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A SEROLOGIC SURVEY OF DOGS FOR BRUCELLA CANIS AND BRUCELLA ABORTUS AND EVALUATION OF MERCAPTOETHANOL MICROAGGLUTINATION TEST

K. Serdar Diker[1] **Nejat Aydın**[2] **Jale Erdeğer**[3]
Mustafa Özyurt[4]

Köpeklerin Brucella canis ve Brucella abortus infeksiyonları üzerinde serolojik bir tarama ve mercaptoethanol mikroaglütinasyon testinin değerlendirilmesi

Özet: *Bu çalışmada, Brucella canis infeksiyonlarının teşhisi için, merkaptoetanol mikroaglütinasyon testinin değerlendirilmesi yapıldı. Ayrıca, üç değişik köpek grubunda Br. canis ve Br. abortus infeksiyonlarının sıklığı incelendi. Br. canis aglütininlerinin saptanması için Merkaptoetanol Tüp Aglütinasyon Testi (ME-TAT), Merkaptoetanol Mikro Aglütinasyon Testi (ME-MAT) ve Mikroaglütinasyon Testi (MAT) karşılaştırıldı, Br. abortus infeksiyonunun teşhisi için mikroaglütinasyon testi kullanıldı. ME-TAT testinde* 1:200 *titrede pozitif reaksiyon, aktif Br. canis infeksiyonunun belirtisi olarak kabul edildi. Bu titre ME-MAT testinde* 1:40 *olarak kabul edildi. ME-TAT ve ME-MAT sonuçları paralellik gösterdi. Br. canis infeksiyonlarının teşhisi bakımından MAT güvenilir sonuçlar vermedi.*

İncelenen 222 *serumun* 14 (% 6.3) *ü* 1:200 *veya daha yüksek titrede pozitif bulundu ve bunlar aktif Br. canis infeksiyonu olarak kabul edildi. Sokak köpeklerinin* % 15.6 *sı, ev köpeklerinin* % 4.5 *inde infeksiyon saptanmasına karşın, askeri hizmet köpeklerinde infeksiyon bulunmadı. Br. abortus infeksiyonları yönünden, birkaç serum çok düşük titrede pozitif reaksiyon verdi ve bu durum infeksiyon belirtisi olarak kabul edilmedi.*

Summary: *The application of micromodification of mercaptoethanol agglutination test to the serologic diagnosis of Br. canis infection was evaluated. The prevalence of antibodies to Br. canis and Br. abortus in three different*

1 Dr., A.Ü. Veterinary Faculty, Microbiology Department, Ankara 06110
2 Doç. Dr., 3 Araş. Gör., A.Ü. Veterinary Faculty, Microbiology Department, Ankara 06110.
4 Vet. Med., KÜVEM, Etimesgut

groups of dogs was also investigated. Sera were compared by MA-TAT (Mercaptoethanol Tube Agglutination Test), ME-MAT (Mercaptoethanol Micro-Agglutination Test) MAT (Micro Agglutination Test) for agglutinins to Br. canis and tested by MAT for agglutinins to Br. abortus. A titer of 1:200 *in ME-TAT was considered as indicative of active Br. canis infection. This titer corresponded to* 1:40 *in ME-MAT. All results of ME-MAT correlated well with those of ME-TAT. MAT for Br. canis infection did not give reliable result.*

Of 222 *sera examined,* 14 (6.3 %) *had a titer of* 1:200 *or more and these were considered as active Br. canis infection. None of the military service dogs were positive for Br. canis whereas* 15.6 % *of stray dogs and* 4.5 % *of pets were positive. In MAT for Br. abortus, a few of sera gave positive reaction at low titer, and these were considered as negative test result.*

