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## *Ornithobacterium rhinotracheale*: An emerging poultry pathogen

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### Abstract

Respiratory infections are the common causes for mortality and morbidity in poultry, which leads to huge economic losses to the poultry industry. *Ornithobacterium rhinotracheale*, is the emerging pathogen and difficult respiratory tract bacteria to isolate and grow in the laboratory. The first report of isolation *O. rhinotracheale* was in 1981 from the respiratory tract of 5 weeks old turkeys with fibrinopurulent airsacculitis and the characterization was done in 1993. The bacterium can be grown on 5-10% sheep blood agar and does not grow on MacConkey agar and Simmons citrate medium. It grows aerobically, micro aerobically and anaerobically, but the best growth occurs in air enriched with 7.5 to 10 % CO<sub>2</sub>. Microscopically, Gram-negative, non-motile, highly pleomorphic, rod-shaped and non-sporulating bacterium. It spreads horizontally by direct and indirect contact through aerosol or drinking water. The outbreaks are inconsistent and can be influenced by a wide range of environmental factors. Symptoms characterized by mild respiratory signs, nasal discharge, swollen sinus, slightly increased mortality and poor performance specially in chicks.

**Keywords:** *Ornithobacterium rhinotracheale*, gram-negative, emerging, poultry pathogen, fibrinopurulent airsacculitis, respiratory infection

### Introduction

Poultry industry is always vulnerable for losses from the respiratory infections by variety of pathogens. Major bacterial pathogens involved in respiratory infections are *E. coli*, *Pasteurella* spp, *Haemophilus* spp, *Mycoplasma* spp. The emerging respiratory pathogen *Ornithobacterium rhinotracheale* is a slow growing, pleomorphic, Gram-negative bacterium of the rRNA superfamily V within the Cytophaga-Flavobacterium-Bacteroides phylum. *Ornithobacterium rhinotracheale* infection, also known as ornithobacteriosis, is a highly contagious disease of avian species, primarily turkeys and chickens, causing respiratory distress, decreased growth, and mortality. The bacterium has been also isolated from chukar partridges, quail, ducks, geese, ostriches and guinea fowl. These are influenced by housing, environmental stressors such as poor management, inadequate ventilation and high ammonia levels, high stocking density, poor litter conditions, poor hygiene, food borne mycotoxins, suboptimal nutrition and concomitant infectious diseases. The severity of the disease is worsen when birds have coexisting infections with other respiratory pathogens. It can be a primary or secondary etiological agent and this depends on strain virulence, adverse environmental factors, the immune status of the flock, and the presence of other infectious agents. ORT co-infection with avian influenza (H9N2), Infectious bronchitis virus and *E. coli* has been reported. However, many of the infections, caused by *O. rhinotracheale* are not recognized as such either because the causative agent cannot be isolated or because investigators are not aware of the possibility that *O. rhinotracheale* can cause infections other than the more well-known respiratory organisms (Thachil *et al.*, 2009 and Pan *et al.*, 2012) [38, 29].

### History

Empel 1998 reported that *O. rhinotracheale* was isolated from the respiratory tract of 5 weeks old turkeys with nasal discharge, facial edema and fibrinopurulent airsacculitis in 1981 and from rooks in 1983. In the year 1987 in Hungary, a bacterium similar to *Pasteurella* spp. isolated from 10 weeks old Pekin ducks with respiratory disease (Empel, 1998 and Chin *et al.*, 2008) [14, 10]. The first report related to the characterization of *O. rhinotracheale* was by Charlton *et al.* (1993) [7].

