Report on isolation and identification of *Ornithobacterium rhinotracheale* from broiler flocks in İzmir, Turkey

Bericht über Isolierung und Identifizierung von *Ornithobacterium rhinotracheale* aus Masthähnchenherden in İzmir, Türkei

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Introduction

Respiratory disease conditions continue to cause heavy economic losses in the poultry industry. They are usually caused by viral, bacterial, mycotic or parasitic agents and the course of the diseases can be influenced by environmental conditions and management failure (JOUBERT et al., 1999). At the beginning of 90’s bacteriological examinations from turkeys and chickens suffering from respiratory distress resulted in isolation of slowly growing, pleomorphic, gram-negative rods. Initially, the bacterium was designated as Pasteurella-like, Kingella-like, Taxon 28 or pleomorphic gram-negative rod before the name *Ornithobacterium rhinotracheale* gen. nov. sp. nov. in the rRNA-superfamily V was suggested (VANDAMME et al., 1994). The infection occurs worldwide and is incriminated as a possible additional cause in respiratory disease complexes. *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).

*O. rhinotracheale* infections have been reported from various European countries (VAN EMPEL and HAFEZ, 1999) as well as from Israel (BOCK et al., 1995), United States (CHARLTON et al., 1993) and Turkey (ERGANIŞ et al., 1999, TURAN and AK, 2002, TURAN and ARCADAG, 2003, TÜRKYILMAZ, 2001). Eighteen distinct serotypes have been identified of which serotype A is most prevalent in chickens (Van Empel and Hafez 1999). The disease spreads horizontally by direct and indirect contact. Vertical transmission is suspected, since *O. rhinotracheale* has been isolated at very low incidence from reproductive organs, hatching eggs, infertile eggs and dead embryos (VAN EMPEL, 1998). There is no known public health significance (ARCHAMBAULT et al., 1999).

Diagnosis of *O. rhinotracheale* on the basis of clinical features and pathological lesions is often difficult since it can be confused with other infectious conditions. Proof of infection therefore must be carried out by isolation and identification of the causative agent (HAFEZ, 1998, 2002).

This paper reports on the isolation and identification of *O. rhinotracheale* from commercial broiler flocks in İzmir, Turkey.

Materials and Methods

Samples

Three different broiler flocks belonging to three different enterprises around İzmir province showed an increase in mortality and decreased feed intake. From each farm 10 dead and 5 live chickens between 38 and 42 days old and showed severe respiratory distress (eye discharge, gasping and dyspnoea.) were collected for necropsy and bacteriological examination.

Bacteriological examinations

At necropsy lungs and tracheas were collected aseptically, streaked on Blood Agar with 10% sheep erythrocytes and MacConkey Agar. The plates were incubated at 37°C under aerobic condition as well as at 37°C under microaerobic conditions for 2-3 days. The biochemical identification was carried out with commercial biochemical test-kit (API 20 NE, Bio-Mérieux, France) as described (VAN EMPEL et al., 1997 and HAFEZ, 1998).

Serological typing

The serological typing of the isolates were carried out using heat stable antigen with known positive antisera in agar gel precipitation Test (AGP) according to the method described by HAFEZ and STING (1999).

Antimicrobial sensitivity of *O. rhinotracheale* isolates

Antimicrobial sensitivity tests were applied to the isolated strains by Kirby-Bauer Disk Diffusion Method described by BAUER et al. (1966). The diameters of the zones of growth inhibition were measured with a ruler. A direct judgement for *O. rhinotracheale* is currently not established. The judgement was carried out according to references mentioned in Table 1.

Results

Course of the disease and necropsy findings

After the onset of clinical signs the mortality rate ranged between 2 to 6% per week. Treatment using erythromycin (Gallimycin Fort) for 5 days at the dose level of 1 g/liter via drinking water resulted in reduction of mortality down to 0.5-1% per week. At necropsy all birds showed severe air-
sacculitis and congestion of the lung accompanied by pneumonia.

**Bacteriological examination**

Out of 90 tested samples (45 trachea and 45 lungs) after 48 hours of incubation 6 samples showed pinpoint grey-white colonies. Gram staining showed gram-negative and pleomorphic rods. Growth was observed under both aerobic or microaerophilic conditions. All isolates produced oxidase but not indole and were (-galactosidase (ONPG) positive, catalase negative and all of them reacted negatively in urease test. All isolates gave the code number 0020004 in the API20-NE test. On the basis of biochemical characteristics, by routine laboratory and API 20NE tests, all 6 isolates were identified as *O. rhinotracheale*.

**Serological typing**

All of the 6 isolates were identified as *O. rhinotracheale* serotype B. Some strains, however showed cross reaction with antisera prepared against serotype E and A. The serotyping results are shown in Table 2.

**Antimicrobial sensitivity of the isolates**

Antimicrobial agents and the numbers of susceptible, intermediate and resistant isolates are shown in Table 3. All isolates were resistant to ampicillin, penicillin, gentamicin, streptomycin, ampicillin/sulbactam and kanamycin. On the other hand all isolates were sensitive to chloramphenicol and tetracycline. Five isolates were sensitive to erythromycin followed by 3 isolates which were sensitive to rifampin and two isolates were found sensitive to clarithromycin.

