DIAGNOSTICS AND THERAPEUTIC STRATEGIES AGAINST BRUCELLOSIS IN COWS

THESIS

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1. INTRODUCTION

India is an agrarian country. A large proportion of the population, especially in the rural areas, depends on agriculture and primarily on animal production. India is largest milk producer in the world (102 million tone in year). Animal resource in the country is threatened by reproductive disorders viz., infertility, retained placenta, abortion, endometritis etc., causing considerable economic losses. Brucellosis has been one of the most important reproductive disease among different livestock species as well as animal handlers. It is an emerging disease since the discovery of *Brucella melitensis* by Bruce in 1887 and the isolation of *Brucella abortus* by Bang in 1897 from aborted cattle (McMahan, 1944).

Brucellosis is found worldwide. It is one of the most serious diseases in developing countries. The rate of infection varies greatly from one country to another and between regions within the country, with highest prevalence in dairy cattle. In India, brucellosis was first reported in 1942 and is now endemic throughout the country (Renukaradhya *et al.*, 2002). In general, risk factors such as unrestricted trade, movements of animals, use of local cattle yards or fairs for trading, sending dry animals back to villages for maintenance, use of semen from unscreened bulls for artificial insemination and poor farm hygiene probably attribute to the spread and transmission of the infection.

The practice of sharing equipment between various farms is also a potential danger. It has also been observed that calves fed on infected milk harbour infection and excrete *Brucella* organisms in their feces for up to 4 weeks after the cessation of feeding. The high rate of isolation of the *Brucella* from the udder and the supra mammary lymph nodes is reflected in the numbers excreted in milk which can vary from a few hundred up to 2,00,000 organisms/ml of milk (Corbel, 1988). Thus, the milk is an important material to be processed for knowing the prevalence of brucellosis in particular area.

Despite the advances made in the diagnosis and therapy, brucellosis is still wide spread and prevalent in many developing countries. Economic losses by brucellosis in animals are due to abortion, premature
births, decrease milk production and due to repeat breeding and may lead to temporary or permanent infertility in infected livestock. Economic losses due to brucellosis in livestock are considerable in India.

The most widely used serological tests for diagnosis of brucellosis in animals are Rose Bengal Plate Test (RBPT), Standard Tube Agglutination Test (STAT) and Enzyme Linked Immuno Sorbent Assay (ELISA). Since, neither a single serological test nor a combination of tests detects all infected animals and also due to high homology among *Brucella* species, the detection of brucellosis remains a major problem.

Bovine brucellosis has become a serious problem in Indian dairy herds because of certain religious, social and animal husbandry practices (Singh *et al*., 2014). Despite extensive studies on different aspect of disease in dairy cattle in past 50 years, data on ‘optimum antibiotic treatment’ for therapeutic management of brucellosis is either not available or is still disputed. This may be due to intracellular localization of *Brucella* and its ability to adapt to the environmental condition encountered in its ‘replicative niche’ e.g. macrophage. Treatment failure and relapse rates are high and depend on the drug combination and high cost of treatment.

In an earlier study, Hashemi *et al.* (2012) found that doxycycline-streptomycine was superior to doxycycline-rifampicin. In human beings, recent trials assessed the effect of quinolone based combination therapy and triple drug regimens. Streptomycin has been replaced by newer amino-glycosides and their effects on brucellosis have not been further reported (Skalsky *et al*., 2008). Finally, the advantage of combination therapy over mono-therapy has not been quantified.

Keeping the above facts in view, the present study was undertaken with the following objectives:

**OBJECTIVES**

1- To find the occurrence of brucellosis in cows.

2- To compare the different therapeutic regimen against brucellosis in cows.
2. REVIEW OF LITERATURE

Bovine brucellosis has a worldwide occurrence and according to the Food and Agriculture Organization (FAO), the World Health Organization (WHO) and the World Organization for Animal Health (OIE), is still one of the most important and widespread bacterial zoonoses in the world (Constable et al., 2017). The prevalence of infection varies considerably among herds, areas and countries. Many countries have made considerable progress with their eradication programs, and some have eradicated the disease. However, in our country, brucellosis is still a serious disease facing the veterinary and medical professions.

The detailed review of literature related to various aspects of brucellosis in cattle is presented in the following sub-headings;

(1) Epidemiology of brucellosis
(2) Therapy against brucellosis

(1) Epidemiology of brucellosis

Kapur and Grewal (1974) reported 2.79% and 3.87% samples of cattle and buffaloes, respectively, as positive by STAT in organized farms and villages in Haryana.

Sharma et al. (1979) carried out sero-epidemiologic investigation on brucellosis by testing 361 (cattle) and 551 (buffaloes) sera in Uttar Pradesh and Delhi and recorded 6.37 % and 4.9 % seropositivity in cattle and buffaloes, respectively.

Akakpo et al. (1984) recorded highest prevalence of brucellosis in cattle of 10 years and above age.

Chatterjee et al. (1985) in West Bengal, screened 20 herds of cattle and 6 of buffaloes using STAT and recorded 20.5% and 19.3% seroprevalence in cattle and buffaloes, respectively.

Chapparo et al. (1990) documented 12.6%, 10.7% and 5.3% prevalence of brucellosis in buffaloes in adult female, sub-adult and juveniles, respectively.
Lodhi et al. (1995) found seroprevalence of brucellosis in adult buffaloes 12.98% and 2.40% through RBPT and STAT, respectively, in Faisalabad, Pakistan.

Ghani et al. (1996) observed higher seroprevalence of disease in female buffaloes above 4 years of age.

Jiwa et al. (1996) reported overall 10.85% seroprevalence of brucellosis in Tanzania. The prevalence rate was minimum in animals kept in local management system, intermediate in organized dairy farms and highest in Ranch.

Agab (1997) documented that brucellosis was more prevalent among the animals above 3 years of age. The cows above 4.5 years of age showed highest prevalence of positive reactors (10.3%) by RBPT.

Spath et al. (1997) noticed overall 7.3% prevalence of brucellosis was noticed in 95 farms in Argentina using buffered plate antigen, 2-mercaptoethanol and tube agglutination tests.

Shome et al. (1998) conducted a sero-epidemiological study in Andaman, 48 samples (23.6%) were RBPT positive and out of these 33 samples (68.75%) showed agglutinating antibodies in Standard tube agglutination test.

Rao et al. (1999) collected 160 serum samples (80 Murrah buffaloes and 80 crossbred cows) with a history of frequent abortions. The samples were subjected to RPAT, STAT and dot-ELISA. They noticed that dot-ELISA gave higher percentage of positive results (16.25% and 31.25%) followed by RPAT (11.5% and 6.25%) and STAT (8.75% and 15.00%) in graded Murrah buffaloes and crossbred cow, respectively.

Chakraborty et al. (2000) screened a total of 141 bovine sera in Assam for brucellosis by RBPT, STAT and I-ELISA. Out of these 79 (56.02%), 71 (50.35%) and 47(33.33%) were found positive by ELISA, STAT and RBPT, respectively.

