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## Epidemiosurveillance of *Brucella* infection in humans, non-ruminants and wildlife from Pakistan perspective (2000-2020)

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**ABSTRACT:** This review aimed at providing an overview of the prevalence and epidemiosurveillance of brucellosis in non-ruminants and humans in Pakistan during 2000-2020. Sero-prevalence of brucellosis has been reported in non-ruminants such as camels, equines, dogs and humans with the range of 0.5-21%, 16.23-62.6%, 9.2-63.8% and 2.0-70% respectively. Non-target species like Avian, reptiles and amphibians were also reported with the prevalence of 2.5%, 24.9% and 25% respectively. Ignorance and indifference make it endemic in ruminants and much-neglected disease in non-ruminants with less or no studies reported in canines. Vaccines are available and being used for ruminants while none is available for non-ruminants, which may serve as an important source of spreading disease in animals and humans. In Pakistan, it is considered as ignored disease in non-ruminants lacking effective policies for control and eradication. This review guides policymakers to draw guidelines regarding brucellosis control and eradication using one health approach.

**Keywords:** Brucellosis; non-ruminants; endemic; zoonosis; epidemiology; Pakistan

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## INTRODUCTION

**B**rucellosis is a zoonotic disease affecting both animals and humans caused by bacteria of the genus *Brucella* (Karthik et al., 2016). This contagious disease poses a heavy economic impact on the livestock industry and also has serious health hazards. *Brucella* is a facultative intracellular, non-motile, non-spore-forming coccobacillus (Shahzad et al., 2017). *B. melitensis*, *B. abortus*, and *B. suis* cause abortion and infertility in domestic animals, while *B. canis* causes infection in canines (Lopes et al., 2010; Godfroid and KaËsbohrer, 2002; Karthik et al., 2016). Camel is considered susceptible to *B. abortus* and *B. melitensis* (Shahzad et al., 2017). Humans can be infected by *B. melitensis*, *B. abortus*, *B. suis* biovars 1-4 and *B. canis* making it a public health concern. *B. melitensis* considered the most pathogenic and invasive species for humans followed by *B. abortus*, *B. suis* and *B. canis* in descending order (Lopes et al., 2010). *Brucella* species and their potential to infect humans are presented in Table 1.

Infected domestic animals are the main source of infection as well as the natural reservoir of these bacteria. They are excreted in milk, urine, semen and fetal fluids of the infected animals and transmitted through the conjunctiva, oral, nasal and sexual routes (Alfattli, 2016). The practice of rearing mixed livestock species can facilitate the spread of the disease (Radostitis et al., 2007). Humans are most frequently infected via direct contact with infected reproductive material,

through inspecting and whipping slaughtered animals and consuming rawmilk (Earhart et al., 2009; Liu et al., 2014). Person-to-person transmission is very rare. Breastfeeding, blood transfusion, organ transplantation, and accidental self-inoculation of *Brucella* vaccine strains can result to disease in humans.

*Brucella* is a facultative intracellular microorganism, which multiplies and escapes the host immune mechanism simultaneously by developing inside phagocytic cells (Gorvel and Moreno, 2002). Maintaining the chronic infection by this pathogen lies in its ability to survive and replicate within the macrophages (Neta et al., 2010; Roop et al., 2004).

The disease is of great economic importance having the potential effects on the production and reproductive status of animals including infertility and cessation of milk production after abortion (Wadood et al., 2009). In animals, the main clinical signs are abortion, low milk production, infertility, weak offsprings and death due to acute metritis and retained fetal membranes. The clinical signs of brucellosis in camels can vary from asymptomatic to abortion. Retention of fetal membranes, infertility, and delayed sexual maturity have been documented. Males may suffer from orchitis and arthritis accompanied by acute lameness (Sprague et al., 2012). In equines, the clinical manifestations of brucellosis are poll-evil and fistulous withers due to the inflammation of supraspinous bursa and connective tissue, leading to abscess formation and fistulation in the affected regions.

