PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS IN PIGS

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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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MANNUTHY, THRISSUR - 680651
KERALA

1998

DECLARATION

I hereby declare that the thesis entitled "**PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS IN PIGS**" 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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CERTIFICATE

Certified that the thesis entitled "**PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS IN PIGS**" is a record of research work done independently by **Sri. Ajay Kumar, V.J.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to him.

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ACKNOWLEDGEMENT

I sincerely express my whole-hearted gratitude to my major advisor Dr. E. Nanu, Professor, Department of Veterinary Public Health for his valuable advice, meticulous guidance, great patience and constant encouragement during the study. I am obliged to him for his stable help and immense support as the chairman of Advisory Committee.

I am grateful to Dr. P. Prabhakaran, Professor and Head, Department of Veterinary Public Health, for his precious suggestions and advice offered to me.

I express my heartfelt gratitude to the members of advisory committee, Dr. V. Jayaprakasan, Associate Professor, Department of Microbiology and Dr. M.R. Saseendranath, Associate Professor, Department of Preventive Medicine for their valuable and constructive criticism and wholehearted help during the study.

I wish to express my sincere thanks to Dr. C. Latha, Dr. B. Sunil, Dr. M. Sunil, Assistant Professors, Department of Veterinary Public Health, Dr. T.V. Viswanathan, Associate Professor, University Pig Breeding Farm, Mannuthy and to Dr. K. Baby, Professor and Head, Department of preventive Medicine I extend my thanks to Dr. Ganesh Pandian, Dr. Narahari and other staff members of MPI, Koothattukulam, to the staff and labourers of pig breeding farms at Kunnamkulam and Angamaly and to the staff members of Saroja Nursing Home, Thrissur. I also express my gratitude towards Dr. Sathyanarayana Rao, Associate Professor, Department of Veterinary Public Health, Veterinary College, Bangalore for the help he extended to me. I also acknowledge the help done by Dr. K.R. Ramanatha, Deputy Director, IAH&VB, Bangalore in procuring the antigens.

Grateful acknowledgements are due to Drs. Reenu John, Vinod, V.K., Deepa Jolly, Bindu Michael, Reni K. Oomen, Dinesh, C.N., Jayan, K.C., Sunil, G., Nandakumar, S., Sanjay, D., Manoj Johnson, George Varghese, Madhu Rajan Mathews, Shihabudeen, Mal Marugan, Jayakumar, Anil Kumar, V.T. and to all my other M.V.Sc. colleagues and friends for their support during my study.

I wish to express my gratitude to Leela, A., Mary, K.A., Karappan, P.K. and Sundaran, K.R., the staff members of the Department of Veterinary Public Health for the valuable assistance offered to me.

I am grateful to the Dean, College of Veterinary and Animal Sciences for providing the essential facilities for research work.

I am thankful to Mr. O.K. Ravindran, C/o Peagles, Mannuthy for meticulous typing of the manuscript.

I am highly indebted to my family whose constant encouragement, help, understanding and spirit throughout the course of my study helped me in the successful completion of the research work.

Last but not least, I express my immense gratitude to each and everyone who helped me, directly or indirectly, for the successful completion of my research programme.

AJAY KUMAR, V.J.

Dedicated To My Parents

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Introduction

INTRODUCTION

Among the livestock, pig is the only species being reared exclusively for meat production. In India, pig production is on the increase for the last four decades. During the year 1990, the production of pork and pork products in the country account for a sum of Rs.150 million. According to the livestock population census of 1996, the pig population in Kerala was 1.43 lakhs. In this part of the country pig is reared, as a means of self employment by the unemployed youth, rearing of a few pigs in the household setting as an additional source of income for the family and also in the form of organised farms.

Pigs suffer from various diseases caused by different etiologic agents. They not only suffer from these diseases but also act as a source of infection for human beings.

Brucellosis is one of the bacterial diseases which affect pigs and has a world wide distribution. The disease has both economic and public health significance. Brucellosis in swine is caused by the various biotypes of *Brucella suis* and it exhibits a wider principal host variability than other Brucellae.

The principal route of transmission in pigs is by ingestion of contaminated materials and venereal transmission also occurs readily. Virulent *Brucella* organisms are highly invasive and capable of penetrating the mucous membrane of the nose, throat, conjunctiva, urogenital tract, epithelium of the teat canal, parenchyma of mammary gland and normal or abraded skin.

The classical manifestations of swine brucellosis are abortion, infertility, orchitis, posterior paralysis and lameness. *Brucella* causes most serious tissue damage in gravid uterus. The organism after localisation in the gravid uterus causes metritis and placentitis in pregnant sows with resulting abortion or still birth depending on the stage of infection. However affected sows seldom have a second abortion and female infected before sexual maturity do not abort. Affected boars usually continue to breed, but may have a reduced fertility.

Human brucellosis caused by *Brucella suis* is one of the serious occupational hazards and has a world wide distribution. The infection is primarily acquired from animals infected with the organism either by direct contact or indirectly through the discharges or inhalation of infected materials or through contaminated animal products. It is an occupational disease of veterinarians, farmers and their

families and also abattoir workers and meat packers who handle carcasses of infected pigs. Occasionally *B. suis* infects cattle, causing milk borne outbreaks in the general population.

Rare instances of person-to-person transmission have been recorded either in circumstances implicating sexual contact or by tissues, including blood and bone-marrow transfer. Laboratory acquired brucellosis is also reported.

The disease is often prolonged and debilitating with a tendency to produce severe complications. Among the various complications, endocarditis causes a high proportion of the fatalities in brucellosis especially that caused by *B. suis*.

Diagnosis of the disease in animals and human beings is essential to establish the existence and extent of the disease among animal and human population. The data obtained from such works will be useful in taking proper control measures. Considering the economic importance and public health significance of porcine brucellosis, the present study was undertaken to assess *Brucella* antibody level in the sera of pigs and animal handlers and also to compare the efficacy of various diagnostic tests in determining the level of *Brucella* antibody in sera of pigs and human beings.

Review of Literature

REVIEW OF LITERATURE

First accurate description of the brucellosis was given by Marston (1863) in his report on Malta fever. Bruce (1887) isolated the causal organism which he subsequently named as *Micrococcus melitensis* from the spleen of a patient who had died of Malta fever. Meyer and Shaw (1920) suggested the name *Brucella* for the organism in honour of Bruce. Brucellae were isolated from aborting swine by Traum (1914). Huddleson (1929) noted that isolate from swine, described by Traum differed from *Brucella abortus* to warrant establishment of a new species, *Brucella suis*.

Polding (1950) conducted a serological survey on brucellosis in India. He found low grade infection in nearly all farms in India and in many villages of the penninsula which lead to abortions of one to 15 per cent per year. In Kerala, the disease was first reported by Raja *et al*. (1959).

Distribution of brucellosis in India

Brucellosis in pigs

Sen *et al*. (1972) screened a total of 408 pig serum samples collected from different parts of India for *Brucella* agglutinins using standard tube agglutination test (STAT). They reported an overall incidence of 13.4 per cent.

Kulshrestha *et al.* (1978) studied the incidence of brucellosis in pigs. They examined 98 serum samples of pigs and reported that one per cent of the samples were positive.

Kumar and Rao (1980) examined 1,103 serum samples of Yorkshire pigs, over six months of age and reported the incidence as 11.37 per cent. They could isolate a strain of *Brucella suis* biotype 2 from a reactor pig.

Chowdhary *et al.* (1983) studied the seroprevalence of porcine brucellosis in Bihar. They collected a total of 241 serum samples which were tested by STAT and reported an overall prevalence of 4.97 per cent. The agglutination titre of positive samples ranged between 80 IU and 1280 IU/ml. One of the serum samples which showed a titre of 80 IU/ml in STAT was reduced to 20 IU/ml, in heat inactivation test.

Sharma *et al.* (1984) reported the prevalence of brucellosis in pigs as 17.24 per cent.

Das (1985) collected 884 and 900 serum samples from boars and sows, respectively. These samples were subjected to plate agglutination test and STAT to detect the presence of *Brucella* agglutinin. Among the 884 samples of boars 26 (2.94%) revealed a positive titre for brucellosis by STAT, whereas 18 (2%) of 900 samples of sows showed a positive titre for brucellosis by the test.

Singh and Narayan (1988) evaluated the prevalence of brucellosis in a pig breeding farm and observed a prevalence of 30.47 per cent. Kalimuddin and Choudhary (1988) studied seroprevalence of brucellosis in pigs and bacon factory workers by STAT. Of the 213 pig serum samples tested, 5.63 per cent was found seropositive and positive titre ranged between 80 and 160 IU/ml. Only one serum sample out of 13 workers was seropositive.

In a serological survey, Ghosh (1989) collected a total of 177 serum samples from four pig farms and subjected to STAT. The test revealed that the serum samples from three farms had an infection varying from 7.2 to 8.8 per cent and the titre ranged between 80 IU/ml and 1280 IU/ml.

Brucellosis in man

Serological evidence of human brucellosis in India dates back to 1897 when Wright and Smith identified it in invalidated soldiers from india.

Kulshrestha *et al.* (1978) made sero-epidemiological studies on human and animal brucellosis in Haryana. A total of 44 human sera were screened and reported that eight of them were seropositive for the disease. Reddy *et al.* (1981) screened 26 workers of a livestock farm in Andhra Pradesh and found that 4 (15.38%) were seropositive.