Mehra et al. (2000) tested serum samples of 877 cows, 349 heifers, 70 buffaloes, from organized farms using STAT and compared out with serum samples of 135 cows, 95 buffaloes, from unorganized farms in
Madhya Pradesh, India to determine the magnitude of bovine brucellosis in Satpura and Madhya. The seroprevalence of brucellosis in organized farms in cows, heifers and buffaloes were 9.6%, 12.6% and 11.4%, respectively, whereas seropositive cows of unorganized farms were only 2.2% vs. 9.4% buffaloes against Brucella.

Darnish and Benkirane (2001) found a seroprevalence of 3.14 and 2.94 percent of brucellosis in cattle and small ruminants, respectively, in Syria during the period 1990-1996.

Sandhu et al. (2001) studied 666 cows and 750 buffaloes to determine the seroprevalence of brucellosis in Punjab, India. Of these, 67 cows and 70 buffaloes were found positive for brucellosis with a prevalence of 10.06 % and 9.33%, respectively.

Maansi and Thapliyal (2002) recorded the prevalence of brucellosis as 23.80%, 28.57%, 19.04% and 11.9%, respectively, with MRT, RBPT, Plate ELISA and STAT.

Renukaradhya et al. (2002) recorded seroprevalence of brucellosis in Gujarat at the rate of 6.6% (247 out of 3750) in cattle and 6.3% (14 out of 222) in buffaloes.

Shringi et al. (2002) tested the serum samples for presence of Brucella abortus agglutinin using rapid plate agglutination test (RPAT), serum tube agglutination test (STAT), heat inactivation test (HIT) and 2-mercaptoethanol tube agglutination test and seropositive results were 38.11%, 35.84%, 33.96% and 31.32% respectively.

Rajesh et al. (2003) studied seroprevalence of brucellosis in 719 cattle of Kerala (India) using RBPT and STAT. Of these, 9 were found positive by RBPT but 5 gave a doubtful reaction, whereas all 14 samples were positive in STAT. They found that the overall seroprevalence was 1.95%. They also concluded that seropositivity was higher in heifers and pregnant animals.
Sarumathi et al. (2003) reported that 12.45% samples collected from the rural sectors and organized farms, were found positive for brucellosis.

Rivera et al. (2003) collected 1,523 milk samples from individual animals and bulk milk belonging to 200 herds in the province of Cundinamarca, Colombia for detection of *B. abortus* antibody and comparative evaluation of the MRT and I-ELISA in cattle milk.

Varasada (2003) reported that 19.76%, 16.57% and 24.12% of cattle were positive for brucellosis by RBPT, STAT and I-ELISA, respectively. Whereas 12.75%, 11.16% and 19.12% buffaloes were positive by RBPT, STAT and I-ELISA, respectively. They also recorded that overall seroprevalence of brucellosis in cattle and buffaloes of Central Gujarat were 16.80%, 14.03% and 22.01% by RBPT, STAT and I-ELISA, respectively.

Barbuddhe et al. (2004) investigated the prevalence of brucellosis in Goa region in organized farms with abortion storms. Out of 107 serum samples tested for brucellosis, 40 (37.38%), 39 (36.45%) and 43 (40.18%) were found positive for antibodies against *Brucella*, by RBPT, STAT and AB-ELISA, respectively.

Chand and Sharma (2004) carried out the screening for brucellosis of serum samples from animals of different farms by ELISA, RBPT and STAT. Eight cattle farms were investigated, of which four belonged to the state government and four to the private sector. None of the animals in four state government cattle farms were detected positive for brucellosis whereas the rest four private cattle farms were positive for brucellosis. An overall prevalence rate of brucellosis on cattle farms was observed to be 26.50% by ELISA, 20.40% by RBPT and 18.87% by STAT.

Erdenebaatar et al. (2004) used ELISA to eliminate false positive amongst RBPT positive sera. They collected 697 serum samples in Mongolia from human and animals in 23 nomadic herds which classified in to three groups as brucellosis endemic (BE), brucellosis suspected (BS) or *Brucella* vaccinated (BV). The 295 sera (43.0%) were found positive by
RBPT. But 206 (69.8%) of these were positive according to ELISA; therefore, 30.2% of the RBPT positive sera found to be false positive.

Gumber *et al.* (2004) analyzed 970 bulk milk samples by AB milk-ELISA and MRT to know the status of bovine brucellosis in Punjab. Of the samples, 218 and 115 were found positive by AB milk-ELISA and MRT, respectively. They also found that the prevalence of brucellosis at village level was 22.5% by milk-ELISA.

Mahato *et al.* (2004) used MRT to detect *Brucella* antibody in individual milk samples of 67 cows and found 24 (35.82%) positive.

Nasir *et al.* (2004) studied seroprevalence of brucellosis using RBPT and STAT in 1473 cattle and 481 buffaloes and 286 cattle and 223 buffaloes from various Government and private livestock farms, respectively. The RBPT recorded the seroprevalence as 14.70% and 15.38% in cattle and buffaloes, respectively, at Government farms and 18.53% and 35.40% in cattle and buffaloes, respectively, at various private livestock farms.

Singh *et al.* (2004) reported maximum proportion (23.7%) of animals positive for brucellosis in 7 years age group followed by 8, 6, 5, more than 9, 4 and 3 years age group.

Kachhawaha *et al.* (2005) screened a total of 859 cattle and 133 buffaloes of organized sector (Goshala and Tabela) and unorganized sector of Jodhpur region using RBPT. The positive samples were subjected to STAT. The prevalence of brucellosis was found much higher in cattle (41.79%) than in buffaloes (25.56%) and also more in cattle of organized sector (Goshala) in comparison to unorganized sector.

Jain *et al.* (2006) recorded higher prevalence of brucellosis in crossbred cattle (12.50%) than indigenous cattle (5.38%) in domesticated ruminant of Garhwal region in Uttaranchal state.

Junaidu and Garba (2006) screened 1711 serum samples from slaughtered cattle in Nigeria. It was observed that 383 (22.38%), 376
(21.97%) and 395 (23.08%) samples were positive for *Brucella* using RBPT, SAT and cELISA, respectively.

Sharma *et al.* (2007) screened 2988 animals in 62 dairy farms/gaushalas of Punjab for brucellosis and 540 (18.07%) were found positive by STAT.

Bhonsle *et al.* (2008) aimed the study to compare the RBPT, STAT and ELISA methods in the diagnosis of brucellosis in animals. Out of total 102 serum sample screened for brucellosis, 12 (11.76%) were found to be positive with RBPT. However, 16 (15.68%) were positive with STAT and ELISA.

Trangadia *et al.* (2009) carried out the investigation of brucellosis in organized dairy farms with history of abortion in India and found 22.18% by ELISA, 13.78% by RBPT and 12.82% by MRT.

Rahman *et al.* (2012) conducted the present study to determine the seroprevalence of brucellosis in dairy cattle using screening test Rose Bengal test (RBT) and the positive sera were further confirmed by indirect-ELISA. For this purpose, a total of 400 serum samples from dairy cows with history of abortion and various reproductive disorders were collected from the Kurigram district of Bangladesh for the detection of *Brucella* antibody. The overall prevalence of brucellosis in dairy cattle was 2.25%. Age-wise seroprevalence was found 3.0% in 2 to 3 years age group and 2.0% in 4 to 8 years age group. The prevalence of brucellosis in indigenous and cross-bred cattle was 3.6% and 1.7%, respectively.

