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SEROPREVALENCE OF BRUCELLOSIS AND ASSOCIATED HEMATO-BIOCHEMICAL CHANGES IN PAKISTANI HORSES

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The aim of this study was to determine the seroprevalence and hemato-biochemical manifestations of brucellosis in horses. Serum samples were screened for *Brucella* antibodies by Rose Bengal plate test (RBPT) and serum agglutination test (SAT). Blood samples were evaluated for hemato-biochemical parameters following standard procedures. Results indicated seroprevalence of brucellosis 20.13 and 16.23% in horses by RBPT and SAT, respectively. Brucellosis does not lead to any significant change in hematological and biochemical parameters in relation to age, sex, body condition and lactation except few parameters. The values of erythrocyte sedimentation rate, neutrophil, basophil and alkaline phosphatase significantly decreased in brucellosis positive animals as compared to healthy animals whereas lymphocytes and alanine aminotransferase were in opposite order. It was concluded from the results that prevalence of brucellosis in horse population is of concern; therefore, control measures should be opted so that its zoonotic threat is curtailed.
Keywords: brucellosis, horses, seroprevalence, hemato-biochemical changes

INTRODUCTION

Brucellosis caused by *Brucella*, is a febrile zoonotic infection and has worldwide distribution among humans as well as animals (Hussain *et al*., 2008; Maadi *et al*., 2011; Akhtar *et al*., 2012), with a high prevalence in Mediterranean countries (Apan *et al*., 2007; Gul and Khan, 2007). Brucella is usually transmitted to humans through the consumption of raw milk and its products (milk cream, butter, and fresh cheese) or through contact with afterbirth products from infected animals (Khorasgani *et al*., 2008; Behzadi and Mogheiseh, 2011; Abubakar *et al*., 2012).

Brucellosis may cause considerable economic losses especially it reduces productivity and leads to abortion which could result in temporary or permanent infertility in affected animals. Death may occur as a result of acute metritis following retained fetal membranes (Radostits *et al*., 2000). Clinically, infection of the supraspinatus bursa (fistulous withers) is the most common manifestation of brucellosis in horses. Infection of the supraatlantal bursa (poll evil) is a less common syndrome of brucellosis. *Brucella* has also been reported as a cause of recurrent uveitis, abortion, and orchitis, but horses appear to be more resistant to infection than cattle, swine, and goats (Megid *et al*., 2010).

The *Brucella* can enter the body through digestive tract, lungs or mucosal layers, penetration of intact skin and spread through blood and lymphatics to any body organ as well as causing localized disease (Lapaque *et al*., 2005). Soon after entry into the body, these organisms are phagocytosed by polymorphonuclear and mononuclear phagocytes (Young, 2005). Once in the blood stream, the organisms are seeded to multiple organs, especially in those rich in reticuloendothelial tissue, such as liver, spleen, skeletal and hematopoietic system (Greenfield *et al.*, 2002) leading to various hemato-biochemical changes in the body (Al-Eissa and AL-Nasser, 1993). Brucellosis usually leads to hemolytic anemia, leukopenia, thrombocytopenia or pancytopenia, neutropenia, lymphocytopenia, eosinophilia, elevated transaminase and alkaline phosphatase (Dimitrov *et al*., 1978; Galanaksis *et al*., 1996; Pourbagher *et al*., 2006). Seroprevalence of brucellosis in cattle and buffaloes (Hussain *et al*., 2008; Munir *et al*., 2010; Abubakar *et al*., 2010), sheep and goats (Masoumi *et al*., 1992; Ghani *et al*., 1995), horses and poultry (Ahmed and Munir, 1995a and b; Wadood *et al*., 2009) has been carried out in Pakistan. However, relatively scarce information on hematological and biochemical findings of this disease in horses is available. Moreover, an association between levels of fibrinogen and brucellosis has never been reported in horses. Hence this study was carried out with the aims to report the seroprevalence, hematological and biochemical changes associated in horses affected with brucellosis in Pakistan.