**Table 1:** Currently described *Brucella* species and their zoonotic potential (Sprague et al., 2012)

Species	Biovars	Animal host	Human disease
<i>Brucella</i> (B.) <i>abortus</i>	1-9	Cattle, bison, buffalo, elk, yak, camel	Yes
<i>B. melitensis</i>	1-3	Sheep, goat, cow, camel	Yes
	3	Nile catfish; dog	
<i>B. suis</i>	1	Horse	Yes (biovars 1-4)
	1,2,3	Pig, wild boar	
	2	European hare	
	4 ( <i>B. rangiferi</i> )	Caribou, reindeer	
	5	Rodents	Yes
<i>B. ovis</i>		Ram	Not reported
<i>B. neotomae</i>		Rodent	Not reported
<i>B. canis</i>		Canines	Yes (rarely)
<i>B. ceti</i>		Whale, dolphin, porpoise	Yes
<i>B. pinnipedialis</i>		Seal	Not reported
<i>B. microti</i>		Common vole, red fox; (soil)	Not reported
<i>B. inopinata</i>		Human	Yes
Baboon isolate		Baboon	Not reported
BO2		Unknown	Yes
Australian rodent strains		Rodents	Yes

Occasionally abortions and other reproductive problems are also reported (Megid et al., 2010). The typical sign in female dogs is late abortion followed by a mucoid, serosanguineous, brownish, or gray vaginal discharge that persists for up to six weeks (Hollett, 2006; Shin and Carmichael, 1999). The clinical signs in males are severe epididymitis, orchitis, and prostatitis (Hollett, 2006; Wanke, 2004). Natural infections of birds with *Brucella* and transmissions of disease from aborting cows to birds were discussed many times in the literature (Shahzad et al., 2018; Wareth et al., 2020). However, birds often show no clinical signs but they do occur, symptoms frequently include enteritis and diarrhea (Wareth et al., 2020). In the case of amphibians, pathologic changes ranging from individual, localized disease manifestations (e.g. subcutaneous abscess, skin lesions, swollen paravertebral ganglia, panophthalmitis) to systemic bacterial infections with high mortality were observed (Mühldorfer et al., 2017). The clinical manifestation of brucellosis in humans is an undulant fever in which the temperature can vary from 37°C in the morning to 40°C in the afternoon. Other symptoms like night sweats, chills and weakness are also reported. Malaise, insomnia, anorexia, headache, arthralgia, constipation, sexual impotence, nervousness, and depression are also common in patients (Megid et al., 2010).

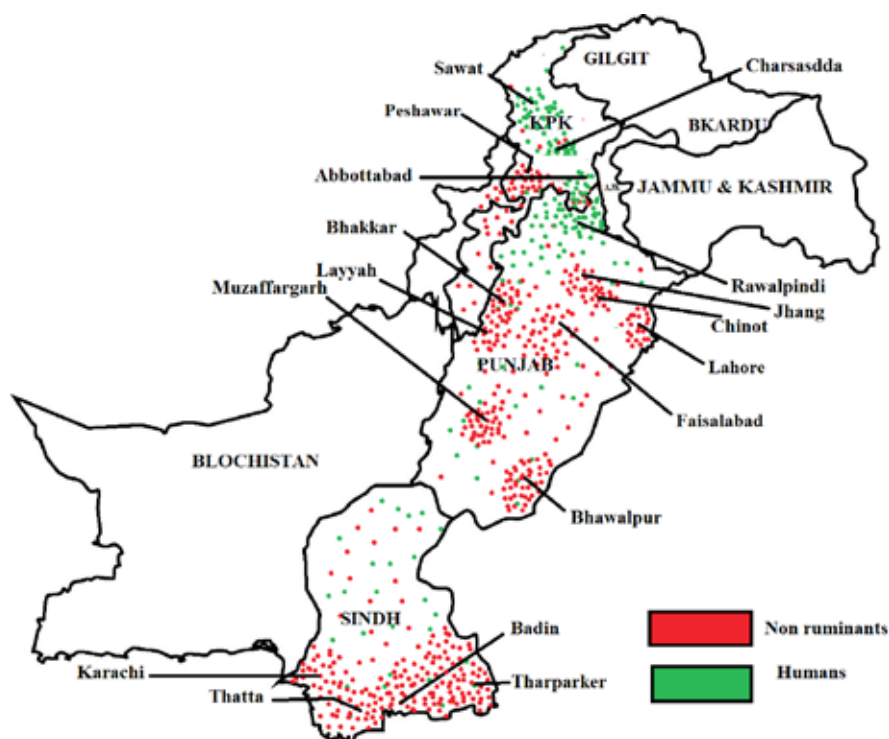
The gold standard test for diagnosis of brucellosis is isolation and identification of the organism. It takes a longer time, which makes this method laborious and time-consuming. Infection risk is a major hurdle in culturing *Brucella* due to its zoonotic potential (Karthik et al., 2014). A presumptive diagnosis can be made by a different serological test like Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Enzyme-Linked immune sorbent assay (ELISA) (Nicoletti, 2007). Other screening tests include Buffered Plate Agglutination Test (BPAT), Milk Ring Test (MRT), Complement Fixation Test (CFT), and Fluorescence Polarization Test (FPT) (Acha and Szyfres, 2003; Godfroid et al., 2010). The use of PCR to identify the *Brucella* species up to biovars level has increased the efficiency of the test. It is an easy, cost-effective and less time-consuming method to identify the organism (Fernando et al., 2010).

