Comparative Serological Studies ON Caprine Brucellosis in and Around Patna

THESIS

SUBMITTED TO THE

RAJENDRA AGRICULTURAL UNIVERSITY

PUSA (SAMASTIPUR) BIHAR

(FACULTY OF POST-GRADUATE STUDIES)

In partial fulfilment of the requirement

FOR THE DEGREE OF

Master of Veterinary Science

IN

MICROBIOLOGY

By

Dr. Akhileshwar Thakur

Registration No. - M/VM/60/2001-2002

Department of Veterinary Microbiology

BIHAR VETERINARY COLLEGE

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P A T N A (BIHAR)

2004

Dedicated to

benevolent adorable

My Parents

DEPARTMENT OF VETERINARY MICROBIOLOGY
BIHAR VETERINARY COLLEGE, PATNA-14
RAJENDRA AGRICULTURAL UNIVERSITY
PUSA (SAMASTIPUR) , BIHAR.

CERTIFICATE-I

This is to certify that thesis entitled ***"Comparative serological studies on caprine brucellosis in and around Patna"*** submitted in partial fulfilment of the requirements for the Degree of **Master of Veterinary Science (Veterinary Microbiology)** of the Faculty of post-graduate studies, Rajendra Agricultural University, Pusa, Samastipur, Bihar is the record of bonafide research work carried out by **Dr. Akhileshwar Thakur** Registration no. M/VMC/60/2001-2002, under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received during the course of this investigation and preparation of the thesis have been fully acknowledged.

16/1/2004

(***B. K. Sinha***)

M.Sc. (AIIMS), Ph.D.

Major Advisor

Chairman & Head

Department of Veterinary Microbiology

Bihar Veterinary College, Patna - 800014

DEPARTMENT OF VETERINARY MICROBIOLOGY
BIHAR VETERINARY COLLEGE, PATNA-14
RAJENDRA AGRICULTURAL UNIVERSITY
PUSA (SAMASTIPUR) , BIHAR.

CERTIFICATE-II

We, the undersigned members of the Advisory Committee of **Dr. Akhileshwar Thakur**, Registration No. M/VMC/60/2001-2002, a candidate for the Degree of Master of Veterinary Science with Major in **Veterinary Microbiology** have gone through the manuscript of the thesis and agree that the thesis entitled ***"Comparative serological studies on caprine brucellosis in and around Patna"*** may be submitted by **Dr. Akhileshwar Thakur** in partial fulfilment of the requirements for the degree.

(*B.K. Sinha*) 16/01/04

Chairman, Advisory Committee

Members of the Advisory Committee :

1. **Dr. S.P. Verma, Chairman and Head,**
   Department of Veterinary Medicine,
   Bihar Veterinary College, Patna-14

S.P. Verma 16.01.04

2. **Dr. S.R.P. Sinha, Assoc. Prof. and Head,**
   Department of Veterinary Parasitology &
   Head, Deptt. of Pathology
   Bihar Veterinary College, Patna-14

16/1/04

3. **Dr. J.N. Singh (Nominee of the D.R.I. cum -Dean P.G. Studies),**
   Assoc. Prof. & Head Department of L.P.T.
   Bihar Veterinary College, Patna-14

16/01/04.

DEPARTMENT OF VETERINARY MICROBIOLOGY
BIHAR VETERINARY COLLEGE, PATNA-14
RAJENDRA AGRICULTURAL UNIVERSITY
PUSA (SAMASTIPUR) , BIHAR.

CERTIFICATE - III

This is to certify that the thesis entitled ***"Comparative serological studies on caprine brucellosis in and around Patna"*** submitted by **Dr. Akhileshwar Thakur**, Registration No. M/VMC/60/2001-2002, in partial fulfilment of the requirements for the Degree of **Master of Veterinary Science (Veterinary Microbiology)** of the Faculty of Post-Graduate studies, Rajendra Agricultural University, Pusa, Samastipur, Bihar, was examined and approved on .22.3..... 2004.

(***B.K. Sinha***)

Chairman, Advisory Committee

Members of the Advisory Committee :

1. **Dr. S.P. Verma, Chairman and Head,**
   Department of Veterinary Medicine,
   Bihar Veterinary College, Patna-14

2. **Dr. S.R.P. Sinha, Assoc. Prof. and Head,**
   Department of Veterinary Parasitology &
   Head, Deptt. of Pathology
   Bihar Veterinary College, Patna-14

3. **Dr. J.N. Singh (Nominee of the D.R.I. cum -Dean P.G. Studies),**
   Assoc. Prof. & Head Department of L.P.T.
   Bihar Veterinary College, Patna-14

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Place – Patna

Date - 16.01.2004

Akhileshwar Thakur

(Akhileshwar Thakur)

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ABBREVIATIONS

AGIT	:	Agar Gel Immunodiffusion Test
AGT	:	Antiglobulin Test
B-A ELISA	:	Biotin-Avidin-ELISA
BAPAT	:	Buffered Acidified Plate Antigen Test
BPAT	:	Buffered Plate Antigen Test.
Br.	:	Brucella
C-ELISA	:	Competitive-ELISA
CFT	:	Complement Fixation Test
CIEPT	:	Counter Immunelectrophoresis.
CSPA	:	Cytoplasmic Soluble Protein Antigen
ELISA	:	Enzyme-linked immunosorbent assay.
I-ELISA	:	Indirect - ELISA
IHAT	:	Indirect Haemagglutination Test.
2-ME	:	2-mercaptoethanol.
MRT	:	Milk Ring Test.
P-ELISA	:	Plate-ELISA
PT	:	Plate Agglutination Test.
RBPT	:	Rose Bengal Plate Test.
RBT	:	Rose Bengal Test.
RPAT	:	Rapid Plate Agglutination Test.
SAT	:	Serum Agglutination Test.
SDTH	:	Skin Delayed Type Hyper sensitivity.
SPAT	:	Standard Plate Agglutination Test.
SSAT	:	Slow Serum Agglutination Test.
STAT	:	Standard Tube Agglutination Test.
TAT	:	Tube Agglutination Test.

CHAPTER - I

INTRODUCTION

INTRODUCTION

Brucellosis is a contagious bacterial anthropozoonosis of worldwide occurrence. It is also widely prevalent in almost every state of India. It is known by various synonyms like Bang's disease, Contagious abortion, Infectious abortion, Undulent fever, Malta fever, Mediterranean fever etc..

David Bruce (1887) isolated the causative agent of Malta fever in man on the Island of Malta and subsequently named the organism *Micrococcus melitensis*. In 1897, Bang in collaboration with Stribolt, isolated a small bacillus from cows suffering from infectious abortion and named it *Bacillus abortus*.

Alice Evans (1918) was the first to recognize the close morphological, biochemical and serological resemblance between the *Micrococcus melitensis* of Bruce (1887) and *Bacillus abortus* of Bang (1897). This view was supported by Bevan (1921-22) and Keefer (1924) and several other workers from many parts of the world (Stableforth, 1959). These closely related organisms have now been placed in Genus Brucella, a name given on the suggestion of Meyer and Shaw (1920) in honour of Sir David Bruce.

The disease is caused by various species of Genus Brucella viz. *Br. melitensis, Br. abortus, Br. ovis, Br. canis, Br. suis and Br. neotomae*. Goats are the principal hosts of *Br. melitensis* and this organism is the chief causative agent of caprine brucellosis. There are three biovars of the *Br. melitensis* organism and these all three types have been isolated in India (Polding, 1942). This facultative intracellular organism is reasonably resistant to environmental influences and under suitable conditions can

survive for over one year in environment. But they are readily killed by the common disinfectants.

Brucellosis in female goat is characterized by late abortion, retained placenta and infertility. The gravid uterus is prediliction sheet for brucella organisms because it contains erythritol, a chemical substance produced by foetus which stimulates the growth of brucella organisms (Cruickshank, 1968). The organism has also prediliction for the udder, lymphnodes, joint capsules, bursae, testicle and accessory male sex glands. Infection in male goat is characterized by depression, fever, septicaemia, arthritis, hygroma, orchitis (which is frequently unilateral) and weight loss.

Br. melitensis is the most envasive and pathogenic for human then other species of the Genus Brucella (Blood *et al.*, 1995). In human it causes 'Malta' or 'Mediterranean' fever which is transmitted by direct contact (even through intact skin also) with infected animals or their aborted tissue materials, ingestion of unpasteurized milk and or milk products from infected animals. Occupational exposure to farmers (cultures that cohabit with their animal), veterinarians, abattoir workers as well as people involved in processing of goat skin may occur. Large number of organisms are excreted at and following parturition providing source of infection.

In India, about 20% of the rural population suffer at any time from pyrexia of unknown origin (PUO) and 1-2% of such pyrexia are due to brucellosis (Chakrabarti, 1997).

Caprine brucellosis is a global problem with great economic and public health implications. It is a disease of great economic importance in small ruminants reared under intensive and semi-intensive system of management and perhaps the most important disease of zoonotic

significance. Brucellosis inflicts heavy economic losses by way of abortion, still birth and early death in young stock. It also results in reduced fertility and loss of milk production. Singh *et al.* (1994) reported that 13.4% kids lost due to abortions and stillbirth in organized goat herds.

The goat (*Capra hircus*) is a versatile animal and is mainly reared in tropical countries like India. With a total population of 118.3 million goats, India ranks first in the world (FAO, 1994) and with a total of 17459 thousand goats, Bihar stands first among the states in our country (Govt. of India, Annual report 1999-2000). Goat is better known as 'poor man's cow' all over the world, 'wet nurse' in Europe, 'Swiss baby's foster-mother' in Switzerland (milch goat) and also 'walking refrigerator' for the storage of milk and can be milked number of times in a day.

Goat is a mini cow which can be easily reared by poor farmers and landless labourers with least investment. We are having 20 well known Indian goat breeds, apart from a number of local non-descript breeds scattered throughout the our country (Banerjee, 1998). The goat contributes about 2.20 million tonnes of milk, 0.47 million tonnes of meat and 0.109 million tonnes of skin annually (FAO, 1994). Thus goat rearing plays an important role to overcome poverty and unemployment by improving socioeconomic conditions of weaker sections of society.

Goat milk has well-dispersed fat globules which are easily digestible by old and young. It has anti-allergic properties also. It is recommended for use in dyspepsia, peptic ulcer and pyloric stenosis. It is preferred to cow milk in liver dysfunction, jaundice, acidosis and insomnia. Goat also plays an important role as a meat producing animal. It contributes about 35% of the total meat production of India (I.C.A.R., 1997) and there is high demand of

goat meat (chevon) in the our country as all communities of people accept to it.

Inspite of vast raw material base, India contributes less than 1% to the world meat production. There is urgent need to tap the world meat export market by establishing modern and hygienic slaughter houses and making zoonosis free particularly brucellosis free herds. If the quality of Indian meat is strictly controlled, the country may boost its meat exports and this will also help in fetching better prices for our produce which is nearly 30% lower than the average world meat export price (Sharma, 1995).

The goats are also an important sources of fresh skin, pashmina and mohair (the valuable textile fibres). The goats are also susceptible to a bovine pathogenic strain of *Br. abortus* and are considered to be a suitable model for the study of bovine brucellosis (Blood, 1995). Thus goat keeping has now assume a key position in the rural development programme in developing countries like India.

Caprine brucellosis is diagnosed either directly through culture of milk, blood, aborted materials, wound or indirectly by assessing the relative amount of Brucella agglutinins in the blood serum and also in milk, vaginal mucous membrane and semen of animals. Various serological tests have been employed for diagnosis of disease from time to time. Among the serological tests used, some of main tests are SAT, RBPT, CFT, IHAT, BPAT and ELISA have been used for serodiagnosis of caprine brucellosis.

The screening animals through serological tests like RBPT, RPAT, STAT, ELISA etc. and eradicating the brucella positive animals have reduced the prevalence of brucellosis in countries like Australia, Germany, Canada, Great Britain, Poland, Newzealand, U.S.A. and Japan. Therefore it

is essential to provide suitable diagnostic serological test for screening the brucella positive goats, which bears reliability, repeatability and specificity and which will also help the veterinarians to know the magnitude of infection. So that on time proper preventive measure can be adopted.

Brucellosis creates the economical losses to the livestock industry and causes hindrance in public health. It has a great zoonotic importance also. Therefore, the work has been undertaken with the following objectives –

(i) To determine the sero-prevalence of caprine brucellosis in and around Patna.

(ii) To evaluate the different serological tests for diagnosis of brucellosis in goat.

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CHAPTER - II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Pinedo *et al.* (1978) screened goats for brucellosis in Spain by Plate agglutination test. 5.7% of goats were positive to brucellosis over the period of 1967-76.

Aguilera *et al.* (1979) tested 10367 samples of goat serum for brucellosis and found that the 8.22% of serum samples had a titre of 25 I.U. per ml, 2.86% had 50 I.U. per ml, 1.13% had 100 I.U. per ml and 1.04% had 200 I.U. per ml by Rapid Plate agglutination test.

Falade and Hussein (1979) tested 250 samples of goat serum for antibodies of *Br. melitensis* by different serological tests. Brucella antibodies were detected in 28 (11.2%) of samples by a combination of the tests. Individual positive results were RBPT-7 (2.8%), SAT-7 (2.8%), 2- ME-4 (1.6%), AGT-14 (5.6%) and the Rivanol test-9 (3.6%).

Polydorou (1979) determined incidence of brucellosis in sheep and goats by intra palpebral allergic skin tests and concluded that this test was reliable for screening purposes.

Waghela *et al.* (1980) compared results from the SAT, AGIT, RBPT and CFT. The RBPT was most sensitive test and AGIT the most specific, when compared with CFT. RBPT and AGIT were useful when facilities for the CFT were unavailable.