Introduction

Brucella canis is well known as a cause of abortion and infertility in bitches, epididymitis and testicular atrophy in male dogs (8, 16). Several studies have indicated that the disease is widely distributed throught the world in many breeds of dogs. There is even serological evidence for infection in the wildlife population and in cat (23, 24). The results of serosurveys of *Br. canis* antibodies in dogs indicated 30.5 % positive reaction in Argentina (21), 2.9 % in Japan (25), 28 % in Mexico (10), 0.3 % in Canada (5), 8.2 % in Brasil (18) and 1 % to 12 % in USA (4, 16, 26). In Turkey ,canine brucellosis due to *Br. canis* was first recognized serologically in 1983 (17). Most infected dogs are free of cilinical signs though many experience reproductive failure and loos vigor. *Br. abortus* can also cause canine infection infrequently (3).

The ability of *Br. canis* to infect human beings has also been established. Sporadic cases of human infection associated with laboratory exposure to cultures and contact with infected dog have been reported (8, 20). There is serologic evidence of human infection due to *Br. canis* in Turkey (9). Laboratory methods are essential to identify the presence of the disease as its cilinical signs may be very varied. Various serological methods have been developed for diagnosis of canine brucellosis: tube agglutination test with or without mercaptoethanol, slide agglutination test, complement fixation and agar-gel diffusion, using *Br. canis* or *Br. ovis* antigen (2, 7, 11). Since *Br.*

canis is naturally mucoid, the standart antigens and test procedures used for the diagnosis of brucella infections caused by smooth brucella, e.g. *Br. abortus* can not be used for the diagnosis of canine disease caused by *Br. canis* (27).

The present report deals with the application of micro-modification of ME-agglutination test to the serologic diagnosis of *Br. canis* infection. The prevalence of antibodies to *Br. canis* and *Br. abortus* in three different groups of dogs has also been investigated.

Materials and Methods

Serum samples: Blood samples were collected from 222 mature dogs, of which 64 were stray dogs, 88 were household pets and 70 were military service dogs. Sex and breed distinction was not included when a blood sample submitted. Each blood sample allowed to clot and after centrifugation, the serum was pipetted into screw-topped vials and stored at – 20 °C until used. Positive (high titer and medium titer) and negative control sera for *Br. canis* and *Br. abortus* were included in each experiment.

Antigens: *Br. canis* ME-TAT antigen was kindly provided by Dr. G.M. Brown (USDA Diagnostic Reagent Section, Ames, Iowa). *Br. abortus* TAT antigen was a product of Pendik Veterinary Research Institute, Istanbul.

Serologic Tests: All control sera were tested by *Br. canis* ME-TAT, ME-MAT, MAT and *Br. abortus* MAT to compare these tests and evaluate most reliable one and any cross-reaction. Number of field samples investigated by *Br. canis* ME-TAT, ME-MAT and *Br. abortus* MAT is shown in Table 1.

Br. canis ME-TAT: The method of "USDA diagnostic reagent section" was used. Diluent for preparing the ME solution and test

Table 1. Number of field sera tested by three procedures

Groups of dogs	Br. canis		Br. abortus
	ME-TAT	ME-MAT	MAT
Stray dogs (n = 64)	64	14	14
Household pets (n = 88)	88	4	4
Military service dogs (n = 70)	70	70	70
Total (n = 222)	222	88	88

antigen was prepared by adding a ratio of 0.6 ml of formalized saline stock solution (10 % v/v) to 99.4 ml of 3.5 % saline solution. ME solution (0.1 M) was prepared by adding a ratio of 0.715 ml 2- Mercaptoethanol (Merck) to 99.285 ml above diluent and pH was adjusted to 8.5. Serum was diluted (two-fold beginning from 1:50) in 1 ml of ME solution. Concentrated antigen was added (4.4 ml) to 95.6 ml of formalized 3.5 % saline, and 1 ml of this test antigen (final concentration = OD 0.9 at 550 nm) was added to each tube. Tubes were incubated at 37 °C for 48 hours.

