SURVEILLANCE OF BRUCELLOSIS IN LIVESTOCK IN RURAL COMMUNITIES OF PUNJAB, PAKISTAN.

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ABSTRACT:- Brucellosis is an emerging animal as well as human health issue in Pakistan. Population based estimation of true prevalence is time occurring in different livestock species and requires to develop prevention and control strategy, that hasn't been done in Pakistan. Active disease surveillance at door steps was conducted to collect blood samples from small and large ruminants of subtropical rural communities in the province of Punjab during January-March, 2015. Total 59665 sera samples from apparently healthy animals were collected and screened by Rose Bengal Plate test and confirmed by Complement fixation test, respectively. After adjustment for test sensitivities and specificities accordingly, and chi square analysis of results, the true prevalence per 1000 animals in buffaloes was (7.86%), significantly (P<0.01) higher than small ruminants (5.84%) and cattle (2.05%). Being a subtropical region, geographically significant (P<0.01) variation was observed in burden and pattern of brucellosis i.e., the highest prevalence was in Central (14.81%) followed by South (0.5%) and northern (0.4%) Punjab. Considering the high mean prevalence and its zoonotic importance it can be devastating if not controlled because of bad management practices, lack of awareness and close contact of farmers with their livestock in Pakistan. Where the socio-economic conditions of rural population does not allow test and slaughter policy, therefore, mass vaccination is recommended to control brucellosis through local government support.

Key Words: Ruminants; Brucellosis; Rural; Prevalence; Pakistan.

INTRODUCTION

In developing countries the brucellosis image is much more erratic. These countries report the maximum number of epidemics and animal losses (WHO, 2011). Brucellosis is a gram negative bacterial disease from genus Brucella. The species infecting livestock are *B. melitensis*, in sheep and goats mainly; *B. abortus* in cattle; *B. suis*, in pigs; and *B. ovis*, in sheep only (Crawford, 1990). In all these natural hosts, Brucellosis is usually represented by reduced fertility, abortion and in ruminants with

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reduced milk production (Crobel, 1988). It's always ranked amongst the most economically vital zoonoses globally (Perry and Grace, 2009 and WHO, 2009). Humans can get the disease through direct contact or by ingestion of contaminated milk of infected animals (Paweska et al., 2002). It is apparent that brucellosis could have a dramatic affect on both animal and human health, and that dynamic attempts should be taken to control it in endemic areas in live-stock, also in the countries where brucellosis is emerging or re emerging (Russo et al., 2009).

Being an agricultural country, Pakistan livestock accounts for 9.4% of its national GDP. Brucellosis have a substantial impact on animal and human health, also on socioeconomic factors, where rural revenue relies mostly on domestic animals farming, land cultivation. In this part of the world people usually live in extreme close proximities with their livestock (Shafee et al., 2011; Maadi et al., 2011). Mixed crop livestock farming system is practiced, where people usually breeds 90% buffaloes and 10% cattle, for milk production and dairy products (Afzal and Naqvi, 2004). The most important output that comes from strategies of control and eradication of brucellosis are financial, due to improvement in livestock productivity and savings in health costs for animals, human's treatment and hospitalization. Therefore, meticulous emphasis should be given on active surveillance identifying the infected animals and removing them from the herd (test and slaughter). Very few studies reporting the prevalence of critical production and zoonotic diseases of livestock in Pakistan have been conducted, which

is key information for the implementation and prioritization of disease control programs.

Therefore, the present population based survey was conducted as a model to understand the epidemiology and review the importance of surveillance strategies for selecting the most suitable strategy for the control of brucellosis in the sub tropical continental lowland region of rural communities in Punjab, Pakistan.

MATERIALS AND METHOD

Study Design and Location

An active surveillance based survey was conducted under the supervision of the Directorate of Animal Disease Reporting and Surveillance, Government of Punjab during January-March 2015 parallel to the ongoing mass vaccination campaign against foot and mouth disease (FMD), Hemorrhagic Septicemia (HS) and Enterotoxaemia. A total of 17061 villages and 55586 households of 36 districts were visited by district diagnostic lab staff members for collecting blood samples (Figure 1). In rural communities of Punjab mostly the intensifying mixed crop livestock system exists. That is a sub tropical continental lowland region of Pakistan geographically.

