BOVINE BRUCELLOSIS IN RELATION TO PUBLIC HEALTH

By

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THESIS

Submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

Faculty of Veterinary and Animal Sciences
Kerala Agricultural University

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I here by declare that the thesis entitled **"BOVINE BRUCELLOSIS IN RELATION TO PUBLIC HEALTH"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society

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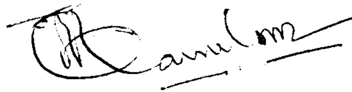

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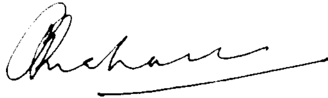

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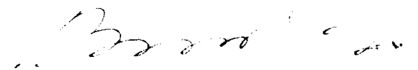

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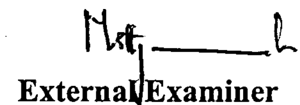

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VINOD. V.K

Dedicated

to

My beloved parents

and

brother

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Introduction

INTRODUCTION

Man domesticated cattle about 6000 years ago. With advancement in civilization, people started rearing cattle for milk production. Even from pre-historic time, cattle rearing for milk became one of the popular means of occupation of the people.

India had a cattle population of 205 million in 1992 and was ranked first in the world. The country is blessed with more than 20 different breeds of cattle. Kerala with a cattle population of 35 lakhs was ranked 13th among the Indian states in 1992.

Milk forms an important source of animal protein to the various strata of the population. The per capita milk consumption in India, in 1995 was 199 g/day whereas in Kerala it was only 181 g/day. In India cattle are reared mainly for milk production. During the year 1997, the country had an annual milk production of 78 million tonnes and was ranked the highest milk producer in the world. Cattle rearing for milk production forms an important means of livelihood to the rural folk.

There are a number of diseases affecting cattle and some of them seriously impair the health of the animal, which result in loss of production and also cause severe economic loss to the farmer. One such disease is brucellosis, a bacterial disease of cattle, which can also affect man. The disease is primarily caused by *Brucella abortus*. It can cause abortion, premature birth, decrease in milk yield or sterility and thus economic loss to farmers.

As early as 1974, Mathur and Sharma estimated the economic loss to India due to brucellosis among cattle and buffaloes. According to them, the annual economic loss to the country due to reduction in milk yield and loss of calves was approximately Rs. 311.47 million.

Brucellosis is an anthropozoonosis. The infection is transmitted to man mainly through the consumption of improperly pasteurized milk of diseased animals and the products derived from such milk. The disease can also be transmitted to various occupational groups such as milkers, animal handlers, slaughter-house workers and veterinarians. Person to person transmission of the disease is rare and hence the control of human infection primarily depends on the control and elimination of the disease in animals. In order to initiate proper control programmes, it is essential to understand the prevalence and magnitude of infection in humans and animals.

Considering the economic importance and public health significance of brucellosis, the present study was under-taken with the aim of assessing the presence and level of brucella antibody in the sera of cattle, animal handlers and general population by serological tests.

Review of Literature

REVIEW OF LITERATURE

Brucellosis is an important bacterial zoonoses which affects both man and various species of animals. Marston (1863) had given an accurate description of the disease in his "Report on Malta Fever". Later in 1887, Bruce isolated the etiological agent of the disease, *Micrococcus melitensis*, from the spleen of the patients who had died of malta fever. *Brucella abortus* was first isolated and described in 1897 by a Danish veterinarian Bernard Bang. He isolated the organism from a cow which had suffered from contagious abortion. Later in 1911, Schroeder and Cotton isolated the organism from infected cows milk. Meyer and Shaw (1920) suggested the genus name "*Brucella*" for the etiological agent of the disease to commemorate David Bruce who had first isolated the organism from human patients in Malta island.

Brucellosis in animals

Cattle

From the available records, it appears that Polding (1950) is the first scientist who undertook an extensive survey on brucellosis among domestic animals in India and established the prevalence of disease in cattle, buffaloes, sheep and goats.

In Kerala, the occurrence of the disease in cattle had long been recognized by Raja *et al.* (1959). During the study, the investigators found that two out of seven cattle belonging to a private farm had the disease. One of these animals was a cow which yielded a serum agglutination titre of 1:640 and the other was a heifer with an agglutination titre of 1:160.

Kataria and Verma (1969) screened 4335 cattle serum samples for brucellosis by tube agglutination test and reported that 5.25 per cent cattle had the disease. The per cent of infection in cows, heifers, bulls and young bulls was 6.5, 11.5, 6.8 and 3.35 respectively.

Kulshreshtha *et al.* (1973a) reported that 14 (2.3 per cent) out of 589 cattle sera were found positive for brucellosis by standard tube agglutination test.

Nag *et al.* (1977) collected serum samples from 50 cows and buffaloes including those which had a history of abortion. The samples were subjected to both standard serum tube agglutination and plate agglutination test to detect the presence of *Brucella* agglutinins. Both the tests registered a seropositivity of 24 per cent for brucellosis.

Purbey and Sane (1978) made an investigation on brucellosis in Dangi breed of cattle. They collected 23 cattle serum samples and examined them by plate and tube agglutination tests. The study revealed that five (21.74 per cent) and four (17.39 per cent) samples were positive for brucellosis by tube and plate agglutination tests, respectively.

Halder *et al.* (1979) studied seroprevalence of brucellosis among cross bred cattle and Hariana breed of cattle. They performed serum tube agglutination test on 2521 serum samples and reported the overall brucella infection in the herd as 4.99 per cent. The percentage of infection in Hariana breed, first generation cross and second generation cross were 7.9, 3.9 and 1.3 respectively.

A serological survey on brucellosis in cattle belonging to four state farms of Nagaland was carried out by Maiti *et al* (1980). They examined 143 serum samples of cattle belonging to four farms and 17 samples of bulls belonging to key village centres by tube agglutination test. The survey revealed that cattle belonging to three farms had brucellosis and the percentage of infection varied from 10.52 to 26.66. Bulls of the key village centres were found free from the disease. The study revealed an agglutination titre of 160 iu/ml in 13 out of 15 positive samples.

Chackraborthy & Kwatra (1980) screened 397 slaughtered bovine serum samples by rapid plate agglutination test and recorded a seropositivity of 14.8 per cent.

Ramachandra *et al.* (1981) examined the serum samples of 13 aborted cows by rapid plate test and standard tube test. Both the tests detected 10 positive cases for brucellosis.

Oberoi and Kwatra (1982) examined 1385 serum samples of cattle belonging to military and semi government farms in Punjab and also from one of the farms of Haryana. The study revealed that 60 (4.33 per cent) samples were positive for brucellosis by tube agglutination test.

Verma (1982) screened 74 serum samples collected from a cattle breeding farm for brucellosis by rapid plate agglutination and standard serum agglutination tests and reported that two were found positive for the disease by both the tests.

During a survey on brucellosis, 410 serum samples of breeding bulls were tested by tube agglutination test and reported that one of these bulls had a *Brucella* agglutinin titre positive for the disease (Reddy *et al.* 1983).

Bermudez and Barriola ~~*et al.*~~ (1983) screened 20665 bovine serum samples for brucellosis by Rose Bengal plate test and recorded a seropositivity of 5.5 per cent.

Rahman *et al.* (1983) subjected serum samples collected from 78 apparently healthy Abortus Bang Ring test positive cows, 14 cows with history of abortion, and 15 cows with retained placenta and genital complications for brucellosis using tube agglutination test and reported that the percentage of infection among them were 60, 78.5 and 60 respectively.

Chatterjee *et al.* (1984) carried out a seroepidemiological study on bovine brucellosis in organised herds in West Bengal. They examined the serum samples of 2675 cows and 367 bulls by standard tube agglutination test and reported the overall seroprevalence of brucellosis in cattle as 20.5 per cent. The seroprevalence of the disease in cows was 21.3 per cent and bulls was 14.1 per cent.

Sharma *et al.* (1984) performed tube agglutination test on serum samples collected from 666 cattle, to detect *Brucella* infection. The study revealed that 58 samples (8.7 per cent) were positive for brucellosis with a diagnostic titre of 80 iu/ml or more.

Babu *et al.* (1985) studied the seroprevalence of brucellosis in cattle. They collected serum samples of 632 Jersey and 226 Ongole breeds of cattle. Standard

tube agglutination test performed on the samples revealed an overall seropositivity of 0.82 per cent for brucellosis.

Manickam and Mohan (1987) investigated brucellosis in cattle. The percentage of seropositivity in apparently healthy cows, cows with history of previous abortion, retention of placenta or infertility by standard plate test was 1.41, 15.32, 7.46 and 2.62 respectively.

Savalgi *et al.* (1987) screened 82 serum samples from cows for brucellosis. They recorded an incidence rate of 12.1 per cent by standard tube agglutination test.

The seroprevalence of brucellosis in the pure Jersey breed of cattle was investigated by Bachh *et al.* (1988). During the study, they found an overall seropositivity of 44.35 per cent by standard tube agglutination test. The prevalence was more in females than in males and in adult age group than in young animals. Cattle in more than one lactation had higher incidence than those in first lactation. Brucellosis in cattle with previous history of abortion and repeat breeders with no history of abortion was 88.9 per cent and 66.7 per cent respectively. The percentage of seropositivity in pregnant cattle was 66.7 and non pregnant cattle was 20.

A serological investigation was carried out to evaluate the incidence of brucellosis in organised dairy farms and breeding bulls in Assam (Barman *et al.*, 1989). They examined the serum samples of 129 cows and 30 bulls using standard tube agglutination test. The percentage of prevalence of brucellosis among cows and bulls were 44.9 and 33.3 respectively.

Mathur *et al.* (1991) studied the seroprevalence of brucellosis in 102 cattle with history of abortion and associated ailments. Standard tube agglutination test revealed that four out of 102 samples were positive for brucellosis. Three of these animals had a serum agglutination titre of 80 iu/ml and one had a titre of 160 iu/ml. Comparatively higher percentage of *Brucella* agglutinins were noted (37.25 per cent) in cattle with a history of abortion at seven to nine months of pregnancy.

Kalimuddin *et al.* (1990) screened serum samples from 101 cows of two organised farms for brucellosis by standard tube agglutination, heat inactivation and 2-Mercaptoethanol tests. The percentage of seropositivity detected by standard tube agglutination, heat inactivation and 2-Mercaptoethanol test were 18.81, 16.83 and 12.87 respectively.

Chandramohan *et al.* (1992) collected serum samples from 115 Zebu cattle with a history of abortion, retained placenta, repeat breeding, orchitis or pyrexia and performed ELISA to detect the presence of *Brucella* antibodies. Of the samples tested, 21 (18.26 per cent) had antibodies positive for brucellosis.

Izigur *et al.* (1992) screened serum samples from 320 cattle suspected for brucellosis using serum agglutination test, Rose Bengal plate test and serum agglutination test with EDTA treated antigen and recorded a seropositivity of 23.8, 21.6 and 13.8 per cent respectively.

Suresh *et al.* (1993) conducted a serological survey on bovine brucellosis in Tamil Nadu. A total of 459 cattle serum samples were collected from cases of reproductive disorders from organised farms and rural areas. Rapid plate

agglutination test and Rose Bengal plate test performed on the samples revealed a seropositivity of 6.51 and 7.2 per cent respectively.

Kenar and Guler (1994) screened serum samples of 569 slaughtered cattle for brucellosis by Rose Bengal plate test and serum agglutination test and recorded a seropositivity of 5.7 and 4.5 per cent respectively.

Ghani *et al.* (1998) carried out a serological study on bovine brucellosis. The study revealed that the prevalence of brucellosis in 240 live cattle examined by standard plate test, standard tube test, Rivanol test and 2-Mercaptoethanol tests were 1.66, 0.83, 0.83 and 0.41per cent respectively. In the case of 240 slaughtered cattle, the prevalence of brucellosis by these tests were 1.25, 1.25, 1.25 and 0.83 per cent. The serum samples from 10 live and 10 slaughtered bulls were found negative by all the above tests.

Buffaloes

Joshi and Prakash (1971) subjected serum samples collected from 300 slaughtered buffaloes to standard tube agglutination test and found that six (two per cent) were positive for brucellosis with an agglutination titre of 80 iu/ml or more.

Kulshreshtha *et al.* (1973 a) tested serum samples from 799 buffaloes for brucellosis and reported that 13.88 per cent were positive for the disease by standard plate agglutination test.

Kulshreshtha *et al.* (1978) examined serum samples from 1244 buffaloes of various farms by standard tube agglutination test for brucellosis and recorded that 4.4 per cent were positive.