Expansion of the animal industry, lack of hygienic conditions on the farm and improper food processing makes brucellosis a public health risk. It spreads from one region to others due to international travel, importation of animals and their derived products. Being a

zoonotic disease, it is considered an occupational hazard for those persons having direct contact with infected animals like farmers, veterinarians, and butchers (Dil et al., 2017). Brucellosis has been eradicated from many developed countries but still endemic in Africa, the Middle East, the Mediterranean, Asia and Latin America (Geering et al., 1995; Refai, 2002). In Pakistan, there is little information on animal and human brucellosis. The epidemiosurveillance and bacteriological isolations of *Brucella* are very scarce. In the last few decades, brucellosis in ruminants has become a focused point for researchers resulting in a huge research gap for other vulnerable species. It is one of the most ignored diseases with respect to non-ruminants in Pakistan. Despite the detection of brucellosis in all domestic and wild animals, Pakistani people lack awareness regarding the zoonotic potential of this disease with their existing habit of raw milk consumption and close contact with infected animals. This review aims to describe the prevalence and epidemiology of brucellosis and encourage interested researchers to understand the brucellosis situation in Pakistan in a better way. For this purpose, available epidemiological data from 2000 to 2020 on non-ruminants and human brucellosis in Pakistan were analyzed using various search engines such as google scholar, Pubmed, Scopus and Web of Science. The geographical distribution of Brucellosis in non-ruminants and humans in Pakistan is illustrated in figure 1.

## CAMEL BRUCELLOSIS

Investigations confirmed the presence of brucellosis in camels. Studies reported that seroprevalence of brucellosis may range from 0.51 % (Ullah 2015) to 21.0% (Baloch et al., 2016) in Pakistan. Fatima et al (2016) examined 200 camel sera using random and multi-cluster sampling from the lower Punjab of Pakistan. They found 5%, 2% and 1.5% sera positive using RBPT, cELISA and real-time PCR respectively. Shehzad et al., (2017) investigated 761 camel serum samples for brucellosis using RBPT and found 3.1% positives. The prevalence of brucellosis in camels belonging to various regions of Pakistan is summarized in table 2. A higher prevalence of brucellosis was recorded in the nomadic production system than in the organized production system. The seroprevalence of brucellosis was higher among adult camels than young ones and also higher in females compared to males (Fatima et al., 2016; Shahzad et al., 2017). With the increase in age, an increase in the level of hormones and erythritol may enhance the growth of



**Fig 1:** Geographical distribution of non-ruminant and human brucellosis in Pakistan

this pathogen (Poester et al., 2013). The high seropositivity of brucellosis was noticed in the animals with poor health status followed by moderate and good health status (Shahzad et al., 2017). The animals having more parity numbers were found more infected compared to the animals with fewer parity numbers (Shahzad et al., 2017). Fatima et al. (Fatima et al., 2016) suggested that brucellosis cases decrease in summer and spring and increase in winter because the pathogen does not survive in hot weather and cannot withstand direct exposure to sunlight. The study also described that sharing of common pastures and water points with infected animals had enhanced the transmission of brucellosis to camels.

### EQUINES BRUCELLOSIS

Very few seroprevalence-based studies of equine brucellosis conducted in Pakistan. The prevalence range of equine brucellosis in Pakistan was reported as 16.23% (Gul et al., 2013) to 62.6% (Safirullah et al., 2014). Wadood et al., (2009) investigated 300 serum samples from equines and used RBPT and SAT. The overall prevalence was 20.7% by RBPT and 17.7% by SAT. All studies about the prevalence of equine brucellosis are presented in table 2. Females were more prone to this disease than males (Gul et al., 2013; Safirullah et al., 2014; Wadood et al., 2009). Wadood et al., (2009) observed that 9.6% of stallions

