SERO CONVERSION STUDIES OF *BRUCELLA ABORTUS* STRAIN RB5I VACCINE IN CATTLE

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Thesis submitted in partial fulfilment of the requirement for the degree of

Master of Veterinary Science

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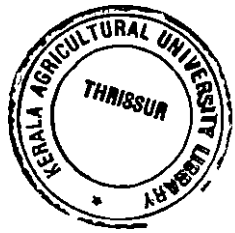

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DECLARATION

I hereby declare that the thesis entitled "**SERO CONVERSION STUDIES OF *BRUCELLA ABORTUS* STRAIN RB51 VACCINE IN CATTLE**" is a bonafide record of research work done by me during the course of research and that this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis, entitled **"SERO CONVERSION STUDIES OF *BRUCELLA ABORTUS* STRAIN RB51 VACCINE IN CATTLE"** is a record of research work done independently by **Nimisha K.N.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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CONTENTS

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Introduction

1. INTRODUCTION

Brucellosis results in devastating productivity of livestock and affecting mankind either directly through infection or indirectly through reduction of productivity among livestock. Ruminants and pigs are heavily infected in many areas of the world, and wild life is not exempt of brucellosis, thus acting as a potential reservoir for domestic livestock and a consequent risk for human beings. Because domestic ruminants and swine are essential to the economy of millions of persons, particularly in under developed and developing countries, brucellosis is a major cause of direct economical losses and a major impediment for trade and exportations.

Brucellosis in domestic animals are characterized by abortion, infertility, retention of placenta and still birth. In humans it is a very painful syndrome and produces various symptoms like chills, sweating, head ache, fatigue and joint pain. It is estimated by the World Health Organization (WHO) that more than 5,00,000 new cases of human brucellosis occur each year (Cutler, 2006).

As per Animal Disease Monitoring and Surveillance (ADMAS) report on long term survey on bovine brucellosis (1994-2002), the disease is wide spread in most parts of the country with cumulative average of 6.8 per cent in bovine. The overall national average incidence of brucellosis in cattle and buffalo were 7.2 per cent and 5.25 per cent respectively. The prevalence of brucella antibodies ranges from 2.7 per cent in Andaman & Nicobar island to a maximum of 63.5 per cent in West Bengal. Great economic losses are caused by the disease because of reproductive problems in animals and loss of man–days in humans in addition to treatment costs. Treatment being unreliable and very cumbersome in humans and imprudent in animals, only way to check the malady is the prevention and control programmes in animals.

It is generally agreed that under most conditions, vaccination is essential for the control of bovine, ovine and caprine brucellosis. Individual identification, strict control of animal movement and trade, 100 per cent vaccine coverage and monitoring of the serological and clinical status are the most relevant requisites to achieve eradication. When prevalence is higher or these requirements are not met, the relevant point is to use the best vaccine in terms of protection. Calf-attenuated vaccine is still the best and most economical option as the other measures are not practicable in India owing to diverse socio-economic, religious and cultural factors.

Attenuated *Brucella abortus* strain 19 and *Brucella melitensis* Rev1 were proven to be effective vaccines against *Brucella abortus* in cattle and against *Brucella melitensis* and *Brucella ovis* in sheep and goats, respectively. However, both vaccines have the disadvantages of inducing O-polysaccharide specific antibodies that interfere with serologic diagnosis of disease, of causing abortion in vaccinated animals under some circumstances, of being pathogenic for human being. Alternate vaccines have been sought for many years, with limited success. However, stable rough *Brucella abortus* mutant RB51 has now emerged as a strong candidate as an alternative vaccine to strain 19 because it provides a degree of protection in cattle comparable to that induced by strain 19.

The present study was conducted with the following objectives:

1. To assess the sero conversion of *Brucella abortus* strain RB51 vaccine in cattle.

2. To study the interference of *Brucella abortus* strain RB51 induced antibody with routine diagnostic tests.

Review of Literature

2. REVIEW OF LITERATURE

2.1 ETIOLOGY

Brucella abortus is a gram negative cocco-bacillary organism having a length of about 0.4 to 2.5μm and breadth about 0.4- 0.6μm (Huddleson, 1943).

Brucella are small, gram negative bacilli or cocco-bacilli, non-spore forming, non-motile and non-acid fast organisms. These are aerobic but may require 5-10 per cent carbon dioxide tension for primary isolation and are commonly seen intracellularly singly or in groups. Delicate translucent colonies of 2-3 mm in diameter grow on blood agar or glucose agar. *Brucella ovis* grows in M (mucoid) form, *Brucella abortus* and *Brucella melitensis* grow at the beginning in S (smooth) form and later dissociate into the R (rough) and M form. The biotypes are differentiated serologically applying specific monosera (M and R) (Stableforth, 1959).

Corbel (1975) isolated the ribosomes from *Brucella abortus* strain 19 and 45/20 and reported that they had some role in the bovine immune responses to *Brucella* infection and vaccination.

The greater stability of *Brucella* in phenol was due to the covalent linkage of protein with lipopolysaccharide molecule (Jones and Berman, 1975).

Riley and Robertson (1984) opined that *Brucella abortus* did not stimulate an effective level of degranulation after ingestion as observed with extra cellular parasite, and that the smooth intermediate strain was more resistant to intraleukoytic killing system than rough strain.

In the infected host, *Brucella abortus* multiplied within the phagosomes of macrophage monocyte lineage cells by inhibiting phago-lysosome fusion (Frenchick *et al.*, 1985).

The lipopolysaccharides (LPS) of wild type strains of *Brucella abortus* and *Brucella melitensis* contained O-Polysaccharide side chains (OPS) that gave colonies a smooth phenotype, while *Brucella ovis* occured in the rough phenotype because its LPS was devoid of OPS (Jimenez de Bagues *et al.*, 1994).

Gupta *et al.* (1995) concluded that the immunoreactivity of *Brucella* S-LPS is associated with distinct protein.

Lord *et al.* (1998) reviewed that *Brucella abortus* was a facultative intracellular parasite that could cause abortion and decreased fertility in animals.

Brucella is an α proteo bacteria causing an infectious disease of mammals that is transmitted to humans. *Brucella* devoid of the OPS is termed rough or R because their colonial surface contracts with the glistening smooth aspect of those carrying S-LPS (Moriyon *et al.*, 2004).

The etiological agents of brucellosis in man is a small, pleomorphic gram negative rod which was first isolated from human clinical cases of undulant fever by David Bruce in1887 (Cutler, 2006).

2.2 EPIDEMIOLOGY

2.2.1 Prevalence

In Madhya Pradesh 4.44 per cent bovine brucellosis was recorded on the basis of serum samples analyzed by tube agglutination test (Kataria and Verma, 1969).

