Outbreak of *Ornithobacterium rhinotracheale* (ORT) infection in chickens in Pakistan

Ausbrüche von *Ornithobacterium rhinotracheale* (ORT) Infektionen bei Hühnern in Pakistan

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Introduction

*Ornithobacterium rhinotracheale* (ORT) was isolated for the first time from 5-week-old turkeys with nasal discharge, facial oedema and fibrinopurulent airsacculitis in Germany in 1981 (Chin et al., 2003). ORT initially known as Pasteurella-like organism was subsequently isolated in 1986 in England from trachea of turkey breeding flocks with mild coughing, general depression, decreased egg production, fibrinous airsacculitis (Hinz et al., 1994) and also from the birds in flocks with high mortality and unilateral pneumonia suggestive of fowl cholera. From chicken breeders, it was initially isolated during 1993 in South Africa from the birds showing mild respiratory signs and mild depression (Van Reek et al., 1994). In contrast to fowl cholera, the disease did not spread from shed to shed, neither mortality nor morbidity was high and birds responded to tetracyclines (Chin and Droul, 1998). Over succeeding years, isolations have been reported from numerous geographical regions including Belgium (Devriese et al., 2001), Brazil (Canal et al., 2005), Canada (Joubert et al., 1999), France (Leroy-Setrin et al., 1998), Germany (Hafez and Sting, 1996), Israel (Bock et al., 1997), Japan (Sakai et al., 2000), Mexico (Soriano Vargas et al., 2002), the Netherlands (Van Empe et al., 1997; Van Veen et al., 2000), South Africa (Travers, 1996), the UK (Van Empe et al., 1997) and USA (Odor et al., 1997). ORT might have probably been present in poultry flocks for many years but due to difficult isolation and identification it might have been missed as a secondary, opportunistic pathogen.

The genus *Ornithobacterium* is a member of the Flavobacteriaceae within the Cytophaga-Flavobacterium-Bacteriodes phylum and represents a rather distinct line of descent within this family (Vandamme et al., 1994). *Ornithobacterium rhinotracheale* is a pleomorphic, Gram negative, non-sporulating bacillus. Using boiled extract antigens (BEAs) and monovalent antisera in the agar gel precipitation (AGP) and enzyme linked immunosorbent assay (ELISA) tests, 18 serotypes (A through R) of *O. rhinotracheale* have been determined. Serotype A was the most prevalent serotype among chicken isolates (94%) and turkey (57%) isolates (Van Empe et al., 1996). However, cross protective immunity against different *O. rhinotracheale* serotypes can be induced by live vaccination (Schuiffel et al., 2005). ORT may grow aerobically, microaerobically or anaerobically, best growth being on blood agar enriched with 7.5–10% CO2. The organism can grow within wide limits of temperature varying from 30 to 42°C. It can readily grow on 5% sheep blood agar and chocolate agar. After 24 hours, small pinpoint colonies less than 1 mm are observed and by 48 hours colonies are approximately 1–2 mm in diameter, circular, opaque to grey and convex with entire edges. It does not grow on MacConkey’s agar and growth is poor on Triple Sugar Iron (TSI) slants. The organisms are catalase negative and oxidase positive. Glucose, galactose, lactose, maltose and fructose are fermented by most of the isolates. Although ORT infection usually occurs in 3–4 weeks old chicken broilers, the breeders are also affected within 24 to 52 weeks of age (Hafez, 1996). There is high mortality, low feed intake, mild respiratory signs, decreased egg production, poor egg shell quality and decreased egg size (Chin and Droul, 1998). However, it is usually said that ORT occurs as an opportunistic pathogen in flocks which have been immunosuppressed by exposure to adenoviral hemorrhagic enteritis and infectious bursal disease or Marek’s disease. Primary virus infections like infectious bronchitis, Newcastle disease and pneumoviruses (TRT) predispose the birds to ORT (Travers, 1996). The birds might be simultaneously affected with other bacteria particularly *Mycoplasma gallisepticum* and *Escherichia coli* (Sakai et al., 2000).

In February 1999, a heavy mortality was recorded in broiler farms around Faisalabad (Central Punjab, Pakistan). There was respiratory involvement and mortality reached even up to 50 percent in some of the cases. A number of antibiotics were tried but the response was quite poor. Within a few weeks, similar problem was also seen around Gujranwala, Lahore, Rawalpindi, Karachi and most of the other broiler raising areas of the country. This created havoc in the industry and most of the poultry farms ceased operating. The present project was designed to elucidate the etiology of the respiratory disease syndrome, various epidemiological factors and response to antimicrobials.