Sample Size Determination

The whole small and large ruminant's inhabitant of rural communities of Punjab was the target population for the present study. Sampling frame was large and small ruminants (N=74.42 million) and sample unit was a single animal. By specifying the values i.e., Population size (N) = 74.42 million, anticipating a hypothesized percent frequency of
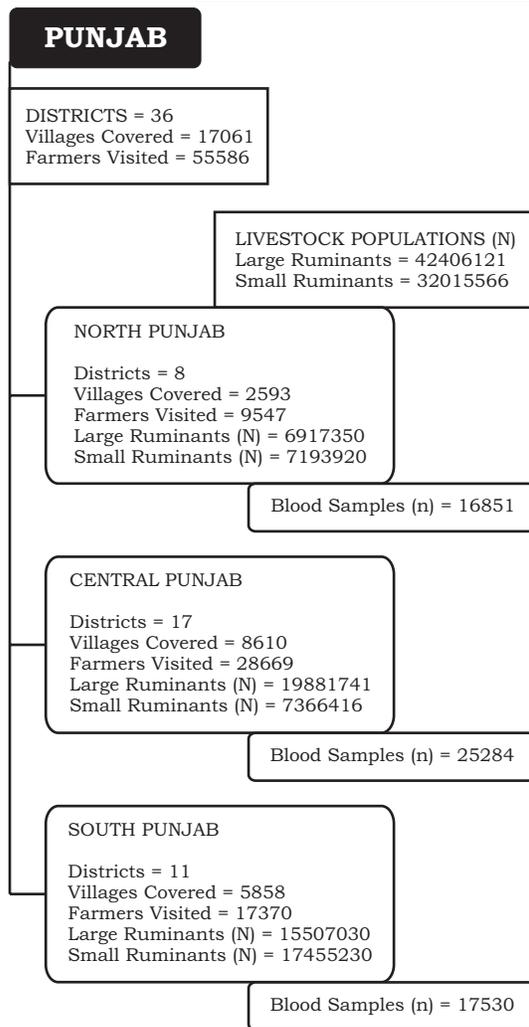
Figure 1. Flow diagram showing number of samples collected with population size from different regions of the Punjab.

outcome factor in the population (p) = 5%+/- 1 (Gul and Khan, 2007), having the absolute precision of 1%.

Using the following formula the sample size was calculated.

Sample
size (n) $=[Np\,(1-p)]/\,[(d^2/Z^2_{1-\alpha/2}(N-1) + p\,(1-p)]$.

Where,

N   =Population size (Null)

$Z^2_{1-\alpha/2}$ =1.96

d   =Precision (0.05)

p   =Estimate prevalence

The desired sample size at a confidence interval of 99.9% was calculated as 7192 for small and large ruminants. The blood samples selected randomly (n=59665) was much higher than the desired sample size. The sampling plan intended the selection of animals on a random basis, provided the farmers approved the samples collection, which the majority (>99%) did. The higher response was due to the free vaccination; their animals were getting in return. Samples were taken only from fully grown animals irrespective of sex and with no stratification.

Serology

Diagnosis of caprine and ovine brucellosis was performed by Rose Bengal plate agglutination (RBPT) and complement fixation test (CFT) as recommended for individual animals and screening flocks by (Robinson, 2003). The European Food Safety Authority (EFSA) recommends the RBPT and complement fixation tests (CFT) as confirmatory for brucella diagnosis in bovines that established comparable performances. RBPT and CFT were performed according to the procedures described by OIE (2008).

Data Entry and Analysis

Once all samples tested, the results were entered into a spreadsheet (Excel 2010; Microsoft Corporation, USA) and a database program (Access 2010; Microsoft Corporation, USA). For districts where serologically positive samples were identified, the apparent point prevalence was adjusted, for the combined serological tests sensitivity and specificity (Table 1) as

Table 1. **Distribution functions used for the estimation of RBPT and CFT sensitivity and specificity for calcu-lation of true prevalence**

Parameter	Probability distribution	References
Sensitivity RBPT(Se1)	β (61, 13)	(Gall and Nielsen, 2004)
Sensitivity CFT (Se2)	Triangular (0.68. 0.81,0.93)	(Qazilbash and Bari, 1997)
Combined Sensitivity	[Se ×Se]	---------
Specificity RBPT	β (1052, 1)	(Stemshorn et al., 1985)

per reported by Rogan and Gladen (1978). To allow for the uncertainty about the tests sensitivities, they were entered as probability distribution (Table 1).

