TÜBİTAK

Short Communication

Turk. J. Vet. Anim. Sci.
2009; 33(2): 147-150

doi:10.3906/vet-0712-29

Detection of *Bordetella avium* by Polymerase Chain Reaction in the Lungs and Tracheas of Turkeys with Pneumonia

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Received: 31.12.2007

Abstract: Lung, trachea, and serum samples obtained from turkeys showing clinical signs of respiratory disease were examined for the presence of *Bordetella avium* infection (bordetellosis) using culture, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR). The material of this study consisted of 2 live turkeys at the age of 24 weeks with respiratory distress, nasal discharge, and cough that had been brought to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Adnan Menderes University. The results of the study show that *B. avium*, the causative agent of bordetellosis, was not isolated from either the lung or trachea, while both trachea and lung samples were positive in PCR. The presence of antibodies against *B. avium* was detected in all serum samples by ELISA. However, sera samples were negative in PCR. In conclusion, it was thought that ELISA is a practical assay while the results of ELISA and PCR were found complementary.

This study reported the presence of *B. avium* from turkeys in Turkey for the first time by PCR. However, further studies should be planned with more materials to understand the epidemiological importance of the disease in poultry in this region.

Key Words: *Bordetella avium*, turkey, isolation, ELISA, PCR

Bordetella avium'un Pnömonili Hindilerin Akciğer ve Trachealarından Polimeraz Zincir Reaksiyonu ile Saptanması

Özet: *Bordetella avium* infeksiyonunun (bordetellozis) varlığı solunum sistemi hastalığı belirtileri gösteren hindilerden alınan akciğer, trake ve serum örneklerinin kültür, enzyme-linked immunosorbent assay (ELISA) ve polimeraz zincir reaksiyonuyla (PCR) incelenmesiyle araştırıldı. Çalışmanın materyalini Adnan Menderes Üniversitesi Veteriner Fakültesi Mikrobiyoloji Anabilim Dalı'na ciddi solunum güçlüğü, burun akıntısı ve öksürük şikâyetleri ile getirilen 24 haftalık yaşta iki adet hindi oluşturmaktadır. Çalışma sonucunda, bordetellozis etkeni olan *B. avium*, akciğer ve tracheadan izole edilemez iken; bu organlar PCR'da pozitif olarak belirlendi. İncelenen her iki serum örneğinde *B. avium*'a karşı oluşan antikorlar ELISA ile saptandı. Bununla birlikte, serum örnekleri PCR'da negatif sonuç verdi. Bu çalışmada ELISA'in pratik bir yöntem olduğu; ELISA ile PCR'nun birbirlerini tamamlayıcı yöntemler olarak kullanılabilecekleri sonucuna varıldı.

Bu çalışma ile Türkiye'de hindilerde *B. avium*'un varlığı PCR ile ilk kez belirlendi. Bununla birlikte, yörede kanatlı populasyonunda hastalığın epidemiyolojik öneminin anlaşılabilmesi için daha fazla sayıda materyal kullanılarak çalışma yapılması gerekmektedir.

Anahtar Sözcükler: *Bordetella avium*, hindi, izolasyon, ELISA, PCR

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Bordetella avium is a gram-negative aerobic bacterium responsible for the avian disease referred to as bordetellosis, a highly contagious upper respiratory disease of young poultry. The micro-organism causes bordetellosis in domesticated turkeys (1) and is an opportunistic pathogen in chickens (2). In field cases only low isolation rates of *B. avium* can be seen. Due to the difficulties experienced in isolating and identifying *B. avium*, many think that this disease may be under-diagnosed (1,3). Because of evidence that suggests that recovered birds may periodically relapse or remain asymptomatic carriers, the need for a sensitive and consistent diagnostic test is significant. In addition to culturing, serological tests were developed to identify *B. avium* infections (4). Serology is also important, and both microagglutination (detects IgM) and ELISA (detects IgG) tests are available. The ELISA test generally detects specific antibodies >2 weeks after infection and has the added benefit of detecting maternal antibody (3-5). Recently, a polymerase chain reaction (PCR)-based assay for the detection of *B. avium* became available (6,7).

Most research to date on the effects of *B. avium* has focused on the turkey because of the economic impact of this disease on the poultry industry (5,8,9). In Turkey, a few serological studies are available related to bordetellosis (9,10), but there is no study available in the literature on detection of *B. avium* by PCR. In the present study, lung, trachea, and serum samples obtained from turkeys showing clinical signs of respiratory disease were examined for the presence of *B. avium* infection (bordetellosis) using culture, enzyme-linked immunosorbent assay (ELISA), and polymerase chain reaction (PCR).