Baby (1978) reported an overall incidence of 2.24 per cent brucellosis among buffalo population in Trichur district.

Sero diagnosis of brucellosis revealed 4.33 per cent and 8.64 per cent infected cattle and buffaloes respectively at organized farms in and around Punjab (Oberoi and Kwatra, 1982).

Sharma *et al.* (1984) observed that the sero prevalence of brucellosis in Uttar Pradesh was highest among equines, followed by that in pigs, cattle, buffaloes, goat and sheep.

Prevalence of brucellosis in milch goats at Bikaner was found to be 11.42 per cent and higher in those goats which were in more than one lactation (25 per cent) while it was found to be 15.38 per cent in first lactation (Kapoor *et al.*, 1985).

The prevalence of brucellosis in lactating cows was less than non lactating and more in pregnant than in non pregnant animals (Bachh *et al.*, 1988).

Lavsen *et al.* (1988) found out that prevalence rate of brucellosis among pregnant cows was higher than in the non pregnant cows.

Pillai *et al.* (1991) identified *Brucella canis* infection of dogs in Madras, the incidence being 2.18 per cent among the canine population.

Bovine serum samples from Zebu cattle and buffaloes with history of abortion, retained placenta, repeat breeding and pyrexia were tested by ELISA for the presence of *Brucella* antibodies. Of the 138 serum samples tested, 26(18.84 per cent) showed positive antibody levels (Chandramohan *et al.*, 1992).

Ghani *et al.* (1998) reported low incidence of brucellosis in Peshawar district of Pakistan after screening of 500 serum samples.

The prevalence of brucellosis among aborted and pregnant buffaloes in Gujarat was 44 per cent and 11.11 per cent respectively (Chauhan *et al.*, 2000).

Sandhu *et al.* (2001) reported sero prevalence of brucellosis among 10.06 per cent of cows and 9.33 per cent of buffaloes respectively, in Punjab.

The overall seroprevalence of brucellosis among cattle and buffaloes in Kerala was recorded as 15.29 per cent (Priya, 2003).

Among different animal species, highest prevalence of brucellosis was recorded in sheep (15.6 per cent), followed by goats (5.4per cent), buffaloes (3.42 per cent) and cattle (2.65 per cent) in Himachal Pradesh (Charanjeet *et al.*, 2004).

Acosta-Gonzalez *et al.* (2006) examined serum samples from 420 equines using Rose Bengal test and Rivanol test and recorded a sero prevalence of only 0.238 per cent in the state of Tamaulipas, Mexico.

2.2.2 Host Factors

2.2.2.1 Age

Chantal and Thomas (1977) found out a high prevalence rate (8.7 per cent) of brucellosis in cattle of 5-10 years of age.

Gray and Martin (1980) recorded higher prevalence (11.23 per cent) of brucellosis in Punjab in a random survey conducted and observed that young animals had a low prevalence of the disease.

Susceptibility to brucellosis was found to be higher in animals above four years of age (Ghani *et al.*, 1998).

Cattle were more resistant to brucellosis before maturity and became more susceptible as they approached breeding age (Radostits *et al.*, 2000).

Leite *et al.* (2003) concluded that susceptibility to brucellosis was associated with sexual maturity rather than age and therefore, sexually immature cattle did not become infected following exposure or recovered quite rapidly.

Priya (2003) concluded that bulls and cows aged more than five years showed the highest sero positivity to brucellosis.

Highest prevalence of brucellosis was observed in animals above twelve years of age (Sharma *et al.*, 2003).

Age wise prevalence of brucellosis revealed higher occurrence in cattle and buffaloes of more than nine years of age. There was low prevalence in animals less than two years of age (Charanjeet *et al.*, 2004).

Dhand *et al.* (2005) reported a high prevalence (11.23 per cent) of brucellosis in Punjab in a random survey conducted and observed that young animals had low prevalence of the disease.

Kaur *et al.* (2006) observed that both cattle and buffaloes in the age group of 4-6 years were highly susceptible to infection of *Brucella abortus.*

2.2.2.2 Breed

Jeyaprakash *et al.* (1999) reported that brucellosis was more in indigenous (66.7 per cent) cows than that in crossbred cows (33.3 per cent).

Apparently no breed resistance to brucellosis was known and all breeds of cattle were susceptible to brucellosis (Radostits *et al.*, 2000).

Among the breeds, pure bred Holstein Friesian bulls and crossbred Jersey female cattle showed the highest seroprevalence in Kerala (Priya, 2003).

Cross bred animals were less resistant to infection and remained under stress due to hot and humid conditions (Sharma *et al.*, 2003).

2.2.2.3 Sex

Bachh *et al.* (1988) reported that the prevalence of brucellosis was more in female than in male. Cattle with previous history of abortion and repeat breeders with no history of abortion were 88.9 per cent and 66.7 per cent sero positive respectively.

Female cattle and female buffaloes showed higher sero prevalence than male cattle and male buffaloes (Priya, 2003).

Sharma *et al.* (2003) observed that sero prevalence of brucellosis was higher in bovine females than in males.

2.2.3 Management

All isolates of *Brucella* organisms from each herd were of same biochemical antigenic type (Nelson *et al.*, 1966).

Sreenivasan (1972) recorded a higher prevalence of brucellosis in big herds resorted to hill grazing and kept in hygienic conditions devoid of direct sunlight.

Bali and Kumi-Diaka (1981) noticed existence of brucellosis in cattle in the livestock investigation and breeding centers in the Northern Nigeria, which was attributed to poor fencing, indiscriminate use of strain 19 vaccine, interaction of cattle with those of nomadic herdsmen and indiscriminate buying in of animals without quarantine.

Agro climatic zones and systems of management practiced in the farms were important risk factors in the occurrence of *Brucella abortus* infection in Sri Lanka (Silva *et al.*, 2000).

Prevalence of brucellosis was found to be higher in unorganized farms than in organized farms in Assam (Hussain *et al.*, 2000).

Kalorey *et al.* (2000) reported high incidence of brucellosis in herds, where cows and goats co-habit and suggested that this might be due to poor management and hygienic practices and association with *Brucella* infected cows.

2.2.4 Transmission

Although brucellosis is acquired most often by ingestion, infection can occasionally follow venereal contact, penetration through skin abrasions, conjunctiva or inhalation and infection occurs when animals make direct or indirect contact with infective excretions (Quinn *et al.*, 1999).

Brucellosis can be transmitted horizontally by the introduction of acutely or latently infected animals into a herd, or vertically *in utero* to calves born from infected dams. Contaminated pasture, fodder, or water is an important source of infection (Radostits *et al.*, 2000).

In humans, infection can result from direct contact with infected animals and can be transmitted to consumers through raw milk and milk products (Amin *et al.*, 2005).