After that Vandamme *et al.* (1994) <sup>[41]</sup> described the phylogenetic position and various genotypic, chemotaxonomic and classical phenotypic characteristics of 21 strains were then described and assigned the name *O. rhinotracheale*. However, this bacterium appears to have been isolated before 1993 (Chin *et al.*, 2008) <sup>[10]</sup>. *O. rhinotracheale* could be isolated repeatedly from cases of airsacculitis and purulent pneumonia in meat turkeys and broiler chickens all over the world (Beek *et al.*, 1994 and Roepke *et al.*, 1998) <sup>[4, 32]</sup>. In India Murthy *et al.* (2008) <sup>[26]</sup>, studied isolation and identification of pathogenic bacteria, with special reference to *Ornithobacterium rhinotracheale* associated with respiratory diseases, isolation was performed from a total of 253 biomaterials collected from 125 layers of 35 commercially reared layer farms in Namakkal of Tamil Nadu state. In total 27 (51.9%), 18 (34.6%), 5 (9.6%) and 2 (3.8%), isolates were identified as *Escherichia coli*, *Ornithobacterium rhinotracheale*, *Pasteurella multocida* and *Haemophilus paragallinarum*, respectively. Nayak *et al.*, 2017 <sup>[28]</sup> conducted study on incidence of gentamicin resistant *O. rhinotracheale*, the isolation was done from 176 nasal swabs, 167 lung pieces and 176 tracheal swabs. The incidence of gentamicin resistance *O. rhinotracheale* were 0.56% from tracheal swabs and 0.59% from lung pieces and no isolates was found positive from nasal swabs.

### Epidemiology

The investigation of the epidemiology of *O. rhinotracheale* is hampered by the difficulties found in culturing *O. rhinotracheale* from infected organs, the brevity of the serological responses after an *O. rhinotracheale* infection and the complexity of the infections in which *O. rhinotracheale* can be involved. *Ornithobacterium rhinotracheale* infection has been reported mostly in broiler chickens and turkeys, and less frequently in other avian species such as pheasants, quails, gray partridges, chukar partridges, red-legged partridges, guinea fowls, ostriches, rooks, pigeons, ducks, geese, and gulls (Empel and Hafez, 1999; Chin *et al.*, 2008 and Moreno *et al.*, 2009) <sup>[12, 10, 25]</sup>. Experimentally *O. rhinotracheale* infection have been reproduced from broiler chickens and turkey.

### Transmission

*Ornithobacterium rhinotracheale* spreads horizontally by direct and indirect contact through aerosol or drinking water. Besides its isolation from ovaries, oviduct, hatching eggs, infertile eggs, dead embryos, and dead-in-shell chickens and turkeys (Empel, 1998) <sup>[14]</sup>, there are circumstantial evidences of vertical transmission in birds affected by ornithobacteriosis. According to experimental reproductions of the disease, the clinical signs are seen 24–48 hours post inoculation (Chin *et al.*, 2008) <sup>[10]</sup>. It has been proven that transmission of *O. rhinotracheale* is possible not only horizontally through aerosols but also vertically through the egg. Because eggs are sent all over the world, these findings make it more easy to understand the relative rapid, world-wide spread of *O. rhinotracheale* infections in the commercial poultry world during the last decade.

### Incubation Period

Experimental inoculation of 22 weeks old turkeys with *O. rhinotracheale* resulted in depression, coughing and decreased feed intake within 24 hours (Sprenger *et al.*, 1998) <sup>[32, 34]</sup>. In 48 hours, turkeys were coughing bloody mucus. Five

days post incubation, the coughing had decreased and the surviving turkeys were less depressed. In experimental infections in 5 weeks old chickens, *O. rhinotracheale* infected the respiratory organs within 2 days post inoculation and clinical signs were seen after 4 days (Empel *et al.*, 1999) <sup>[13]</sup>.

### Clinical Signs

The severity of clinical signs, the duration of the disease and the mortality caused by *O. rhinotracheale* outbreaks are extremely variable and can be influenced by a wide range of environmental factors (Chin *et al.*, 2008) <sup>[10]</sup>. Rahimi and Banani (2007) studied on a respiratory disease observed in the chickens of a large broiler farm in Kermanshah province, west of Iran in 2005. Relatively severe respiratory signs started with sneezing at 27 days of age. The disease lasted up to the end of fattening period and accompanied by increased mortality (13.6%). At postmortem examination, tracheitis, airsacculitis and pneumonia were obvious. Serologic examinations were negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. On virological examinations, virulent infectious bronchitis virus (IBV), avian influenza virus (AIV) and virulent Newcastle disease virus (NDV) could not be isolated. Histopathologic examinations showed no pathognomonic lesion typical for infectious laryngotracheitis. On bacteriologic examinations, *Ornithobacterium rhinotracheale* (ORT) was isolated from trachea, lungs and air sacs of the affected birds.

### Broiler chickens

Signs generally seen at 3 to 6 weeks of age. Mild respiratory signs (sneezing, nasal discharge) beginning around 3–4 weeks discharge, swollen sinus, slightly increased mortality, poor performance and condemnation rates at processing are typical of infection in young chickens (Chin *et al.*, 2008) <sup>[10]</sup>.

