CHAPTER V

SUMMARY

Bovine brucellosis, caused by *Brucella abortus*, is a serious zoonotic disease manifested by reproductive disorders such as abortions, infertility, retention of placenta, stillbirth and calf loss in animals, and results in huge economic losses to dairy farmers. During the present study, stratified sampling technique was used to draw a simple random sample from the population of animals. In the first stratum zones were selected followed by districts and finally dairy units were selected from selected district. A total of 2345 whole blood samples (1730 cattle and 615 buffaloes) and 9 aborted material samples were collected from the selected animals. All sera were screened by RBPT.

Four different serological techniques viz. Rose Bengal Plate Agglutination Test (RBPT), Micro Agglutination Test (MAT) which is actually a miniaturization of Standard Tube Agglutination Test, modified Micro Agglutination Test (mMAT) and indirect Enzyme Linked Immunosorbent assay (I-ELISA) were evaluated on 900 sera drawn from the total 2345 samples.

The samples collected for the purpose of molecular diagnosis of Brucellosis using PCR included abomasal contents of 9 aborted foetuses and 200 whole blood/serum samples. Three types of PCRs were used, for serum samples genus specific PCR was used. Aborted material were first analysed using genus specific PCR and latter on species differentiation was done by *B. abortus* specific primers. Bruce ladder PCR was used for differentiation of vaccine and field strains. The conditions for genus specific PCR were standardised using serum as starting material, and 8µl volume of sample DNA was found to be optimum per 25µl of the
reaction. The PCR products obtained were confirmed by allowing them to electrophoretically migrate in 1.5% agarose gel containing ethidium bromide. The electrophoresis run was allowed for 60 minutes at 80 volts, the gel was viewed under UV-transilluminator and the products were confirmed by their specific size.

For identification of risk factors associated with the occurrence of brucellosis among dairy animals (cattle and buffaloes), a pretested structured questionnaire was designed to include questions about factors known or thought to influence the spread of Brucella infection. Personal visits were made to the dairy units for both, collection of samples and introducing the questionnaire to farmers.

For observing the impact of managemental practices on the control of brucellosis ten dairy farms were selected from the total of 39 dairy units visited. The farmers were given information regarding the etiology, transmission, clinical signs, diagnosis, prevention, control and various risk factors associated with the occurrence of brucellosis in animals. The dairy units were followed throughout the study period. Samples were collected at the beginning and at the end of the study to observe the impact of managemental practices on the prevalence of the disease within these dairy herds.

Seroprevalence of brucellosis in the Punjab state was estimated on the basis of results obtained by RBPT. The overall seroprevalence of brucellosis was 20.89. Of the total of 2345 animals tested, percent seroprevalence in sub-mountain zone (Zone I), central zone (Zone II) and arid irrigation zone (Zone III) was found to be 10.55%, 22.62% and 13.56% respectively. The highest percent seroprevalence was observed in Fateh Garh district (48.29%) followed by Sangrur (47.82%) and least in Mansa, Ropar and Barnala (0.00). The central zone is the most fertile and has large-sized dairy farms; as a result, maximum sales and purchases of animals occur in this zone
of the state. Cow slaughter is banned in the state, hence the dairy farmers mostly sell *Brucella* positive animals in cattle markets and the disease gets transmitted to another farm. Furthermore, farmers usually do not screen the animals against brucellosis prior to purchase, consequently the introduction of a single infected animal at the farm leads to a storm of abortions in the herd.

An overall sero-prevalence of 23.12% and 14.63% was observed in cattle and buffaloes respectively. Cattle were found to be apparently more susceptible to infection compared to buffaloes and the difference was statistically significant (p ≤ 0.01). The breed wise prevalence of infection was as follows; Sahiwal 3.07% (2/65), HF cross 23.54% (398/1665) in cattle and in buffalo it was 14.74% (83/563) in Murrah and 13.46% (7/52) in Niliravi. A statistically significant difference (p ≤ 0.01) in susceptibility to infection between Sahiwal and HF cross was observed. There was an apparent difference in susceptibility to infection between Murrah and Niliravi buffaloes but it was statistically non significant (p ≥ 0.05). The prevalence of infection in female cattle was 23.70% (399/1683) and in female buffaloes it was 15.08% (89/590). In bulls and buffalo bulls the prevalence was 2.12% (1/47) and 4% (1/25). The difference between the two sexes with respect to susceptibility to infection in cattle was statistically significant (p ≤ 0.01). But in case of male and female buffaloes the differences were statistically non significant (p ≥ 0.05). The prevalence of the disease was higher in animals of age group of >7 years followed by animals in the age groups of 5-7 and 3-5 years, and least in < 3 years of age. The differences in disease prevalence among these four age group were statistically significant (p ≤ 0.01), with animals in the age group of > 7 years being most susceptible. 7.77% cattle and 3.64% buffaloes had a history of abortion. The seroprevalence of infection was significantly (p ≤ 0.05) higher in animals with a
history of abortion than in those without such histories. During the study, retention of placenta was noticed in 66 animals in which 55 were cows and 11 were buffaloes. 39 out of 55 cattle and 7 out of 11 buffaloes were found positive for brucellosis.