Baby and Paily (1979) investigated the incidence of brucellosis in 1026 buffaloes in Kerala. They reported that the percentage of positive reactors among the male and female buffaloes was 2.13 and 2.28 respectively by standard tube agglutination test with an overall incidence of 2.24 per cent in the population tested.

Mathur *et al.* (1979) screened serum samples collected from 77 buffaloes for brucellosis by standard tube agglutination test. They reported that 1.3 per cent were positive for brucellosis.

Oberoi and Kwatra (loc. cit) examined serum samples from 601 buffaloes for brucellosis. The study revealed that 52 (8.65 per cent) were positive for brucellosis by tube agglutination test.

Chatterjee *et al.* (1984) tested serum samples from 807 buffaloes by standard tube agglutination test for brucellosis and reported a seroprevalence of 19.3 per cent.

Babu *et al.* (loc. cit) studied the seroprevalence of brucellosis in buffaloes. Standard tube agglutination test performed on 726 serum samples revealed that two (0.28 per cent) were positive for the disease.

Savalgi *et al.* (loc. cit) serologically screened 464 buffaloes of organised farms for brucellosis using standard tube agglutination test and recorded an incidence rate of 2.1 per cent.

Suresh *et al* (loc. cit) screened 284 buffaloe serum samples collected from cases of reproductive disorders. Rapid plate agglutination test and Rose Bengal plate test of the samples revealed a seropositivity of 5.9 and 8.4 per cent respectively.

Swine

Kulshreshtha *et al.* (1978) studied the seroprevalence of brucellosis in different species of animals. Standard tube agglutination test performed on 91 porcine serum samples revealed a seropositivity of one per cent for the disease.

Mathur *et al.* (1979) examined 110 pig serum samples by standard tube agglutination test for brucellosis and recorded a prevalence rate of 3.6 per cent.

Kumar and Rao (1980) screened serum samples of 1103 Yorkshire pigs by serum agglutination test for brucellosis. They recorded an incidence rate of 11.33 per cent.

Choudhary *et al.* (1983) studied the seroprevalence of porcine brucellosis in Bihar. They screened the serum samples of 241 pigs for brucellosis by standard tube agglutination test and reported an overall prevalence of 4.97 per cent.

Kalimuddin and Choudhary (1988) investigated the seroprevalence of brucellosis in pigs. They examined serum samples collected from 213 pigs brought for slaughter at a bacon factory by serum tube agglutination test and found that 5.63 per cent were positive for the disease.

Lateef *et al.* (1989) made an attempt to study the seroprevalence of brucellosis among country pigs in AndhraPradesh. They performed rapid plate agglutination, standard tube agglutination and 2-Mercaptoethanol tests on 299 serum samples. A total of three samples (one per cent) have shown seropositivity by rapid plate agglutination, standard tube agglutination and 2-Mercaptoethanol tests.

Ghosh (1989) carried out a serological survey of brucellosis in organised pig farms. He examined 177 serum samples by standard tube agglutination test and recorded an overall seroprevalence of 5.4 per cent.

Sheep and goat

Joshi and Prakash (loc. cit) studied the seroprevalence of brucellosis in sheep and goat in Delhi. Out of 800 sheep and 950 goat sera tested for brucellosis by standard tube agglutination test, 0.88 per cent of sheep and 1.57 per cent of goats were positive.

Mahakur and Panda (1972) undertook a study to assess the seroprevalence of brucellosis in sheep and goats and reported that two out of 100 serum samples tested were positive for brucellosis by standard tube agglutination test.

Kumar *et al.* (1976) studied the incidence of brucellosis among goats. They examined 10420 serum samples by serum agglutination test and reported that 0.33 per cent were positive for the disease.

Kulshreshtha *et al.* (1978) tested serum samples from 128 sheep and 749 goats for brucellosis and observed that 1.5 per cent of sheep and 1.6 per cent of goats were positive for the disease by tube agglutination test.

Stephen *et al.* (1978) investigated the seroprevalence of brucellosis in sheep and goat by employing standard tube agglutination test on 82 sheep and 125 goat serum samples. They recorded the per cent of seropositivity as 14.63 in sheep and 15.2 in goat.

Sreemannarayana (1980) examined 926 serum samples of goats by standard tube agglutination test and recorded an overall incidence of 2.15 per cent.

Panjarathinam (1983) reported that standard tube agglutination test performed on 357 goats and 44 sheep serum samples revealed a seropositivity of 10.64 and 11.36 per cent respectively.

Sharma *et al.* (loc. cit) screened 3472 goats and 607 sheep serum samples collected from various farms in UttarPradesh and reported that 2.9 per cent goats and 1.8 per cent sheep were positive for brucellosis by tube agglutination test.

Kapoor *et al.* (1985) subjected 174 goat serum samples to standard tube agglutination test and found that 1.92 per cent were positive for brucellosis.

Ghosh and Verma (1985) investigated the incidence of brucellosis among sheep and goat in Nagaland. They examined the serum samples of 189 goats and 65 sheep by standard tube agglutination test and recorded an overall incidence rate of 6.29 per cent.

Bandey *et al.* (1989) conducted seroepidemiological studies on brucellosis in exotic sheep in Kashmir valley. They examined 8034 serum samples of unvaccinated sheep by tube agglutination test and recorded a prevalence rate of 3.46 per cent.

Masoumi *et al.* (1992) studied the seroprevalence of brucellosis in sheep and goat in Lahore area. They examined 500 goat and 532 sheep serum samples by standard tube agglutination test and recorded an overall seroprevalence of three per cent in goats and 1.69 per cent in sheep.

Sharma *et al.* (1995) analysed the serum samples collected from aborted sheep and found that all of them gave positive reaction to the standard serum agglutination test with titres ranging from 1:160 to 1: 640.

Katochi *et al.* (1996) examined 102 serum samples of rams by Kolmer's complement fixation test for detection of antibodies for *Brucella ovis*. The test revealed an overall prevalence of 5.88 per cent for *Brucella ovis* infection.

Kumar *et al.* (1997) performed Rose Bengal plate test (RBPT), standard tube agglutination test (STAT), complement fixation test (CFT) and dot. enzyme linked immunosorbent assay (Dot. ELISA) on serum samples from 289 goats and 255 sheep. In goats the incidence was 1.73, 1.38, 7.26 and 18.33 per cent respectively on the basis of RBPT, STAT, CFT and Dot. ELISA while in sheep the incidence was 9.00, 4.31, 27.48 and 10.58 per cent respectively on the basis of RBPT, STAT, CFT and Dot. ELISA.

Dogs

Stephen *et al.* (loc. cit) tested serum samples from 11 dogs for brucellosis by tube agglutination test and reported that one (9.09 per cent) was positive for the disease.

During an investigation Pillai *et al.* (1991) examined 640 serum samples of dogs by 2-Mercaptoethanol tube agglutination test and reported a seropositivity of 2.18 per cent for brucellosis.

Srinivasan *et al.* (1992) studied the seroepidemiology of canine brucellosis in Madras city. Out of 460 serum samples screened by 2-Mercaptoethanol tube agglutination test, 1.96 per cent had *Brucella canis* antibodies.

Horses

Investigation on brucellosis in equines had been done in India. Srivastava *et al.* (1983) screened 25 sera samples from aborted mares by standard tube agglutination test and reported that ten were positive for brucellosis.

Sharma *et al.* (1984) studied the seroprevalence of brucellosis in equines in UttarPradesh. They recorded a seropositivity of 17.39 per cent in 569 animals screened.

Yadav *et al.* (1991) conducted a study to asses the seroprevalence of brucellosis in horses, mules and donkeys. They examined the serum samples of 2173 horses, 166 donkeys and 56 mules by standard tube agglutination test and reported that 185 horses, six donkeys and four mules were positive for brucellosis.

Man

Singh and Saxena (1964) screened 2020 human sera samples obtained from cases of pyrexia of unknown origin and reported that 0.9 per cent of samples had *Brucella* agglutinins.

Koshi and Myers (1967) performed a total of 8010 blood cultures and 724 *Brucella* agglutination tests and detected 20 cases positive for the disease (six by culture and serology, two by culture alone and 12 by serology alone). They opined

that the discrepancy between high prevalence of brucellosis among animals and man may be due to the low infectivity of *Brucella abortus* to man compared to *Brucella melitensis*.

Joshi and Prakash (loc. cit) tested 1410 human sera collected from cases of pyrexia of unknown origin by standard tube agglutination test and reported that 3.9 per cent of the sera had an agglutination titre positive for brucellosis.

Mahakur and Panda (loc. cit) screened 215 sera of patients with pyrexia of long duration and 800 serum samples from afebrile cases for brucellosis by standard tube agglutination test and recorded that 3.9 per cent of samples from pyrexia of long duration and two per cent of samples from afebrile cases were positive for brucellosis.

Stephen *et al.* (loc. cit) examined a total of 406 human sera samples obtained from 255 males and 151 females for brucellosis. The standard tube agglutination test revealed that 11.73 per cent of males and 10.6 per cent of females were positive for brucellosis.

Singh (1982) examined human sera collected from 217 cases of pyrexia of seven to 10 days duration for the presence of *Brucella* agglutinins by standard tube agglutination test. The study revealed that eight (3.6 per cent) were positive for the disease with an agglutination titre of 80 iu/ml or more.

Verma (loc. cit) examined 32 human serum samples collected from pyrexia of unknown origin by rapid plate agglutination and standard tube agglutination test and reported that none of the samples were positive for brucellosis by the above tests.

Rahman *et al.* (loc. cit) studied the seroprevalence of brucellosis among human beings. They found that 12 (15 per cent) out of 80 serum samples collected from dairy workers or milkers were positive for brucellosis. Nine (12.85 per cent) out of 70 serum samples of cowboy and agricultural workers who had close contact with animals had *Brucella* agglutinin titre positive for the disease. The serum samples of 40 agricultural workers with no direct contact with animals did not yield an agglutination titre positive for brucellosis.

Umapathy *et al.* (1984) examined the serum samples collected from 376 apparently healthy rural population, 644 human patients admitted with pyrexia of unknown origin (PUO) and 75 apparently healthy persons working in *Brucella abortus* infected farm to detect the presence of *Brucella* agglutinins. The study revealed that 1.2 per cent of the human patients admitted with PUO had *Brucella* agglutinins positive for brucellosis by standard tube test whereas in apparently healthy persons working in *Brucella abortus* infected farm, the per cent of seropositivity was 24. None of the serum samples from apparently healthy rural population was found positive for brucellosis by standard tube test.

Sharma *et al.* (1984) examined 2473 human serum samples collected from various medical colleges and hospitals for seroprevalence of brucellosis by tube agglutination test. They recorded that 14 (0.56 per cent) were found positive for brucellosis with a diagnostic titre of 80 iu/ml or more.

Kapoor and Rao (1984) examined serum samples of six persons rearing goats positive for brucellosis by serum plate agglutination test and observed that two

samples were positive for brucellosis. Tube agglutination test of these two samples revealed an agglutination titre of 40 iu/ml and 20 iu/ml.

Kapoor *et al.* (loc. cit) studied the seroprevalence of brucellosis in human beings. Tube agglutination test performed on 101 serum samples collected from patients with pyrexia of unknown origin revealed that 2.97 per cent were positive for the disease.

Savalgi *et al.* (loc. cit) investigated the incidence of brucellosis among the staff working in a *Brucella* infected dairy farm and reported that four (20 per cent) out of 20 staff's serum was found positive for brucellosis by standard tube agglutination test.

Zeftawi *et al.* (1986) screened serum sample collected from 745 abattoir workers for brucellosis using plate agglutination test and found that 1.7 per cent were positive for the disease.

Koshi *et al.* (1988) tested 2415 sera from patients suspected to have brucellosis and 12541 sera from Widal negative patients with pyrexia of unknown origin and reported that 49 patients from the former group and 43 from the later group were found positive for brucellosis by tube agglutination test. They opined that use of *Brucella melitensis* antigen for tube test help to detect more subjects infected with *Brucella melitensis.*

Kalimuddin and Choudhary (loc. cit) tested serum samples from 13 workers of a bacon factory by serum agglutination test and found that one was positive for *Brucella* infection with a titre of 80 iu/ml.

Kalimuddin *et al* (loc. cit) conducted standard tube agglutination, heat inactivation and 2- Mercaptoethanol ~~Mercaptoe~~thanol tests on 11 serum samples collected from attendants of a dairy farm and found that one sample was positive for brucellosis by all the above tests.

Masoumi *et al.* (loc. cit) screened 522 human serum samples for brucellosis and recorded an overall prevalence of 0.95 per cent by serum agglutination test.