### In commercial layers and broiler breeders

*O. rhinotracheale* infection is most commonly seen at the age of 20 to 50 weeks especially during peak production. Mortality is slightly increased, feed intake decreased. Mild respiratory signs are presents. There may be decreased egg production, poor egg shell quality, misshapen and decreased egg size. Sudden death with or without respiratory signs has been reported in chickens with nervous signs (Chin and Charlton, 2008) <sup>[9]</sup>.

### Co-infection

The role of *O. rhinotracheale* as the primary pathogen is still uncertain. Generally, most of the losses are due to co infection with respiratory tract bacteria or viruses or any environmental stress. Roussan *et al.* (2011) <sup>[33]</sup> conducted a cross-sectional study from November 2008 to July 2010 in commercial broiler flocks in southern (n = 50) and northern (n = 50) areas of Jordan, to determine the flock-level prevalence of *Ornithobacterium rhinotracheale* (ORT) and *Mycoplasma synoviae* (MS) infections. Tracheal swabs were collected from commercial broilers with respiratory disease and tested by polymerase chain reaction. In total, 21% (95% CI: 18–45%) and 25% (95% CI: 20–51%) of commercial broiler flocks were positive for ORT and MS, respectively. In many reported cases of affected broiler chickens and turkeys, *O. rhinotracheale* infection played an associated role with other respiratory pathogens such as *Escherichia coli*, *Bordetella avium*, *Streptococcus zooepidemicus*, *Mycoplasma gallisepticum*, *Mycoplasma synoviae*, *Chlamydophila psittaci*,

Newcastle disease virus (Travers, 1996), Avian influenza virus, Avian metapneumo virus, Infectious bronchitis virus and *Cryptosporidium* spp. (Pan *et al.*, 2012) <sup>[29]</sup>.

## Classification and characteristics

### Morphology

*Ornithobacterium rhinotracheale* is a Gram-negative, non-motile, highly pleomorphic, rod-shaped, and non-sporulating bacterium of the rRNA superfamily V within the Cytophaga-Flavobacterium-Bacteroides phylum. Genus *Ornithobacterium* belongs to family *Flavobacteriaceae* (Vandamme *et al.*, 1994) <sup>[41]</sup>. When cultured on solid media, the bacterium appears as short, and plump rods measuring 0.2-0.9 µm in width and 0.6-5 µm in length and less frequently as long filamentous rods or club-shaped rods (Chin and Charlton, 2008) <sup>[9]</sup>. No structures such as pili, fimbriae and plasmids or properties such as specific toxic activities have been reported for the species (Empel and Hafez, 1999) <sup>[12]</sup>.

### Growth requirements

The use of 5-10% sheep blood agar plate is recommended for isolation and optimal growth of the causing agent. *Ornithobacterium rhinotracheale* does not grow on MacConkey agar, Simmons citrate medium (Chin *et al.*, 2008) <sup>[10]</sup>. The bacterium grows aerobically, micro aerobically and anaerobically, but the best growth occurs in air enriched with 7.5 to 10 % CO<sub>2</sub> at 37°C (Chin and Charlton, 2008) <sup>[9]</sup>. Tryptic soy agar, BHI broth, Nutrient agar and PPLO are the other alternative media for its growth.

### Colony morphology

On sheep blood agar 24 hours post-incubation, *O. rhinotracheale* develops pin-point colonies smaller than 0.1 mm in diameter. After 48 hours, the colonies are approximately 1 to 2 mm in diameter, gray to gray-white, circular and convex with an entire edge and some isolates from chickens have a reddish glow. Cultures of *O. rhinotracheale* have a distinct smell similar to that of butyric acid (Empel and Hafez, 1999; Chin and Charlton, 2008) <sup>[12, 9]</sup>. Because of the resistance to gentamicin and polymyxin B observed in 90% of *O. rhinotracheale* field isolates (Vandamme *et al.*, 1994) <sup>[41]</sup>, 5 µL/mL of each antibiotic is recommended to be added to blood agar media for selective isolation of this bacterium. The use of 10 µg of gentamicin per ml of blood agar medium has also been suggested to isolate *O. rhinotracheale* from contaminated samples (Chin and Charlton 2008) <sup>[9]</sup> also proposed the use of blood agar plates without antibiotic to prevent missing 10% of the antibiotic susceptible isolates. *Ornithobacterium rhinotracheale* was first identified as a non-hemolytic microorganism (Empel and Hafez, 1999) <sup>[12]</sup>. However, the presence of extensive and unusual β-hemolytic activity has been recently reported among field isolates after the 48 hours period following incubation at room temperature (Gornatti Churria *et al.*, 2011) <sup>[22]</sup>.

