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Detection and Prevalence Antibodies Against *Ornithobacterium rhinotracheale* (ORT) by ELISA in Broiler Chicken Farms in Guilan Province, Iran

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Abstract: The objective of this study was to estimate the true prevalence of seropositive broiler chicken farms against *Ornithobacterium rhinotracheale* (ORT) in Guilan province, Iran. In this study 32 flock and 640 serum sample collect from broiler chickens farms 30- 35 and 40 - 45 days were examined by ELISA test and SPSS ver.18 statistical analaysis for the detection of antibody against *Ornithobacterium rhinotracheale* in broiler chicken serum. Result showed that 10 flock (30.44%) of were positive, 7 flock (21.74%) were suspect and 15 flock (47.82%) were negative Base on statistical analysis and total of 640 serum samples taken, 331 (84.51%) samples were negative, 147 (22.85) samples were suspect and 162 (25.31) samples were positive. And sample collected in 30-35 days 24 sample (7.4%) were positive, 30 sample (9.5%) were suspect and 266 sample (83.1%) were negative. In 40-45 days 178 sample (55.6%) were poistive, 77 sample (24.3%) suspect and 65 sample (20.1%) were negative. This study indicated that prevalence of ORT antibody to the extent was high in the commercial broiler chicken in guilan province population. between Antibody titers and broiler age there is positive correlations significantly ($p<0.05$).

Key words: *Ornithobacterium rhinotracheale* · Antibody prevalence · Broiler chicken · Guilan province

INTRUDUCTION

Respiratory problems are one of the main disorders leading to economic losses in poultry farms worldwide. They may be induced by various bacterial and viral agents, either alone or in combination [1]. Diverse pathogens have been recognized as causing respiratory diseases, acting either in a primary or secondary role. *Ornithobacterium rhinotracheale*, a lately reported pathogen, is a Gram negative, pleomorphic, rod-shaped very slow growing, bacterium of the rRNA superfamily V that was originally identified by bacterium associated with respiratory disease, growth retardation, mortality and decreased egg production in poultry [2,3]. and subsequently named by [4]. Initially, the bacterium was designated a Pasteurella-like, Kingella-like or pleomorphic Gram-negative rod (PGNR) and also the name TAXON 28 was used before 1994, when the name *ORT* gen. nov. sp. nov. was suggested for this species [4]. Serotyping in chickenshas revealed that the majority of isolates are of serotype A and that 95% of strains belong to the four major serotypes A, B, D and E. Turkey isolates seemed to be more heterogeneous, being distributed among the serotypes [35]. *ORT* can cause highly infectious diseases in poultry, but the severity of clinical symptoms, duration of the disease and mortality has been described to be highly variable [5]. *ORT* can be a primary or secondary etiological agent depending on strain virulence, adverse environmental elements, immune condition of the flock and presence of other contagious agents [5]. Differences in virulence between strains, however, have been noted [6]. Molecular epidemiologic studies suggest that this bacterium was recently introduced to domestic poultry from wild bird populations [7]. Clinical disease in broilers is less severe than in turkeys and involves sneezing, rhinitis, pneumonia, tracheitis, airsacculitis and facial oedema [8, 9] but the bacterium can also disseminate to other sites of the body resulting in local pathology such as hepatitis, meningitis and joint-infections [10, 11]. This infectin can cause highly contagious diseases in poultry, but the severity of clinical signs, duration of the disease and mortality has been found to be extremely variable [12,13].

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ORT can be a primary or secondary etiological agent depending on strain virulence, adverse environmental factors, immune state of the flock and presence of other infectious agents [13]. The majority of reported cases highlight the effects of *ORT* in meat-type poultry. *ORT* infections are common in broilers at 3–6 weeks of age and in broiler breeders 20–50 weeks of age. Mortality is variable and relatively low in uncomplicated cases. There can be a drop in egg production, decrease in egg size and poor eggshell quality. Fertility and hatchability are unaffected in many cases [14,15]. Diagnostic tests are routinely utilized for poultry-health prevalence studies and ideally, True Prevalence (TP) should be estimated from Apparent Prevalence (AP) by modifying with test Sensitivity (Se) and Specificity (Sp). Absence of knowledge of or disregard for test errors (i.e. false positives and negatives) can lead to unsuitable sample size calculations for studies, misclassification of diseased and non-diseased conditions and prejudiced estimates of measures of result in risk factor studies. The advantage of the serological tests over bacteriological examination is that antibodies persist for several weeks after infection and the bacterial shedding is short [16]. Serological examination for detection of antibodiescould be carried out using the slide agglutination test prepared from different serotypes [17] enzyme-linked immunosorbent assay (ELISA) tests [18,19] or dot-immunobinding assay (Dot-Iba) [20]. Because *ORT* is difficult to identify, use of a reliable identification method is of importance. PCR assays were shown to be useful for identification purposes recently [16]. There are reports of *ORT* infections in the United States, Germany, South Africa, The Netherlands, France, Belgium, Hungary, Japan, United Kingdom, Turkey and Iran (1,2,3,6,21-26). The aim of this study was to detect *ORT* antibodies by ELISA from serum samples collected from commercially broiler chickens flocks in Guilan province of Iran region showing respiratory disease symptoms.