Verma (1982) conducted a preliminary serological survey for brucellosis in Manipur. During his study he collected 32 human sera of individuals with pyrexia of unknown origin (PUO). The samples were tested by rapid plate agglutination and standard serum agglutination tests and reported that none of the samples yielded a positive titre for brucellosis.

Panjarathinam (1983) tested 805 serum samples of women who had abortion and could detect *Brucella* agglutinin in 52 samples by STAT. Of the samples tested, 50 were positive by Rose Bengal Plate test (RBPT) and 11 were positive by Rapid plate test (RPT). Panjarathinam and Gulrajani (1983) tested 180 serum samples from professional blood donors and 88 samples from inpatients for *Brucella* agglutinins. Among the professional blood donors, 4.4 per cent samples had a titre of 80 IU/ml.

Rahman *et al.* (1983) studied the seroprevalence of brucellosis among human who were in close contact with animals in Bangladesh. Among 190 samples examined, 21 (11.05%) were found positive. Sharma *et al.* (1984) tested the serum samples from human for *Brucella* agglutinin. They reported that 0.56 per cent human samples were positive. Vanhoye (1983) and Russo *et al.* (1984) conducted studies among veterinary students and reported the occurrence as 10 and 13 per cent, respectively.

Umapathy *et al.* (1984) carried out a sero-epidemiological study on human brucellosis among rural population, hospitalized patients and persons working in *Brucella abortus* infected farms in and around Bangalore. The per cent of rural population revealed the presence of *Brucella* agglutinins in their sera by indirect haemagglutination test was 10.6. The test revealed a seropositivity of 3.9 per cent in hospital patients and 46.7 per cent in persons working in *B. abortus* infected farm.

Kapoor *et al.* (1985) conducted a study on seroprevalence of brucellosis in human beings in Bikaner. Among 101 patients with Pyrexia of unknown origin (PUO), 2.97 per cent had a *Brucella* agglutinin diagnostic titre of 80 IU/ml or more per ml of serum by STAT. A higher prevalence rate was found in females than in males. Savalgi *et al.* (1987) investigated the incidence of brucellosis among working staff in an infected farm and reported an incidence rate of 20 per cent among the 20 working staff. They also reported the isolation of *B. melitensis* biotype 1 from animals and human.

Koshi *et al.* (1988) reported a *Brucella* agglutinin titre of 640 IU/ml of serum in a patient with PUO and could isolate *B. melitensis* from the bone marrow of the patient.

Kalimuddin *et al.* (1990) conducted STAT, HIT and 2-ME test on 11 sera sample from the dairy farm attendants and

reported that one of these sample was positive for all the tests performed. In a study on seroprevalence of brucellosis Masoumi *et al.* (1992) tested 522 serum samples of human and recorded an overall prevalence of 0.45 per cent by serum agglutination test. Higher prevalence was recorded in males (1.27%). The per cent of seroprevalence in 20-29 yr age group, butchers and persons consuming raw milk was 1.86, 8.33 and 5.4 respectively. The study also revealed that the villagers had a higher prevalence rate (2.48%) as compared to the urban dwellers (0.27%). Mathai *et al.* (1996) performed ELISA on 23 serum samples of patients with prolonged fever and found that 39.1 per cent of these samples were positive for brucellosis.

Brucellosis in cattle

Among the various species of livestock in India, extensive studies on bovine brucellosis were made as compared to other species of animals. Mathur (1968) isolated 74 strains of *B. abrotus* from cows and buffaloes from organised farms. Buth and Manchanda (1972) reported that the per cent of seropositivity for brucellosis in cows and buffaloes to be 0.96 and 0.55, respectively.

Kulshresthra *et al.* (1978) screened 1539 cattle and 1224 buffaloes for brucellosis using serological tests. Eight

per cent of cattle and 4.4 per cent of buffaloes were found positive.

Baby (1978) conducted a study on the incidence of brucellosis in buffaloes in Thrissur (Kerala). An overall incidence of 2.24 per cent was reported on the basis of STAT.

Ramachandra *et al.* (1981) made bacteriological study of 13 aborted foetuses and was able to isolate *B. abortus* from 10 of these foetuses. He also noticed a positive correlation between isolation of *Brucella* and results of immunoserological studies using sera and milk. Reddy *et al.* (1981) examined the serum samples from various species of animals and human for *Brucella* agglutinin and reported that five (4.08%) out of 144 murrah buffaloes and 25 (19.68%) out of 145 cattle revealed *Brucella* agglutinins. Buffaloes showed comparatively less sero positivity and the rate of infection was higher in females of both group of animals.

Verma (1982) tested 74 serum samples of individual cattle by RPT and STAT and reported that only two of them had *Brucella* agglutination titer positive for the disease. Butchaiah and Khera (1982) studied the prevalence of *Brucella* abortion among the various species of livestock in organised farms located at 3 widely separated geographical locations and found that eight out of 28 cows and one out of six buffaloes were seropositive. Sharma *et al.* (1984) conducted serological

survey on brucellosis in cattle and buffaloes. They found that 8.7 per cent of cattle and 5.71 per cent of buffaloes were positive for the disease.

Babu *et al.* (1985) studied the seroprevalence of brucellosis among pure bred cattle and buffaloes. They collected serum samples from 632 jersy and 226 ongole breeds of cattle and also 726 samples from murrah buffaloes. Among the samples tested, five of the jersey cows, one ongole cow and two murrah she buffaloes had positive titre for brucellosis.

Papang *et al.* (1987) screened 208 serum samples of cows from organised livestock farms to detect the presence of *Brucella* agglutinin. They found that 15 of these animals had a serum titre positive for the disease. Manickam and Mohan (1987) conducted an epidemiological investigation on *Brucella* abortus infection in milch cattle and reported an overall prevalence rate of 3.3 per cent.

Bachh *et al.* (1988) made an attempt to study the seroprevalence of brucellosis among exotic cattle in Kashmir. They examined 115 serum samples collected from unvaccinated exotic cattle herds and reported that the per cent of seropositivity was 44.35. They also observed that the prevalence was more in females than in males and in adult age group than in young animals. Seroprevalence of brucellosis in

an organised cattle farm in Assam was studied by Barman *et al.* (1989). Among the 129 serum samples which were collected from animals with symptoms of the disease, 44.9 per cent of samples yield a seropositive titre for brucellosis.

Chandramohan *et al.* (1992) studied the incidence of bovine brucellosis in an endemic area. They collected 115 serum samples of zebu cattle and 23 samples of buffaloes. The per cent of cattle and buffalo serum samples positive for the disease with was ELISA 18.84 and 21.74, respectively. In a serological survey on bovine brucellosis, Suresh *et al.* (1993) collected 459 serum samples of cattle with reproductive disorders and were subjected to various serological tests. They reported that 9.37 per cent of cattle serum and 10.92 per cent of buffalo serum were positive for brucellosis. Ghani *et al.* (1994) performed standard plate test, STAT, rivanol test and 2-ME test on serum samples collected from 500 healthy and 500 slaughter cattle and the per cent of seropositivity for brucellosis obtained by corresponding tests in these groups was 2, 1.2, 0.8, 0.4 and 2, 2, 1.8 and 1.14 respectively.

Sharma and Saini (1995) studied seroprevalence of brucellosis in various species of farm animals in Punjab. They reported the per cent prevalence of brucellosis among cattle and buffaloes as 8.69 and 14.61, respectively.

Brucellosis in sheep and goat

Kulshrestha *et al.* (1978) screened 128 sheep and 749 goat serum samples for brucellosis and reported that 1.5 per cent sheep and 1.6 per cent goats had brucellosis.

Sreemannarayana (1980) collected a total of 926 serum samples of goat and subjected to rapid plate test and STAT. The study revealed an overall incidence rate of 2.15 per cent. Reddy *et al.* (1981) reported that 13.58 per cent of 589 sheep serum samples tested were positive for brucellosis. Butchaiah and Khera (1982) performed a study on Brucellosis in various species of animals and reported that 47 out of 120 sheep and 14 out of 32 goats showed sero-reaction for brucellosis.

Panjarathinam (1983) collected 44 sheep and 357 goat sera and the samples were subjected to serological test to detect *Brucella* agglutinin. He reported that 37 of 357 goats and five of the 44 sheep were positive for the disease. Sharma *et al.* (1984) reported that 11.53 per cent goats and 7.84 per cent sheep serum samples were positive for brucellosis.

Ghosh and Verma (1985) studied the incidence of brucellosis among sheep and goat in Nagaland. They tested the serum samples from 189 goats and 65 sheep and reported that the overall incidence rate was 6.29 per cent. Bachh *et al.* (1987) conducted serological investigation on brucellosis

among exotic sheep and goats in Kashmir. They found 5.55 per cent of 294 sheep and 2.22 per cent of 46 goats seropositive for brucellosis.

Parvaiz *et al.* (1988) studied seroprevalence of brucellosis among sheep in Bikaner and recorded an overall incidence of 4.94 per cent of the 557 sheep serum screened. Ghosh and Nanda (1988) reported the seroprevalence of brucellosis as 13.29 and 6.95 per cent of the 316 goat serum samples tested by Rose Bengal plate test and standard tube agglutination test.

Bandey *et al.* (1989) made sero-epidemiological studies on brucellosis in exotic sheep in Kashmir valley. They collected 8034 serum samples of sheep during 1979 to 1986. The samples revealed an overall prevalence of 3.46 per cent seropositivity with STAT.