RESULTS AND DISCUSSION

Being novel in the history of Pakistan, this survey does have some deficiencies in it. But, data venia, if we consider the resources available with the Livestock and Dairy Development (L & DD), Punjab, the short time frame for execution of this mission, the harsh weather, logistics management, the difficult terrains and high personal risk zones/hostile environments particularly for lady veterinarians then it's true worth is revealed. Surveillance can provide essential scientific information for informing decision-makers, stakeholders and consumers, and drive the most suitable strategy to limit brucellosis in susceptible hosts. The present study was conducted in the rural Punjab, the biggest province of Pakistan in terms of agricultural land availability, livestock population and human population. In rural communities of Punjab mostly intensifying mixed crop livestock system exists. This part of the country is a sub tropical continental lowland region geographically where the summer season remains extremely hotter and longer.

The point prevalence of the present study was calculated per 1000 animals of the target population. The serological true point prevalence of brucellosis recorded in some of the districts surveyed in this study was below 1 % or in some cases even zero prevalence was reported with a 99.99 % confidence. Only a few farmers practice vaccinations. Other studies conducted in sub-tropical plane regions of Punjab frequently reported mean prevalence ranging 10 % -21% in bovines (Nasir et al., 2004; Saleha et al., 2014). The low prevalence and or even zero prevalence here, is astonishing. Brucellosis is endemic in Pakistan and the production, animal husbandry and management practices were found almost correlating throughout the province. The estimated true point prevalence in buffaloes in the present study was 7.86%, significantly (P<0.01) higher than small ruminants (5.84%) and cattle (2.05%). These results are almost comparable with the reported results of (Ali et al., 2013), but are in contrast to those of (Nasir et al., 2004). The elevated cases of brucellosis in buffaloes may be due to their susceptibility to infectious diseases in hotter climates as comp-ared to other livestock species i.e., cattle and small ruminants as reported by Khan et al. (2015). The higher prevalence of brucellosis in buffaloes is highly comparable with that of (Fosgate et al., 2011) reporting on the basis of difference in brucella epidemiology in buffaloes and cattle. The higher prevalence may be due to adjustment for

tests sensitivities and specificities. Present results were in agreement to those of (Rahman et al., 2011) for the prevalence of brucellosis in cattle. The prevalence detected in small ruminants of (5.84%) was much higher than previously reported by (Nasir et al., 2000) among goats (0.4%) and sheep (1.93%) in Punjab region. Considering the elevated prevalence detected in the present study in small ruminants is alarming. The Directorate of Breed Improvement, L & DD, Punjab, needs to screen out all the animals with history of abortion or retained placenta or orchitis or epididymitis in collaboration with District Disease Diagnostic Laboratories. The higher prevalence in the previous studies is not comparable with the present study that may be due to the study design and sampling technique where these studies were conducted in herds with the histories of reproductive disorders. The aim of the present study was to review significance of such surveillance studies when evaluating the true prevalence of brucellosis based on population and to select the most apposite strategy for the control of brucellosis in this area. In the context of poor farmer's socio economic and disease epidemiological conditions an effective test and slaughtering policy will not be possible to be implemented here therefore a mass vaccination is recommended for the control of brucellosis in both small and large ruminants. It was concluded that the prevalence of brucellosis estimated in the present study in apparently healthy animals is an alarming sign especially in small ruminants that provides obvious evidence that it's a serious concern in Pakistan. There-fore there is an urgent need to devise policies for control and prevention of brucellosis in Pakistan.

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AUTHORSHIP AND CONTRIBUTION DECLARATION

S.No	Author Name	Contribution to the paper
1.	Ms. Ghazala Nawaz	Conceived the idea, and wrote the Manuscript
2.	Mr. Muhammad Nawaz Malik	Data Collection and Field work
3.	Mr. Muhammad Hassan	Overall management of the article,Technical input
4.	Mr. Fraz Munir Ahmad	Data Collection and Field work
5.	Mr. Ali Abdullah Shah	Wrote Abstract, Methodology
6.	Mr. Farooq Iqbal	Data entry in and prepared data sheet for statistical analysis
7.	Mr. Shinawar Waseem	Did SPSS Analysis
8.	Dr. Zahida Fatima	Technical input at every step, overall Management of the article
9.	Mr. Amjad Khan	Conclusion, Technical input, helped in finalizing the manuscript