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Ornithobacterium Rhinotracheale (ORT) Infection in Poultry

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Abstract: *Ornithobacterium Rhinotracheale* (*O. Rhinotracheale*, ORT) infections can cause acute highly contagious diseases in poultry, which can be associated with high economic losses due to an increase in mortality rates, condemnation rates, drop in egg production or due to a decrease of the performance results. Up to now, ORT has not been found to be of public health significance. *Ornithobacterium Rhinotracheale* (ORT) is a pathogen best known for causing respiratory tract infections, such as airsacculitis and pneumonia, in birds all over the world. ORT can be a primary or secondary etiological agent depending on strain virulence, adverse environmental factors, the immune state of the flock, and the presence of other infectious agents). The pathogen may cause systemic diseases such as hepatitis, joint lesions, and cerebrovascular pathology or could lead to economic losses due to growth retardation and the rejection of carcasses for consumption. *Ornithobacterium Rhinotracheale* (ORT) has been firstly isolated from broiler chickens**).** Recently ORT has been isolated from ducks, goose, ostrich, pheasants, pigeons, quails, rook and turkey. In many countries of the world, ORT has been incriminated as a possible additional causative agent in respiratory disease complex in poultry. The organism causes substantial financial losses due to high rates of condemnation up to 50% in slaughtered affected flocks *Ornithobacterium Rhinotracheale* was defined as Gram-negative, highly pleomorphic, non-motile, non sporulating bacteria Ornithobacterium Rhinotracheale (ORT) Infection in Poultry.
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Introduction

Respiratory disease conditions are continuing to cause heavy economic losses in the poultry industry by increased mortality rates, increased medication costs, increased condemnation rates at slaughter, drops in egg production, reduction of egg shell quality, and decreased hatchability. The severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by many factors such as a virulence and pathogen city of the infectious agent as well as by many environmental factors. Many infectious agents can cause respiratory diseases in poultry such as fungi **(Akan et al., 2002; França et al., 2012),** viruses **(Alexander, 2000; Boroomand et al., 2012; Chansiripornchai et al., 2007; Higgins, 1971; Ignjatovic et al., 2002; Ip et al., 2012; Lee et al., 2012; McFerran and Adair, 1977; Swayne et al., 2001)** and bacteria **(Blackall, 1999; da Rocha et al., 2002; Noormohammadi et al., 2002; Pruimboom et al., 1996).**

Since December 1991 a respiratory disease with different clinical causes has been observed in poultry flocks in different countries **(Charlton et al., 1993; Du Preez, 1992; Hafez et al.,1993; van Beek et al., 1994)**. Bacteriological examinations have resulted in isolation of slowly growing, pleomorphic gram-negative rod (PGNR). Initially, the bacterium was designated as Pasteurella-like, Kingella-like, Taxon 28, or pleomorphic gram-negative rod before the name Ornithobacteriumrhinotracheale gen. nov.sp. nov.in the rRNA-Superfamily V was suggested **(Hafez and Vandamme, 2011; Vandamme et al., 1994).** Currently infections with *Ornithobacteriumrhinotracheale* (*O.rhinotracheale,* ORT) occur worldwide and O. rhinotracheale is incriminated as a possible causative agent in respiratory disease either alone (mono-causal) or in synergy with different other micro-organisms (multicausal). Moreover non-infectious factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, and high ammonia level are concurrent causes that increase the severity of the disease **(Bock et al., 1997; Chin et al.,2003., Vandamme et al., 1994).**

O. rhinotracheale has been isolated from chickens, chukar partridges, ducks, geese, guinea fowl,

gulls, ostriches, partridges, pheasants, pigeons, quail, rooks, and turkeys **(van Empel and Hafez, 1999)** Currently 18 different serotypes designated A to R have been identified **(Chin et al., 2008; vanEmpel and Hafez, 1999**). Serotyping can be done with reference antisera using agar gelprecipitation test (AGP) or enzyme linked immunosorbent assay (ELISA) **(Hafez and Sting,1999; van Empel et al., 1996; Vandamme et al., 1994).** However, AGP is the method of choice for serotyping. Since clinical signs and post-mortem lesions of *O. rhinotracheale* infections are not sufficiently specific to allow diagnosis, laboratory methods are needed for definite diagnosis. While detection of nucleic acids using polymerase chain reaction (PCR) is reliable and fast **(Hassanzadeh et al., 2010)**.