Sharma and Saini (1995) reported that the per cent of seropositivity for brucellosis among the farm reared sheep and goat in Punjab was 15.45 and 1.75, respectively.

Brucellosis in dogs

In India *B. canis* was first isolated by Pillai *et al.* (1991). They attempted to isolate the organism from five seropositive dogs by haemoculture and could isolate two

strains of the organism. They recorded the incidence of brucellosis as 2.18 per cent of 640 dog serum samples tested. Srinivasan *et al.* (1992) studied the sero-epidemiology of canine brucellosis in Madras city. Out of 460 sera samples screened, nine had *B. canis* antibodies. The study also concluded that there was no breed or sex specificity in case of seropositivity.

Brucellosis in other species of animals

Investigation on brucellosis in other species of animals had been conducted and reported from India. Boro *et al.* (1981) collected 21 serum samples of mithuns and subjected to STAT after heat inactivation of the sera at 56°C for 30 minutes. They reported that the samples from two each of the bulls and cows and three heifers had *Brucella* agglutinin titre positive for brucellosis.

Srivastava *et al.* (1983) examined 25 serum samples from aborted mares and found that 10 samples had a *Brucella* agglutinin titre of 80 IU and above. Yadav *et al.* (1992) studied seroprevalence of brucellosis in equines in some states of the country. Of the 2395 samples collected, 195 reactors were found.

Diagnostic methods

Confirmatory diagnosis of brucellosis in human and animals primarily depends upon isolation and identification of the etiologic agent. A variety of serologic and allergic tests are also used for the diagnosis of the disease. Each of these tests has its own merits and demerits. However, considering the ease of performing the test and the time taken by it, many laboratories and researchers prefer serological tests for the diagnosis of the disease.

Standard tube agglutination test (STAT)

Wright and Smith (1897) discovered STAT. Grinsted (1909) first used it to diagnose the disease in cattle. At present it remains as the most commonly used serological test for the diagnosis of brucellosis.

Plate or rapid agglutination test

Huddleson (1920) had used the plate or rapid agglutination test since 1920 as a routine test for the diagnosis of brucellosis and found that it gave satisfactory results with serum, whey and whole blood.

Milk ring test (MRT)

Fleischauer (1937) was the first one to introduce the milk ring test. It has great value as a screening test in large dairy herds.

Flocculation test

Sachweh (1935) and Meinicke (1938) described flocculation reactions for blood or whey in the diagnosis of brucellosis.

Complement fixation test (CFT)

Larson (1912) introduced CFT for the diagnosis of brucellosis. The test is very specific, but it is very laborious and hence very rarely used for the diagnosis of the disease under field conditions.

Dried antigen for agglutination

Lerche and Roots (1946) and Hauduroy and Tanner (1952) described the use of freeze dried *Brucella* antigens for the diagnosis of the disease. The reconstituted freeze dried antigen gave same result as that of the original antigen. The use of freeze dried antigen has the advantage that a large amount of similar antigen can be centrally prepared and economically stored for long periods.

Coombs' test

This test was designed by Coombs *et al*. (1945). The test brings about agglutination of cellular antigens that have attached to the non-agglutinating antibody from a previous negative agglutination test.

Blocking test

The test (Griffitts, 1947) is carried out by setting up an agglutination test in an ordinary way and if it is negative, adding a drop of positive serum of moderate titre, mixing and re-incubating overnight.

Rapid cup agglutination test

The test was described by Jameson (1957). Concentrated antigen and serum diluents are mixed in depressions in a perspex plate which is then placed at 37°C for one and half hours and read by an oblique transmitted light against a black background.

Vaginal mucus test

The test was described by Kerr (1955) who found that it was not positive after vaccination by S19 strain, so a reaction indicates infection with virulent organisms.

Gel diffusion precipitin test

The test was described by Bruce and Jones (1958). Many research workers use this as a supplementary diagnostic method along with other tests.

Surface fixation test

Castaneda (1950, 1953 and 1954) described a surface fixation method to test serum on filter paper.

Fluorescent antibody method

According to fifth report of Joint FAO/WHO (1971) expert committee on brucellosis, the fluorescent antibody test provides an accurate means for differential diagnosis.

Rose Bengal plate test

This is a modification of acid plate test introduced by Rose and Roepke (1957) who noted that the antibody activity of non-specific agglutinins is destroyed at a low pH (3.6).

Card test

The test satisfies the need for rapid sensitive accurate test for screening any herd especially in range areas (Nicoletti, 1967).

Indirect haemagglutination test

Becht (1958) observed that by extraction with acetic acid at 100°C, followed by precipitation with alcohol, a substance could be demonstrated in *Brucella abortus* which could sensitize the cattle erythrocytes which in turn were specifically agglutinated by sera from cattle with brucellosis. This test was found valuable in the case of sera which reacted doubtfully in slow agglutination test.

Antibody neutralization test

Chernysheva and Aslangan (1975) found this test suitable to detect antigen in water, milk or tissues.

Allergic tests

McFadyean and Stockman (1912) studied this test as a means of detecting *Brucella* infection in cattle. Intradermal test in the case of humans were also used.

Heat inactivation test

Amerault *et al.* (1961) developed this test for differentiating specific and non-specific agglutination in bovine brucellosis since then it was used in other species of animals also.

2-mercaptoethanol test

Anderson *et al.* (1964) studied the relation of mercaptoethanol stability to complement fixation in case of *Brucella* agglutinating antibodies. The compound dissociates 1gM pentamer and destroys its agglutinating activity. Nicoletti (1969) found that 2-mercapto ethanol test could identify 97 per cent of infected cattle.

Rivanol test

In principle, the test is the same as that of 2-MET. This was demonstrated by Erdem and Unel (1969).

Ethylene diamine tetra acetate (EDTA) agglutination test

This test was done by Nielsen *et al.* (1979). The agglutination was performed in the presence of EDTA. So the agglutination due to EDTA laile non-specific agglutinins can be differentiated.

Comparison of serological tests

According to the Joint FAO/WHO (1986) expert committee on brucellosis, STAT is the most widely used procedure for the measurement of anti-brucella antibodies, although many other more sensitive and specific tests are described. Many of the research workers had attempted more than one test in the

sero-diagnosis of brucellosis and compared the results obtained.

Panjarathinam (1983) performed Rapid plate test (RPT), RBPT, STAT, coombs test, complement fixation test (CFT), indirect bacterial haemagglutination test and gel diffusion test on 805 serum samples collected from women who had abortion. From the findings of the study he concluded that RPT was inferior to STAT and RBPT was very rapid and as efficient as STAT. Chowdhary *et al.* (1983) carried out a study on seroprevalence of Porcine brucellosis in Bihar found that the sample which had a titre of 80 IU/ml in STAT showed a titre of 20 IU/ml in HIT.

Stemshorn *et al.* (1985) made a comparison of standard serological tests for the diagnosis of bovine brucellosis in Canada. On a sample of 167 culture positive cattle, the sensitivities of the test were; CFT:79 per cent, Buffered plate antigen test:75.4 per cent, RBPT:74.9 per cent, CARD:74.3 per cent; SPT:73.1 per cent, STAT:68.9 per cent and 2-MET:59.9 per cent. It was an interesting finding that all the tests combined detected only 82 per cent of these infected cattle.

Das and Paranjape (1987) compared *Brucella* stabilized antigen plate test (BSPT) and RBPT in rapid diagnosis of brucellosis and found that BSPT was having more merits. In a

study on seroprevalence of brucellosis among goats in Tripura, Ghosh and Nanda (1988) observed that maximum number of reactors were obtained by milk ring test (MRT), followed by RBPT and then by STAT.

Chand and Sadana (1988) compared counterimmuno-electrophoresis (CIE) to STAT and RBPT. In the study it was found that CIE and RBPT yielded same results in culturally positive ones, but in same culturally positive ones, STAT reaction was weak.

Kalimuddin *et al.* (1990) screened 101 serum samples of cows and found that 19, 17 and 13 were positive with STAT, HIT and 2-MET, respectively. Shrivastava *et al.* (1991) made a comparison of dot-enzyme linked immunosorbent assay (dot-ELISA) with RBPT and STAT for the sero-diagnosis of bovine brucellosis. They found that dot-ELISA was a more suitable screening test for brucellosis as it was simple, economical, rapid and highly sensitive. Kulkarni *et al.* (1991) made a comparison of ELISA, STAT and RBPT in diagnosis of bovine brucellosis and found that the ELISA was more specific in detecting the brucellosis. Nicoletti and Tanya (1993) made a similar study in bovines and concluded that there is no advantage in using primary binding assays rather than simple buffered antigen agglutination procedures to detect cows infected with a field strain of *B. abortus*.

Barbuddhe *et al.* (1994) compared dot-ELISA with conventional serological tests for the diagnosis of ovine brucellosis. Out of the 77 field serum samples screened, three, four, seven, two and 17 were positive by RBPT, STAT, CFT, indirect haemolysis test (IHLT) and dot-ELISA, respectively.

Ghani *et al.* (1994) performed different serological tests on a total of 1000 samples and found that SPT was most sensitive followed by STAT, Rivanol-test and 2-MET. Barbuddhe *et al.* (1995) made a comparison of different serological tests in the diagnosis of caprine brucellosis. Of the 75 field serum samples screened RBPT, STAT and IHLT could not find any positive ones whereas two and one were positive to CFT and lgG dot-ELISA, respectively.

