Pathobiology of Naturally Occurring Uncomplicated Ornithobacterium Rhinotracheale Infection in Layer Chicken

S. Sivaseelan*, G.A. Balasubramaniam, P. Balachandranand and R. Madheswaran
Department of Veterinary Pathology, Veterinary College and Research Institute, Namakkal - 637 002, Tamil Nadu, India

Abstract
Pathobiology of uncomplicated Ornithobacterium rhinotracheale (ORT) infection in layer chicken was evaluated in 30 commercial layer chicken farms. Ornithobacterium rhinotracheale organism was confirmed by its growth characteristics on agar media and typical morphology on Gram’s staining. Commonly encountered concurrent respiratory virus and bacteria were ruled out. Polymerase chain reaction technique was used to rule out Mycoplasma gallisepticum. Newcastle disease and Infectious bronchitis were screened by haemaggutination and inhibition tests and bacterial diseases like Colibacillosis, Pasteurellosis and Infectious coryza were ruled out by culture and staining techniques. Uncomplicated cases of ORT revealed mild sinusitis with crusts in the external nares, mild subcutaneous swelling of face, catarrhal rhinitis and tracheitis, airsacculitis and unilateral or bilateral pneumatic changes of the lungs. Foamy, whitish, yogurt like exudates with strands of fibrin was observed in the abdominal airsacs. Gross and histopathological changes were observed throughout the respiratory tract and seven per cent mortality was recorded in the uncomplicated cases.

Key words: Ornithobacterium rhinotracheale, Uncomplicated infection, Pathology, Chicken.

Introduction
Ornithobacterium rhinotracheale (ORT) causing respiratory disease in broiler chickens is a gram-negative, pleomorphic, rod-shaped bacterium (Vandamme et al., 1994). The disease is common in broiler chicks at 3 - 6 week of age and in broiler breeders at 20 - 50 week of age (Allymetir, 2006).

Several concurrent infections of ORT with other respiratory infections have been reported. Dual infection with Newcastle disease virus and ORT in broilers caused more severe respiratory lesions and higher mortality rates than in birds with only Newcastle disease and the pathogenicity of NDV was enhanced when it occurred with ORT infection (Travers, 1996). Concomitant ORT and E. coli infections in chicken broilers were reported earlier (El-Shukon et al., 2002).

When experimentally inoculated, ORT caused more severe lesions when there was a concurrent infection with respiratory viruses or bacteria (Back et al., 1997; Van 1997; Van Empel and Hafez, 1999; Van Veen et al., 2000)

This paper deals with the pathobiology of uncomplicated ORT

*Email: pathologysiva@yahoo.co.in
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infection in layer chicken in order to explore the exact pathological effects caused by ORT alone, excluding the complementary or synergistic role of other commonly occurring concurrent respiratory diseases in layer chicken.

**Materials and methods**

This study was conducted for one year period, in which 30 commercial layer flocks (strength varied from 10,000 to 50,000 birds) with the history and symptoms of ORT were investigated. Necropsy was carried out on recently died chicken carcasses and ailing birds. Five per cent of samples from each farm such as trachea, lungs, airsacs, spleen, proventriculus, intestine, ceecal tonsils and swabs of infraorbital sinus exudates, heart blood and liver were collected.

**Diagnosis of Ornithobacterium rhinotracheale**

Samples such as trachea, lungs, airsacs, liver, and swabs of heart blood and infraorbital sinus exudate were utilised for the confirmation Ornithobacterium rhinotracheale by their growth characteristics on sheep blood agar media (M/s Hi Media laboratories, Mumbai, India) and Gram’s staining. Growth on sheep blood agar with gentamicin discs was also performed to assess the gentamicin resisting nature of O. rhinotracheale organism.

**Screening of CRD**

The most commonly encountered concurrent infection, CRD was ruled out by collecting trachea and airsac pieces in Frey’s medium and subjecting the Mycoplasma gallisepticum DNA to PCR confirmation.

