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Sero-prevalence of Brucellosis in Cattle and Related Human Population in District Malakand, Khyber Pakhtunkhwa, Pakistan

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Abstract

Brucellosis is one of the most widespread and contagious bacterial zoonotic diseases in the world posing a major threat to human health and animal husbandry. Sero-prevalence of brucellosis was investigated in District Malakand, Khyber Pakhtunkhwa, Pakistan. A total of 886 blood samples were collected from animals (n=484) and humans (n=402). A total of 58 (11.98%) animal samples and human samples 25 (6.21%) were found positive for Brucellosis. The seropositive percentage was higher in buffaloes (12.02%) than in cows 7/60 (11.66%). In buffaloes, more females (12.07%) were found infected with brucellosis as compared to males (11.62%). Similarly, female cows showed higher seropositivity (13.20%) than male cows (0). The highest seroprevalence (19.23%) was observed in the age group 6–8 years. Seropositive animals with a history of previous abortion were (77.77%) while seropositive animals with no abortion history were (6.69%). In humans, prevalence percentage was greater in males (6.94%) than in females (5.81%). The age group 37-48 years showed the highest seroprevalence (9.91%). Aborted females showed higher seropositivity (45%) as compared to non-aborted females (2.52%). Sero-prevalence was recorded in 6.38% of individuals with animal contact and in 3.84% of individuals with no animal contact. Raw milk consumers (6.46%) and non-consumers (3.22%) were also found positive for Brucellosis. Different risk factors that may influence the prevalence of Brucellosis need to be considered for the control of the disease and to minimize its spreading in the population.

Keywords: Brucellosis, zoonotic, seroprevalence, risk factors

Резюме

Бруцелозата е една от най-разпространените и заразни бактериални зоонози в света, която представлява сериозна заплаха за човешкото здраве и животновъдството. Сероразпространението на бруцелозата е изследвано в област Malakand, Khyber Pakhtunkhwa, Пакистан. За експериментите са събрани общо 886 кръвни проби от животни (n=484) и хора (n=402). Общо 58 (11.98%) проби от животни и 25 (6.21%) проби от хора са положителни за бруцелоза. Процентът на серопозитивните животни е по-висок при биволите (12.02%), отколкото при кравите 7/60 (11.66%). При биволите са открити повече женски животни (12.07%), заразени с бруцелоза, в сравнение с мъжките (11.62%). По подобен начин кравите покават по-висока серопозитивност (13.20 %), отколкото воловете (0). Най-висока серопревалентност (19.23%) се наблюдава във възрастовата група 6-8 години. Серопозитивните животни с анамнеза за предишен аборт са 77.77%, докато серопозитивните животни без анамнеза за аборт са 6.69%. При хората, процентът на разпространение е по-висок при мъжете (6.94%), отколкото при жените (5.81%). Възрастовата група 37-48 години показва най-висока серопревалентност (9.91%). Абортиралите жени показват по-висока серопозитивност (45%) в сравнение с неабортиралите жени (2.52%). Серопревалентност е регистрирана при 6.38% от лицата, които са имали контакт с животни, и при 3.84% от лицата, които не са имали контакт с животни. Сред консуматорите (6.46%) и неконсуматорите (3.22%) на сурово мляко също са установени положителни за бруцелоза. За да се контролира заболяването и да се сведе до минимум разпространението му сред населението трябва да се контролират различните рискови фактори, които могат да окажат влияние върху разпространението на бруцелозата.

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Introduction

Brucellosis is a zoonotic disease and is also called Malta fever, Cyprus or Mediterranean fever, intermittent typhoid, Rock fever of Gibraltar, and undulant fever (Al Dahouk *et al*., 2003). Brucellosis is caused by *Brucellae* (genus *Brucella*), which are gram-negative coccobacilli, facultative intracellular and aerobic bacteria (Foster *et al*., 2007). Human brucellosis is a very widespread zoonotic disease with over 500 000 annual new cases (Pappas *et al*., 2006). Globally, it is identified as the second most zoonotic problem (Cutler and Whatmore, 2003). Brucellosis is a widespread disease in the developing world and is considered to be a serious issue in nearly 86 countries (Tadesse, 2016; Khan and Zahoor, 2018). Humans may become infected by consumption of unpasteurized milk or dairy products, through direct contact with infected animals and their secretions (Khan and Zahoor, 2018). Animals may become infected by inhaling organisms or through the conjunctiva. Important sources of infection include aborted fetuses, placental membranes, and uterine discharges. They can also be infected by ingesting contaminated food and water (Khurana *et al*., 2021).