### Antigenic structures

Eighteen serotypes (A to R) have been differentiated because of the results observed in enzyme linked immunosorbent assays (ELISAs) and agar gel precipitation tests using boiled extract antigens and monovalent antisera (Chin *et al.*, 2008) <sup>[10]</sup>. Relationships are seen between the geographic origin of the *O. rhinotracheale* isolates and their serotype. From the

eighteen serotypes, serotype A is predominant among the chicken-isolates (96%) and the most frequent (54%) among the turkey isolates, which are more heterogeneously divided. Up to now there is no explanation for these differences in distribution but it has been shown that serotype A and C strains from chickens and serotype B, D and E strains from turkeys have a similar virulence for both chickens and turkeys. So, there is no indication of any host specificity of the serotypes. A possible explanation may be found in the different breeding practices in the chicken and turkey industries.

## Pathology

### Gross pathology

The most common macroscopic findings in broiler chickens are unilateral pneumonia, pleuritis and abdominal airsacculitis with foamy, white yogurt-like exudate (Chin *et al.*, 2008) <sup>[10]</sup>. Other respiratory lesions, such as catarrhal tracheitis and bilateral exudative pneumonia (Gornatti Churria *et al.*, 2012) <sup>[21]</sup>, has also been found in chickens affected by ornithobacteriosis. Condemnation rates of 60% in broilers at slaughter due to airsacculitis in 84% of the birds examined and due to pericarditis and pneumonia in a few birds have been reported to be associated with *O. rhinotracheale* infection (Veen *et al.*, 2000) <sup>[43]</sup>. In addition, more than one third of the respiratory lesions in broiler chickens at slaughter age have been reported to be caused by *O. rhinotracheale* infection, indicating the wide distribution of this bacterium in the broiler industry of Europe (Veen *et al.*, 2005) <sup>[42]</sup>. Uncommon lesions such as subcutaneous edema of the skull with severe osteitis and osteomyelitis together with encephalitis without the involvement of the respiratory tract have been described in 28 days old broiler chickens (Goovaerts *et al.*, 1998) <sup>[20]</sup>. In turkeys, unilateral and bilateral consolidations of lungs due to pneumonic or bronchopneumonic lesions with fibrinous exudate of the pleura have been found (Tabatabai *et al.*, 2010). Mild or severe tracheitis, fibrinosuppurative thoracic and/or abdominal airsacculitis, pericarditis and peritonitis have also been described in turkeys. Swelling of the liver and spleen, degeneration of the heart muscle and infection of vertebrae and joints has been observed in some cases of *O. rhinotracheale* infection in turkeys (Chin *et al.*, 2008) <sup>[10]</sup>.

### Histopathology

As it is primarily a respiratory infection histological lesions are observed in lungs, pleura and air sacs. Lung lesions caused by *O. rhinotracheale* are similar to those produced by *Pasteurella multocida* (Fletcher *et al.*, 2008) <sup>[17]</sup>. They are characterized by large and coalescing areas of necrosis centered in the lumen of parabronchi, filled with degenerated and necrotic heterophilic infiltrate or fibrinous exudate. Collections of fibrin with macrophages and heterophils occupying the interstitial tissues and air passages are also found. According to Fletcher (2010) <sup>[17]</sup>, fibrinoheterophilic diffuse pneumonia in turkeys is suspected to be caused by *O. rhinotracheale* infection. Pleura and air sacs can be thickened with interstitial fibrin, diffuse heterophilic infiltrate, necrotic foci and fibrosis (Chin *et al.*, 2008) <sup>[10]</sup>.

