

A REPORT OF A BRUCELLOSIS OUTBREAK FROM CENTRAL INDIA

Sunant K. Raval, Joice P. Joseph*, Aatur Shah, R.S. Joshi and B.B. Bhanderi**ABSTRACT**

During an outbreak of brucellosis in Chikhodra village of Gujarat, India, a study was performed to find the seroprevalence of brucellosis in two farms in the affected area. All animals (104) were screened using the rose bengal plate agglutination test. Thirtyfour (32.69%) animals were found serologically positive for this test and positive samples were subjected to standard tube agglutination test for finding antibody titer against brucellosis. Twentyeight animals had titer >320 IU; titer values of 160 and 80 IU were present in two animals each. Hematological values were compared statistically between seropositive and a control group. It was found that there was a statistical reduction of leukocytes, platelets and mean corpuscular volume in affected animals compared to control animals.

Keywords: brucellosis, rose bengal plate agglutination test, standard tube agglutination test, hematology, seroprevalence

INTRODUCTION

Brucellosis is named after Sir David Bruce, who in 1886 isolated the causative agent from a soldier in Malta, where the disease caused

considerable morbidity and mortality among British military personnel. This disease occurs in cattle in most parts of the world. Brucellosis was first recognized in India in 1942. It occurs in cows, buffaloes, sheep, goats, pigs, dogs, and humans. Economic losses due to this disease are considerable in an agrarian country such as India. Outbreaks occur in heifers; older cows become infected but do not abort. Etiological agent is a facultative intracellular organism, and persistent infection is a characteristic feature of this disease. Since it is a zoonotic disease, prevalence of this infection among cattle is dangerous to human beings also. The important aim of this study was to find out the overall seroprevalence of brucellosis in two farms which were found positive for milk ring test.

MATERIALS AND METHODS

This study was carried out during an outbreak of brucellosis (July 2010) at Chikhodra village in Anand district of Gujarat, India. Milk samples from two farms where this study was carried out were already proved positive for the milk ring test. Blood samples were collected from all animals in these farms. Serum was separated from those samples and was subjected to heat treatment to avoid nonspecific reactions (56°C for 30 minutes).

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Rose Bengal Plate Agglutination Test

All the procedures were carried out as described by Morgan *et al.* (1978). Before performing the test, the antigen and sera were brought to room temperature. One drop of serum (30 μ l) was put on a glass slide using a micropipette. The antigen bottle was shaken well to ensure homogeneous suspension and one drop of antigen (30 μ l) was added to the serum on the slide. The serum and antigen were then mixed quickly using separate spreader for each serum sample. The slide was held over a white surface and rocked gently from side to side for 4 to 5 minutes. Samples showing definite clumping were considered positive for brucellosis. In negative samples, the mixture remained homogenous without formation of any clumps.

Standard Tube Agglutination Test

The standard tube agglutination test (STAT) was performed according to Alton *et al.* (1975). All sera samples were tested as five dilutions. Five tubes were placed in a rack. Phenol saline 0.8 ml was taken in first tube and 0.5 ml in all other tubes. Serum 0.2 ml was added in the first tube and mixed well and 0.5 ml of diluted serum was transferred to the second tube and mixed thoroughly. Then, 0.5 ml from the second tube was transferred to the third tube. This process was continued up to the fifth tube, and 0.5 ml was discarded from the last tube after mixing. To each tube *Brucella abortus* antigen 0.5 ml was added and was mixed thoroughly. This provided a final dilution of 1:10, 1:20, 1:40, 1:80 and 1:160 etc. A control tube was set up to simulate 50 percent clearing by mixing 0.50 ml antigen with 1.50 ml of phenol saline in an agglutination tube. All the tubes were then incubated at 37°C for 20 h. Results were read after incubation. The reciprocal of the highest serum dilution showing 50 percent

or more agglutination (50 % clearing) was taken as the titer of the serum. The titer so obtained was converted into International Unit (I.U.) of *Brucella* antibody activity by multiplying with 2 as recommended by the joint FAO/WHO expert committee on Brucellosis.

Hematology

Hematological parameters such as hemoglobin (Hb), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC), packed cell volume (PCV), platelets count (PLT), mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were measured from K3EDTA added blood samples using a Medonic CA 620 (Merck) blood auto analyzer.

Statistical analysis

Hematological values were analyzed by standard statistical procedure described by Snedecor and Cochran (1992) and were expressed as mean (\pm SE).

RESULTS AND DISCUSSION

Serological examinations

Out of 104 serum samples tested using the rose bengal plate agglutination test (RBPT), thirty-four were found positive. Those samples found positive for RBPT were subjected to STAT to find the titer of antibody against *Brucella* infection in the affected animals. Results of STAT are as shown in Table 1.

Antibody detection in paired serum samples is not recommended during an outbreak because of the length of time required to confirm a diagnosis (Radostits *et al.*, 2006). The rose bengal test is an

inexpensive as well as quick test. It is an excellent test for large-scale screening of serum samples for brucellosis. Even though it gives highly sensitive results, it is not a highly specific test. So positive results of RBPT has to be confirmed using some other tests. In this study, positive results of RBPT were further confirmed using STAT. All samples found positive by RBPT were confirmed by STAT except one which was negative for this test. Combined results of RBPT and STAT are shown in Table 2.