2.3 CLINICAL SIGNS

Mathur (1971) reported that abortions due to brucellosis were found to occur most often at the eighth month of gestation, followed by seventh and ninth months.

Fensterbank (1978) reported hygroma in a male calf having congenital *Brucella abortus* infection.

Roberts (1986) described the occurrence of retention of placenta due to placentitis and cotyledonitis in animals suffering from brucellosis.

Brucella agglutinins were present in cattle with previous history of abortion and also in repeat breeders with no history of abortion (Bachh *et al.*, 1988).

Maiti *et al.* (1999) reported arthritis in *Brucella* infected new born calves.

Brucella abortus infection causes abortion storm in first calf heifers after fifth month of pregnancy and orchitis, epididymitis and synovitis in bulls (Radostitis *et al.*, 2000).

The Brucella infection tends to localize in the reticulo-endothelial system and genital tract with abortion in the females and epididymitis and orchitis in males as the most common clinical manifestations (Walker, 2002).

Brucellosis is an important cause of abortion in several species of domestic animals, causing sometimes temporary or permanent infertility and great economic losses (Leite *et al.*, 2003).

Clinical manifestations of brucellosis are abortion, infertility, retention of fetal membrane, still birth and calf loss in animals (Kahn, 2005).

2.4 DIAGNOSIS

2.4.1 Isolation of organism

Sevalgi *et al.* (1987) isolated *Brucella abortus* biotype1 from animals and human beings in Dharwad.

Forbes (1990) reported that *Brucella abortus* biovar 4 was isolated from 14 dogs from the farms with *Brucella* infected cattle, of which 10 dogs were serologically positive.

Verma *et al.* (2000) isolated *Brucella abortus* biotype 3 from aborted cows and indicated that isolation and identification of *Brucella* from clinical samples and morbid materials was possibly the most reliable method.

2.4.2 Serological Diagnostic Tests

2.4.2.1 Rose Bengal plate test (RBPT)

Acidified plate test inhibited nonspecific agglutinins than specific agglutinins at a pH of 3-4 (Roepke *et al.*, 1957).

The acidified plate agglutination test appeared to have a higher degree of accuracy on serum from infected vaccinated cattle than on serum from infected non vaccinated cattle (Lambert and Amerault, 1962a).

Rose Bengal plate test could be used as a quick reliable diagnostic test in the diagnosis of *Brucella* infection under field condition (Kalorey *et al.*, 2000).

Sandhu *et al.* (2001) reported prevalence of 10.06 per cent, 9.33 per cent and 1.18 per cent *Brucella* antibodies in cattle, buffaloes and goat respectively, using RBPT.

Rose Bengal plate test was found to have higher sensitivity, negative predictive value and accuracy than Standard tube agglutination test and 2-Mercaptoethanol test (Priya, 2003).

Charanjeet *et al.* (2004) reported that sensitivity of RBPT was found to be 75 per cent, where as specificity was 98.3 per cent.

Rose Bengal plate test was more efficient than Standard tube agglutination test in all species (Thakur and Thapliyal, 2004).

2.4.2.2 Milk ring test (MRT)

Milk samples from infected cows were constantly negative to ring test when the fat globules were of uniform and predominantly small size, and invariably positive when the fat globules showed a marked difference in size (Ogonowski,1955).

Roepke *et al.* (1957) noticed marked variations between quarter milk titres and also in the relative levels of agglutinins in the blood. These variations suggested that specific brucella agglutinins in the milk did not result from blood agglutinins, but from udder infection and also indicated the presence of a barrier which prevented the free movement of the agglutinins.

Milk ring tests were reliable only during the period of normal lactation. During the last six to eight weeks of drying off period and in colostrum, seroglobulins bearing the seroagglutinins were present in a higher concentration (Kerr, 1960).

Kapur and Singh (1967) recommended abortus bang ring as a screening test for *Brucella* infection in individual animals and found 86 per cent correlation between Standard tube agglutination test and Milk ring test in buffaloes.

Mathur (1971) reported that milk ring test was a simpler method of judging the extend of brucellosis among cows of a farm.

Buth and Manchanda (1972) carried out MRT on pooled milk samples from lactating cows and buffaloes in 24 villages around Karnal. The positive results were confirmed by tube and plate serum agglutination test.

2.4.2.2 Standard tube agglutination test (STAT)

Kataria and Verma (1969) reported an over-all incidence of 4.44 per cent bovine brucellosis after analyzing 6438 serum samples by STAT.

Lewkowiez (1973) found that during pregnancy, the highest titres were observed in the third month and the lowest in the fifth month in STAT.

According to Kalimuddin *et al.* (1990) STAT was not sufficient for the diagnosis of brucellosis, three other serological tests *viz.,* Complement fixation test, 2-Mercaptoethanol test and Heat inactivation test were also essential.

Among the conventional serological tests, STAT was found to have a relative sensitivity and specificity of 28.57 per cent and 17.14 per cent respectively in the diagnosis of ovine brucellosis (Barbuddhe *et al.*, 1994).

Standard tube agglutination test was more reliable, sensitive, less time consuming and could be easily applied in the sero epidemiology of bovine brucellosis (Ghani, 1995).

The tube agglutination test has become the standard method recommended for collection of quantitative information on immune responses, and is the most frequently used confirmatory serological test (Lucero and Bolpe, 1998).

Sensitivity of STAT was found to be 81.81 per cent following screening of 238 serum samples (Agarwal and Batra, 1999).

Charanjeet *et al.* (2004) compared STAT, RBPT, and Avidin-Biotin ELISA with regard to their sensitivity and specificity in the diagnosis of brucellosis and recorded 66 per cent sensitivity and 98.5 per cent specificity for STAT.

Ramani Pushpa and Punya Kumari (2005) stated that STAT was more appropriate when compared to RBPT to detect the prevalence of brucellosis.

2.4.2.4 2-Mercaptoethanol test (2-MET)

Stemshorn *et al.* (1985) reported 99.8 per cent specificity for 2-MET based on screening 1051 sera from brucellosis free herds and 59.9 per cent sensitivity based on 167 culture positive cattle sera.

Pillai *et al.* (1991) analyzed 640 dog serum samples using 2-MET and Counter immuno electrophoresis test and reported that 2-MET and CIE showed good correlation. None of the sera negative for 2-MET was found positive by CIE.

Shringi *et al.* (2002) reported a sensitivity and specificity of 90.74 and 92.22 per cent respectively for 2-Mercaptoethanol test, after evaluating the efficacy among various serological tests.

Priya (2003) reported a specificity of 100 per cent for 2-Mercaptoethanol test after comparing with STAT and RBPT.