## Diagnosis

### Sample collection

From the bird showing respiratory symptoms serum sample and nasal swab must be collected from live birds while at the



time of postmortem Nasal swab, tracheal pieces, lung pieces and air sac in bacterial transport media with or without gentamicin is generally preferred. Microscopically, Gram-negative, non-motile, highly pleomorphic, rod-shaped and non-sporulating bacterium. When cultured on solid media, the bacterium appears as short and plump rods measuring 0.2-0.9 µm in width and 0.6-5 µm in length and less frequently as long filamentous rods or club-shaped rods. Isolation and Identification of the organism is performed on blood agar. No growth on Mac Conkey agar. Biochemical they are oxidase, catalase, Vogues-Proskauer, urease, arginine dehydrolase and carbohydrates like glucose, mannose, lactose, maltose and sucrose are positive. Lysine decarboxylase, ornithine decarboxylase, phenylalanine deaminase, indole production, H<sub>2</sub>S, sorbitol and dulcitol are negative.

Back *et al.*, 1998<sup>[3, 32, 34]</sup> performed the serum plate agglutination test (SPAT). Serum samples from chickens and turkeys experimentally infected were tested and antibodies against *O. rhinotracheale* were detected by SPAT in both avian species, whereas the serum samples from not exposed birds remained negative. Vega *et al.*, (2008)<sup>[44]</sup> tested the hemagglutinating activity of serotypes A to I of *O. rhinotracheale* reference strains by using red blood cells from 15 different species, including avian, mammal, fish and human erythrocytes and concluded that rabbit erythrocytes were suitable to test *O. rhinotracheale*. Chernyshev *et al.* (2011)<sup>[8]</sup> reported the hemagglutinating activity of 19 Russian isolates of *O. rhinotracheale* strains with chicken and sheep erythrocytes.

ELISAs have been developed using different serotypes and extracted antigens of *O. rhinotracheale*. Field surveys using these ELISAs or commercial ELISA kits (available in Europe) have been useful for monitoring flocks and the diagnosis of *O. rhinotracheale* infections. Erganiş *et al.* (2002)<sup>[16]</sup> developed a dot immunobinding assay (DIA) and compared it with agglutination assays by testing serum samples from turkeys with respiratory signs and the authors concluded that the sensitivity of the DIA appeared to be lower than the agglutination assays studied. In Iran, Allymehr (2006)<sup>[1]</sup> carried out serological surveys and described 44.2% of positive serum samples from chickens against *O. rhinotracheale* in west Azerbaijan province. Ghanbarpour and Salehi (2009)<sup>[19]</sup> found lower percentages of positivity among serum samples of broiler chickens (31.9%) in south-eastern Iran. In Argentina, Uriarte *et al.* (2010)<sup>[40]</sup> analyzed a total of 739 serum samples from broiler chicken and broiler breeder flocks located in Buenos Aires and Entre Ríos provinces and found 345 positive serum samples. The statistical analysis demonstrated higher possibilities of seropositivity among breeders. Nayak *et al.* (2017)<sup>[28]</sup> screened 166 serum samples of poultry suffering from respiratory infection in Jabalpur by Idexx ORT antibody test kit. An overall 8.43% of poultry serum samples were detected positive for ORT antibodies.

### Molecular diagnosis

PCR assays are more sensitive, rapid and specific for identification purposes. The primer targeting 16S rRNA gene amplify a 784 bp fragment of *O. rhinotracheale*, but not of other closely-related bacteria with which *O. rhinotracheale* could be confused (Empel and Hafez, 1999)<sup>[12]</sup>. Besides the use for identification purposes, PCR assays have also been optimized for the demonstration of *O. rhinotracheale* in, eggs, faeces and dust or tissue samples and can therefore be useful in epidemiological studies. Moreno *et al.* (2009)<sup>[25]</sup>,