2.3 Taxonomy of the genus Ornithobacterium

O. rhinotracheale belongs to the phylum *Bacteroidetes,* class *Flavobacteria,* order *Flavobacteriales* family Flavobacteriaceae, and the Genus *Ornithobacterium* **(Vandamme etal., 1994)**. Initially *Riemerellacolumbina, Ornithobacteriumrhinotracheale* and *Coenoniaanatina* were recognized in the of long-term studies on the etiology of respiratory tractinfections in birds as phenotypically unusual isolates **(Segers et al., 1993).** Moreover, the reclassification of the organism known as *Pasteurellaanatipestifer* or *Moraxella anatipestifer* as *Riemerellaanatipestifer* (Segers et al., 1993) triggered a series of taxonomic studies leading to the stepwise characterization and description of *O. rhinotracheale* **(Vandamme et al., 1994),** as well as *C. anatine* and *R. columbina* **(van Empel and Hafez, 1999).** The formal description of *O. rhinotracheale*led to a lot of studies and researches on this bacterium because of its acknowledgement as an economically important pathogen in turkey and chicken husbandry. Nowadays *Riemerella, Ornithobacterium* and *Coenonia* are thought to belong to the same major phylogenetic linage, now known as the family Flavobacteriaceae **(Bernardet and Bowman, 2006; Bernardet et al., 2002; Bernardet et al., 1996).**

Etiology and Colony Morphology

O. rhinotracheale is a Gram-negative, non-motile, pleomorphic, rod-shaped, non-sporulatingbacterium. Its colonies are characterized by a circular, grey to grey- white color, sometimes with a reddish glow **(van Empel and Hafez, 1999)**. They are convex with an entire edge **(Devriese et al., 2001; Erganis et al., 2002; Murthy et al., 2008; Roepke et al., 1998; van Empel et al., 1997).** Usually it is considered to be not haemolytic, but recently β-hemolyticactivity has been revealed in field isolates in North America **(Tabatabai et al., 2010)** and Latin America **(Churria et al., 2011).**

No special structures or properties such as pili, fimbriae, or plasmids could be detected **(Leroy-Setrin et al., 1998; van Empel and Hafez, 1999).** For growth of the organism incubation on 5-10% sheep blood agar for at least 48 hours under micro-aerophilicconditions (5-10% CO2) at 37°C is required **(Chin et al., 2008; Erganis et al., 2002; van**

Empel et al., 1997; van Empel and Hafez, 1999). No growth occurs on MacConkey agar, Endo agar, Gassner's agar, Drigalski agar, and Simmon's Citrate media **(Chin et al., 2008).**

Moreover, at the first isolation, most *O. rhinotracheale* cultures show a big difference in size of colonies from 1 to 3 mm after 48 hours of incubation; after subcultivation for 2-3 times, the colony size becomes more uniform **(van Empel and Hafez, 1999).** After several subcultivations, some strains may be adapted to growth under aerobic conditions, although growth is always significantly better under microaerobic conditions **(van Empel and Hafez, 1999).** In liquid media, *O. rhinotracheale* are very variable in length (0.6 to 5 μm) and often form clusters, which can hold up to several thousands of organisms, but which can easily b edisrupted **(van Empel and Hafez, 1999).** Not all strains of *O. rhinotracheale* will grow equally in liquid media and a rich medium such as Todd Hewitt broth or Brain Heart Infusion broth supplemented with serum, is required. *O. rhinotracheale* can be suppressed by overgrowth by less fastidious bacteria in contaminated samples including *E. coli* **(van Empel and Hafez, 1999).** The G+C content of the genome of *O. rhinotracheale* strains is between 37 and 39 % **(Vandamme et al., 1994)**.