Ghani (1995) studied the incidence of brucellosis in samples from a total of 2000 animals. Maximum number of positive samples were identified by SPT followed by STAT, Rivanol test and 2-MET.

Materials and Methods

MATERIALS AND METHODS

In the present investigation, a total of 255 pig and 250 human blood serum samples were collected and tested to detect the presence of *Brucella* agglutinins. Of the pig serum samples collected, 31 were from pigs reared in University pig breeding farm of Kerala Agricultural University, Mannuthy. Forty samples were collected from pigs reared in Angamaly pig breeding farm and 14 samples from pig breeding farm, Kunnamkulam. Among these pigs, three were males and 82 were females. Serum samples were also collected from 170 pigs slaughtered in the abattoir of Meat Products of India, Koothattukulam. Among these 98 samples were collected from males and 72 from female pigs.

One hundred and twenty two human serum samples were collected from the general population, whose sera were collected for the diagnosis of various other disease conditions in a private nursing home at Thrissur. Of these samples, 65 were from females and 57 were from males. Eighty eight serum samples, consisted of 56 male and 32 female students of the fifth year undergraduate students of the College of Veterinary and Animal Sciences, Mannuthy were also collected. Twenty samples each were collected at random from the veterinary surgeons and pig farm workers.

Collection and storage of serum

About seven millilitres of blood was collected aseptically from the ear vein of each farm fed pigs with a disposable syringe and needle. The blood was transferred into a clean dry test tube and allowed to coagulate it in a slanting position at room temperature. Afterwards it was kept overnight in the refrigerator. On the following day sera from each tube was transferred into a sterile clean dry test tube and it was centrifuged at 2000 rpm for five minutes. The clear supernatent serum was transferred into sterile screw capped vial and added a drop of 1:10000 Merthiolate solution per millilitre of serum. These were stored at -20°C for serological studies.

Blood samples from the pigs slaughtered in the abattoir were collected at the time of sticking. Human samples were collected aseptically from the radial vein with a sterile disposable syringe and needle. The collection of blood, processing and storage of serum from slaughtered pigs and human were carried out in the same way as that of the blood samples collected from the farm fed pigs.

Antigens

Brucella abortus coloured and plain antigens were purchased from the Institute of Animal Health and Veterinary Biologicals, Hebbal, Bangalore.

Test procedures

The serum samples collected from pigs and human were subjected to each of the following tests to detect the presence of *Brucella* agglutinins.

1. Plate agglutination test

Plate agglutination test of the serum samples was performed following the procedure described by Alton *et al.* (1975) Rose Bengal coloured antigen was used. In order to carry out the test one drop of serum (25 μl) was measured with a sampler onto an opaque plastic white tile and 25 μl of antigen was added to it. 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, the sample showing any degree of visible agglutination was considered positive and the others were considered negative by the test.

2. Standard tube agglutination test (STAT)

The test was done using standard *Brucella abortus* antigen following the procedure described by Alton and Jones (1967). In order to carry out the 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) so as to form the serum dilutions of 1:5, 1:10, 1:20, 1:40 and 1:80. To prepare this dilutions of test serum, 0.8 millilitre of the phenol saline was added to the first agglutination tube and 0.5 ml each to the remaining four tubes. Followed by this 0.2 ml of the serum under test was added to the first tube, mixed thoroughly and 0.5 ml was transferred to the second tube. Mixing and transferring of the contents of second to fifth tube was carried out as in the case of first tube; from the fifth tube 0.5 ml of the diluted serum was discarded. To each of these tube containing diluted serum, 0.5 ml of *Brucella abortus* standardized plain antigen was added and contents in the tube 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 was set up to simulate 50 per cent clearing by mixing 0.25 ml plain antigen to 0.75 ml phenol saline. The tubes containing the test samples and control tube were incubated at 37°C for 24 hr. At the end of the period of incubation the result of the tests were read and recorded. In recording of the

results, the degree of agglutination was determined by reading the degree of clearing without shaking the tubes. The highest serum dilution showing 50 per cent or more agglutination was taken as the end point titre of the serum. The test serum showing a titre of 1:40 dilution or above was considered positive, a titre of 1:20 dilution was regarded as a suspicious reaction and a titre less than 1:20 indicated negative reaction. Any test serum sample which revealed over 50 per cent agglutination in 1:160 dilution, the test of the sample was repeated with higher doubling dilutions to find out the correct titre of the sample and the result was recorded.

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 serum sample to be tested was subjected to heat inactivation by keeping the serum in a water bath maintained at a temperature of 56°C for 30 minutes. The test procedure and reading of the results were carried out as in the case of STAT.

4. 2-mercaptoethanol test (2-MET)

The 2 ME test was done according to the procedure described by Alton *et al.* (1975). The *Brucella abortus* plain antigen was made phenol free in the laboratory. In order to

make the antigen phenol free 100 ml of antigen was mixed thoroughly and transferred to a clean sterile centrifuge tube and centrifuged at 3000 rpm for 15 minutes. After this the cell free supernatent solution was removed using a pasteur pipette. The cells were reconstituted in sterile normal saline and centrifuged as before. The washing of cells with normal saline was carried out twice. Then the cells were finally reconstituted in normal saline solution which was made upto 100 millilitre. Instead of phenol saline 0.1 molar solution of 2 ME in saline solution was used as the diluent. This was made in the laboratory by making up 7.07 ml of 14.139 mol solution of 2 ME in normal saline into one litre. The diluent was stored at 4°C and prepared freshly in every week. The serum dilution, addition of antigen, period and temperature of incubation and the reading of the test were same as that of STAT.

5. **Ethylene diamine tetra acetate agglutination test (EAT)**

The test was performed based on the procedure described by the Joint FAO/WHO (1986) expert committee on Brucellosis. A 10 m molar solution of EDTA disodium salt was prepared in phosphate buffered saline with a pH 7.2 and used as the diluent to perform the test. Except for the use of this as diluent, the test procedure was same as that for STAT.

Results

RESULTS

Serum samples from 170 slaughter pigs, 85 farm fed pigs and 250 human were subjected to rose bengal plate test (RBPT), standard tube agglutination test (STAT), heat inactivation test (HIT), 2-mercaptoethanol test (2-MET) and EDTA agglutination test (EAT). The results of the above tests on 255 pig serum samples are shown in Table 1.

Table 1 Seropositivity revealed by pig serum samples with different tests for brucellosis

Sex	No. of samples	No. of samples positive by each test				
		RBPT	STAT	HIT	2-MET	EAT
Male	101	13 (12.87)	17 (16.83)	16 (15.84)	12 (11.88)	12 (11.88)
Female	154	21 (13.64)	27 (17.53)	25 (16.23)	20 (12.99)	21 (13.64)
Total	255	34 (13.33)	44 (17.25)	41 (16.08)	32 (12.55)	33 (12.94)

Figures in parenthesis indicate per cent

A comparison of seropositivity of the samples revealed in each of the above test are shown in Fig.1. The dotted line indicates the percent of samples positive by all the tests. Figure 2 reveals the distribution of the number of samples having the highest titre in STAT, HIT, 2 MET and EAT. The

