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Prevalence of *Babesia equi* and *Babesia caballi* in Horses by Serological Methods in The Mediterranean Sea and the Black Sea Regions of Turkey

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Abstract: The aim of this study was to determine the prevalance of *Babesia equi* and *Babesia caballi* by serologic (IFAT and CFT) and microscopic examination in the Mediterranean Sea and the Black Sea regions. For this purpose, sera and microscopic slides were prepared and examined from 168 horses randomly chosen from 8 different cities in these regions in year of 2003. Microscopic slides stained with Giemsa were examined for *Babesia* species, and the agents were not detected. In the serological tests performed, seropositivity was found by IFAT in the Mediterranean Sea region to be between 12.72% and 44.44% for *B. equi*, and between 0% and 30% for *B. caballi*; while CFT revealed a prevalence between 5% and 12.72% for *B. equi*,between 0% and 1.81% for *B. caballi*. In the Black Sea region, on the other hand, seropositivity was found by IFAT between 0% and 20.68% for *B. equi*, and between 0% and 12.5% for *B. caballi*; and by CFT to be between 0% and 12.06% for *B. equi*, and none for *B. caballi*. The differences between the results of the tests were evaluated statistically, and IFAT was more sensitive and specific as compared to CFT and microscopic examination.

Key words: Horse, *Babesia equi, Babesia caballi*, IFAT, CFT, Turkey

INTRODUCTION

Babesiosis is a protozoan disease caused by *Babesia* species in vertebrated animals. The disease is caused by *Babesia equi* and *Babesia caballi* in horses[1].

Unlike the other *Babesia* species, schizogony development stage in lymphoblastoid cells was reported to be as in *Theileria* species in development of Babesia equi[2].It was reported that *Babesia equi* is much more pathogenic than *B. caballi*, as *B. equi* damaged the blood pattern more severely, and it took longer for this pattern to turn normal than it did for B. caballi[1] .

The aim of this study was to examine prevalence of *Babesia* species in the horses in the Mediterranean Sea and The Black Sea regions of Turkey.

MATERIALS AND METHODS

The material of this study consisted of 168 horse sera and microscopic slides supplied from The Mediterranean Sea and The Black Sea regions within the year of 2003. Peripheric blood microscopic slides were prepared from tail tips from randomly chosen horses, and the blood samples were collected from the jugular veins into vacuumed tubes.The smear slides were stained with Giemsa. The sera were isolated in the laboratory, and stored at -20°C until use. Antigens (12-well IFA substrate slide) Lot BK-12- 030320, used in the Indirect Fluorescent Antibody Test (IFAT), were acquired from Fuller Laboratories Fullerton, CA USA. Anti-horse IgG (whole-molecule) FITC conjugate, no. F3762, by Sigma was used as conjugate, with a 1:32 dilution. The Complement Fixation Test (CFT) was performed according to the USDA National Veterinary Services Laboratories Testing Protocol[3], and as described by Alton *et al.*,[4].

Statistical significance of the differences between the results of the tests was examined by X^2 (chi square) test, and their accordance by Kappa test.

RESULTS

Blood smear slides from 168 horses in 8 different cities in The Mediterranean Sea and The Black Sea regions were examined microscopically for *Babesia spp.* parasites, and sera samples were examined by IFAT and CFT, for antibodies against *B. equi* and *B. caballi.*

As can be seen in Table, in this study, prevalence of antibodies specific to *B. equi* was detected to be 23.52%, and that for *B. caballi* detected to be 15.29% by IFAT, and CFT revealed prevalence of antibodies specific to *B. equi* to be 11.76%, and that for *B. caballi* to be 1.17% in the Mediterranean Sea region. The prevalence for *B. equi* specific antibodies was 15.66%, while that for *B. caballi* was 9.63% by IFAT, and rate of antibodies against *B. equi*

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Table 1: The distribution of seropositiveness by IFAT and CFT to the cities