MATERIALS AND METHODS

Samples Collection: In this study total of 640 blood samples were obtained from 32 broiler flocks. The birds were two aged 35 and 45 days were examined for the presence of antibodies against ORT. The samples were taken regardless of the presence of any signs of respiratory infections in the studied flocks.

Serology: The serum samples were tested for antibodies to *ORT* by the enzyme-linked immunosorbent assay (ELISA). The indirect ELISA was carried out using a modification of the method described previously by [19]. The ELISA test was performed using a commercial kit (BioCheck, Inc., the Netherlands), which was able to determine antibodies of 12 serotypes (A–M) of *ORT*. The antigen was diluted 1: 100 with 0.06 M carbonate-bicarbonate buffer (pH 9.6) and 100 µl was added to each well of plate. The plates were covered and held at 4°C for 18 hours. The plates were washed three times with PBST (Phosphate buffered saline 0.01 M, 0.15 M NaCl, 0.05% Tween 20, pH 7.2). For neutralization of polysteren non-adsorption of antigen (Blocking step), 100 microliters of PBS supplemented 1% Bovine Serum Albumin (BSA) was added to each well [27]. The plates were placed on a low speed, continous shaker and incubated at 37°C for 1 hour. Then, the plate was washed again three times as above. All serum samples (diluted 1: 100) were tested in duplicate and the plates were incubated at 37°C for 1 hour. After the plates were washed again 100 µl of a 1: 5 000 dilution (in sample dilution buffer) of goat anti-chicken immunoglobulin G peroxidase-labelled antibody as conjugate (Nordic Laboratories, Tilburg, The Netherlands) was added to each well and incubated at 37°C for 1 h and washed again. Then 100 µl of chromogen substrate was added to each well. The plate was incubated at room temparature for 20 minutes. The optical density (OD) was measured on ELISA reader in 405 nm. Results were determined by calculating the sample to positive (S/P) ratio.

Serotyping: For serotyping of ORT isolates, Rapide Slide Agglutination Test (RSA) were done using standard antisera [28] against ORT antigen.

RESULTS

In this investigation 640 serum samples and 32 flock obtained from broiler chickens, Origin and numbers of flocks, samples and age of chickens are shown in Table 1. Optical densities were determined in an ELISA reader and processed with software from Biochek. According to the test, three categories of the results had been established. The negative, suspect and positive results are in the titer range 424, 425–1431 and 1432, respectively,Table 2. Result showed that 10 flock (30.44%) of were positive, 7 flock (21.74%) were suspect and 15 flock (47.82%) were negative Base on statistical analysis,Table 3. and total of 640 serum samples taken, 331 (84.51%) samples were negative, 147 (22.85) samples were suspect and 162 (25.31) samples were positive,Table 4. Broiler in the 30 – 35 days, 24 (7.4 %) samples were positive, 30 sample (9.5 %) were suspect and 266 (83.1%) showed were negative result, Table 5. Broiler in the 40 – 45 days 178 samples (55.6 %) were positive, 77 (24.3%) samples were suspect and 65 samples (20.1 %) were negative too, Table 6.

Table 1: Description of sampled of the broiler chicken flocks and serologic test results in Guilan province, Iran

Number of Flock	Age of Birds (Days)	Number of Smples	ELISA result
1	33	20	Negative
2	35	20	Negative
3	44	20	Positive
4	45	20	Positive
5	34	20	Suspect
6	30	20	Negative
7	43	20	Positive
8	33	20	Negative
9	32	20	Negative
10	44	20	Positive
11	32	20	Negative
12	33	20	Negative
13	41	20	Suspect
14	33	20	Negative
15	43	20	Positive
16	32	20	Negative
17	44	20	Suspect
18	31	20	Suspect
19	43	20	Negative
20	30	20	Negative
21	45	20	Positive
22	31	20	Suspect
23	43	20	Suspect
24	44	20	Positive
25	43	20	Suspect
26	44	20	Positive
27	41	20	Negative
28	43	20	Positive
29	32	20	Negative
30	44	20	Positive
31	33	20	Negative
32	35	20	Negative

Table 2: *Ornithobacteruim rhinotracheale* standard titer

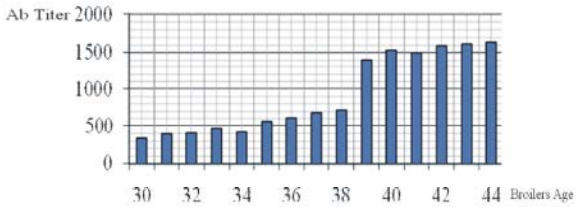
Ab	Mean Titer	S/P
Negative	< 424	< 0.4999
Suspect	425-1431	0.500-999
Positive	>1432	>1

Table 3: Result of all serum sample collected from flocks in Guilan province of Iran