The key feature of the genus *Brucella* is its potential to survive both in phagocytic and non-phagocytic cells (Celli and Gorvel, 2004). There is a temporary fusion of the *Brucella*-containing vacuole with the lysosome that leads to the subsequent release of the lysosomal proteins (Starr *et al*., 2008). A virulence factor, lipopolysaccharides (LPS) of *Brucella* helps in its initial survival in macrophages (Lapaque *et al*., 2005). *Brucellae* are facultative intracellular pathogens causing infection by entering macrophages and escaping the mechanisms of macrophage-induced host protection (Adams, 2002; Gorvel and Moreno, 2002). Clinical manifestations in humans include flu-like symptoms, weight loss, high fever, night chills, fatigue, headache, arthritis, abortion in pregnant females, and orchitis in males (Khan and Zahoor, 2018). Clinical signs may be seen within 1 to 3 weeks or several months after bacterial exposure (Hasanjani Roushan and Ebrahimpour, 2015).

Brucellosis is a reproductive disease responsible for great economic loss in bovines by causing abortion, death of young ones, stillbirth, birth of weak calves, delayed calving, male infertility, and reduction in milk production (Abubakar *et al*., 2011; Maadi *et al*., 2011). The basis of laboratory diagnosis of human brucellosis is the isolation of *Brucella* species from blood cultures and the identification of specific antibodies using serological tests (Roushan *et al*., 2010). Although many serologic tests have been developed, the commonly used method remains the tube agglutination test (Park *et al*., 2012). For the diagnosis of human brucellosis, different PCR tests targeting various gene loci have been effectively used (Navarro *et al*., 2004). *Brucella* DNA in milk samples was detected using PCR-based methods (Hamdy and Amin, 2002). In adults, effective therapy is a 6-week regimen of orally administered doxycycline 100 mg/d, along with intramuscular administration of streptomycin 1 g/d for the first 2-3 weeks (Ariza *et al*., 2007).

Prevention of human brucellosis cases depends on disease control by vaccination, slaughter of infected animals, and opting for hygienic measures (Godfroid *et al*., 2010). A number of vaccines have been available for animals over the years, among which live attenuated *Brucella* vaccines are the most effective (Blasco, 2006). The occurrence of brucellosis is highly reduced by effective eradication movements in the European Union, and many countries have become brucellosis-free (Pappas *et al*., 2006). The aim of the present study was to investigate the seroprevalence of brucellosis in cattle and in-contact humans in District Malakand, Khyber Pakhtunkhwa, Pakistan, and to study the risk factors responsible for the maintenance and spread of the disease.

Materials and Methods

This study was performed in the Malakand district according to the guidelines of the Helsinki Declaration (Rickham, 1964). The study was approved by the Ethical Committee of the University of Malakand, Lower Dir, Khyber Pakhtunkhwa, Pakistan**.** Participants were contacted and visited at different areas of district Malakand for data collection using proforma.

Blood collection

Peripheral blood samples were taken aseptically after obtaining written informed consent from the individuals. A total of 886 blood samples (402 human and 484 animals) were collected and investigated for seroprevalence of *Brucella abortus.* To allow clotting for serum separation, the blood samples were kept at room temperature (25-30°C) for about 25 minutes. Then these samples were kept overnight at 4°C. The samples were centrifuged at 2000 rpm, 25°C for 15 minutes to extract the serum. The isolated serum samples were labeled and stored in Eppendorf tubes at -20°C till serological analysis.

Febrile antigen slide agglutination test

Febrile antigen direct test was used for screening of brucellosis in animal and human serum samples. The test was performed using commercial febrile antigen kits obtained from Laboratory Diagnostics Co., Inc. Morganville, USA. Briefly, 40 µl of serum sample was poured on a glass slide and one drop of antigen suspension was added to it. The serum and antigen were mixed thoroughly with the help of a stirrer for four minutes. The slide was observed for agglutination. The same procedure was repeated for both positive and negative control sera.