Mathai *et al.* (1996) examined serum samples of 23 patients who were clinically suspected for brucellosis, 26 patients with prolonged fever and 17 persons as control to detect the presence of *Brucella* agglutinins by enzyme linked immunosorbent assay. The per cent of positive cases in these groups were 39.1, 26.9 and zero respectively.

Serological diagnostic tests

The ideal method for the diagnosis of human and animal brucellosis is the isolation and identification of the causative organism. However, it is difficult to have 100 per cent isolation success. Isolation requires various types of media, chemicals and equipments and also it is laborious and time consuming. Moreover in this process, the handling of live bacteria can cause disease in laboratory workers. During the last few decades many serological tests have been developed to diagnose the disease. Commonly used serological tests are standard tube agglutination test, Rose Bengal plate test, Heat inactivation test, 2-Mercaptoethanol test and EDTA agglutination test.

Standard tube agglutination test (STAT)

Wright and Smith (1897) developed this test which is widely used in the diagnosis of brucellosis. Grinsted (1909) first used this test in the diagnosis of bovine brucellosis. Although the test identifies majority of infected animals, it is not 100 per cent effective and fails to differentiate antibodies produced due to infection and recent vaccination. The test may be negative in the early stage of infection and in old longstanding chronic cases (Morgan, 1967). Prozone and other blocking phenomena, particularly in high titre serum samples are reported to cause false negative reactions in tube agglutination test (Cho & Ingram, 1972). For the reasons outlined, supplementary tests are used on samples that give suspicious titres on the STAT (Sutherland, 1980).

Rose Bengal plate test (RBPT)

Rose Bengal plate test is a single dilution serum agglutination test (Sutherland, 1980). The antigen consists of *Brucella* cells stained with Rose Bengal and suspended in an acidic buffer having pH 3.65. It has been suggested that acidic buffer inhibits non specific agglutinins (Rose and Roepke, 1957). Perusal of the current literature shows that RBPT has been used either as a screening test (Morgan, 1969) or as a definitive test (Nicoletti, 1969). False positive reactions to RBPT have been reported (Alton *et al.* 1975a). Allan *et al.* (1976) showed that the RBPT detects I_gM antibodies more efficiently than I_gG_1 or I_gG_2 antibodies. As I_gM is produced in response to vaccination (Beh, 1974), vaccinal I_gM may account for the false positive reactions in RBPT (Sutherland, 1980). A small number of false negative reactions in

the serum from animals later found to be infected has also been reported (Miller *et al.* 1973; Lapraik *et al.* 1975).

Heat inactivation test (HIT)

The test is based on the observation that two types of *Brucella* agglutinins were found in cattle sera and could be differentiated on the basis of stability at 65°C for 15 minutes (Morgan, 1967 and Corbel, 1985). A high correlation between the percentage of heat labile agglutinins and percentage of fast sedimenting agglutinins in serum collected from heifers artificially infected with *Brucella abortus* was recorded by Rose *et al.* (1964). Amerault *et al.* (1961) opined that HIT could be used to clarify the status of suspect herd when there is no other evidence of brucellosis.

Ethylene diamine tetraacetate agglutination test (EAT)

The *Brucella* agglutinating activity present in the sera of a high proportion of uninfected cattle reacting to tube agglutination test, is labile in the presence of the chelating agent EDTA disodium salt. These EDTA labile agglutinins are associated mainly with I_gM molecules, although in a small proportion of animals, I_gG may also be involved. These immunoglobulins attach to *Brucella* cells via the F_c region of their 7s subunits and not via the antibody-combining site, located on their Fab portion. This interaction is blocked by EDTA. (Joint FAO / WHO (1986).

2-Mercaptoethanol test (MET)

The test was based on the observation that, the activity of I_gM or 19s antibodies was destroyed after the serum had been treated with mercaptoethanol, whilst the activity of I_gG or 7s was not so affected [Morgan (1967) and Anderson *et*

al. (1964)]. The test differentiates antibodies resulting from vaccination and those from infection and is of value in detecting chronic carrier animals.

The other serological tests used in the diagnosis of brucellosis includes Complement fixation test (Larson, 1912), Plate agglutination test (Huddleson, 1920), Milk ring test (Fleischauer, 1937) Coombs test (Coombs *et al.*, 1945), Vaginal mucus test (Kerr, 1955) Rivanol test (Morgan, 1967), CARD test (Alton *et al.*, 1975b) and Enzyme linked immunosorbent assay (Sutherland *et al.*, 1986).

Comparison of serological tests

Nicoletti (1969) compared serological tests used in the diagnosis of bovine brucellosis and reported that standard tube agglutination test, acidified plate antigen test, Rivanol precipitation plate agglutination test, complement fixation test, Individual ring test and 2-Mercaptoethanol test detected 52, 95, 96, 98, 89 and 97 per cent respectively, of the infected cattle as positive.

Kulshreshtha *et al.* (1973b) compared titres shown by standard tube agglutination and Mercaptoethanol inactivation tests employed in the diagnosis of bovine and human brucellosis and recorded no definite correlation in titres. This had been substantiated by the fact that in acute form of the disease, the macroglobulins (I_gM) which are sensitive to the reducing action of mercaptoethanol were present, while in chronic form macroglobulins disappear and microglobulins (I_gG) which are resistant to reducing action of mercaptoethanol remain.

Alton *et al.* (1975a) evaluated complement fixation, serum agglutination, and Rose Bengal Test in the diagnosis of bovine brucellosis and opined that Rose Bengal

test is most useful as a screening test. They also recorded that complement fixation test was far superior to the serum agglutination test as a diagnostic test.

Kumar *et al.* (1984) compared disulphide bond reduction test and standard tube agglutination test and suggested that performing the above two tests simultaneously help to clarify doubtful cases of brucellosis in cattle, sheep and buffaloes.

Stemshorn *et al.* (1985) compared various serological tests used in the diagnosis of bovine brucellosis and recorded the sensitivity of complment fixation test, buffered plate antigen test, Rose Bengal plate test, card test, standard plate agglutination test, standard tube agglutination test and 2-Mercaptoethanol test as 79, 75.4, 74.9, 74.3, 73.1 68.9 and 59.9 per cent, respectively.

Nowlan and Geus (1985) compared serum agglutination test with EDTA modified antigen and standard serum agglutination test in the diagnosis of bovine brucellosis and observed that animals which had *Brucella* agglutinins as a result of exposure to *Brucella abortus* donot have EDTA labile agglutinins.

Kalimuddin *et al.* (1990) evaluated standard tube agglutination, heat inactivation and 2-Mercaptoethanol test in the diagnosis of bovine and human brucellosis and concluded that heat inactivation test and 2-Mercaptoethanol test can be used to reduce non specific *Brucella* agglutinins.

Kulkarni *et al.* (1991) compared ELISA, standard agglutination test and Rose Bengal plate test in the diagnosis of bovine brucellosis and found that ELISA was

more specific compared to standard agglutination test and Rose Bengal plate test in detecting *Brucella* antibodies.

Ghani *et al.* (1994) compared standard plate test, standard tube agglutination test, Rivanol test and 2-Mercaptoethanol test in the diagnosis of bovine brucellosis and found that standard plate test was more sensitive compared to other tests.

Serological cross reactions

Serological cross reactions have been demonstrated between smooth *Brucella* species and *Escherichia coli* 0:116 and 0:157, *Francisella tularensis*, *Salmonella* serotype of Kauffmann-whitegroup N (0:30 Antigen), *Pseudomonas maltophila*, *Vibrio cholerae* and *Yersinia enterocolitica* serogroup 0:9. Exposure to these bacteria by oral or parenteral routes can provoke diagnostically significant titres of antibodies cross reacting with *Brucella*. Differentiation of cross-reaction is reported to be difficult by agglutination test especially in the case of *Yersinia enterocolitica* 0:9 antigen., but immuno diffusion, enzyme linked immuno sorbent assay and cross absorption procedures are claimed to be useful (Corbel, 1985).

Diagnostic titres for brucellosis

Alton and Jones (1967) recommended that a standard tube agglutination titre of 80 to 100 iu/ml can be taken to indicate infection in non vaccinated cattle and that a titre of 40 to 50 iu/ml is regarded as suspicious and requires re-test after about three weeks. The FAO/WHO expert committee on brucellosis (1971) in their fifth report, recommended that in cattle, the minimum diagnostic level of agglutinins in the serum should be 100 iu/ml for non vaccinated cattle and a titre of 50 iu/ml is regarded as

suspicious in them. In animals vaccinated with strain 19, minimum diagnostic level is 200 iu/ml and a titre of 100iu/ml is considered as suspicious. In the diagnosis of bovine brucellosis, standard tube agglutination titre of 80 iu/ml had been taken as positive and 40 iu/ml as suspicious by various Indian workers Kulshreshtha *et al.* (1973a), Nag *et al*.(1977), Baby and Paily (1979), Mathur *et al.* (1979), Chatterjee *et al.* (1984), Sharma *et al.* (1984), Bachh *et al.* (1988) and Kalimuddin *et al.* (1990).

A standard tube agglutination titre of 80 iu/ml have been suggested as positive, 40 iu/ml as doubtful and 20 iu/ml or low as negative in human by Alton and Jones (1967). The above criteria was followed by workers like Joshi and Prakash (1971), Mahakur and Panda (1972), Soni (1976), Stephen *et al.* (1978), Singh (1982), Kapoor *et al* (1985) and Savalgi *et al.* (1987).

In 2-Mercaptoethanol test an agglutination titre of 80 iu/ml was taken as positive and 40 iu/ml as suspicious for brucellosis in human and bovine sera by Alton *et al.* (1975 b). Kumar *et al.* (1984) also considered an agglutination titre of 80 iu/ml or above as diagnostically significant in disulphide bond reduction test. Das and Mukherjee (1984) followed the above criteria in 2-Mercaptoethanol test performed with porcine sera.

The reading and recording of the result of EDTA agglutination test with bovine sera is done in the same way as that of standard tube agglutination test. [Joint FAO/WHO expert committee on brucellosis (1986), Macmillan and Cockrem (1985) and Nowlan and Geus (1985)].

Materials and Methods

MATERIALS AND METHODS

In this serological survey, 1233 bovine serum samples and 747 human serum samples were collected and tested to detect the presence of *Brucella* agglutinins. Of the bovine serum samples, 82 were from various organised farms of Kerala Agricultural University consisting of Regional Agricultural Research Station, Kumarakom (10), Livestock Research Station, Thiruvizhamkunnu (59) and Rice Research Station, Pattambi (13). Twenty three serum samples were collected from a private dairy farm of Kannur. All the above samples were collected from cows. The remaining 1128 samples were collected from cattle slaughtered at Municipal Slaughter House, Kuriachira, Trichur. Among these samples, 610 were collected from male and the remaining 518 samples were from females.

During the investigation, 733 human serum samples were collected from private nursing homes in Trichur. Of these samples, 406 were from males and 327 from females. Apart from these, 10 samples were collected from veterinary surgeons, two from animal attendants and two from slaughter house workers.

Collection and storage of serum

Blood samples from cows of organised farms were collected from jugular vein using disposable syringe and needle. About 10 ml of blood was collected from each cow, transferred into a clean dry test tube and allowed to coagulate in a slanting position at room temperature. The clotted blood was allowed to stand at room temperature for about two hours, then the clot was slightly dislodged from the side of the test tube with a sterile loop and was kept overnight in a refrigerator. On the

following day, sera from each blood sample was transferred to a sterile clean dry test tube and centrifuged at 2000 rpm for five minutes. The clear supernatant serum of individual animal was transferred to a sterile screw capped vial, added a drop of 1:10,000 merthiolate solution per millilitre of serum and stored at -20°C for further study.

Blood sample from each of the slaughtered animals were collected at the time of bleeding. Human blood samples were collected aseptically from the radial vein with a sterile disposable syringe and needle. The method of separation of serum samples from the slaughtered bovines / human beings, the addition of mertholate and storage was the same as that of serum samples from organised farms.

Serological tests

1. **Rose Bengal plate test (RBPT)**

All serum samples of bovine and human were subjected to plate agglutination test following the method described by Alton *et al.* (1975). In this test, one drop of serum (25 µl) was mixed with an equal quantity of *Brucella abortus* Rose Bengal coloured antigen on an opaque plastic white tile. The serum and antigen were mixed with a spreader and the tile was rocked by hand for four minutes. At the end of the period, samples which showed any degree of visible agglutination were considered positive for *Brucella* agglutinins and others regarded as negative.

