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Short Communication

Detection of Antibodies Produced in Quails (*Coturnix coturnix japonica*) against *Mycoplasma gallisepticum* with Different Serological Tests

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Abstract: The objectives of this study were to isolate and identify *Mycoplasma gallisepticum* in quails with clinical and pathological signs of chronic respiratory disease (CRD), and to compare the sensitivity and specificity of commonly used serological tests that are used for the detection of *M. gallisepticum* antibodies. The study included 20 Japanese quails 10-weeks old with respiratory distress, nasal discharge, mortality and swollen infraorbital sinuses. There was no bacteriological agent isolated. All of the 20 sera samples obtained from the quails were evaluated with AGID, RSA, HI, and ELISA; 2 (10%), 5 (25%), 10 (50%), and 17 (85%) sera samples were positive, respectively. In conclusion, for detecting *M. gallisepticum* antibodies in quails the use of ELISA, which is a very sensitive test, together with other conventional tests was beneficial.

Key Words: *Mycoplasma gallisepticum* antibodies, serologic tests, quail

Bıldırcınlarda (*Coturnix coturnix japonica*) *Mycoplasma gallisepticum*'a Karşı Oluşan Antikorların Çeşitli Serolojik Testlerle Saptanması

Özet: Bu çalışmada klinik ve nekropsi bulgularına göre kronik solunum yolu (CRD) infeksiyonundan şüphelenilen bıldırcınlarda *Mycoplasma gallisepticum*'u (MG) izole ve identifiye etmek; MG infeksiyonlarında oluşan antikorlarının saptanmasında en çok kullanılan serolojik testlerin sensitiviteve ve spesifitelerini karşılaştırmak amaçlanmıştır. Çalışmanın materyalini solunum güçlüğü, infraorbital sinüslerde şişkinlik, mortalite, göz-burun akıntısı gibi şikayetlerle getirilen 10 haftalık 20 bıldırcın oluşturdu. Bakteriyolojik incelemelerde etken izolasyonu yapılamadı. Serumların hepsi AGID, RSA, HI ve ELISA ile değerlendirildi. Sırasıyla 2 (% 10), 5 (% 25), 10 (% 50) ve 17 (% 85) pozitif serum saptandı. Bıldırcınlarda MG antikorlarını saptamada konvansiyanel testlerle birlikte ELISA gibi duyarlılığı yüksek bir yöntemin kullanılmasının faydalı olabileceği sonucuna varıldı.

Anahtar Sözcükler: *Mycoplasma gallisepticum* antikorları, serolojik testler, bıldırcın

Mycoplasma gallisepticum is the most economically significant mycoplasma pathogen affecting poultry and has a worldwide distribution. *M. gallisepticum* infection is commonly designated as chronic respiratory disease (CRD) in chickens and infectious sinusitis in turkeys. Infection may also occur in pheasants, partridges, and peafowl (1-3). Routine detection of *Mycoplasma* spp. infection depends on isolation of the organism, with subsequent identification of the isolates or detection of specific antibodies in sera (4). Although some basic biochemical tests can be helpful in preliminary classification of *Mycoplasma* spp. isolates, definitive identification must be performed by serological tests. Several serological tests have been used to detect *M. gallisepticum* antibodies, but their specificity and sensitivity have been lacking to some degree in all of them (4). The most commonly used serological tests for *M. gallisepticum* are the rapid serum agglutination (RSA) test, hemagglutination inhibition (HI) test, agar gel immunodiffusion (AGID) test, and enzyme-linked immunosorbent assay (ELISA) (4,5). Because of its high sensitivity and ease of use, ELISA represents a potential replacement for the AGID, RSA, and HI tests (5).

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The objectives of this study were to isolate and identify *M. gallisepticum* in quails with clinical and pathological signs of CRD and to compare the sensitivity and specificity of the commonly used serological tests (AGID, RSA, HI, ELISA) used for the detection of *M. gallisepticum* antibodies.

