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SEROPREVALENCE AND ISOLATION OF *ORNITHOBACTERIUM RHINOTRACHEALE* IN BROILER FLOCKS IN MAZANDARAN PROVINCE, NORTH OF IRAN

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Summary

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The aim of this study was to determine the prevalence of seropositive broiler flocks against *Ornithobacterium rhinotracheale* (ORT) and biochemical identification of this bacterium in Mazandaran province, north of Iran. Tracheal and serum samples were collected from 45 broiler flocks at farms and slaughter houses. From 450 tracheal samples, 12 (2.6%) ORT isolates were identified using biochemical tests. Of the 450 serum samples, 320 (71.1%) samples were positive for antibodies to ORT by ELISA. Results of this study indicated the presence of ORT and high prevalence of antibodies against ORT infection in north of Iran.

Key words: broilers, ELISA, Iran, *Ornithobacterium rhinotracheale*, seroprevalence

INTRODUCTION

Respiratory infections are among the most serious disease affecting poultry and cause heavy economic losses in the poultry industry worldwide. Various pathogens have been identified as causing respiratory disease, acting either in a primary or secondary role. *Ornithobacterium rhinotracheale* (ORT) is a gram-negative, pleomorphic, rod-shaped bacterium associated with respiratory distress, mortality, and decreased growth in chickens and turkeys (Vandamme *et al.*, 1994; Chin *et al.*, 2008). *Ornithobacterium rhinotracheale* is recognised as a primary or secondary etiological agent depending on strain virulence, adverse environmental elements, immune condition of the flock, and presence of other contagious agents in Europe, Africa, Middle East, Asia, Far East and South America (Bisgaard *et al.*, 2008; Van Empel *et al.*, 2008). Studies in some parts of Turkey have shown different seroprevalence of ORT in broiler flocks (Turan & Ak, 2002; Ozbey *et al*., 2004). Refai *et al*., (2005) found that between 20.3% and 77.7% of serum samples from broiler flocks in Egypt were positive for ORT antibodies when using two different ELISA kits. Chansiripornchai *et al*., (2007) randomly examined 17 broiler farms (19 flocks) in Thailand. The seropositive flocks were 63% and the sera analysis showed that the individual 280 broiler serum antibody responses were 19.6% positive. Canal *et al*., (2003) collected 1550 sera related to 50 broiler flocks during the slaughter time in southern Brazil. The prevalence of ORT antibodies was 63.83%, but in each individual flock only 6.52% of the birds were positive. Diagnosis of ORT infections must be approved by isolation and identification of

the bacteria and/or detection of antibodies using serological examination such as slide agglutination test and ELISA (Hafez, 1998; Erganis *et al.*, 2002; Ozbey *et al.*, 2004).

The presence of ORT in poultry farms in Iran has been confirmed over the last few years. Allymehr (2006) examined 463 serum samples from 50 broiler flocks in west Azarbaijan province, northwest of Iran. The result showed that 41 broiler flocks (82%) were positive for ORT. Ganbarpour & Salehi (2009) reported the seroprevalence of ORT in the southeast of Iran: 134 (31.9%) out of 420 serum samples or 17 (81%) out of 21 broiler flocks were positive.

The aim of this study was to detect ORT antibodies by ELISA from serum samples collected from commercially reared chicken flocks in Mazandaran province, north of Iran and to isolate and biochemically identify the ORT isolates from bacteriological samples.

MATERIALS AND METHODS

Sample collection

In this study a total of 250 tracheal and serum samples were collected from 25 commercially reared chicken flocks, with or without respiratory signs (10 trachea and serum samples per each flock). In addition trachea samples and serum samples were collected from 200 broilers (from 20 flocks) on the slaughter line of 4 abattoirs located in Mazandaran province, north of Iran. In the present study, the age of sampled broiler flocks was between 18 and 52 days. The samples were taken from broiler strains including Ross, Cobb, Arbor Acres and Arian, but Ross was the dominant strain. Population density differed from 5,500 to 13,000 in each flock.

