

# EPIDEMIOLOGICAL INVESTIGATION ON OUTBREAK OF BRUCELLOSIS AT PRIVATE DAIRY FARMS OF SINDH, PAKISTAN

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**ABSTRACT:** Brucellosis is one of the drastic diseases of zoonotic significance. Brucellosis is a global challenge not limited to Asia. As a result, economic losses are escalating due to the burden posed by Brucellosis in the investigated area. The present study was conducted to estimate the prevalence of brucellosis in cattle and buffaloes of Sindh, Pakistan. Blood samples were collected from (n=1200) animals (dairy cattle and buffaloes) of different age (2-7 years) and sex from 10 different districts of Sindh, Pakistan where no vaccination against brucellosis is practiced and were subjected to indirect ELISA for detection of *Brucella* antibodies. The overall mean prevalence was 18.16% with prevalence in unorganized is higher organized farms. In absence of any vaccination presence of circulating antibodies against *Brucella* in all age group of animals indicated the natural circulation of infection in the state. Based on the findings, the disease is seems to be endemic in the area, perhaps, due to partial or no vaccination. Also, the area presents poor bio-security measures and management. It is therefore, warranted to adopt good surveillance system for early identification of the brucellosis outbreak and appropriate measure for further control transmission of the brucellosis. Study indicated an urgent need of policy for prevention and control of brucellosis in dairy animals.

**Keywords:** Brucellosis, Prevalence, Privet dairy farms, Bio Security, Sindh

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## INTRODUCTION

Bovine brucellosis is the one of the most dangerous zoonotic disease which prevalence is throughout all over the world (Makita et al., 2011), and after this the most important is rabies (Shafee et al., 2011). This disease is distributed in livestock almost all over the world (Anka et al., 2014). Annually approximately 60 thousand new cases are reported at hospital in human, worldwide, is re-emerging with significant veterinary and public health concern (Manish et al., 2013). In cattle, brucellosis is generally caused by the *B. abortus* and *B. melitensis* (Anka et al., 2014). Most of the *Brucella* strains are highly pathogenic for the human population (Goni et al., 2008) as indicated by the presence of *Brucella melitensis* and *Brucella abortus* in pregnant women (Khan et al., 2001) and *Brucella* in man (Mai et al. 2013). It is also called bang diseases (Durrani et al., 2015). Bovine brucellosis causes serious economic losses in the cattle and buffalo, as a consequences of the late abortion, stillbirths, slaughtering of the infected animals, reduction in meat and milk production (Calistri et al., 2013), low fertility and cost of replacement of the animals (Shafee et al., 2011).

Cattle and buffalo are large ruminants which are mostly considered for dual purpose milk and meat purpose. Buffalos are also known as "black gold" (Soomro et al., 2014). Cattle and buffalos play key role in GDP of Pakistan via producing milk and meat (Soomro et al., 2014). Such animals are also play a significant role in draught. Due to very poor hygiene condition and poor management and care these animals are facing very serious several health problems, such as brucellosis and colibacillosis diseases, which are economical loss (Mailk et al., 2013). An aerobic gram-negative bacterium is the causative agent of brucellosis which belongs from genus *brucella* (Yousaf et al., 2015). In human being the brucella organism was affirmed as the primary cause of brucellosis, which are transmitted from cattle/buffalo or goat, sheep's through contamination or other air borne pathogens (Buhari et al., 2013). In males it causes orchitis and epididymitis while abortion in the females (Anka et al., 2014). Preventive measure can be adopted by minimizing animal to animal transmission. The predominant sources of transmission occurs via mucous membrane, contact with the contaminated material, inhalation, milk etc (Mai et al., 2013). In farm animals the main reported genus cause of brucellosis are *brucella melitensis* and *brucella abortus* (Karaca et

et al., 2007). Compared to other microbes, *Brucella* has the capability to localize in supra mammary lymph nodes and mammary glands of infected animals (Calistri et al., 2013). This pathogenic process is a powerful signal for down regulation of the immune system.

In Sindh, cattle and buffaloes are indigenous animals. Different breeds of cattle and buffaloes are kept in large and small scale. Sindh is a border Province, where animals are brought from other cities/provinces (i.e., Karachi, Quetta and Punjab Province etc) without bio security or appropriate measure of quarantine, it may be cause of very serious and several infectious diseases such as Tuberculosis, Anthrax and Brucellosis. Accurate diagnosis, quarantine and proper culling of the infected animals from the herd are required for control and prevention of the disease.

## MATERIALS AND METHODS

### Study Area

Cross-sectional studies were conducted in 10 districts (Hyderabad, Tando Muhammad Khan, Karachi, Mir Pur Khas, Khair Pur, Larkana, Thatta, Jamshoro, Rani Pur and Kashmor) of Sindh, Pakistan. The climate of the investigated area is humid subtropical with dry winter type and the vegetation is tropical dry and low rainfall of 750-1,100 mm/year, with RH (relative humidity) (25-45%), and normal annual temperature (20 °C to 48 °C).