2. **Standard tube agglutination test (STAT)**

All serum samples of bovines and human were subjected to standard tube agglutination test, following the method described by Alton *et al.* (1975b). In this test

two fold dilutions of each test serum samples was made with phenol saline (0.85 per cent sodium chloride solution containing 0.5 per cent phenol) to form the serum dilutions of 1:5, 1:10, 1:20, 1:40 and 1:80. In order to prepare this dilutions of test serum, 0.8 ml of phenol saline was placed in the first tube and 0.5 ml in each succeeding tube. 0.2 ml of the serum under test was transferred to the first tube, mixed thoroughly with the phenol saline already there and 0.5 ml of the mixture was carried over to the second tube from which after mixing 0.5 ml was transferred to the third tube and so on. This process was continued until the last tube, from which after mixing 0.5 ml of the diluted serum was discarded. To each of these tubes containing diluted serum, 0.5 ml of *Brucella abortus* standardized plain antigen was added and contents in the tubes were mixed thoroughly by rolling the tube in between the palm. The final dilution formed in the tubes were 1:10, 1:20, 1:40, 1:80 and 1:160 of the test serum. A control tube simulating 50 per cent clearance was prepared by mixing 0.25 ml of plain antigen and 0.75 ml of phenol saline. All the tubes were incubated at 37°C for 24 hours. After the incubation period, the results of the test were read and recorded. While recording the results, the titre of agglutination was determined by reading the degree of clearing without shaking the tubes. The highest serum dilution showing 50 per cent agglutination or more was taken as the end point titre of the serum. Serum sample showing a titre of 1:40 dilution (80 iu/ml) or above was considered as positive, a titre of 1:20 dilution (40 iu/ml) was regarded as suspected reaction and a titre less than 1:20 indicated negative reaction. In the case of serum samples revealing over 50 per cent agglutination in 1:160 dilution, the test was repeated with higher doubling dilutions to find out correct titre of the sample.

All bovine and human samples which were positive either, in Rose Bengal plate test or standard tube agglutination test or both the tests were subjected to heat inactivation test, 2-Mercaptoethanol test and EDTA agglutination test.

3. **Heat inactivation test (HIT)**

Heat inactivation test was performed following the principle described by Amerault *et al.* (1961). Before carrying out the test, each sample to be tested was subjected to heat inactivation by keeping the serum in a water bath maintained at a temperature of 65°C for 15 minutes. The heat inactivated serum was centrifuged at 2000 rpm for five minutes and the supernatant was collected for the test. The test procedure and reading of the result was carried out as in the case of STAT.

4. **2-Mercaptoethanol test (MET)**

The 2-Mercaptoethanol test was performed according to the procedure described by Alton *et al.* (1975). In this phenol free *Brucella abortus* plain antigen was used. To make the antigen phenol free, 100 ml of the antigen was mixed thoroughly and transferred to a clean sterile centrifuge tube and centrifuged at 3000 rpm for 15 minutes. The cell free supernatant solution was removed, the cells reconstituted in sterile normal saline and centrifuged as before. The washing and centrifuging of the cells were carried out twice. The cells were finally reconstituted in normal saline solution and made upto 100 ml. During the test 0.1 molar solution of 2-Mercaptoethanol in normal saline solution was used as the diluent. This was prepared by making up 7.07 ml of 14.139 mol solution of 2-Mercaptoethanol to one litre with normal saline. The solution was stored at 4°C and prepared fresh every two to three

weeks. The serum dilution, addition of antigen, period and temperature of incubation and reading of the test were same as that of standard tube agglutination test.

5. **Ethylene diamine tetraacetate agglutination test (EAT)**

The test was performed based on the procedure described by Joint FAO/WHO (1986) Expert Committee on Brucellosis. A 10 m mol/l solution of EDTA disodium salt in phosphate buffered saline of pH 7.2 was used as diluent. The test procedure and reading of the results were carried out as in the case of standard tube agglutination test.

Results

RESULTS

During the present investigation, 1233 serum samples of bovines which consisted of 610 males and 623 females and 747 human serum samples comprising of 420 males and 327 females were screened for brucellosis by RBPT and STAT. The samples positive by both or either of these tests were further subjected to HIT, MET and EAT. The number of bovine and human serum samples which were positive for brucellosis are given in the table 1.

Table 1. Bovine and human serum samples positive for brucellosis by serological tests

Tests	Cattle		Human	
	Male N = 610	Female N=623	Male N=420	Female N=327
RBPT	41	47	6	5
STAT	37	44	4	4
HIT	32	34	3	3
MET	26	27	3	3
EAT	27	30	3	3

N= Number of serum samples screened

Among cattle, the seroprevalence of the disease was higher in females compared to males. Highest seropositivity was detected both in males and females by RBPT and least by MET. In human beings, females recorded a comparatively higher seroprevalence of disease than males. RBPT recorded the highest seroprevalence for the disease.

In bovines (slaughtered and farm fed), RBPT and STAT recorded a seroprevalence of 7.14 and 6.57 per cent respectively, while the per cent of

seropositivity revealed by HIT, MET and EAT were 5.35, 4.29 and 4.62 respectively. In slaughtered bovines, RBPT, STAT, HIT, MET and EAT recorded a seroprevalence of 7.27, 6.65, 4.63, 4.29 and 4.91 per cent respectively.

Among human serum samples, RBPT and STAT recorded a seroprevalence of 1.47 per cent and 1.07 respectively, while HIT, MET and EAT recorded the seropositivity as 0.8 per cent.

Slaughtered animals

The serum samples of 610 slaughtered male bovine were tested for brucellosis by serological tests. The number of serum samples positive for brucellosis are given in table 2.

Table 2. Slaughtered male bovine serum samples positive for brucellosis by serological tests

Tests	No. of samples		Overall per cent positive
	Tested	Positive	
RBPT	610	41	6.72
STAT	610	37	6.07
HIT	41	32	5.25
MET	41	26	4.26
EAT	41	27	4.43

Of the 26 MET positive samples, EAT and HIT recorded a suspicious reaction in two and one serum samples respectively. So only 23 (56.1 per cent) out of 41 RBPT positive serum samples were found positive for brucellosis by all the tests employed in this study. Among the screening test, RBPT detected a seroprevalence of 6.72 per cent in this study group. STAT could detect only 6.07 per cent samples as

positive for the disease. MET detected the least number of serum samples, 26 (4.26 per cent) as positive for brucellosis. The per cent of serum samples positive for the disease by HIT and EAT were 5.25 and 4.43 respectively.

Of the 41 samples positive by RBPT, 32 (78.1 per cent), 26 (63.4 per cent) and 27 (65.85 per cent) samples had an agglutination titre positive for the disease by HIT, MET and EAT respectively. STAT revealed that only 37 samples had an agglutination titre positive for the disease. Of this, 32 (86.5 per cent), 26 (70.3 per cent) and 27 (72.97 per cent) samples were found positive for the disease by HIT, MET and EAT respectively.

The agglutination titres revealed by STAT, HIT, MET and EAT are shown in table 3.

Table 3. Agglutination titres of slaughtered male bovine serum samples

Agglutination titre (iu/ml)	Serological tests			
	STAT	HIT	MET	EAT
20	26	5	8	5
40	15	4	7	9
80	17	19	20	16
160	10	8	5	7
320	6	4	-	3
640	3	-	-	-
1280	-	-	-	-
2560	-	-	-	-
5120	-	-	1	1
10240	-	1	-	-
20480	1	-	-	-
Total positive	37	32	26	27

Agglutination titre in STAT ranged from 20 iu/ml to 20480 iu/ml. One (2.7 per cent) of the serum samples positive for brucellosis had an agglutination titre of

20480 iu/ml. Of the positive serum sample 45.95 per cent, 27.02 per cent, 16.2 per cent and 8.11 per cent revealed an agglutination titre of 80 iu/ml, 160 iu/ml, 320 iu/ml and 640 iu/ml respectively.

Agglutination titres of the serum sample ranged between 20 iu/ml and 10240 iu/ml in HIT. The highest agglutination titre, 10240 iu/ml was revealed by 3.13 per cent of the HIT positive samples. An agglutination titre of 80 iu/ml was seen in 59.4 per cent of positive samples. Of the positive serum samples, an agglutination titre of 160 iu/ml and 320 iu/ml was revealed by 25 per cent and 12.5 per cent respectively.

In MET, agglutination titres of the serum sample ranged from 20 iu/ml to 5120 iu/ml. Of the positive samples, 3.85 per cent had an agglutination titre of 5120 iu/ml, 76.9 per cent had an agglutination titre of 80 iu/ml, and 19.2 per cent had a titre of 160 iu/ml.

Agglutination titres of the serum samples ranged from 20 iu/ml to 5120 iu/ml in EAT. Of the 27 positive serum samples 3.7 per cent had an agglutination titre of 5120 iu/ml while 59.3 per cent had a titre of 80 iu/ml. It was observed that 25.9 per cent and 11.1 per cent of positive samples had an agglutination titre of 160 iu/ml and 320 iu/ml respectively.

Result of serological tests on 518 serum samples of slaughtered females are shown in table 4.

Table 4. Seropositivity revealed by serum samples of slaughtered females for brucellosis

Tests	No. of samples		Overall per cent positive
	Tested	Positive	
RBPT	518	41	7.92
STAT	518	38	7.34
HIT	43	31	5.98
MET	43	26	5.02
EAT	43	28	5.4

Two RBPT negative serum samples recorded a positive reaction by STAT. So 43 serum samples were subjected to HIT, MET and EAT. It was found that two MET positive samples gave a suspicious reaction in EAT. So only 24 (58.5 per cent) out of 41 RBPT positive samples were found positive for brucellosis by all the tests. RBPT detected the highest number of samples as positive for brucellosis and recorded a seroprevalence of 7.92 per cent. MET detected only 5.02 per cent of serum samples as positive for the disease. The per cent of serum samples positive for brucellosis by STAT, HIT and EAT were 7.34, 5.98 and 5.4 respectively.

Among the 41 samples positive for the disease by RBPT, only 31 (75.6 per cent) were found positive by HIT. The number of samples positive by MET and EAT were 26 (63.4 per cent) and 28 (68.3 per cent) respectively. Out of the 38 samples positive for the disease by STAT, 31 (81.6 per cent), 26 (68.4 per cent) and 28 (73.7 per cent) samples recorded an agglutination titre positive for the disease by HIT, MET and EAT respectively.

The agglutination titres revealed by female bovine serum samples in STAT, HIT, MET and EAT are shown in table 5.

Table 5. Agglutination titre shown by serum samples of slaughtered female bovines by serological tests

Agglutination titre (iu/ml)	Serological tests			
	STAT	HIT	MET	EAT
20	21	7	9	7
40	19	5	8	8
80	15	17	20	16
160	11	11	4	9
320	8	2	1	2
640	3	1	1	1
1280	1	-	-	-
Total positive	38	31	26	28

In STAT, the agglutination titre of the serum sample ranged between 20 iu/ml and 1280 iu/ml. In HIT, MET and EAT, the agglutination titres of the test serum varied from 20 iu/ml to 640 iu/ml.

In STAT, only 2.6 per cent of the positive samples had an agglutination titre of 1280 iu/ml. The per cent of samples with an agglutination titre of 80 iu/ml, 160 iu/ml, 320 iu/ml and 640 iu/ml was 39.47, 28.9, 21.1 and 7.9 respectively.

In HIT, 3.23 per cent of the positive samples had an agglutination titre of 640 iu/ml. Of the 31 positive samples, 54.84 per cent, 35.48 per cent and 6.45 per cent recorded an agglutination titre of 80 iu/ml, 160 iu/ml and 320 iu/ml respectively.

The highest titre of 640 iu/ml in MET was recorded by 3.85 per cent of the positive samples. The percentage of samples with an agglutination titre of 80 iu/ml, 160 iu/ml and 320 iu/ml was 76.92, 15.38 and 3.85 respectively.

The highest agglutination titre observed in the EAT was 640 iu/ml. Of the EAT positive samples 3.57 per cent had the above titre. An agglutination titre of 80 iu/ml was revealed by 57.14 per cent of the samples. A titre of 160 iu/ml was found in 32.14 per cent of the positive samples, whereas 7.14 per cent of the samples had a titre of 320 iu/ml.

Statistical analysis of the data revealed that none of the test employed could detect a statistically significant difference in the seroprevalence of the disease between male and female slaughtered animals.

Farm fed animals

One hundred and five serum samples collected from farms were subjected to various serological tests. Out of this, 82 samples collected from the cows maintained at three regional research stations of Kerala Agricultural University were found to be free from the disease. The results of various serological tests on the remaining 23 serum samples collected from cows belonging to a private dairy farm are shown in table 6.

