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Detection of Antibodies Produced against *Ornithobacterium rhinotracheale* and *Bordetella avium* by Enzyme-Linked Immunosorbent Assay in Hens and Turkeys in Aydın Province, Turkey

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Abstract: Respiratory diseases are very common in poultry. *Bordetella avium* and *Ornithobacterium rhinotracheale* are among the major microorganisms responsible for these diseases. The study was conducted to investigate the seroprevalence of *O. rhinotracheale* and *B. avium* in Aydın. Two hundred seventy-six chicken and 360 turkey serum samples were collected from poultry with respiratory disease suspicion in poultry enterprises around the city and samples were then examined for the presence of *B. avium* and *O. rhinotracheale* by commercial ELISA kits. The results revealed that 66.3% of chicken and 11.1% of turkey serum samples were positive for *O. rhinotracheale* and 29.1% of turkey serum samples were positive for *B. avium*. Presence of *O. rhinotracheale* and *B. avium* antibodies was serologically detected for the first time in hens and turkeys in Aydın province, Turkey.

Key Words: *Ornithobacterium rhinotracheale*, *Bordetella avium*, poultry, ELISA

Türkiye'de Aydın Yöresinde Tavuk ve Hindilerde *Ornithobacterium rhinotracheale* ve *Bordetella avium*'a Karşı Oluşan Antikorların ELISA ile Saptanması

Özet: Solunum Sistemi Hastalıkları (SSH) kanatlı hayvanlarda sık görülen infeksiyonlardır. *Bordetella avium* ve *Ornithobacterium rhinotracheale* kanatlı hayvanlarda SSH hastalıklarına neden olan önemli bakteriyel etkenlerdendir. Bu çalışma, Aydın yöresinde *O. rhinotracheale* ve *B. avium*'un seroprevelansinin araştırılması amacı ile yapıldı. Serum örnekleri Aydın ilindeki işletmelerde bulunan solunum sistemi şikayeti olan 276 tavuk, 360 hindiden alındı ve bu serumlar ticari ELISA kitleri ile incelendi. *O. rhinotracheale* infeksiyonu yönünden tavukların % 66,3'ü, hindilerin % 11,1'i; *B. avium* infeksiyonu yönünden hindilerin % 29,1'i pozitif olarak tespit edildi. Böylece, Aydın yöresindeki tavuk ve hindilerde *O. rhinotracheale* ve *B. avium* antikorlarının varlığı ilk kez bu çalışma ile serolojik olarak belirlendi.

Anahtar Sözcükler: *Ornithobacterium rhinotracheale*, *Bordetella avium*, kanatlı hayvanlar, ELISA

Introduction

Respiratory diseases cause heavy economic losses in poultry. *Ornithobacterium rhinotracheale* is a recently described pathogen and it is associated with respiratory diseases characterized with decreased growth rate and increasing mortality rate in poultry (1-3). It is a slow growing, pleomorphic, Gram-negative, rod-shaped bacterium of the rRNA superfamily V and is relatively a new bacterium associated with poultry diseases worldwide. *O. rhinotracheale* has been isolated from the chicken, chukar partridge, duck, goose, guinea, fowl, gull, ostrich, pheasant, pigeon, quail, rook and turkey

(1,2). Diagnosis of *O. rhinotracheale* infection is based on its isolation and identification, serologic tests and polymerase chain reaction (PCR). Using boiled extract antigens (BEAs) and monovalent antiserum in the AGP test, 18 different serotypes of *O. rhinotracheale* have been distinguished (3,4). Using BEAs in an enzyme linked immunosorbent assay (ELISA), *O. rhinotracheale* cannot only be serotyped but can also be distinguished from other relevant, potentially pathogenic Gram-negative rods and from other species which *O. rhinotracheale* could be confused with. In ELISA, cross reactions within the serotypes of *O. rhinotracheale* do occur, mainly among the serotypes A, B, D, E, I and O. Serology allows

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detection of antibodies against a pathogen long after infection, whereas bacterial strains can only be isolated during active infection (3-5).

Bordetella avium is the causative agent of bordetellosis, which is commonly referred to as turkey coryza. Bordetellosis is a highly contagious disease of the upper respiratory tract of turkeys characterized by abrupt onset of sneezing, oculonasal discharge, growth depression and predisposition to other infectious diseases (6,7). *B. avium* causes bordetellosis in domesticated turkeys (8) and is an opportunistic pathogen in chickens (9) with as high as 80%-100% morbidity rate and usually less than 10% mortality rate (8). In order to control the disease, various serological procedures such as microagglutination (MA) test and ELISA have been developed to detect *B. avium* antibodies in infected or vaccinated turkeys. Due to its sensitivity and rapid determination of the disease agent, ELISA has become the most preferred serologic test in poultry laboratories processing lots of sera (9-11).

Studies concerning the detection of the *B. avium* and *O. rhinotracheale* are very limited in Turkey and so far no study has been conducted to determine the presence of these agents in Aydin. Therefore, the aim of the study was to determine whether or not *B. avium* (in turkeys) and *O. rhinotracheale* (in both chickens and turkeys) antibodies were present by ELISA in poultry in Aydin.