Materials and methods

A new disease malady mainly characterized by respiratory distress and high mortality was observed in poultry farms around Faisalabad. Various reported farms (n=25) with similar problem were visited and farm data pertaining to drinking system, type of feed, system of rearing, previous disease

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problems, treatment and its response, vaccination and biosecurity measures adopted was obtained. The birds were autopsied and various organs including liver, lungs, trachea, spleen and bird’s heads were procured for isolation studies. Samples were transported to the laboratory on ice and processed immediately for bacterial isolation studies.

For isolation and identification of various bacterial entities, different tissues were cultured on Nutrient broth (Difco), Nutrient agar (Difco), Blood agar (Difco), MacConkey’s agar (Difco), Typtose Soya agar, PPLO broth (Oxide) and PPLO agar (Oxide). After 24 to 48 hours of incubation at 37°C, various colonies were picked and detailed biochemical investigations were undertaken (Cruickshank, 1975; Quin et al., 1994). PPLO broth and agar were observed up to a week. Various isolates were subjected to oxidase and β-galactosidase tests for confirmation.

The susceptibility of the isolates to eleven different antibacterials was determined by the Kirby-Bauer disc diffusion method described by Bauer et al. (1966). The commercially available antibiotic discs included lincomycin, neomycin, kanamycin, norfloxacin, chloramphenicol, tetracycline, doxycycline, furazolidone, flumequine, enrofloxacin and oxalic acid. The studies were continued in the subsequent years (2000–2006) and the information present in a tabulated form and in a descriptive way.

Table 1. Biochemical characterization of Ornithobacterium rhinotracheale (ORT) infection in chickens in Pakistan

<table>
<thead>
<tr>
<th>Tests</th>
<th>Results</th>
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<tbody>
<tr>
<td>Indole</td>
<td>-</td>
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<tr>
<td>Voges-Proskauer test</td>
<td>+</td>
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<tr>
<td>Methyl red test</td>
<td>V</td>
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<tr>
<td>Urease</td>
<td>+</td>
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<tr>
<td>Oxidase</td>
<td>+</td>
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<tr>
<td>Nitrate reduction test</td>
<td>-</td>
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<tr>
<td>β-galactosidase</td>
<td>+</td>
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<tr>
<td>Catalase test</td>
<td>-</td>
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<tr>
<td>Growth on nutrient agar</td>
<td>No Growth</td>
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<tr>
<td>Growth on MacConkey agar</td>
<td>No Growth</td>
</tr>
<tr>
<td>TSI</td>
<td>No Growth</td>
</tr>
<tr>
<td>Trypticase soya agar</td>
<td>Poor Growth</td>
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<tr>
<td>Blood agar</td>
<td>Growth</td>
</tr>
<tr>
<td>Glucose</td>
<td>-</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
</tr>
<tr>
<td>Zylase</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
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<tr>
<td>Arabompsse</td>
<td>-</td>
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<tr>
<td>Inositol</td>
<td>-</td>
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<tr>
<td>Lysine Decarboxylase</td>
<td>-</td>
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<tr>
<td>+</td>
<td>Positive reaction</td>
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<tr>
<td>-</td>
<td>Negative reaction</td>
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<tr>
<td>V</td>
<td>Variable reaction</td>
</tr>
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</table>

Results

Respiratory disease syndrome was recorded in February 1999 around Khurrianwala, a poultry-raising town, 22 kilometers east of Faisalabad, Punjab, Pakistan. Gradually its spread to various other poultry raising areas around Faisalabad was reported. Later on, a similar problem was seen around Gujranwala, Lahore, Rawalpindi, Karachi and other parts of the country.

In most of the affected flocks, purulent nasal discharge, swelling of head, weakness and respiratory distress were noted. On postmortem, congestion and consolidation of lungs (uni-lateral or bi-lateral), congestion of liver and enlargement of spleen were the consistent lesions observed. Variable mortality was recorded in different affected flocks, the mortality started suddenly and it peaked within 4–5 days and then gradually declined. Mortality ranged from 15 to 50% on various farms.

During 1999, the problem was recorded in 23 broiler flocks and two layer flocks. The number of birds in these affected broiler flocks (23) ranged from 1200 to 10,000 between 16 to 38 days of age. On the affected layer flocks, the number of birds was 1250 and 2000 with respective age of 28 and 70 days.