Nitu *et al.* (2013) carried out the seroprevalence study of brucellosis in Chhattisgarh state. A total of 250 serum samples (176 cattle and 74 buffaloes) were screened for presence of *Brucella* antibodies by RBPT, STAT and Indirect ELISA. The overall seroprevalence of brucellosis in Chhattisgarh state of India by RBPT, STAT and I-ELISA was 13.0% 19.8% and 31.2% respectively in cattle whereas 16.2%, 14.8% and 20.2% respectively in buffaloes. Cattle of >6 years age group showed highest seroprevalence followed by 4-6 years and lowest in 0-2 years age group. On
the contrary, buffaloes of 4-6 years age group showed highest seroprevalence followed by >6 years age group. Seroprevalence was higher in crossbred than indigenous cattle and more in female animals in cattle and buffaloes.

Patel et al. (2014) studied prevalence and risk factor’s analysis of bovine bcellosis in peri-urban areas under intensive system of production in Gujarat, and stated that the overall herd and animal prevalence in urban areas was 33.70% and 11.90%, respectively.

Sarkar et al. (2014) examined the antibodies for Brucella using the Milk Ring Test (MRT) and Rose Bengal Test (RBT). Overall 2.62 % of milk samples were positive according to MRT, while 2.06 % of the serum samples were positive to the RBT. Only 6 (1.13 %) of the samples were positive for both tests. Out of 312 samples only 10 (3.20 %) were positive to MRT while 8 (2.06%) were positive to RBT in Holstein Friesian cross on the other hand out of 221 samples only 4 (1.80%) were positive to MRT while 3 (1.35%) were positive to RBT in Sahiwal cross. The prevalence of brucellosis was significantly higher in the age group of > 5 years than other age groups on both test. Based on parity, significantly higher prevalence (MRT 2.93% and RBT 2.44%) were obtained in parity 3-5 in comparison to other parity group.

Gupta et al. (2016) conducted seroprevalence of Brucella infection amongst the cattle population in and around Rewa district (Madhya Pradesh) using RBPT and c-ELISA. Out of 50 samples tested, 42 % by RBPT and 20 % by c-ELISA were detected to be positive for brucellosis.

Subedi et al. (2016) collected 92 blood samples of cattle for detection of Brucella antibodies using RBPT test and the positive samples were further retested by Indirect ELISA test through ID Vet iELISA kit 2016. The samples which showed positive on both tests were confirmed as seropositive. Chi-square test and Fisher Exact test was used to find out the association between various variables. The result showed that 14.13% (13/92) and 10.86% (10/92) sample were positive by RBPT and iELISA test respectively. There was no significant prevalence differences on location, age
Comparing abortion with prevalence of Brucellosis, there was significant differences in the result of both RBPT and iELISA test. Inferring from this result, there is association between the abortion and occurrence of Brucellosis. The higher significant prevalence differences was according to the time of abortion where higher seropositivity was obtained in the cattle aborted on 5-7th month of pregnancy and cattle of 3rd parity.

**Therapy against brucellosis**

Fensterbank (1976) studied 45 brucella infected cows, 15 to 30 days following abortion. Eighteen cows were slaughtered and autopsied for examination of Brucella in the organs and lymph nodes. The remaining 27 cows were treated during two and one-half months and then slaughtered after undergoing a treatment with 0, 1, 2 or 3 intraperitoneal injections of oxytetracycline dissolved in 100 ml of physiological saline. The treatment modified only slightly the natural evolution of antibody titers. The levels of infection were similar for all cows which received no treatment. Cows treated with oxytetracycline had less severe infection than the non treated animals and four were infection-free at slaughter. The level of infection of treated cows was independent of the treatment regime. The advantages of treating non pregnant cows were to reduce the level of infection and risk of abortion.

Milward et al. (1984) evaluated various chemotherapeutic regimens in 48 culture-positive dairy cows. Cessation of shedding of Brucella abortus from udder secretions and absence in selected tissues at necropsy were criteria of success. A combination of a long acting oxytetracycline and streptomycin eliminated Brucella in 10 of 14 (71.4%) cows. Two cows that were retreated with the same regimen also became culture-negative. Other treatment regimens, including the use of liposome-encapsulated antibiotics, were less successful.

Nicoletti et al. (1985) recorded the decrease of one dilution in tube agglutination test of six cows and increase in the titre among four cows in
both group treated with oxytetracycline alone or combined with streptomycin and no change in the titre was observed in 18 cows and concluded that titre test was of limited value in short term evaluation of therapeutic regimens.

Guerra and Nicoletti (1986) obtained eight isolates of *Brucella abortus* from cows before and after they were treated with oxytetracycline and streptomycin. The susceptibility to these antibiotics was determined by broth-dilution minimal inhibitory and minimal lethal concentrations. Differences were not found in the minimal lethal concentrations of oxytetracycline or streptomycin in isolates obtained from cows before and after they were treated. This indicates that treatment failures in the cows were not the result of development of resistance to the antibiotics by *Brucella abortus*.

Barman (1991) also reported that brucella infected cows which received long acting oxytetracycline and streptomycin recovered completely from joint ill.

Jimenez de Bagues et al. (1991) aimed a clinical trial to see the effect of antibiotic therapy against brucellosis. Treatment of 16 reactor cows with a combination of oxytetracycline and streptomycin and strain 19 vaccination of the remaining 79 seronegatives cows from a *Brucella melitensis* infected herd stopped the transmission of brucellosis within the herd, even though reactor and non reactor cows were kept together for 2 years. The antibiotic treatment produced cessation of excretion in 5 out of 11 *Brucella melitensis* excretor cows and diminished the number of *Brucellae* to the environment.

Radwan et al. (1993) conducted a clinical study using various therapeutic regimens against brucellosis. Regimen a (tested on 35 cows) consisted of LA-OTC 25 mg/kg administered intramuscularly every 3 days for 42 days, ST 25 mg/kg intramuscularly daily for 8 days, and OTC-IMI 20 ml/teat daily for 4 days. Regimen B (tested on 53 cows) was similar to regimen A, except that ST was administered every 2 days for 16 days and OTC-IMI every 2 days for 8 days. Both regimens were equally effective in eliminating *Brucella* organisms from all cows involved in the tests and no relapses were recorded. However, regimen C, which was similar to regimen
A, except that ST was administered every 3 days for 24 days and OTC-IMI every 3 days for 12 days, resulted in the elimination of Brucella organisms from only 30 (91%) of 33 cows.

Mahato and Sharma (2002) reported that simultaneous treatment with long acting oxytetracycline and streptomycin in brucella positive pregnant animal resulted birth of healthy calves in 8 out of 10.

Kumar et al. (2005) studied therapeutic aspects of brucellosis and found that after treatment with long acting oxytetracycline, 3 cows showed two dilution decrease and 2 showed one dilution decreases in the serotitre. No change in serotitre was observed in one cow.