MATERIALS AND METHODS

This study was tailored and executed keeping all the national and institutional legislations regarding animal protection and welfare of laboratory animals as laid down by the Ethics Committee, University of Agriculture, Faisalabad, Pakistan.

Moreover, Directorate of Graduate Studies, University of Agriculture, Faisalabad, Pakistan approved the synopsis.

***Experimental Animals*:** This study comprised of 308 horses of both sexes which were selected randomly from the periphery of Faisalabad city and the University of Agriculture Clinics. Information about age, parity and body condition for each animal was recorded. A blood sample from each animal was collected with and without anticoagulant. Samples collected with anticoagulant (EDTA) were used for hematological studies. Samples collected without anticoagulant were used for serum extraction. Collected serum was stored at –20˚C for serodiagnosis and biochemical studies.

***Diagnostic Tests*:** For serodiagnosis, Rose Bengal plate test (RBPT) and serum agglutination test (SAT) were performed following procedures described previously (Aldomy *et al*., 2009). Blood samples collected with EDTA were analyzed for total erythrocyte and leukocyte counts (TEC and TLC), differential leukocyte counts (DLC), hemoglobin (Hb) concentration, packed cell volume (PCV) and erythrocyte sedimentation rate (Khaliq and Rahman, 2010). Plasma proteins were determined by Goldberg refractrometer and fibrinogen by Schalm method (Benjamin, 1978). For the determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) commercially available kits (Cat # DIG80, DIG60, GIA30, respectively) were used following the instructions provided by the manufacturer (Diasis Diagnostik, Istanbul, Turkey).

***Statistical Analysis*:** Animals under observation were divided into various groups on the basis of age (1-5, 6-10, 11-15 years), sex (male, female), lactation status (lactating, non-lactating) and body condition (good, fair, poor). Chi-square test was applied to know the difference in seroprevalence in various groups formed on the basis of age, sex, lactation status and body condition. Unpaired *t* test was applied to know the difference in various hematological and biochemical parameters among brucellosis sero-positive and sero-negative animals. Data on hematological and biochemical parameters in relation to age and body conditions were subjected to one way analysis of variance using computer software Minitab.

RESULTS

The seroprevalence of brucellosis was found to be 20.13 and 16.23% by RBPT and SAT, respectively and non-significant difference (χ^2 value = 1.088; P=0.297) in between these two tests was found (Table 1). In relation to sex, the seroprevalence of brucellosis was higher in mares as compared to stallions, but this difference was non-significant (χ^2 value = 1.434; P=0.2311). Seroprevalence of brucellosis in relation to age (χ^2 value = 2.832; P=0.2430) and body condition (χ^2 value = 3.541; P=0.1700) was not significantly different. Prevalence of the disease was highest in the 6-10 years of age group, followed by 11-15 years and 1-5 years of age.

Table 1. Seroprevalence of brucellosis in horses (n = 308) based on diagnostic tests

Tests	Positive Samples	
	Number	%
RBPT	62	20.13
SAT	50	16.23
χ^2 Value	1.088	
P value	0.297	

The disease was also more prevalent in poor and fair conditioned horses. The seroprevalence in relation to lactation was significantly high (χ^2 value = 18.511; P=0.0001) in non-lactating mares as compared to lactating mares (Table 2).

Table 2. Seroprevalence of brucellosis in horses on the basis of different parameters.

Parameters	Animals Tested	SAT +ve		χ^2 Value	P Value
		number	%		
Sex					
Male	36	3	8.33	1.43	0.231
Female	272	47	17.28		
Age (Years)					
1-5	75	8	10.66	2.83	0.243
6-10	153	31	20.26		
11-15	80	11	13.75		
Body Condition					
Poor	48	10	20.83	3.54	0.170
Fair	75	17	22.66		
Good	185	23	12.43		
Lactation					
Lactating	218	24	11.00	18.5	0.000
Non-Lactating	54	23	42.59		