			IFAT				CFT			
			B. equi		B. caballi		B. equi		B. caballi	
Region	City	Sera	Number	%	Number	%	Number	%	Number	%
Mediterranean Sea										
	Antalya	55	7	12.72	5	9.09	7	12.72	1	1.81
	Adana	20	8	40	6	30	1	5	-	-
	Mersin	9	4	44.44	2	22.22	1	11.11	-	-
	K.Maraþ	1	1	100	-	-	1	100	-	-
Total		85	20	23.52	13	15.29	10	11.76	1	1.17
Black Sea										
	Tokat	58	12	20.68	7	12.06	7	12.06	-	-
	Kastamonu	11	-	-	-	-	-	-	-	-
	Bolu	6	1	16.66	-	-	-	-	-	-
	Düzce	8	-	-	1	12.5	-	-	-	-
Total		83	13	15.66	8	9.63	7	8.43	-	-

Table 2: The distribution of IFAT and CFT seropositiveness according to the regions

	IFAT-CFT		IFAT-CFT	
	B. equi		B .caballi	
Regions	Number	%	Number	%
Mediterranean Sea	8	4.76	1	0.59
Black Sea	7	4.16	-	-
Total	15	8.92	1	0.59

was found to be 8.43% and that for *B. caballi* was found to be 0% by CFT in the Black Sea region.

As evident in Table 2, fifteen of the 168 (8.92%) horses examined by both tests were positive for *B. equi*, and 1 (0.59%) of them for *B. caballi*. Seropositivity for *B. equi* was higher both in the Mediterranean Sea region and The Black Sea by both IFAT and CFT. Again examination by either tests revealed no seropositivity for *B. caballi* in the Black Sea region .

As can be seen in Table 3, comparison of IFAT results by CFT results revealed that, while 18 sera were positive for *B. equi* by IFAT appeared to be negative by CFT, 2 samples found positive for *B. equi* by CFT gave negative results by IFAT: Again, while no positivity for *B. caballi* was evident in 20 samples found positive for *B. caballi* by IFAT, the 1 sera found positive for *B. caballi* by CFT also proved positive by IFAT. Examination of IFAT and CFT results in terms of *B. equi* revealed that while 15 of the 168 horses were found positive by both tests, 133 horses were detected to be seronegative by IFAT and CFT. Similarly, 147 of the 168 horses were detected to be seronegative by both tests.

As evident in Table 4, mixed positivity by IFAT was detected in 10 sera, while 1 sera revealed mixed positiveness by CFT in the Mediterranean Sea. The difference in the results of the two tests were statistically evaluated, and IFAT was found to be more sensitive and specific compared to CFT.

DISCUSSION

It was reported that the best and the most reliable method for the diagnosis of babesiosis in horses was microscopic examination, although it is difficult to detect, but that the chance of detect the cause in subclinical infections by that way[1]. In this study, no parasites were detected in microscopic examinations. This finding is in accordance with the earlier results[5-9].

In this study, prevalence of antibodies specific to *B. equi* was detected to be 23.52%, and that for *B. caballi* detected to be 15.29% by IFAT, and CFT revealed the prevalence of antibodies specific to *B. equi* to be 11.76%, and that for *B. caballi* to be 1.17% in the Mediterranean Sea region. The prevalence for *B. equi* specific antibodies was 15.66%, while that for *B. caballi* was 9.63% by IFAT, and rate of antibodies against *B. equi* was 8.43% and that for *B. caballi* was 0% by CFT in the Black Sea region.

Differences between regions were statistically significant ($p<0.0001$). The prevalence of seropositivity for *B. equi* in the Mediterranean Sea region varied between 12.72% and 44.44%, and that for *B. caballi* to varied between 0% and 30% by IFAT, while it varied between 5% and 12.72% for *B. equi* by CFT, and for *B. caballi* varied between 0% and 1.81%. In the Black Sea region, on the other hand, IFAT revealed the prevalence of seropositivity for *B. equi* to vary between 0% and 20.68%, and that for *B. caballi* between 0% and 12.5%; while CFT revealed a prevalence of seropositivity for *B. equi* varied between 0% and 12.06%, with no *B. caballi* detected.