The study included 20 Japanese quails 10-weeks old with respiratory distress, nasal discharge, and swollen infraorbital sinuses that were examined at the Faculty of Veterinary Medicine Microbiology Laboratory, Adnan Menderes University, Turkey. Lung and trachea samples for microbiological examinations, and blood samples for serologically examinations were taken from quails necropsied in aseptic conditions. Cultures were grown on general (blood agar) and selective (PPLO broth and agar) mediums from suspected lungs and tracheas (4). For serologic tests, preparation of antiserum was carried out on rabbits in accordance with a method described by Timms (6). Standard *M. gallisepticum* antigen (Intervet International, Holland) was used with the RSA test for the detection of *M. gallisepticum* antibodies in the collected sera. The RSA test was conducted with crystal violet stained *M. gallisepticum* antigen. For *M. gallisepticum* hemagglutination (HA) and HI antigen, the S6 strain of *M. gallisepticum* was used. For this, the *M. gallisepticum* strain was propagated in PPLO broth supplemented with 10% fresh yeast extract and 20% horse serum. Penicillin and thallium acetate were added to the medium when needed. The S6 strain of *M. gallisepticum* was incubated for 24 h at 34 °C in a humidified CO_2 incubator. The culture contained 10^9 colony-forming units (CFU) ml^{-1} and was used as an antigen in the HA and HI tests (6,7). HA and HI tests were performed as described by Matsuo et al (8). The AGID test was performed in a medium that contained 8% NaCl and 1.25% Noble Agar (Difco) (9). The antigen that was produced for HI and HA tests was frozen at –20 °C, thawed for 10 min, disrupted by sonicating (Sonic, 300 Dismembranator, Fisher Scientific, 20,000 rpm) for 10 min, stored in a refrigerator for 2-3 h for precipitation, and then the supernatant was used as the AGID test antigen (9). The AGID test was performed as described by Nonomura and Yoder (9). The commercial *M. gallisepticum* ELISA (KPL, Maryland, US) was conducted according to the manufacturer's instructions.

In this study there was no bacteriological growth in any of the lung and tracheal samples. In all, 20 serum samples were examined individually with RSA, HI, AGID, and ELISA.

For the 20 sera samples, the rate of positive test results varied according to test: 2 (10%) with AGID, 5 (25%) with RSA, 10 (50%) with HI, and 17 (85%) with ELISA. The serological test results are given in Table 1.

The sensitivity and specificity of ELISA to AGID, RSA, and HI tests in quail sera are presented in Table 2.

The sensitivity of AGID, RSA, and HI was 11%, 29%, and 58%, respectively, and the specificity of all 3 tests was 100% (Table 2).

The clinical symptoms of *M. gallisepticum* infection are rales, cough, respiratory distress, inflammation, and swelling of sinuses (4). In the present study, the quails that were examined in the laboratory exhibited respiratory distress, coughing, sneezing, and swollen infraorbital sinuses. Bacteriological isolation was negative for all samples that were taken from lungs and tracheas. Since there was no bacteriological isolation, it was

Table 1. Serological test results.

Sera No	RSA	HI (titer)	AGID	ELISA
1	+	-	-	+
2	+	+ (1/160)	-	+
3	+	+ (1/160)	-	+
4	-	-	-	-
5	+	+ (1/160)	-	+
6	-	-	-	+
7	-	-	-	+
8	+	+ (1/160)	+	+
9	-	-	-	+
10	-	+ (1/320)	+	+
11	-	+ (1/80)	-	+
12	-	-	-	+
13	-	+ (1/160)	-	+
14	-	-	-	+
15	-	-	-	+
16	-	+ (1/80)	-	+
17	-	+ (1/160)	-	+
18	-	+ (1/80)	-	+
19	-	-	-	-
20	-	-	-	-
TOTAL	5 (25%)	10 (50%)	2 (10%)	17 (85%)

Table 2. Specificity and sensitivity of AGID, RSA, and HI tests.