A non-significant difference was observed in total erythrocyte counts (TEC), packed cell volume (PCV), hemoglobin (Hb) concentration and total leukocyte counts (TLC) in apparently healthy and brucellosis positive horses (Table 3). However, a significant difference was observed in erythrocyte sedimentation rate (ESR) (P=0.0001), neutrophils (P=0.0125), lymphocytes (P=0.0031), monocytes (P=0.0267) and basophils (P=0.0504) counts in apparently healthy and brucellosis positive horses. ESR, neutrophils and basophils were lower in positive animals as compared to healthy animals whereas lymphocytes were in the opposite order (Table 3). In negative and brucellosis positive horses, the total and differential leukocyte counts (TLC and DLC) were not significantly different in relation to age groups and sex. However, a significant (P=0.040) difference was noted in monocyte counts in brucellosis positive horses in relation to age groups as these were

Table 3. Hemato-biochemical findings in brucellosis positive and negative horses.

Parameters	Abbreviation	Units	Seropositive (n = 50)	Seronegative (n = 258)	Two tailed P value
Total erythrocyte counts	TEC	10^{12}/L	5.94 ± 1.89	6.03 ± 2.74	0.9886
Packed cell volume	PCV	%	35.86 ± 7.91	37.17 ± 8.12	0.9445
Hemoglobin concentration	Hb	g/dL	11.21 ± 1.75	10.74 ± 1.82	0.9112
Erythrocytes sedimentation rate	ESR	mm/hr	92.44 ± 14.76*	100.17 ± 11.48	0.0001
Total leukocyte counts	TLC	10^9/L	8.25 ± 2.79	7.55 ± 2.91	0.1182
Neutrophils	Neu	%	35.50 ± 19.57*	41.68 ± 15.13	0.0125
Lymphocytes	Lym	%	52.94 ± 19.06*	45.32 ± 16.03	0.0031
Monocytes	Mon	%	4.89 ± 0.72	4.32 ± 1.78	0.0267
Eosinophils	Eos	%	10.78 ± 3.49	12.15 ± 6.97	0.1761
Basophils	Bas	%	0.21 ± 0.40*	0.36 ± 0.51	0.0504
Total proteins	TP	g/dL	8.57 ± 0.78	8.49 ± 0.92	0.5651
Fibrinogen	Fib	g/L	2.87 ± 3.08	2.89 ± 2.86	0.9644
Alanine aminotransferase	ALT	U/L	28.18 ± 5.71*	25.63 ± 6.04	0.0062
Aspartate aminotransferase	AST	U/L	236.82 ± 22.80	228.90 ± 42.17	0.1977
Alkaline phosphatase	ALP	U/L	92.36 ± 13.84*	97.72 ± 16.36	0.0308

Value (mean ± SD) bearing asterisk in a row differ significantly.

significantly higher in aged horses (Table 4). Neutropenia (P=0.013) and lymphocytosis (P=0.047) were observed in 6-10 years brucellosis positive horses (Table 4). According to the body condition, in apparently healthy horses, TLC were the highest (P=0.050) in horses having poor body condition (8.59 ± 2.59 X 10^9/L), followed by fair (6.18 ± 2.58 X 10^9/L) and good (6.79 ± 2.10 X 10^9/L). It was observed that TLC increased significantly (P=0.019; from 6.30 ± 0.85 to 8.30 ± 3.34 X 10^9/L) in non-lactating brucellosis positive mares whereas it was reverse in lactating (P=0.040; from 10.27 ± 3.29 to 6.49 ± 1.91 X 10^9/L; data not shown in Table). Among biochemical parameters, total proteins (TP), fibrinogen (Fib), and aspartate aminotransferase (AST) did not differ, whereas alanine aminotransferase (ALT) (P=0.0062) and alkaline phosphatase (ALP) (P=0.0302) differ significantly among brucellosis positive and apparently healthy horses (Table 3). ALT and ALP was higher and lower in seropositive horses, respectively. There was non-significant difference in TP and Fib values in relation to age (TP, P=0.956; Fib, P=0.266) and lactation (TP, P=0.507; Fib, P=0.737) in brucellosis positive horses.