Nitu et al. (2013) performed the study to assess the therapeutic efficacy of combination of long acting oxytetracycline and streptomycin in brucellosis infected cattle. The therapeutic study of brucella infected animals revealed that long acting oxytetracycline and streptomycin combination had a significant decrease in the antibody titre on the 30th day of post treatment.

Singh et al. (2014) treated the dairy cattles suffering from bovine brucellosis. Cows naturally infected were treated in two phases, cow in phase I of the therapeutic schedule A were given streptomycin, isoniazid and rifampicin with tetracyclines and cows in therapeutic schedule B were given streptomycin and rifampicin with enrofloxacin for 15 days. In phase II, maintenance schedule A was given isoniazid and rifampicin with long acting tetracycline and in therapeutic schedule B cows were given isoniazid and rifampicin for 15 days with one shot of bayrocin. Of 27 cows treated with 2 therapeutic schedule, 12 become pregnant and 10 (83.3%) had normal calving.
3. MATERIAL AND METHODS

The present work on diagnostics and therapeutic strategies against brucellosis in cows was carried out in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur during the period from October 2016 to April 2017.

Animals

A total of 200 lactating cows were screened for brucellosis from private Birla dairy farm Satna, Jain and Yadav dairy farms Jabalpur. The information pertaining to age, parity, history of abortion and vaccination status of individual animal was recorded.

Table 01: Source and number of cows screened for brucellosis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of farm</th>
<th>Animal screened</th>
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<tbody>
<tr>
<td>1</td>
<td>Birla Dairy Farm, Satna</td>
<td>132</td>
</tr>
<tr>
<td>2</td>
<td>Jain Dairy Ukhry, Jabalpur</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>Yadav Dairy Katangi, Jabalpur</td>
<td>38</td>
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<tr>
<td></td>
<td>TOTAL</td>
<td>200</td>
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</tbody>
</table>

Sample Collection

Milk

The udder was thoroughly washed and cleaned with potassium permagnate solution (1:1000) and dried with clean cloth. Teat opening was disinfected with 70% of ethyl alcohol. After discarding few drop of milk, approximately 5 ml of milk from each animal was collected in sterile screw capped plastic vials and transported on the ice to the laboratory for Milk ring test (Plate 01).
**Serum**

About 9 ml of blood was collected aseptically from the jugular vein of properly restrained animal in a vacuette with serum clot activator on day 0 pre treatment and days 15, 30 and 45 post treatment. The vacuettes were kept in upright position at room temperature for 2 hours. The separated serum was collected in a screw capped plastic vial and transported to the laboratory were stored at -20°C till further use (Figure 2a & b). Collected serum sample were subjected to Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT) (Plate 02a & b).

**Testing of samples**

**Milk**

**Milk Ring Test (MRT)** – also known as Abortus Bang Ring Test (ABRT). This test was employed to test milk from *Brucella* infected cattle herd.

The antigen was procured from biological products division, Indian Veterinary Research Institute Izzatnagar (U.P.)

**Antigen**

The antigen is a suspension of pure growth culture of smooth *Brucella abortus* strain 99 stained with 2,3,5 triphenyl tetrazolium chloride suspended in 0.85% saline containing 1% glycerol and 1% phenol (Plate 3a).

**Methodology**

Antigen and milk samples were brought to the room temperature prior to the performing the test. About 30-50 µl of antigen was added to the 2 ml of milk in a narrow test tube and mixed thoroughly. The tubes then were incubated at 37°C for 1 hour together with the positive and negative working standard.

**Interpretation**

A strongly positive reaction was indicated by formation of dark pink ring above a white milk column. The test was considered to be negative if the pink color of the underlying milk exceeds that of cream layer.
Serum

**Rose Bengal Plate Test (RBPT)**

The antigen was procured from biological products division, Indian Veterinary Research Institute, Izzatnagar (U.P.)

**Antigen**

Rose Bengal plate test (RBPT) antigen is an 8% suspension of pure, smooth, killed cells of *Brucella abortus* strain 99 phenolised and stained with Rose Bengal dye. It is buffered at pH 3.65 using lactic acid buffer (Plate 3b).

**Methodology**

The RBPT was performed following the procedure of Morgan *et al.* (1969). Serum samples and RBPT antigen were brought to the room temperature and then one drop (0.03 ml) of serum was taken on a clean, dry and non greasy glass slide by micropipette. The antigen bottle was shaken well to ensure homogenous suspension and then one drop (0.03 ml) the antigen was added. The antigen and serum were mixed thoroughly with the spreader and then the slide was rotated for four minute and then final reading was taken.

**Interpretation**

Definite clumping/agglutination was considered as positive reaction, where as no clumping/agglutination was considered as negative.

**Standard Tube Agglutination Test (STAT)**

The antigen was procured from biological products division, Indian Veterinary Research Institute, Izzatnagar (U.P.)

**Antigen**

*Brucella* SAT antigen is a suspension of a pure smooth culture of *Brucella abortus* strain 99 in phenol saline (Plate 3c).
Methodology

The standard tube agglutination test was performed according to Weybridge technique (Alton et al., 1975). All the serum samples were tested up to minimum of nine dilutions. For high titred sera, more dilutions were prepared in order to achieve end point titer. In brief, eleven agglutination tubes were placed in a rack. Further, 0.8 ml of 0.5 per cent phenol saline was taken in a first tube and 0.5 ml in rest of the tubes. 0.2 ml of serum was added in the first tube, mixed well and 0.5 ml of diluted serum transferred to the second tube. The process was continued up to the ninth tube and 10th tube was kept for control tube, 0.5 ml was discarded from the last tube after mixing. Then 0.5 ml B. abortus plain antigen was added to each tube and mixed thoroughly. This provided a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160 and so on. Considering the special significance of 50 per cent end point, a control tube was set up to simulate 50 per cent clearing by mixing 0.25 ml antigen with 0.75 ml of 0.5 per cent phenol saline in an agglutination tube. All the tubes were incubated at 37ºC for 24 hour.

Table 02: Procedure for standard tube agglutination test

<table>
<thead>
<tr>
<th>Tube No.</th>
<th>1</th>
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<th>3</th>
<th>4</th>
<th>5</th>
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<th>7</th>
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<th>9</th>
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<th>11</th>
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<tbody>
<tr>
<td>Tube No.</td>
<td></td>
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</tr>
<tr>
<td>a. 0.5%Phenol saline</td>
<td>0.8</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<td>0.5</td>
<td>0.5</td>
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<tr>
<td>b. Test Serum</td>
<td>0.2</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
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<tr>
<td>Mixed thoroughly and transferred until tube no. 9 discarded 0.5 ml from tube no. 11 i.e. discard tube.</td>
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<tr>
<td>c. Brucella abortus plain antigen</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>d. Final dilution</td>
<td>1:10</td>
<td>1:20</td>
<td>1:40</td>
<td>1:80</td>
<td>1:160</td>
<td>1:320</td>
<td>1:640</td>
<td>1:1280</td>
<td>1:2560</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Interpretation

The results were compared with the control. The highest dilution of the serum which showed 50 per cent agglutination was taken as end titre. The titre so obtained was expressed in unit system by doubling of the serum titre as International Unit (IU) per ml of serum. The antibody titre of 1:80 (160 IU / ml) and above was taken as positive for brucella.
Therapeutic regimen

Selection of the cows for the therapeutic regimen was done on the basis of serological tests. The positive cases of brucellosis were randomly divided in to four groups having six cows in each group. However, six healthy cows were used as negative control.