**DNA extraction**

One ml of sample cultured in Frey’s medium was centrifuged at 10,000 x g for 20 min twice and the pellet was washed with 70 per cent ethanol. The pellet was resuspended with 50 µl of Tris EDTA buffer and boiled for 3 - 5 minutes to release the DNA. The extracted DNA was stored at – 20 °C until use.

**Polymerase chain reaction Primers**

The following forward and reverse primers were used for the amplification of target sequence (530 bp) of M. gallisepticum.

Forward primer

5’ - AAC ACC AGA GGC GAA GGC GAG G - 3’

Reverse primer

5’ - ACG GAT TTG CAA CTG TTT GTA TTG G - 3’

The following mixture of materials was subjected to PCR (Kiss et al., 1997) in a thermal cycler (Eppendorff).

Master Mix : 25 µl (dNTPs, Taq polymerase and PCR buffer)
Forward primer : 1 µl (40 picomols)
Reverse primer : 1 µl (40 picomols)
DNA template : 2 µl
DNase free water to make up to 50 µl

The reaction consisted of initial denaturation for 5 min at 95 ºC followed by 35 cycles of denaturation at 94 ºC for 30 seconds, annealing at 55 ºC for 30 seconds and extension at 72 ºC for 30 seconds with
final extension at 72 °C for 10 min. The PCR products were separated on 1.5 per cent agarose gel in 1X TAE buffer containing ethidium bromide 50 µg per ml at 100 volts for 45 minutes to one hour.

**Diagnosis of other concurrent respiratory virus and bacteria**

Tissue homogenate of pooled samples of trachea, lung, liver, spleen, proventriculus, intestine, ceecal tonsils and kidney were subjected to haemaggutination and inhibition tests to identify Newcastle disease virus and Infectious bronchitis virus (Alexander, 1988).

The following culture media from M/s Hi Media laboratories, Mumbai (India) were prepared as per instructions of the manufacturer and used for the isolation of below mentioned bacteria associated with respiratory diseases in chicken:

- *Escherichia coli* – MacConkey and Eosin Methylene Blue (EMB) agar
- *Pasterurella multocida* – Brain Heart Infusion (BHI) agar
- *Avibacterium paragallinarum* – Sheep blood agar and Chocolate agar

Samples such as trachea, lungs, airsacs, liver, and swabs of heart blood and infraorbital sinus exudate were utilized for the confirmation of these bacteria by their growth characteristics on agar media and Gram’s staining of organisms.

**Pathology**

After recording the gross lesions, a transverse section of tissue approximately 0.5 cm in thickness was taken from infraorbital sinus, trachea and lungsof birds. Airsacs were as such removed. Tissue pieces were fixed in 10 per cent buffered neutral formalin and trimmed to a thickness of about 3 mm. The tissues were dehydrated, cleared and embedded in paraffin in a routine manual processing. Tissues were cut at 5 µm thicknesses, mounted on glass slides, stained with haematoxylin and eosin and covered with coverslips for histopathological examinations (Bancroft and Stevens, 1996).

**Results**

**Disease investigation**

Though ORT occurred with other respiratory illness in 12 farms (40 %) out of 30 investigated, exclusive occurrence of ORT was observed only in three farms (10 %). Seven percent average mortality was recorded in exclusive ORT outbreaks.

**Diagnosis of Ornithobacterium rhinotracheale**

Non-haemolytic, grey to greyish white, opaque, convex, very small, circular colonies with a diameter of 1-2 mm and with butyrous odour were observed on sheep blood agar after 48 h of incubation at 37 oC under anaerobic conditions. Growth on sheep blood agar with gentamicin was also noticed (Fig. 1).

![Fig.1 Growth of ORT on sheep blood agar showing gentamicin resistance](image-url)
Smears prepared from the colony revealed highly Pleomorphic Gram-Negative Rods designated as PGNR.

**Diagnosis of other concurrent respiratory virus and bacteria**

Concurrent infection of Newcastle disease, Infectious Bronchitis, CRD, Colibacillosis, Pasteurellosis and Infectious coryza were ruled out in the farms under report.

