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Short Paper

Isolation of *Ornithobacterium rhinotracheale* from the chickens of a broiler farm in Kermanshah province, west of Iran

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Summary

On June 2005, a respiratory disease was observed in the chickens of a large broiler farm in Kermanshah province, west of Iran. 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 post-mortem examination, tracheitis, airsacculitis and pneumonia were obvious. Serologic examinations were negative for *Mycoplasma gallisepticum* and *Mycoplasma synoviae*. On virologic 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. Based on clinical, post-mortem and laboratory findings, ORT could be probably the primary cause of respiratory disease on this farm.

Key words: *Ornithobacterium rhinotracheale*, Broiler, Respiratory disease

Introduction

Respiratory infections are the most serious group of diseases of poultry, accompanied by heavy economic losses. Various pathogens have been identified as etiologies of respiratory disease, acting either primarily or secondarily. *Ornitho-bacterium rhinotracheale* (ORT), named by Vandamme *et al*. (1994), has been associated with respiratory disease, increased mortality, retarded growth and decreased egg production in avian species. ORT is a Gram-negative, pleomorphic, rod-shaped, and non-motile bacterium (Vandamme *et al*., 1994). So far, no special structures or properties, such as pili, fimbriae, plasmids or specific toxic activities, have been reported within the species (Leory-Setrin *et al*., 1998). ORT can cause highly contagious disease in poultry, but the severity of clinical signs, duration of the disease, and mortality has been found to be extremely variable (Van Empel and Hafez, 1999). There are reports of ORT infections in the United States, Germany, South Africa, the Netherlands, France, Israel, Belgium, Hungary, Japan, the United Kingdom, Turkey and Iran (Charlton *et al*., 1993; Hafez, 1994; Hinz *et al*., 1994; Van Beek, 1994; Dudouyt *et al*., 1995; Travers, 1996; Joubert *et al*., 1999; Banani *et al*., 2000; Sakai *et al*., 2000; Banani *et al*., 2002; Turan and Ak, 2002; Allymehr, 2006). Isolation of ORT, for the first time in Kermanshah province, west of Iran, from the chickens of a broiler farm with respiratory disease is reported here.

Materials and Methods

On June 2005, a respiratory disease was

observed in the chickens of a large broiler farm in Kermanshah, west of Iran. Totally, 72,000 broilers were kept in six windowless houses (12,000 birds per house). All houses were equipped with central heaters, pad cooling systems and ventilators to provide the optimum environmental conditions for the broiler chickens. The birds were fed commercial corn-soybean meal diets. The lighting program was 23 hrs light and 1 hr dark. All chickens of the farm were vaccinated against Newcastle disease, infectious bronchitis, avian influenza (H9N2) and infectious bursal disease.

During the disease outbreak, the birds were observed for clinical signs. A complete necropsy was carried out on at least 10 newly-died birds of each house, and samples of trachea, lungs, air sacs and hearts of five birds per house were collected for histo-pathologic examination.

Serum plate agglutination (SPA) tests were done on 10 sera per house to detect the possible involvement of *Mycoplasma gallis-epticum* (MG) and *Mycoplasma synoviae* (MS). Approximately 0.02 ml of serum collected from each affected bird and 0.03 ml of specific stained antigens for MG and MS (Intervet, Boxmeer, Holland) were mixed on a glass plate. The plate was rotated for 2–3 min; the tests were then examined for visible clumping (Kleven, 1998).

Virus isolation procedures in embryonated chicken eggs followed standard protocols (Easterday *et al*., 1997; Alexander, 1998). Antibiotic media were used to make 20% w/v suspensions of finely minced tissues and organs. Tracheal and faecal swabs were placed in sufficient antibiotic medium to ensure full immersion. The suspensions were held at room temperature for 1–2 hrs and then centrifuged at 1000 g for 10 min. Each of five specific-pathogen-free (SPF) 9–11-day embryonated chicken eggs (Valo, Lohmann, and Cuxhaven, Germany) was inoculated with 0.2 ml of the supernatant fluid into allantoic cavity. The eggs were placed at 37°C and candled regularly. The allantoic/amniotic fluid was harvested, and assayed by a haemagglutination (HA) test. The presence of avian influenza virus (AIV) or Newcastle disease virus (NDV) in any haemagglutinin-positive samples could be confirmed or discounted by haemagglutination-inhibition (HI) testing with specific antisera (Intervet, Boxmeer, Holland). Sample collection and processing for infectious bronchitis virus (IBV) followed the procedures, as previously described in detail (Gelb and Jackwood, 1989; De Wit, 2000).

On bacteriologic examination, samples from the trachea, lungs and air sacs of five affected birds of each house were streaked onto 5% sheep blood agar with 10 μg/ml of gentamicin. Plates were incubated at 37°C under 5–10% CO_2 atmosphere for 24–48 hrs (Chin and Droual, 1997). Colonies, which were circular and small (1–3 mm in diameter), opaque to greyish, and non-haemolytic were selected (Vandamme *et al*., 1994).