Bacteriological examinations

Tracheal swabs were streaked on MacConkey and sheep Blood agar with 10 µg/ml gentamicin. The plates were incubated at 37°C under aerobic conditions as well as at 37°C under microaerobic conditions in a candle jar for 2–3 days (Chin *et al.*, 2008). Colonies, which were circular and small (1–3 mm in diameter), opaque to grayish and non-haemolytic were then selected (Vandamme *et al.*, 1994). Colonies were stained by Gram's method and identified with biochemical methods as followed: catalase, nitrate reduction, H_2S production in triple sugar iron (TSI), ornithine decarboxylase, growth on MacConkey, indole, urease, oxidase, nitrate, carbonhydrate fermentation tests such as glucose, fructose, galactose, maltose, lactose (Chin *et al.*, 2008).

Serological examinations

The antibody titre was determined using the FlockChek® *O. rhinotracheale* antibody test kit (IDEXX Laboratories, Westbrook, Maine). The ELISA test and analysis of the results was performed according to the manufacturer's recommendations. Briefly, samples were diluted in a 1/500 ratio and the OD was measured on an ELISA microplate reader (SFRI IRE96, France) at 650 nm. Results were determined by calculating the sample to positive (S/P) ratio. Samples with S/P ratios of 0.4 or less were considered negative, and samples having S/P values higher than 0.4 (titres higher than 844) were considered positive.

Statistical analysis

Statistical analysis between ORT serum titers and the rate of isolation were performed by SPSS software and chi-square exact test.

RESULTS

Culture

A total of 450 tracheal swab samples were obtained from 45 broiler chicken flocks in Mazandaran province, north of Iran. ORT was isolated from 12 (2.6%) swab samples. The biochemical properties of 12 ORT isolates are shown in Table 1. All isolates showed the same biochemical characteristics with no deviation between them.

Table 1. Biochemical characterisation of ORT isolates

Reaction	Result
Oxidase	+
Catalase	–
Urease	+
Indole	–
Lysine decarboxylase	–
Fructose	+
Lactose	+
Maltose	+
Galactose	+
Glucose	+
Nitrate reduction	–
TSI	–
Growth on MacConkey agar	–

ELISA

Of the 450 serum samples obtained from 45 broiler chicken flocks, 320 (71.1%) samples were positive for the presence of antibodies to ORT by ELISA. All of the flocks with respiratory signs were serologically positive. Details of broiler flocks with bacteriological and serological results are shown in Table 2. There was no significant difference between flock size and isolation of ORT; the age of birds was neither a significant factor in ORT isolation.

There was no significant difference between the rate of isolation and flock ORT titers ($P>0.05$).

DISCUSSION

Mazandaran province is located in north of Iran beside the Caspian Sea. The climate is temperate as an effect of Alborz Mountainous climate and the Caspian Sea. Being adjacent to the Caspian Sea, the climate of the province is highly humid (relative humidity is between 40 to 100%) and average temperature is 17.5 ^{o}C. It may provide optimal condition for ORT and affects the high prevalence of this bacterium. As clinical signs and post-mortem lesions of ORT infection are similar to other bacterial and virus infections, accurate diagnosis must be substantiated by direct detection or isolation of the causative bacteria and /or indirectly through the detection of antibodies using serological examination (Van Empel & Hafez, 1999; Hafez, 2002).

In this study 5 out of 25 broiler flocks showed respiratory signs such as sneezing, coughing and nasal and ocular discharge by the time of sampling and all of them were serologically positive. This finding is in agreement with result of Canal *et al.*, (2003) who found a positive correlation between the presence of respiratory signs and antibodies to ORT, although the reverse correlation was not significant. Furthermore, 9 out of 20 broiler flocks sampled at slaughterhouses were serologically positive. This finding indicates that apparently healthy flocks can be carriers of the disease.

The presence of antibodies against *O. rhinotracheale* could be detected by ELISA in one-day-old birds and in egg

Table 2. Characteristics of sampled broiler flocks with bacteriologic and serologic results