**Gross pathology**

Uncomplicated cases of ORT revealed mild sinusitis with crusts in the external nares, mild subcutaneous swelling of face, catarrhal rhinitis and tracheitis, airsacculitis and unilateral or bilateral pneumonic changes of the lungs. Unilateral pneumoniawas more frequently noticed than bilateral type. Foamy, whitish, yogurt like exudates with strands of fibrin was observed in the abdominal airsacs (Fig. 2).

**Fig. 2 Foamy, whitish, yogurt like exudate observed in the abdominal airsacs**

In few cases, cut section of the lungs showed whitish miliary nodules ranging from 2-3 mm in diameter.

**Histopathology**

Focal deciliation and necrosis of surface epithelial cells of trachea were observed in acute cases, whereas long standing cases showed necrosis and sloughing of both surface as well as glandular epithelium.

Fibrinopurulent pneumonia characterised by collection of fibrin admixed with macrophages and heterophils within the lumen of air capillaries and interstitial septa, and parabronchi were observed. Secondary bronchi showed epithelial hyperplasia and accumulation of fibrin and heterophils mixed fibrinopurulent exudate. Epithelial necrosis and sloughing, subepithelial massive infiltration of mononuclear cells, and thickened connective tissue were noticed in airsacs (Fig. 3).

**Fig.3 Airsac revealing epithelial necrosis, subepithelial massive infiltration of mononuclear cells, and thickened connective tissue**

**Discussion**

**Disease investigation**

More prevalence (40 %) of combined infections of ORT with other respiratory infections when compared to uncomplicated ORT outbreaks (10 %) observed in this study coincides well with the earlier reports (Travers, 1996; El-Sukhon et al., 2002) and indicates that combined infections are higher than uncomplicated occurrence of ORT.

**Diagnosis of Ornithobacterium rhinotraeale**

The growth characteristics observed on sheep blood agar are similar to the earlier reports (Zorman-Rois et al.,...
Growth on sheep blood agar with gentamicin indicated the gentamicin resisting nature of O. rhinotracheale (Hafez et al., 1993). Smears prepared from the colony revealed highly pleomorphic rods (Gopalakrishnamurthy et al., 1998; Van Empel and Hafez, 1999). These observations clearly confirm the etiology as ORT.

**Diagnosis of other concurrent respiratory virus and bacteria**

Concurrent infection of Newcastle disease, Infectious Bronchitis, CRD, Colibacillosis, Pasteurellosis and Infectious coryza were ruled out in the farms under report by using standard diagnostic techniques. These diseases were ruled out to ascertain that the observed pathobiology is pertaining only to ORT infection.

**Gross pathology**

The gross lesions observed in the sinus, trachea and airsacs in this study are in agreement with the earlier findings (Van Empel and Hafez, 1999).

Unilateral pneumonia with whitish miliary nodules of the lungs noticed in few cases concurred with the earlier observation (Joubert et al., 1999). Airsacculitis was mostly observed in abdominal airsacs than thoracic ones. On the contrary Joubert et al., (1999) reported more involvement of thoracic airsacs. Cloudy airsacs without foamy exudate noticed in the affected farms might be the early lesions when compared to the whitish yoghurt like exudate commonly observed in ORT affected birds later. Severe airsacculitis with more exudate observed might be the reason for cause of death owing to the fact that occlusion of airsacs will cause asphyxiation due to failure of bellowing activity that regulates respiration in birds.

**Histopathology**

In exclusively ORT affected cases, the microscopic changes observed coincided well with the earlier findings (Odor et al., 1997; Gopalakrishnamurthy et al., 1998).

Epithelial sloughing, subepithelial massive infiltration of mononuclear cells and thickened connective tissue noticed in the airsacs supports the gross lesions, which could cause loss of bellowing activity of affected airsacs to bring about asphyxiation and death.

It is concluded that, in addition to concurrent infection, exclusive uncomplicated ORT outbreaks have been recorded in this study. Seven per cent mortality recorded in exclusive ORT outbreaks indicates that this disease is not an innocent disease and suitable prophylaxis and treatment strategies should be formulated to contain the outbreaks.

**References**