### Collection of samples

In this study, blood samples from (n=1200) animals (of various sex, age and place) including (n=600) from unorganized and (n=600) from organized farms. Animals were kept in separate groups include adults, heifers, and young stock. All the animals at the farm were stall fed. No animal at the farm was vaccinated against the brucellosis. It was ensured very hygienic condition during blood sample collection by applying antiseptic solution on jugular vein of animals. About 5cc blood sample were collected from each and every animals and it was brought to laboratory on ice. The serums were collected by centrifugation at 3,000 rpm for 5 min. Samples were cooled immediately in ice jar as it was collected to transport for diagnostic laboratory. While at laboratory such samples were stored at -20 °C for as described by (Soomro et al., 2014).

### Epidemiology Study

Descriptive epidemiology and retrospective cohort study was conducted. In descriptive epidemiology, the herd demographic and management practices were recorded. In retrospective cohort study risk factors associated with the *bovine brucellosis* were investigated. A questionnaire was designed, pre-tested and interviews of owner/employees were conducted. The attendants at farm were also investigated for any sign and symptoms related to the *brucellosis* and farm record was reviewed.

### Sero Isolation of anti brucella antibodies

Rose Bengal Plate Test (RBPT) is standard key test for brucellosis in blood (Soomro et al., 2014). The serum samples were analyzed by Rose Bengal Plate Test and using commercially available kit (IDEXX brucellosis, USA) of enzyme linked immune sorbent assay-ELISA (OIE Manual 2004).

### Analyzing Results

The data were analyzed descriptively and analytically by using the Epi Info version 7. For the univariate analysis, frequencies of variables and measures were calculated.  $P < 0.05$  was considered to be statistically significant.

## RESULT AND DISCUSSION

A total (n=1,200) blood samples were collected throughout 10 districts of Sindh, n=76 (12.67%) were positive for brucella *abortus* antibodies in organized dairy farm, whereas 142 (23.67%) were regarded as positive in unorganized dairy farm for *B. abortus* antibodies. Hence overall prevalence percentage in Sindh was documented too much (18.16%) in 10 districts (Table 1), while unorganized farm are much more infected as compare to the organized dairy farm due to their best management, stress free environment, proper medication and well diet ration. As noted, there was no quarantine measures adopted for new animals that are likely to introduce into the farm. The bovine brucellosis outbreak occurred in the month of May 2014. Brucellosis cases were high among human and cattle population between months of March and September (Lee et al., 2013).

In each district total 120 samples were collected from organized (n=60) and unorganized (n=60) dairy farm. District wise details of prevalence of brucellosis in dairy farms are as showed in (Table 2).

**Table 1 - Comparison of Organized Dairy Farms & Un-organized Dairy Farm of Sindh.**

Farms Details	Total Sample	Positive Result Sample	% +Ve Sample Result
Organized Dairy Farms	600	76	12.67%
Un-Organized Dairy Farms	600	142	23.67%
Total	1200	218	18.16%

**Table 2 - Comparison of Organized Dairy Farms & Un-organized Dairy Farm districts wise of Sindh**

District	Organized Dairy Farm		Unorganized Dairy Farm	
	No. of Positive Sample	Positive Result %	No. of Positive Sample	Positive Result %
Hyderabad	07	11.66	12	20
Tando M. Khan	12	20	13	21.67
Karachi	03	05	15	25
Mir Pur Khas	05	8.33	11	18.33
Khair Pur	04	6.67	17	28.33
Larkna	03	05	09	15
Thatta	09	15	18	30
Jamshoro	08	13.33	13	21.67
Rani Pur	12	20	16	26.67
Kashmor	13	21.67	18	30
TOTAL	76	12.67	142	23.67

The farm management (Biosecurity and housing) was very poor because the waste material was not properly disposed especially the aborted material which is the source of the infection. It's may be due to lack of awareness. There was no proper disposal of the aborted material at farms in district Hyderabad (Soomro et al., 2014). There were no proper fences or boundary wall around the farm. Rodents and dogs were also present at the farm.

The prevalence with multiple risk factors and different herds always has the possibility of false positive results affecting the final outcome of the study. However, the sensitivity of particular test in any study model reduces the chances of false positivity. It is evaluated from the current study that a wide spread presence of brucellosis in dairy cattle and buffalos are documented in these selected districts of Sindh. Presences of *brucella* antibodies are mostly found in non-vaccinated. The prevalence in un-organized farms is at alarming level. Moreover, animal owners/farmers in this area are in close contact with these animals, and consumption of raw milk and improper handling of aborted materials is frequent. Thus brucellosis is not only the cause of reproductive and production losses but also may be the potential biohazard in this region. Thus, from the current study the more study regarding brucellosis in this area are suggested epidemiological investigations to find out the link between animal and human brucellosis. To get control on this disease and can get control against such dangerous disease.

## CONCLUSION

Present study is summarized that the management conditions of the farm were not organized. The area was not fenced for stop the rodents and stray dogs, waste material not properly disposed, no separate pens for aborted and sick animals, poor quality of drinking water, feed not stored properly and no proper treatment of the animals. The workers in the farm working without biosecurity measures. There was no surveillance system working in the areas to identify the brucellosis outbreak.

A good surveillance system is important to early identify brucellosis outbreak and take immediate measure for further control and transmission of the brucellosis. Vaccination and quarantine of newly introduced animals should be regularly practiced. A strict biosecurity measures should be adopted at the farm. The waste materials should be properly handled to reduce the challenges of brucellosis challenges from the potential source of infection.

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## Conflict of interests

The authors declare that they have no conflict of interest with respect to the research, authorship, and/or publications of this article.

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