



Original Research Article

Epidemiological Study of Brucellosis in Equines of District Peshawar Khyber Pakhtunkhwa Pakistan

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ABSTRACT

Keywords

Brucellosis;
age;
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prevalence;
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An epidemiological study was conducted at different regions of district Peshawar for the prevalence of brucellosis in different species of equines. A total of 500 blood samples from equines (horses 196, donkeys 267 and mule 37) were collected during the year January 2012 to December, 2012 and were examined to investigate the prevalence of the disease. The prevalence of brucellosis and distribution by gender, age and health condition were calculated by chi square test for comparison. Sera were subjected to SPAT and PCR tests for detection of *Brucella* infection in equines. The incidence of disease was high in horses (71.93%) as compared to donkeys and mules 63.67 and 5.4%. The passive surveillance / prevalence rate was high in female of horses and donkeys 63.82% and 58.23% as compared to male of horses and donkeys 36.17% and 41.76% respectively. Overall prevalence rate of brucellosis according to age was high in horses, donkeys and mules with brucellosis involvement 78 (55.31%), 87 (51.17%) and 2 (5.4%), ranging in age from 5-11 years, while least cases were observed in equines, ranging in age from 1-2 year. Over all brucellosis prevalence in horses, donkeys and mules was high in poor health animals 58.15%, 49.41% and 5.4% as compared to good health animals 10.63%, 17.05 and 0%. These results suggest that horses and donkeys from the Peshawar regions can be considered as potential reservoirs for these infectious agents for other species and may contribute to the disease spreading and to direct and indirect contamination of humans.

Introduction

Brucellosis was first recognized as disease affecting human on the island of Malta in the early 20th century (Greering *at el.*, 1995). Brucellosis occurs naturally in domestic animals. It is still an important

public health problem throughout the world, but principally, and in particular, in the Mediterranean region, including Turkey, the Arabian Peninsula, the Indian subcontinent, Mexico, and parts of Central

and South America (Young 1995, Colmenero *et al.*, 1996). Human infection is contracted from infected animals and closely linked to poor animal husbandry methods, feeding habits, and hygiene standards (Pascual *et al.*, 19984). The disease typically attacks young and middle-aged adults, with a low incidence among infants and elderly patients Wise (1980). Brucellosis is a disease of great economic importance, as it adversely affects the productive and reproductive potential of animals in terms of, loss of young ones, infertility and reduction or complete cessation of milk after abortion (Radostits *at el.*, 2000).

The population of horses in Pakistan is 0.34 million while that of mules and Asses is 0.16 and 4.27 million, respectively. The contribution of Khyber Pakhtunkhwa in the horse population is 22% while in mules and asses it is 43 and 13%, respectively Livestock Census (2006). The disease is caused by *Brucella abortus* species in horses.

The aim of the present study was to determine the distribution of brucellosis in equines in district Peshawar from different angles like sex, age and health, and to advise the remedies for awareness, control and bio-safety measures in the area

Materials and Methods

The study was conducted on equines in District Peshawar in already available template of I, II, III and IV was used for this study. Total 500 blood samples from equines (horses 197, donkeys 267, and mule 37) were investigated for this purpose. A questionnaire, regarding investigation of animal age, sex, body condition and management system adopted, was filled for each animal by

interviewing the owner of the animal. Based on systemic disease distribution of brucellosis a total 500 blood samples, 112 from town I, 156 from town II, 118 from town III and 114 from town IV were collected.

A 5 ml blood sample was collected from each animal from jugular vein through disposable sterilized syringe. After collection, the blood samples were kept in slant position in cold chain box and transported to Veterinary Research Institute Peshawar. Serum separated from all blood samples by centrifugation at 3000 rpm for 10 minutes and stored at -20°C in deep freezer till use.

All samples were screened out for the presence of *Brucella abortus* and *Brucella melitensis* specific antibodies. The samples were tested using Serum Plate Agglutination Test (SPAT) and Polymerase Chain Reaction Test (PCR).

Statistical Analysis

The data was statistically analyzed using chi-square method for the comparison of means of the species and T.test was used for the means comparison of the two tests.