Table 03: Various therapeutic regimen in different treatment groups for brucellosis in cows

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of cows</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td>6</td>
<td>Inj. Oxytetracycline (Long acting) @20 mg/kg b.wt I/m every 3&lt;sup&gt;rd&lt;/sup&gt; day (7 dose)</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td>6</td>
<td>Inj. Marbofloxacin (Long acting) @10mg/kg b.wt I/m every 3&lt;sup&gt;rd&lt;/sup&gt; day (7 dose)</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td>6</td>
<td>Inj. Enrofloxacin (Long acting) @10mg/kg b.wt I/m every 3&lt;sup&gt;rd&lt;/sup&gt; day (7 dose)</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (Positive control)</td>
<td>6</td>
<td>No treatment</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt; (Negative control)</td>
<td>6</td>
<td>No treatment</td>
</tr>
</tbody>
</table>

NOTE: Common treatment was given to the groups T1, T2 and T3 which includes Inj. Dihydrostreptomycin @ 20 mg/kg b.wt sid i/m for 15 days, Tab. Rifampicin and Isoniazid (450mg+300mg) @ 6 tab sid for 21 days and Hepato protective liquid (Liv 52 @100ml) was given to each animal under treatment.

Therapeutic response study

Therapeutic response of the treatment was assessed on the basis of results of antibody sero titre of all the brucella infected cows under the treatment using STAT on days 0 (pre treatment) and 15, 30 and 45 (post treatment).

Statistical analysis

To know the effect of treatment, the data were analyzed using analysis of variance with two way classification and Duncan’s Multiple Range Test (Snedecor and Cochran, 1994).
4. RESULTS

The present study was carried out to know the prevalence of brucellosis in cows by detecting antibodies in milk and serum samples by employing MRT, RBPT and STAT, and to compare the different therapeutic regimen in brucellosis. A total of 200 cows from three dairy farms at different location were screened over a period of 07 months i.e. from October 2016 to April 2017.

Epidemiology of brucellosis in cows

For epidemiological study, a total of 200 (comprised of 68 indigenous and 132 crossbred) cows were screened for occurrence. The occurrence of brucellosis in cows was studied by MRT, RBPT and STAT (Plate 4a, b and c).

Overall occurrence of brucellosis

Out of 200 samples, 27, 36 and 52 samples were found positive for brucellosis using MRT, RBPT and STAT, respectively. The overall occurrence of the brucellosis in cows was 13.50% by MRT, 18.00% by RBPT and 26.00% by STAT (Table 04 and Figure 01).

Table 04: Overall occurrence of brucellosis in cows

<table>
<thead>
<tr>
<th>Cows screened</th>
<th>Brucella positive cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MRT</td>
</tr>
<tr>
<td>200</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>(13.50)</td>
</tr>
</tbody>
</table>

Figure in parenthesis ( ) indicate per centage

Origin wise occurrence of brucellosis

Out of 132 crossbred cow's milk and sera samples were tested by MRT, RBPT, and STAT and found to be positive for brucellosis 24 (18.18%), 30 (22.72%) and 45 (34.09%) respectively. While in indigenous cows, out of 68 milk and sera samples tested; 3 (4.41%), 6(8.82%) and 7 (10.29%) by MRT, RBPT and STAT respectively showed seropositivity for brucellosis. The results are shown in table 05 and figure 02.
Table 05: Occurrence of brucellosis in indigenous and crossbred cows

<table>
<thead>
<tr>
<th>Description</th>
<th>Cows screened</th>
<th>Brucella positive cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MRT</td>
</tr>
<tr>
<td>Indigenous cows</td>
<td>68</td>
<td>3 (4.41)</td>
</tr>
<tr>
<td>Cross bred cows</td>
<td>132</td>
<td>24 (18.18)</td>
</tr>
</tbody>
</table>

Figure in parenthesis ( ) indicate percentage

Age wise occurrence of brucellosis

Age wise occurrence of brucellosis has been presented in table 06 and figure 03. Cows up to 2 years of age showed no seropositivity for serological tests (RBPT and STAT). Cows above 6 years age group showed the highest occurrence (24.46% by MRT, 31.91% by RBPT and 46.80% by STAT) followed by 4-6 years age group (8.53%, 12.5% and 16.66% by MRT, RBPT and STAT respectively). Whereas lowest occurrence of brucellosis was reported in 2-4 years age group of cows viz. 4.76%, 7.14% and 9.52% by MRT, RBPT and STAT, respectively.

Table 06: Age wise occurrence of brucellosis in cows

<table>
<thead>
<tr>
<th>Age of cows in years</th>
<th>Cows screened</th>
<th>Brucella positive cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MRT</td>
</tr>
<tr>
<td>0-2</td>
<td>40</td>
<td>-</td>
</tr>
<tr>
<td>2-4</td>
<td>42</td>
<td>2 (4.76)</td>
</tr>
<tr>
<td>4-6</td>
<td>24</td>
<td>2 (8.53)</td>
</tr>
<tr>
<td>6 and above</td>
<td>94</td>
<td>23 (24.46)</td>
</tr>
</tbody>
</table>

Figure in parenthesis ( ) indicate percentage
Parity wise occurrence of brucellosis

Cows of 6th and above 6th parity revealed the highest occurrence (35.71% by MRT, 50.00% by RBPT and 71.42% by STAT) followed by 4th parity cows (25.00% and 33.33%, 41.66% by MRT, RBPT and STAT respectively). However, the cows of 3rd parity showed lower occurrence i.e. 20.83% and 25.00% by MRT and RBPT respectively, while the lowest occurrence was reported in the cows of 5th parity (14.28% and 19.04%, 28.57% by MRT, RBPT and STAT respectively). Cows of 1st and 2nd parity did not reveal seropositivity for serological tests (MRT, RBPT and STAT). The results are shown in table 07 and figure 04.

Table 07: Parity wise occurrence of brucellosis in cows

<table>
<thead>
<tr>
<th>Parity of cows</th>
<th>Cows screened</th>
<th>Brucella positive cows</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MRT</td>
</tr>
<tr>
<td>1</td>
<td>50</td>
<td>0 (0.00)</td>
</tr>
<tr>
<td>2</td>
<td>32</td>
<td>2 (6.25)</td>
</tr>
<tr>
<td>3</td>
<td>24</td>
<td>3 (12.50)</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>6 (25.00)</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>6 (14.28)</td>
</tr>
<tr>
<td>6 and above</td>
<td>28</td>
<td>10 (35.71)</td>
</tr>
</tbody>
</table>

Figure in parenthesis () indicate percentage

Therapeutic study

A total of 24 brucella infected cows, positive for serological tests i.e. MRT, RBPT and STAT were randomly divided into 4 group’s viz. T1, T2, T3 and T4, each comprised of 6 cows. Cows of group T1, T2, T3, were treated by long acting antimicrobials at 72 hours interval for 7 occasions. Cows of group T4 received no treatment and served as positive control. Six apparently
healthy cows (Brucella seronegative for MRT, RBPT and STAT) grouped as T5 (negative control). Serotitres of individual cow of different groups during observation periods are presented in the table 08 and the mean of serotitres are presented in table 09 and figure 05.