Bandnjevic and Bajrovic (1981) studied 2212 blood samples from men, cattle, pigs, horses, sheep and goats for brucellosis diagnosis by agglutination, complement fixation and rose bengal tests, confirmed that the latter was satisfactorily sensitive. The result of the three tests showed close

correlation, closer between rose bengal and agglutination test than between rose bengal and complement fixation test (CFT).

Badnjevic and Nevjestic (1981) tested 1653 serum samples from human beings, cattle, sheep, swine, horses and goats by card test, agglutination and CF-test for detecting the sensitivity of the card test in diagnosis of brucellosis reactors in man and animals. There was close correlation between the results of the three tests, especially between the card test and agglutination test. The correlation was best in negative cases.

Falade (1981) tested 705 serum samples of goat by different serological tests. 44 samples were positive by the serum agglutination test, 61 by coombs antiglobulin test, 46 by the 2- mercaptoethanol test, 52 by the rivanol test and 2 by the rose bengal test out of the total 705 serum samples.

Falade (1981) isolated *Br. melitensis* biotype 1 from 9 of the 53 aborted goat fetuses and from 19 of the 269 milk samples positive in the ring test. *Br. abortus* biotype 1 was isolated from the vagina of one of five goats which had recently aborted and from seven milk samples.

Falade *et al.* (1981) tested 189 serum samples of goat by the Rose Bengal, Standard agglutination and Rivanol test for antibodies of *Br. abortus* organism. The antibodies of Br. abortus detected in 17 (9%) of the goat serum samples by a combination of the above tests.

Giantzis (1981) conducted serum agglutination test (SAT), complement fixation Test (CFT) and rose bengal test (RBT) in 2848 bovine and 592 ovine serum samples for the adaptation of the RBT for the diagnosis of brucellosis. The results of the RBT paralleled those of the CF-test, with 2.4 and 3.2% of the bovine and ovine samples positive in the CFT being

negative in the RBT and 1 and 4.7% of those negative in the CFT being positive in the RBT.

Weber *et al.* (1981) compared the specificity and sensitivity of the slide agglutination and tube agglutination test. It was concluded that the slide agglutination test is a simple and rapid method for the serological diagnosis of *Br. canis* infection in dogs.

Bale *et al.* (1982) tested 1774 serum samples of goat by Rose Bengal Plate Test (RBPT), Serum Agglutination Test (SAT) and Complement Fixation Test (CFT) for the seroprevalence of caprine brucellosis. 287 (16.1%) goats were positive by RBPT, 29 samples had antibody titres 50 I.U. and above by SAT and 8 by CFT. Based on antibody titres of 50 I.U. and above, the prevalence of brucellosis in goats were 0.5-1.6%.

Butachaiah and Khera (1982) studied animals at three farms in widely separated areas for brucellosis. On all farms, abortion had occurred. *Br. melitensis* was isolated from four aborting and one in nine apparently healthy goats on one farm. 14 of 32 goats were positive to the serum agglutination test for *Br. abortus*.

Fadda and Sanna (1982) observed a decline in the number of outbreak of brucellosis in animals and the number of cases in man from 1970-1980 in Sardina. In 1980 only a single outbreak in goats was reported.

Focken and Muller (1982) detected the infection rate of brucellosis in goats as 3.8% during 1975-1977 in Portugal.

Andreani *et al.* (1983) tested 340 goats serum sample for brucellosis by slow serum agglutination test. 18 (5%) of goats were positive to brucellosis.

Choudhary *et al.* (1983) tested 241 serum samples of pigs for brucellosis in Bihar by standard tube agglutination test using *Br. abortus* antigen (strain 99) during 1979. Titres of 80 I.U. of agglutinins and above were considered positive, those with a titre of 40 I.U. were considered to be suspicious. 12 (4.97%) samples were positive and 10 (4.14%) samples were suspicious. The prevalence varied from 10.52% in Nawada district to 3.96% in Hazaribagh. All 14 pigs from Darbhanga were negative.

Falade (1983) compared the usefulness of RBPT, STAT, Heat inactivation and Rivanol tests in the diagnosis of caprine brucellosis. There was good correlation between tests when all were negative. The RBPT, although less sensitive in the diagnosis of caprine brucellosis was effective in vaccinated goats. The usefulness of the Rivanol (ethacridine) test was doubtful.

Falade and Ezenwane (1984) studied 256 sheep and 20 goats serum samples for brucellosis. 15 (5.4%) reacted to the serum tube agglutination test (STAT), using *Br. abortus* antigen, at titre of 1/10+ to 1/40+, but all were negative to the RBPT and rose bengal capillary tests using *Br. abortus* and *Br. melitensis* antigens. Of 55 sheep and 119 goats slaughtered in Ibadan, 16 (9.2%) were positive to the SAT at titre of 1/10++ to 1/40+, but only 4 of these were positive to both rose bengal tests. Capillary test was more time consuming. It was concluded that there is no satisfactory rapid test for brucellosis in small ruminants.

Jeblawi *et al.* (1985) tested 64 goats serum samples for brucellosis by Serum agglutination and Rose-Bengal card tests. All the tested goats were negative to brucellosis.

Kapoor *et al.* (1985) studied the seroprevalence of brucellosis in goats in Bikaner. Antibodies to *Br. abortus* were present in 10 of 174 goats, including 2 of 104 male goats sent for slaughter and 8 of 70 lactating females.

Pisanu (1985) examined 45 young goats for brucellosis, 15 gave positive low agglutination tests.

Stemshorn *et al.* (1985) compared the sensitivity of the standard serological tests for the diagnosis of brucellosis. The relative sensitivity of the six agglutination tests gave the following ranking: BPAT>RBPT>CARD>SPAT>STAT. The 2-MET (2-mercaptoethanol test) ranked between the BPAT (Buffered Plate Antigen Test) and RBPT (Rose Bengal Plate Test) or between the RBPT and CARD depending on the analysis used. The use of the BPAT as a screening test is recommended provided that a test of high specificity and sensitivity such as the CFT is used to confirm the reactions.

Walker *et al.* (1985) studied the usefulness of an Enzyme-linked immunosorbent assay (ELISA) for detection of antibodies to *Br. ovis* in sheep, using an autoclave-extracted soluble antigen. ELISA was found sensitive and specific.

Zaghloul and Kamel (1985) tested 73 goats serum samples for antibodies of *Br. melitensis* by rose bengal test and by the tube agglutination test. None of 73 goats were positive to caprine brucellosis.

Zowghi and Ebadi (1985) examined 110817 sheep and goats serum samples by Serum agglutination test (SAT) and Rose Bengal Plate Test (RBPT) for brucellosis. 14.7% of sheep and goats were positive during 1970-1984.

Fensterbank (1986) reported that *Br. melitensis* infection was still present in 17 countries of the 48 countries studied.

Chukwu (1987) tested 604 serum samples of goats for antibodies of brucella organisms by tube agglutination test and rose bengal test. 27 (4.5%) samples gave positive and 67 (11%) suspicious reaction to the tube agglutination test, while 17 (2.8%) were positive to the rose bengal test.

Huang (1987) examined 221 serum samples of sheep by a B-A-ELISA (biotin-avidin-ELISA) and an ELISA for antibodies to brucellosis and the results were compared with those of routine serological examinations. It was demonstrated that the detection rate of the BA-ELISA, ELISA, Tube agglutination test, CFT and RBPT were 95.3% (60/63), 82.5% (52/63), 48.8% (20/41), 41.5% (17/41) and 39.0% (16/41) respectively. The specificity of the BA-ELISA was good and its sensitivity was 4 to 8 times greater than that of the ELISA and 226 times greater than that of the CFT. The BA-ELISA was considered to be a promising method for the serological diagnosis of ovine brucellosis.

Lord *et al.* (1987) tested 2939 serum samples of goat for brucellosis. 365 (12.4%) samples gave positive and 416(14%) suspicious reactions for brucellosis. Only a few cases of abortion and other clinical signs attributable to brucellosis were observed.

Lorenzo and Pennimpede (1987) compared the result of rose bengal test with tube agglutination test and rapid plate test for detection of brucellosis. The usefulness of the rose bengal test confirmed as an adjunct to the two other tests.

Mahajan and Kulshreshtha (1987) tested serum samples from 75 ewes that had aborted and 373 healthy sheep for antibodies to *Br. melitensis* B_{115}

rough antigen and *Br. abortus* smooth antigen. 56 of the aborted ewes and 76 of healthy sheep had antibody titres to smooth antigen and 21 aborted ewes and 18 healthy sheep had titres to rough antigen. Most samples with high titres to smooth antigen had little or no antibody to rough antigen.

Ghosh and Nanda (1988) collected 319 milk samples and 316 serum samples of goats from 2 farms and 6 villages in Tripura and examined for brucellosis using Milk Ring Test (MRT), RBPT and the Standard tube agglutination test (SAT). The percent of positive reactors was 13.79% by the MRT, 13.29% by the RBPT and 6.95% by the SAT.

Karaman and Guler (1988) compared the results of the tube agglutination test, ring test, complement fixation test and rivanol test in serological studies on brucellosis. The ring test results were similar to those of the tube agglutination test, whilst the rivanol and CFT were of equal value in differentiating vaccination and infection titres.

Mustafa and Corbel (1988) isolated *Br. melitensis* biovar 1 and biovar 2 from sheep and goats serum sample in Libya.

Pappous and Hontou (1988) tested 407 goats serum samples from 16 infected flocks for brucella antibodies by rose bengal and complement fixation test (CFT). 131 and 150 goats were positive to these tests respectively. It was concluded that in some cases the CFT may be more sensitive than the RB test and the use of both tests were recommended in infected sheep and goat flocks.

Boargob and Muhammed (1989) investigated the prevalence of brucellosis in 11 sheep and goat flocks using RBPT, Counterimmunelectrophoresis (CIEPT), SAT and CFT. Positive animals were detected in three flocks consisting of 21, 31 and 216 animals, the

morbidity being 25%, 81% and 35% respectively. All goat sera and 96.6% of sheep sera positive in RBPT were positive in CFT. RBPT recommended as a single test for the detection of ovine and caprine brucellosis.

Kiel and Khan (1989) observed 11.6% incidence of brucellosis in sheep and goats in Saudi Arabia.

Lako *et al.* (1989) studied 15 sheep infected experimentally with *Br. melitensis* in Yugoslavia. 13 (86.6%) were positive in RBPT, 14 (93.3%) in the slow and rapid agglutination tests and all 15 to the indirect-immunofluorescence and ELISA methods. The control animals were negative to all the tests.

Marin *et al.* (1989) compared sensitivity and specificity of the CFT, Gel diffusion and ELISA tests for the diagnosis of *Br. ovis* infection. The best sensitivity was obtained with the ELISA (97.4%) and CFT (92.7%). All the tests were 100% specific when testing serum samples from rams free of *Br. ovis*.

Al-Delaimia and Ali (1990) tested 696 serum samples of healthy sheep for brucellosis. 52 (7.4%) were positive for brucellosis by SAT and 64 (9.19%) by RBT (rose bengal test). Of the 30 sheep that had aborted 19 (62%) were positive by RBT and 22 (77%) by the SAT. Thirteen strains of *Br. melitensis* biotype-3 were isolated from vagina of sheep that had aborted.

Toma (1990) reported 182 (0.33%) Brucellosis infected goat herds in France.

Mahajan and Kulshreshtha (1991) studied 647 serum samples of sheep comparatively for brucellosis by Standard tube agglutination test, RBPT and Counter- immunelectrophoresis (CIEP) test. No individual test could detect the 13 known positive reactors (the fetuses of which yielded *Br. melitensis*)

but by combination of two tests all 13 were positive. The SAT detected more reactors during the early stages of infection. All these tests may be carried out in a field laboratory at very low cost.

Ahl *et al.* (1993) conducted serological studies on 161 goats serum samples from 9 herds to determine the evidence for the presence of Brucella antibodies in goats. They observed that the seroprevalence (at suspect or reactor titres level) for *Br. melitensis* antibodies was 2.5% for goats.

Blasco *et al.* (1994a) compared the efficacy of different Rose Bengal and Complement Fixation antigens for the diagnosis of *Br. melitensis* infection in sheep and goats. They observed that the all rose bengal antigens and the CF test had 100% specificity when testing brucella free or unvaccinated brucella free animals. But, there were large differences in sensitivity between the rose bengal antigen with sera from culture-positive sheep. The CFT was less sensitive than the rose bengal test when testing culture positive sheep. When testing sera from animals belonging to infected flocks with antigens according to European Union rule, no great differences were observed in the sensitivities of the two tests.

Dessai *et al.* (1995) tested serum samples of 653 sheep, 630 goats and 102 men to determine the incidence of brucellosis by using RPAT and RBPT. The incidence due to *Br. abortus* and *Br. melitensis* were differentiated by using their respective plain antigens in standard tube test. The incidence of brucellosis was 4.9, 7.6 and 5.9% respectively in sheep, goats and men.

Fidanci *et al.* (1995) studied the 976 bovine serum samples for the comparison of efficacy of an ELISA and other serological techniques in detection of antibodies to *Br. abortus*. They found 49 (5%), 17 (1.7%) and

15 (1.5%) positive samples by ELISA, serum agglutination (SAT) and RBPT respectively. The ELISA was recommended for use in brucella eradication programmes in Turkey.

Reichel *et al.* (1996) found six foci of brucella infection during an extensive serological survey involving 6266 goats carried out in most of the district of the KwaZulu-Natal province. The prevalence in positive herds varied between 17% and 100% and all infected herds fell within a 50 km radius.