Fig.1 COMPARISION OF SERO-POSITIVITY OF PIG SERUM IN DIFFERENT SEROLOGICAL TESTS

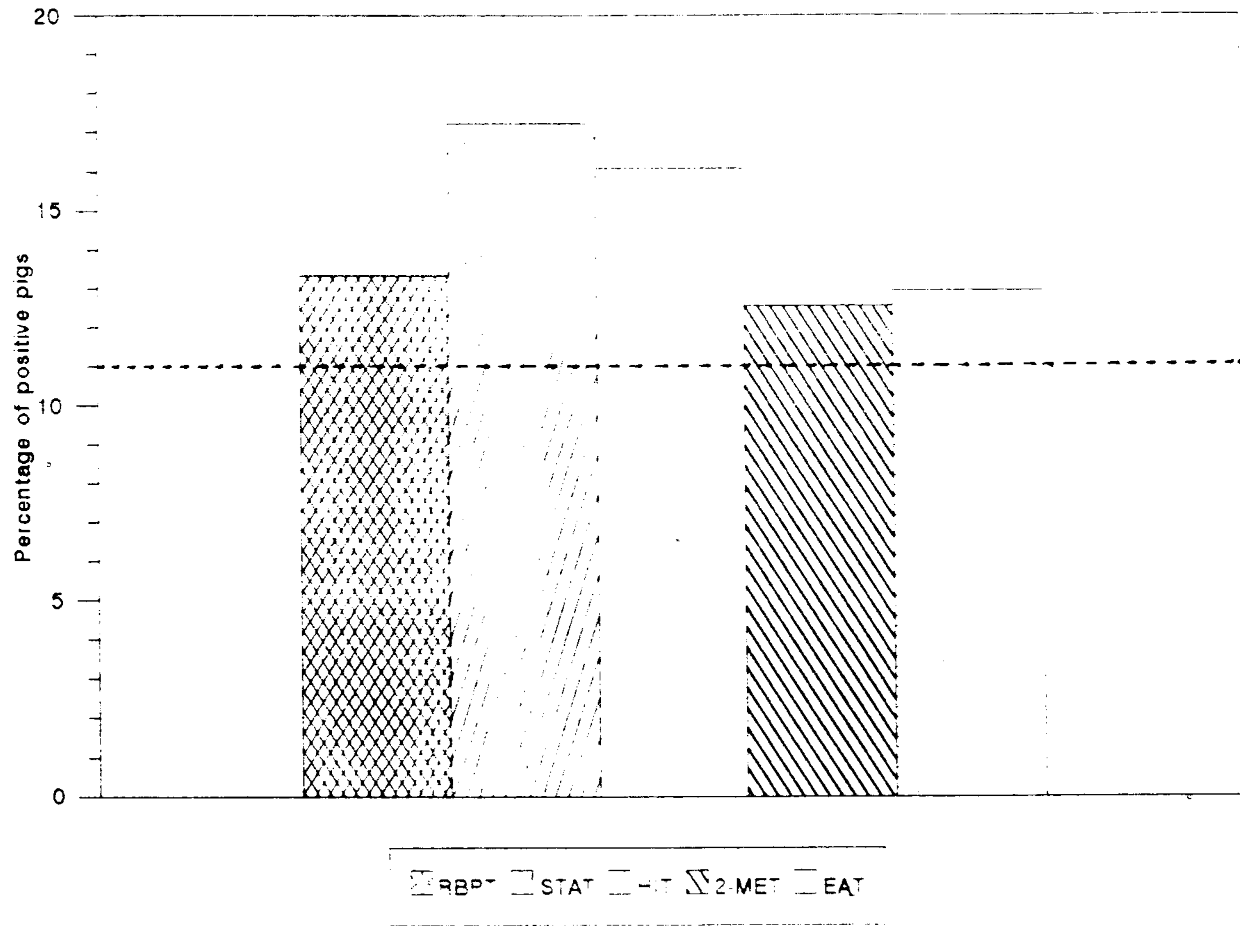
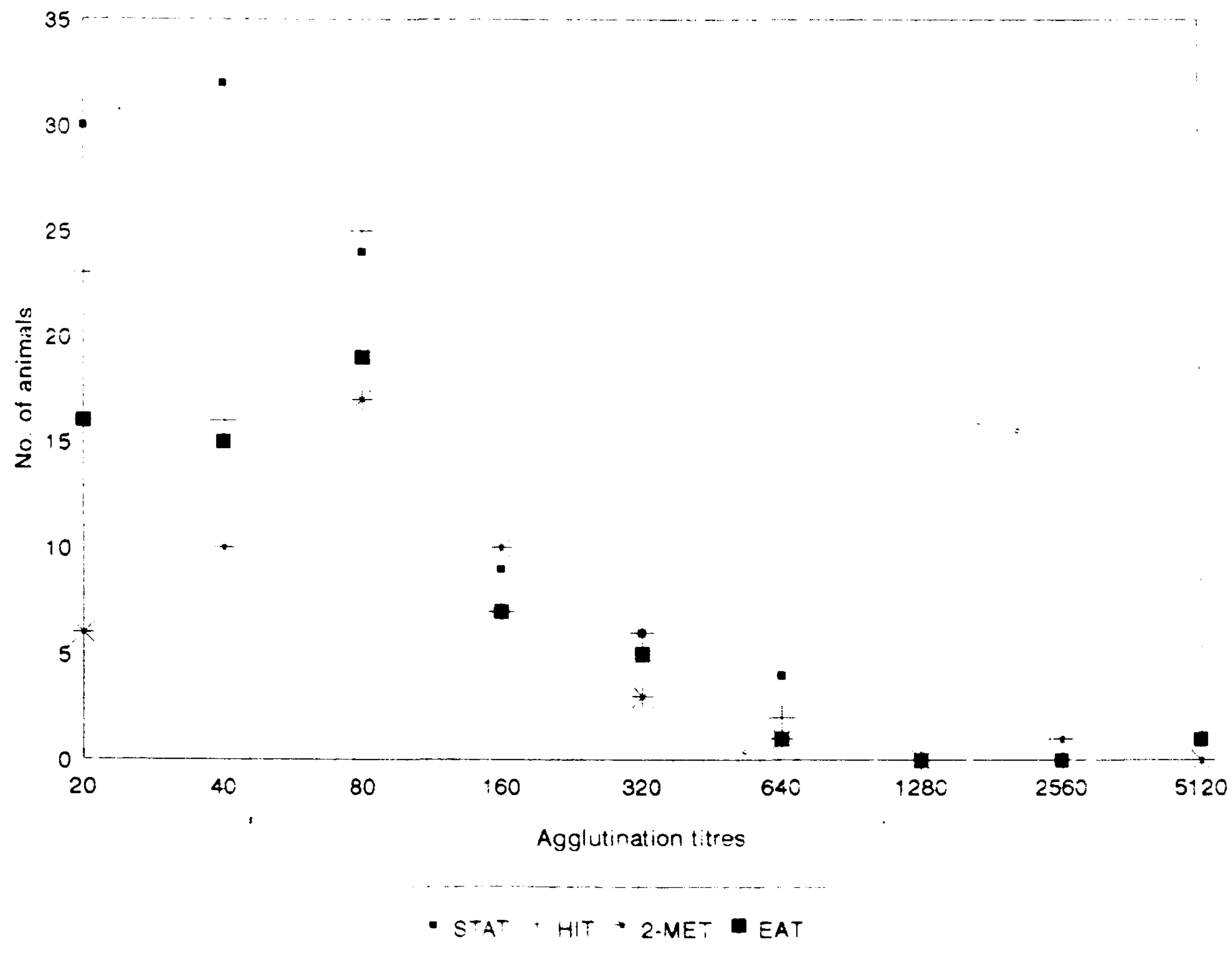


Fig.2 DISTRIBUTION OF AGGLUTINATION TITRES OF PIG SERUM IN VARIOUS SEROLOGICAL TESTS



titre of samples in these test ranged between 20 IU/ml and 5120 IU/ml. The agglutination/agglutination titre revealed by individual serum samples of pig and human are given in Appendix 1.

The results of RBPT, STAT, HIT, 2-MET and EAT on serum samples from slaughtered pigs are given in Table 2.

Table 2 Seropositivity revealed by slaughtered animals with different tests for brucellosis

Sex	No. of samples	No. of samples positive by each test				
		RBPT	STAT	HIT	2-MET	EAT
Males	98	13 (13.26)	17 (17.35)	16 (16.33)	12 (12.24)	12 (12.24)
Females	72	14 (19.44)	20 (27.78)	18 (25.00)	15 (20.83)	15 (20.83)
Total	170	27 (15.88)	37 (21.76)	34 (20.00)	27 (15.88)	27 (15.88)

Figures in parenthesis indicate per cent

In RBPT, 13 (13.26%) of the 98 samples from male pigs and 14 (19.44%) of the 72 samples from female pigs revealed agglutination reaction positive for brucellosis. The STAT revealed that 37 out of 170 animals had a positive titre for brucellosis. Among the 98 male pig samples tested, 17 were found positive for the disease by STAT. The agglutination

titre in the test ranged from 20 IU/ml and 5120 IU/ml. Only one of these animals had a titre of 5120 IU/ml. The *Brucella* agglutinin titre of 640 IU/ml, 320 IU/ml, 160 IU/ml and 80 IU/ml was revealed by two (2.04%), four (4.08%), two (2.04%) and eight (8.16%) of the samples tested, respectively. A serum titre of 40 IU/ml was showed by seven (7.14%) of the 98 samples tested by STAT.

Out of the 72 female pig serum samples tested by STAT, 20 samples were positive for *Brucella* agglutinins. Agglutination titre of the samples ranged between 20 IU/ml and 640 IU/ml. The highest serum titre, 640 IU/ml, was revealed by two (2.78%) of the samples tested. Two (2.78%) of the samples tested had a titre of 320 IU/ml, whereas five (6.94%) samples revealed a titre of 160 IU/ml. The lowest titre, 80 IU/ml, which is indicative of a positive reaction for brucellosis was shown by 11 (15.28%) of the samples tested. Nine (12.5%) of the 72 samples had a titre suspicious for brucellosis.

The overall seropositivity of the slaughtered pig serum samples was 20 per cent of the 170 samples tested by HIT. Of the 98 male samples subjected to the test, 16 were found positive. The titre ranged between 20 IU/ml and 2560 IU/ml. Only one of these animals revealed the titre of 2560 IU/ml. Samples from four (4.08%) animals each had a titre of 320 IU/ml and 160 IU/ml, respectively. Seven (7.14%) of these

samples revealed that a titre of 80 IU/ml. The samples from 3 (3.06%) animals had a titre suspicious for brucellosis.

The *Brucella* agglutinin titre of 72 female pig serum samples ranged from 20 IU/ml and 640 IU/ml by HIT. The samples from two (2.78%) animals had an aggglutination titre of 640 IU/ml whereas another two animals revealed a titre of 320 IU/ml. An agglutination titre of 160 IU/ml and 80 IU/ml was revealed by three (4.17%) and 11 (15.28%) of 72 samples tested, respectively. Thus 18 were positive by the test. Eight (11.1%) of the samples tested gave an agglutination titre suspicious for brucellosis.

The serum samples from 170 slaughtered pigs subjected to 2-MET revealed an overall seropositivity of 15.88 per cent. One of the 98 samples of male pigs tested revealed a titre as high as 2560 IU/ml. The *Brucella* agglutinin titre of 80 IU/ml, 160 IU/ml and 320 IU/ml was revealed by four (4.08%), five (5.1%) and two (2.04%) of the samples tested. Serum samples of four (4.08%) animals revealed a titre suspicious for brucellosis.

