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Short Communication

Epidemiological Investigation of Outbreak of Brucellosis at Private Dairy Farm, Central Punjab-Pakistan

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Abstract | Brucellosis is one of the drastic disease of zoonotic significance. The disease is not only endemic to Pakistan but also to other South Asian countries. As a result, economic losses are escalating due to the burden posed by Brucellosis in the investigated area. The present study included herd demographic, management characteristics and risk factors as variables. Total of 66 cattle were identified at Pindi Bhatian dairy farm, Punjab Pakistan. Information on the risk factors was collected by filling the questionnaire from dairy farm workers. The blood sample was collected from 66 female cattles age range of 1.5 to 6 years on average. The serum was analyzed through Rose Bengal Test (RBPT) and i-ELISA. Out of the total, 42 samples positive (63.64%) with the RBPT and 38 (57.58%) samples were positive with the iELISA indicating the sensitivity of this test Crossbreed (22 cases) vs 16 cases in the Friesian breed. Relative Risk (RR) towards *Brucella* antigen. By comparison, the number of iELISA positive cases were high in for the breed (Crossbreed and exotic breed) was (p=0.1855; CI (95%).The animals with 6 years age (n=20 cases) were found to be high vs. 1.5 years. As for as abortion is concerned, it was found only in the 6 animals during the 3rd trimester irrespective of the age. 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 biosecurity 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.

Keywords | Brucellosis, Biosecurity, Demographic, Risk factors, Retrospective

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Bovine brucellosis in livestock is distributed almost all over the world (Anka et al., 2014). It is a contagious disease and can affect the human population. It is considered as one of world's most widespread zoonotic disease (Makita et al., 2011) and second most important after rabies (Shafee et al., 2011). It is prevalent in the Africa, Middle East, Asia, Central and South America (Bakhtullah et al., 2014). It is caused by Gram negative bacteria of genus *Brucella* (Anka et al., 2014) and has six main species such as *Brucella suis, Brucella abortus, Brucella ovis, Brucella melitensis, Brucella canis* and *Brucellaneotomae* (Makita et al., 2011). 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 meltensis* and *Brucella abortus*in pregnant women (Khan et al., 2001) and *Brucella* in man (Mai et al. 2013).

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). 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). 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.

The developed nations has fortunate enough in controlling and eradication of *Bovine brucellosis*, however, it remains a significant problem for the developing countries (Mai et al., 2013). The incidence rate of brucellosis in Pakistan is escalated in recent years, 21% to 26% (Shafee et al., 2011). It is important to investigate the outbreak occurred at the Pindi Bhatian dairy Farm, Punjab, Pakistan. As according to the history it was suspected for the bovine brucellosis. It is a zoonotic disease; therefore, it is essential to protect the human population and to control the disease for further spreading to other animals as it causes high economic loss to the farmer.

The study was conducted at a private dairy farm in Central-Punjab, Pakistan. Total numbers of animals investigated were 220 in numbers. Farm has one (1.0) acre covered area. In this area farm to farm distance was approximately 0.5 km. Animals were kept in separate groups include adults, heifers, and young stock. All the animals at the farm were stall fed (n=220). No animal at the farm was vaccinated against the brucellosis. The farm employees were also investigated and it was found that one attendant had signs and symptoms related to the *Brucella* infection.

Selection of case was depending upon the animal characterized by one or more of following signs: abortion, orchitis, retained placenta, arthritis, epididymitis, and excretion of the organisms in milk and in uterine discharges (OIE, 2004).

Descriptive epidemiology and retrospective cohort study was conducted. In descriptive epidemiology, the herd demographic and managemental 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.

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, 2004).

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-value < 0.05 was considered to be statistically significant. Relative Risk (RR) was calculated to determine the risk factors associated with the bovine brucellosis with 95 % Confidence Interval (CI).

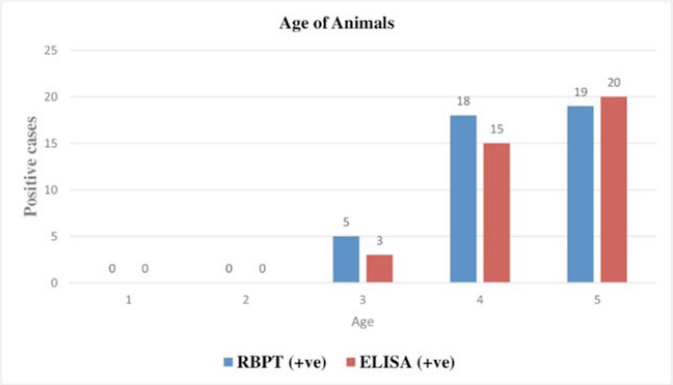

Figure 1: Sensitivity of RBPT and ELISA towards number of positive samples and age
RBPT: Rose Bengal Plate Test antigen; **ELISA:** Enzyme Linked Immunosorbent Assay; **AI:** Artificial Insemination

Total 66 samples tested with RBPT, and then same samples again confirmed with ELISA. There were 42 (63.64%) samples positive with the RBPT and 38 (57.58%) samples were positive with the ELISA (Figure 1 and 2). All the animals were females having 1.5 to 6 years. The highest numbers of cases were positive for animals with 6 years age (20 cases). The abortion was recorded only in the 6 animals (3rd trimester). There were three breed of animals at the farm, crossbreed, Friesian and Jersey. The highest number of positive cases were in Crossbreed (22 cases) while 16 cases were recorded in Friesian breed.