The materials were 2 live turkeys with severe respiratory distress obtained from a turkey flock in Aydın province. The first clinical signs (eye and nasal discharge, gasping, rhinitis, cough, and dyspnoea) appeared at about 14-15 weeks of age. The outstanding clinical feature of animals was the conspicuous deep dry honking cough that continued for 15-20 days. The turkeys were treated with 0.04 g/bird enrofloxacin (Baytril) for 5 days via drinking water. The clinical history of this flock was excellent and these animals had never received any vaccination. From the animals that were still coughing at 24 weeks of age 2 were necropsied. At necropsy, lung, trachea, and blood samples were collected from the animals for PCR, bacteriological, and serological tests.

The standard *B. avium* 002 strain was obtained from Dr. Y.M. Saif (Food Animal Health Research Program Wooster, Ohio, USA) and used for positive control in PCR.

After necropsy, lungs and tracheas were collected aseptically and immediately streaked on blood agar with 7% sheep blood and MacConkey agar. Lung and trachea samples used in the bacteriological assay were stored at -20 °C. The frozen samples were used for PCR later. Sera were obtained from whole blood and kept at -20 °C until used in ELISA and PCR later.

Serum samples were analysed by commercial ELISA kit (KPL, Maryland, US) following standard procedures. The samples were diluted 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.

For PCR, DNA extraction from standard *B. avium* strain (positive control for PCR), internal organs (trachea and lungs), and sera was performed. DNA extraction from the standard *B. avium* strain was performed by standard boiling method. Whole-cell lysates used as templates were prepared by suspending a colony, ~1.5 mm in diameter or the equivalent, in 10 µl of water. The mixture was boiled for 10 min, placed on ice until chilled, and centrifuged at 16,000 xg for 1 min to pellet cell debris. Supernatant (2.5 µl) was used as the template in each PCR (7).

DNA extraction from internal organs was performed using a commercial NucleoSpin Tissue mini column (Macherey-Nagel, Düren, Germany) as recommended by the manufacturer.

DNA extraction from sera was performed using a DNA extraction kit (Fermentas) as recommended by the manufacturer.

B. avium-specific primer sequences were obtained from the previous report by Savelkoul et al. (6). The sequence of the forward primer was modified by deletion of the two 5' bases due to inconsistencies at these 2 positions between the report and the corresponding *B. avium* sequence deposited in GenBank by the same authors (accession no. X74117). The corrected primers are as follows: the forward primer chosen was 5'CGGCGTCAACACATACTCTTGAT 3' (1184-1206). The reverse primer was 5'AGGGAGGTCAGATAGCTCTAGAAT

3' (1707-1684). This primer set generates an amplicon of approximately 524 bp. Presence of bacterial DNA was confirmed using universal primers as indicated in previous studies (7,11,12).

Amplification reactions were carried out in an Eppendorf thermal cycler (Eppendorf AG, Hamburg, Germany). PCR experiments were carried out in a total volume of 25 µl and the following selected conditions were used: 1 U AmpliTaq polymerase (Applied Biosystems, Foster City, CA, USA), 2.5 µl 10X Taq buffer II (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 5% dimethyl sulphoxide (DMSO), 1.5 mM $MgCl_2$, 0.5 µM primers, and 200 µM deoxynucleoside triphosphates, and 2.5 µl of template sample DNA in a final volume of 25 µl. Amplification was achieved with an initial denaturation step at 95 °C for 5 min, followed by 35 cycles at 95 °C for 30 s, and 50 °C for 30 s and 30 s at 72 °C, followed by a final extension step of 5 min at 72 °C (7). Ten microlitres of PCR products were separated on a 2% agarose gel with 0.5 µg/ml ethidium bromide. The DNA fragments were visualised by UV illumination and photographed with Polaroid film. The molecular sizes of the PCR products were compared with a 250 bp DNA ladder.

After 72 h of incubation on blood agar at 37 °C no bacteriological growth was seen. At necropsy all birds showed severe airsacculitis, tracheitis, and congestion of the lung accompanied by pneumonia. Serum from both turkeys tested was positive for antibodies against *B. avium* by ELISA.

The serological test results were confirmed by PCR. Both suspicious trachea and lung samples were positive in PCR. Approximately 524 bp amplification product was obtained, corresponding to the expected size. However, sera samples were negative in PCR (Figure). No amplified products were obtained from the negative control.