2.4.2.5 Complement Fixation Test (CFT)

Lambert and Amerault (1962b) concluded that CFT was effectively used to identify non infected cattle, however, it was not as efficient as the other test in detecting early infection.

Dohoo *et al.* (1986) evaluated the efficacy of five serological tests *viz.*, Buffered plate antigen test, Standard tube agglutination test, Complement fixation test, Hemolysis in Gel Test and Indirect-ELISA in sera from 1208 cattle in brucellosis free herds, 1578 cattle in reactor herds of unknown infection status and 174 cattle from which *Brucella abortus* had been cultured and stated Complement fixation test as an appropriate confirmatory test having high specificity.

Agarwal *et al.* (1999) used CFT for the detection of brucella antibodies in sheep and goat and reported a sensitivity of 97.9 per cent and specificity of 89.8 per cent.

2.4.2.6 Enzyme Linked Immunosorbent Assay (ELISA)

Compared with indirect ELISA, competitive ELISA appeared to amplify the difference between responses of *Brucella abortus* strain 19 vaccinated and infected cattle (Nielsen *et al.*, 1989).

Cloeckaert *et al.* (1992) suggested that the antibody response to outer membrane proteins (OMPs) was different from one animal to another. A combination of several OMPs would be necessary for detection of all infected animals that had anti-LPS antibodies. The antibody response against brucella OMPs in bovine brucellosis by Immuno blot analysis and Competitive ELISA indicated the potential usefulness of the OMPs as diagnostic antigens.

Barbuddhe *et al.* (1994) compared Dot-ELISA with conventional serological tests and opined that Dot-ELISA was more sensitive than any other test and could be able to detect antibodies present during the early stage of infection.

Dot-blot assay was highly specific in detecting antibody titers during the first eight weeks after vaccination with strain RB51.Calfhood vaccination of cattle with 10^{10} CFU of strain RB51 might not induce antibody responses that could be differentiated from non-vaccinated cattle on the RB 51 dot-blot assay (Olsen *et al.*, 1997).

Agarwal and Batra (1999) reported that inhibition ELISA had a sensitivity of 92.04 per cent and was more convenient to perform as the testing protocol required no alteration with the change of animal species.

Renukaradhya *et al.* (2001) reported that the over all specificity and sensitivity of Avidin- Biotin ELISA were 98.8 per cent and 98.2 per cent respectively.

An indirect ELISA was developed to identify *Brucella Abortus* strain RB51-specific antibodies using mouse monoclonal antibody specific for bovine IgG_1.This test was relatively easy to perform, suitable to automation and standardization across multiple laboratories, and correctly identified RB51 inoculated animals (Colby *et al.*, 2002).

Priya (2003) reported that Avidin- Biotin ELISA detected 245 positive reactors (15.29 per cent) out of the total 1602 serum samples tested and suggested that ELISA could be used as a confirmatory diagnostic test.

The over all specificity and sensitivity of Avidin-Biotin ELISA for the detection of antibodies to *Brucella abortus* was found to be 98.8 per cent and 98.2 per cent respectively (Charanjeet *et al.*, 2004).

Gall *et al.* (2006) developed a simple, rapid, field-adapted indirect ELISA for the detection of antibodies to *Brucella abortus* in whole blood and serum samples. This assay detected antibodies in approximately 15 min or less.

2.4.3 Molecular Diagnosis

2.4.3.1 Polymerase Chain Reaction (PCR)

The AMOS-PCR assay is a multiplex primer assay that uses a five-primer cocktail identifies *Brucella abortus* strain19 and RB51. Identification is based on the number and size of six products amplified by PCR (Bricker and Halling, 1995).

PCR can be complementary to classical serological tests for the detection of *Brucella* species infection in buffaloes, especially in the initial phase, when the immune response of the animal is not detectable (Guarino *et al.*, 2000).

Leal-klevezas *et al.* (2000) used PCR to diagnose goat brucellosis in milk and blood samples. Results showed that 86 per cent of blood samples were positive on PCR test, while 60 per cent were positive on the serological test and it was concluded that sensitivity of PCR was higher than RBPT and blood culture and could be used for rapid identification of *Brucella* strains.

Cortez *et al.* (2001) detected *Brucella* DNA from aborted fetuses and four of the 54 culture negative samples using PCR.

Chavarria *et al.* (2006) isolated *Brucella abortus* strain 19 in cow milk samples using PCR. Also suggested that PCR assays could be used to identify strain 19 and strain RB51 and distinguish them from other *Brucella* species and biotypes.

2.5 VACCINES

2.5.1 *Brucella abortus* Strain 19

Mathur (1971) reported that vaccination with strain 19 in calf hood did not give 100 per cent guarantee against abortions, although it reduced number of abortion gradually to a very large extent.

Barton and Lomme (1980) stated that reduced dosage for strain 19 vaccination, coupled with improved tests and infected herd management techniques, had minimized the problems associated with adult vaccination.

In case of cross bred calves maximum concentration of IgM and IgG_1 were detected on 15^{th} and 30^{th} day of strain 19 vaccination respectively and IgG_1 peaked on 15^{th} day. Immunoglobulin G_2 antibody was produced in much higher quantities than either IgG_1 or IgM (Das and Mulbgal, 1982).

Misra *et al.* (1982) suggested that exotic and cross bred calves should be vaccinated at the age of six months and these became serologically negative for vaccinal titres at a period of ten months.

Das and Mulbgal (1983) concluded that following strain 19 vaccination, IgM was the first antibody to appear followed by IgG class and was more reactive than IgG in STAT and Complement fixation test.

Venkatesha and Upadhye (1987) reported that calves three to five weeks of age vaccinated subcutaneously with strain 19 were positive for rapid plate test up to two months and at third month majority were negative. Agglutinating antibody response

and complement fixing antibody response following vaccination with strain 19 were related to age at vaccination.

The persistence of post-vaccinal titers that interfere with serological test interpretation was one of the most important disadvantages of the vaccinal strain 19 of *Brucella abortus* (Lord *et al.*, 1998).

Controlled experiments had demonstrated that the *Brucella abortus* strain 19 in cattle was a useful vaccine and abortion caused by strain 19 was low but that of Rev1 could be higher, particularly in association with some of its variant (Moriyon *et al.*, 2004).

2.5.2 *Brucella abortus* Strain RB51

The O-polysaccharide (OPS) deficient RB51 was produced by serial passages of virulent *Brucella abortus* strain 2308 on rifampin supplemented trypticase soy agar (Schurig *et al.*, 1991).

Brucella abortus strain RB51 vaccine provided a degree of protection in cattle comparable to that induced by strain 19 without the disadvantages of it (Cheville *et al.*, 1993).

Jimenez de Bagues *et al.* (1994) documented that vaccination with strain RB51 not only conferred protection against *Brucella abortus*, but also provided cross protection against *Brucella melitensis* and *Brucella ovis*.