Results

A total of 886 samples (402 humans and 484 animals) from different areas of the Malakand district were examined for *B. abortus* antibodies. The overall prevalence of brucellosis in animals and humans was 11.98% and 6.21%, respectively.

Brucellosis in animals

A total of 484 animals including 60 cows and 424 buffaloes from different regions of the District were screened for *brucella* antibodies. Among them, 7 (11.66%) cows and 51 (12.02%) buffaloes were found infected with brucellosis. The seropositive percentage was higher in buffaloes than in cows. The total number of female buffaloes and male buffaloes was 381 and 43 respectively. More female buffaloes 46 (12.07%) were found positive for brucellosis as compared to male buffaloes 5 (11.62%). The total number of female cows and male cows was 53 and 7 respectively. Similarly, female cows showed higher seropositivity 7 (13.20%) than male cows (0). There were four age groups of animals, from 1.5 to 10 years. In the age groups 1.5-2 years, 3-5 years, and 6-8 years, the prevalence of brucellosis was 10.08%, 10.91% and 19.23% respectively. None of the animals was positive in the age group 9-10 years. The highest seroprevalence (19.23%) was observed in the age group 6-8 years. Seropositive animals with a history of previous abortion were 28/36 (77.77%), while seropositive animals with no abortion history were 30/448 (6.69%) (Table 1).

Brucellosis in humans

A total of 402 humans including 258 females and 144 males were screened for the presence of *brucella* antibodies. The percentage of male individuals 10 (6.94%) with brucellosis was greater as compared to females 15 (5.81%). Humans were classified into five age groups, from 9 to 63 years. In the age groups 13-24 years, 25-36 years, and 37-48 years, seroprevalence of brucellosis was 5.71%, 5.35% and 9.91%, respectively. The highest seroprevalence (9.91%) was found in the age group of 37-48 years. There were no positive cases in the age groups 9-12 years and 49-63 years. Out of 258 screened females, 20 were abortive and 238 were non-abortive. Higher seropositivity was reported in aborted females 9 (45%) than the non-aborted females 6 (2.52%). Among 402 humans, 376 were in contact with animals and 26 had no animal contact. In-contact individuals 24 (6.38%) and 1 (3.84%) with no contact were found positive for brucellosis. Raw milk users 24/371 (6.46%) and non-users 1/31 (3.22%) were tested positive for brucellosis (Table 2).

Table 1. Sero-prevalence of animal brucellosis based on various factors in Malakand district

Parameters	Category	Total samples	Positive samples	Percentage (%)
Species	Buffalo	424	51	12.02
	Cow	60	7	11.66
Buffalo	Female	381	46	12.07
	Male	43	5	11.62
Cow	Female	53	7	13.20
	Male	7	0	0
Age (years)	1.5-2	119	12	10.08
	3-5	284	31	10.91
	6-8	78	15	19.23
	9-10	3	0	
Abortion history	Abortive	36	28	77.77
	Non-abortive	448	30	6.69

Table 2. Sero-prevalence of human brucellosis based on various factors in Malakand district

Parameters	Category	Total samples	Positive samples	Percentage (%)
Gender	Male	144	10	6.94
	Female	258	15	5.81
Age (years)	9-12	9	0	0
	13-24	70	4	5.71
	25-36	168	9	5.35
	37-48	121	12	9.91
	49-63	34	0	0
Abortion history	Abortive	20	9	45
	Non-abortive	238	6	2.52
Animal contact	In-contact	376	24	6.38
	No contact	26	1	3.84
Use of raw milk	Users	371	24	6.46
	Non-users	31	1	3.22

Discussion

Brucellosis is a worldwide zoonotic disease, caused by *Brucellae,* resulting in great economic loss in endemic areas and serious disorders in affected patients (Yagupsky and Baron, 2005). Human transmission is usually via the consumption of raw milk and by contact with after-birth products of infected animals (Khorasgani *et al*., 2008). The present study was designed to investigate the prevalence of brucellosis in animals and in-contact individuals. The effect of different risk factors of brucellosis was studied.