Abdel Kader (1997) tested 16285 blood samples collected from goats from 4-regions. Brucellosis sero prevalence using RBPT, BAPAT, TAT and Rivanol test were found to be 0.33, 0.33, 0.15 and 0.30% respectively.

Dessai and Krishnappa (1997) studied on the EDTA-labile non-specific agglutination reactions in the diagnosis of ovine and caprine brucellosis. It was concluded that the addition of EDTA to the *Br. abortus* and *Br. melitensis* antigen preparations reduced the antibody titres two to four folds, without significantly reducing the number of positive test results. Tests with the modified antigen preparations (with EDTA) showed more non-specific reactions against *Br. melitensis* and *Br. abortus*.

Mac Millan (1997) tested serum samples by RBT and ELISA methods and observed that the relative sensitivity of the antigens varied but all showed 100% specificity with brucellosis free unvaccinated sheep and goats. There appeared to be a difference in sensitivity of nearly 5% between the least and the most sensitive antigen.

Prahlad *et al.* (1997) studied the prevalence of brucellosis in goats. They tested 289 goats sera samples and brucella was diagnosed in 5,4,21 and 53 goats using RBPT, STAT, CFT and dot-ELISA respectively. Incidence

was highest in Rajasthan (29.7%) followed by U.P. (29.0%) and Punjab (15.8%).

Abuharfeil and Abo-Shehada (1998) compared the results of RBT, ELISA and CFT for *Brucella melitensis* infection in sheep. They have concluded that the ELISA is the most specific and sensitive method, because no false negative results were recorded. They have also mentioned that a single test is not sufficient to confirm the diagnosis of brucellosis and a combination of two tests is recommended, preferably RBT and ELISA.

Bercovich *et al.* (1998) concluded that screening sheep for brucellosis with ELISA improves brucella diagnosis as compared with SAT, CFT, RBPT and SDTH (skin delayed type hyper sensitivity) when 132 sheep serum samples were tested.

Gea *et al.* (1998) conducted a serodiagnostic survey in Argentina, to determine seroprevalence of caprine brucellosis by the Buffered plate antigen (BPA), RBPT and 2-mercaptoethanol tests. Only 4% of the samples from pregnant goats gave positive result in the BPA test while these samples were negative in the other tests. All other samples were negative in all tests. It was concluded that the brucellosis is not present in the region tested.

Jacques *et al.* (1998) compared efficacy of ELISA test to conventional tests (RBPT and CFT). It was concluded that the ELISA is a good screening test for detection of brucella in sheep.

Mirza *et al.* (1998) screened goat flocks in Pakistan by using RBPT. 1613 goats serum samples were tested for brucellosis. 8.6% of goats were positive to brucellosis.

Russo and Monzon (1998) studied 532 goats serum samples taken from 55 goat herds in Argentina. Reactors were found in 20 of the 55 herds of goats with prevalence of brucellosis in these herds ranging from 10-27%.

Singh *et al.* (1998) obtained 473 serum samples from Jamunapari, Barbari and Beetal goats of 143 villages in 7 district of U.P., Punjab, 142 samples from Government farms and 380 samples from goats slaughtered at an abattoir in Agra. All samples were tested for Brucella antibodies by dot-ELISA, STAT and micro-CFT. Incidence of brucellosis was 0.8% in the village flocks, 4.9% in organized farm and 7.1% in the goats slaughtered at the abattoir.

Abela (1999) studied the epidemiology and control of *Br. melitensis* in Malta and found that the herd prevalence was 23% in 1987 which fell to less than 1.5% by 1993. The prevalence of brucellosis again rose to 13% in 1995. Large herds and herds with small ruminants were most at risk to brucellosis infection.

Aboud-Dutra *et al.* (1999) tested sera obtained from 430 horses between June and December 1995 and from 202 goats between August and December1995 for brucellosis by plate agglutination test. 10 (2.3%) horses, but no goats, had titres $\geq$ 1:100. The seroprevalence was highest in horses in urban areas.

Agarwal and Batra (1999) compared efficacy of ELISA with that CFT, plate ELISA and agglutination tests on 50 goats serum. The percentage agreement between the inhibition ELISA and CFT was 94.53% and between the inhibition ELISA and plate ELISA, 90.75%. The sensitivity of the ELISA was 92.04% and that of the agglutination test was 81.81%.

Agarwal *et al.* (1999) tested 1666 goats serum samples by dot-ELISA for brucellosis and results were compared with SAT and CFT. The dot-ELISA has a sensitivity 97.9% and specificity 89.8%. Similarly, SAT has a sensitivity 75.5% and specificity 84.7%.

Aparicio *et al.* (1999) studied on the sensitivity and specificity of the modified Card test for diagnosis of caprine brucellosis with two different concentrations of antigens (3% and 8%), the sensitivity was 79% for the 8% cellular concentration antigen and 98% for the 3% cellular concentration antigen. Specificity was 100% with both antigens. It was concluded that the diagnosis of brucellosis using 8% cellular concentration of antigen was not adequate due to its low sensitivity. The use of a 3% cellular concentration of antigen was recommended for diagnosis of caprine brucellosis by Card test.

Mahajan *et al.* (1999) studied the 208 goats serum samples to determine the seroprevalence of brucellosis in Himachal Pradesh. Brucellosis seroprevalence in goats using Standard tube agglutination test (SAT) was found to be 18.64%.

Mrunalini and Ramasastry (1999) observed that the seroprevalence in goats were 7.0% in Andhra Pradesh, when 2010 goats serum samples screened for caprine brucellosis using Plate Tube Agglutination Test.

Robles *et al.* (1999) conducted a serological survey in Neuquen province, Argentina to determine the prevalence of caprine brucellosis. They have tested 831 goats serum samples by BPAT and modified RBPT. They observed that all the results were negative, suggesting that crossbred goats in Neuquen were free of brucellosis.

Samatrino *et al.* (1999) evaluated the performance of the indirect ELISA (I-ELISA) and competitive ELISA (C-ELISA) for the diagnosis of

brucellosis in comparison to conventional serological tests routinely used in Argentina. 3500 serum samples from brucella-free herds, from vaccinated cattle and from naturally infected cattle were tested by BPAT, RBT, 2-ME test, CFT, I-ELISA and C-ELISA. Sensitivity and specificity of the BAPAT, RBT, I-ELISA and C-ELISA were determined relative to the 2-ME and the CFT. The C-ELISA was considered suitable for eliminating most serological reactions of vaccinated animals and was more specific than the other tests.

Seddek (1999) observed that the seroprevalence in goats were 1.1, 1.1, 0.74 and 1% when 3872 serum samples of goats from 5 locations were screened for caprine brucellosis by using RBPT, BAPAT, TAT and Rivanol tests respectively.

Shehu *et al.* (1999) tested 1010 goats serum sample to determine the seroprevalence of brucellosis. They found 4.75% prevalence of brucellosis in goats. There was no significant difference between dilution ratio of 1:10, 1:20 and 1:40.

Ucan *et al.* (1999) compared different serological tests used in diagnosis of brucellosis in horses. The 130 serum samples from horses from Turkey were examined for brucellosis by using RBPT, Plate agglutination test (PT), Serum agglutination test (SAT) and CFT. 49 serum samples (37.6%) were positive by PT, 39 (30%) had SAT titre of 1:10 to 1:20 and none was positive by RBPT and CFT. It was concluded that all horses were serologically negative for brucella infection.

Singh *et al.* (2000) compared dot-ELISA with the SAT, micro- CFT and p-ELISA for field use in screening herds of goats against brucellosis. The result of dot-ELISA correlated well with those of plate-ELISA (p-ELISA), dot-ELISA founded more suitable and rapid test for screening large number of goats in the field.

Sting and Ortmann (2000) compared the sensitivity and specificity of ELISA test with slow serum agglutination test (SSAT) and CFT for diagnosis of brucellosis in animals. They observed ELISA showed higher sensitivity than SSAT and CFT. The serological comparison of 630 goats sera from brucellosis free herds showed similar specificities for both CFT and ELISA, however specificity of the SSAT was significantly lower being 96.2% in goats. Examination of serum samples from 1796 goats demonstrated suitability of ELISA for routine diagnosis.

Rana *et al.* (2002) isolated *Brucella melitensis* biovar-1 from the vaginal swabs and serum sample of goats. They have also characterized this organism on the basis of cultural and biochemical characteristic.

Shringi, *et al.* (2002) evaluated performance of different serological tests on 148 bovine serum samples for diagnosis of brucellosis. The sensitivity percent of tests were 86.76, 87.09 and 95.58 by RPAT, STAT and ELISA tests respectively.

Gupta *et al.* (2003) evaluated the diagnostic potential of cytoplasmic soluble protein antigens (CSPA) in detecting brucella antibodies in goats. Serum samples (522) were tested with serum agglutination test (SAT), *Br. melitensis* CSPA based ELISA and the samples which were positive in *Br. melitensis* 'CSPA' based ELISA were retested in *Br. abortus* CSPA based ELISA. Out of these, 23 (4.4%), 52 (9.9%) samples were seropositive in SAT and *Br. melitensis* 'CSPA' based ELISA. Out of 52 samples positive in *Br. melitensis* CSPA based ELISA, only 32 were found positive in *Br. abortus* 'CSPA' based ELISA. Sero surveillance of abortion cases with these tests were also supportive of the above results.

MATERIALS AND METHODS

ANTIGENS :

The Brucella standard tube agglutination test (STAT) antigen, Rapid plate agglutination test (RPAT) antigen and Rose Bengal Plate test (RBPT) antigen along with positive antisera were procured from Biological Product Division, Indian Veterinary Research Institute, Izatnagar, Bareilly.

Brucella SAT antigen is a suspension of a pure smooth culture of *Brucella abortus* strain 99 in phenol saline.

Brucella abortus coloured antigen is the stained (with crystal violet and brilliant green) suspension of *Brucella abortus* strain 99 used for RPAT.

RBPT antigen is a 8% suspension of pure, smooth, killed cells of *Br. abortus* strain 99 phenolised and stained with Rose Bengal dye. It is buffered at pH 3.65 using lactic acid buffer.

Culture media were obtained from Hi-media laboratory, Pvt. Mumbai.

COLLECTION OF SAMPLES :

In the present study altogether 524 goats belonging to slaughter houses, Organised farms, Veterinary hospitals and Private breeders in and around Patna were taken to determine the sero-prevalence of caprine brucellosis. From all the goats 5 ml blood was collected by sterilized disposal syringe taking all the aseptic precautions. This was immediately replaced into sterilized test tube, which was kept in standing position at an angle of 45^0 for 3 to 4 hrs. After clotting of the blood the serum was separated and collected in a sterilized centrifuge tube with the help of sterilized Pasteur pipette and centrifuged at 2000-3000 r.p.m. for 10 to 15 minutes. The

supernatant was collected by sterilized Pasteur pipette into a screw capped vial and were properly labelled and kept in the deep freezer at – 20^{0}C till use.

Before testing all the serum samples were heat inactivated at 56^{0}C in a waterbath for 30 minutes.

Sample for culture : A total of 136 samples from suspected goats were collected. The vaginal discharge from suspected non-vaccinated goats (having history of abortion, retension of placenta and infertility) and stomach content of aborted kids were collected aseptically for the culture of the brucella organisms and from each goat the blood sample was also collected and serum was separated. The serum samples were labelled properly with identity mark of its respective culture sample and positive sera samples (of culture positive goats) were used for comparative serology. In case of the aborted kids the sera samples from their respective dams were collected for comparative serological studies. Another 25 sera samples were also collected from apparently healthy goats having no history of infertility or abortion etc. for comparison.

Serological Tests :

All the serum samples of goats were screened for the prevalence of brucella antibody by RPAT, RBPT and STAT.

Rapid Plate Agglutination Test (RPAT) : The test was performed by using technique described by WHO (1967).

A drop of serum was placed on a clean and dry glass plate. After that a drop of Brucella antigen was added, mixed and tilted to and fro then examined for an agglutination. A control test on a same slide with antigen and saline solution only was performed for each sample. Agglutination was

observed by naked eye. The positive sample showed an agglutinated mass on the slide within 1 to 2 minutes.

Rose Bengal Plate Test (RBPT) :

This test was performed following the procedure described by Alton *et al.* (1975) and the literature supplied by I.V.R.I., Izatnagar, along with R.B.P.T. antigen.

Antigen and sera stored at 4^0C was removed from refrigerator, shacked well and kept at room temperature for ½ hour. One drop (approx. 0.03ml) of antigen was placed on the clean and dry glass plate. After that with the help of micropipette one drop (approx. 0.03ml) of serum was placed along with side mixed of (but not into) the antigen. Antigen and serum were thoroughly mixed with toothpicks. Plates were rotated for 3 minutes and immediately reading recorded. A known positive and negative serum was included in each day's test. Agglutination was examined in good light and the test was read as positive (any degree of agglutination) negative (no agglutination).

Standard Tube Agglutination Test (STAT) :

The technique described by Alton *et al.* (1988) and the literature supplied by I.V.R.I., Izatnagar, along with *Br. abortus* SAT antigen was used. The STAT was carried out as a 10 tubes test in doubling dilution from 10 to 5120. For each sample ten Wasserman tubes were set up in a test tube rack. In the first tube 0.8 ml and in the rest nine tubes 0.5 ml of phenol-saline (0.5%) was added. 0.2 ml of the serum was added in the first tube and mixed well and from this 0.5 ml was transferred to the second tube and the process was continued upto the tenth tube from which 0.5 ml was discarded. In all the ten tubes 0.5ml of standard Brucella SAT antigen was added and mixed

by shaking. A control with known positive serum was set up for each batch of the test. Antigen control tubes corresponding to no, 25%, 50%, 75% and 100% agglutination were set up for comparing the result of test samples. 50% agglutination were considered as end point.