Hematology

The mean hematological values of infected and control group are as shown on Table 3. Mean corpuscular volume, platelet count and total leukocyte count of the infected group were significantly decreased ($P < 0.05$) compared to the control group. Figure 1 depicts the percentage of animals suffering from different hematological conditions.

Even though hematological studies of brucellosis in human are adequate in number, there are very few in cattle. Results of this study showed that mean corpuscular volume, platelet count and total leukocyte count of the infected group significantly decreased ($P < 0.05$) compared to control group. Similar observation has been reported in human brucellosis also (Crosby *et al.*, 1984). Other nonsignificant conditions noted in the affected animals such as leukocytosis, anemia, lymphocytopenia and pancytopenia were also observed in human beings by many scientists (Crosby *et al.*, 1984; Kadri *et al.*, 2003). Lymphopenia has been recorded in *Brucella* infected camels also (El-Boshy *et al.*, 2009).

Multiple possible mechanisms are responsible for thrombocytopenia and leukocytopenia in brucellosis (Crosby *et al.*, 1984).

Hemophagocytosis, disseminated intravascular coagulation, direct damage of bacteria to platelets, granulomatous lesions of the bone marrow, bone marrow suppression, hypersplenism, and immune-mediated damage are thought to be the major reasons for abnormal hematology. Krauss (2003) pointed hemophagocytic lymphohistiocytosis as the reason for leucopenia, thrombocytopenia and anemia. Increase and decrease of total leukocyte count in blood may depend on the stage of infection in animals. Pancytopenia in brucellosis is also multifactorial in origin and is attributed to hypersplenism and bone marrow involvement (Abdi-Liae *et al.*, 2007). Marked pancytopenia or isolated deficits can be attributed to diffuse intravascular coagulation, hemophagocytosis, or immunologically mediated cellular destruction (Pappas *et al.*, 2005). Bacteremia occurring while brucellosis explains granulocytosis noted in some affected animals. In the later stages of the infection, lymph nodes may develop chronic granulomatous lymphadenitis, leading to lymphoid depletion (Radostits *et al.*, 2006). This may result in lymphocytopenia in many of the affected animals.

The herd characteristics and the results of the first herd test may be used as predictors of the potential presence or absence of *Brucella abortus* in herds with reactors to the tube agglutination test. The presence of only single suspicious reactor on the first test is a reliable predictor of lack of infection. The presence of one or more positive reactors on the first herd test is a reliable predictor of the presence of infection. Presence of more seropositive animals indicates high prevalence of brucellosis in these two farms.

Table 1. Results of the standard tube agglutination test.

Titer (IU)	No. of animals	Interpretation of titer
>320	28	Positive
160	2	Positive
80	2	Positive
40	1	Doubtful
20	1	Negative

Table 2. Combined results of the rose bengal plate agglutination test and the standard tube agglutination test.

Test	Total	Serologically Positive	Doubtful	Serologically Negative	Percentage of Seroprevalence
RBPT	104	34	-	-	32.69%
STAT	34	32	1	1	30.77%

Table 3. Hematological values of infected and control animals.

Parameters	Brucellosis positive animals	Control animals
TEC (Millions / μ l)	6.07 \pm 0.31	6.10 \pm 0.26
MCV (c μ μ)	44.60 \pm 0.85*	46.99 \pm 0.75
PLT (Thousand / μ l)	115.76 \pm 13.13*	128.82 \pm 16.63
TLC (Thousand / μ l)	6.13 \pm 0.70*	7.36 \pm 0.72
Hb (g%)	9.41 \pm 0.50	9.78 \pm 0.34
MCH (μ g)	15.50 \pm 0.25	16.15 \pm 0.25
MCHC (%)	34.81 \pm 0.20	34.32 \pm 0.20
Lymphocyte (%)	43.73 \pm 3.42	45.53 \pm 5.11
Mid cells (%)	11.50 \pm 0.95	10.32 \pm 0.92
Granulocytes (%)	44.76 \pm 3.53	43.56 \pm 4.71

*P<0.05 Significant parameter.

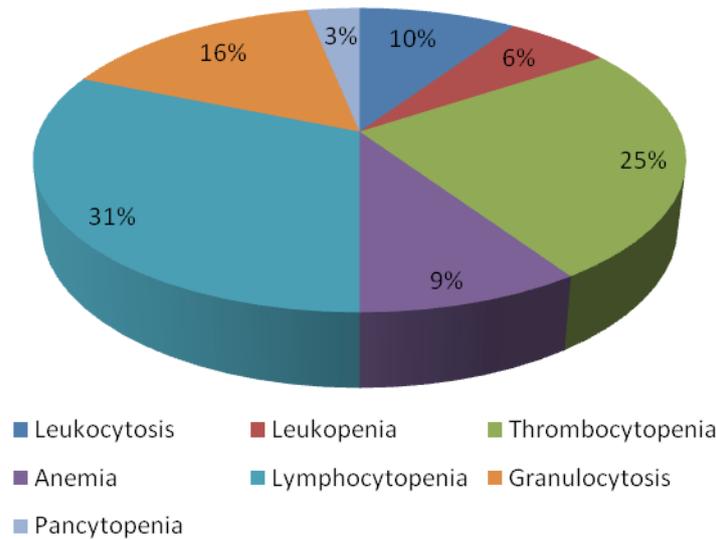


Figure 1. Percentage of animals suffering from different hematological conditions.

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