and 17.7% mares were found infected with brucellosis. The higher prevalence in mares might be due to their close association with reproductive discharges passed after abortion or parturition by infected mares which can infect the healthy ones. Sexually matured animals are more susceptible to *Brucella* infection than sexually immature animals of either sex (Radostitis et al., 2007). Higher seroprevalence was found in those groups with the age of greater than 5 years than those groups with the age of less than or equal to 4 years (Gul et al., 2013; Safirullah et al., 2014; Wadood et al., 2009). Brucellosis prevalence 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 (Gul et al., 2013). The low prevalence can be explained on the basis that young animals may harbor the organism without expressing any detectable antibodies until their first parturition or abortion. Generally, the disease was more prevalent in animals of poor health condition while less in healthy animals (Safirullah et al., 2014; Wadood et al., 2009). Wadood et al., (2009) observed that 9.7%, 13.0% and 20.0% of seropositivity rate was found in good, fair and poor conditioned animals respectively. Wadood et al., (2009) also documented that desi breed was highly infected with brucellosis followed by Thoroughbred, Crossbred and Arabian horses in descending order. This study also revealed that chances of brucellosis increase with the increase of parity number in mares.

**Table 2:** Prevalence of brucellosis based on various diagnostic tests in non-ruminants and humans of Pakistan (2000-2020)

Year of studying	Diagnostic method	Camel	Equine	Dogs	Human	Avian	Reptiles	Amphibian	References
2001-2002	SAT	775 (1.8)							(Siddiqui, 2016)
2008	RBPT				300 (14)				(Ali <i>et al.</i> , 2013)
	ELISA				300 (11)				
2008	ELISA				360 (21.7)				(Mukhtar and Kokab, 2008)
2009	RBPT		300 (20.7)						(Wadood <i>et al.</i> , 2009)
	SAT		300 (17.7)						
2012	SPAT, PCR		500 (62.6)						(Safirullah <i>et al.</i> , 2014)
2012-2013	SPAT				300 (3.66)				(Ahmad <i>et al.</i> , 2017)
	STAT				300 (2)				
	PCR				300 (2.66)				
2013	RBPT	100 (21)							(Baloch <i>et al.</i> , 2016)
	SAT	100 (21)							
	cELISA	100 (13)							
2013	RBPT, SAT				262 (6.9)				(Hussain <i>et al.</i> , 2018)
2013	RBPT				429 (5.8)				(Ali <i>et al.</i> , 2016)
2013	RBPT		308 (20.13)						(Gul <i>et al.</i> , 2013)
	SAT		308 (16.23)						
2014	SPAT				200 (10)				(Perveen and Raqebullah, 2015)
	PCR				200 (7.5)				
2014	SAT				95 (38.94)				(Asif <i>et al.</i> , 2014)
	PCR				95 (14.7)				
2014-2015	RBPT				446 (10.1)				Saddique <i>et al.</i> , 2019
	qPCR				446 (5.8)				
2015	RBPT	387 (0.51)							(Ullah, 2015)
	PCR	387 (0)							
2015-2016	SAT			87 (9.2)					Jamil <i>et al.</i> , 2019
	ELISA			87 (10.3)					
	qPCR			87 (1.15)					
2015-2016	SAT			94 (63.8)					
2016	RBPT	200 (5)							(Fatima <i>et al.</i> , 2016)
	cELISA	200 (2)							
	qPCR	200 (1.5)							
2016	RBPT, ELISA				250 (16)				(Sultan Ali <i>et al.</i> , 2018)
2016	RBPT					79 (2.5)	34 (24.9)	4 (25)	(Shahzad Ali <i>et al.</i> , 2018)
2016	SPAT				73 (24.6)				Khan <i>et al.</i> , 2018
	RBPT				73 (6.84)				
	PCR				73 (12.3)				
2016	SPAT				50 (30)				Khan <i>et al.</i> , 2018
	RBPT				50 (4)				
	PCR				50 (18)				
2017	SPAT				200 (6)				(Khan <i>et al.</i> , 2017)
	PCR				200 (2)				
2017	RBPT	761 (3.41)							(Shahzad <i>et al.</i> , 2017)
2017	SAT				70 (70)				(Malik <i>et al.</i> , 2018)
2018	SPAT				100 (23)				Maria Saif <i>et al.</i> , 2018
2018	RBPT				183 (8)				(Waheed <i>et al.</i> , 2018)
	cELISA				183 (13)				
	PCR				183 (33)				

Figures in parenthesis indicate percentage and outside show total no. of samples examined