The tubes were incubated at 37^0C for 20 hours and then examined by ordinary transmitted light and result was recorded.

The results of the test were expressed in the International Unit. To expressed the unit system, double of the serum titre was taken as total number of International unit (IU) per ml of serum, 80 IU per ml or above was considered positive for Brucellosis in unvaccinated goat. Actual titre of the antibodies in the serum was obtained by serial dilution of the serum. The test was repeated with diluted serum as described above.

An antibody titre of 1:40 and above was considered as positive, 1:20 as doubtful and 1:10 as negative (Topley and Wilson, 1999).

Culture and Identification :

The culture was done by following the method described by Rana *et al.* (2002).

Brucella selective media (Hi-media)

+

Antibiotic

+

Horse Sera – 10%

The samples (vaginal discharge and stomach content of aborted kids) collected for culture were inoculated to Brucella selective media (Hi-media) plates and incubated for 24 hrs. at 37^0C. These plates were examined for any growth.

Each characteristic colonies identified by its colony character, morphological character and biochemical characters as described by

Cruickshank (1968) and Cowan and Steel (1970). Each colony was obtained in specified form on a nutrient agar slant for further examinations.

(i) Staining reaction and Morphology :

Smear was prepared from all types of representative colonies appearing on a plate as well as from those on which there was no apparent growth. These were stained by Gram's method and examined under oil immersion lens. Gram-negative organisms with the following morphological characters were looked for :- coccoid or coccobocillary rods with some what sharpened ends, lying singly or in groups.

(ii) Hydrogen sulphide production test :

The isolates were grown in peptone water and a 10% lead acetate paper was inserted between plug and tube in such way that part of the paper should hang inside the tube. After incubation at 37^0C the tube was examined for blacking of paper daily for seven days.

(iii) Urease production test :

Christensen's urea medium slant was inoculated heavily with the culture. The tubes were incubated at 37^0C and examined at two hours, four hours and after overnight incubation. Negative tubes were observed daily for 4 days in order to detect any delayed reaction given by the members of the certain groups other than Proteus. Ureas positive cultures produced a purple pink colour due to a change in the colour indicator.

(iv) CO_2 requirement test :

The culture was inoculated into the Brucella selective media (Hi- media) and incubated at 37^0C for 24 hours aerobically.

(v) Serological test :

The isolates primarily identified as *Brucella melitensis* were further tested by rapid agglutination test using Brucella antisera which were procured from IVRI, Izatnagar.

A drop of normal saline solution was put on a clean dry glass plate to this, a loop full of culture was added and mixed thoroughly. After that a drop of antisera was added, mixed and examined for an agglutination. A control test on a same slide with antigen and saline solution only was performed for each sample. Agglutination was observed by naked eye. The positive sample showed an agglomerate on the slide.

Statistical Analysis :

The prevalence of Brucella melitensis infection in blood serum of goat was recorded. The data thus obtained on compilation of results were statistically analysed using "Chi-square" test. The sensitivity percent of the serological tests were evaluated as per Ruppaner *et al.*, (1980).

Sensitivity denotes the ability of a test to correctly detect the presence of disease or infection.

$$\text{Sensitivity \%} = \frac{\text{No. of diseased or infected animals showing a positive test}}{\text{Total no. of diseased or infected animals}} \times 100$$

Specificity denotes the ability of a test to correctly detect the non-diseased (non-infected) or healthy status of animals in herds.

$$\text{Specificity \%} = \frac{\text{No. of non- diseased or non- infected animals showing a negative test}}{\text{Total No. of animals not diseased or not infected}} \times 100$$

(Thapliyal and Misra, 1996)

CHAPTER - IV

RESULTS

RESULTS

Rapid Plate Agglutination Test : The result of Rapid Plate Agglutination Test of goat sera samples for *Brucella melitensis* has been presented in Table-I. Out of 176 male goats 5 goats were found positive for *Br. melitensis*, while out of 348 female goats, 23 goats were found positive for *Br. melitensis*. Thus 2.84% male and 6.609% female goats gave the positive reaction for *Br. melitensis* by RPAT. Chi-square test showed non-significant difference so far the occurrence of *Br. melitensis* between male and female goats are concerned.

Rose Bengal Plate Test : Table – II revealed the result of Rose Bengal Plate Test of the goat sera samples for Brucella antibodies. Out of 176 male goats 5 goats were found positive for *Br. melitensis* while 23 goats among 348 female goats tested, showed the positive test for *Br. melitensis*. Thus, 2.840% male and 6.609% female goats found to be positive for the *Br. melitensis*. However, statistically non-significant difference was observed, for the occurrence of *Br. melitensis* in between male and female goats.

Standard Tube Agglutination Test : The result of Standard Tube Agglutination Test of the goat sera samples has been depicted in Table - III. Five among the 176 male goats and 22 among the 348 female goats found to be positive for *Br. melitensis* giving the percentage of 2.84% and 6.321% in male and female goats respectively. Statistically non-significant difference was observed to the positive reaction for *Br. melitensis* between male and female goats.

Distribution of Brucella antibodies : Table-IV showed the distribution of Brucella antibodies among positive sera samples of goat. None of the sera

samples of male goat shown the positive reaction upto the 1 : 80 antibody titre level while maximum number of goats that is two gave positive reaction at the titre level 1:160 and above that titre level equal number of male goat gave the positive reaction at corresponding titre level upto 1 : 1280. While for the female goats upto 1:40 titre level none of them found the positive for Brucella antibodies. Two female goats gave positive reaction at the titre level 1:80. Eight female goats shown the positive test for Brucella antibodies at the titre level 1:160 that is the highest number of positivity among the all antibody titre level. Five female goats showed the positive test for 1:320 titre level, four goats for 1:640, two for 1:1280 and one for 1:2560 Brucella antibodies titre level.

Incidence from different sources :

The result of incidence of caprine brucellosis from different sources has been presented in Table-V. Amongst different sources from which goat sera were collected (Table-V), the highest incidence (6.91%) was observed in the goats slaughtered at different slaughter houses, followed by organized farms (5.55%), veterinary hospitals (4.87%) and private breeders/rural areas (2.14%).

Culture of Brucella : The steps for the culture and identification of *Br. melitensis* has been shown in Table-VI and the result of the culture of Brucella organism is presented in the Table – VII. Out of 136 samples (vaginal discharge and abomasum content of kids) from suspected goats and aborted kids 42 samples were found to be culture positive for Brucella, while all 25 sera samples from apparently healthy goats were found to be culture negative. The Brucella, colonies were found-round, 0.5 to 1mm in diameter,

convex, entire edged, transluscent and semitransparent, glistening, smooth surfaced and slightly grayish white by reflected light.

Gram's staining reaction revealed that the organisms were Gram-negative, morphologically coccobacillary roads with somewhat sharpened ends and lying singly or in groups.

Biochemical Test :

The result of biochemical tests for confirmation *of Br. melitensis* on culture positive samples has been presented in Table – VIII. For growth it requires an aerobic environment and enriched media such as trypticase 50 g in blood agar but not containing 5-10% CO_2. *Brucella melitensis* usually not produces H_2S. Ureas activity was variable with *Brucella melitensis* organism.

Comparative serology : Table - IX showed the result of comparative Brucella agglutinin reactivity of the caprine sera in serological tests for evaluation of the serological tests.

Out of 42 culture positive samples 36 sera samples were found to be positive by RPAT, 36 sera samples by RBPT and 37 sera samples were found to the positive by STAT. Thus the sensitivity of these three tests were found to be 85.71%, 85.71% and 88.09% respectively. These all three serological tests gave negative result on all 25 culture negative goats sera sample. Hence, the specificity of all these tests were observed to 100%.

Table – I : Showing the result of Rapid Plate Agglutination Test of the goat sera samples for *Brucella melitensis*.

Sl. No.	Details of sera samples examined group	Details of sera samples found positive				
		Total no. of sera examined	No. found to be positive	No. found to be negative	% positive	Chi-square values
1.	Male	176	5	171	2.840	
2.	Female	348	23	325	6.609	$\chi^2_{1df} = 3.28$ N.S.
	Total	**524**	**28**	**496**	**5.343**	

Parenthesis shows percentage.

N.S. – Non-significant (Row total x column total)

Tabulated value of χ^2_{1df} at 0.05 LS = 3.84.

Fig. 1 : Histogram showing the result of Rapid Plate Agglutination Test of the goat sera samples for Brucella melitensis.

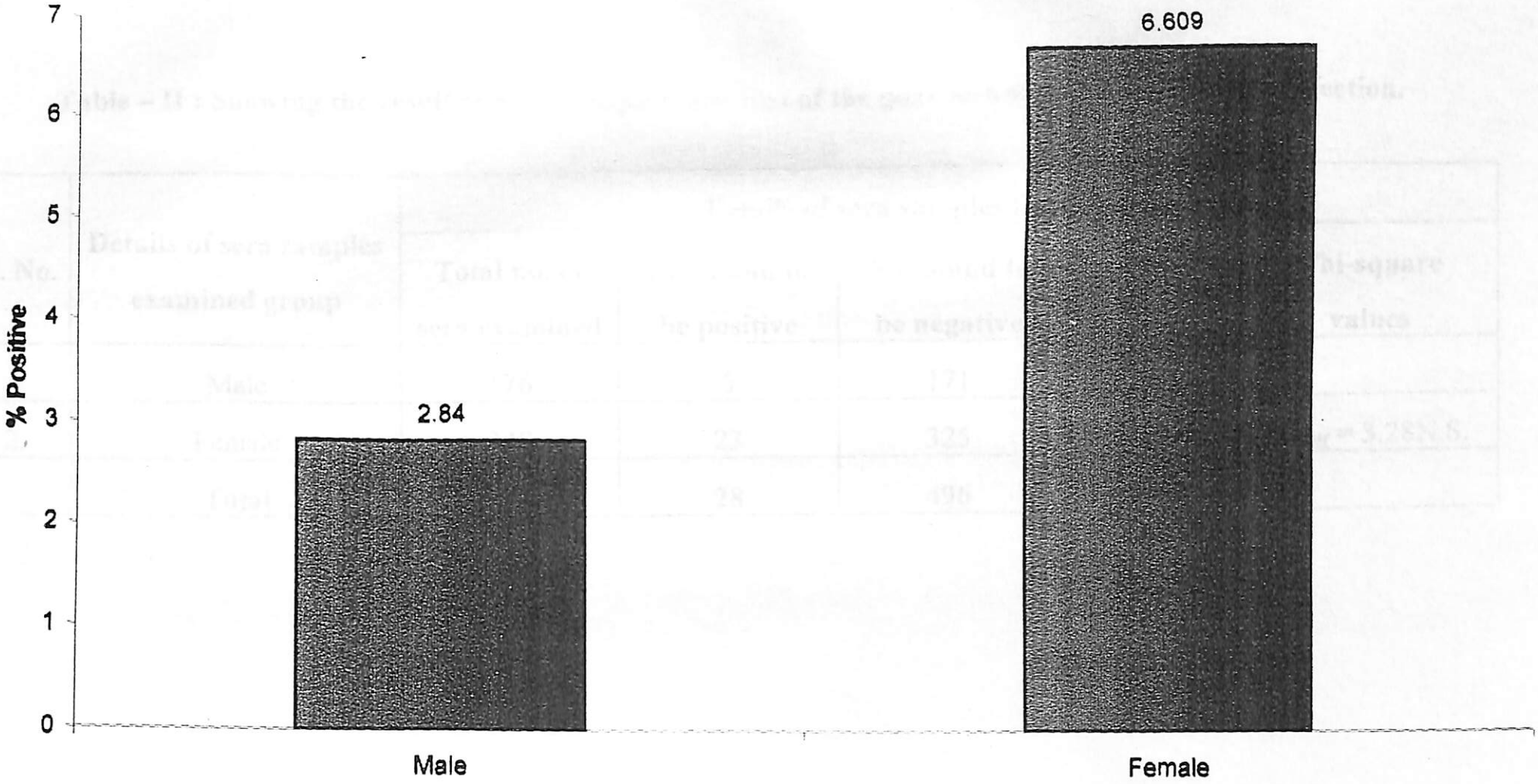


Table – II : Showing the result of Rose Bengal Plate Test of the goat sera samples for Brucella infection.

Sl. No.	Details of sera samples examined group	Details of sera samples found positive				
		Total no. of sera examined	No. found to be positive	No. found to be negative	% positive	Chi-square values
1.	Male	176	5	171	2.840	
2.	Female	348	23	325	6.609	$\chi^2_{1df} = 3.28$N.S.
	Total	**524**	**28**	**496**	**5.343**	

Fig. 2 : Histogram showing the result of Rose Bengal Plate Test of the goat sera samples for Brucella infection.