Different commercial feeds such as Hi-Tech, Punjab, National, Blue Star, Ravi, Lahore and Ani were used on these farms. The chicks were procured from a number of sources, from various hatcheries having different breeds. It was observed that neither the source of feed nor the breed/breeding company had any effect on the prevalence and severity of the disease, as the problem was recorded in birds of all breeds and being maintained on different feeds. Management on 60% (15) of the farms was poor, it was satisfactory on 24% (6) and good on 16% (4) of the farms. Relatively more cases were recorded in birds maintained under poor management conditions. Different antibacterials like neomycin, doxycycline, norfloxacin, trimethoprim and enrofloxacin had been tried but with poor response. The birds had been vaccinated against Newcastle disease and infections bursal disease during 1st and 2nd week of age, respectively. All the broiler flocks had also been vaccinated against hydropericardium syndrome around 14 to 18 days of age.

Biochemical characterization

Initially the problem was suspected for mycoplasmosis, therefore, various organs were cultured in PPLO broth and agar but no change of colour was observed in the inoculated media up to 7 days. This negated the possibility of mycoplasmosis. On blood agar, typical colonies, 1–2 mm in diameter, circular, opaque to grey and convex with round edges were seen. These colonies were picked and streaked on MacConkey’s agar, Nutrient agar and Tryptose soya agar. From many of the flocks, there was no growth on nutrient and MacConkey’s agar. However, on tryptose soya agar, pinpoint, round circular colonies were observed. The isolates grew poorly on TSI slants.

The organisms were Gram-negative cocco-bacilli. Clinical signs, typical colonies and bacterial morphology of the organism were suspicious for Ornithobacterium rhinotracheale as the causative organism. Various biochemical tests including indole, catalase, nitrate reduction, urease, methyl red, oxidase, β-galactosidase, lysis decarboxylase and sugar fermentation tests were performed for confirmation. The results of these various tests are summarized in Table 1. Biochemical characteristics revealed that the iso-

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tative organism (16 isolates) was *Ornithobacterium rhinotracheale*. During the subsequent years, from 2000 to 2006, ORT was recorded in 84 broiler and 8 layer flocks in and around Faisalabad and other surrounding districts. The number of broilers in these affected flocks (84) ranged from 2500 to 16000 between the age of 20 to 42 days. In layer flocks although similar clinical signs and postmortem lesions were recorded but the severity of the disease and mortality was relatively less. Respiratory distress appeared to be relatively less intense and digestive system involvement seemed to be more common. Typical consolidation of lungs which was observed in 1999 has been recorded only in a few flocks. Congestion of liver seemed to be increased with the passage of time. Mortality due to ORT has been decreased during the subsequent years. Mortality during 2006 in affected broiler flocks (12) ranged from 4–20% and in layer flocks (2) 8 and 14%. During the years 2000–2006, 54 isolates of ORT were isolated from various affected broiler and layer flocks.

**Antimicrobial sensitivity**

Antimicrobial sensitivity of 70 isolates of *Ornithobacterium rhinotracheale* was performed and the results are briefly summarized in Table 2. Lincospectrin (Spectinomycin + Lincomycin) and doxycycline were most effective as all the 70 isolates were susceptible to these drugs. Most of the isolates were susceptible to Chloramphenicol (46) and Kanamycin (42). Neomycin and flumequine were effective against almost 50% of the isolates of ORT. Out of 70 isolates, 48 *Ornithobacterium rhinotracheale* isolates revealed resistance against Tetracycline and most of isolates (65) were resistant to norfloxacin, enrofloxacin (64) and oxalinic acid (67). All the isolates were resistant to furazolidone.

**Discussion**

Important respiratory diseases of poultry in Pakistan include Newcastle disease, infectious bronchitis, infectious laryngotracheitis, hydropericardium syndrome, avian influenza, pasteurellosis, infectious coryza and mycoplasmatis. These diseases have a great economic impact in poultry industry. There are a number of stress factors and infectious agents which compromise immune competence in poultry. Generalized immuno-suppression also affects respiratory tract immunity, involving both humoral and cellular immune responses. The most important immunosuppressive agent being infectious bursal disease (IBD) virus, which results in reduced local immune response of the respiratory tract (Deshmukh and Survash, 1998).

