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A Comparative Study of Detection of *Bordetella avium* Antibodies in Turkeys by ELISA, SPAT, and AGID Test

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Abstract: The aims of this study were to develop a serum plate agglutination test (SPAT) antigen and agar gel immunodiffusion (AGID) test antigen for the serological detection of turkeys that have been exposed to *Bordetella avium*; to compare the sensitivity of commercial enzyme-linked immunosorbent assay (ELISA) with SPAT, and AGID test, and to survey *B. avium* antibodies in turkey flocks in Aydın, Turkey. For these purposes, serum samples collected from 300 turkeys were examined by ELISA, SPAT, and AGID test. The seroprevalence was 44%, 28%, and 15% for ELISA, SPAT, and AGID test, respectively. This study revealed that ELISA was the most sensitive test for the detection of *B. avium* antibodies; however, it was determined that SPAT and AGID test can also be used in screening for *B. avium* antibodies.

Key Words: *Bordetella avium*, ELISA, SPAT, AGID

Hindilerde *Bordetella avium* Antikorlarının ELISA, SPAT ve AGID Testleri ile Belirlenmesi Üzerine Karşılaştırmalı Bir Araştırma

Özet: Bu çalışmanın amaçları *Bordetella avium*'a maruz kalmış hindilerin serolojik olarak saptanması için serum pleyt aglutinasyon test (SPAT) ve agar jel immunodiffuzyon (AGID) test antijenlerinin geliştirilmesi, ticari enzimle işaretli immün deneyi (ELISA) ile SPAT ve AGID testinin duyarlılıklarının karşılaştırılması, Aydın'da hindi sürülerinde *B. avium* antikorlarının serolojik olarak taranmasıdır. Bu amaçla, 300 hindiden toplanan serum örnekleri ELISA, SPAT ve AGID test yöntemleriyle incelendi. Seroprevalens ELISA, SPAT ve AGID testi için sırasıyla % 44, % 28, % 15 olarak belirlendi. Çalışmada, *B. avium* antikorlarının tespit edilmesinde en duyarlı testin ELISA olduğu; bununla birlikte, SPAT ve AGID testinin de *B. avium* antikorlarının belirlenmesinde kullanılabileceği tespit edildi.

Anahtar Sözcükler: *Bordetella avium*, ELISA, SPAT, AGID

Introduction

Bordetella avium is the causative agent of turkey coryza, which is a highly contagious upper respiratory tract disease of turkeys. The diagnosis of bordetellosis depends on clinical symptoms, lesions in the respiratory tract, isolation of *B. avium* from the upper respiratory tract, and serological tests. The most important problem in making the diagnosis is to distinguish *B. avium* from other *Bordetella*-like microorganisms. In this context, serological diagnosis is preferable because of its short time requirement and ease of use (1,2).

In order to detect *B. avium* antibodies, various serological procedures, such as micro agglutination test

(MAT) (3), antibody-based indirect fluorescence test (IFAT) (4), and enzyme-linked immunosorbent assay (ELISA) were developed (5-7). Ocak (8) reported that ELISA, MAT, and IFAT were sensitive in the indirect diagnosis of *B. avium* infections. Although the price of the ELISA kit was considered a disadvantage, it was the most preferable serological test in poultry laboratories because of its sensitivity and rapid characteristics (7,9).

For rapid diagnosis and surveillance studies of this disease, practical serological tests would be of great benefit. In this study, serum plate agglutination test (SPAT) was developed for rapid serological detection of turkeys exposed to *B. avium*. The test relies on the

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agglutination of bacteria and is biased towards the detection of IgM antibodies. For this reason, SPAT was suitable for acute stage diagnosis of infections. Interpretation of the SPAT was visual, and there was no difficulty in detection of the positive and negative samples (10). There were no studies in the literatures on the determination of antibodies produced for *B. avium* with agar gel immunodiffusion (AGID) test. The test relies on the precipitation of soluble bacterial antigens and is biased towards detection of IgG antibodies (11). For this reason, AGID was suitable for diagnosis of the infections in chronic stages.

This study was conducted to develop the SPAT and AGID test antigens for rapid serological detection of turkeys exposed to *B. avium*, to compare the commercial ELISA with SPAT and AGID test, and to conduct a serosurvey of *B. avium* in turkey flocks in Aydin, Turkey.