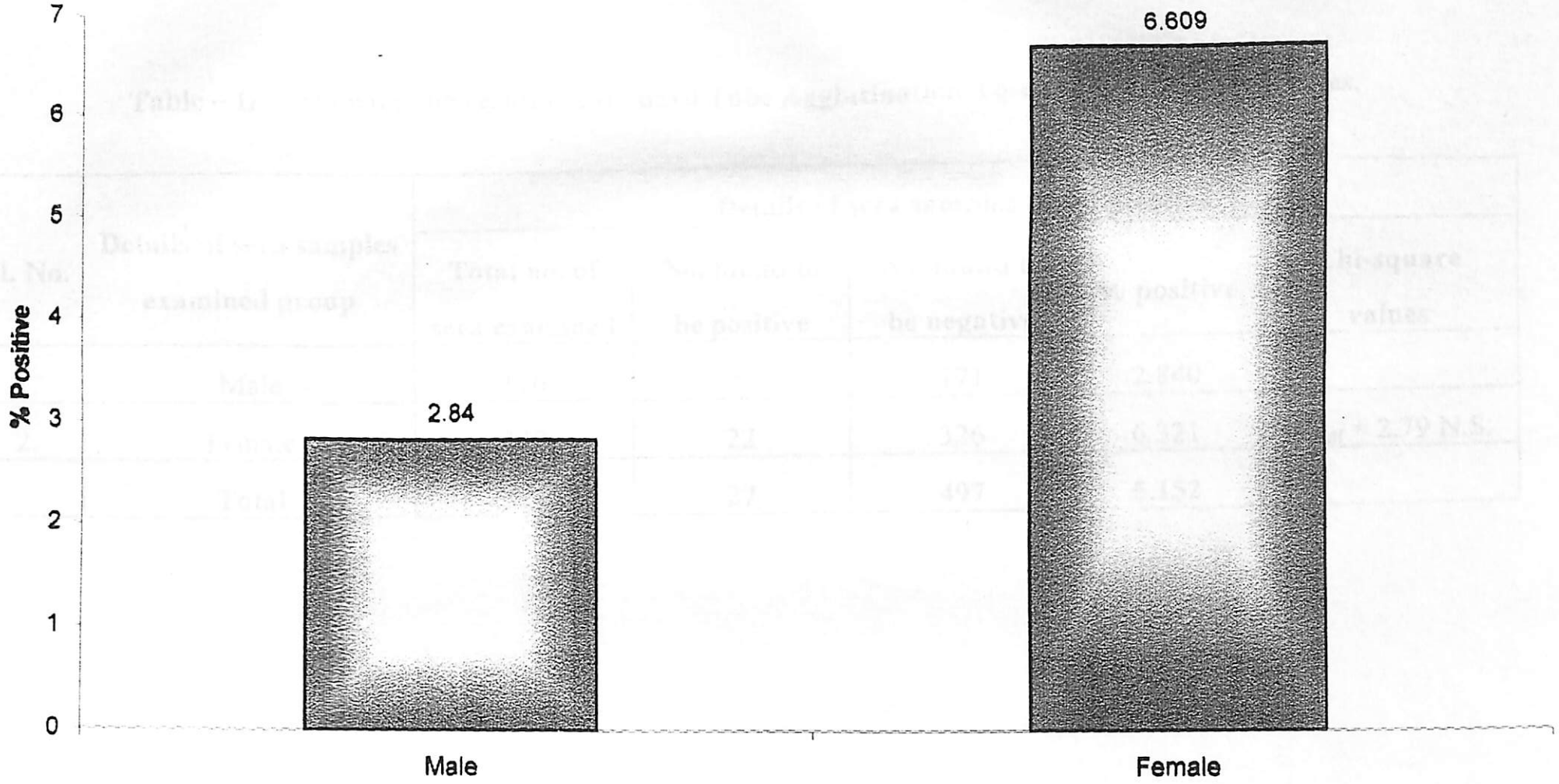


Table – III : Showing the result of Standard Tube Agglutination Test of the goat sera samples.

Sl. No.	Details of sera samples examined group	Details of sera samples found positive				
		Total no. of sera examined	No. found to be positive	No. found to be negative	% positive	Chi-square values
1.	Male	176	5	171	2.840	
2.	Female	348	22	326	6.321	$\chi^2_{1df} = 2.79$ N.S.
	Total	**524**	**27**	**497**	**5.152**	

Fig. 3 : Histogram showing the result of Standard Tube Agglutination Test of goat sera samples.

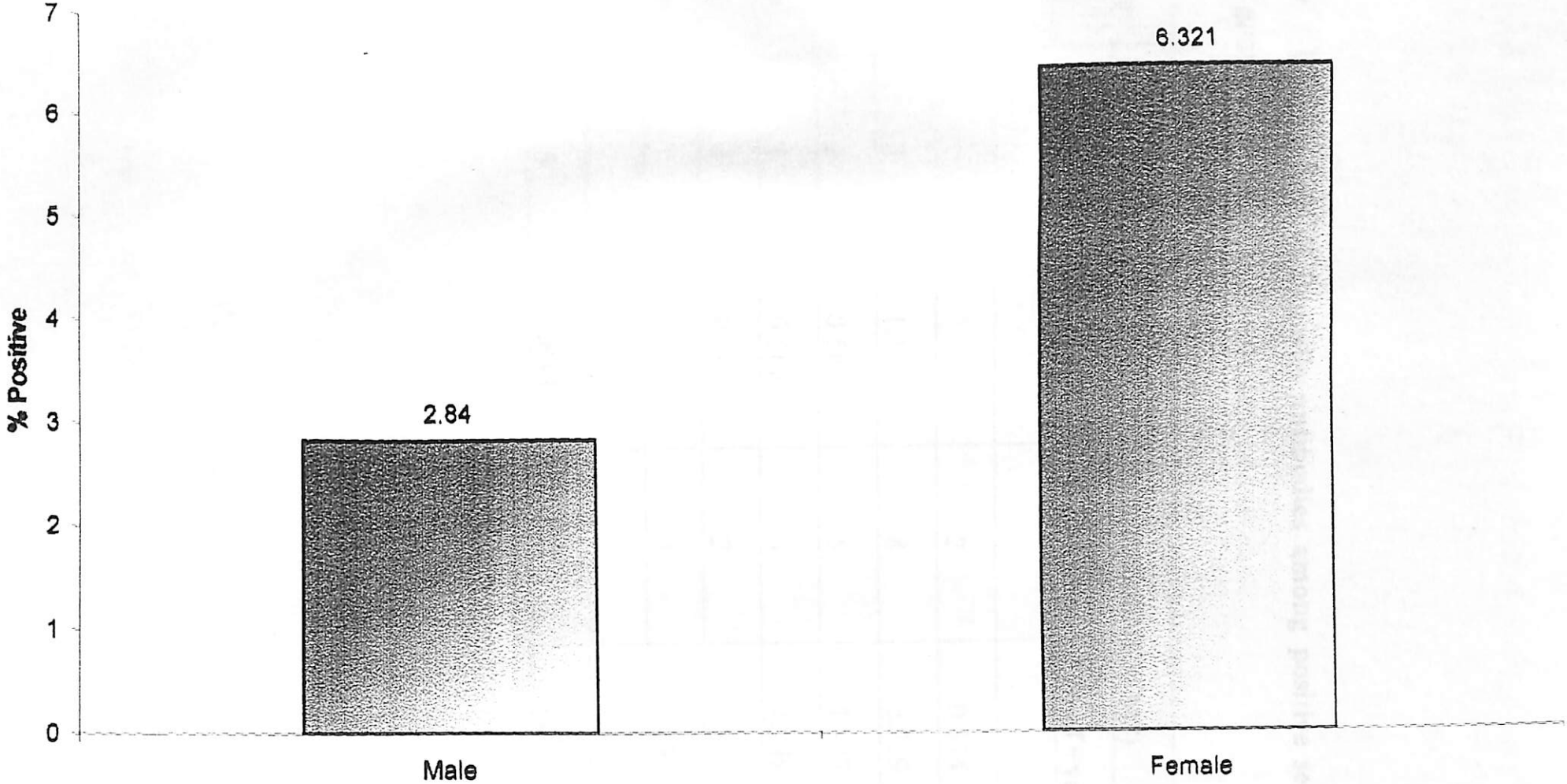


Table - IV : Distribution of Brucella antibodies among positive sera samples of goat.

Antibody Titre	Male (n = 176)		Female (n = 348)	
	No. of +ve	% of +ve	No. of +ve	% of +ve
1 : 40				
1 : 80			2	0.574
1 : 160	2	1.136	8	2.299
1 : 320	1	0.568	5	1.437
1 : 640	1	0.568	4	1.149
1 : 1280	1	0.568	2	0.574
1 : 2560			1	0.287
1 : 5120				
Total	**5**	**2.840**	**22**	**6.321**

Fig. 4 : Histogram showing distribution of Brucella antibodies among positive sera samples of goat.

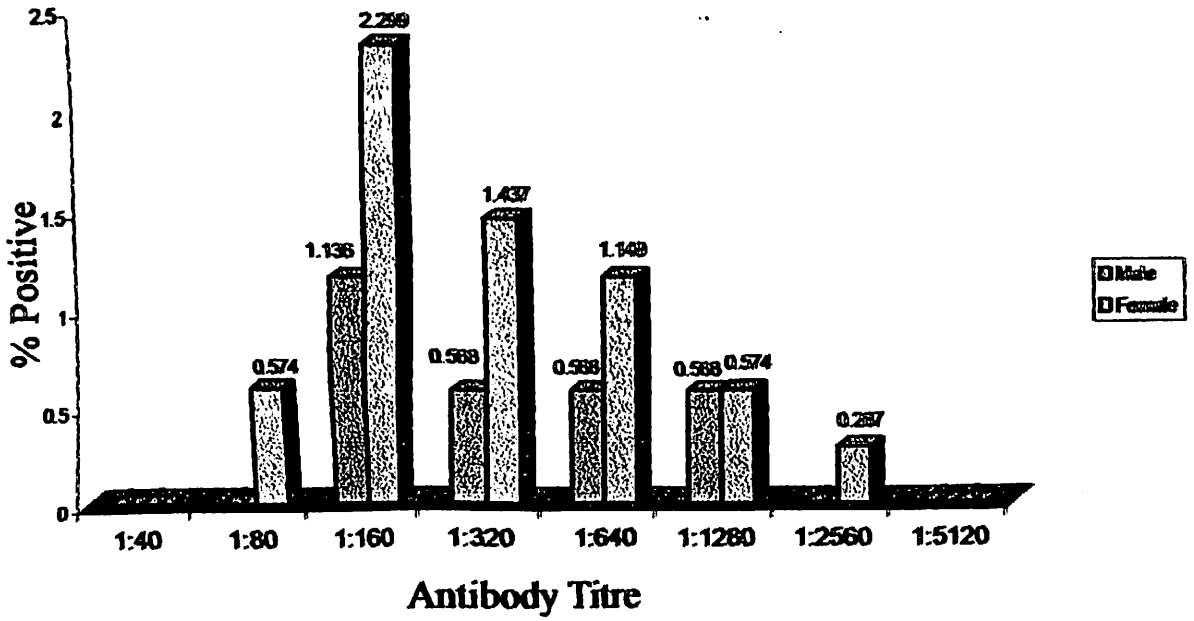


Fig. 5 : Graph showing distribution of Brucella antibodies among positive sera samples of goat.

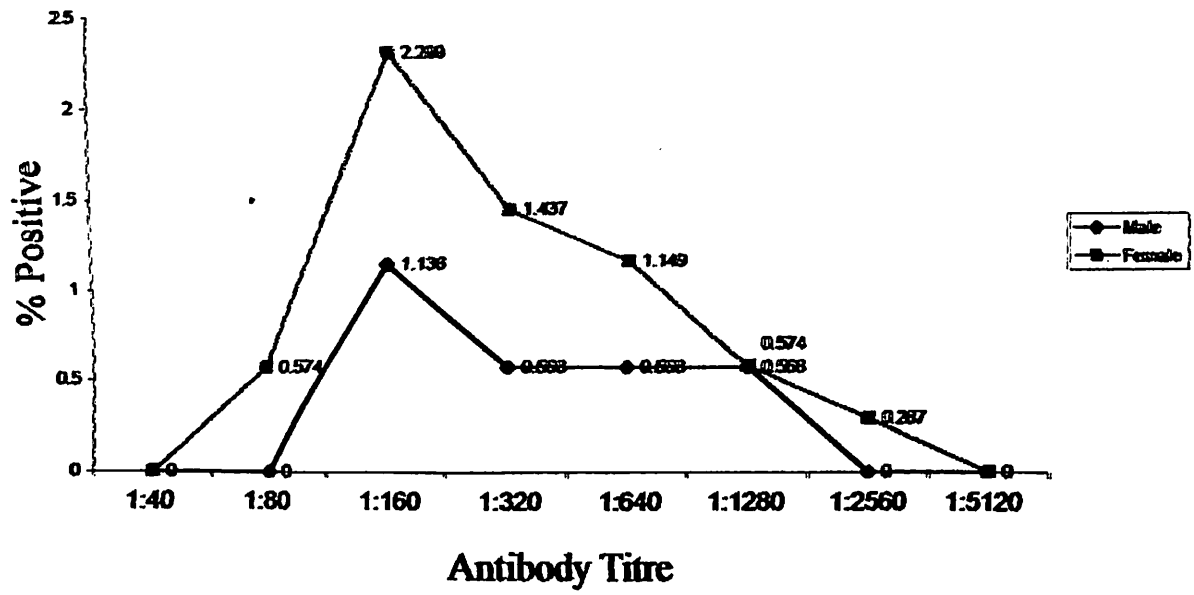


Table – V : Showing the incidence of Brucellosis in goats from different sources.

Sl. No.	Source of samples	No. of samples collected	No. of samples positive in STAT	Percentage positive samples
1.	Organised farms	126	7	5.55
2.	Veterinary Hospitals	41	2	4.87
3.	Slaughter houses	217	15	6.91
4.	Private breeders and rural areas	140	3	2.14
	Total	**524**	**27**	**5.152**

Fig. 6 : Histogram showing the incidence of of Brucellosis in goats from different sources.