Brucellosis in animals

The low percentage of seropositive animals in the age group of 1.5-2 years can be explained by the higher immunological tolerance of young animals to the causative agent of brucellosis. Infected young animals may remain undetectable by diagnostic tests, including serology until they give birth or abort. A higher percentage in older animals may be related to the fact that susceptibility to *B. abortus* increases with advancing sexual maturity. As reported by Kazi *et al*. (2005), high brucellosis prevalence is related to sexual maturity in older animals. The lack of positive serological reaction in the group of 9-10-year-old animals may be due to the small number of samples examined, which was only 3. The higher seropositive percentage in buffaloes than in cows may be due to the fact that buffaloes are known to be more susceptible to brucellosis than cows, and may be due to the genetic predisposition of the species. Some breeds of cattle may also be more resistant to *B. abortus*. Similar results were also reported by (Sharma and Saini, 1995; Kumar *et al*., 2005), who demonstrated a relatively high prevalence in buffaloes than in cattle. Higher prevalence in females is due to reproductive tract infection that provides a reservoir for the propagation of the bacteria. As suggested by Ali *et al*. (2013), the higher percentage of females from rural areas was due to the fact that infected females spread the disease as they remained in contact with the animals.

The higher percentage in aborted animals is associated with the fact that brucellosis is one of the main causes of abortion in animals, and also because the immunological reaction of the organism is stronger in case of reinfection. This agrees with the reports of Saini *et al*. (1992) who recorded 38.18% of abortions in cattle and 41.8% in buffaloes with brucellosis.

Brucellosis in humans

As brucellosis is a zoonotic disease, contact or more interaction with animals would increase the chances of infection. The higher percentage of brucellosis in men than in women is because usually men were found closer to animals, hence found with greater susceptibility to the disease. Similar observations were recorded by Kadri *et al*. (2000), who found that the high ratio in men is related to their professions as mostly they deal with animal husbandry in rural areas. We can attribute the higher percentage of seropositive women with a history of previous abortion to the fact that abortion is the possible outcome of brucellosis, and also because of the stronger immunological reaction of the organism in case of reinfection. The result was supported by Khan *et al*. (2001) who reported that

during pregnancy, brucellosis is associated with a considerable risk of spontaneous abortion. The higher prevalence in the age group 37-48 years is associated with increasing age. As people age, susceptibility to B. abortus also increases, especially in the most active childbearing and working age group (37-48 years), while the groups under 12 and over 49 years are seronegative for *B. abortus*. These findings correlate with the studies of Al Dahouk *et al*. (2007) who reported 16% positive cases with less than 20 years of age and high incidence in people with 60–69 years of age. The majority of the positive cases (6.38%) were attributed to direct animal contact while only 3.84% of positive humans were without any contact. Handling of aborted fetuses and placentas without precaution can be considered as a reason for infection. Our findings correlate with the observations of Min *et al*. (2005) who reported that human brucellosis was found to be related to touching infected calves or placental materials in Korea. Raw milk consumption was significantly more in positive individuals than in negative ones. Similar findings have been documented by Pandey *et al*. (2012) who revealed frequent use of raw milk and a lack of awareness about zoonosis in Nepalese society. The same result was obtained in a study on female brucellosis patients in Egypt by Sabah *et al*. (2008), who suggested that nearly 87% had a history of raw milk use. The present study is in alignment with the earlier observations by Samartino (2002) who reported poor veterinary services to be a risk factor for brucellosis in Argentina.

Conclusion

Most of the positive cases may be due to the lack of awareness of the population about brucellosis in the area we studied, poor hygiene practices, insufficient sanitary measures, and veterinary services. This study can be used as a basis for future more serious studies of the prevalence of brucellosis in the country. Regular routine screening of animals should be carried out and brucellosis control programs should be implemented. Greater awareness is needed among people raising animals and consuming dairy and meat products to minimize the spread of the disease to animals and humans. In addition, integrated approaches are needed to combat this pathogen and control its possible outbreak in the community. The Integrated approach will include participation of the governmental and non-governmental organization along with the human population to improve the human and livestock health status.

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