Materials and Methods

Serum Samples: All samples were collected from poultry enterprises in and around Aydin. Blood samples of 276 chickens and 360 turkeys with suspected respiratory disease were drawn into a sterile plastic tube and permitted to clot. Serum samples were separated and stored in microfuge tubes at -20 °C until use.

Chicken Serum Samples: The serum sample set included 21 flocks with ages ranging from 1 to 86 weeks. Chicken serum samples were collected between October 2003 and June 2003. Five to twenty-one serum samples were collected from commercial chicken flocks. Breeding type, age, clinical signs, number of serum samples tested and ELISA results are given in Tables 1 and 2.

Out of 21 flocks from which blood samples were taken, 7 were broilers and 14 were layer breeders. Two hundred seventy-six serum samples were analyzed for the presence of antibodies against *O. rhinotracheale* by using a commercial ELISA kit according to the manufacturer's recommendations of Hafez et al. (5).

Serology: Serum samples were tested for antibodies against *O. rhinotracheale* by ELISA. *O. rhinotracheale* ELISA kit (Biocheck, Gouda, The Netherlands) was used to measure the concentration of antibody against *O. rhinotracheale* in the serum. The test and decision-making procedures were carried out according to the

Table 1. Description of the broiler flocks.

Number of enterprises	Age (weeks)	Clinical findings	Number of samples tested	ELISA result (Number of <i>O. rhinotracheale</i> positive serum samples)
1	9	a, b	12	7
2	1	a, b	10	-
3	2	e	5	5
4	6	a	10	6
5	12	f	5	5
6	35	a, b, d	11	11
7	3	a, b, c	10	-
TOTAL			63	34

a: Retarded growth b: Dyspnoea c: Lachrymation d: Lack of appetite e: Clinical symptom unseen
 f: No information was obtained h: High mortality

Table 2. Description of the layer flocks.

Number of enterprises	Age (weeks)	Clinical findings	Number of samples tested	ELISA result (Number of <i>O. rhinotracheale</i> positive serum samples)
1	43	a, b	15	13
2	86	h	5	5
3	10	b	5	4
4	44	a, b	18	17
5	16	a, b, c, d	11	11
6	26	a, b, c, d	13	11
7	35	a, b, c, d	15	10
8	20	*	25	20
9	43	*	19	11
10	42	a, b, c	21	10
11	14	a, b, c, d	15	6
12	42	a, b, c, d, h	20	10
13	12	*	16	11
14	15	e	15	10
TOTAL			213	149

a: Retarded growth b: Dyspnoea c: Lachrymation d: Lack of appetite e: Clinical symptom unseen
 *: No information was obtained h: High mortality

manufacturer's recommendations as follows: first, samples were diluted at 1/100 ratio and assayed as duplicated samples. Optical density (OD) of the samples was measured by ELISA reader (Organon Teknika Reader 530, version 1.20) at 405 nm. The judgement of the ELISA readings was made after calculating the sample to positive ratio (S/P). Samples having 1.0 S/P or higher were considered positive while samples with 0.499 S/P or less were considered negative. S/P between 0.500 and 0.999 were considered suspected.

Turkey Sera Samples: Turkey serum samples were collected between October 2003 and July 2003 from turkey enterprises. The sample set included 11 flocks with ages ranging from 5 to 56 weeks. All eleven flocks were breeders. Thirty to forty-five serum samples per flock were collected from commercial chicken flocks. A total of 270 turkey serum samples were analyzed for the presence of antibodies against *O. rhinotracheale* by ELISA kit (Biocheck, Gouda, The Netherlands) according to standard procedures recommended by Hafez et al. (5). Three hundred sixty turkey serum samples were also analyzed for the presence of antibodies against *B. avium* using an ELISA kit (KPL, Maryland, US) according to the manufacturer's

recommendations. Clinical signs, number of tested serum samples and ELISA results are given in Table 3.

Serology: *B. avium* ELISA was carried out according to the manufacturer's recommendations as follows: first, samples were diluted at 1/100 ratio and assayed as duplicated samples. OD of the serum samples was measured by ELISA reader (Organon Teknika Reader 530, version 1.20) at 405 nm. The judgement of the commercial ELISA was made after calculating the S/P ratio. Samples with 1.0 or greater S/P ratio were considered positive.

Results

Antibodies were detected serologically with ELISA kits for *O. rhinotracheale* (chicken and turkey) and *B. avium* (Turkey). The results showed that 66.3% of chicken serum samples and 11.1% of turkey serum samples were positive for *O. rhinotracheale* and 29.1% of turkey serum samples were positive for *B. avium*. Thus, *O. rhinotracheale* and *B. avium* antibodies were detected by ELISA for the first time in Aydın. *O. rhinotracheale* and *B. avium* ELISA results are given in Table 4.

Table 3. Description of the turkey flocks.