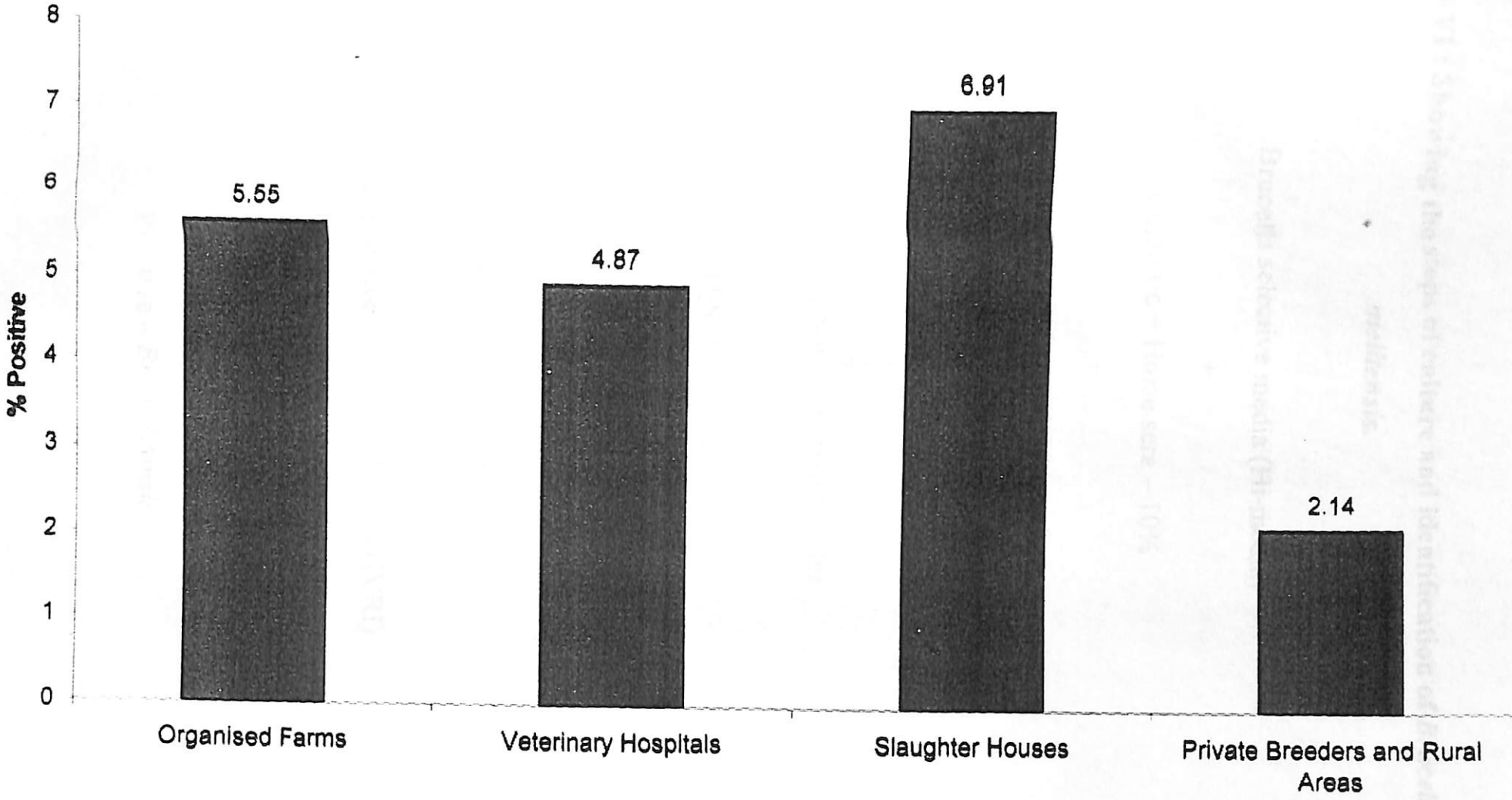


Table – VI : Showing the steps of culture and identification of *Brucella melitensis.*

Brucella selective media (Hi-media)

+

Antibiotic + Horse sera – 10%

+

Inoculation of material (samples)

↓

Incubated for 24 hrs. at 37^0C

↓

After 24 hrs. colony, Peptone water

↓

H_2S (Negative)

Urease (Variable)

Aerobic (Positive)

↓

Antisera (Positive) for *Br. melitensis* (IVRI)

↓

Slide Agglutination Test

↓

Positive – *Br. melitensis*

Table – VII : Showing the result of culture of Brucella organism.

Type of samples used for culture	Total no. of samples used for culture	No. of samples found to be culture positive (Shown growth of colonies on selective media)	% positive
Vaginal discharge and stomach content of aborted kids.	136	42	30.88

Table – VIII : Showing the result of biochemical tests for confirmation of *Br. melitensis* on culture positive samples.

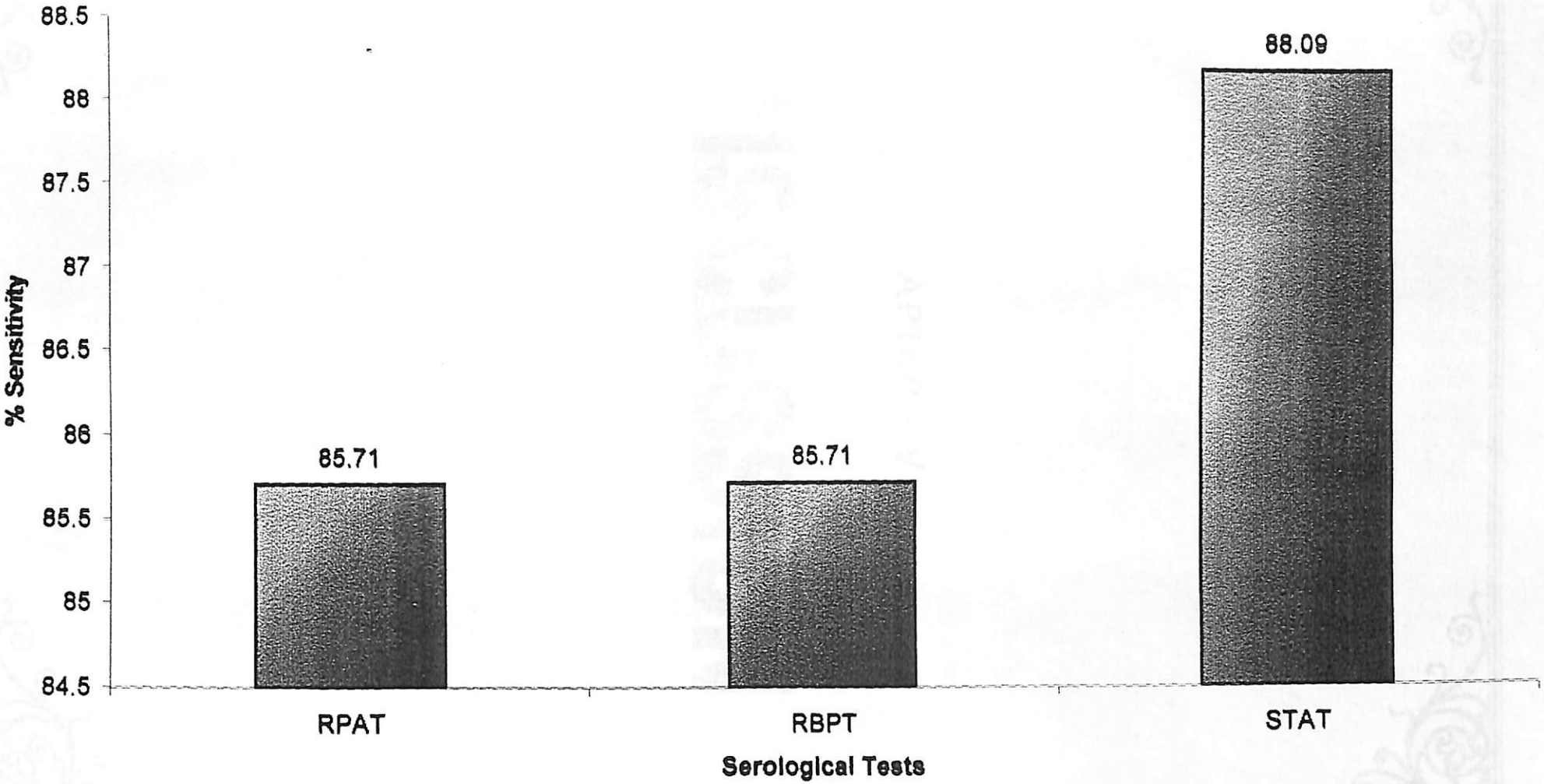
No. of culture positive samples tested	No. of samples found to be positive by biochemical tests	Biochemical tests (shown by *Br. melitensis*)		
		H_2S	Urease	Aerobic
42	42	Negative	Variable	Positive

Table - IX : Showing the result of sensitivity of serological tests on goat serum samples for comparative evaluation.

Sl. No.	Particulars	Serological Tests		
		RPAT	RBPT	STAT
1.	No. of serum samples tested from infected goats	42	42	42
2.	No. of test positive serum samples	36	36	37
3.	No. of test negative samples	6	6	5
4.	Sensitivity percent	85.71	85.71	88.09

♦♦♦♦♦♦

Fig. 7 : Histogram showing the result of sensitivity of serological tests on goat sera samples for comparative evaluation.



CHAPTER - V

DISCUSSION

DISCUSSION

The sheep and goats occupy an important place in the agricultural economy of India, utilizing the uncultivable waste lands and weeds from the field.

The problem of abortion and infertility could reasonably be considered as a national problem as it affects economy of poor and weaker sections of people.

During last 3 to 4 decades, brucellosis in small ruminants has gained much attension because of their role in spread of infection to cattle and human beings (Rafyi and Afshar, 1987).

Brucellosis, a world-wide prevalent contagious bacterial zoonotic disease (Mahajan *et al.* 1999) was first reported in man (David Bruce, 1887) on Malta Island. *Brucella melitensis* was the usual causative agent. Caprine brucellosis is also caused by *Br. melitensis*. The disease in female goat is characterized by late abortion, infertility and retension of placenta. In male goat it causes fever, septicaemia, arthritis and orchitis leading to heavy economic losses in goat industry. Brucella organisms can also infect human population resulting into Malta fever or Undulent fever. Thus brucellosis has great economical and public health importance.

The organism *Brucella melitensis* which is small, Gram negative, coccobacilli, non-motile and non-sporeforming bacteria is reasonably resistant to environmental influences but are readily killed by the common disinfectants (Merchant and Packer, 1999). It is transmitted by direct contact with infected materials and ingestion of milk and milk products from infected animals. Occupational exposure occurs in the people associated with

animals rearing, management, treatment and working in the animal products processing units.

Br. melitensis is found to be most envasive and pathogenic for human then other species of the Genus Brucella, viz. *Br. abortus, Br. ovis, Br. suis and Br. canis.* Brucellosis remains a most serious zoonosis in area of the world where *Br. melitensis* is enzootic in goats and sheep and the resulting disease in humans is severe and long lasting (Blood *et al.*, 1995).

Despite long standing efforts for control, eradication and vaccination programmes, brucellosis remains a leading zoonotic disease in India (Shakya *et al.* 1995). The present investigation reports the incidence of disease among goats in and around Patna of Bihar.

Many aspects of brucellosis in goats, an infection caused by *Br. melitensis* remain unknown, unclear, or controversial specially the diagnosis despite a considerable increase in knowledge in recent years. One of the most critical and controversial points concerning the serological diagnosis of *Br. melitensis* infection is related to which Brucella species and biovars are used in the production of diagnostic antigen. The antigens used in the rose bengal test (RBPT), complement fixation test (CFT) and serum agglutination test (SAT) are made with *Br. abortus* biovar 1 (an A dominant strain) that means that, theoretically infections due to M dominant strain (*Br. melitensis* biovar 1) could be misdiagnosed (Alton *et al.* 1975).

An important problem affecting the sensitivity of these tests concerns the standardization of antigens. These standardization conditions, which seem to be suitable for diagnosis of *Br. abortus* infection in cattle, are not adequate for the diagnosis of *Br. melitensis* infection in goats (Blasco *et al.* 1994a, 1994b and Ray, 1979).

There is no scientific agreement on what should be the nature and characteristics of a universal antigen for diagnosing brucellosis due to smooth Brucella. Therefore further research is needed to clarify the practical importance and interest of using species-specific diagnosing antigens for the different serological tests (Gupta *et al.* 2003).

Presently, the incidence of abortions in caprine population in Bihar showed a rising trend, thus it is essential to provide suitable diagnostic test for diagnosis of brucellosis in goats, which bears reliability, repeatability and specificity. It is planned to evaluate early developed serological tests like Rapid Plate Agglutination Test (RPAT), Rose Bengal Plate Test (RBPT) and Standard Tube Agglutination Test (STAT).

Numerous works on the various aspects of caprine brucellosis, such as isolation of *Br. melitensis* (Falade, 1981; Rana *et al.*, 2002), demonstrating the Brucella agglutinin in the blood sera by adopting different serological tests (RPAT, RBPT, STAT and ELISA) were reported from time to time. There are three biovars of *Br. melitensis* organism and all these three biovars have been isolated in India (Polding, 1942).

Several reviews of prevalence of caprine brucellosis have been published by various workers from abroad (Abdel Kader, 1997; Gea *et al.*, 1998; Mirza *et al.*, 1998; Russo and Monzon, 1998; Robles *et al.*, 1999; Seddek, 1999) and from India (Kapoor *et al.*, 1985; Ghosh and Nanda, 1988; Prahlad *et al.*, 1997; Singh *et al.*, 1998; Mrunalini and Ramasastry, 1999 etc.) time to time.

The percent seroprevalence of brucellosis in goats were recorded from abroad as, 11.6% in Soudi Arabia (Kiel and Khan, 1989); 2.5% in U.S. Vergin islands (Ahl *et al.*, 1993); 8.6% in Pakistan (Mirza *et al.*, 1998);

10-27% in Argentina (Russo and Monzon, 1998); 4.75% in Bauchi district, Nigeria (Shehu *et al.*, 1999) etc.