In 2 MET, the *Brucella* agglutinin titre of 72 female pig samples ranged between 20 IU/ml and 640 IU/ml. One of the samples had a titre of 640 IU/ml and in another one it was 320 IU/ml. The number samples which had an agglutination titre

of 80 IU/ml and 160 IU/ml was 8 (11.1%) and 5 (6.9%), respectively. Three of the samples tested revealed a titre of 40 IU/ml.

The 170 samples of slaughtered pigs tested by EAT revealed an overall seropositivity of 15.88 per cent. The agglutination titre of 98 samples from male pigs varied from 20 IU/ml and 5120 IU/ml. Only one of these samples revealed the highest titre. Three (3.06%) samples showed a titre of 320 IU/ml whereas four (4.08%) samples each had a titre of 160 IU/ml and 80 IU/ml, respectively. Six (6.12%) of the samples showed a titre of 40 IU/ml.

Among 72 serum samples from female pigs 15 were seropositive by EAT. One of the samples revealed a titre as high as 640 IU/ml. The samples from two (2.78%) pigs had a titre of 320 IU/ml. Three (4.16%) of these samples showed an agglutination titre of 160 IU/ml and in nine (9.18%) samples it was 80 IU/ml. A titre suspicious for brucellosis was revealed by eight of these samples.

The results of RBPT, STAT, HIT, 2-MET and EAT on 85 serum samples of farm fed pigs consisting of 82 females and three males are given in Table 3.

Table 3 Seropositivity revealed by farm animals with different tests for brucellosis

Sex	No. of samples	No. of samples positive by each test				
		RBPT	STAT	HIT	2-MET	EAT
Males	3	-	-	-	-	-
Females	82	7 (8.54)	7 (8.54)	7 (8.54)	5 (6.10)	6 (7.32)
Total	85	7 (8.24)	7 (8.24)	7 (8.24)	5 (5.88)	6 (7.06)

Figures in parenthesis indicate per cent

The samples from three male pigs did not reveal positive *Brucella* agglutinin titre in any of the above tests. In RBPT seven (8.54%) of the 82 samples of the female pigs showed agglutination positive for brucellosis.

STAT revealed an overall seropositivity of 8.24 per cent. The highest serum titre revealed by the serum samples was 160 IU/ml. Among the seven female samples positive for brucellosis, two (2.44%) had a titre of 160 IU/ml and five (6.1%) had a titre of 80 IU/ml. Among the samples, 16 (19.51%) had a titre suspicious for brucellosis.

In heat inactivation test, the per cent of seropositivity revealed by the 82 serum samples of farm fed female pigs was 8.54. The HIT titre in the samples ranged from 20 IU/ml to 80 IU/ml. Seven (8.54) of these samples had a titre of 80 IU/ml.

The 2-mercaptoethanol test on 82 female pig serum samples showed seropositivity of 6.10 per cent. All samples positive for brucellosis had a *Brucella* agglutinin titre of 80 IU/ml. Three of these samples had an agglutinin titre suspicious for brucellosis.

The overall seropositivity for brucellosis among the 82 serum samples of farm fed female pigs was 7.32 per cent by EAT. Among these samples, six had a titre of 80 IU/ml. But three of these samples revealed a titre suspicious for brucellosis.

The results of RBPT, STAT, HIT, 2-MET and EAT done on 250 human serum samples are shown in Table 4.

Table 4 Seropositivity revealed by human serum samples with different tests for brucellosis

Sex	No. of samples	No. of samples positive by each test				
		RBPT	STAT	HIT	2-MET	EAT
Male	140	1 (0.71)	1 (0.71)	1 (0.71)	1 (0.71)	1 (0.71)
Female	110	3 (2.73)	3 (2.73)	3 (2.73)	3 (2.73)	3 (2.73)
Total	250	4 (1.60)	4 (1.60)	4 (1.60)	4 (1.60)	4 (1.60)

Figures in parenthesis indicate per cent

One hundred and twenty two human serum samples collected from the general population were subjected to RBPT, STAT, HIT, 2-MET and EAT and the results are given in Table 5.

Table 5 Seropositivity revealed by human samples from general population with different tests for brucellosis

Sex	No. of samples	No. of samples positive by each test				
		RBPT	STAT	HIT	2-MET	EAT
Males	57	1 (1.75)	1 (1.75)	1 (1.75)	1 (1.75)	1 (1.75)
Females	65	2 (3.07)	2 (3.07)	2 (3.07)	2 (3.07)	2 (3.07)
Total	122	3 (2.45)	3 (2.45)	3 (2.45)	3 (2.45)	3 (2.45)

Figures in parenthesis indicate per cent

Two of the 65 samples from females and one of the 57 samples from males showed agglutination indicative of brucellosis by RBPT. The overall per cent of samples revealed agglutination indicative of the disease was 2.45 by STAT. The only one positive sample out of 57 male samples had a titre of 80 IU/ml by RBPT, STAT, HIT, 2-MET and EAT. Among the 65 female samples, two were found seropositive for brucellosis by RBPT, STAT, HIT, 2-MET and EAT. The STAT revealed a titre of 160 IU/ml and 320 IU/ml in these samples. The remaining tests, HIT, 2-MET and EAT, in these samples showed a titre of 80 IU/ml and 160 IU/ml correspondingly.

Serum samples from 88 final year veterinary students were tested by RBPT, STAT, HIT, 2-MET and EAT and the results are given in Table 6.

Table 6 Seropositivity revealed by samples from veterinary students with different tests for brucellosis

Sex	No. of samples	No. of samples positive by each test				
		RBPT	STAT	HIT	2-MET	EAT
Males	56	-	-	-	-	-
Females	32	1 (3.13)	1 (3.13)	1 (3.13)	1 (3.13)	1 (3.13)
Total	88	1 (1.14)	1 (1.14)	1 (1.14)	1 (1.14)	1 (1.14)

Figures in parenthesis indicate per cent

Out of the 32 female samples examined, only one was found positive with all the tests. The sample revealed a titre of 80 IU/ml by the tests except RBPT. None of the samples from male students revealed agglutination reaction by the tests.

Serum samples from 20 veterinarians (6 females and 14 males) and 20 pig farm attendants (7 females and 13 males) were screened serologically for *Brucella* agglutinins using RBPT, STAT, HIT, 2-MET and EAT. None of the samples yielded a positive reaction with any of the tests.

Discussion

DISCUSSION

Among the livestock, pig is the only one species reared exclusively for meat production. In Kerala, pig is being reared by small farmers as a means of supplementary occupation to increase their income and also reared on an industrial basis in farms. Pigs act as a reservoir/carrier of many zoonotic disease agents and is one of the animal sources for human brucellosis. *Brucella suis* is the primary agent which causes brucellosis in pigs. The agent is capable of causing severe disease in human than that caused by all other species of Brucellae except *B. melitensis*. The important aspect of the disease is two fold, viz., the public health significance and the economic loss to the animal industry. The significance of brucellosis thus includes not only direct or indirect transmission of the disease from infected animals to human and consequent illness, physical incapacity and loss of manpower, but also the serious diminution of much needed foodstuffs, especially animal proteins, which are essential to human health and well being.

The confirmatory diagnosis of the disease can be done by isolation and identification of the causative agent. As this method is not always successful and being very difficult, the serological methods are adopted for the diagnosis of the disease. At present no vaccine is commercially available for

the control of the disease in pigs. The only practical method of control still remains to be the removal of seropositive pigs from the herd.

During the present investigation 255 serum samples from pigs and 250 from human were tested by RBPT, STAT, HIT, 2-MET and EAT. Among the pig samples screened, 26 (10.26%) had revealed agglutination/agglutination titre positive for brucellosis by all the above tests. Four (1.6%) of the human samples also revealed a similar reaction. The serum of slaughtered pigs were collected from the Bacon factory Koothattukulam. All these pigs were brought from different parts of Kerala, which were reared by individual farmers at their household and/or reared in organised farms. In this group of pigs the overall seropositivity by RBPT was 15.88 per cent. Per cent of animals seropositive by STAT was 21.76. The overall seropositivity found in slaughtered pigs in the present investigation was higher as compared to the 14.3 per cent reported by Sen *et al.* (1972) and 5.63 per cent reported by Kalimuddin and Choudhury (1988).

The rate of infection found in the males and females among the slaughtered animals were higher with that of the two per cent of females and 2.94 per cent of male samples from slaughtered pigs recorded by Sen *et al.* (1972). Kalimuddin and Choudhury (1988) also recorded that the seropositivity in

slaughtered female pigs (9.43%) was higher than in males (4.37%). The higher sero-prevalence of brucellosis among slaughtered pigs observed in the present study may be viewed seriously since the disease can be transmitted to human during the handling of infected animals at various stage of slaughter and also through their products.

Heat inactivation test could detect 20 per cent of 170 samples from slaughtered pigs as positive for brucellosis. 2-mercapto ethnol test, EAT and RBPT revealed 15.88 per cent of the samples as positive for the disease. Out of the 170 samples a doubtful agglutination titre of 40 IU/ml was observed in 16 9.41%), 11 (6.47%), 7 (4.12%) and 14 (8.23%) samples by STAT, HIT, 2 MET and EAT, respectively.

The samples which revealed negative reaction by STAT did not show a doubtful and positive reaction by other tests used in this study. Similarly other tests did not produce higher agglutination titre than STAT for the given sample. Thus STAT was found more sensitive when compared to other tests.