Transmission

The infection spreads horizontally by direct and indirect contact through aerosols and/ or drinking water (van Empel and Hafez, 1999). *O. rhinotracheale* infection appears to have become endemic and can affected every new restocking even in previously cleaned and disinfected houses, especially in areas with intensive poultry production as well as in multipleage farms **(Hafez, 1996; van Empel and Hafez, 1999)**. The survival of *O. rhinotracheale* at lower temperatures may be associated with the higher incidence of its infection in poultry during winter months **(Lopes et al., 2002b). Heeder et al. (2001)** examined the seroprevalence of *O. rhinotracheale* within a commercial ***layer*** population. Of the pullet flocks examined, 43% and 52% were positive by SPAT and ELISA, Respectively The prevalence of *O. rhinotracheale* antibody is high in the commercial layer population, suggesting that this respiratory pathogen can easily spread through multiple-age layer farms from older flocks to newly housed pullet flocks. Surveillance of exposure to *O. rhinotracheale*

infection in the field has shown that prevalence of the infection is higher during winter months. In addition, **Amonsin et al. (1997)** raised the hypothesis that *O. rhinotracheale* might be introduced to domesticated poultry flocks from wild bird populations. The results obtained by **Gutzer et al. (2011)** support the above mentioned hypothesis. Vertical transmission is suspected, since some reports on the isolation of *O. rhinotracheale* at very low incidence from reproductive organs, hatching eggs, infertile eggs, and deadembryos were published **(Back et al., 1998; El-Gohary, 1998; Tanyi et al., 1995)**

Experimentally *O. rhinotracheale* infection was established by three routes of inoculation: intravenous, intratracheal and intranasal. Tissue samples from several organs (airsacs, brain, intestine, kidney, liver, lung, ovary, oviduct, spleen and trachea) were collected on days 3, 7 and 14, and were examined for the presence of *O. rhinotracheale* by cultural isolation and *in situ* detection by immunofluorescent antibody assay (IFA). *O. rhinotracheale* was recovered from ovaries and oviducts on days 3 and 7 after inoculation and again from the oviduct on day 14 by cultural isolation. By IFA *in situ* detection, *O. rhinotracheale* was recovered from all ovaries and oviducts on day 3 and day 7 after inoculation. This may be due to the ability of the turkey's immune response to clear the infection from most of the tissues by day 14. The isolation of the organism from ovaries and oviducts in this experiment supports the possibility of the vertical transmission. If this transmission does occur, one would assume that it might happen in the acute stage of the infection **(Back et al., 1998)**

Clinical signs

Clinical signs in ***broilers*** generally appear between the 3rd and 4th week of age with a mortality rate of 2-10 %. The clinical signs identified are depression, decrease in food intake, reduced weight gains, transient nasal discharge, and sneezing, followed by facial edema **(Cauwerts et al., 2002; Du Preez, 1992; Odor et al., 1997; van Beek et al., 1994).** Sudden deaths of young chickens due to *O. rhinotracheale* infection of the brains and the skull with weakened skull-bones can also found. Moreover, subcutaneous edema over the cranium with a severe bacterial osteitis without respiratory tract infection was also described **(van Empel et al., 1999).** Furthermore, especially in older turkeys and chickens *O. rhinotracheale* was shown to spread to other body sites, causing arthritis, osteitis, andosteomyelitis that may develop with the formation of a purulent, exudates found in the joints of lame birds **(Chin et al., 2008).** In ***broiler breeders*** the disease primarily affects the birds at the peak of production or shortly before entering production, mostly between 24th and 52nd week of age. Before the main symptoms are detected, a slight increase in mortality and decrease in feed intake maybe observed. The first signs are mild respiratory distress. The symptoms are generally accompanied by a drop in egg production, decrease in egg size, and poor egg shell quality. Fertility and hatchability are unaffected in many cases (Hafez, 1996). Clinical signs in layer flocks are similar to that found in breeder flocks **(Sprenger et al., 2000a).**