In the present studies, on blood agar, bacterial colonies were 1–2 mm in diameter, circular, opaque to grey in colour with round circular edges. Visible growth was recorded within 24–48 hours at 37°C. Smears were prepared and stained with Gram’s method. Gram negative short cocco-bacilli were observed. Respiratory diseases caused by Gram negative coccobacilli mainly include pasteurellosis, infectious corzya and colibacillosis. On MacConkey’s agar, lactose fermenting colonies were also observed within 24 hours of incubation at 37°C. Biochemically this organism was confirmed as *E. coli*. Various biochemical tests were performed to confirm the bacterial isolates which did not grow on MacConkey’s and nutrient agar. Colony morphological characteristics and biochemical parameters did not confirm any of the above mentioned bacteria. However, the epidemiological pattern of outbreak and gross pathological lesions did not confirm *E. coli*. This organism is also normally present in gastrointestinal tract (GIT) and is usually opportunistic and comes as secondary invader, so the role of *E. coli* might be as secondary invader (Chin and Drouil, 1998). All these findings and considerations negated the possibility of above mentioned bacterial diseases and on the basis of biochemical characteristics, the isolates were identified as *Ornithobacterium rhinotracheale*. *Ornithobacterium rhinotracheale* (ORT) was first isolated during 1993 in South Africa from broilers showing mild respiratory signs and growth depression (Van Beek et al., 1994). ORT infections occur naturally in chickens and turkeys and mortality rates usually range from 2–11%. Mild respiratory signs beginning around 3–4 weeks of age, slightly increased mortality and higher condemnation rates are typical of infection in young chicks (Mante, 1999). During the investigation period, mycotoxin levels of the feeds were also quite high, that may be an important predisposing factor potentiating high morbidity and mortality.

For the control of ORT, there are many reports on the use of autogenous vaccines (Van Empel et al., 1996). Autogenous vaccines were tried in some of these flocks. Although it did not give excellent response in all the flocks,
however, in most (42) of the flocks, response was good and mortality was reduced.

*Ornithobacterium rhinotracheale* is reported to be resistant to furazolidone, kanamycin, norfloxacin and enrofloxacain (Chin and Droul, 1998). In-vitro studies revealed that the isolates in the present outbreaks were susceptible to Lincopectrin (Lincomycin + Spectinomycin), doxycycline and neomycin. It was observed in California that *Ornithobacterium rhinotracheale* were susceptible to spectinomycin and erythromycin but resistant to quinolone group (Chin and Droul, 1998).

Although the disease has subsided timely, however, there is every chance of outbreaks in stressful conditions so we should not forget and be well equipped for the control of this malady. For future control measures, bacteria should be isolated from different areas and various isolates should be used for the preparation of refined vaccines. For monitoring purposes, various antigens have been prepared from the prevalent isolates. However, along with all these efforts, we have to educate our farmers particularly for adopting proper vaccination programs and biosecurity measures.

**Summary**

A wide spread respiratory problem was recorded in broiler chickens of different age groups in various poultry raising areas of Pakistan. There was high mortality as well as morbidity and poor response to antimicrobials. It created havoc in the poultry industry and many farms were closed. The present investigations were initiated to ascertain the etiology of the syndrome, its various epidemiological factors and susceptibility to various antimicrobials.

Affected flocks revealed chronic whitish diarrhea. Consistent gross pathological lesions recorded were congestion and consolidation of lungs, hemorrhages in trachea, congestion and hemorrhages in liver, splenomegaly, and necrotic foci on the kidneys. Mortality in various flocks ranged from 3.7–50%. Detailed isolation and biochemical studies revealed that *Ornithobacterium rhinotracheale* was responsible for this wide spread respiratory problem. This is the first report of chicken *Ornithobacterium rhinotracheale* in Pakistan. Antibacterial sensitivity revealed that most of the isolates were sensitive to lincopectrin (Lincomycin + Spectinomycin), doxycycline and neomycin. Many of the isolates showed resistance to kanamycin, norfloxacin, enrofloxacain, tetracycline and chloramphenicol.

**Key words**

*Ornithobacterium rhinotracheale*, ORT, respiratory syndrome, etiology, resistance to antimicrobials

**Zusammenfassung**

Auszüge von *Ornithobacterium rhinotracheale* (ORT) Infektionen bei Hühnern in Pakistan


**Stichworte**

*Ornithobacterium rhinotracheale*, ORT, Atemwegsyndrom, Ätiologie, Antibiotika-Resistenz

**References**


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