No. of flocks	Flocks size	Age (days)	Number of sampled chickens		Number (%) positive	
			Bacteriology	Serology	Bacteriology	Serology
1	7000	32	10	10	0	5
2	6500	38	10	10	0	7
3	10800	27	10	10	0	6
4	11000	41	10	10	1	10
5	7000	34	10	10	0	7
6	8700	34	10	10	0	6
7	13000	28	10	10	1	10
8	10000	31	10	10	0	6
9	6600	18	10	10	0	6
10	9000	33	10	10	1	10
11	7000	41	10	10	0	7
12	6500	25	10	10	0	6
13	5500	27	10	10	1	10
14	12000	31	10	10	1	10
15	7500	19	10	10	0	6
16	13000	27	10	10	0	5
17	6500	29	10	10	0	6
18	6500	20	10	10	1	9
19	9200	38	10	10	0	8
20	8800	22	10	10	0	7
21	6000	23	10	10	0	8
22	5500	19	10	10	0	7
23	12000	26	10	10	1	10
24	10500	35	10	10	0	5
25	10000	43	10	10	1	10
26	6600	52	10	10	1	9
27	5500	46	10	10	0	7
28	11200	50	10	10	0	6
29	11800	51	10	10	0	7
30	12700	50	10	10	0	8
31	8000	48	10	10	0	6
32	9000	43	10	10	1	7
33	8700	47	10	10	0	6
34	9100	42	10	10	0	6
35	10000	55	10	10	1	10
36	11300	53	10	10	0	7
37	13000	46	10	10	0	6
38	7000	45	10	10	0	6
39	6500	50	10	10	1	10
40	8000	47	10	10	0	0
41	9500	55	10	10	0	7
42	12000	43	10	10	0	6
43	10500	51	10	10	0	7
44	7000	48	10	10	0	6
45	8000	49	10	10	0	6
Total			450	450	12 (2.6%)	320 (71.1%)

yolks, as well as in birds with clinical signs, confirming that the ELISA can be useful for diagnostic purposes. Antibody titres peak at 1 to 4 weeks after a field infection, but decline rapidly thereafter, indicating that serum samples for flock screening should be taken at different ages (Van Empel & Hafez, 1999).

In this study, ORT was isolated from 2.6% of the samples. This proportion is lower than other researchers' results, such as: 9.8% in Markazi province, Iran (Ghaemmaghami *et al.*, 2007), 8.8% in Northern and Middle Jordan (El-Sukhon *et al.*, 2002) and 11.46% in Turkey (Turan & Ak, 2002), but is in agreement with 3.5% in south-eastern Iran (Ghanbarpour & Salehi, 2009), 2.27% in Guilan province, north of Iran (Asadpour *et al.*, 2008), 1% in Ahvaz, South of Iran (Jamshidian & Mayahi, 2008), 1.5% in Elazig province, East of Turkey (Ozbey *et al.*, 2004), 0.4% (Erganis *et al.*, 2002), and 1.2% in Turkey (Turkyilmaz, 2005). ORT can normally be isolated only at an early stage of the infection and attempts to recover it at a later stage often fail. In contaminated samples ORT can be masked easily by an overgrowth of other bacteria such as *E. coli*, *Proteus* spp. or *Pseudomonas* spp. and therefore cannot be detected in routine investigations. Conversely, serology is useful for flock monitoring or as an aid in the diagnosis of ORT (Hafez, 2002; Chin *et al.*, 2008).

In this study, serum samples were positive in 71.1% of samples by ELISA. These findings are higher than results recorded in Iran by Ghanbarpour & Salehi (2009) and Allymehr (2006) (31.9%, and 44.2%, respectively), but are in agreement with the results reported by Refai *et al.*, (2005) from broiler flocks in Egypt and by Turan & Ak (2002) from broiler flocks in Turkey (77.7% and 64.4%, respectively). Another study in Iran reported that 97.63% of serum samples were positive for ORT antibodies by ELISA (Keleidari *et al.*, 2008).

In a field study using a sensitive technique for confirmation, ORT was found to be involved in 70% of cases with respiratory signs in broiler chickens, while through bacteriology and/or serology only 30% of the cases could be related to ORT (Pattison *et al.*, 2008). In the current study ORT was isolated from a low percentage of ELISA positive broiler flocks. In Brazil, ORT strains were isolated from 16% of the ELISA positive broiler flocks (Canal *et al.*, 2005). The difference may be related to the age of the examined chickens and sample collection approach. ORT excretion and antibody response may also be affected by a number of factors such as antibiotic therapy and vaccination. The effect of antibiotic therapy on the serologic response to ORT remains unclear (Hafez, 2002; Ozbey *et al.*, 2004).

In conclusion, the results of this study indicated for the first time the presence of ORT and high prevalence of antibodies against ORT infection in Mazandaran province. Determination of different serotypes of ORT can useful in disease prevention programme. Future studies on the current topic are therefore necessary.

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