In ***turkeys*** outbreaks mostly have been observed in male birds over 14 weeks of age, however in many cases young pouts up to the 2nd week of age could also found to be affected **(Hafez et al., 1993; van Beek et al., 1994).** The mortality ranges between 1 and10 % during the acute phase (8 days). Initial symptoms are coughing, sneezing, and nasal discharge followed in some cases by severe respiratory distress, dyspnoea, prostration, sinusitis, and arthritis. The symptoms are accompanied by a reduction in feed consumption and water intake **(Chin et al., 2008; Hafez, 2002)**. **Szalay et al. (2002)** observed nervous manifestations in one flock of 5-week-old pouts and in three 16- to 20-week-old turkey flocks. The symptom was accompanied by increased mortality and was found to be associated with fibrinopurulent inflammation of the cranial bones and meningitis. The bacterium could be isolated from these lesions. In turkey breeder flocks clinical signs are accompanied mostly by slightly increased mortality, drops in egg production (2-5%), and increases in the number of unsettable hatching eggs **(Chin et al., 2003; Chin et al., 2008; de Rosa et al., 1996; Hafez et al., 1993; vanBeek et al., 1994).**

Gross lesions

The lesions in broilers include pneumonic lungs, pleuritis, and airsacculitis. In the air sac accumulation of creamy, "yoghurt-like" exudates could be observed (Charlton et al., 1993; vanEmpel and Hafez, 1999 In turkeys lesions generally were localized in the lungs and include edema and uni-orbilateral consolidation of the lungs with fibropurulent exudates. Pericarditis, airsacculitis, peritonitis, and enteritis could be detected. In some cases, swelling of the liver and spleen plus degeneration of heart muscles have been seen **(Hafez et al., 1993; Hinz et al., 1994; Roepke et al., 1998; van Beek et al., 1994; van Empel et al., 1996; van Empel and Hafez,1999).**

Synergism with other avian pathogens

In turkeys, infection was aggravated by the prior administration of TRT virus or ND virus isolates **(Marien et al., 2005; van Empel et al., 1996)**, *Bordetellaavium* **(Droual and Chin,1997),** *Mycoplasma gallispeticum* and /or *E. coli* **(de Rosa et al., 1996; Marien et al., 2007)**.

In order to clarify the role of other avian pathogens in the course of *O. rhinotracheale* infection, further serological surveillance for antibodies against

O. rhinotracheale, TRT, *Chlamydophilapsittaci* were carried in turkey flocks. Results showed an interaction between *O. rhinotracheale* and other pathogens **(Hafez, 1998, 2002; van Loock et al., 2005)**. On the other hand, a concomitant infection with *Mycoplasma synoviae* did not show an obvious effect on mortality rates nor on the antibody response against *O. rhinotracheale* in turkeys **(Zorman-Rojs et al., 2000).**

Marien (2007) used an experimental groups of 15 susceptible 3-week-old turkeys. These animals were inoculated oculonasally with TRT subtype A, *E. coli* O2:K1 and *O. rhinotracheale*, with a 3 days interval between viral and bacterial inoculation and approximately 8 hours between the two bacterial inoculations. Macroscopic findings were comparable between the experimental groups. The lesions of all groups were serous to seromucous exudates in the turbinates and sinuses, as well as hyperaemia of the turbinate sand the trachea. As mentioned before, some bacteria including.

Bordetellaavium and *E.coli* have also been suspected to induce the establishment of *O. rhinotracheale* infections, but nevertheless respiratory viral infections are more important, because they lead to more severe respiratory lesions and higher mortality rates than bacterial infection **(Marien et al., 2007; Marien et al., 2005; Marien et al., 2006).** In broilers, infection was aggravated by the prior administration of ND virus, and to a lesser extent by prior administration of Infectious Bronchitis (IB) virus or a chicken-TRT virus isolate, in particular with regard to development of airsacculitis and pneumonia. Without the virus no airsacculitis or pneumonia was seen in these studies **(van Empel et al., 1996; Odor et al., 1997)**. Also in field cases viruses had influence on *O. rhinotracheale* infections **(Travers, 1996).**

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