Cattle vaccinated with *Brucella abortus* strain RB51 failed to produce antibodies that could be detected by conventional serological tests that were used to diagnose bovine brucellosis (Stevens *et al.*, 1994).

Stevens *et al.* (1995) reported that cattle vaccinated with *Brucella abortus* strain 19 or strain RB51 had similar immune responses, but unlike strain 19, strain RB51 did not induce positive results in the standard tube agglutination test used to diagnose brucellosis in cattle.

Brucella abortus strain RB51 vaccine was protective at doses comparable to those of strain 19 in calves 3-10 months of age, but immunogenicity and failure to induce antibodies that interfered with the serologic diagnosis of field infections of *Brucella abortus* made strain RB51 an effective vaccine (Cheville *et al.*, 1996).

Palmer *et al.* (1996) reported that strain RB51 was less abortifacient than strain 19, following intravenous inoculation of pregnant cattle. Only one of four heifers delivered prematurely, whereas strain 19 led to 100 per cent abortion.

It was observed that pregnant cattle could be safely vaccinated with *Brucella abortus* strain RB51 without subsequent abortion and placentitis. Further more, strain RB51 was immunogenic in pregnant cattle, resulting in humoral and cell mediated immune responses, but did not interfere with serologic diagnosis of field infections (Palmer *et al.*, 1997).

Elzer *et al.* (1998) reported that cattle vaccinated orally with the rough variant *Brucella abortus* strain RB51 developed significant protection against abortion and colonization after virulent challenge exposure and did not produce OPS-specific antibodies.

Experiments with a mouse model showed that vaccination with strain RB51 provided protection against challenge with heterologous *Brucella* species including *Brucella melitensis* (Adone and Ciuchini, 1999).

Caspel *et al.* (2000) suggested that viability of *Brucella abortus* strain RB51 could be readily maintained during storage as a lyophilized or liquid vaccine.

Revaccination of strain 19 calf hood-vaccinated pregnant cattle with *Brucella abortus* strain RB51 appeared to be a safe procedure with no diagnostically negative consequences (Samartino *et al.*, 2000).

Uzal *et al.* (2000) observed that multiple vaccinations with *Brucella abortus* strain RB51 had not induced sero conversion to brucellosis surveillance test.

Calves vaccinated subcutaneously with reduced dose of strain RB51 afforded absolute protection and performed better than strain 19 (Moriyon *et al.*, 2004).

Olsen *et al.* (2004) suggested that *Brucella abortus* strain RB51 was safe in black bears and was unlikely to be more pathogenic in bears than field strains.

Leal-Hernandez *et al.* (2005) reported that revaccination with a reduced dose of *Brucella abortus* strain RB51 in endemic zone did not cause abortion and protected 94 per cent of animals against field infection, but caused atypical responses to conventional serological tests.

Brucellosis vaccination of elk with *Brucella abortus* strain 19 or RB51 did not induce robust and persistent cellular immunologic responses even after booster vaccination. As with most other facultative intra cellular pathogens, cell-mediated immunity is believed to play a crucial role in long-term protection against *Brucella* (Olsen *et al.*, 2006).

Materials and methods

3. MATERIALS AND METHODS

The present study was carried out at the Department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during October 2006 to April 2007.

3.1 GLASSWARE AND REAGENTS

All glassware used were of either Borosil or Vensil brand and chemicals were of analytical or guaranteed reagent grade.

3.1.1 Sterilization of glassware and plastic ware

New test tubes were kept over night in potassium dichromate solution (Potassium dichromate Crystals-80mg, Concentrated sulphuric acid-250ml, Distilled Water-750ml).

The test tubes were washed twice in ordinary tap water and once in distilled water. The test tubes were dried and sterilized by keeping in hot air oven at 160 ^{0} C for one hour, after plugging with non absorbant cotton.

Plastic ware were sterilized by autoclaving at 121 ^{0}C for 15 minutes at 15 lbs pressure.

3.2 EXPERIMENTAL ANIMALS

Calves of four months and above age and sero negative for brucellosis were selected for the study from Kerala Agricultural University Livestock Farm, Mannuthy.

A total of 46 animals were selected which were apparently healthy and free from clinical illness. They were grouped into three at random as follows.

Group I : Twenty one animals

Group II .: Ninteen animals

Group III : Six animals

3.3 VACCINES

Two different Brucella vaccines were used for the study (Plate 1)

Vaccine 1: *Brucella abortus* strain RB51(SRB51)- It is a live attenuated rough strain, derived from the virulent smooth strain *Brucella abortus* 2308.

Vaccine 2: *Brucella abortus* strain19 (S19) - It is a live attenuated smooth strain.

3.4 BIOLOGICALS AND REAGENTS

Rose Bengal Antigen:- It is a suspension of pure smooth cultures of *Brucella abortus* strain 99 in phenolised saline, the bacteria being coloured by addition of rose bengal dye.

Brucella abortus plain antigen:- It is a suspension of smooth culture of *Brucella abortus* strain 99 in phenol saline.

3.5 VACCINATION OF ANIMALS

3.5.1 Group I

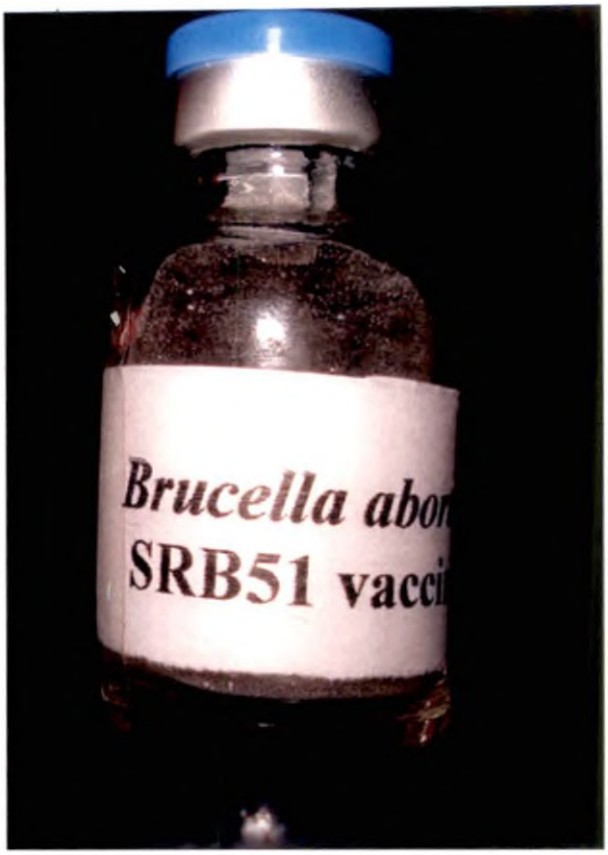
All the 21 calves were vaccinated with two ml (1.8×10^{10} CFU / dose) of *Brucella abortus* strain RB51 vaccine subcutaneously.