**Discussion**

*O. rhinotracheale* can usually be isolated from trachea, tracheal swabs, lung and air sacs (HAFEZ, 1998; SZALAY et al., 2002). Samples for bacterial culture should be collected at early stage of the disease. *O. rhinotracheale* can be identified using immuno-histochemical staining as well as polymerase chain reaction (VAN EMPEL et al., 1999; HUNG and ALVARADO, 2001). In field trials, using a sensitive immuno-histochemical staining, it was found that *O. rhinotracheale* was the cause of 70% of the cases with respiratory symptoms in broiler chickens, while through bacteriology and/or serology only 30% of the cases could be connected to *O. rhinotracheale* (VAN EMPEL et al. 1999; VAN VEEEN et al. 2000). Currently 18 serotypes of *O. rhinotracheale* designated A to R exist. Neither the origin nor the serotype of the *O. rhinotracheale* strains effect its pathogenicity (VAN EMPEL and HAFEZ, 1999). There was no information on the presence of the *O. rhinotracheale* serotype B in the Aegean region of Turkey. However, ERGANI et al. (1999) reported that *O. rhinotracheale* strains have been isolated from two commercial pullets in Konya. From those, the one
was serotype B and the others could not be serotyped. In the present investigation 6 *O. rhinotracheale* isolates belonging to serotype B were isolated from commercial broiler flocks located in the Aegean region. These results show that different *O. rhinotracheale* strains exist in Turkey.

In the present investigation mortality rates between 2 to 6% per week were observed after the onset of clinical signs. Mortality ranging between 0.5 and 2% per day associated with *O. rhinotracheale* infection in the 4-6 weeks-old broiler flocks has been reported in Hungary (Tarnyi et al., 1995). However, the severity of clinical signs, duration of the disease and mortality are extremely variable and are influenced by many environmental factors such as poor management, inadequate ventilation, high stocking density, poor litter conditions, poor hygiene, high ammonia level, concurrent diseases and the type of secondary infection (Hafez, 1996).

*O. rhinotracheale* strains were found susceptible for tetracycline and chloramphenicol and all isolates were resistant to ampicillin, penicillin, gentamicin, streptomycin, ampicillin/sulbactam, and kanamycin. Türkvilmaz, (2001) reported on the sensitivity of three isolates, which were found to be susceptible for danofloxacin, lincomycin, amoxicillin, amoxicillin /clavulonic acid, oxitetracycline, neomycin, cepaperoxone sulbactam, ampicillin sulbactam and tetracycline. The isolates investigated by Ergani et al. (1999) in Turkey were susceptible for oflaxacine, erythromycin, lincomycin, amoxicillin, amoxicillin /clavulonic acid.

In Germany and The Netherlands most *O. rhinotracheale* isolates are resistant to the enrofloxacin antibiotic (Hafez et al., 1993; Van Beek et al., 1994), whereas in France, Belgium and Israel most isolates are sensitive (Bock et al., 1998; Debrie et al., 1995; Duedouyt et al., 1995; Roger and Leorat 1997). In Canada pure *O. rhinotracheale* could be isolated from enrofloxacin-treated birds (Joubert et al., 1999). Van Veen (2003) tested strains originating from field cases of diseased broiler flocks from The Netherlands isolated in the period 1996–1999. The sensitivity of *O. rhinotracheale* strains significantly decreased over the years.

MALIK et al. (2003) examined *in vitro* antibiotic resistance profiles of 125 isolates of *O. rhinotracheale* strains isolated from turkeys in Minnesota during 1996-2002. A majority of isolates was sensitive to clindamycin, erythromycin, spectinomycin, and ampicillin. Resistance against sulfachloropyridazine decreased from 1996 to 2002, but an increase in resistance was seen against gentamicin, ampicillin, trimethoprim sulfa, and tetracycline. The resistance against penicillin remained constant from year to year. Soriano et al. (2003) determined the minimal inhibitory concentrations of 10 antimicrobial drugs for Mexican isolates using microdilution system and found a marked resistance trend. The susceptibility of *O. rhinotracheale* to amoxicillin, enrofloxacin and oxytetracycline was variable. However, consistent higher minimal inhibitory concentrations values were obtained for gentamicin, fosfomycin, trimethoprim, sulfamethazine, sulfamerazine, sulfaquinoxaline and sulfachloropyridazine.

Generally the sensitivity of *O. rhinotracheale* strains may show differences by regions. It may be due to the inherent genetic differences between them or to their exposure to antibiotics used in a specific region or farm over a period of time. In general the sensitivity pattern of *O. rhinotracheale* depends on the source of the strain and the routinely used drugs in an area (Van Empel and Hafez 1999). In addition, the most published results regarding to the sensitivity tests are based on disc diffusion test and are difficult to compare (Archambault et al., 1999; Ergani et al., 1999; Malik et al., 2003).

*O. rhinotracheale* infection seems to circulate in poultry enterprises in Turkey leading to severe economic losses, and several serotypes were detected, however, the serotype B strains were seldom.

### Summary

Respiratory diseases cause heavy economic losses in poultry enterprises in Turkey leading to severe economic losses, and several serotypes were detected, however, the serotype B strains were seldom.
commercial broiler flocks located in Izmir, Turkey was investigated. Six *Ornithobacterium rhinotracheale* strains were isolated and all of them were found to belong to serotype B. *In vitro* the isolates were sensitive to tetracycline and chloramphenicol. All isolates were resistant against ampicillin, penicillin, gentamicin, streptomycin, ampicillin/sulbactam and kanamycin. The Serotype B strains were seldom isolated in this country. Further study on the pathogenicity of isolated serotype B under experimental condition is required and in progress.

**Key words**

*Ornithobacterium rhinotracheale*, serotyping, antimicrobial sensitivity, respiratory disease, broiler, Turkey (Country)

**Zusammenfassung**

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**Stichworte**

*Ornithobacterium rhinotracheale*, Serotypisierung, Sensitivität, Atemwegserkrankungen, Broiler, Türkei

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NCCLS: National Committee for Clinical Laboratory Standards, 1999: Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. NCCLS, 19, No. 11, M31-A.


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Arch. Geflügelk. 3/2006