Table 6. Seropositivity revealed by farm fed cows with different tests for brucellosis

Tests	No. of samples		Over all per cent positive
	Tested	Positive	
RBPT	105	6	5.7
STAT	105	6	5.7
HIT	6	3	2.86
MET	6	1	0.95
EAT	6	2	1.9

Only one (16.67 per cent) out of six RBPT positive serum samples was found positive for the disease by all the tests. Both RBPT and STAT recorded the highest seroprevalence. These tests revealed that 5.7 per cent of the samples tested were positive for the disease. The per cent of samples positive for the disease by HIT, MET and EAT were 2.86, 0.95 and 1.9 respectively.

Out of the six samples each positive by RBPT and STAT, three (50 per cent) were found positive for the disease by HIT, while only one (16.7 per cent) had a positive titre by MET. Of the six samples positive by RBPT and STAT, two (33.3 per cent) samples had an agglutination titre positive for the disease by EAT.

The agglutination titres revealed by STAT, HIT, MET and EAT are shown in table 7.

Table 7. Agglutination titre shown by serum samples of farm fed cows by serological tests

Agglutination titre (iu/ml)	Serological tests			
	STAT	HIT	MET	EAT
20	3	2	5	3
40	2	1		1
80	5	2		1
160				
320				
640				
1280		1	1	1
2560	1			
Total positive	6	3	1	2

The highest agglutination titre was observed in STAT. An agglutination titre of 2560 iu/ml in STAT was recorded by 16.67 per cent of the positive samples, while 83.3 per cent recorded a titre of 80 iu/ml.

Agglutination titres ranged from 20 iu/ml to 1280 iu/ml in HIT, MET and EAT. In HIT, 33.3 per cent of the positive samples had a titre of 1280 iu/ml, whereas 66.7 per cent revealed a titre of 80 iu/ml.

MET could detect only one sample as positive for brucellosis. The sample had an agglutination titre of 1280 iu/ml.

Of the two EAT positive serum samples one had an agglutination titre of 1280 iu/ml and the other had a titre of 80 iu/ml.

Human serum samples

A total of 733 human sera collected from hospitals were subjected to serological tests to detect the presence of *Brucella* agglutinins. The results of 406 male human serum samples tested are given in table 8.

Table 8. Human male serum samples positive for brucellosis by serological tests

Tests	No. of samples		Overall per cent positive
	Tested	Positive	
RBPT	406	6	1.47
STAT	406	4	0.99
HIT	6	3	0.74
MET	6	3	0.74
EAT	6	3	0.74

Only three (50 per cent) out of six RBPT positive samples gave positive reaction for all the serological tests. Among the serological tests, RBPT and STAT detected a seropositivity of 1.47 per cent and 0.99 per cent respectively. The other serological tests viz., HIT, MET and EAT registered a seropositivity of 0.74 per cent for the disease.

Of the six RBPT positive samples, three (50 per cent) samples each were found positive for the disease by HIT, MET and EAT. Among the four samples positive by STAT, three (75 per cent) each had an agglutination titre positive for the disease by HIT, MET and EAT.

The agglutination titres of the serum samples in STAT, HIT, MET and EAT are shown in Table 9.

Table 9. Agglutination titres shown by human male serum samples

Agglutination titre (iu/ml)	Serological tests			
	STAT	HIT	MET	EAT
20	8	2	3	3
40	2	1	-	-
80	1	2	3	2
160	2	1	-	1
320	1		-	-
Total positive	4	3	3	3

The agglutination titre varied between 20 iu/ml and 320 iu/ml in STAT. Of the four positive samples, 25 per cent revealed a titre of 320 iu/ml while an agglutination titre of 80 iu/ml and 160 iu/ml was revealed by 25 per cent and 50 per cent of the positive samples respectively.

In HIT and EAT, agglutination titre ranged from 20 iu/ml to 160 iu/ml. In HIT, the highest agglutination titre of 160 iu/ml was recorded by 33.3 per cent of the positive samples, whereas 66.7 per cent registered an agglutination titre of 80 iu/ml.

In EAT, 66.7 per cent and 33.3 per cent of the positive samples recorded an agglutination titre of 80 iu/ml and 160 iu/ml respectively.

In MET, agglutination titre ranged from 20 iu/ml to 80 iu/ml. All the three positive samples recorded an agglutination titre of 80 iu/ml by this test.

Three hundred and twenty seven serum samples of females were tested for brucellosis by serological tests. The results of these tests are given in table 10.

Table 10. Seropositivity revealed by human female serum samples by serological tests for brucellosis

Tests	No. of samples		Overall per cent positive
	Tested	Positive	
RBPT	327	5	1.53
STAT	327	4	1.22
HIT	5	3	0.92
MET	5	3	0.92
EAT	5	3	0.92

All the tests gave a positive reaction in three (60 per cent) of the RBPT positive serum samples. An overall seropositivity of 1.53 per cent and 1.22 per cent was recorded for brucellosis by RBPT and STAT respectively. A seropositivity of 0.92 per cent was recorded by HIT, MET and EAT.

In RBPT, only five had an agglutination titre positive for the disease. Of these, three (60 per cent) were found positive for the disease by HIT, MET and EAT. Out of the four samples positive for the disease by STAT, three (75 per cent) samples each had an agglutination titre positive for the disease by HIT, MET and EAT.

The agglutination titres of human female serum samples revealed by STAT, HIT, MET and EAT are given in table 11.

Table 11. Agglutination titres of human female serum samples

Agglutination titre (iu/ml)	Serological tests			
	STAT	HIT	MET	EAT
20	11	2	2	2
40	1	-	-	-
80	1	1	2	1
160	1	1	1	1
320	1	1		1
640	1			-
Total positive	4	3	3	3

STAT recorded the highest agglutination titre of 640 iu/ml in 25 per cent of the positive samples. Twenty five per cent each of the samples had an agglutination titre of 80 iu/ml, 160 iu/ml, and 320 iu/ml.

The highest agglutination titre recorded in HIT was 320 iu/ml and was seen in 33.3 per cent of the HIT positive samples. The test recorded an agglutination titre of 80 iu/ml and 160 iu/ml in each 33.3 per cent of the positive samples.

In MET, 66.7 per cent of the positive samples recorded an agglutination titre of 80 iu/ml while, 33.3 per cent revealed a titre of 160 iu/ml.

In EAT, an agglutination titre of 80, 160 and 320 iu/ml was revealed by each 33.3 per cent of the positive samples.

Statistical analysis of the data revealed that none of the tests employed could detect a statistically significant difference in the seroprevalence of disease between males and females.

Of the 14 serum samples collected from veterinary surgeons, animal attendants and slaughter house workers, none was found positive for brucellosis by RBPT and STAT.

Discussion

DISCUSSION

During the present investigation, 1233 serum samples of bovines and 747 samples of human were screened by RBPT and STAT to detect the presence of *Brucella* agglutinins. The samples which had shown *Brucella* agglutinins either by RBPT or STAT or by both were subjected to HIT, MET and EAT. Of the serological tests, RBPT could detect *Brucella* agglutinins in 7.14 per cent (88) of the bovine serum samples, where as MET recorded only 4.29 per cent (53) of the samples as positive for brucellosis. Only 3.89 per cent (48) of the bovine serum samples were found positive for brucellosis by all the serological tests. Among human sera, RBPT detected 1.47 per cent (11) samples as positive for the disease. HIT, EAT and MET identified 0.8 per cent (six) samples each as positive for brucellosis. Only 0.8 per cent (six) of the samples were found positive for the disease by all the tests. None of the serological tests employed could detect a statistically significant difference in the seroprevalence of disease between males and females in both animals and man.

Slaughtered animals serum samples

During the investigation, RBPT detected an overall seroprevalence of 7.27 per cent. An almost similar observation was made by Kenar and Güler (1994), where a seroprevalence of 7.5 per cent was recorded by RBPT.

STAT could detect an overall seroprevalence of 6.65 per cent, which is much lower than that of the 14.85 per cent recorded by Chackraborthy and Kwatra (1980). However the observation of the study is much higher than that of 5.7 per cent

observed by Kenar and Güler (1994) and 1.25 per cent reported by Ghani *et al.* (1998).

Heat inactivation test of the serum samples revealed an overall seroprevalence of 4.63 per cent. The findings of the study was much higher compared to 0.83 per cent recorded by Ghani *et al.* (1998).

The per cent of seropositivity observed by EAT and MET was 4.91 and 4.29 respectively. This result could not be compared due to paucity of literature.

Farm fed animals

In the farm fed animals, RBPT recorded an overall seroprevalence of 5.7 per cent which was much lower as compared to 21.74 per cent reported by Purbey and Sane (1978) and 7.2 per cent reported by Suresh *et al.* (1993). However Bermudez and Barriola (1983) recorded an almost similar observation where a seroprevalence of 5.5 per cent was observed.

STAT revealed an overall seroprevalence of 5.7 per cent. The findings of the study were much lower than that of the 20.5 per cent reported by Chatterjee *et al.* (1984) and 8.7 per cent observed by Sharma *et al.* (1984). However the present observation was almost similar to that reported by Halder *et al.* (1979) who recorded an overall seroprevalence of 4.99 per cent. The seroprevalence observed in the study was higher as compared to 4.33 per cent reported by Oberoi and Kwatra (1982), 0.83 per cent reported by Ghani *et al* (1998) and 0.82 per cent reported by Babu *et al.* (1985).

A seropositivity of 2.8 per cent was recorded by HIT. This observation was very low as compared to 16.83 per cent reported by Kalimuddin *et al.* (1990).

A seroprevalence of 0.95 per cent was recorded by MET which was much lower than the 12.87 per cent reported by Kalimuddin *et al.* (1990).

The per cent of seroprevalence observed in EAT was 1.9 but Izigur *et al.* (1992) recorded a seroprevalence of 13.8 per cent by EAT which was very high as compared to present observation.

The prevalence of brucellosis was found to be more in slaughtered animals as compared to farm fed animals. Usually animals showing ill health and poor reproductive performance are send for slaughter. Such ill health and poor reproductive performance in animals may also be due to brucellosis. These may be the reason for higher rate of occurrence of the disease in slaughtered animals.

Human beings

In the present study 747 human serum samples were subjected to RBPT and revealed a seropositivity of 1.47 per cent, which was almost similar to 1.7 per cent reported by Zeftawi *et al.* (1986).

The seropositivity observed in STAT was 1.07 per cent. A similar observation was also made by Umapathy *et al* (1984), where they recorded the seroprevalence as 1.2 per cent. However the seroprevalence observed in this study was much higher as compared to seroprevalence recorded by Sharma *et al.* (1984) who had observed a seroprevalence of 0.56 per cent. In comparison with the observation of the present study, a higher seroprevalence of 3.9 per cent, 3.6 per cent and 2.97 per cent was

reported by Joshi and Prakash (1971), Singh (1982) and Kapoor *et al.* (1985) respectively.

An overall seroprevalence of 0.8 per cent was recorded by HIT, which is almost similar to that of 0.91 per cent reported by Kalimuddin *et al.* (1990).

In MET, the percentage of seroprevalence observed was 0.8 which is almost similar to 0.91 per cent recorded by Kalimuddin *et al.* (1990).

EAT revealed an over all seropositivity of 0.8 per cent for brucellosis. This result could not be compared due to lack of literature.

In the present study, a higher prevalence rate was observed in females (1.22 per cent) as compared to males (0.99 per cent). Kapoor *et al.* (1985) also recorded a higher prevalence of brucellosis among females (4.4 per cent) compared to males (1.78 per cent). Contrary to the observation of the present study, Stephen *et al.* (1978) recorded a higher prevalence of brucellosis in men (12.95 per cent) than in women (11.73 per cent). The higher prevalence rate of the disease among females might be due to the fact that chances of exposure to infectious agent is more for them due to the frequent involvement in various animal husbandry activities.

In the study, the veterinary surgeons, animal attendants and slaughter house workers were found free from brucellosis but a high prevalence 20 per cent of infection in working staff was reported by Savalgi *et al.* (1987). Kalimuddin *et al.* (1990) recorded a seroprevalence of 0.11 per cent in animal attendants. For the validation of the present finding there is a need to screen a larger number of samples obtained from occupational group.

RBPT recorded a seropositivity of 7.14 per cent for brucellosis in bovines. Weidmann (1991) opined that the test is relatively sensitive and gives few false negative reactions, but may produce false positive reaction in vaccinated animals. Ability of the test to respond to an early infection may be the reason for high sensitivity (Morgan *et al.*, 1969).

A seroprevalence of 6.57 per cent was recorded by STAT. Even though *Brucella* antigen used in this test react with all antibodies against *Brucella* organisms such as I_g A, I_g M, I_gG and I_gG_2 (Joint FAO/WHO, 1986) the test may give negative or doubtful reaction in early stages [Morgan (1967) and Weidmann (1991)].