In apparently healthy horses, a non-significant difference

Table 4. Hemato-biochemical findings in Brucellosis positive horses in relation to age

Parameters	Age (Years)			P value
	1-5 (n = 8)	6-10 (n = 31)	11-15 (n = 11)	
Hematological Parameters				
TEC	5.48 ± 1.21	5.98 ±2 .73	6.62 ± 0.49	0.832
PCV	31.50 ± 7.41	35.50 ± 3.78	46.00 ± 15.55	0.124
Hb	11.60 ± 0.74	11.10 ± 2.48	10.80 ± 1.69	0.881
ESR	115.75 ± 23.44	91.67 ± 29.65	108.00 ± 2.83	0.377
TLC	6.97 ± 2.06	8.22 ± 3.39	10.97 ± 1.09	0.309
Neu	49.04 ± 2.72	24.67 ± 16.56*	65.00 ± 0.00	0.013
Lym	41.96 ± 4.94	62.57 ± 17.66*	25.00 ± 0.00	0.047
Mon	4.10 ± 0.24	4.25 ± 0.40	4.69 ± 0.43*	0.040
Eos	8.53 ± 1.54	12.14 ± 3.97	8.00 ± 0.00	0.401
Bas	0.48 ± 0.67	0.16 ± 0.40	0.00 ± 0.00	0.639
Biochemical Parameters				
TP	9.25 ± 1.01	8.20 ± 0.49	8.40 ± 0.56	0.123
Fib	0.30 ± 0.42	4.40 ± 3.24	0.60 ± 0.56	0.154
ALT	25.11 ± 2.25	25.85 ± 1.20	39.45 ± 1.87*	0.001
AST	246.98 ± 27.53	232.17 ± 22.55	229.51 ± 26.67	0.624
ALP	446.76 ± 64.41	495.82 ± 84.57	413.76 ± 27.79	0.384

Value (mean ± SD) bearing asterisk in a row differ significantly.

was observed in all biochemical parameters in relation to age, sex and body condition except in ALT values which increased (39.45 ± 1.87 U/L) significantly (P=0.001) in aged horse as compared values in other age groups. In relation to body condition in brucellosis positive horses, a significant (P=0.050) difference was noted in TP (9.60 ± 0.01, 9.10 ± 0.98 and 8.14 ± 0.39 g/dL in poor, fair and good animals, respectively) and ALP levels (374.20 ± 0.00, 525.36 ± 73.84 and 436.34 ± 42.54 U/L in poor, fair and good, respectively; data not shown in Table). ALT level had a significant difference (P=0.050) in brucellosis positive non-lactating (25.21 ± 1.86 U/L) and lactating (32.65 ± 7.97 U/L) mares.

DISCUSSION

The definitive diagnosis of brucellosis is carried out through the isolation and identification of the organism but this has several drawbacks including the slow growth of *Brucella*, the low sensitivity depending upon the stage of the disease, the culture medium, the species of *Brucella* involved and the culture technique employed. Hence, serological tests remain important tools for the rapid diagnosis of the disease (Abubakar *et al*., 2010; Ali *et al*., 2011). The main serological test used for brucellosis diagnosis is the RBPT which is a screening test (Blasco *et al*., 1994; Ehizibolo *et al*., 2011; Sikder *et al*., 2012). The RBPT sensitivity is high (>99%) but specificity is low (Barroso *et al*., 2002). SAT is the test used for quantification of the antibodies (Munoz *et al*., 2005; Gul and Khan, 2007), as it is a standard method for the diagnosis of the brucellosis (Lucero and Bolpe, 1998). The sensitivity and specificity of the SAT test is 95.6 and 100.0%, respectively (Memish *et al*., 2002).

Seroprevalence of brucellosis in the present study was 20.13% in horses by RBPT and 16.23% by SAT. In accessible literature, seroprevalence has been reported to vary from 0 to 60.6% (Omer *et al*., 2000; Solmaz *et al*., 2004; Acosta-Gonalez *et al*., 2006). A large difference in prevalence could be due to geographical differences, close contact between domesticated and wild animals, the differences in population intensity or husbandry system being practiced and diagnostic tests applied (Baek *et al*., 2003; Acosta-Gonalez *et al*., 2006; Gul and Khan, 2007).