	ELISA (+)	ELISA (-)	TOTAL
*AGID (+)	2	-	2
*AGID (-)	15	3	18
*TOTAL	17	3	20
** RSA (+)	5	-	5
** RSA (-)	12	3	15
**TOTAL	17	3	20
***HI (+)	10	-	7
***HI (-)	7	3	13
***TOTAL	17	3	20

* Specificity and sensitivity of AGID. Sensitivity: 11%, Specificity: 100%.
** Specificity and sensitivity of RSA. Sensitivity: 29%, Specificity: 100%.
*** Specificity and sensitivity of HI. Sensitivity: 58%, Specificity: 100%.

Table 3. Specificity and sensitivity of RSA test.

	HI (+)	HI (-)	TOTAL
RSA (+)	4	1	5
RSA (-)	6	9	15
TOTAL	10	10	20

Specificity and sensitivity of RSA. Sensitivity: 40%, Specificity: 90%.

thought that these quails might have been previously treated with antibiotics. From anamnesis it was learned that these quails had received antibiotic therapy (Enrofloxacin) for 5 days before sample collection; therefore, it can be surmised that the lack of bacterial isolation was most probably due to the antibiotic treatment. The most typical necropsy findings are mucosal and catarrhal discharge and exudate formation in the trachea, nose, infraorbital sinuses, and increased air sac thickness (4). In the present study the most common findings in necropsied quails were exudate formation in the trachea, inflamed sinuses, and increased air sac thickness.

The difficult and time-consuming characteristics of isolation and identification of *M. gallisepticum* infections have made serological tests mandatory (4,6). The RSA test has been used by many researchers to determine specific antibodies in animal sera (10). The RSA test usually determines Ig M antibodies that can be seen in sera 1 week after the start of infection (10). The HI test has been used as an affirmation test in CRD control programs and has a very high specificity (4,5). This test generally determines Ig G antibodies that can be seen approximately 2 weeks following the start of infection (10). Of the 20 sera samples tested in the present study, 5 (25%) with RSA test and 10 (50%) with HI test were found to be positive. Of the 5 RSA test positive reactions, 1 was found to be negative by HI test, and of 15 sera samples with a negative RSA test reaction, 6 were positive by HI test. The RSA test had a sensitivity of 40% and a specificity of 90%. These findings are consistent with other reports that the HI test has lower sensitivity and higher specificity than the RSA test (5,10). However, the fact that one sera sample had a positive reaction with the RSA test and a negative reaction with the HI test demonstrates that this poultry infection was in the acute stage when the sample was taken. Similarly, as 6 sera samples had a positive reaction with the HI test and a negative reaction with the RSA test, this demonstrates that this poultry infection was in the chronic stage.

One of the other serological tests used for the determination of antibodies in *M. gallisepticum* infections is the AGID test. Esendal (11) found 51 (5.67%) of 900 chicken sera to be positive by the AGID test. In the present study 2 (10%) of 20 sera samples were positive according to the AGID test. These findings show that the AGID test was inferior to the other serologic tests in determining *M. gallisepticum* antibodies. Avakian et al. (5) reported that ELISA was sensitive and specific to RSA and HI tests. With a commercial ELISA, 3 of the 20 sera samples in the current study were defined as negative, while 17 were positive. The sensitivity of AGID, RSA, and HI tests was 11%, 29%, and 58%, respectively. The specificity of ELISA to these serologic tests was 100%. Because 3 ELISA negative sera samples were also defined as negative by the other tests, it can be said that these birds did not have sufficient antibody levels to be detected or they had no infection. The fact that some sera samples gave negative reactions in AGID, RSA, and HI tests, but gave positive reactions with ELISA could have been because the commercial ELISA kit was capable of determining lower levels of antibodies.

In this study *M. gallisepticum* infection was serologically determined for the first time in quails in the Aydın region of Turkey. The results suggest that other bird species in the region are also at risk of infection. It was revealed that the control and rapid diagnosis of *M. gallisepticum* infection is very important for the poultry industry. However, isolation of *M. gallisepticum* infection is a very difficult and time-consuming process, and in some cases it is not possible to isolate this microorganism from all materials. For that reason, it is recommended that serological tests should be performed along with isolation and identification of *M. gallisepticum* to return better results.

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