Clinical findings	Number of samples tested	ELISA results (Number of the <i>O. rhinotracheale</i> positive serum samples)	ELISA results (Number of the <i>B. avium</i> positive serum samples)
*	30	7	-
*	30	3	-
One day old	30	-	-
One day old	30	-	-
*	30	2	5
*	30	2	7
*	30	1	28
*	30	2	21
*	30	2	14
*	45	Not done	25
*	45	Not done	5
TOTAL	360	19	105

*: No information was obtained

Table 4. ELISA results of *O. rhinotracheale* and *B. avium*.

	Chicken Serum Samples (Total: 276)		Turkey Serum Samples (Total: 360)	
	<i>O. rhinotracheale</i>	<i>B. avium</i>	<i>O. rhinotracheale</i> (270 serum samples)	<i>B. avium</i> (360 serum samples)
The number of positive serum samples	Broiler (Total: 63 serum samples)	Not performed	30 (11.1%)	105 (29.1%)
	Layer (Total: 213 serum samples)			
	Total: 183 serum samples (66.3%)			
The number of positive serum samples for both <i>O. rhinotracheale</i> and <i>B. avium</i>			1	

As Table 4 shows, 66.3% of chicken serum samples and 11.1% of turkey serum samples were positive for *O. rhinotracheale* antibody and 29.1% of turkey serum samples were positive for *B. avium*. The results of most of the flocks showed high titers for *O. rhinotracheale* and *B. avium*. One serum sample was positive for both *O. rhinotracheale* and *B. avium*. All suspected sera samples (5 serum samples in chicken, 7 serum samples in turkeys for *O. rhinotracheale*; 6 serum samples in turkeys for *B. avium* were suspected) were evaluated as negative.

Discussion

In this study, presence of *O. rhinotracheale* and *B. avium* as respiratory disease was investigated serologically by ELISA in Aydin. Previous studies revealed that *O. rhinotracheale* 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 *O. rhinotracheale* (4, 5). Turan and Ak (12) also worked on the seroprevalence of *O. rhinotracheale* in Marmara and Western Black Sea

regions of Turkey and found that 65% of the serum samples coming from different flocks were positive. In our study, 66.3% serum samples tested positive for *O. rhinotracheale* from 21 flocks in and around Aydın. The results of this study were fairly similar to the findings of previous studies conducted in Turkey and Europe (4,5, 12).

In this study, ELISA test results showed that all layer flocks were positive whereas two breeder flocks were negative for *O. rhinotracheale*. A previous study conducted by Heeder et al. (13) reported that layer flocks had greater serologic prevalence for *O. rhinotracheale* than pullets (100% vs. 52%). In this study, presence of *O. rhinotracheale* in pullet (10-20 weeks of age) and layer (26-86 weeks of age) serum samples were 71% and 69%, respectively. These findings were parallel with the results reported by Turan and Ak (12). The presence of *O. rhinotracheale* antibody in pullet serum samples was higher than the number reported by Heeder et al. (13) and this could be due to the placing pullet flocks into multiple-age layer populations where they become exposed to *O. rhinotracheale* at earlier ages.

Previous reports also showed that transfer of maternal antibody was possible in *O. rhinotracheale* infections (14). Two turkey flocks were investigated for maternal antibody presence in day-old turkey chicks in the study and all serum samples were detected negative. The lack of maternal antibodies in our samples may be due to the fact that either the turkey flocks had not been exposed to the organism or maternal antibodies were not transferred to the chicks in detectable quantities.

Frequency of *O. rhinotracheale* infection incidence may vary depending on the season and is usually common in winter months (15). In the current study, collection of the serum samples took place mostly in winter and this may have an affect on the number of samples that tested positive.

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Comparing ELISA, MA and avidin-biotin-enhanced dot-immunobinding (AB-DIB) assays, Tsai and Saif (11) reported that ELISA was the most sensitive for detecting maternal antibodies for *B. avium*. Barbour et al. (16) developed a specific ELISA for *B. avium* and they found perfect positiveness for this agent. Ocak (17) investigated 1550 serum samples by ELISA for *B. avium* and found that 22.9% of serum samples were positive. In our study, 29.1% serum positiveness was detected and the results of these studies can be considered parallel with each other and give us an idea of the frequency of *B. avium* disease in cases with respiratory system symptoms.

Working on 170 bacterial isolates from birds with air sac infection from 100 poultry enterprises, El-Sukhon et al. (2) reported that 9 isolates were positive for *O. rhinotracheale* (5.3%), 2 isolates were positive for *B. avium* and only 2 samples (1.2%) tested positive for both *B. avium* and *O. rhinotracheale*. One serum was positive for both *B. avium* and *O. rhinotracheale* in this study. The results of this study were similar to the findings of the previous study (2).

In conclusion, presence of *O. rhinotracheale* and *B. avium* antibodies was serologically detected for the first time in hens and turkeys in Aydın. With this study, it has been determined that *B. avium* and *O. rhinotracheale* were threats to the hen and turkey population in Aydın. It has been thought that similar studies should be continued on defining the role of *B. avium* and *O. rhinotracheale* as pathogenic agents in hens and turkeys in Aydın.

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