In the present study, the seroprevalence of brucellosis in different sexes was not significant. There are controversial reports regarding the prevalence of brucellosis in relation to sex of the animal, as some research reported significantly higher prevalence in females than in males (Solmaz *et al*., 2004), whereas intermittent bacteremia in the mares but not in the stallions with *Br. abortus* infections has been reported (MacMillan *et al*., 1982). Still some reports indicate that *Brucella* antibody titers are not associated with sex (Muma *et al*., 2006; Wadood *et al*., 2009).

The prevalence of brucellosis was highest in the 6-10 years age group (20.26%) followed by 13.75 and 10.66% in 11-15 and 1-5 years age groups with non-significant difference (χ^2 value = 2.832; P=0.2430). The seroprevalence appears to be associated with age, as low prevalence in young stock has been reported than the adults (Ahmed and Munir, 1995b). Increased prevalence of brucellosis among older animals might be related to sexual maturity with the advancing age (Kazi *et al*., 2005). The tropism of *Brucella* to the male or female reproductive tract was thought to be due to erythritol, which stimulates the growth of the organism. Erythritol, a sugar alcohol synthesized in the ungulate placenta and stimulates the growth of virulent strains of *Br. abortus*, has been credited with the preferential localization of this bacterium within the placenta of ruminants (Smith *et al*., 1962; Radolf, 1994).

In the present study, no significant differences were noted in hematological values of healthy and infected horses in relation to age, sex and body condition. However, monocytes were significantly higher in brucellosis positive animals (Table 2) and also in relation to age groups in case of brucellosis positive horses its percentage was higher in older horses (Table 3). In the published literature, there is one view that *Br. abortus* does not lead to any consistent change in hematological or biochemical values (MacMillan *et al*., 1982), however, some others have reported that brucellosis infected animals suffer from severe monocytosis (Chahota *et al*., 2003; Kokoglu *et al*., 2006). Monocytosis in brucellosis could be attributed to the presence of tissue debris in the uterus, as natural uterine cleaning is hampered owing to retention of placenta in brucellosis.

In the present study, neutropenia (P=0.013) and lymphocytosis (P=0.047) were observed in 6-10 years brucellosis positive horses as has also been reported (Chahota *et al*., 2003; Kokoglu *et al*., 2006; Abdi-Liae *et al*., 2007). However, the mechanisms that cause neutropenia are not completely understood. Infections caused by Gram negative bacteria, particularly those resulting in septic shock, may cause extreme neutrophilia on the one hand but neutropenia on the other. The latter results from mature cells being mobilized from the marrow faster than the proliferation rate. The neutropenia in this instance is associated with a bone marrow picture of extreme left-shift or maturation arrest (Class, 1996).

There were no significant differences in the biochemical parameters of brucellosis positive and negative horses in the present study except for ALT and ALP which were higher and lower in seropositive horses, respectively. An increase in ALT has been reported in cases of brucellosis (Vilaseca *et al*., 1978; Dimitrov *et al*., 1978; Sabharwal *et al*., 1995; Nagoev *et al*., 2001). It was interesting to note that ALT level increases as the age advances. ALT elevation could be associated with hepatic necrosis (Benjamin, 1978).

***Conclusion*:** From this study it was concluded that the overall seroprevalence of brucellosis is 20.13% by RBPT

and 16.23% by SAT. Brucellosis does not lead to any significant change in hematological and biochemical parameters in relation to age, sex, body condition and lactation. However, increase in monocytes, alanine aminotransferase (in relation to age, body condition and lactation) and alkaline phosphatase (in relation to body condition) were observed. To control the disease in horses, routine screening of the animals must be carried out and the movement of animals from one stud to the other must be controlled. Male animals which are particularly used for the breeding purpose must be brucellosis free. The pet animals like dogs, which are present on the studs must also be screened for brucellosis on routine basis.

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