Br. canis ME-MAT: All diluents and procedure were same of ME-TAT except that sera were diluted in a total volume of 0.1 ml test reagents in microplate trays (two-fold beginning from 1:5) and plates were incubated at 37 °C for 24 hours and 4 °C for 3 hours. Control sera were also tested by using a more concentrated (OD 2.0 at 550 nm) and compared with standart test antigen (OD 0.9 at 550 nm).

Br. canis MAT: All procedures were same of ME-MAT except that ME was not used for preparing diluents.

Br. abortus MAT: Two-fold dilutions of sera were prepared in a volume of 0.05 ml and 0.05 ml of test antigen was added to each well. The reaction was read after incubation at 37 °C for 18 hours.

Positive reaction at 1:200 or more dilution in ME-TAT was considered as sero-positive. The results of other tests for *Br. canis* were evaluated after comparing with ME-TAT since no established criteria was available for these tests.

Results

Comparison of titers of control sera and some of the positive field sera tested by three procedures for antibodies to *Br. canis* and one method for antibodies to *Br. abortus* are shown in Table 2. As comparing the results for *Br. canis*, positive reaction at 1:200 titer in ME-TAT corresponded to 1:40 titer in ME-MAT. Positive reaction at 1:40 or more in ME-MAT was considered as positive test result. All results of ME-MAT correlated with those of ME-TAT. When comparing the antigen concentration in ME-MAT, the titer of positive result with 0.9 OD antigen was higher than with 2.0 OD antigen. In *Br. canis* MAT, positive control sera had very high titer (1:10240) but negative control sera also gave high titer (1:80).

Table 2. Comparison of test results of four procedures

Type of sera examined	Br. canis					Br. abortus MAT
	ME-TAT	ME-MAT		MAT		
		0:9[a]	2:0	0:9	2:0	
Br. canis control						
Positive (High titer)	1:800	1:160	1:80	1:10240	1:2560	0
Positive (Medium titer)	1:200	1:40	1:40	1:10240	1:640	0
Negative	0	0	0	1:80	1:80	0
Br. abortus control						
Positive	1:10	1:5	–[b]	1:40	–	1.320
Negative	0	0	–	1.20	–	1:5
Field sera						
No. 1	1:200	1:40	–	-	-	0
No. 2	1:400	1:160	–	–		0
No. 3	1:800	1:160	–	–	–	0
No. 4	1:200	1:40	–	-	-	0
No. 5	1:200	1:80	–	–	–	0

(a: optical density at 550 nm; b: Not tested)

The incidence of sero-positive dogs of three different groups for *Br. canis* and *Br. abortus* infection is shown in Table 3.

Table 3. Prevalence of sero-positive results in three different groups of dogs for Br. canis and Br. abortus

Groups of dogs	No. of examined/positive (%)	
	Br. canis	Br. abortus
Stray dogs	64/10 (15.6)	14/0 (0.0)
Household pet dogs	88/4 (4.5)	4/0 (0.0)
Military Service dogs	70/0 (0.0)	70/0 (0.0)
Total	222/14 (6.3)	88/0 (0.0)

Of 222 sera examined, 14 (6.3 %) had a titer of 1:1200 of more in ME-TAT and these were considered as active *Br. canis* infection. None of the military service dogs were positive for agglutinins to *Br. canis* whereas 15.6 % (10 of 64) of stray dogs and 4.5 % (4 of 88) of pets were positive.

Of 88 sera examined for antibodies to *Br. abortus*, 4 sera (4.5 %) were positive at 1:5 titer and 4 sera (4.5 %) were positive at 1:10 titer. These reactions such a low titer not considered as positive result.

Significant cross-reaction was not observed between antigens and antisera of *Br. canis* and *Br. abortus*.

