

The lung sections revealed severe congestion, hemorrhages and serous exudation in the parabronchial lumen (Fig. 8); while some sections showed disorganization of the primary and secondary bronchial epithelium. Walls of affected bronchi were infiltrated with large numbers of inflammatory mononuclear cells and exudates.

Diagnosis

Out the 24 cases of clinically ILT suspected cases, 10 nos. could be confirmed for ILT by demonstrating the several syncytial cells with intranuclear eosinophilic inclusion bodies in the sloughed off epithelial cells of tracheal mucosa.

DISCUSSION

In the present study period, higher incidences of ILT were seen in 3-6 and 6-9 weeks old birds followed by a lower incidence in 9-12 weeks old birds, while birds in the age groups of 1-3 and above 12 weeks were not affected. This result is in agreement with earlier workers (Bagust *et al*., 1986; Hitchner and Winterfield, 1960; Fahey *et al*., 1960) who describes that all ages of chickens are affected but chickens older than 3 weeks are most susceptible to ILTV. The percent morbidity (20-40%) and mortality (2-5%) recorded during the present study indicates the moderately milder form of the disease. Two researchers[6,7] describe that the severe forms of ILT cause high morbidity of 90%-100% and mortality of 5%-70%, whereas some researchers (Sellers *et al*., 2004; Pulsford and Stokes, 1953; Raggi *et al*., 1961) express that the mild form may cause morbidity as low as 5% and very low mortality 0.1%-2%. The present study showed that all the incidences of the disease were found to occur only in winter season. This might be due to the strong dry wind and extreme cold weather prevailing in this region which predisposes the birds to get this kind of air-borne infections.

The present clinical signs recorded in the ILT affected birds such as conjunctivitis, ocular discharge, mouth breathing, gasping, respiratory rales and coughing of blood are in agreement with the earlier reports (Barhoom and Dalab, 2012; Aziz, 2010; Russell, 1983; Zellen *et al*., 1984; Chacon *et al*., 2007). Some birds showed great respiratory distress by extending their neck with prolong difficult inspiration through wide open beak, expectoration of bloody mucus and high mortality, while some exhibited mucoid tracheitis, sinusitis, unthriftiness and low mortality. Similar observations have been described (Hidalgo, 2003; Srinivasan *et al*., 2012). In addition to the respiratory signs, some of the birds were cyanotic before death, which supports the signs described by earlier researchers (Preis *et al*., 2013). The gross changes commonly recorded in the present study included mucoid tracheitis, laryngitis and severe hemorrhages in the trachea which was filled with blood-mixed mucus leading to obstruction. Similar changes have been described by several workers (Hidalgo, 2003; Barhoom and Dalab, 2012; Aziz, 2010). Some birds showed mucoid exudates blocking the lumen of cranial part of the trachea and also had conjunctivitis with intense hyperemia, oedema and sinuses with caseous exudates. These findings support the previous reports (Preis *et al*., 2013).

The changes in the lungs observed in the present study is in agreement with a researcher (Aziz, 2010) who found that the lungs of affected birds were reddened and their cut surfaces revealed white or yellow exudates within large airways (bronchi). Most of the tracheal sections revealed severely congested and hemorrhagic mucosa which showed the presence of syncytial cells with intranuclear inclusion bodies, while some sections had complete desquamation of the mucosa. There were infiltration of lymphocytes and plasma cells in the mucosa and sub-mucosa. Many of the sections showed presence of varying amounts of exudates composed of fibrin, heterophils, inflammatory mononuclear cells, sloughed epithelial cells; syncytial cells and red blood cells on the mucosal surface and also in the lumen. These microscopic changes of the present study are in accordance with the earlier reports of many researchers (Hidalgo, 2003; Srinivasan *et al*., 2012; Preis *et al*., 2013; Aziz, 2010; Guy *et al*., 1992; Russell , 1983; Seifried, 1931; VanderKop, 1993; Ebrahimi *et al*., 2001; Nair *et al*., 2008; Hinshaw *et al*., 1931). Most of the lung sections showed severe congestion, hemorrhages and serous exudation in the parabronchial lumen, while some revealed disorganization of the primary and secondary bronchial epithelium. Walls of affected bronchi were infiltrated with large numbers of inflammatory mononuclear cells and exudates. Similar microscopic changes have been described (Aziz, 2010; Russell , 1983; Seifried , 1931; Ebrahimi *et al*., 2001; Hinshaw *et al*., 1931).

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