Before treatment (on day zero) the serotitre of groups T1, T2, T3 cows on STAT ranged 640-1280 IU giving an average of 1173.33 ± 106.67 IU, whereas that of group T4 (positive control) was ranged 320-1280 IU with an average of 853.33±196.68 IU. However, no seropositivity was measured in the cows of group T5 (negative control) during the entire period of observation.

**GROUP T1**

Treatment with long acting Oxytetracycline, Dihydrostreptomycin, Rifampicin and Isoniazid combination resulted in decrease in serotitre of group T1 cows when compared with zero day (pre treatment). However, the mean serotitre on different treatment intervals was significantly decreased (i.e. 136.66±100.72 IU, 56.66±20.92 IU and 33.33 ±4.22 IU on days 15\textsuperscript{th}, 30\textsuperscript{th} and 45\textsuperscript{th}). During different observation period, decrease in serotitre ranged 20-40 IU on 15\textsuperscript{th} day in all cows except one cow (640 IU). On 45\textsuperscript{th} day of treatment serotitre of two cows were found normal (20 IU) and another four cows were found (40 IU). On the basis of serotitre on days 15\textsuperscript{th} and 30\textsuperscript{th} the recovery was 83.33% (5/6) and on day 45\textsuperscript{th} post treatment, there was 100% recovery in the Brucella infected cows.

**GROUP T2**

Treatment with long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid combination resulted in decrease in serotitre of group T2 cows when compared with zero day (pre treatment). The mean serotitre on days 15\textsuperscript{th} (80.00±48.17 IU) and 30\textsuperscript{th} (56.66±20.92 IU) were significantly lower in comparison to day Zero. However, on day 45\textsuperscript{th} post treatment, serotitre was slightly increased in comparison to days 15\textsuperscript{th} and 30\textsuperscript{th} although the serotitre was significantly lower in comparison to day Zero. During the observation period, decrease in serotitre ranged 20-40 IU on 15\textsuperscript{th} day in all cows except one cow (320 IU). On day 30\textsuperscript{th} the serotitre of that one cow was decreased 160 IU. Moreover, the serotitre on day 45\textsuperscript{th} of treatment, that one cow again showed marked increased (i.e. 640 IU) in serotitre. Although the serotitre of two cows were found normal (20 IU) and another
three cows had (40 IU). On the basis of serotitre, 83.33% (5/6) recovery was obtained during entire period of treatment.

**GROUP T₃**

Treatment with long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid combination resulted in decrease in serotitre of group T₃ cows when compared with zero day (pre treatment). After treatment the mean serotitre value was significantly lower i.e. 146.66±53.30 IU on day 15th when compared with zero day (pre treatment). However the mean serotitre on day 30th (200.00±97.98 IU) and on day 45th (306.66±112.03 IU) was slightly higher in comparison to day 15th post treatment. During the observation period, decrease in serotitre ranged 40-320 IU on day 15th. On day 45th of the treatment the serotitre of all the cows (ranged 80-640 IU) was increased when compared to the serotitre on day 15th. On the basis of serotitre, only two cows (33.33%) showed the cure on days 15th and 30th. However, on day 45, all cows of this group had serotitre above normal limit (1:40 IU).

**GROUP T₄**

On other hand, on day zero the serotitre of group T₄ (untreated) cows on STAT ranged 320-1280 IU. The serotitre of three cows showed increasing trends and three rest remain unchanged on 15th day, while on 30th day four out of six cows showed escalating trend in the serotitre and two rest remain unchanged in comparision to zero day. On 45th day serotitre ranged 1280-2560 IU showed the strong serotitre.

Response to therapy was evaluated on the basis of results of STAT on pre and post treatment (days 0, 15th, 30th and 45th). The cows of group T₁ (received combination of long acting Oxytetracycline, Dihydrostreptomycin, Rifampicin and Isoniazid) showed better recovery on day 15th post treatment except one cow. Although better recovery (100%) in all the cows reported on day 45th. The cows of group T₂ (received combination of long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid) showed recovery on day 15th except one cow and that remain infective during the entire period of treatment rather the serotitre was increased on day 45th of treatment and overall 83.33% (5/6) recovery was noticed. The cows of group T₃ (received combination of long acting Enrofloxacin, Dihydrostreptomycin,
Rifampicin and Isoniazid) showed decreasing trend in serotitres (2/6) on 15\textsuperscript{th} day of treatment however, the five cows showed increased serotitre on day 45\textsuperscript{th} when compared with serotitre of cows on days 15\textsuperscript{th} and 30\textsuperscript{th} post treatment.

Therefore, combination of long acting Oxytetracycline, Dihydrostreptomycin, Rifampicin and Isoniazid found to be most efficacious followed by combination of long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid and least being combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid.

**Table 08: Pre and post treatment sero-titre (IU) of brucella infected cows**

<table>
<thead>
<tr>
<th>Group</th>
<th>Interval (days)</th>
<th>Sero-titre of brucellosis in individual cow</th>
</tr>
</thead>
<tbody>
<tr>
<td>T\textsubscript{1}</td>
<td>0</td>
<td>640</td>
</tr>
<tr>
<td>Oxytetracyline (LA) + Dihydrostreptomycin + Rifampicin + Isoniazid</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>20</td>
</tr>
<tr>
<td>T\textsubscript{2}</td>
<td>0</td>
<td>1280</td>
</tr>
<tr>
<td>Marbofloxacin (LA) + Dihydrostreptomycin + Rifampicin + Isoniazid</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>T\textsubscript{3}</td>
<td>0</td>
<td>1280</td>
</tr>
<tr>
<td>Enrofloxacin (LA) + Dihydrostreptomycin + Rifampicin + Isoniazid</td>
<td>15</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>320</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>640</td>
</tr>
<tr>
<td>T\textsubscript{4}</td>
<td>0</td>
<td>1280</td>
</tr>
<tr>
<td>Positive Control</td>
<td>15</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>1280</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>1280</td>
</tr>
<tr>
<td>T\textsubscript{5}</td>
<td>0</td>
<td>Nil</td>
</tr>
<tr>
<td>Negative Control</td>
<td>15</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Nil</td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>Nil</td>
</tr>
</tbody>
</table>
Table 09: Mean values of serum antibody titre in *Brucella* infected cows in different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Observation period (in days)</th>
<th>Pre-treatment</th>
<th>Post treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>15th</td>
</tr>
<tr>
<td>T&lt;sub&gt;1&lt;/sub&gt;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>1173.33&lt;sup&gt;aA&lt;/sup&gt; ± 106.67</td>
<td>136.66&lt;sup&gt;bB&lt;/sup&gt; ± 100.72</td>
</tr>
<tr>
<td>T&lt;sub&gt;2&lt;/sub&gt;</td>
<td></td>
<td>1173.33&lt;sup&gt;aA&lt;/sup&gt; ± 106.67</td>
<td>80.00&lt;sup&gt;bB&lt;/sup&gt; ± 48.17</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt;</td>
<td></td>
<td>1173.33&lt;sup&gt;aA&lt;/sup&gt; ± 106.67</td>
<td>146.66&lt;sup&gt;bB&lt;/sup&gt; ± 53.30</td>
</tr>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt;</td>
<td>Positive Control</td>
<td>853.33&lt;sup&gt;bcC&lt;/sup&gt; ± 196.68</td>
<td>1173.33&lt;sup&gt;abB&lt;/sup&gt; ± 106.67</td>
</tr>
<tr>
<td>T&lt;sub&gt;5&lt;/sub&gt;</td>
<td>Negative Control</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Mean values with different superscript lowercase (between treatment), uppercase (between interval) vary significantly (p≤0.05)
5. DISCUSSION