The percent seroprevalence of caprine brucellosis in Indian states recorded were, 5.747% in a total of 174 goats and 1.923% in 104 male goats in Bikaner (Kapoor *et al.*, 1985); 6.95% in the 6 villages of the Tripura (Ghosh and Nanda, 1988); 29.7% in Rajasthan, 29% in U.P. and 15.8% in Punjab (Prahlad *et al.*, 1997); 7.6% in the Bangalore and Bidar area of Karnataka (Dessai *et al.*, 1995); 0.8% in the village flocks, 4.9% in the organized farm and 7.1% in the goats slaughtered at the abattoir of the seven district of U.P. and Punjab (Singh *et al.*, 1998); 7.0% in the Andhra Pradesh (Mrunalini and Ramasastry, 1999) etc.

Besides sera, brucella organism and brucella antibody have also been demonstrated in different biological fluids such as milk (Lord *et al.*, 1987), vaginal discharge (Al-Delaimia and Ali, 1990), aborted foetal stomach content (Mahajan and Kulshreshtha, 1991) and also in lymphnode (Lord *et al.*, 1987) etc.

Diagnosis of caprine brucellosis is generally done either directly through culture of milk, blood, aborted materials or indirectly by assessing the relative amount of Brucella antibodies in the blood serum, milk, vaginal discharge and semen of the male goats.

No specific serological test for *Br. melitensis* infection of goats have been developed, and it is widely assumed that the serological test used for *Br. abortus* infection in cattle are also adequate for the diagnosis of *Br. melitensis* infection of goats (Gupta *et al.*, 2003).

Confirmatory diagnosis of brucellosis based on isolation is bersome (Ray, 1979), and therefore different serological tests play a or role in diagnosis of brucellosis.

Serological monitoring has assumed an importance in predicting umulation of unusual number of susceptible individuals. These tools are safe to use, more so when the disease has zoonotic implication hajan *et al.*, 1999).

As the vaccination in goats against brucellosis has not been carried out ihar. Thus the presence of antibodies in caprine serum is the reflection of and present infection of brucella in the goat population.

As the growth of brucella organism on the culture media required ger duration and due to the fastidiousness nature of organism and the ential hazard of growing the organism, one has to depend on the nonstration of antibodies against brucella organism in these cases (Blood *l.*, 1995). Some serological test are also found to be easy to perform and id too.

Indeed, for the last few decades serological procedures are frequently plied to detect specific antibodies to infectious agents and have become cornerstone in disease diagnosis. These serological tests manifest idence of specific antibodies more so when isolation of causative agent is t possible either due to non-viability, less number of infectious agents in ssues or any flaw in collection and preservation of tissue samples. Such ro-surveys are important to find out disease profile or distribution, so that itable control measures are undertaken to circumvent losses (Verma *et al.*, 98). The commonly used serological tests for this purpose is RPAT, MRT, BPT, STAT, CFT and ELISA.

Confirmatory diagnosis of brucellosis based on isolation is cumbersome (Ray, 1979), and therefore different serological tests play a major role in diagnosis of brucellosis.

Serological monitoring has assumed an importance in predicting accumulation of unusual number of susceptible individuals. These tools are also safe to use, more so when the disease has zoonotic implication (Mahajan *et al.,* 1999).

As the vaccination in goats against brucellosis has not been carried out in Bihar. Thus the presence of antibodies in caprine serum is the reflection of past and present infection of brucella in the goat population.

As the growth of brucella organism on the culture media required longer duration and due to the fastidiousness nature of organism and the potential hazard of growing the organism, one has to depend on the demonstration of antibodies against brucella organism in these cases (Blood *et al.,* 1995). Some serological test are also found to be easy to perform and rapid too.

Indeed, for the last few decades serological procedures are frequently applied to detect specific antibodies to infectious agents and have become the cornerstone in disease diagnosis. These serological tests manifest evidence of specific antibodies more so when isolation of causative agent is not possible either due to non-viability, less number of infectious agents in tissues or any flaw in collection and preservation of tissue samples. Such sero-surveys are important to find out disease profile or distribution, so that suitable control measures are undertaken to circumvent losses (Verma *et al.,* 1998). The commonly used serological tests for this purpose is RPAT, MRT, RBPT, STAT, CFT and ELISA.

As the recent status of the caprine brucellosis in Bihar is not known, hence in the present investigation, it was tried to find out the seroprevalence of caprine brucellosis in goats in and around Patna (Bihar). For this altogether 524 goat sera samples were screened for the presence of antibody against Brucella organism. The various serological tests viz. RPAT, RBPT and STAT were also evaluated for its sensitivity and specificity for the diagnosis of caprine brucellosis.

ELISA test was found highly sensitive and specific for the diagnosis of Brucellosis in animals (Jacques *et al.*, 1998; Agarwal *et al.*, 1999; Agarwal and Batra, 1999; Samatrino *et al.*, 1999; Singh *et al.*, 2000; Sting and Ortmann, 2000; Sarumathi *et al.*, 2003). In present investigation ELISA test could not carried out due to some unavoidable reason.

Though CFT and ELISA test found to be very sensitive and specific test, but it requires sophisticated laboratory which is not always available in infected area and are costly too (Blood *et al.*, 1995). Therefore, in the present investigation other suitable serological tests were evaluated for their usefulness in our conditions.

In present investigation for demonstration of antibody against Brucella in goat sera, RPAT, RBPT and STAT were performed as per the standard method described by WHO (1967), Alton *et al.* (1975) and Alton *et al.* (1988) respectively and the guideline provided by I.V.R.I., Izatnagar, along with Brucella antigens.

Twenty eight sera samples out of 524 goat sera samples showed positive reaction to RPAT and RBPT, 27 sera samples showed brucella positive reaction to the STAT. The highest number of goats positive to brucella infection were observed at 1 : 160 titre level (Table IV).

In the present investigation serological tests on 524 goat sera revealed over all prevalence of caprine brucellosis to the extent of 5.343% by RPAT and RBPT and 5.152% by STAT, which is in close agreement with the finding of Singh *et al.* (1998), where the over all prevalence of infection in the organized farm and in the goats slaughtered at the abattoir was 4.9% and 7.1% respectively. The finding of present investigation on seroprevalence of caprine brucellosis is also in the close agreement with the finding of Pinedo *et al.* (1978), Andreani *et al.* (1983), Kapoor *et al.* (1985), Dessai *et al.* (1995), Mrunalini and Ramasastry (1999) and Shehu *et al.* (1999), where the recorded prevalence of caprine brucellosis were 5.7%, 5%, 5.747%, 7.6%, 7.0% and 4.75% respectively.

The sex wise prevalence of Brucella antibody in the present investigation showed 2.840% and 6.609% seropositivity in male and female goats by RPAT and RBPT and 2.840% and 6.321% male and female goats by STAT respectively (Table I,II and III). However, the statistical analysis revealed non-significant difference of Brucella seropositivity between male and female goats. These findings indicate equal involvement of both the sexes of goat to the caprine brucellosis. The finding on prevalence of brucellosis in male and female goat by Kapoor *et al.* (1985) is nearer to the findings of present investigation as they reported 5.747% prevalence in female and 1.923% in male goats.

Amongst different sources from which goat sera were collected (Table V), the highest incidence (6.91%) was observed in the goats slaughtered at different slaughter houses, followed by organized farms (5.55%), veterinary hospitals (4.87%) and private breeders/rural areas (2.14%). Singh *et al.* (1998) reported similar findings on incidence of caprine brucellosis from

different sources, where they observed highest incidence (7.1%) in the goats slaughtered at the abattoirs, followed by organized farms (4.9%) and lowest incidence (0.8%) in the village flocks.

The highest prevalence of caprine brucellosis recorded in the present studies in the goats belonging to different slaughter houses (6.91%) is probably due to the slaughtering of majority of infertile and culled goats at different abattoirs of Bihar.

The higher incidence of brucellosis in organized farms (5.55%) as compared to village or rural area flocks (2.14%) may be attributed to more density of animals in organized farms, resulting into the spread of disease by contact and contamination of environment, bedding, food, etc. due to excretion of large number of organisms at and following parturition from infected goats (Blood *et al.*, 1995). Different stress factors may also contribute to increased susceptibility of animals towards infection due to release of naturally occurring corticosteroids which are strongly immunosuppressive in nature.

In an attempt to analyse the factors responsible for the dissemination of the infection, Idem (1947) found a direct correlation between rainfall and occurrence of brucellosis in animals. Infection was more in the farm animals in areas where total rainfall was high. The rate of village infection was negligible in semi desert areas but considerable high in humid coastal belts.

Adopting systematic brucellosis eradication programme, some region of the world attained either very low level of infectivity or some what considered as free from brucellosis in the animals. While working on the incidence of brucellosis among the farm animals in Assiut Governorate, Zaghloul and Kamel (1985) found that none of the 73 goats sera were

positive by the RBPT and STAT. Gea *et al.* (1998) tested serum samples from 274 pregnant goats, 82 female goats which had aborted upto 30 days previously and 38 castrated males in the Serrana del Sur zone of the province of Corodoba, Argentina by BPAT, RBPT and 2-ME test for sero-diagnosis of caprine brucellosis. Although 11 (4%) of the samples from pregnant goats were positive in the BPAT but these samples were negative in the other tests. All other samples were negative in all tests. They concluded that the brucellosis was not present in the region tested. Robles *et al.* (1999) conducted a serological survey for *Br. melitensis* infection in crossbred goats of Neuquen province, Patogonia, Argentina by using BPAT and RBPT. All the results were negative, suggesting that crossbred goats in Neuquen are free of caprine brucellosis.

The epidemiology and control of *Br. melitensis* in Malta was analysed using herd test data made available by the veterinary service of Malta (Abela, 1999). The eradication scheme commenced in 1987 with the introduction of a "test and slaughter' scheme using the RBPT. Herds registered with Malta Dairy Products Limited (MDP) showed a herd prevalence of 23% in 1987 which fell to less than 1.5% by 1993. Prevalence rose to 13% in 1995. Herds not delivering milk to MDP showed an initial herd prevalence of 4% which fell below 1% in 1994, remaining under 2% in 1995. It was noticed that the large herds and herds with small ruminants were most at risk to brucellosis infection. The seasonal fluctuation of prevalence was apparent. It was suggested that increased inforcement of regulations and motivation of farmers would accelerate eradication of brucellosis in Malta.

In Bihar, the scenery of brucellosis in pigs were recorded by Choudhary *et al.* (1983). They found 4.97% seropositive samples among a total of 241 pigs to the porcine brucellosis. While according to the National Agriculture Technical Project (NATP) scheme annual report (2002), (Department of Vet. Microbiology, Bihar Veterinary College, Patna) the sero prevalence of brucellosis recorded in cattle and buffaloes were 17.73 % and 9.17 % respectively.

In present investigation 5.152% (by STAT) brucellosis in goats were recorded in and around Patna localities. Our finding on goats are nearer to the finding of Chaudhary *et al.* (1983) on pigs while much lower than the findings revealed by NATP annual report on cattle and buffaloes. Thus it is clear by above instance that the seroprevalence of brucellosis in the goats and pigs seems to be lower than the cattle and buffaloes in Bihar.

The variation in the sero-prevalence of brucellosis among the animals may be due to the various factors such as environmental factors, breed, species, age, sex, etc. of animals. Besides these factors the method of sampling, type of serological tests employed, genetic factor of the animals and other factors also attributed to the variation in percent prevalence of antibodies to brucella.

The abortion during late pregnancy found most obvious sign in goats and sheep (Blood *et al.,* 1995). The primary reason of abortion in infected goats during late pregnancy may be due to considerably higher concentration of Brucella organisms in the placental and foetal fluid of uterus. This leads to ulcerative endometritis of the intercotyledonary space. The allantochorion, foetal fluids and placental cotylendons are next to invade and villi destroyed. So, the abortion takes place (Blood *et al.,* 1995).

Due to placentitis and cotyledonitis in brucella infection, placenta does not detach i.e. retention of placenta occurs in brucella infection (Roberts, 1997).

The comparative serological studies were conducted by various workers, viz. Fidanci *et al.* (1995); Aparicio *et al.* (1999); Singh *et al.* (2000); Sarumathi *et al.* (2003) etc. on brucellosis for evaluation of efficacy of different serological tests applied for the diagnosis of brucellosis.

During the present study 136 samples (uterine discharge and stomach content of aborted kids for culture and sera of these goats for comparative serology) collected from suspected goats having history of abortion, infertility, and retention of placenta for the culture purpose, 42 samples showed growth of Brucella colonies on the Hi-media agar. The morphological and staining characterization and the different biochemical tests applied for identification revealed these 42 samples positive for *Brucella melitensis*. The culture positive sample's respective serum sample was used for evaluation of serological tests (RPAT, RBPT and STAT) and all 25 sera samples collected from apparently healthy goats for comparative serology found negative for *Brucella melitensis*.

The results of the culture of brucella organism has been depicted in Table –VII. It is evident from Table – VII that percentage of brucella positivity (30.88%) was very high. It was probably due to fact that for the culture purpose the samples were collected from the clinically sick goats i.e. goats showing the symptoms of metritis and infertility and having history of abortion and retention of placenta and from aborted kids.