Colonies with characteristics of ORT were stained by Gram's method, identified biochemically to confirm the main phenotypic traits, and antigenically tested by agar gel precipitation (AGP), as previously described (Van Empel *et al*., 1997). The standard anti-ORT antibodies were kindly supplied by Dr. Silim (Faculty of Veterinary Medicine, University of Montreal, Canada). The biochemical characterization was performed with oxidase, catalase, Mac-Conkey, arginine, lysine, ornithine, phenylalanine, urea, indole, H_2S, Vogues-Proskauer, and carbohydrate fermentation. On carbohydrate fermentation tests, tubes containing phenol red broth, supplemented with 1% (w/v) glucose, mannose, lactose, sucrose, sorbitol, maltose, and dulcitol were each inoculated with ORT-suspected overnight cultures. All inoculated tubes were incubated at 37°C for 24–48 hrs and observed or tested for biochemical characterizations (Chin and Droual, 1997).

Results

Relatively severe respiratory signs started with sneezing at 27 days of age. The affected birds showed nasal exudates, wet eyes, swelling of infraorbital sinus, and gasping. The disease lasted up to the end of fattening period. The clinical signs accompanied by increased mortality (13.6%) and poor performance parameters. At post-mortem examination, tracheitis, air-sacculitis and pneumonia were obvious. The most

striking feature was a foamy white exudate in the trachea and air sacs.

On histopathologic examinations, the pericardia were thickened and infiltrated with macrophages and heterophils. Thickened and oedematous air sacs showed heterophilic infiltration. The lungs were congested, and infiltrated with macrophages and heterophils. There were foci of necrosis within the lumen of parabronchi. Tracheitis was characterized by congestion, and infiltration of heterophils and macrophages in the epithelium and lamina propria. There was no pathognomonic lesion typical for infectious laryngotracheitis (ILT).

Serological examinations were negative for MG and MS. On virological examinations, virulent IBV, AIV and virulent NDV could not be isolated. On bacteriologic examinations, it was possible to obtain circular, grey to grey-white colonies. Gram-negative, pleomorphic, and rod-shaped bacteria were observed. Bacteria isolated from 53% of the samples taken from trachea, lungs, and air sacs of the affected broilers showed biochemical (Table 1) and antigenic characteristics of ORT.

Table 1: Results of the biochemical tests used to identify the ORT isolated from broiler in Kermanshah province

Test	Result
Oxidase	+
Catalase	-
Growth on MacConkey	-
Arginine dehydrolase	+
Lysine decarboxylase	-
Ornithine decarboxylase	-
Phenylalanine deaminase	-
Urease	+
Indole production	-
H_2S	-
Vogues-Proskauer	+
Acid from carbohydrates:	
Glucose	+
Mannose	+
Lactose	+
Sucrose	+
Sorbitol	-
Maltose	+
Dulcitol	-

Discussion

Based on serologic, virologic and histopathologic examinations, the main causative agents of avian respiratory disease (MG, MS, IBV, AIV, NDV and ILT) could not be incriminated as the etiological factor(s) of the disease on this farm. Avian pneumovirus (APV) infections can be confused with diseases resulting from infections with other organisms such as *Bordetella avium*, ORT, and *Mycoplasma* spp. (Gough, 2003). However, APV infection is more common in turkeys; it is less clearly defined and may not always be associated with clinical signs in chickens (Cook, 2000). ORT could be isolated from the trachea, lungs and air sacs of the affected broilers of the farm.

ORT has been incriminated as a possible causative agent in the respiratory disease complex (Van Empel and Hafez, 1999). Several pathogens are indicated as possible causes of respiratory diseases, either alone, in synergy with other micro-organisms or along with non-infectious factors, such as climatic conditions and management-related problems (Van Empel and Hafez, 1999).

On the farm where the study was performed, relatively severe respiratory signs were started at 27 days of age. ORT has been shown to be associated with considerable losses in broiler chickens of 28 days and older (Goovaerts *et al*., 1998). It is clear that ORT can cause acute highly contagious disease in poultry (Van Empel and Hafez, 1999). The isolate obtained in our study resulted in bacteria compatible with ORT and for the first time are been recognized in Kermanshah province, west of Iran. There are reports of ORT infections in other parts of country (Banani *et al*., 2000; Banani *et al*., 2002; Allymehr, 2006). The investigation of the epidemiology of ORT infections is hampered by the difficulties found in culturing ORT from infected organs, the brevity of the serological responses after an ORT infection and the complexity of the infection in which ORT can be involved. It has been proven that transmission of ORT is possible not only horizontally through aerosols but also vertically through the egg (Van Empel and Hafez, 1999). These findings make it easier to understand the relative rapid spread of ORT infections in the commercial poultry flocks in Iran.

Based on clinical, post-mortem and

laboratory findings, ORT could be the primary cause of respiratory disease on the farm. Further works are necessary to generate information about the economical losses due to ORT, molecular characterization, antimicrobial susceptibility, pathogenicity and eventually vaccine strains.

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