A battery of four serological tests viz RBPT, I-ELISA, MAT and mMAT were evaluated for their sensitivities and specificities. I-ELISA was taken as the reference test against which the rest of the tests were evaluated. A total of 260 samples were found positive by RBPT, 352 samples were positive by I-ELISA, 281 by MAT and 262 by mMAT. Substantial agreement was observed among all the three test combinations. The present study has shown that the commonly used conventional sero-diagnostic tests for brucellosis may not be absolutely reliable. I-ELISA was found to be most sensitive. Fifty *Brucella* positive animals were followed for four months and samples were collected at monthly intervals. The titre end points were determined using MAT. Some of the animals were having low titres at the beginning, which rose over the period of time, while others had very high titres which declined with time. This may be due to the fact that some animals with low titres would have been in the early phase of infection, while those with very high titre would have passed this stage. All the animals were positive in RBPT throughout this period.

PCR detected amplicons of 193-bp in 68 sera and 6 samples from aborted foetuses. For large-scale field screening, identification of *Brucella* by genus-specific PCR tends to be simple and adequate. The sensitivity and specificity of diagnostic assays can influence effective prevention and control of zoonosis. A total of 81 samples were positive by RBPT, 102 samples were positive by I-ELISA, 85 by MAT, 79 by mMAT and 68 by PCR. Substantial degree of agreement was observed between I-ELISA/RBPT, I-ELISA/MAT ($k = 0.72$), and I-ELISA/mMAT. While as
least degree of agreement was observed between I-ELISA and PCR. All the 6 positive aborted foetal materials were identified to be positive for *B. abortus*. None of these was positive for *B. melitensis*. PCR using DNA from six *B. abortus* strains amplified five fragments of 1682, 794, 587, 450 and 152 bp in size. PCR with *B. abortus* S19 DNA did not produce 587bp fragment common to *Brucella* strains tested.

Over all farm level prevalence of infection was 84.61% (33/39). In this study, from the results of the questionnaire and from direct observations of all the farms it becomes clear that several factors can be considered as potential risk factors which increase the risk of an animal being infected with *B. abortus* these include no precaution taken regarding visitors (p < 0.05, OR = 0.07), no disinfection of farm premises (p < 0.05, OR = 0.147), female introduction without testing (p < 0.01, OR = 18), farm replacement (p < 0.05, OR = 0.22), breeding method (p < 0.05, OR = 0.22), serological testing (p < 0.05, OR = 0.22), attitude with positive animals (p < 0.01, OR = 13.33), culling of affected animals (p < 0.01, OR = 0), knowledge of farmer (p < 0.05, OR = 0) and herd size (p < 0.05, OR = 2.125).

For observing the impact of managemental practices on control of brucellosis ten dairy farmers were selected. Out of these ten farmers 9 could bring down the prevalence of the disease to a very low level. From the results it is evident that educating the farmers may prove very fruitful for combating this disease which has assumed alarming prevalence in India in general and Punjab in particular.

There are various constraints that impede in the control of this disease in India, important among these are, religious sentiments attached with the slaughter of cattle, lack of compensation for the farmers for disposing off the animals in a legal way and lack of education and awareness of dairy farmers regarding the disease.
Therefore, it is essential to impart education to the dairy farmers regarding the disease and its socio-economic and public health impact, so that farmers are able to combat this disease without incurring economic loses.

**Conclusions:-**

1. Based on the 2345 serum samples tested, the overall sero-prevalence of brucellosis was found to be was 20.89%.

2. The highest sero-prevalence was observed in central plain zone, followed by arid irrigation zone and the least in sub-mountain.

3. Highest percent seroprevalence was observed in Fateh Garh Sahib District followed by Sangrur and least in Mansa, Ropar and Barnala districts, respectively.

4. Cattle were found to be more susceptible to infection compared to buffaloes.

5. Cross bred cattle were found to be more susceptible to infection than indigenous cattle. Among buffaloes all the breeds were equally susceptible to infection.

6. Sero-prevalence of the disease was higher in animals of age group >7 years and least in < 3 years of age.

7. More positive samples could be detected by I-ELISA compared to RBPT, MAT & mMAT.

8. PCR may be used for detection of infection in those cases which are negative by serological assays (false negative) using serum as starting material.

9. Bruce ladder PCR provides easy and convenient way to differentiate the vaccine strain from the field strain.

10. Use of EDTA in MAT reduces non specific agglutination.

11. Risk factors which were found to increase the risk of brucellosis:
• No precaution taken to visitors.
• No disinfection of farm premises.
• Lack of testing and quarantine of newly purchased animals
• Breeding method (AI)
• Attitude with positive animals.
• Lack of knowledge of farmer about disease.