## CANINE BRUCELLOSIS

The first-ever report on *B. canis* and *B. abortus* in dogs of Pakistan reported by Jamil et al., (2019) showed the 9.2% and 10.3% serological prevalence of brucellosis in dogs of Faisalabad district of Pakistan by SAT and ELISA respectively. Only one Elisa positive sample being founded positive for *B. abortus* through real-time PCR. They also reported 63.8% seroprevalence in dogs of Bahawalpur district of Pakistan with none of the sample was positive by Elisa and real-time PCR. One-year-old stray dogs were found positive against *B. canis* with poor body conditions. Moreover, *B. abortus* was detected from wounds present on the animal body. These findings highlight a risk of disease transmission from stray, wild and domestic dogs to livestock and humans and vice versa.

## AMPHIBIANS, REPTILES AND BIRDS BRUCELLOSIS

To date, the epidemiology of *Brucella* infections in cold-blooded hosts is largely unknown. Shahzad et al., (2018) examined 117 blood samples from birds, amphibians and reptiles collected from the Sindh (Karachi) and Punjab (Pattoki) provinces of Pakistan. They found 11.11% samples seropositive for *Brucella* antibodies. More specifically, 25% avian, 29.4% reptiles and 25% amphibian samples were found seropositive using RBPT. In avian species, 6.25% peafowl and 9.1% Indian blue rock pigeons were diagnosed positive against brucellosis. In the case of amphibians and reptiles, 25% of Indian bullfrog and 32.3% yellow-spotted mud turtles were seropositive for *Brucella* antibodies respectively. These animals had no clinical signs of disease but could be a non-target species of brucellosis and can serve as a potential source of disease spread in marine, ground and flying birds. They can also pose a great risk to zoo laborers, veterinarians and shopkeepers.

## HUMAN BRUCELLOSIS

The prevalence of brucellosis in humans may range from 2.0% (Ahmad et al., 2017) to 70% (Malik et al., 2018) in Pakistan. Waheed et al., (2018) collected 183 blood samples of occupationally exposed humans and tested using RBPT, cELISA and PCR and results revealed 8%, 13% and 33% samples positive respectively. Asif et al., (2014) examined 95 blood samples collected from veterinary professionals, livestock farmers and butchers. They found 38.94% and 14.7% of sample positives using SAT and PCR respectively. All prevalence-based studies are illustrat-

ed in table 2. Generally, it is found to be more common in males as compared to females. Sultan Ali et al., (2018) observed that the prevalence of brucellosis was higher in males (24%) than females (8%). In contrast, few studies reported that the disease is more common in females as compared to males (Ahmad et al., 2017; Khan et al., 2017; Malik et al., 2018). It was suggested that age constituted an important epidemiological risk factor for human brucellosis. Mukhtar and Kokab, (2008) reported that the age group of 51-60 years had the maximum seropositivity. While, Malik et al., (2018) documented that the majority of *Brucella*-positive individuals belonged to the age group 21-40 years. In another study, the highest prevalence of brucellosis was found in the age group ranging from 40-60 (Perveen and Raqeebullah, 2015). Young people showed higher prevalence than older because they were more engaged in working with livestock and also exposed to other occupational risks. In a study, 5.8% of pregnant Pakistani women were found to be seropositive and indeed women from rural areas were more often seropositive than those from urban areas (Ali et al., 2016). This study further revealed that pregnant women consuming raw milk were more often seropositive (76.5%) compared to those never consuming raw milk (2.9%). Sultan Ali et al., (2018) reported that individuals living in rural areas were 2.3 times more likely to be *Brucella* seropositive as compared to urban areas. Malik et al., (2018) found brucellosis among patients presenting with nonspecific symptoms. Symptoms were malaise, headache, insomnia, and fever. Lack of education and proper awareness about health and diet is the major reason for infections in the area. When the presence of infection was measured with education level, 83% of total patients were found to be illiterate (Ahmad et al., 2017). A high prevalence of *Brucella* infection was reported in those people having direct contact with animals and/or their products. The slaughterhouse workers are generally more susceptible to contract brucellosis by virtue of their direct exposure to viscera, gravid uterus and fetal membranes of infected animals (Mukhtar and Kokab, 2008). This study further revealed that brucellosis was found to be more common among individuals who had been involved in calf deliveries and had handled placenta. It is a significant finding that raw milk is a constant source of disease spread to farmers, milking men and general users. Disease prevalence was more in people associated with milking activities possibly due to the use of raw milk (Waheed et al., 2018).