Özbey and Muz (13) reported information on the presence of *B. avium* in chickens from Turkey. These researchers examined the lungs of 250 chickens with pneumonia in Elazığ and they reported isolation of *B. avium* from 3 (1.2%) samples. In our investigation, *B. avium* was not isolated from lung or trachea samples. However, we report the first detection of *B. avium* by PCR from turkeys' internal organs (lungs and trachea) in

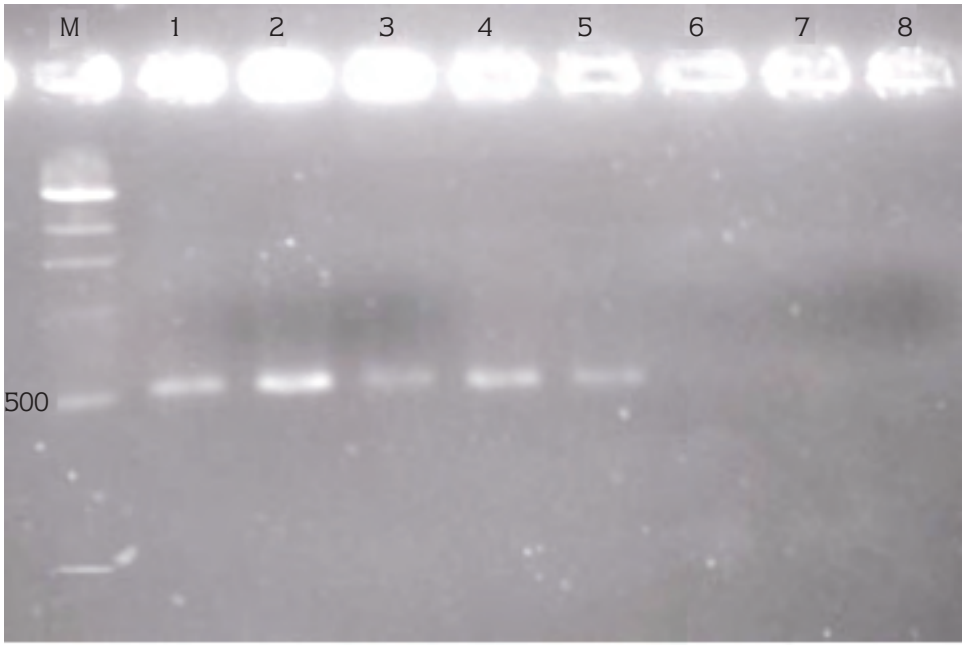

Figure. Identification of DNA amplified fragments by agarose gel electrophoresis (7). An amplicon of 524 bp was obtained. Lanes: M = 500-bp DNA ladder; Lane 1 = Trachea DNA from patient 1, Lane 2 = Lung DNA from patient 1, Lane 3 = Trachea DNA from patient 2, Lane 4 = Lung DNA sample from patient 2, Lane 5 = Positive control (Standard *B. avium* 002 strain DNA), Lane 6 = Serum DNA from patient 1, Lane 7 = Serum DNA from patient 2, Lane 8 = Negative control without DNA.

Turkey. The reason for the failure of the bacteriological isolation from the turkeys could be the administration of antibiotic for 5 days before the animals were brought to the laboratory.

Respiratory diseases and economic losses related to *B. avium* infection in turkeys in Aydın were studied by Türkyılmaz and Kaya (10), who reported that there was an urgent need for a readily applicable test to detect infected birds, and a flock surveillance system for monitoring turkeys for *B. avium* infection would be useful.

It was reported that ELISA was the most sensitive serologic test for the detection of antibodies for *B. avium* (4,14). There is a paucity of data in the literature about this subject in Turkey. The first investigation was carried out by Ocak (9). In that study, a total of 1550 serum samples from broilers and turkeys were examined and 22.9% of the samples were seropositive by ELISA. Moreover, it has been reported that 29.1% of the samples from turkeys were seropositive in Aydın district by ELISA (10). In the present study, it was determined that the serum samples from the 2 turkeys were seropositive, but positive results were not obtained from PCR carried out using DNA extracted from these serum samples. The negative PCR from ELISA-positive sera reflects the absence of bacteria but the presence of *B. avium*-specific antibodies in the blood.

This study reported the first demonstration of *B. avium* by PCR in turkeys from Turkey. The results presented here show that the complementary use of ELISA and PCR as rapid screening tests shows considerable promise for the diagnosis of bordetellosis in turkeys. However, further studies are required to understand the epidemiological importance of this disease in the poultry population of this region.

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