Statistical difference observed in *B. equi* was found significant by both IFAT ($p<0.0001$) and CFT ($p<0.001$). Statistical difference in *B. caballi* was also significant ($P = 0.17$). In the Mediterranean Sea region, no significant

Table 3: The comparison of IFAT and CFT results

	IFAT B. equi (+)	IFAT B. equi (-)	IFAT B. caballi(+)	IFAT B. caballi(-)
CFT B.equi (+)	15	2		
CFT B.equi (-)	18	133		
CFT B.caballi(+)			1	-
CFT B.caballi (-)			20	147

Table 4: The geographic distribution of mixed positiviess by IFAT and CFT

	Mediterranean Sea	Black Sea	Total
Mixed positivies by IFAT	7	3	10
Mixed positivies by CFT	1	0	1

difference was present in total between IFAT and CFT in *B. equi* ($p>0.225$), while a significant difference was evident for B. caballi ($p<0.009$). In the Black Sea region, no significant difference was present in total between IFAT and CFT for *B. equi* ($p>0.744$), while a significant difference was evident for *B. caballi* ($p<0.0001$).

Fifteen of the 168 (8.92%) horses examined by both tests were positive for *B. equi*, and 1 (0.59%) of them for *B. caballi*.Seropositivity for *B. equi* was higher both in the Mediterranean Sea region and The Black Sea by both IFAT and CFT. Again, examination by either tests revealed no seropositivity for *B. caballi* in the Black Sea region .

Comparison of IFAT results by CFT results revealed that, while 18 sera were found positive for *B. equi* by IFAT appeared to be negative by CFT, 2 samples were found positive for *B. equi* by CFT gave negative results by IFAT. Again, while no positivity for *B. caballi* was evident in 20 samples found positive for *B. caballi* by IFAT, the 1 sera was positive for *B. caballi* by CFT also proved positive by IFAT. Examination of IFAT and CFT results in terms of *B. equi* revealed that while 15 of the 168 horses were found positive by both tests, 133 horses were detected to be seronegative by IFAT and CFT. Similarly, 147 of the 168 horses were detected to be seronegative by both tests.

Mixed positivity by IFAT was detected in 10 sera, while 1 sera revealed mixed positiveness by CFT in the Mediterranean Sea. The difference in the results of the two tests were statistically evaluated, and IFAT was found to be more sensitive and specific compared to CFT.

It is known that the use of serological tests are commonly used, especially for the detection of carrier horses, besides blood microscopic slides for diagnosis of piroplasmosis in single clawed animals[9-11]. *B. equi* infections are reported to be more common in the world, as compared to *B. caballi* infections[1]. Tenter and Friedhoff[12], and Weiland[9] have used IFAT and CFT in diagnosis of Babesia infections in horses. Although they have detected seronegativity by CFT on from 2-3 months following experimental infections, they have reported that these horses were still positive when they were studied by IFAT. Furthermore, they have observed that *B. equi* antibodies remained in high titer by IFAT and CFT, compared to *B. caballi* antibodies.

Among 3765 horses in Europe, 105 were seropositive for *B. equi*, 8 horses for *B. caballi*, and 10 horses for both species. All the sera were also tested by IFAT, where all that were CFT positive were also observed to be positive by IFAT; and besides , 26 of the sera that were suspected in CFT applications were seropositive for *B. equi* and *B. caballi*[12]. In the study with 23 horses which appeared healthy in Argentina, all were detected to carry specific antibodies for *B. equi*, and 18 of them were seropositive for *B. equi* by CFT[8]. In Israel, 361 sera samples collected from 361 horses were examined by IFAT, and one third of the horses were seropositive for *B. equi*[13].