Results and Discussion

Anti *B. abortus* and *B. melitensis* antibodies were found in 141 out of 196 horses (71.93%), 170 out of 267 donkeys (63.67%) and 02 out of 37 mules (5.4%) with SPAT and PCR (Table I). In our study, positive cases of 141 out of 196 in horses (51 male and 90 female), 170 out of 267 donkeys (71 male and 99 female) and 02 out of 37 mules of both sexes were found positive for the disease brucellosis (Table II).

Overall prevalence rate of brucellosis according to age in horses, donkeys with brucellosis involvement occurred 78 (55.31%), 87 (51.17%) and in mules 2 (5.4%), ranging in age from 5-11 years, while least cases were observed in horses, donkeys 41(29.07), 60 (35.29%) and in mules 0 (0%) ranging in age from 1-2 year respectively (Table III). brucellosis prevalence in horses, donkeys and mules were found in poor health animals (58.15%), 49.41% and 5.4% and in good health animals it was 10.63%, 17.05 and 0% (Table IV).

The prevalence of brucellosis in equine of district Peshawar was studied serologically as well as through molecular characterization. In this study four different towns of district Peshawar were included. For serological diagnosis, Serum Plate Agglutination Test (SPAT) while for molecular characterization Polymerase Chain Reaction (PCR) was used during the study. Even though Hirsh and Zee (1999) have reported that male animals are less susceptible to *Brucella* infection due to the absence of erythritol, other authors (Waghela *et al.*, 1978; Abu-Damir *et al.*, 1984; Abbas *et al.*, 1987) reported equal distribution of *Brucella* antibodies between both sexes. On the contrary, Bekele (2004) from Ethiopia, Yagoub *et al.*, (1990) and Agab *et al.*, (1994) from Sudan, and Ajogi and Adamu (1998) from Nigeria revealed the likelihood of occurrence of infection is higher in female than male animals. Same results were recorded in the present study. Relatively higher susceptibility of she-camels could be due to the fact that they have more physiological stresses than the males Walker (1999). Equines are possibly shedder of *Brucella* and may be a potential source of infection for other animals and man (Ocholi *et al.*, 2004; Refai 2002).

Higher seroprevalence was found in those groups with age of greater than 5 years than those groups with age of less than and equal to 4 years. Sexually matured animals are more prone to *Brucella* infection than sexually immature animals of either sex (Radostits *et al.*, 2007). On the other hand, it is also true that younger animals tend to be more resistant to infection and frequently clear an established infection, although latent infections can occur (Walker, 1999; Quinn *et al.*, 2004). This may be due to the fact that sex hormones and erythritol, which stimulate the growth and multiplication of *Brucella* organisms, tend to increase in concentration with age and sexual maturity (Radostits *et al.*, 2007). In our study, the higher rate of brucellosis seroprevalence was detected in females in comparison to males. Although no particular susceptibility of female horses for brucellosis was mentioned in the literature, the seroprevalence of brucellosis was detected two-folds higher in females compared to males in camels (Refai 2002) In another study performed in cattle and buffaloes, seroprevalence of brucellosis detected in males and in females were 3.6 % and 4.9 % respectively (Silva *et al.*, 2000).

(Wadood *et al.*, 2009) also studied sex wise sero-prevalence of brucellosis in horses and found 9.67 and 17.7% in male and female, respectively. In our study the prevalence of the disease was found higher in both male and females as compared to the findings of the authors. The disagreement of our study with (Wadood *et al.*, 2009) could be due to health status, age, sex and area. Maximum people in the present study area are poor, keeping only old age 1 mares for tanga pulling/ transportation purposes with open wounds. The difference in the prevalence of the disease in different age group animals was

Table.1 Prevalence of brucellosis in equines in district Peshawar through SPAT and PCR

Species	No of sample tested	SPAT Positive		PCR Positive		Total # of positive (SPAT+PCR)	Prevalence Rate %
		#	%	#	%		
Horses	196	78	39.79	63	32.14	141	71.93
Donkey	267	97	36.32	73	27.34	170	63.67
Mule	37	01	2.7	01	2.7	02	5.4
Total	500	176	35.2	137	27.4	313	62.6