An investigation about a disease helps to understand the distribution and epidemiology of the disease status. Brucellosis results in huge economic losses in the countries where it is prevalent, but the true magnitude of the disease is not known. In India, brucellosis costs around Rs. 350 million in the form of food, animals and labor losses (Kunen, 1994). In a campaign against brucellosis, it is impossible to apply only one method which would suit every country. The “test and slaughter” method combined with calf hood vaccination, no doubt contributes to considerable reduction in the number of infected herds; however, for economic reasons, this method cannot be applied in all cases. An eradication campaign through slaughter and compensation is not within the economic scope of developing countries, especially where it is difficult to change agricultural customs or social habits (Radwan et al., 1993). On the other hand, mass vaccination of infected herds protects only uninfected animals without altering the course of infection. The infected vaccinated animals continue to present a serious public health risk. Livestock producers in India and in many other developing countries cannot afford the traditional “test and slaughter” approach used in developed countries. An effective, practical and safe antibiotic therapy would be of enormous benefit to producers in these countries as an alternative to slaughter of infected animals of high value. Although this has been the goal of several workers, none has been fully successful.

The present study was carried out to know the occurrence of brucellosis in cows by detecting antibodies in milk and serum samples by employing MRT, RBPT and STAT, and to compare the different therapeutic regimen in brucellosis. A total of 200 cows from three dairy farms at different location were screened over a period of 07 months i.e. from October 2016 to April 2017.

**Epidemiology of brucellosis in cows**

The study of epidemiology of brucellosis in cows was conducted by screening the cows for the presence of *Brucella* antibodies in milk and serum samples. For serodiagnosis of brucellosis MRT, RBPT and STAT were used.
Overall occurrence of brucellosis

The overall occurrence of the brucellosis in cows during October 2016 to April 2017 was 13.50% by MRT, 18.00% by RBPT and 26.00% by STAT. The results of the study are in agreement with findings of Chapparo et al. (1990) who have reported the overall 12.6% seroprevalence of brucellosis. Varasada (2003) documented overall prevalence of brucellosis as 19.76% by RBPT and 16.57% by STAT. Chakraborty et al. (2000) reported comparatively higher seroprevalence of brucellosis as 50.35% by STAT and 33.33% by RBPT in West Bengal.

In contrast to the present findings, Rahman et al. (2012) reported the overall prevalence of brucellosis in dairy cattle as 2.25%. Sarkar et al. (2014) also documented lower seroprevalence of brucellosis as 2.62% by MRT and 2.06% by RBPT.

The present findings indicate the presence of brucellosis in the cows. Serological methods of diagnosis are very useful in supporting the diagnosis and are useful particularly in epidemiological investigation. The serological tests employed in the present investigation were MRT, RBPT and STAT, which are simple, easy to perform and less time consuming. The variation in the prevalence rate of brucellosis might be attributed to the fact that diagnostic tests varied between studies. Moreover, there may be difference in managential conditions, climate study, Deigned and study methods used. Due to wide variation in the number of sample tested by different workers in different part of the world, comparison in this regard would be of little value.

Breed wise occurrence of brucellosis

Breed wise occurrence of brucellosis was comparatively higher (i.e. 18.18%, 22.72% and 34.09% by MRT, RBPT, and STAT) in crossbred cows in comparison to indigenous cows (i.e. 4.41%, 8.82% and 10.29% by MRT, RBPT and STAT).

Our findings corroborated the findings of Jain et al. (2006), who reported higher prevalence in crossbred cattle (12.50%) in comparison to the
indigenous cattle (5.38%) of sub Himalayan Kumaon region. Rahman et al. (2012) also recorded the higher prevalence in crossbred cattle (3.6%) than indigenous 1.7 percent. This shows that the indigenous cows are comparatively resistant to bovine brucellosis and crossbred cows are less adapted to the hot and humid climate including management practices of the particular region. The intensive use of artificial insemination (A.I.) in crossbred animals may be a contributing factor for higher prevalence of brucellosis.

**Age wise occurrence of brucellosis**

In the present study cows up to 2 years of age showed no seropositivity for serological tests (RBPT and STAT). Cows above 6 years age group showed the highest occurrence (24.46% by MRT, 31.91% by RBPT and 46.80% by STAT) followed by 4-6 years age group (8.53%, 12.5% and 16.66% by MRT, RBPT and STAT respectively). Whereas lowest occurrence of brucellosis was reported in 2-4 years age group of cows viz. 4.76%, 7.14% and 9.52% by MRT, RBPT and STAT respectively.

These findings correlate with the work of Singh et al. (2004), Jain et al. (2006) and Nitu et al. (2013) who also reported the higher prevalence of brucellosis in animals in 6-8 years age group. Sarkar et al. (2014) recorded the significantly higher prevalence of brucellosis in above 5 years age group. On the contrary, Rahman et al. (2012) observed the higher seroprevalence of brucellosis in dairy cattle in 2 to 3 years of age group (3.0%) when compared with 4 to 8 years age group (2.0%).

Higher prevalence of brucellosis in animals above 4 years might be due to the fact that this is the most suitable age for breeding. It might also be due to the fact that there is a marked decrease in immune status with the advancement of age.

**Parity wise occurrence of brucellosis**

The parity wise occurrence of brucellosis revealed the highest occurrence (35.71% by MRT, 50.00% by RBPT and 71.42% by STAT) in cows of 6th and above 6th parity. Cows of 1st and 2nd parity did not reveal seropositivity for serological tests (MRT, RBPT and STAT). The results of this
study are in partial agreement with the findings of Sarkar et al. (2014), who reported higher prevalence of brucellosis in cattle in 3 to 5 parity comparison to other parity groups. Subedi et al. (2016) recorded the significantly higher prevalence of brucellosis in cattle of 3rd parity. The higher prevalence of brucellosis in females may be due to the preferential localization of Brucella organisms in uterus having erythritol which stimulates growth of these organisms (Bala and Siddhu, 1982).

Therapeutic study

Response to therapy was evaluated on the basis of results of antibody serotitre of Brucella infected cows pre and post treatment (days 0, 15th, 30th and 45th). The cows of group T1 (received combination of long acting Oxytetracycline, Dihydrostreptomycin, Rifampicin and Isoniazid) showed better recovery on day 15th post treatment except one cow. Although better recovery in all the cows reported on day 45th. The cows of group T2 (received combination of long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid) showed recovery on day 15th except one cow and that remain infective during the entire period of treatment rather the serotitre was increased on day 45th of treatment. The cows of group T3 (received combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid) showed decreasing trend in serotitres on 15th day of treatment however, the five cows showed increased serotitre on day 45th when compared with serotitre of cows on days 15th and 30th post treatment.