RBPT was negative in two STAT positive cases with an agglutination titre of 80 iu/ml. Morgan *et al.* (1969) also recorded a negative reaction in RBPT where the titre of the serum was 100-160 iu/ml. This may be due to denaturation of acid labile nonspecific agglutinins by low pH of Rose Bengal antigen.

HIT recorded 5.35 per cent of the bovine samples as positive for brucellosis. Seventy five per cent of the RBPT positive and 81.5 per cent of the STAT positive samples gave a positive reaction in HIT. The test recorded a negative reaction in 5.3 per cent (two) of the 37 STAT positive samples with an agglutination titre of 80 iu/ml. Twenty one STAT positive samples had a titre of 160 iu/ml. Of this, 71.4 per cnt (15) samples had a titre of 80 iu/ml in HIT. In STAT, 14 serum samples revealed a titre of 320 iu/ml but 92.9 per cent (13) of these samples had a titre of 160 iu/ml in HIT. In HIT, the titre value was lowered to 50 per cent in all samples having a titre of 640 iu/ml, 1280 iu/ml, 2560 iu/ml and 20480 iu/ml but in one case, STAT titre of 640 iu/ml was reduced to 160 iu/ml. Reduction in titre by heat inactivation could be

attributed to the alteration or modification or inactivation or destruction of the heat labile nonspecific agglutinins present in the bovine sera (Corbel, 1985). According to him the heat labile agglutinating activity is associated with poorly defined macro and microglobulin serum components.

EAT revealed an overall seroprevalence of 4.62 per cent for brucellosis. The test detected 64.8 per cent of the RBPT positive and 70.4 per cent of the STAT positive samples as positive for the disease. Thirty seven STAT positive samples had an agglutination titre of 80 iu/ml but six (16.2 per cent) were found negative for the disease in EAT. In STAT, 21 samples had a titre of 160 iu/ml. Thirteen (61.9 per cent) of these samples revealed a titre of 80 ii/ml in EAT. Fourteen samples showed a titre of 320 iu/ml in STAT. When these samples were subjected to EAT, 50 per cent (seven) had a titre of 160 iu/ml, while 42.9 per cent (six) had a titre of 80 iu/ml. In STAT one sample had an agglutination titre of 1280 iu/ml and another had a titre of 2560 iu/ml. When these samples were subjected to EAT, their titre was found to be reduced by half. One serum sample with a STAT titre of 20480 iu/ml recorded a titre of 5120 iu/ml in EAT. The reduction in titre in EAT may be due to the fact that, sodium salt of EDTA used in EAT blocks the attachment between *Brucella* cell antigen and EDTA labile nonspecific antigen via the Fc region of their 7s sub units (Joint FAO/WHO, 1986).

MET detected a seroprevalence of 4.29 per cent for brucellosis. The test recorded a positive reaction in 60.2 per cent of RBPT and 65.4 per cent of STAT positive cases. Of the 37 STAT positive samples with an agglutination titre of 80 iu/ml, 35.1 per cent (13) recorded a negative reaction in MET. An agglutination titre

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of 80 iu/ml was recorded in 90.5 per cent (19) of the 21 STAT positive samples with a titre of 160 iu/ml. The agglutination titre was reduced to half in 28.6 per cent (four) of the 14 samples with a STAT titre of 320 iu/ml and to one fourth in 71.4 per cent (10) samples. Out of six STAT positive samples with a titre of 640 iu/ml, the titre was reduced to 320 iu/ml in 16.7 per cent (one) samples and to 160 iu/ml in 66.3 per cent (five) samples. One serum sample each with a STAT titre of 1280 iu/ml, 2560 iu/ml and 20480 iu/ml registered a titre of 640 iu/ml 1280 iu/ml and 5120 iu/ml in MET.

The reduction in agglutination titre of the samples in MET could be due to the treatment of serum with 2-Mercaptoethanol, a sulphhydryl reducing agent, which dissociates I_gM pentamer and reduces its agglutinating activity without affecting that of I_gG isotypes. Anderson *et al.* (1964) found that mercaptoethanol susceptible antibodies are macroglobulins (I_gM). The test gives fewer false positive reaction compared to STAT and RBPT. This may be the reason for the detection of least number of samples as positive for disease by the test.

Among human serum samples, RBPT and STAT recorded a seroprevalence of 1.47 per cent and 1.07 per cent respectively.

Both HIT and EAT recorded the overall seroprevalence as 0.8 per cent. Both the tests recorded an agglutination titre positive for brucellosis when corresponding STAT titre was 160 iu/ml or more.

MET recorded an overall prevalence of 0.8 per cent for brucellosis. Serum samples with STAT titres of 640 iu/ml, recorded agglutination titres of 160 iu/ml in

MET, while those with STAT titres of 320 iu/ml and 160 iu/ml, reduced their titre to 80 iu/ml by MET.

Among human serum samples, HIT, MET and EAT detected the same samples as positive for brucellosis which indicate an active infection in these individuals, becausein proven cases of brucellosis the predominant and occasionally the only antibody present in the serum is I_gG.

Tentative inference made in the present study is, that in general, when STAT titre was 160 iu/ml or above, the other serological tests employed, also registered a positive reaction. The test showing the highest per cent of samples as positive for the disease was RBPT, followed by STAT, HIT, EAT and MET.

Morgan *et al.* (1969) and Alton *et al.* (1975a) observed that RBPT is better than STAT as a screening test. Stemshorn *et al.* (1985) and Barbuddhe *et al.* (1994) recorded higher sensitivity for RBPT compared to STAT. The observation of the present study is in agreement with the above finding.

The observation of a negative reaction in HIT or MET in a number of serum samples positive by STAT in this study is in agreement with the finding of Kalimuddin *et al.* (1990), Ghani (1998) and Ghani *et al.* (1994).

Macmillan and Cockrem (1985) recorded lesser number of positive cases in EAT compared to STAT, which is in agreement with the present study.

Serum samples of slaughtered bovines revealed that of the 88 RBPT positive samples, 66 (75 per cent), 53 (60.2 per cent) and 57 (64.8 per cent) samples had positive agglutination titre for brucellosis by HIT, MET and EAT respectively. Of

the 81 STAT positive serum samples, 66 (81.5 per cent), 53 (65.4 per cent) and 57 (70.4 per cent) samples were found positive for the disease by HIT, MET and EAT respectively. In the case of farm fed animals, out of six samples each positive by RBPT and STAT, three (50 per cent), one (16.7 per cent) and two (33.3 per cent) registered a positive reaction by HIT, MET and EAT respectively. In the case of human beings, of the 11 samples positive by RBPT, six (54.5 per cent) each had an agglutination titre positive for the disease by HIT, MET and EAT respectively. STAT revealed that eight human serum samples had an agglutination titre positive for the disease. Of this, six (75 per cent) each had an agglutination titre positive for the disease by HIT, MET and EAT respectively. It was observed that, of the RBPT positive and STAT positive cases, HIT recorded maximum number of positive cases followed by EAT and MET. Considering sensitivity, specificity and ease in performing the test, it is suggested that a combination of RBPT and EAT can be used in the diagnosis of bovine brucellosis as compared to RBPT and STAT.

According to Faine (1982) the presence of three per cent or more carrier animals in the population has to be viewed seriously from epidemological point. The result of screening test revealed a seroprevalence of 7.1 per cent for the disease.

Results of the above investigation and a review of literature clearly reveal that brucellosis is an endemic disease in India. Although it is an occupational disease, reports of human cases are comparatively less, probably due to the unwillingness of the physician to consider brucellosis as an alternative diagnosis in many illnesses. Therefore a proper liaison between medical and veterinary professionals is an essential prerequisite for the diagnosis, treatment and control of the disease.

Lack of co-operation on the part of the farmers is a major stumbling block in the control of brucellosis in animals. The prospect of economic losses through the elimination of infected animals and the inconvenience caused by repeated testing has contributed to this scenario. Enlightening farmers about the long term advantages of control and providing adequate compensation may help in abolishing the disinclination of farmers towards adopting control measures.

Summary

SUMMARY

A serological survey was carried out to assess the presence of *Brucella* agglutinins in the serum of both animals and man. During the study, serum samples were collected from slaughtered bovines (1128), cows belonging to organised farms (105), human beings attending private clinics (733), veterinary surgeons (10), animal attendants (two) and slaughter house workers (two). Among slaughtered bovines 610 were males and 518 were females and of the human serum samples, 406 were from males and 327 were from females. All slaughtered bovine serum samples were collected from Municipal Slaughter House, Kuriachira, Trichur. Cows belonging to organised farms consisted of 10 from Regional Agricultural Research Station, Kumarakom, 59 from Livestock Research Station, Thiruvizhamkunnu, 13 from Rice Research Station, Pattambi, and 23 from a private dairy farm at Kannur.

All the serum samples were screened by Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT). The samples which showed a positive reaction, either by RBPT or STAT or both were subjected to Heat Inactivation Test (HIT), EDTA Agglutination Test (EAT) and 2-Mercaptoethanol Test (MET).

RBPT detected 41 (6.72 per cent) positive cases in slaughtered males of which 32 (78.1 per cent), 26 (63.4 per cent) and 27 (65.85 per cent) samples had an agglutination titre positive for brucellosis by HIT, MET and EAT respectively. STAT recorded 37 (6.07 per cent) positive cases of which 32 (86.5 per cent), 26 (70.3 per cent) and 27 (72.97 per cent) were found positive for the disease by HIT, MET and EAT respectively. An overall seroprevalence of 5.25 per cent, 4.26 per cent and 4.43 per cent was recorded in HIT, MET and EAT respectively.

Among slaughtered females 41 (7.92 per cent) was found positive for brucellosis by RBPT, of which, 31 (75.6 per cent), 26 (63.4 per cent) and 28 (68.3 per cent) samples had an agglutination titre positive for the disease by HIT, MET and EAT respectively. STAT recorded 38 (7.34 per cent) positive cases of which 31 (81.6 per cent), 26 (68.4 per cent) and 28 (73.7 per cent) were found positive for the disease by HIT, MET and EAT respectively. The overall seroprevalence recorded by HIT, MET and EAT was 5.98 per cent, 5.02and 5.4 per cent respectively

None of the serological tests employed could detect a significant difference in the seroprevalence of disease between male and female slaughtered animals.

Of the 105 samples from organized farms, six (5.7 per cent) was found positive for brucellosis by both RBPT and STAT. Out of these six samples, three (50 per cent), one (16.7 per cent) and two (33.3 per cent) were found positive for the disease by HIT, MET and EAT respectively. The overall seroprevalence recorded by HIT, MET and EAT was 2.86 per cent, 0.95 per cent and 1.9 per cent respectively.

Among bovine serum samples RBPT detected *Brucella* agglutinins in a maximum number of 88 (7.14 per cent) serum samples. Followed by this, STAT, HIT, EAT and MET recorded a positive reaction in 81 (6.57 per cent), 66 (5.35 per cent), 57 (4.62 per cent) and 53 (4.29 per cent) samples respectively. Only 48 (3.89 per cent) serum samples were found positive for brucellosis by all the above tests. Considering sensitivity, specificity and ease in performing the test, it is suggested that a combination of RBPT and EAT can be used in the diagnosis of bovine brucellosis.

Of the male human serum samples, six (1.47 per cent) revealed a positive reaction in RBPT while STAT could detect only four (0.99 per cent) samples as positive. HIT, MET and EAT detected three (0.74 per cent) samples each as positive for the disease. Of the six RBPT positive samples, three (50 per cent) samples each were found positive for the disease by HIT, MET and EAT. Among the four samples positive by STAT, three (75 per cent) each had agglutination titre positive for the disease by HIT, MET and EAT, respectively.

Among the samples from human females, five (1.53 per cent) had an agglutination positive for brucellosis by RBPT. Only four (1.22 per cent) samples recorded an agglutination titre positive for the disease by STAT. Three (0.92 per cent) samples each revealed an agglutination titre positive for the disease by HIT, MET and EAT. Out of the four samples positive for the disease by STAT, three (75 per cent) samples each had an agglutination titre positive for the disease by HIT, MET and EAT respectively. In RBPT, only five had agglutination titre positive for the disease. Of these three (60 per cent) samples each were found positive for the disease by HIT, MET and EAT respectively. Serological tests employed in the present study could not detect a statistically significant difference in the seroprevalence of disease between males and females. None of the serum samples collected from veterinary surgeons, animal attendants and slaughter house workers were found positive for brucellosis.

The study clearly establishs that *Brucella* infection is prevalent in man and animals. There is a need for establishing effective disease control measures both in animals and human beings and to have a closer liaison between veterinary and medical professionals.