The sera collected from farm animals were subjected to all the tests used in the study. But none of the three samples from male animals revealed a positive titre for the disease. However, the exact picture about the disease in farm fed male pigs will be clear only after testing large number of animals. The overall per cent of reactor pigs in RBPT, STAT

and HIT was 8.24. The overall seropositivity observed by STAT was high as compared to 11.33 per cent recorded by Kumar and Rao (1980). The per cent of seropositivity in the study was lower with that of 5.6 per cent reported by Ghosh (1989). 2-mercapto ethanol test could detect 5.86 per cent of the samples as seropositive but EAT found 7.06 per cent. The number of animal serum samples which revealed an agglutination titre of 40 IU/ml by STAT, HIT and 2-MET was 16, five and three, respectively. Ethylene diamine tetra acetate agglutination test also revealed three samples with the titre 40 IU/ml. The overall prevalence found in this study was slightly higher as compared to the 4.97 per cent reported by Chowdhary *et al.* (1983).

In the present study, the occurrence of disease is more in slaughter animals. Sometimes animals showing ill health and poor reproductive performance are send for slaughter. There is also a chance that *Brucella* infected animals are send for slaughter, either knowingly or unknowingly. These may be the reason for high rate of occurrence of the disease in slaughtered animals.

During the study 170 slaughtered pig serum and 85 farm fed live pig serum samples were tested by RBPT, STAT, HIT, 2-MET and EAT. Out of the 255 samples, 26 (10.20%) were found positive for the disease by all these tests. STAT detected 44

(17.25%) of the samples as seropositive. The reason for the higher sensitivity with STAT could be attributed to the fact that all antibodies against *Brucella* organism such as IgA, IgM, IgG and IgG_2 react with *Brucella* antigen used in STAT (Joint FAO/WHO, 1986). The test also give reaction with various non-specific agglutinins found in animals which are not infected with *Brucella* organisms.

Followed by STAT, HIT detected 41 (16.08%) out of 255 pig serum as positive for brucellosis. On examination of the result it is clear that three of the samples which had positive *Brucella* agglutination titre in STAT had reduced it's titre as doubtful. Reduction in the titre was also observed in some samples which produced 40 IU/ml by STAT. A closer examination of the test result indicate that such reduction in agglutination titre after heat inactivation of sera was mostly seen in samples with titre of 80 IU/ml or below by STAT. This reduction in titre on heat inactivation of the sera could be attributed to the fact that the alteration or modification or inactivation or destruction of the heat labile non-specific agglutinins present in the sera of pigs. The heat labile agglutinating activity has been associated with poorly defined macroglobulin and microglobulin serum components (Corbel, 1985).

Of the various rapid slide or plate agglutination tests used for the detection of *Brucella* antibodies in the serum of infected animals, the most effective is Rose Bengal plate test. Out of 255 pig serum samples tested by RBPT, 34 (13.33%) were found positive. In this test, the number of samples which showed a positive reaction were lower than that of STAT and HIT. This lower seropositivity could be attributed to the use of stained, acidified, buffered, *Brucella* antigen with a low pH (3.65 or 4.0) which might have nullified the effect of acid-labile non specific agglutinins which are associated with poorly defined macroglobulin and microglobulin serum components. Thus the number of samples which showed positive reaction by the test indicate that acid-labile agglutinating components are more than that of the heat labile components.

Ethylene diamine tetra acetate agglutination test could detect 33 (12.94%) of 255 pig serum as positive for brucellosis. The test was pushed back to the fourth place, comparatively, in identifying the positive samples. Eleven of the STAT positive samples were found negative by EAT. This difference between STAT and EAT in the detection of *Brucella* antibodies in the serum of pigs can be attributed to the fact that the sodium salt of EDTA used in the EAT blocks the agglutination between *Brucella* cell antigen and the EDTA-labile non-specific agglutinins by inactivating the

latter. However, Joint FAO/WHO (1986) suggested that when tube agglutination test is employed in testing animals for the purpose of international trade, the agglutination reactions caused by EDTA labile agglutinins should be avoided.

Only 32 (12.55%) of the 255 samples produced positive reaction by 2-MET. A closer examination of the result of the present study indicate that 12 of the serum showed positive reaction by STAT were found negative by 2-MET. This could be due to the treatment of serum with 2-mercaptoethanol, a sulphydryl reducing agent, which dissociates the IgM pentamer and reduces its agglutinating activity without affecting that of the IgG isotype. Therefore, it can be regarded that the 32 samples which revealed a positive reaction by 2-MET had active infection. Further, most of the non-specific agglutinins are attached to IgM group of antibodies. The above reasons might be attributed to the detection of least number of samples as positive for the disease by the test.

The distribution of serum samples which reveals the highest titre in STAT, HIT, 2-MET and EAT are showed in Fig.1. The maximum number of samples showing agglutination titre was observed in STAT and minimum in 2-MET. It was observed that when the titre of a sample in one test was more than 80 IU/ml then the titre of the sample was almost similar in other tests also. That is, at higher titres, the chances of non-specific

agglutination are less. From this study it was observed that when a sample have a titre of 160 IU/ml and above in STAT, such sample always revealed a titre positive for brucellosis in other tests used in this study.

The percentage of samples positive by different tests are given in Fig.2. STAT revealed highest per cent of samples as positive for the disease. Followed by this test, in the order of preference of test which showed the highest per cent of samples as positive for the disease was HIT, RBPT, EAT and 2-MET. The per cent of samples positive by all the tests used in this study is demarcated by a dotted line.

Chowdhury *et al.* (1983) reported a higher sensitivity for STAT as compared to HIT. The findings of the present investigation confirms the observation made by Chand and Sadana (1988) and Barbuddhe *et al.* (1994), whereas Stemshorn *et al.* (1985) and Ghosh and Nanda (1988) observed that RBPT was more sensitive than STAT.

The efficacy of STAT compared to HIT and 2MET observed in the investigation was similar to that observed by Kalimuddin *et al.* (1990), Ghani *et al.* (1994) and Ghani (1995). The result of the present study shows that pig sera contain considerable amount of EDTA labile non-specific antibodies. Nielsen *et al.* (1979) also reported the presence of such non-specific antibodies in the sera of cattle.

On the basis of the observations made in this study it is suggested that a combination of RBPT and EAT may be used for the diagnosis of porcine brucellosis as compared to RBPT and STAT. EAT is preferred over 2-MET and HIT since it is easy to perform and the interference with non-specific agglutinins in the test is too low. Whereas as STAT gives too many non-specific reactions.

Human sera collected from general population revealed an overall seropositivity of 2.45 per cent which indicate the existence of disease in the general population. The prevalence observed in the study is slightly lower to the recorded 2.97 per cent (Kapoor *et al.*, 1985) and higher in comparison with the 0.95 per cent recorded by Masoumi *et al.* (1992). In the present investigation, a high prevalence was found in women (3.09%) than that observed in men (1.75%). This finding is in accordance with the observation made by Kapcor *et al.* (1985). Contrary to the finding in the present study, Masoumi *et al.* (1992) reported a high prevalence of the disease in males. The higher prevalence in females may be attributed to the fact that females are more frequently engaged than the males in various animal husbandry activities like watering and feeding of animals, cleaning of animal and animal shed, and also in preparation of meat and meat products. Thus the chances of exposure to infectious agent is more for them.

A prevalence of 1.14 per cent was observed in the veterinary students while the veterinarians and pig farm attendants were found free of the disease. This observation in the study differ from those of Vanhoye (1983) and Russo *et al.* (1984) who reported 13 and 10 per cent prevalence in students, respectively. Contrary to the observation made in the study, Masoumi *et al.* (1992) recorded that the students were free of the disease. Veterinarians and animal attenders in the study group were found free of the disease against a high prevalence of 20 per cent of working staff (Savalgi *et al.*, 1987) and 0.11 per cent of animal attendants (Kalimuddin *et al.*, 1990). This low prevalence of the disease in veterinary students and the absence of it is veterinarians and animal attendants might be attributed to the low prevalence of disease in farm animals, lesser contact with infected animals as well as high hygienic standard maintained by them. However, exact presence and extend of the disease in this group can only be found out by doing the tests in large number of samples.

Control of the brucellosis should be an attractive economic proposition to the farmers and others engaged in animal production. Unfortunately, in practice, the prospects of immediate losses through elimination of infected animals and the inconvenience caused by repeated testing may outweigh in the mind of farmer than the long term advantages of control

at a remote date in future. Thus it is necessary to explain to all the concerned, the rationale and the advantages of control programme, especially the continuing economic benefits, and elimination of serious risk to human beings, including health of farmer himself, of his family, and of other animal handlers. This is the main task to be met and made possible by brucellosis control health education programmes.

Summary

SUMMARY

Brucellosis is an important bacterial disease affecting swine which has great economic importance, since it causes abortion, still-birth, sterility and other complications in pigs and also has a public health significance as it affects human beings. The present study was undertaken to assess the presence and extent of brucellosis in slaughtered and farm reared pigs, animal handlers and people belonging to the general population by Rose Bengal plate test (RBPT), Standard tube agglutination test (STAT), Heat inactivation test (HIT), 2-mercaptoethanol test (2-MET) and by Ethylene diamine tetra acetate agglutination test (EAT). An attempt was also made to compare the efficiency of the above serological tests in detecting Brucella agglutinin in the serum of pigs and human beings.