### Policy, response and control strategies

Treatment of brucellosis is not effective in animals. Career animals should be quarantined to limit the further spread and infected ones need culling based on screening and confirmatory tests (Falagas and Bliziotis, 2006). Brucellosis can be treated using a multi-drugs approach but treatment failure and relapse rates are very high (Pal et al., 2017). Antibiotic treatment can be used for genetically superior animals but due to uncertain outcomes, it is not recommended (Radostitis et al., 2007). Human treatment is possible and effective if this disease is diagnosed at an early stage and the patient gets effective drugs for an adequate length of time. A combination of doxycycline, rifampin, sulphamethoxazole, and trimethoprim are being used for treatment in humans (Alp et al., 2006; Khuri-Bulos et al., 1993; Yilmaz et al., 2004).

Most of the countries, particularly developed states, follow test and cull policy for infected animals to limit the spread of this disease among animal and human populations. This policy is not practicable in Pakistan due to limited resources to compensate the farmers for the slaughtering of infected animals (Mukhtar, 2010). Measures need to be followed for regular screening of the herd using RBPT, MRT, and PAT. Farmers should use screening tests while purchasing new animals from the market, if any animal is found positive, then avoid buying such an animal. A national eradication program has yet to propose for brucellosis in the country. The main hurdles limiting the control of brucellosis are the security of the country, shortage of funds, laboratory facilities and trained manpower. A human vaccine against brucellosis is not developed yet, therefore, the control of human brucellosis can only be possible by keeping animals brucellosis-free (Ning et al., 2013). In order to reduce the brucellosis burden in humans, mass vaccination not only in ruminants but also in non-ruminants can increase the resistance to infection in animals. To date, the brucella vaccine for dogs is not commercialized yet and trials are being conducted at the research level. Preventive measures for Brucella infection in dogs include spaying or neutering, giving antibiotics for several months, and frequent blood tests to monitor treatment progress. Pasteurization of milk and dairy products is another preventive measurement for humans. The development of powerful tactics is necessary to raise awareness in people about brucellosis, its zoonotic potential, the economic impact on the livestock industry and preventive measurements using extension services, leaflets, posters

and other mass media. Mass vaccination not only in ruminants but also in non-ruminants can increase the resistance to infection in animals. Seroprevalence and epidemiology-based studies at the country level can help policymakers to propose a brucellosis eradication program in the country. Brucellosis control and eradication program should be initiated as in Egypt and Palestine (Eltholth et al., 2017; Awwad et al., 2018). In Egypt, two strategies are used. One is to test the animals and cull the infected ones based on positive serological tests. Another strategy is vaccination of the animal population. In Palestine, the brucellosis control program includes mass vaccination of animals and strengthening of their institutions for controlling and monitoring the disease.

### CONCLUSION

Brucellosis poses a significant impact on human and animal health as well as socio-economic impact, particularly in those countries where rural income relies largely on livestock production and dairy products. Sharing of pastures, water and feeding points with infected animals should be avoided. High stocking density, the introduction of untested livestock animals from the market, lack of quarantine policy, and mixing of different species at the same farm are associated with a high prevalence of brucellosis in non-ruminants of Pakistan. In humans, males are found more susceptible as compared to females due to their occupational risks. The rural population of Pakistan harbors more infection than urban due to consumption of raw milk. Screening animals for brucellosis in villages and slaughterhouses is necessary and further attempts should be made to control this disease. Non-ruminants spread disease to humans as people are engaged at animal farms, treating animals and providing assistance in births. People keep dogs as a pet in their homes and they can be a source of infection, highlighting the need to screen pets on a routine basis. Detection of brucellosis in non-target species (reptiles, amphibians and birds) indicates the diverse host range of *Brucella* in Pakistan and increases the risk of infection not only for livestock animals but also for workers engaged with farm and wild animals. Vaccination in dairy animals is successfully being used but there is a dire need of time to vaccinate non-ruminants which may serve as an infection source for humans and animals. Programs to educate the agricultural people about brucellosis and food hygiene are needed to reduce the disease incidence. Due to the high prices of animals, the test and slaughter



policy is not an effective approach for the eradication of brucellosis in Pakistan. Testing, isolation, and management of infected animals in a quarantine system is the only viable approach to limit the spread of brucellosis. However, the impact of such policy in Pakistan has yet to be demonstrated.

#### **CONFLICT OF INTEREST STATEMENT**

None declared.

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