3.5.2 Group II

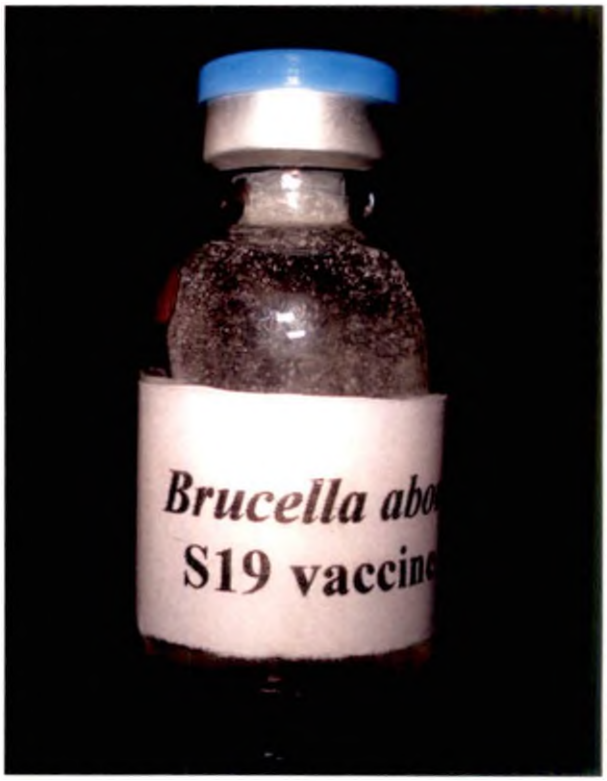
All the 19 calves were vaccinated with one ml (1×10^{10} CFU /dose) of *Brucella abortus* strain RB51 vaccine subcutaneously.

3.5.3 Group III

All the six calves of this group were vaccinated with two ml (4×10^{10} CFU /dose)of *Brucella abortus* strain 19 vaccine subcutaneously.



A



B

3.6 CLINICAL OBSERVATION

All the animals were observed for any febrile reaction and other clinical signs two days before and one week after vaccination.

3.7 COLLECTION OF BLOOD

All the calves were exsanguinated before vaccination. Five ml of blood was collected aseptically by jugular puncture using an 18G sterile hypodermic needle, into test tubes of 15 ml capacity. The tubes were labelled and kept in a slanting position for clotting. Later, the clots were disrupted and the test tubes were transferred to refrigerator and kept overnight at 4^0C.

Next day, sera were centrifuged at 1000×g for 10 min and transferred to cryo vials after heat inactivation at 56^0C for 30 min in water bath to inactivate non- specific agglutinins. The vials were stored at -20^0C, which formed the zeroth day samples.

All the calves were bled at weekly interval during the first month and there after at monthly interval for a period of six months from the date of primary vaccination.

3.8 ROSE BENGAL PLATE TEST (RBPT)

Method described by Alton and Jones (1967) was followed

3.8.1 Materials

a. Porcelain Slab

b. Spreaders

c. Test Sera Samples

d. Coloured Antigen

3.8.2 Test Procedure

b) The clean porcelain plate was placed on a table with ruled side upper most. Both serum and antigen were brought to room temperature.

c) One drop of serum was mixed with equal quantity of *Brucella abortus* strain 99 Rose Bengal coloured antigen on porcelain slab.

d) The serum and antigen were mixed with a spreader and was gently rocked by hand for about three minutes.

e) The results were analyzed on the basis of the size of the particle and the speed with which they appeared.

3.8.3 Interpretation

Time of reaction	character of reaction	grading	suggested meaning
Instantaneous	Large Particulation	+++	Strong Positive
2-3 minutes	Medium Particulation	++	Moderate Positive
4-5 minutes	Powdery Particulation	+	Weak Positive
10 minutes	Slight Particulation	±	Doubtful
–	No Particulation	_	Negative

3.9 STANDARD TUBE AGGLUTINATION TEST (STAT)

The test was performed based on the method described by Alton and Jones (1967).

3.9.1 Materials

a. Agglutination tubes and racks

b. Normal saline

c. Test serum

d. *Brucella* known positive serum

e. *Brucella* known negative serum

f. *Brucella abortus* strain 99 plain antigen

3.9.2 Procedure

a) Six agglutination tubes were set up in an agglutination rack and added 0.8 ml of normal saline to the first tube and 0.5 ml to other tubes.

b) Added 0.2 ml of test serum to the first tube. Mixed well and transferred 0.5 ml to tube no: 2. Repeated two fold dilution to tube no: 6 and then discarded 0.5 ml from the sixth tube. Thus the dilution in each tubes were 1:5, 1:10, 1:20, 1:40, 1:80 and 1:160

c) 0.5 ml of *Brucella abortus* plain antigen was added to each tube and mixed well so that final dilution of serum resulted will be 1:10, 1:20, 1:40, 1:80, 1:160 and 1:320

d) Control tubes were also set as follows.

1) Positive control with 0.5 ml known positive serum and 0.5 ml of *Brucella abortus* plain antigen.

2) Negative control with 0.5 ml of known negative serum and 0.5 ml of *Brucella abortus* plain antigen.

3) Fifty per cent control with 0.75 ml normal saline and 0.25 ml of *Brucella abortus* plain antigen.

4) Antigen control with 0.5 ml *Brucella abortus* plain antigen and 0.5 ml of normal saline.

e) All the tubes were incubated at 37^0 C, over night.

f) The degree of agglutination was determined by observing the clarity of supernatant without shaking the tubes. The highest serum dilution showing 50 per cent or more agglutination and fifty per cent clearing was taken as the end point.

To express in unit system, twice the serum titre showing 50 per cent agglutination was taken as International Units (IU) per ml of serum.

3.9.3 Interpretation

Cattle, Buffaloes - 80 IU or above: Positive.
40 IU: Doubtful

3.10 INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

The test was performed based on the method described by Colby *et al.* (2002).

3.10.1 Materials

a. ELISA plates

Flat bottom 96 well ELISA plates (TARSON) were used as the test plates.