References

REFERENCES

Allan, G.S., Chappel, R.J., Willianson, P. and Mc Naught, D.J. (1976). A quantitative comparison of the sensitivity of the serological tests for bovine brucellosis to different antibody classes. *J. Hyg. Camb.* **76**: 287-298.

Alton, G.G. and Jones, L.M. (1967). Laboratory techniques in brucellosis. World Health Organisation. Monograph series. No.55, pp. 43-52.

Alton, G.G., Maw, J., Rogerson, B.A. and Mc Pherson, G.G. (1975a). The serological diagnosis of bovine brucellosis: An evaluation of complement fixation, serum agglutination and Rose Bengal tests. *Aust. Vet. J.* **51** (2): 57-63.

Alton, G.G., Jones, L.M. and Pietz, D.E. (1975b). Laboratory techniques in brucellosis. 2nd ed. World Heath Organisation. Monograph series. No. 55, pp. 112-113.

Amerault, T.E., Manthei, C.A., Goode, E.R. and Lambert, G. (1961). A heat inactivation test for differentiating specific and nonspecific agglutination reaction for bovine brucellosis. *Am. J. Vet. Res.* **22** (5): 564-569.

Anderson, R.K., Jenness, R., Brumfield, H.P. and Gough, P. (1964). *Brucella* agglutinating antibodies: Relation of mercaptoethanol stability to complement fixation. *Science.* **143** (3612): 1334-1345.

Babu, Y.H., Sudhakar, R., Srinivas, C.S. and Khan, M.A. (1985). Seroprevalence of brucellosis in pure breeds in organised farms. *Indian Vet. J.* **62** (2) : 175-176.

Baby, K. and Paily, E.P. (1979). Seroepizootology of brucellosis in buffaloes in Kerala. *Kerala J. Vet. Sci.* **10** (2): 187-192.

Bachh, A.S., Nowsheri, M.A., Rashid, A., Raina, A.K. and Wani, S. (1988). Seroprevelence of brucellosis in exotic cattle in Kashmir. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **9** (1): 23-27.

Bandey, S.D., Parvez, S. and Bandey, S. (1989). Sero-epidemiological studies on brucellosis in exotic sheep in Kashmir valley. *Indian. J. Anim. Sci.* **59** (2): 213-215.

Bang, B. (1897). The etiology of contagious abortion. Cited in Brucellosis in man and animals. Revised Edition. Commonwealth Fund, New York, 1943. pp.1.

Barbuddhe, S.B., Yadava, V.K. and Singh, D.K. (1994). Comparison of DOT-ELISA with conventional serological tests for diagnosis of ovine brucellosis. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **15** (1 & 2): 1-4.

Barman, M.N., Ahmed, K., Saikia, G.K. and Boro, B.R. (1989). Seroprevalence of brucellosis in organised cattle farms of Assam (India). *Indian J. Anim. Hlth.* **28**(2): 99-102.

Beh, K.J. (1974). Quantitative distribution of *Brucella* antibody amongst immunoglobulin classes in vaccinated and infected cattle. *Res. Vet. Sci.* **17**: 1-4.

Bermudez, J. and Barriola, J. (1983). Comparison of the result of serological test for bovine brucellosis in Uruguay. *Veterinaria Uruguay.* **19** (84): 29-35.

Bruce, D. (1887). Notes on the discovery of a micro-organism in Maltafever. Practitioner. 39 pp. 161. Cited in Brucellosis in man and animals. Revised Edition. The Commonwealth Fund, New York, 1943. pp.1.

Chackraborthy, A. and Kwatra, M.S. (1980). Serological study on bovine brucellosis and its histopathology in reproductive organs of non pregnant slaughtered cows. *Indian J. Path. Microbiol.* **23** (2): 125-128.

Chandramohan, C.P., Ramadass, P. and Raghavan, N. (1992). Studies on bovine brucellosis in an endemic area. *Indian Vet. J.* **69** (7): 581-583.

Chatterjee, A., De, B.N., Bidyanta, J., Chakraborthy, M., Mondal, P. and Sen, G.P. (1984). Sero-epidemiological studies on bovine brucellosis in organised herds in West Bengal. *Indian J. Anim. Sci.* **55** (4): 249-252.

Cho, H.J. and Ingram, D.G. (1972). Mechanism of prozone formation in agglutination reaction. *Can. J. Microbiol.* **18**: 449-456.

Choudhary, S.P., Singh, S.N., Narayan, K.G. and Kalimuddin. Md. (1983). Seroprevalence of porcine brucellosis in Bihar. *Indian Vet. J.* **60** (5): 336-338.

Coombs, R.R.A., Mourant, A.E. and Race, R.R. (1945). *Brit. J. Exp. Path.* **26**: 255. Cited in Infectious diseases of animals, Vol. I. Butterworths Scientific Publications, London, 1959. pp.68.

Corbel, M.J. (1985). Recent advances in the study of *Brucella* antigens and their serological cross reactions. *Vet. Bull.* **55** (12): 927-942.

Das, A.M. and Mukherjee, S.M. (1986). Use of supplementary serological tests in the diagnosis of porcine brucellosis. *Indian Vet. J.* **63**(12): 1054-1056.

Faine, S. (1982). Guidelines for the control of leptospirosis. World Health Organisation. Geneva. pp.88.

Fleischauer, G. (1937). *Berl. Tierarztl. Wschr.* **53**: pp.527. Cited in Infectious diseases of animals. Vol. I. Butterworths Scientific Publications, London, 1959. pp.65.

Ghani, M., Zeb, A., Siraj, M. and Naeem, M. (1994). Comparative studies on standard plate, standard tube agglutination, Rivanol and 2-Mercaptoethanol tests in the diagnosis of brucellosis. *J. Vet. Anim. Sci.* **25** (2): 115-119.

Ghani, M., Zeb, A., Siraj, M. and Naeem, M. (1998). Sero-incidence of bovine brucellosis in Peshwar district of Pakistan. *Indian. J. Anim. Sci.* **68** (5): 457.

Ghosh, S.S. (1989). Incidence of brucellosis in organised pig farms in north eastern hill region. *Indian Vet. Med. J.* **13** (1): 52-53.

Ghosh, S.S. and Verma, P.C. (1985). Incidence of brucellosis in sheep and goat in Nagaland. *Indian. Vet. J.* **62**(5): 339-340.

Grinsted, F. (1909). The agglutination test for the diagnosis of infectious abortion in cattle. Maanedsskrift for Dyrlaeger. **21**: pp.395. Cited in Brucellosis in man and animals. Revised edition. The Commonwealth Fund, NewYork, 1983. pp.214.

Halder, S.K., Choudhary, M.R., Dutta, S. and Sen, G.P. (1979). Bovine brucellosis in relation to cross breeding in organised farm. *Indian Vet. J.* **56** (1): 76-78.

Huddleson, I.F. (1920). *Tech. Bull. Mich. Agric. Exp. Sta.* No. **49**. Cited in Infectious diseases of animals. Vol. I. Butterworths Scientific Publications, London, 1959. pp.66.

Izigur, M., Akay, O., Arda, M. and Erdeger, J. (1992). Use of agglutination tests at 65°C and the EDTA for diagnosis of bovine brucellosis. *Veterinary Fakultesi Dergisi*. **39** (1-2): 191-200.

Joint FAO/WHO (1971). Expert Committee on Brucellosis. Report on fifth session.

Joint FAO/WHO (1986). Expert Committee on Brucellosis. Report on sixth session. *Tech. Rep. Series*. 740.

Joshi, D.V. and Prakash, O. (1971). Brucellosis in man and animals. *Indian J. Path. Bact.* **14** (2): 96-101.

Kalimuddin, Md and Choudhary, S.P. (1988). Seroprevalence of brucellosis in pigs and bacon factory workers. *Indian Vet. Med. J.* **12** (9): 190-192.

Kalimuddin, Md., Arora, A.K. and Sinha, A.K. (1990). Evaluation of serodiagnostic methods for brucellosis in dairy cattle and their attendants. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **11** (3 & 4): 130-133.

Kapoor, P.K. and Rao, K.L. (1984). *Brucella* agglutinins in human beings rearing goats positive for brucellosis. *Indian. J. Anim. Sci.* **54** (6): 584-585.

Kapoor, P.K., Sharma, S.N. and Rao, K.L. (1985). Seroprevalence of brucellosis in goats and human beings in Bikaner (Rajasthan). *Indian. J. Comp. Mirobiol. Immunol. Infect. Dis.* **6** (2 & 3): 96-101.

Kataria, R.S. and Verma, G.P. (1969). Bovine brucellosis in Madhya Pradesh (A serological study). *Indian Vet. J.* **46** (1):13-16.

Katochi, R.C., Joshi, V.B., Sharma, M., Batta, M.K. and Nagal, K.B. (1996). Seroprevalence of *Brucella ovis*, *Brucella melitensis* and *Chlamydia psittaci* in rams. *Indian J. Anim. Sci.* **66** (11): 1030-1031.

Kenar, B. and Guler, L. (1994). Brucellosis antibodies in serum samples taken from cattle slaughtered at the EBK abattoir in Konya. Hayvan Asilardotless Kontrol Merkezi Mudurlugu Dergisi. **18** (32): 61-66.

Kerr, W.R. (1955). *Brit. Vet. J.* **111**: 169. Quoted in Vet. Rec. **70**: 504.

Koshi, G. and Myers, R.M. (1967). Report on twenty bacteriologically diagnosed cases of brucellosis encountered in routine laboratory service over a period of ten years. *Indian J. Med. Sci.* **21**: 89-98.

Koshi, G., Sulochana, J., Pulimood, B.M., Cherian, A.M., John, L., Swami, S.K. and Mathai, K.V.C. (1988). Protean manifestations of brucellosis encountered at Vellore. *Indian J. Med. Res.* **88** (10): 322-329.

Kulkarni, S.B., Knot, J.B., Sherikar, A.A. and Joshi, U.M. (1991). Detection of brucella antibodies in bovines by ELISA and its comparison with the standard agglutination and Rose Bengal plate tests. *Indian. Vet. Med. J.* **15** (12): 256-260.

Kulshreshtha, R.C., Kalra, D.S., Arora, R.G. and Gupta, R.K.P. (1973a). Prevalence of brucellosis among cattle and buffaloes in Haryana state. *Indian. Vet. J.* **50** (3): 228-230.

Kulshreshtha, R.C., Atal, P.R. and Wahi, P.N. (1973b). A study on serological tests for the diagnosis of human and bovine brucellosis. *Indian J. Med. Res.* **61** (11): 1471-1477.

Kulshreshtha, R.C., Kalra, D.S. and Kapur, M.P. (1978). Seropidemiological studies on brucellosis in animals and man in Haryana state. *Indian. Vet. J.* **55** (1): 1-3.

Kumar, P., Singh, D.K. and Barbuddhe, S.B. (1997). Serological evidence of brucellosis in sheep and goats. *Indian J. Anim. Sci.* **67** (3): 180-182.

Kumar, R. and Rao, C.V.N. (1980). Porcine brucellosis in Tamil Nadu. *Indian Vet. J.* **57** (1): 1-4.

Kumar, R., Quader, S.A. and Arunachalam, T.N. (1976). Brucellosis in goats. *Indian Vet. J.* **53** (7): 493-494.

Kumar, S., Kulshreshtha, R.C. and Chandiramani, N.K. (1984). Comparison of disulphide bond reduction and standard tube agglutination tests for the diagnosis of brucellosis. *Indian J. Anim. Sci.* **54** (2): 208-210.

Lapraik, R.D., Brown. D.D., Mann, H. and Brand, T. (1975). Brucellosis: a study of five calves from reactor dams. *Vet. Rec.* **97**: 52-54.

Larson, W.P. (1912). The complement fixation reaction in the diagnosis of contagious abortion of cattle. *J. Infectious. Dis.* **10**: 175. Cited in Brucellosis in man and animals. Revised Edition. The Commonwealth Fund, NewYork, 1943. pp. 214.

Lateef, M.A., Babu, Y.H., Rao, K.S., Rao, P.V.R. and Alam, S.H. (1989). Seroprevalence of porcine brucellosis among country pigs in Andhra Pradesh. *Indian Vet. J.* **66** (1): 86-87.

Macmillan, A.P. and Cockrem, D.S. (1985). Reduction of non specific reactions to the *Brucella abortus* serum agglutination test by the addition of EDTA. *Res. in Vet. Sci.* **38**: 288-91.

Mahakur, A.C. and Panda, G.K. (1972). Incidence of brucellosis in and around Burla. *Indian. J. Med. Sci.* **26** (12): 826-830.