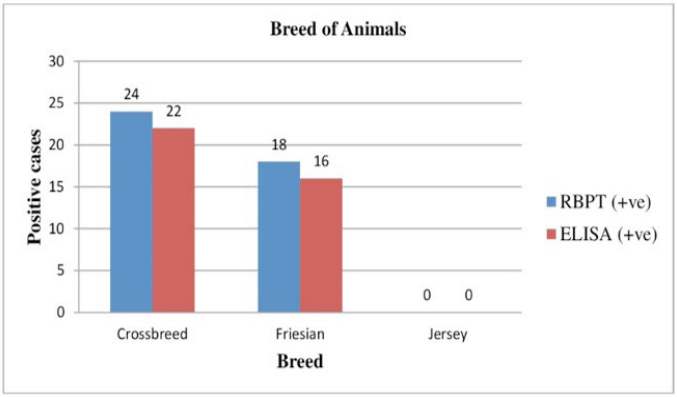

Figure 2: Sensitivity of RBPT and ELISA towards breed

All (66) animals at the farm were stall fed. The source of water for drinking purpose was tube well. No animal at the farm was vaccinated against the *Brucella* vaccine. One attendant had signs and symptoms of Brucellosis. In this area farm to farm distance was approximately 0.5 km. All the animals were purchased from the market. The farm man-

agement was very poor because the waste material was not properly disposed especially the aborted material which is claimed to be the source of the infection. There were no proper fences or boundary wall around the farm. Rodents and dogs were also present at the farm. The worker from other farms usually visited this farm that further aggravate the potential threat of *Brucella* spreading.

As noted, unfortunately, there was no quarantine measures adopted for new animals that are likely to introduce into the farm. Relative Risk (RR) for the breed (Crossbreed and exotic breed) causing brucellosis was 0.7676 (p-value=0.1855) {CI (95%) = 0.4368-1.3490} (Table 1).

Table 1: Risk Factors for the bovine brucellosis

Bread	Cariables			
	%	RR	p-value	CI (95%)
Crossbreed	22/35 (62.28)	0.7676	0.1855	0.4368 -1.3490
Exotic breed	16/31 (51.61)			

RR: Relative Risk; **CI:** Confidence Interval

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). There were 42 samples positive (63.64%) with the RBPT and 38 (57.58%) samples were positive with the ELISA. Our present findings are in harmony with Shafee et al. (2011) who investigated 86 samples of cattle and found 17 (20%) positive with i-ELISA in Pakistan; while another study reported 191 (45.80%) positive animals for the bovine brucellosis with i-ELISA (Jagapur et al., 2013), though not in the same region.

Anti-Brucella antibodies were detected by the RBPT and ELISA in 56 (10.18%) and 44 (8%) of cattle respectively (Hussain et al., 2008). All the animals were females of 1.5 to 6 years. The highest numbers of cases were positive for animals having 6 years age (20 cases). The anti-*Brucella* antibodies were associated with the increasing age in the bovine (McDermott et al., 1987). Although, our sample size was low but still number of vaccinated animals were found zero. Similarly, a recent study investigated a total of 749 herds, 74 herds were not vaccinated against the bovine brucellosis (Borba et al., 2013).

One attendant had signs and symptoms related to the *Brucella*. The prevalence of the brucellosis among the farm workers was found to be 10% (Hussain et al., 2008). In this area farm to farm distance was approximately 0.5 km. There was an attack rate of 28.1% in herd which was neighbouring to the herd with the primary outbreaks (Abernethy et al., 2011), and 21% of cattle were purchased from the outside of the veterinary district (Cowie et al., 2014). All the animals were purchased from the market. There were 3.1% animals found seropositive for brucellosis which were newly introduced to the farm (Stringer et al., 2008).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 information. 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. Dog and cats were present in the 76 bovine brucellosis seropositive herds (Borba et al., 2013).

Present study was concluded 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 very important to early identify the 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 because it is a 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.

AUTHORS' CONTRIBUTION

Muhammad Kaleem was the principle investigator and Aneela Zameer Durrani was the supervise. Muhammad Arif Rizwan coordinated sampling; Muhammad Asif Arain and Zohaib Ahmed Bhutto helped in article review; Muhammad Saeed analysed the data statistically; Khushal

Khan Kasi helped in epidemiological designs while Umar Bacha coordinated in sampling.

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