Materials and Methods

Field Serum Samples

All serum samples were collected from commercial turkey enterprises in Aydin. Samples from 300 turkeys with symptoms of respiratory disease constituted the material for this study. Blood samples were placed in sterile plastic tubes for 30 min for clotting. After the clotting the serum was separated with a sterile glass stick. The clot was precipitated after centrifugation at 1500 rpm for 10-15 min. The separated clot was distributed to sterile tubes with a sterile pipette. Sera were then separated and stored in microfuge tubes at -20 °C until used. Serum samples were collected from December 2003 to April 2004. The samples were taken from 9 flocks ranging from 3 to 35 weeks in age. The 9 flocks from which blood samples were obtained were meat-type. Serum samples (30-45) from each flock were collected for the study.

Commercial ELISA

Serum samples were analysed by commercial ELISA kit (KPL, Maryland, USA) following standard procedures. Samples were diluted to 1/100 and assayed in duplicate. The optical density (OD) was measured with an ELISA reader (BioTek ELx808) at 405 nm. The evaluation of the commercial ELISA was performed by calculating the sample to positive ratio (S/P). Samples with 1.0 S/P or greater were defined as positive.

Standard *B. avium* Strain

Standard *B. avium* strain for preparing the SPAT and AGID test antigens was obtained from the Department of Microbiology, Faculty of Veterinary Medicine, Ankara University, Turkey.

Preparation of the Antisera

Antisera for *B. avium* were prepared in specific-pathogen-free (SPF) chickens. Birds that were 4 weeks old were inoculated via the intranasal route with 0.5 ml 10^7 colony-forming units (CFU)/ml of *B. avium* culture. The birds were then boosted intramuscularly at 5 and 6 weeks of age with 1 ml of formalin-killed *B. avium* culture, containing 10^9 CFU/ml. At the 7th week of age, blood samples were obtained and sera were separated and stored in microfuge tubes at -20 °C until used (10).

Preparation of the Negative Control Sera

Preparation of the negative control sera was performed in 4-week-old SPF chickens. After 4 weeks, the birds were slaughtered. Blood samples were placed in sterile plastic tubes and permitted to clot; then sera were separated and stored in microfuge tubes at -20 °C until used.

Development of SPAT to Detect Antibodies to *B. avium*:

Standard *B. avium* strain was used for preparing antigen. To prepare antigen for the SPAT, microorganisms were grown on sheep blood agar for 24 h at 37 °C in aerobic conditions. Maximum growth of *B. avium* in 48 h was obtained by using heavy inoculums. The cells were harvested by scraping the bacterial growth and were then diluted with distilled water. The bacteria were inactivated with 0.8% formalin and incubated for 3 h at 37 °C in aerobic conditions. The cells were harvested by centrifugation at 3000 xg for 10 min, and pellets were suspended in distilled water (10). To determine the optimal cell concentration for the SPAT, serial 2-fold dilutions of the antigen were made and titrated with a constant volume of known positive *B. avium* serum. The highest dilution of antigen that produced clear agglutination with positive serum, and no agglutination with negative serum, was chosen for further use. One milliliter of 1:100 diluted Rose Bengal dye in distilled

water was added to each 100 ml of the antigen. For the SPAT, 25 µl antigen and 25 µl sera were mixed on a slide. The slide was rotated and the presence or absence of agglutination within 1 or 2 min was recorded. A serum sample was considered positive when clear agglutination was seen and the absence of agglutination was interpreted as negative for *B. avium* antibodies (10).

Preparation of AGID Test Antigen

AGID test antigen was prepared as described by Gil-Turnes et al. (12). The microorganism was grown on 7% sheep blood agar for 48 h at 37 °C in aerobic conditions. The cells were harvested by scraping the bacterial growth and suspending the cells in distilled water. The suspension was frozen and thawed 10 times and centrifuged at 2500 xg for 15 min. The supernatant, including 0.01% merthiolate, was kept at 4 °C until used.

AGID Test

The test was carried out in petri dishes with 1.5% Noble agar, 8.5% NaCl, and 0.1% thimerosal in accordance with the method described by Van Empel et al. (13). A hexagonal pattern was cut into the agar layer that consisted of a central well surrounded by 6 peripheral equidistant wells approximately 5 mm from the central well. The diameter of each well was 3 mm. The central well was filled with antigen extract and the peripheral ones with two-fold dilutions of field serum samples. The petri dishes were incubated for at least 72 h in a moist chamber at 37 °C and were then observed for precipitation lines under UV light.

Standardization of AGID Test

Two-fold dilutions of both antiserum and antigen were made. AGID test was performed by placing antigen dilutions into the central well and positive serum dilutions into the peripheral wells, and then examining for precipitation. After the standardization, it was determined that the antigen that was diluted in half can be used as test antigen. Field sera were evaluated using these antigen dilutions.