Present findings are similar to the observations made by Milward et al. (1984), Barman (1991), Mahato and Sharma (2002), Kumar et al. (2005), Nitu et al. (2013) and Singh et al. (2014). Moreover, these scientists documented the variable pattern of the recovery using broad spectrum antibiotics, singly or in combination with various schedules. However, these schedules had limited success in complete cure of infection. Several chemo-therapeutic agents have been employed in recent decades for the treatments of Brucella abortus infection in cows; however none of these has been entirely successful (Singh et al., 2014).
Dose and duration of application of selected antibiotics in the present study indicate that the long term therapy and the drug combinations used, yielded better results than several other regimens. The oxytetracycline was used as it is capable of penetrating intracellularly and inhibits bacterial protein synthesis at the level of ribosomes. The long acting oxytetracycline was selected to save the time and effort and to provide long lasting oxytetracycline concentration in blood plasma, as one injection gave and effective concentration of 0.6µg/ml for 3 days (Nicoletti et al.,1989). Streptomycin was also used, because it is known to inhibit protein synthesis in gram negative bacteria. Furthermore, streptomycin acts synergistically with oxytetracycline to inhibit growth of *Brucella abortus* within bovine cell culture *in vitro*. Isoniazid and rifampicin combination is commonly used as anti tubercular drug for the treatment of tuberculosis in human being at therapeutic levels. Isoniazid is bacteriocidal against actively growing intracellular and extracellular *Mycobacterium tuberculosis* organisms. Rifampicin have good intracellular diffusion and in vitro bactericidal activity on *Brucella*, and is effective in experimental brucellosis in mice. It was therefore legitimate to use rifampicin in cows.

In conclusion, when treatment protocols were strictly observed, the combination of long acting Oxytetracycline, Dihydrostreptomycin, Rifampicin and Isoniazid found to be most effective. On the basis of serum antibody titre by STAT in brucella infected cows, the recovery was 100% on day 45th of treatment. However, this recovery may be for limited period. To insure the 100% recovery, there must be observation on antibody serotitre of the same cows after next calving and the antibody serotitre by STAT should be less than 1:80 IU. Consequently, at the present time this treatment would probably be of limited value in breeding animals. However, further research may yield a regimen which could be useful for the treatment of brucellosis.
6. SUMMARY, CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK

6.1 SUMMARY

Brucellosis is one of the most serious diseases in developing countries. The rate of infection varies greatly from one country to another and between regions within the country, with highest prevalence in dairy cattle. In India, brucellosis was first reported in 1942 and is now endemic throughout the country. In general, risk factors such as unrestricted trade, movements of animals, use of local cattle yards or fairs for trading, sending dry animals back to villages for maintenance, use of semen from unscreened bulls for artificial insemination and poor farm hygiene probably attribute to the spread and transmission of the infection. Despite the advances made in the diagnosis and therapy, brucellosis is still widespread and prevalent in many developing countries. Economic losses by brucellosis in animal are due to abortion, premature births, decrease milk production and due to repeat breeding and may lead to temporary or permanent infertility in infected livestock. Economic losses due to brucellosis in livestock are considerable in India.

The present work on diagnostics and therapeutic strategies against brucellosis in cows was carried out in the Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, Nanaji Deshmukh Veterinary Science University, Jabalpur during the period from October 2016 to April 2017. For epidemiological study a total of 200 lactating cows were screened for brucellosis from private Birla dairy farm Satna, Jain and Yadav dairy farms Jabalpur. The information pertaining to age, parity, history of abortion and vaccination status of individual cow was recorded. For screening of brucellosis, milk and sera samples were collected and tested by MRT, RBPT and STAT. For therapeutic study, a total of 24 Brucella infected cows positive for serological tests i.e. MRT, RBPT and STAT were randomly divided into 4 groups viz. T₁, T₂, T₃ and T₄, each comprised of 6 cows. Cows of group T₁, T₂, T₃, were treated using long acting antimicrobials at 72 hours interval for 7 occasions. Cows of group T₄ received no treatment and served
as positive control. Six apparently healthy cows (brucella seronegative for MRT, RBPT and STAT) grouped as T5 (negative control).

The overall occurrence of the brucellosis in cows was recorded as 13.50% by MRT, 18.00% by RBPT and 26.00% by STAT. Higher occurrence of brucellosis was found in crossbred cows 18.18% by MRT, 22.72% by RBPT and 34.09% by STAT, in comparison to indigenous cows i.e. 4.41% by MRT, 8.82% by RBPT and 10.29% by STAT. Age wise highest occurrence (24.46% by MRT, 31.91% by RBPT and 46.80% by STAT) recorded in cows above 6 years age while no seropositivity was observed in cows up to 2 years of age. However, the highest occurrence (35.71% by MRT, 50.00% by RBPT and 71.42% by STAT) was revealed in cows of 6th and above 6th parity.

Response to therapy was evaluated on the basis of results of antibody serotitre of *Brucella* infected cows pre and post treatment (15th, 30th and 45th). The combination of long acting Oxytetracycline, Dihydrostreptomycin, Rifampicin and Isoniazid found to be most effective followed by combination of long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid and least being combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid.
6.2 Conclusions

- The overall occurrence of the brucellosis in cows was recorded as 13.50% by MRT, 18.00% by RBPT and 26.00% by STAT.
- Higher occurrence of brucellosis was found in crossbred cows in comparison to indigenous cows.
- Age wise highest occurrence recorded in cows above 6 years of age while no seropositivity was observed in cows up to 2 years of age.
- Parity wise highest occurrence was revealed in cows of 6th and above 6th parity.
- Combination of long acting Oxytetracyclin, Dihydrostreptomycin, Rifampicin and Isoniazid was found to be most effective followed by long acting Marbofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid combinations.
- Combination of long acting Enrofloxacin, Dihydrostreptomycin, Rifampicin and Isoniazid was reported to be least effective.
6.3 Suggestions for further work

The present study was planned looking into the limited time period and feasibility of work. However, future studies are warranted in present topic in the following areas:

- Greater sample size from different regions would increase the accuracy for study of occurrence of \textit{Brucella} in M.P.

- For drawing specific conclusion, study could be undertaken in large number of bovines (cattle and buffaloes) in organized and unorganized farms covering larger areas.

- For studying therapeutic aspects of the disease, a large number of Brucella infected animals should be included and monitored for larger period. Some new drug like nanoparticulate doxycycline can be tried for this.

- The residue of antibiotics in milk of treated animals should be investigated to determine accurately when milk can be safely consumed following completion of the treatment.
7. REFERENCES


Guerra, M.A. and Nicoletti, P. (1986). Comparison of the susceptibility of Brucella abortus isolate obtained before and after were treated With oxytetracycline and streptomycin. American Journal of Veterinary Research, 47(12): 2612-2613