Maiti, C.R., Ghosh, S.S., Roy, D.J., Sen, G.P. and Sharma, R.H. (1980). Survey of bovine brucellosis in cattle breeding farms in Nagaland. *Indian J. Anim. Hlth.* **19:** 83-86.

Manickam, R. and Mohan, M. (1987). Epidemiological studies on *Brucella abortus* infection in milch cattle. *Indian Vet. J.* **64** (7): 546-549.

Marston, J.A. (1863). Report on fever (Malta). Army Medical Report. **3**: 486. Cited in Infections diseases of animals. Vol. I. Butterworths Scientific Publications, London, 1959. pp.53.

Masoumi, J.P., Sheikh, M.A., Ahmad, R., Naeem, M., Ahmed, M. and Hussain, I (1992). Seroprevalence of brucellosis in sheep, goats and man in Lahore area. *Indian J. Dairy. Sci.* **45** (6): 298-299.

Mathai, E., Singhal, A., Verghese, S., Lima, D.D., Mathai, D., Ganesh, A., Thomas, K. and Moses, P. (1996). Evaluation of ELISA for the diagnosis of brucellosis. *Indian J. Med. Res.* **103** (6): 323-324.

Mathur, K., Tomer, V.N.S. and Purohit, A.K. (1991). Study on the seroprevalence of toxoplasmosis, Q fever and brucellosis in cattle with history of abortion and associated ailments. *Indian. J. Anim. Hlth.* **30**(2): 138-140.

Mathur, K.N., Bhargava, S.C. and Khanna, V.K. (1979). Seroprevalence of animal brucellosis in and around Bassi (Jaipur), Rajasthan. *Indian J. Microbiol.* **19** (3): 107-109.

Meyer, K.F. and Shaw, E.B. (1920). *J. Infect. Dis.* **51**: 72. Cited in Infections diseases of animals. Vol. I, Butterworths Scientific Publications, London, 1959. pp.54.

Miller, J.K., Nettleton, P.F. and Robertson, A.M. (1973). Evaluation of a two-channel automated system for the sero-diagnosis of brucellosis. *Vet. Rec.* **92**: 492-496.

Morgan, W.J.B. (1967). The serological diagnosis of bovine brucellosis. *Vet. Rec.* **80**: 612-621.

Morgan, W.J.B. (1969). Brucellosis in animals: diagnosis and control. *Proc. R. Soc. Med.* **62**: 1050-1052.

Morgan, W.J.B., Mackinnon, O.J., and Cullen, G.A. (1969). The Rose Bengal plate agglutination test in the diagnosis of brucellosis. *Vet. Rec.* **85**: 636-641.

Nag, N.C., Kanjilal, B.C. and Ray, J.P. (1977). Brucellosis in cows and buffaloes in West Bengal. *Indian. J. Anim. Hlth.* **16**(2): 89-90.

Nicoletti, P. (1969). Further evaluation of serologic test procedures used to diagnosis brucellosis. *Am. J. Vet. Res.* **30** (10): 1811-1816.

Nowlan, P.F. and Geus, H.D. (1985). Use of EDTA modified antigen in the serodiagnosis of bovine brucellosis. *Vet. Rec.* **116**: 13.

Oberoi, M.S. and Kwatra, M.S. (1982). Serodetection of bovine abortions at organised farms in and around Punjab. *Ind. J. Comp. Microbiol. Immunol. Infect. Dis.* **3** (2): 101-102.

Panjarathinam, R. (1983). Animal brucellosis in and around Ahmedabad. *Indian J. Microbiol.* **22** (3): 147-149.

Pillai, M.T., Nedunchelliyan, S. and Raghavan, N. (1991). Serological and bacteriological detection of *Brucella canis* infection of dogs in Madras. *Indian Vet. J.* **68**: 399-401.

Polding, J.B. (1950). Brucellosis in India. *Indian Vet. J.* **72** (2): 158-170.

Purbey, L.N. and Sane, C.R. (1978). Brucellosis and its consequential effects in a herd of Dangi breed of cattle. *Indian J. Anim. Hlth.* **17**(1): 21-25.

Rahman, M.M., Choudhary, T.I.M.F.R., Rahman, A. and Haque, F. (1983). Sero-prevalence of human and animal brucellosis in Bangladesh. *Indian Vet. J.* **60** (3): 165-168.

Raja, C.K.S.V., Neelakantan, C.P. and Nair, D.V.P. (1959). Incidence of brucellosis in Kerala. *Indian Vet. J.* **36** (11): 537-539.

Ramachandra, R.N., Ravindranath, N., Taranath, D.M. and Sastri, G.N.S. (1981). A report of brucellosis in a dairy herd. *Indian Vet. J.* **58** (9): 741-742.

Reddy, P.R., Ramulu, M., Rao, P.V.R. and Rao, S.V. (1983). *Brucella* reactors in breeding bulls in AndhraPradesh. *Indian Vet. J.* **60** (4): 329-330.

Rose, J.E. and Roepke, M.H. (1957). An acidified antigen for the detection of non specific reactions in the plate-agglutination test for bovine brucellosis. *Am. J. Vet. Res.* **18**: 550-555.

Rose, J.E., Lambert, G. and Roepke, M.H. (1964). Ultra centrifugation and heat inactivation studies on seroagglutinins of pregnant heifers artificially infected with virulent *Brucella abortus*. *Am. J. Vet. Res.* **25**: 329-332.

Savalgi, V., Haemshetter, B.M., Basavaish, P. and Uttappa, I.M. (1987). An outbreak of *Brucella melitensis* in cattle, buffaloes and human beings. *Indian. J. Comp. Microbiol. Immunol. Infect. Dis.* **8** (4): 173-174.

Schroeder, E.C. and Cotton, W.B. (1911). *Ann. Rep. Bur. Anim. Industr. U.S. Dep. Agric.* 139. Cited in Infections diseases of animals. Vol. I. Butterworths Scientific Publications, London, 1959. pp.53.

Sen, G.P. and Joshi, T.P. (1976). Comparison of allergic reaction with serological tests for diagnosis of swine brucellosis. *Indian Vet. J.* **53**: 241-246.

Sharma, M., Batta, M.K., Asrant, R.K., Katochi, R.C., Joshi, V.B. and Nagal, K.B. (1995). *Brucella melitensis* abortions among organised sheep farms in north west states of India. *Indian J. Anim. Sci.* **65** (8): 874-875.

Sharma, V.D., Yadav, M.P., Singh, V.B. and Singh, S.B. (1984). Prevalence of agglutinins to *Coxiella burnetti* and *Brucella abortus* in man and animals in UttarPradesh. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **5** (1): 12-16.

Singh, B. and Saxena, S.N. (1964). Brucellosis as a cause of pyrexia of unknown origin in Delhi. *J. Ind. Med. Ass.* **43** (4): 164-168.

Singh, U.K. (1982). *Brucella* agglutinins in patients of pyerexia. *Indian J. Microbiol.* **22** (3): 182-184.

Soni, J.L. (1976). Serological evidence of human brucellosis in MadhyaPradesh. *Indian Vet. J.* **53** (11): 841-847.

Sreemannarayana, C. (1980). Incidence of brucellosis in goats in Guntur (A.P). *Indian Vet. J.* **57** (2): 175-176.

Srinivasan, V.K., Nedunchelliyan, S. and Venkataraman, K.S. (1992). Seroepidemiology of canine brucellosis in Madras city. *Indian Vet. J.* **69** (11): 978-980.

Srivastava, N.C., Gupta, B.R. and Kumar, A.A. (1983). A note on the prevalence of *Brucella* agglutinins in horses. *Indian J. Comp. Microbiol. Immunol. Infect. Dis.* **4** (1): 48.

Stemshorn, B.W., Forbes, L.B., Eaglesome, M.D., Nielson, K.H., Robertson, F.J. and Samagh, B.S. (1985). A comparison of standard serological tests for the diagnosis of bovine brucellosis in Canada. *Can. J. Comp. Med.* **49**: 391-394.

Stephen, S., Indrani, R. and Rao, K.N.A. (1978). Brucellosis in coastal Kamataka. *Indian J. Microbiol.* **18** (1): 28-31.

Suresh, S., Ramkrishna, J., Saseendranath, M.R., Tresamol, P.V. and Bhat, M.N. (1993). Sero survey of bovine brucellosis in Tamil Nadu. A recent study. Cheiron. **22**: 1-7.

Sutherland, S.S. (1980). Immunology of bovine brucellosis. *Vet. Bull.* **50** (5): 359-365.

Sutherland, S.S., Evans, R.J. and Bathgate, J. (1986). Application of an enzyme linked immunosorbent assay in the final stages of a bovine brucellosis eradication programme. *Aust. Vet. J.* **63** (12): 412-415.

Umapathy, B.L., Nagamani, M., Bhat, P. and Keshavamurthy, B.S. (1984). Seroepidemiological studies on human brucellosis in and around Bangalore. *Indian J. Microbiol. Immunol. Infect Dis.* **5** (3): 83-87.

Verma, N.D.C. (1982). A preliminary serological survey of brucellosis in Manipur. *Indian. J. Comp. Microbiol. Immunol. Infect. Dis.* **3** (4): 224-225.

Weidmann, H. (1991). Survey of means now available for combating brucellosis in cattle production in the tropics. *Anim. Res. Dev.* **33**: 99-111.

Wright, A.E. and Smith, F.C. (1997). Lancet 1: 656: Cited in Infectious diseases of animals. Vol. I. Butterworths Scientific Publications, London, 1959. pp.66.

Yadav, M.P., Singh, B.K. and Uppal, P.K. (1991). Sero-prevalence of brucellosis in equines in some of the states in India. *Indian J. Anim. Sci.* **62** (1): 41-42.

Zeftawi, A.N.M., Issa, A.L.M., Bekair, S.I., Radwan, A.D. and Hafez, S.M. (1986). Epidemiology of brucellosis among abattoir workers in Saudi Arabia. *Annal. Saudi. Med.* **6** (4): 29-31.

BOVINE BRUCELLOSIS IN RELATION TO PUBLIC HEALTH

By

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ABSTRACT OF A THESIS

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ABSTRACT

A serological survey was undertaken to assess the extent of brucellosis in bovines and humans. Serum samples from 1233 bovines and 747 human were collected. These samples were screened by Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT). The samples which showed a positive reaction, either by RBPT or STAT or both were subjected to Heat inactivation test (HIT), 2- Mercaptoethanol test (MET) and EDTA agglutination test (EAT).

Of the 610 slaughtered male bovine serum samples screened, 41 (6.72 per cent) was found positive by RBPT and 37 (6.07 per cent) by STAT. HIT, MET and EAT detected positive reaction in 32 (5.25 per cent), 26 (4.26 per cent) and 27 (4.43 per cent) samples respectively. A total of 23 samples were positive by all the above tests.

Among the 518 slaughtered female bovine serum samples, RBPT detected 41 (7.92 per cent) samples as positive while only 38 (7.34 per cent) samples were found positive by STAT. HIT, MET and EAT detected positive reaction in 31 (5.98 per cent), 26 (5.02 per cent) and 28 (5.4 per cent) samples respectively. Twenty four serum samples were found positive for the disease by all the above tests. None of the serological test employed could detect a statistically significant difference in the seroprevalence of disease between males and females.

Of the serum samples collected from 105 farm fed cows, six (5.7 per cent) samples were found positive for brucellosis by both RBPT and STAT. The number of samples found positive by HIT, MET and EAT were three (2.86 per cent), one

(0.95 per cent) and two (1.9 per cent) respectively. Only one sample revealed a positive reaction for the disease by all the above serological tests.

Among the 406 human male serum samples collected, six (1.47 per cent) revealed an agglutination reaction positive for the disease by RBPT while only four (0.99 per cent) showed an agglutination titre positive for the disease by STAT. HIT, MET and EAT detected three (0.74 per cent) samples each as positive for the disease. Three samples revealed a positive reaction in all the above serological tests. Of the 327 human female serum samples screened, RBPT and STAT recorded a positive reaction in five (1.53 per cent) and four (1.22 per cent) samples, respectively. Three samples (0.92 per cent) each were found positive by HIT, and MET and EAT. Only three samples were found positive for all the serological tests used in this study. It was observed that serological tests employed in this study could not detect a statistically significant difference in the seroprevalence of disease between males and females. None of the serum samples collected from veterinary surgeons (10), animal attendants (two), and slaughter house workers (two) were positive for the disease.

Of the serological tests employed in this study, RBPT detected the highest number of samples as positive followed by STAT, HIT, EAT and MET. It was also observed that, of the RBPT and STAT positive cases, HIT recorded maximum number of positive cases followed by EAT and MET. The reason for difference observed in the agglutination titre of the serum samples by the above tests were discussed.