Sample collected from flocks	%	Result
10	30.44%	Negative
7	21.74%	Suspect
15	47.82%	positive
32	100	Result

Table 4: Result of all serum sample collected

Number of Sample	%	Result
331	84.51%	Negative
147	22.85%	Suspect
162	25.31%	positive
640	100	Total



Graph 1: Relationship between ages and Ab titer in broiler chickens

Table 5: Result of serum sample in age of 30-35 days

Number of sample	%	Result
24	7.4%	Positive
30	9.5%	suspect
266	83.1%	Negative
320	100	Total

Table 6: Result of serum sample in age of 40-45 days

Number of sample	%	Result
178	55.6%	Positive
77	24.3%	suspect
65	20.1%	Negative
320	100	Total

The results of this study was the increased of age, number of suspect cases and positive for the disease increased. In other words that the increasing age of the broilers and the increased prevalence of infection, there is a significant relationship, Graph 1.

DISCUSSION

The use of ELISA for the detection of antibodies against to *ORT* has been reported [13, 29]. The serotype specificity of the ELISA is a disadvantage, although a number of commercial ELISAs have been described to have high sensitivity to various *ORT* serotypes [30, 31]. Self made ELISA as well as two commercial available ELISA kits are to detect antibodies against all tested ORT serotypes. In addition examination of serum samples collected from commercial flocks in all three systems showed similar results on flock bases using these ELISA tests, how ever some minor variations on sample bases [32]. In other study, was the first to examine the seroprevalence of *ORT* within a commercial egg layer population. Serum samples collected from egg production companies were examined by serum plate agglutination test (SPAT) and outer membrane protein-based enzyme-linked immunosorbent assay (ELISA). Results show that 90% of layer flocks were positive by SPAT and 100% by ELISA. Of the pullet flocks examined, 43% and 52% were

positive by SPAT and ELISA, respectively [31]. The first documented isolation and identification of *ORT* in Iran was made from 4-week-old broilers in 2000 [22]. In this study, 32 flock and 640 serum sample from broiler chickens farms 30- 35 and 40 - 45 days were examined by ELISA test for the detection of antibody against *ORT* in chicken serum in Guilan province from north of Iran. Results showed 10 flock (30.44%) of were positive, 7 flock (21.74%) were suspect and 15 flock (47.82%) were negative Base on statistical analysis. and total of 640 serum samples taken, 331 (84.51%) samples were negative, 147 (22.85) samples were suspect and 162 (25.31) samples were positive. And sample collected in 30-35 days 24 sample (7.4%) were positive, 30 sample (9.5%) were suspect and 266 sample (83.1%) were negative. In 40-45 days 178 sample (55.6%) were poistive, 77 sample (24.3%) suspect and 65 sample (20.1%) were negative. This study indicated that prevalence of *ORT* antibody to the extent was high in the commercial broiler chicken in guilan province population. between Antibody titers and broiler age there is positive correlations significantly ($p<0.05$). According to other studies, in relation to age and the prevalence of *ORT* infection, the most critical time for the clash with the infection in broilers between the ages of 3 to 4 weeks and above the fact that it is stationary loss maternal Antibody through vaccination is sufficient to establish safety. Since vaccination against this infection in Broiler breeder does not take place in Iran, With increasing age in broilers, can increase the risk of infection and antibody against this infection was more caused considerable damage to its industry poultry. The results of this study was the increased of age, number of suspect cases and positive for the disease increased. In other words we can say that the increasing age of the broilers and the increased prevalence of infection, there is a significant relationship. In other studies revealed that *ORT* was the cause of 70% of the total cases with respiratory signs in broiler chickens while bacteriologic or serologic tests revealed that only 30% of the cases could be connected to *ORT* [33, 34]. also worked on the seroprevalence of *ORT* in Marmara and Western Black Sea regions of Turkey and found that 65% of the serum samples coming from different flocks were positive. our study showed, 25.31% serum samples tested positive for *ORT* from 640 samples. The results of this study were somewhat similar to the findings of another studies conducted in Turkey and Europe [23,33,34]. This study All broiler flocks tested were 15 (47.82%). for the *ORT* antibody, but another study the 68% positive flocks found in southern Brazil [35] and higher than the 26% found in broiler flocks in Germany [19]. The high prevalence of positive broiler flocks may be because of the continuous introduction of the agent from their parent flocks [36]. The detection of antibodies in the broiler breeders may lead to the transmission of the bacteria to broilers through the eggs [34]. In contrast, serum positive breeders may passively protect their progeny at the beginning of their life [13], although broilers may become susceptible to clinical disease sometime later [37]. The lower prevalence of *ORT* antibodies in broiler chickens in Guilan province, may be serological cross-reaction with other bacteria, influenced by differences in age, environmental factors and local strains of the bacteria that spread slowly. The results revealed that age might influence the seroprevalence of *ORT* in Guilan province. The broiler chickens, raised over a short period, had been found to display seroconversion to a lesser degree than those of the broiler breeder raised over a longer period. Overall, these numbers show a high prevalence of *ORT* antibodies in broiler chickens in guilan province (Iran).

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