A total of 255 serum samples were collected from pigs. Among this, 170 were collected from pigs slaughtered at Meat Products of India, Koothattukulam. Samples were collected from University Pig Breeding Farm, Mannuthy (31) and from Government Pig Breeding Farms at Angamaly (40) and Kunnamkulam (14). One hundred and twenty two human samples were collected from those belonging to general population. Serum samples were also collected from 88 final year veterinary students, 20 veterinarians and 20 pig farm attendants.

Out of the 98 male pig samples among the 170 slaughtered animals, 13 (13.26%) were positive with RBPT. Standard tube agglutination test revealed 17 (17.35%) samples with a positive titre while only 16 (16.33%) had a diagnostic titre for the disease by HIT. Twelve (12.24%) samples each were positive with 2-MET and EAT. Among the 72 female pig samples 14 (19.44%) were positive by RBPT. Twenty (27.78%) samples revealed positive titre with STAT whereas only 18 (25%) had a titre positive for the disease by HIT. Of the samples, 15 (20.83%) had a diagnostic titre for brucellosis by 2-MET and EAT.

The 85 samples from farm animals consisted of three male and 82 female pigs. The sera of male pigs did not show an agglutination titre positive for brucellosis by any of the tests. Seven (8.54%) out of the 82 female pig samples showed an agglutination positive for the disease by RBPT. Standard tube agglutination test and HIT also revealed seven (8.54%) positive samples. Only five (6.10%) samples had revealed a titre positive for the disease by both 2-MET and EAT.

All the tests used in the study gave a positive reaction with 26 (10.2%) pig serum samples. Standard tube agglutination test detected the highest number of samples (44) as positive for brucellosis. Followed by this in the order of preference of the tests which detected the highest number of

samples positive for the disease were HIT (41), RBPT (34), EAT (33) and 2-MET (32). The observation in the study indicate that non-specific agglutinins present in the sera can influence mostly the results of STAT, as compared to other serological tests used in the study. Based on the findings of the present study it is suggested that EAT can be used as a complementary test for clarification of suspicious or marginally positive results in STAT or RBPT.

Among the human sera from general population, one out of 57 males and two out of 65 females were found positive for brucellosis with all the tests used in the study. Among the serum samples of 88 final year veterinary students subjected to the tests, only one out 32 lady students was found positive with all the tests. All the samples collected from veterinarians and pig farm attendants were found negative for brucellosis. The overall existence of the disease in different categories of pigs and people from the general population warrants the urgent need for evaluation of the existence of the disease in various classes and categories of people as well as animals; to establish effective control of the disease, both in animals and human beings, and thereby preventing the economic loss due to animal and human disease and also to protect the public health.

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PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS IN PIGS

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree of

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1998

ABSTRACT

Brucellosis is one of the bacterial diseases which affect pigs and produce severe economic loss to the farmers since it causes abortion, still birth, sterility and other complications in pigs. The disease in pigs is also a threat to public health because the etiologic agent can be readily transferred from infected pigs to human beings and can cause the disease in them. Considering the above facts, a serological study was undertaken to assess the extent of the disease present in pigs as well as human beings. During the investigation, serum samples from 255 pigs and 250 human were collected and were subjected to Rose Bengal Plate test (RBPT), Standard tube agglutination test (STAT), Heat inactivation test (HIT), 2-mercaptoethanol test (2-MET) and by Ethylene diamine tetra acetate agglutination test (EAT), to detect the presence Brucella agglutinin in these sera.

One hundred and seventy samples were collected from slaughtered pigs of which 37 (21.76%) samples gave a positive titre with STAT but only 34 (20%) samples had a positive titre by HIT. Twenty seven (15.88%) out of 170 samples gave positive test reaction by RBPT, 2-MET and EAT.

Eighty five samples were collected from the farm reared pigs. Seven (8.24%) of these samples revealed positive

agglutination reaction by RBPT, STAT and HIT. 2-mercaptoethanol test could detect only five (5.88%) of their samples as positive for brucellosis whereas six (7.06%) samples showed positive test reaction by EAT. Out of the 255 serum samples collected from pigs, 26 (10.2%) were found positive with all the tests.

Among the 250 human serum samples screened, four of them were found positive for the disease with all the tests. Of the positive samples one male and two female samples were obtained from general population. One of the 88 samples collected from veterinary students was found positive by the tests used in this study. Twenty samples each collected from veterinarians and pig farm attendants were found negative for the disease.

Out of all the tests used in this study, STAT was able to detect maximum number of reactors, followed by HIT, RBPT, EAT and finally by 2-MET. The reasons for differences in seropositivity with different tests on the same sample were discussed.

Appendix

Appendix-I

Agglutination/agglutination titres shown by the serum samples of pigs and human in different serological tests

Samples from slaughtered male pigs

Sl. No.	RBPT	STAT (IU/ml)	HIT (IU/ml)	2-MET (IU/ml)	EAT (IU/ml)
1.	+	640	160	160	160
2.	+	640	320	320	320
3.	-	40	20	-	20
4.	-	40	-	-	20
5.	+	160	160	80	80
6.	+	320	320	160	320
7.	+	5120	2560	2560	5120
8.	-	80	80	40	40
9.	+	320	320	160	160
10.	-	40	20	20	40
11.	+	320	160	160	160
12.	-	20	20	-	-
13.	-	40	40	-	
14.	-	20	20	-	
15.	-	20	20	-	-
16.	-	20	-	-	-
17.	-	20	-	-	-
18.	-	20	20	20	20
19.	-	40	40	40	40
20.	-	20	-	-	-
21.	-	20	-	-	
22.	-	20	20	-	20
23.	-	40	20	-	20
24.	-	20	-	-	-
25.	+	80	80	80	80
26.	-	20	-	-	-
27.	-	20	-	-	-
28.	-	80	20	20	20
29.	-	20	20	-	-
30.	-	20	-	-	-
31.	-	20	20	-	20
32.	-	20	-	-	-
33.	+	80	80	40	80
34.	-	20	20	20	20
35.	-	20	20	-	-
36.	-	80	80	80	40
37.	+	320	320	320	320
38.	+	160	160	160	160
39.	-	80	80	80	40
40.	-	80	80	-	40
41.	+	80	80	40	80
42.	+	40	40	-	-
43.	-	-	-	-	-

Samples from slaughtered female pigs

Sl. No.	RBPT	STAT (IU/ml)	HIT (IU/ml)	2-MET (IU/ml)	EAT (IU/ml)
1.	+	160	160	160	80
2.	+	80	80	80	80
3.	-	40	40	-	20
4.	-	20	20	-	-
5.	+	160	160	160	160
6.	+	160	160	160	80
7.	-	40	40	-	40
8.	-	80	80	40	40
9.	-	40	20	-	20
10.	+	320	320	160	320
11.	+	640	640	320	640
12.	-	40	40	-	40
13.	-	20	-	-	-
14.	-	80	80	40	80
15.	-	40	40	-	20
16.	-	40	20	-	-
17.	+	160	80	80	80
18.	-	20	20	-	20
19.	-	20	20	-	20
20.	-	20	-	-	-
21.	-	40	20	20	40
22.	-	40	40	-	20
23.	-	80	80	80	80
24.	-	80	40	20	-
25.	-	80	40	40	80
26.	+	80	80	-	40
27.	-	20	-	-	20
28.	-	20	-	-	-
29.	-	20	-	-	20
30.	-	20	-	-	-
31.	+	320	320	160	160
32.	-	80	80	80	40
33.	+	40	40	-	40
34.	-	80	80	80	80
35.	+	640	640	640	320
36.	+	80	80	80	40
37.	+	160	80	80	160
38.	+	80	80	80	80

Samples from farm fed female pigs

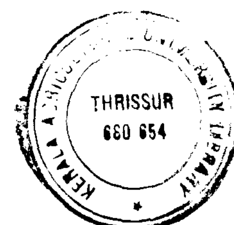
Sl. No.	RBPT	STAT (IU/ml)	HIT (IU/ml)	2-MET (IU/ml)	EAT (IU/ml)
1.	-	40	-	-	-
2.	+	160	80	80	80
3.	-	40	40	40	40
4.	-	80	80	-	80
5.	-	40	-	-	-
6.	-	40	-	-	-
7.	-	40	-	-	-
8.	+	80	80	40	40
9.	-	40	40	-	-
10.	-	40	-	-	-
11.	-	40	40	-	-
12.	-	40	-	-	
13.	-	40	40	40	40
14.	-	40	-	-	
15.	+	40	-	-	-
16.	+	80	80	80	80
17.	-	20	-	-	-
18.	-	40	40	-	-
19.	-	20	20	-	-
20.	-	40	20	-	-
21.	-	20	-	-	-
22.	+	80	80	80	80
23.	-	40	20	-	-
24.	+	160	80	80	80
25.	-	40	20	-	-
26.	+	80	80	80	80

Samples from human (male)

Sl. No.	RBPT	STAT (IU/ml)	HIT (IU/ml)	2-MET (IU/ml)	EAT (IU/ml)
1.	+	80	80	80	80

Samples from human (female)

Sl. No.	RBPT	STAT (IU/ml)	HIT (IU/ml)	2-MET (IU/ml)	EAT (IU/ml)
1.	+	160	80	80	80
2.	+	320	160	160	160
3.	+	80	80	80	80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