genotyped *O. rhinotracheale* strains obtained from Spanish red-legged partridges with neurological signs, otitis and cranial osteomyelitis, following adaptations of previously reported pulse field gel electrophoresis (PFGE) assays. The PFGE patterns were examined and the genetic relationship among isolates was evaluated. An epidemiologically unrelated French strain of *O. rhinotracheale* from a pheasant was added. The study revealed indistinguishable macrorestriction patterns of the *O. rhinotracheale* Spanish strains with the enzymes *Apal* and *SmaI* and no relation was noted with the restriction pattern of the French isolate. Thieme *et al.* (2016)<sup>[39]</sup> conducted a study to establish a multi locus sequence typing (MLST) scheme for ORT that could easily be used by other laboratories and allows for worldwide comparison of sequence data. For this purpose, 87 ORT strains from different poultry hosts, geographical origins, years of isolation and serotypes were included in the analysis to identify correlations. Fourteen different sequence types (ST) were found. The most common ST1 was identified in 40 ORT strains from turkeys and chickens on 4 continents and in 3 different European countries. Together with ST9, both STs represented over three quarters (77%) of ORT strains used in the MLST analysis and included strains of frequently cross-reacting ORT serotypes A, E and I. Nine STs were only represented by one ORT strain and might indicate possible avian host, disease or serotype-specific relationships. In contrast, discrepancies between serotype and phylogenetic relatedness were clearly demonstrated by ORT strains that belonged to identical serotypes but differed in their ST. The overall identified low genetic diversity among strains isolated from turkeys and chickens independent of host and geographical origins suggests that ORT has only recently been introduced into domestic poultry and dispersed worldwide.

### Control and prevention

The best strategy for the control or prevention of *O. rhinotracheale* infection is probably vaccination, because most worldwide *O. rhinotracheale* isolates have acquired resistance against the antibiotics regularly used in poultry. However, in spite of the availability of autogenous vaccines, economic losses related to *O. rhinotracheale* infections in the poultry industry are estimated in hundreds of millions of dollars annually in the United States. Bacterins, live vaccines, and subunit recombinant vaccines have been developed and reported, with variable results for the control of experimental and natural infections associated with *O. rhinotracheale* (Empel and Bosch, 1998 and Murthy *et al.*, 2007)<sup>[15, 27]</sup>. Injectable and inactivated vaccines were found to be impractical for commercial broiler flocks, whereas, autogenous bacterins were successfully used for the control of *O. rhinotracheale* outbreaks in turkeys in Israel. Some authors obtained high maternal antibodies titers. Murthy *et al.* (2007)<sup>[27]</sup>, conducted studied on the effect of vaccination of chickens with different inactivated vaccines against experimental *Ornithobacterium rhinotracheale* challenge was investigated. Eight different vaccines, with different inactivating substances (Formalin and thiomersal) and with or without adjuvant (mineral oil, alum and aluminum hydroxide gel), were produced. The bacterin in mineral oil adjuvant induced the highest serologic response and a significant decrease of lesions such as air sacculitis and pneumonia in vaccinated birds compared with the unvaccinated challenge control birds. The study showed that vaccination of layer chicken at the

eighth week followed by a booster dose at the 12<sup>th</sup> week of age can effectively protect against *O. rhinotracheale* infections. Commercial vaccines are available in the market although not in India.

### Treatment

*Ornithobacterium rhinotracheale* infections have become more common in the poultry industry and the treatment with antibiotics has become less effective due to an increased pathogenicity, an increased burden of infection, and/or an increased level of acquired antibiotic resistance. The treatment of *O. rhinotracheale* infections with antibiotics is more worsened because of the variable susceptibility of strains. *O. rhinotracheale* can acquire reduced susceptibility or resistance against antibiotics such as amoxicillin, ampicillin, doxycycline, enrofloxacin, flumequine, gentamicin, lincomycin, trimethoprim, sulfonamide, tetracycline and tylosin. Susceptibility can be dependent on the regime used by the poultry industry in various geographical locations. For example, in countries where eggs are regularly dipped in an antibiotic such as enrofloxacin almost all isolates will show resistance to that antibiotic (Malik *et al.*, 2003) [24]. In India, *O. rhinotracheale* field isolates were resistant to amikacin, cloxacillin, trimethoprim sulfa, gentamicin, metronidazole, triple sulfa and sensitive to amoxicillin, ampicillin, chloramphenicol, ciprofloxacin, doxycycline, enrofloxacin, erythromycin, oxytetracycline and penicillin G. Susceptibility against cephalixin, norfloxacin, pefloxacin, streptomycin and furazolidone was variable (Murthy *et al.*, 2008) [26].

### Conclusions

*Ornithobacterium rhinotracheale* is an emerging bacteria of poultry from respiratory tract infection, worldwide. It is a slow growing organism with specific requirement and difficult to grow in laboratory. Serologically serotype specificity is a problem. More sensitive, specific and rapid molecular test is used for confirmatory diagnosis. ORT infection can induce higher economic losses and mortality if co infection with H9N2 avian influenza virus or other respiratory infection is present. More research should be conducted on these bacteria.

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