In this study, the prevalence of *B. equi* antibodies in the Mediterranean Sea and Black Sea regions was higher than that for *B. caballi* by both serological methods (IFAT and CFT).

Our results are in parallel with earlier studies[1,7-9,12]. Some investigators[5,6,9-12] have reported that, starting from 2-3 months following the acute infection, particularly the antibodies against *B. caballi* became undetectable by CFT, but that these horses remained positive by IFAT for a long period.

In our study, IFAT was considered to be more sensitive and specific as compared to CFT, and this result was in accordance with results of earlier studies[6,7,9-12].

REFERENCES

1. Levine, N.D., 1985. Vet. Protozool. Iowa State University Press Ames 1th Edition.
2. Schein, E., G. Rehbein, W.P. Voigt and E. Zweygarth, 1981. *Babesia equi* (Laveran 1901) Development in horses and in lymphocyte culture. Tropenmed Parasitol., 32: 223-227.
3. National Veterinary Services Laboratories Testing Protocol., 1997. Complement fixation test for detection of antibodies to *Babesia caballi* and *Babesia equi*- microtitration test. USDA Animal and Plant Health Inspection Service. Ames, IA.
4. Alton, G.G., M.J. Lois, R.D. Angus and J.M. Verger, 1988. Diagnostic Test Procedures. In: Techniques for the Brucellosis Laboratory. Institute National De La Recherche Agronomique, 147, rue de l'Université, 75007 Paris.

5. Frerichs, W.M., A.A. Holbrook and A.J. Johnson, 1969. Equine piroplasmosis: Complement fixation titers of horses infected with *Babesia caballi*. Am. J. Vet. Res., 30, 5 697-702.
6. Aicher, B.M., 1984. On possibilities to differentiate equine *Babesia equi* and *B.caballi* infections by means of CFT, IFAT and ELISA. Aus dem ýnstitut für Verglechende Tropenmedizin und Parasitologie der Tierarztlichen Fakültat der Universitat München.
7. Avarzed, A., D.T. De Waal, I. Igarashi,, A. Saito, T. Oyamada, Y. Toyoda, and N. Suzuki, 1997. Prevalance of equine piroplasmosis in Central Mongolia. Onderstepoort J. Vet. Res., 64:141.
8. Holman, P.J., T. Becu, E. Bakos, G. Polledo, D. Cruz and G.G.Wagner, 1998. *Babesia equi* field isolates cultured from horse blood using a microcentrifuge method. J. Parasitol., 84, 4, 696-699.
9. Weiland, G., 1986. Species-Specific serodiagnosis of equine piroplasma infections by means of Complement Fixation Test (CFT), Immunofluorescence (IIF) and Enzyme-Linked Immunosorbent Assay (ELISA). Vet. Parasitol., 20: 43-48.
10. Kuttler, K.L., W.L. Goff, C.A. Gýpson and B.O. Blackburn, 1988. Serologic Response of *Babesia equi*- Infected horses as measured by Complement Fixation and Indirect Fluorescent Antibody Tests. Vet. Parasitol., 26: 199-205.
11. Weiland, V.G., B.M. Aicher and J. Boch, 1984. Serodiagnostic unk Therapiekontrolle der pferdepiroplasmose mit KBR unt IFAT. Berl Münch Tierarztl Wschr, 97: 341-349.
12. Tenter, A.M. and K.T. Friedhoff, 1986. Serodiagnosis of experimental and natural *Babesia equi* and *B. caballi* infections. Vet. Parasitol., 20: 49-61.
13. Shkap, V., I. Cohen, B. Leibovitz, Savitsky, E. Pipano, G. Avni, S. Shofer, U. Giger, L. Kappmeyer and D. Knowles, 1998. Seroprevalance of *Babesia equi* among horses in Israel using competitive inhibition ELISA and IFA assays. Vet. Parasitol., 76: 251-259.