In the present investigation during the comparative serology, out of 42 culture positive samples 36 samples gave positive reaction and 6 samples

gave negative reaction by RPAT and RBPT, while 37 samples gave positive reaction and five samples proved negative to Brucella antigen by STAT. Thus the sensitivity of these tests was found to be 85.71%, 85.71% and 88.09% respectively. Our finding of sensitivity of these serological tests are in close agreement with the finding of Weber *et al.* (1981) where sensitivity of RPAT was 87.1% and Shringi *et al.* (2002) where the recorded sensitivity of these tests were 86.76% for RPAT and 87.09% for STAT. Each serum sample of the 25 non-vaccinated, apparently healthy goats shown negative reaction to the RPAT, RBPT and STAT. Hence, these all tests were found to be 100% specific in the culture negative samples. The finding of specificity of present study is in close agreement with the finding of Marin *et al.* (1989) and Aparicio *et al.* (1999) where the specificity of there tests were 100% in negative cases of brucellosis.

Waghela *et al.* (1980) found RBPT most sensitive test and AGIT the most specific test when the results from SAT, AGIT, RBPT and CFT were compared. They have also concluded that the RBPT and AGIT were useful when facilities for the CFT were unavailable. Stemshorn *et al.* (1985) compared the agglutination and CFT with respect to specificity, sensitivity and relative sensitivity for diagnosis of bovine brucellosis. Based on 1051 sera from brucellosis – free herds, the specificity of the tests was 99.2% for STAT, 99.3% for the plate agglutination test and 100% for RBPT. On a sample of 167 culture positive cattle the sensitivities of the test were RBPT-74.9%, RPAT-73.1 and STAT-68.9%. While evaluating the diagnostic value of various serological tests for detecting brucellosis in 15 experimentally infected sheep with *Br. melitensis,* Lako *et al.* (1989) found that the 13 (86.6%) sheep were positive in RBPT and 14 (93.3%) in the slow and rapid

agglutination test. Thus they found SAT more sensitive then RBPT. In present work also same trend of sensitivity was obtained.

Aparicio *et al.* (1999) evaluated the modified card test for diagnosis of caprine brucellosis. The sensitivity of the test was determined using sera from *Br. melitensis* culture positive goats. The sera from the goats, negative to brucellosis and from a brucellosis-free area, were used for specificity determination. Similar procedure was adopted in the present study for the evaluation of serological tests. Sting and Ortmann (2000) compared the specificity of slow agglutination test and found 81.1% in cattle and 96.2% in goats from brucellosis free herds. Thus species variation was shown by slow agglutination test in term of specificity of the test.

Stableforth (1959) noted that many infected sheep and goats, however, may not show a positive reaction to tube agglutination test, though they may be positive to other tests. He also mentioned that some of the factors influencing the result of agglutination test quantitatively are : number of organisms present in the antigen and their sensitivity, roughness which increases the titre and gives non-specific reaction, presence of dissolved agar leading to increased sensitivity, temperature and the period of incubation and the presence of haemoglobin in the serum which is responsible for nonspecific reaction. Ferreira de Abreu and Mario (1960) claimed reliable result by centrifuging the haemolysed blood samples and precipitating the haemoglobin by phenol. Lal and Bakshi (1960) noted that a large number of animals having a history of abortion did not show positive sero-agglutination reaction with brucella antigen, but microscopical, cultural and serological examinations at the time of abortion indicated that most of the abortions were caused by brucella.

Literature revealed that the STAT detects more number of seropositive goats than RBPT (Chukwu, 1987), in contrast to above finding Ghosh and Nanda (1988) and Abdel Kader (1997) found that the STAT detects less number of Brucella sero-positive goats than RBPT when used on a certain number of goat sera.

The similar variation in the detection of number of seropositive goats by these tests were observed in present investigation also, where in seroprevalence estimation RPAT and RBPT detected more number of the positive goats i.e. 5.343 and 5.343 percent respectively than STAT (5.152%). While during comparative serology the STAT detected more number of positive goats than the RPAT and RBPT. This variation may be due to that the STAT detects more reactors during the early stages of infection (Mahajan and Kulshreshtha, 1991) and also due to the various factors described by Stableforth (1959).

Rao *et al.* (1999) observed that RPAT gave a high percentage of positivity (11.5% and 16.25%) than STAT (8.75% and 15.00%) in graded Murrah buffaloes and crossbred cows respectively, when compared the efficacy of RPAT and STAT for the detection of antibodies to brucella in bovines.

Thus Rapid Plate Agglutination Test (RPAT) appeared to be an efficient screening test for brucellosis as also supported by Acharya and Panda (1985) and Shakya *et al.*, (1995).

A comparison between serological tests for *Br. melitensis* infection revealed that a single test is not sufficient to confirm the diagnosis of brucellosis and thus a combination of two tests are recommended (Mahajan and Kulshreshtha, 1991; Abuharfeil and Abo-Shehada, 1998). In present

investigation also as the sensitivity of these tests (RPAT, RBPT and STAT) were found not identical, therefore for confirmative sero diagnosis of caprine brucellosis a combination of two tests either RPAT and STAT or RBPT and STAT found useful. All these tests may be carried out in a field laboratory at very low cost as also reported by Mahajan and Kulshreshtha (1991).

On comparative serological studies of caprine brucellosis Pappous and Hontou (1988) found that the RBPT, as a good test for screening of the goat herds for brucellosis. This test was also found effective in vaccinated goats (Falade, 1983). In a comparative investigation between RPAT and STAT the RPAT found a simple and rapid method for the serological diagnosis of *Br. canis* infection in dogs (Weber *et al.*, 1981). On comparison of the Tube agglutination test, Rapid plate agglutination test and Rose Bengal tests in 1467 cattle, confirmed the usefulness of the Rose Bengal test as an adjunct to the two other tests (Lorenzo and Pennimpede, 1987). Evaluation of RBPT by Badnjevic and Bajrovic (1981) confirmed that the RBPT found satisfactorily sensitive and there was close correlation between RBPT and agglutination test.

In the present studies all the serum samples which were positive for sero-prevalence of caprine brucellosis by STAT were also positive by RBPT and RPAT. This suggests that RBPT and RPAT can be used as a rapid screening test for brucellosis in field condition, as also reported by Ghani (1995).

Though all sera samples which were positive by STAT were also positive by RPAT and RPBT, but one more serum sample which was found to be positive by RPAT and RBPT was not positive by STAT (dil. 1: 10 only). Thus STAT ruled out false positive serum sample which shown

positive by the RPAT and RBPT. Verma *et al.* (1998) also observed that RBPT i.e. a quick field test may show some false positive reactors. This fact revealed the usefulness of the STAT as a more satisfactory sero-diagnostic test.

In this way it was observed that screening the herds by RPAT or RBPT and finally testing by STAT gave more satisfactory results in sero-diagnosis of caprine brucellosis.

Jimenez de Baques (1990) also found that there were no one serological test that would accurately identify an infected goat from a non-infected goat.

A combination of tests and tests carried out on several occasions may increase the accuracy of detection of infected animals and the similar views were also reported by Renoux (1961) and Mahajan and Kulshreshtha (1991).

Bihar being an agrarian state, a large number of people frequently come in direct or indirect contact with goats. They are also at high risk of aquiring the infection due to their occupational activities. Besides them the veterinarians, abattoir workers are also at high risk of infection to brucellosis. Heavy economical losses due to caprine brucellosis adversely affects the rural economy of our state where the goat, a "poor man cow" acts as a chief source of income for landless labourers and people residing below poverty line. Thus, caprine brucellosis pose a great public health and economical problem in Bihar.

In the present study the presence of brucella antibodies in the goats clearly reveals that brucella infection is present in goats in the region tested of the Bihar.

As the goats have been slaughtered at different abattoirs are coming from different parts of the state, thus the findings of present study on these goats may show the incidence of brucellosis in goats of this state as a whole.

Further the infection is present in the goats belonging from organized farms, being slaughtered at different slaughter houses in and around Patna, goats arrived at veterinary hospitals as well as goats maintained at rural areas.

The higher economic losses to the farmers result due to kids lost, increased duration between two consecutive kidding, reduced milk production and also due to poor weight gain.

The treatment is unlikely to be effective economically or therapeutically in animals although tetracycline gives variable degree of remedy. The test and slaughter, quarantine of newly purchaged goats, periodical serological screening of herds, regular culling of brucella positive goats, vaccination of young stocks of 3 to 8 months of age, maintaining cleanliness and hygienic condition and educating the people may reduce the prevalence of disease considerably in both goats and human beings (Blood *et al.,* 1995).

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CHAPTER - VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Brucellosis is known as a contagious bacterial disease of world wide occurrence and also reported from all over the world including India. Caprine Brucellosis, usually caused by *Br. melitensis*, creates serious problem in the goat and human population due to its zoonotic importance and causes heavy economic losses.

The rearing of goats play an important role to over come poverty and unemployment by improving socio-economic conditions of weaker sections of society in India.

The present studies have been taken-up to work out the extent of infection of Brucella in goats in and around Patna and to evaluate different serological tests used for diagnosis of Brucellosis in goats.

Antigens, used for serological tests, viz. RPAT, RBPT and STAT were procured from Biological Product Division, I.V.R.I., Izatnagar. Culture media were obtained from Hi-media laboratory, Pvt. Mumbai.

Altogether 524 goat blood samples were collected from different sources, viz. slaughter houses, organized farms, veterinary hospitals and private breeders in and around Patna for sera samples. Another 136 samples of vaginal discharge from suspected sick goats and stomach content of aborted kids were also collected for culture purposes and blood samples from these goats were also collected for evaluation of serological tests. Twenty five blood samples from apparently healthy goats were collected for comparative study.

The RPAT, RBPT and STAT were performed with their respective antigens on all 524 sera samples as per the standard methods described by

WHO (1967), Alton *et al.* (1975) and Alton *et al.* (1988) respectively to know the prevalence of infection. The results of these tests were recorded and converted into International Unit.

The RPAT and RBPT revealed 28 (5.343%) sera samples positive to Brucella antibodies, whereas by STAT, 27 (5.152%) sera samples found positive to Brucella agglutinin.

The sex wise analysis of Brucella infection revealed that 2.84% male and 6.609% female sera samples were found to be positive to RPAT and RBPT. Where as 2.84% male and 6.321% female found positive to STAT. Statistical analysis revealed that sex in goat does not play any significant role in the rate of prevalence of brucellosis as no statistical significant difference was recorded between male and female goats.

The incidence of caprine brucellosis from different sources showed highest prevalence (6.91%) in the goats slaughtered at different slaughter houses, followed by the goats belonging to the organized farms (5.55%), veterinary hospitals (4.87%) and lowest in rural areas (2.14%).

Out of 136 culture samples 42 samples were found to be positive for Brucella growth. Where as, all 25 blood samples from apparently healthy goats were found to be culturally negative.

Out of 42 culture positive sera, the 36 sera samples were found positive by RPAT and RBPT and 37 sera samples found to be positive by STAT. Hence, the sensitivity of these tests were found to be 85.71%, 85.71% and 88.09% respectively.

Each serum sample of the 25 non-vaccinated, apparently healthy goat showed negative reaction by RPAT, RBPT and STAT. Hence, these all tests were found to be more specific on the culture negative samples.

The treatment is unlikely to be effective economically or therapeutically, although tetracycline gives variable degree of remedy. The test and slaughter, quarantine of newly purchased goats, periodical serological screening of herds, vaccination of young stocks of 3 to 8 months of age, maintaining cleanliness and hygienic condition and educating the people may reduce the prevalence of disease considerably in both goats and human being.

Therefore, on the basis of above observations following conclusions can be drawn :

(i) The Brucella seropositive goats were present in the studied area (5.343% by RPAT and RBPT and 5.152% by STAT).

(ii) The sero-prevalence in female goats (6.609% by RPAT and RBPT and 6.321% by STAT) were higher than male (2.840% by RPAT, RBPT and STAT) goats.

(iii) The RPAT and RBPT showed similar results on seroprevalence in both male (2.840%) and female (6.609%) goats respectively.

(iv) The STAT exhibited little lower (5.152%) seropositivity of caprine brucellosis than RPAT and RBPT (5.343%).

(v) The goats belonging to slaughter houses and organized farms shown higher seroprevalence i.e. 6.91% and 5.55% respectively while the least seroprevalence (2.14%) was observed in rural unorganized farms.

(vi) The cultural, morphological, staining, biochemical and serological characterization showed very high rate of seropositivity (30.88%) among the suspected sick goats.

(vii) The sensitivity of STAT (88.09%) exhibited higher than the sensitivity of RPAT (85.71%) and RBPT (85.71%) respectively.

(viii) The RPAT and RBPT showed usefulness in screening the goat herds for brucella seroprevalence.

(ix) The combination of two tests either RPAT and STAT or RBPT and STAT exhibited usefulness in confirmatory diagnosis.

(x) All these three serological tests (RPAT, RBPT and STAT) showed 100% specificity on culture negative non-vaccinated goats.

(xi) All these serological tests showed easy to perform at low cost.

(xii) The control measures have been discussed.

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APPENDIX

APPENDIX

APPENDIX - I

PEPTONE WATER :

Composition -

Peptone	-	1 gm.
Sodium Chloride	-	0.5 gm.
Distilled Water	-	100 ml.

Adjust pH 7.2 and sterilize at 121°C for 15 minutes.

APPENDIX - II

UREA MEDIUM (CHRISTENSEN'S MEDIUM) :

Composition -

Solution A

Peptone	-	0.1 gm.
Glucose	-	0.1 gm.
Sodium Chloride	-	0.5 gm.
Mono-potassium phosphate	-	0.2 gm.
Phenol red	-	0.0012 gm.
Agar	-	2.0 gm.
Distilled Water	-	100 ml.

Sterilize at 115°C for 15 minutes.

Solution B

Urea	-	2 gm.
Distilled water	-	100 ml.

Sterilize by Seitz filtration.

Before use, melt agar medium (solution A), cool to 50°C and add 0.5 ml. of urea solution (solution B) and make slants.