Statistical Analysis

Seropositivity of ELISA and the other 2 tests was analysed by McNemar test. Kappa test was also used to investigate the consistency of ELISA with SPAT and AGID tests (14,15).

Results

In this study, antibodies were detected serologically by using commercial *B. avium* ELISA kit and the results were compared those of SPAT and AGID tests (Table 1).

ELISA Results: ELISA results showed that 44% of turkeys (132 of 300 sera) were positive.

SPAT Results: SPAT results showed that 28% of turkeys (84 of 300 sera) were positive.

AGID Test Results: AGID results showed that 15% of turkeys (45 of 300 sera) were positive.

None of the 1-day-old turkeys were found positive by all 3 tests. The other age groups had a higher prevalence as determined by ELISA than with SPAT and AGID tests.

Table 1. Results of ELISA, SPAT, and AGID test of turkey sera.

Age	Number of samples tested	Number of positive samples		
		ELISA	SPAT	AGID
One day old	30	-	-	-
One day old	30	-	-	-
2 weeks	30	14	6	8
5 weeks	30	20	12	6
6 weeks	30	26	17	8
10 weeks	30	28	18	10
15 weeks	45	26	20	6
30 weeks	45	10	9	1
35 weeks	30	8	2	6
TOTAL	300	132	84	45
Seropositive		(44%)	(28%)	(15%)

Statistical Analysis: Consistency of ELISA with SPAT was statistically significant ($P < 0.001$). The difference between ELISA and AGID test was statistically significant ($P < 0.001$).

Specificity and sensitivity of SPAT and AGID tests to ELISA for seropositivity for *B. avium* in turkey sera are given in Tables 2 and 3.

Discussion

In the present study, *B. avium* infections were investigated serologically with commercial ELISA kit and the results were compared with those of SPAT and AGID test.

Bordetellosis (turkey coryza) is a highly contagious upper respiratory disease, primarily affecting turkeys at 2-6 weeks of age (1,2). It was found that the antibody against *B. avium* was common for all age groups except for 1-day-old turkeys. Results of the present study are in agreement with those of previous studies (1,2).

It was reported that ELISA was the most sensitive test for the detection of antibodies for *B. avium* (7). Barbour et al. (9) developed a specific ELISA for *B. avium* and found perfect positivity for this agent. Ocak (8) investigated 1550 serum samples and found 22.9% seropositivity. In this study, seropositivity was 33.3%, and this result parallels previous studies (7-9).

Respiratory diseases and economic losses related to *B. avium* infection in turkeys in Aydın were studied by

Türkyılmaz, who reported that there was an urgent need for a readily applicable test to detect infected birds, and that a flock surveillance system for monitoring turkeys for *B. avium* infection would be useful (16). The antigen used in the present SPAT was a whole-cell antigen. Back (10) has developed a SPAT antigen for the diagnosis of *O. rhinotracheale* infection in poultry. Slide agglutination test antigen, prepared with the same method in that study, was used for the diagnosis of the *B. avium* infections in turkeys in the present study. It was concluded that this method was useful for this purpose. The AGID test is used for routine diagnosis of poultry serum samples as a serological test. Nevertheless, diagnosis by AGID test is time consuming (11,12). In recent years, ELISA has been preferred to AGID test.

In the present paper, it was determined that SPAT was more sensitive than AGID test. One of the reasons for the lower sensitivity of AGID test could be that it high efficiency in the determination of IgG antibodies. It was concluded that SPAT and AGID test could be used for the diagnosis of the *B. avium* infections in turkeys, though the sensitivity of these tests were lower than ELISA.

Consequently, our study showed that the seroprevalence of *B. avium* antibodies were high in commercial turkey enterprises in Aydın. Although SPAT and AGP test could be used in the screening for *B. avium* infections, the study revealed that ELISA was the most sensitive test.

Table 2. Specificity and sensitivity of SPAT compared to ELISA for seropositivity for *B. avium* in turkey sera.

	SPAT (-)	SPAT (+)	TOTAL
ELISA (-)	168	0	168
ELISA (+)	48	84	132
TOTAL	216	84	300

Kappa: 0.66 ($P < 0.001$),
Sensitivity: 77%; Specificity: 100%

Table 3. Specificity and sensitivity of AGID test compared to ELISA for seropositivity for *B. avium* in turkey sera.

	AGID (-)	AGID (+)	TOTAL
ELISA (-)	168	0	168
ELISA (+)	87	45	132
TOTAL	255	45	300

Kappa: 0.36 ($P < 0.001$),
Sensitivity: 65%; Specificity: 100%

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