Discussion and Conclusion

Serologic testing a relatively simple method for diagnosis of *Br. canis* infection in dogs. There is no complete agreement, however, on the best serologic test to use. Each author claims that his test is better than others. The ME-TAT and SAT are the most commonly used procedures since they are simple and reproducable. The SAT is accurate when the results are negative, but less accurate when results are positive (62.5 % sensitive) (6). In 14.5 % of positive SAT reaction, Hubbert et al (16) failed to confirm the results by ME-TAT. The ME-TAT enables detection of infection and eliminate most "false positive" results. The veterinary use of ME-TAT for testing canine serum is based on the observation that IgM antibody in dogs is of no significance for infectivity (15). Some investigators suggest an ME-TAT titer of 1:100 as indicative of *Br. canis* infection (14, 19). Others, including World Health Organization Commission on brucellosis, require an ME-TAT titer of 1:200 or higher for positive results (1,15). In this study it has been accepted a titer of 1:200 as positive for canine brucellosis, in accordance with the WHO commission recommendation.

Previous experience with microagglutination procedure led us to choose a modification of technique previously reported (22). The result of ME-MAT which was modified in this study, correlated well with the results of ME-TAT. It was also observed that ME-MAT has some advantages. ME-TAT requires clearing of the supernatant fluid within 48 hours to be positive. In ME-MAT this period was shortened to 24 hours. Other advantage of ME-MAT is that it needs less reagent and serum than in ME-TAT, to perform. The original ME-TAT requires large amount of antigen. Experiments with ME-MAT in which positive control sera for *Br. abortus* were used have showed that *Br. canis* antigen does not cross-react with antiserum to *Br. abortus*.

The reason of very high titer obtained in MAT with positive control sera for *Br. canis* antibodies may be non-specific agglutination. Some authors also pointed out that "false positive" reaction due to non-specific agglutination was main disadvantage of TAT (11). Since negative control serum had also relatively high titer in this test, it is not a reliable test for diagnosis of canine brucellosis due to *Br. canis*.

Other purpose of the present study was to determine the prevalence of agglutinins to *Br. canis* and to *Br. abortus*. This survey demonstrated a prevalence of *Br. canis* antibodies indicative of active infection to be approximately 6 times greater in stray dogs than in non-stray dogs. This difference is presumed to be related to an increased oppurtinity of the stray group for exposure through multiple breedings and other contacts with infected dogs, as compared with the more restricted movement and decreased oppurtinity for exposure of the non-strays. Most of the other workers have also indicated that the prevalence of positive serologic results in stray dogss is considerably higher than in non-stray dogs (12, 13).

One of the difficulties in making valid comparative evaluations of the results of *Br. canis* sero-survey has been the lack of standart procedures and test reagents. It is also difficult to compare percentages reported, due to differences in evaluation of the titers obtained. Our finding of 15.6 % stray dogs with ME-TAT titer of 1:200 or more is one of the largest percentage reported from all over the world. Infection rates reported for Mexico (10) and Louisiana (16) have been greater and smaller, respectively.

Although incidence of *Br. canis* infection in house-hold pets is not as high as stray dogs, these animals are most likely a potential for human infection. Recent evidence suggets that the prevalence of this disease in human, as well as its zoonotic potential, may be greater than suspected (22). The diagnosis of human infection is difficult, because routinly used *Br. abortus* antigen does not cross-react with agglutinins to *Br. canis*. This may lead to misdiagnosed or underdiagnosed human cases. The demonstration of two cases of human brucellosis due to *Br. canis* in Turkey shows a possible transmission from dogs to human and indicates the importance of subject (9).

Most of the sera tested were found negative in *Br. abortus* MAT, only a few of them had low titers. These sera were from dogs of urban area where dogs can not feed with aborted fetuses infected with *Br. abortus*. This may explain why dogs have low titers of agglutinins to *Br. abortus*. On the other hand, it has also been reported that the lack of clinical signs produced and the variable agglutinin response after experimental infection indicates a marked resistance of the dogs to infection due to *Br. abortus* (3).

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