3.10.2 Reagents

a. Coating buffer (Carbonate-Bicarbonate buffer) pH 9.6

Sodium carbonate	1.5g
Sodium bicarbonate	2.9g
Distilled water	1000ml

(First dissolved the reagents in 800ml distilled water and adjusted the pH to 9.6. Made up to 1000ml)

b. Phosphate Buffered Saline (PBS) pH 7.2-7.6

i) Stock solution (10x)

Sodium chloride	80g
Potassium chloride	2g
Disodium hydrogen phosphate	14.4g
Potassium dihydrogen phosphate	2.4g
Distilled water	1000ml

ii) Working solution (1x)

PBS stock solution (10x)	1000ml
Distilled water	9000ml

c. Phosphate Buffered Saline Tween-20 (PBS-T)

Tween-20	0.5ml
PBS (1x)	1000ml

d. Blocking buffer

Bovine gelatin	1g
PBS-T	100ml

e. Citrate buffer (Substrate buffer) pH 5.0

Citric acid	5.11g
Disodium hydrogen ortho phosphate	7.3g
Distilled water	1000ml

(First dissolved the reagents in 800 ml of distilled water and adjusted the pH to5.0. Then made up to 1000ml)

f. i) Substrate solution

Tetra methyl benzidine (Sigma)	1 tablet
Citrate buffer	10ml

ii) Activated substrate solution

30% Hydrogen peroxide	0.003ml
Substrate solution	20ml

g. Reaction stopper solution (1.25M H_2SO_4)

Conc. Sulphuric acid	68ml
Distilled water	1000ml

3.10.3 Biologicals

a. Antigen

Acetone killed, lyophilized, *Brucella abortus* RB51 antigen, stored at 4^0C was used. The lyophilized contents of the vial was reconstituted with one ml of distilled water and stored at 4^0C.

b. Conjugate

Horse radish peroxidase conjugated recombinant protein A/G (PIERCE) was used at a working dilution of 1in 20000 in PBS-T.

c. Control sera

i) Antigen negative control: Consisted of two wells that were not coated with RB51 antigen stock solution.

ii) Antibody negative control: Consisted of two wells containing all appropriate reagents except serum. Hundred microliters of PBS-T were used instead of the diluted serum.

iii) Negative serum control: Serum samples obtained from cattle prior to inoculation with RB51 from Bidaj Dairy farm.

iv) Positive serum control: Positive serum was obtained from cattle, 30 days after immunization with RB51 from Bidaj Dairy farm.

Control sera were diluted 1:300 in PBS-T

3.10.4 Procedure

a) Coating of microplates

A working dilution (1:500) of RB51 antigen was prepared in coating buffer. Added 100µl of diluted antigen to all the wells except the antigen negative control wells. Sealed the plates and incubated in a humidified chamber at 4^0C over night.

b) Washing the plates

The antigen coated microplate was removed from the chamber and the contents were discarded by inverting the microplate. Then it was washed four times with freshly prepared PBS-T using a semi automated plate washer. For each washing, wells were filled approximately with 250µl of PBS-T. After the fourth washing, the plate was inverted and tapped on an absorbent towel to fully remove the washing buffer from the wells.

c) Addition of blocking buffer

After washing, the plate was blocked by addition of 200µl of 1% Bovine gelatin to each well and incubated at 37^0C for one hour.

d) Addition of test and control sera

Following incubation, the contents were discarded. The plate was washed with PBS-T four times and dried by tapping on an absorbent towel. The control sera were diluted 1:300 using PBS-T, where as the test sera were diluted 1:5000. Hundred microliters of diluted sera were dispensed into respective wells in microplate in duplicate except the antibody negative wells. The plate was covered and incubated for one hour at 37^0C.

e) Addition of conjugate

After one hour of incubation, microplate was removed and contents were discarded. The plate was washed with PBS-T four times. Then added 100µl of the working dilution of protein A/G HRP conjugate to all wells of the microplate and incubated at 37^0C for one hour.

f) Addition of substrate

After one hour of incubation, removed the plate from the incubator and discarded the contents. Washed four times with PBS-T and plate was dried. Hundred microliter of activated solution of substrate chromogen was added. The plate was kept at room temperature for 10 minutes.

g) Addition of stopper solution

To all 96 wells of microplate, 100µl of the 1.25M H_2SO_4 was added.

h) Reading of the plates

The optical density (O.D.) values were assessed using a multi-scan spectrophotometer at a wave length of 450 nm.

3.10.5 Interpretation

Percent positivity = 100×[Specific OD of test serum ÷ Specific OD of positive control]

Where:

Specific OD of test serum = Mean OD of test serum – Mean OD of blank

and

Specific OD of positive control = Mean OD of positive control – Mean OD of blank

A negative cutoff value was determined as described in the following equation

Negative cutoff value for pre inoculation sample

= Maximum specific OD of pre inoculation sample – Specific OD of negative control

Negative cutoff value for post inoculation sample

= Specific OD of negative control + Negative cutoff value of pre inoculation sample

Where:

Specific OD of negative control = Mean OD of negative control – Mean OD of blank

Any serum sample with a specific OD value above this cutoff was considered to have a significant antibody response in this test.

3.10.6 Statistical analysis

Statistical analyses of the results obtained were done by students paired t-test as per Snedecor and Cochran (1994).

Results

4. RESULTS

All the serum samples collected from cattle belonging to group I, group II and group III were subjected to Rose Bengal plate test and Standard tube agglutination test. The serum samples of group I and group II animals were also subjected to indirect enzyme linked immunosorbent assay. All the animals were observed for their activities and rectal temperature two days before and one week after vaccination.

4.1 CLINICAL OBSERVATIONS

4.1.1 Group I

4.1.1.1 Temperature

Rectal temperatures were recorded during morning and evening hours two days before and one week after vaccination. The rectal temperatures were normal before vaccination i.e. 101.5 to101.8^0 F (normal range: 101.5-102^{0}F). The mean rectal temperature in the morning, day after vaccination was 103.3^{0}F and in the evening 103.7^{0}F. Second day, morning rectal temperature recorded was103.6^{0}F and by evening it became 104.5^{0}F. On third day following vaccination, temperature came down to the normal level i.e. 101.8^{0}F in the morning and in the evening the mean temperature recorded was 102.9^0 F. On the fourth day also, the morning temperature (101.6^{0}F) was with in the normal range and in the evening 101.7^{0}F. For the next three days also temperature remained with in the normal range (Table 1)

4.1.1.2 Clinical signs

Out of the 21 animals, 18 (85.7 per cent) developed swelling at the inoculation site on the second day of inoculation. Gradually it was reduced and became normal within one week. No other clinical manifestation could be noticed.

4.1.2 Group II

4.1.2.1 Rectal temperature

In the group II also, the rectal temperatures were normal before vaccination (101.6-101.9^{0}F). But there was an increase in the rectal temperature following vaccination. First day mean rectal temperature during morning and evening hours were 103.1^{0}F and 104.4^{0}F respectively. Second day also temperature remained the same. On third day the temperature was reduced to 101.9^{0}F and 103.6^{0}F in the morning and evening respectively. On fourth day, morning and evening temperature was further reduced to 101.7^{0}F and 103^{0}F respectively. There after temperatures recorded were with in the normal range. (Table. 2)

4.1.2.2 Clinical signs

In group II also, out of 19, 14 animals (73.7 per cent) developed swelling at the inoculation site, following vaccination. But reduced to normal condition within one week. Animals were quite active and with good appetite.