P. value 0.00

Table.2 Sex Wise Prevalence of Brucellosis in Equines in District Peshawar

Species	Horse Positive		Donkey Positive		Mule Positive		Total Sample Positive	
	SPAT	PCR	SPAT	PCR	SPAT	PCR	SPAT	PCR
Male	33 (23.4)	18(12.76)	29 (17.05)	42 (24.70)			62	60
Female	50 (33.46)	40 (28.36)	67 (39.41)	32 (18.82)			117	72
No Sex					01(2.7)	01(2.7)	01	01
Total	83 (58.86)	58 (41.13)	96 (56.47)	74 (43.52)	01 (2.7)	01 (2.7)	180	133

P. value 0.619

Table.3 Age Wise Prevalence of Brucellosis in Equine of District Peshawar using SPAT and PCR.

Species	Horse Positive # (%)		Donkey Positive # (%)		Mule Positive # (%)		Total Sample Positive	
	SPAT	PCR	SPAT	PCR	SPAT	PCR	SPAT	PCR
Year								
1-2	29 (20.56)	12 (8.51)	25 (14.7)	35 (20.58)			54	47
2-5	09 (6.38)	13 (9.21)	19 (11.17)	04 (2.35)			28	17
5-11	31 (21.98)	47 (33.33)	49 (28.82)	38(22.35)	01 (2.7)	01 (2.7)	81	86
Total	69 (48.93)	72 (51.06)	93 (54.7)	77 (45.29)	01 (2.7)	01 (2.7)	163	150

P. value 0.619

Table.4 Body Condition Wise Prevalence of Brucellosis in Equine of District Peshawar Using SPAT and PCR

Body condition	Horse Positive # (%)		Donkey Positive # (%)		Mule Positive # (%)		Total sample	
	SPAT	PCR	SPAT	PCR	SPAT	PCR	SPAT	PCR
Good	9 (6.38)	6 (4.25)	17 (10)	12 (7.05)			26	18
Fair	24 (17.02)	20 (14.18)	31 (18.23)	26 (15.29)			55	46
Poor	45 (31.91)	37 (26.24)	49 (28.82)	35 (20.58)	01 (2.7)	01 (2.7)	95	73
Total	78 (55.31)	63 (44.68)	97 (57.05)	73 (42.94)	01 (2.7)	01 (2.7)	176	137

P. value 0.75

statistically significant and numerically it was found more in older age groups of animals. The animals included in this study were in different level of body health condition. Broadly, they were categorized according to health score formula in three different classes having good, fair and poor health condition. Generally, the disease was more prevalent in animals of poor health condition while less in healthy animals. The difference in the prevalence of the disease in animals of different health condition was non significant but it may be considered as significant because the P value (P=0.075) is more close to the standard value. The finding of the present study is very close to that of (Wadood *et al.*, 2009) who investigated the disease in different age groups and different body condition in horses. Highest sero-prevalence was recorded in horses of above 05-11 years of age. It is clear from the results of both studies that the disease was more prevalent in horses of old age group and of poor body condition.

The seroprevalence recorded in the present study revealed that brucellosis is a widespread and established disease in district Peshawar. The risk factors

identified for the presence and transmission of the disease from animal to animal were sex, age, body condition, herd size, contact with other ruminants, parity, reproductive problems and physiological status. How-ever, according to the statistical analysis, advancement in age, poor health condition and contact with other ruminants were found to be the major risk factors for the transmission of the disease from horses, donkeys to horses, donkeys and mules as well as from area to area. Moreover, higher seropositivity was recorded in female, matured, pluriparous, pregnant, abortive and lactating mares, she- donkeys which contributed for transmission of the disease. Traditional husbandry and poor management practices, mixing with other animals and unrestricted movement of horses and donkeys were thought to support spread of the disease in the study area. Therefore, a strategic plan should be developed to support in decreasing the chance of contact of animals at different situations and to keep only few healthy and fertile good quality breeds of horses, donkeys and mules per herd as well as on individual basis with immunization campaigns, and public health education on modern animal husbandry and disease

prevention techniques should be imparted continuously.

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