4.1.3 Group III

4.1.3.1 Temperature

In this group, the mean temperature recorded in the morning was 103.3^0 F and 103.6^0 F in the evening for the first day after vaccination, which were normal (101.4-102.1^{0}F) before vaccination. On second day, it became 104^0 F in the morning and 103.8^0 F in the evening. On third day temperature was reduced to 102.9^0 F in the morning but it became 102.2^0 F in the evening. On fourth day, morning rectal temperature recorded was 102.2^0 F and 102^0 F in the evening. Fifth day onwards temperature became normal (Table.3).

4.1.3.2 Clinical signs

All the vaccinated animals (100 per cent) of this group developed swelling on the second of vaccination at the inoculation site but found to be reduced by fourth day and became normal after one week. Animals were quite active and with good appetite throughout the observation period.

Comparison of mean rectal temperature of group I, II & III in the morning and evening were shown in Fig. 1and 2 respectively.

4.2 ROSE BENGAL PLATE TEST (RBPT)

4.2.1 Group I

Animals belonging to this group were negative for RBPT at any stage of the study period.

4.2.2 Group II

Animals belonging to this group also were negative for RBPT at any stage of the study period.

4.2.3 Group III

On day zero, all the serum samples were negative for agglutinating antibodies. On day seven, serum samples showed moderate positivity (++). RBPT revealed strong positivity (+++) by day14 and 21. On day 28, RBPT revealed moderate positivity (++). By day 60, it became weak positive (+) and became doubtful (±) by day 90. Day 120 onwards no detectable antibody response could be found out.(Plate.2)

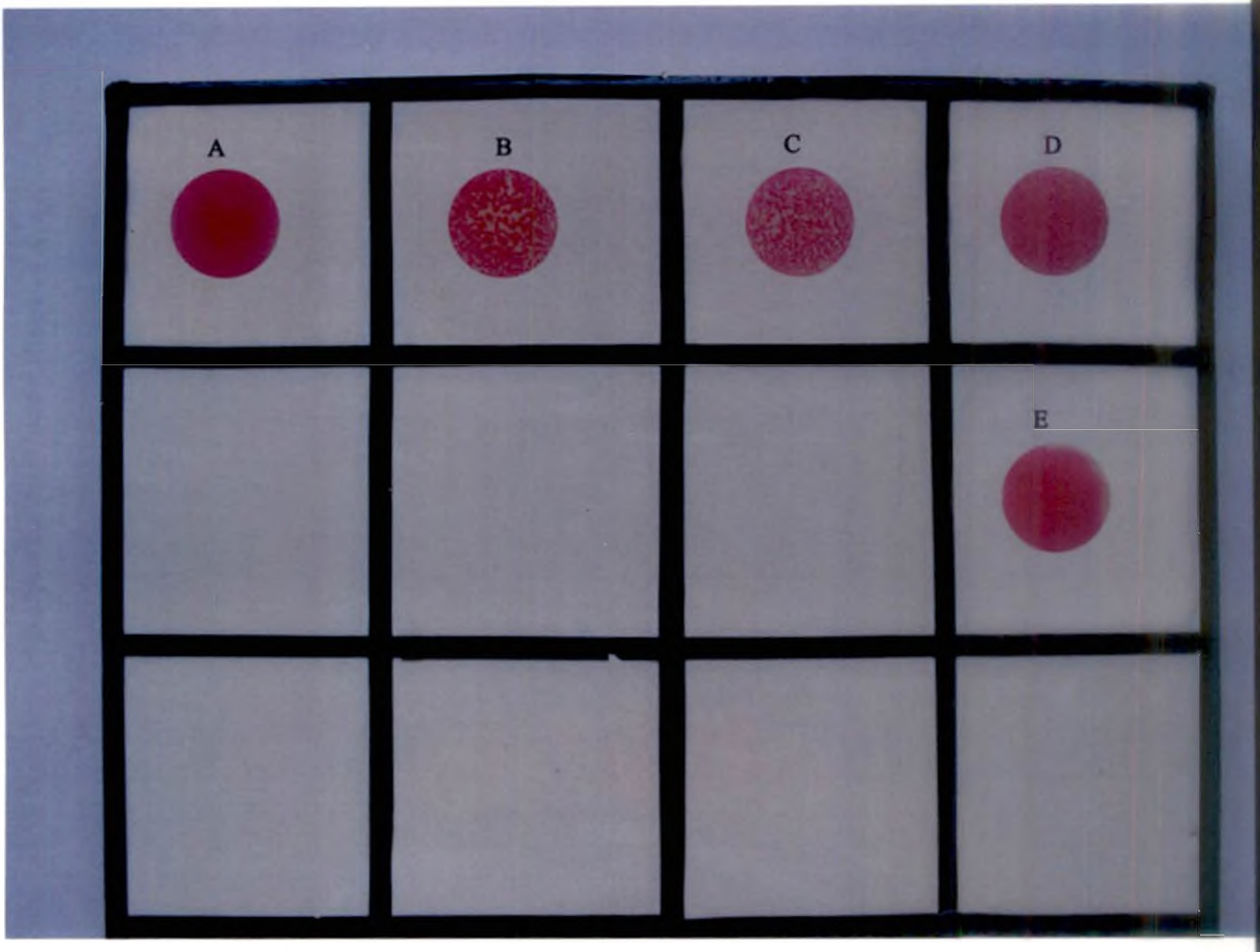


Plate. 2 Rose Bengal Plate Test
A-Negative
B-Strong Positive
C-Moderate Positive
D-Weak Positive
E- Doubtful

4.3 STANDARD TUBE AGGLUTINATION TEST (STAT)

4.3.1 Group I

Animals of this group did not produce measurable serum antibody response in STAT throughout the study period.

4.3.2 Group II

Animals of this group also did not produce any measurable serum antibody response in STAT throughout the study period.

4.3.3 Group III

Out of six animals, all exhibited highest serum antibody titres on 14th day of vaccination in the STAT except one which showed highest titre on the 21st day. Antibody titre started decreasing from 3rd week onwards and undetectable by 120th day except two animals, which showed antibody response till 180th day. The antibody titres to strain19 are presented in Table 4 and Fig. 3 (Plate 3).

4.4 INDIRECT ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

4.4.1 Sero conversion produced following vaccination in two different groups

4.4.1.1 Group I

The percent positivity values of all the animals belonging to group I from day zero to 180th day of vaccination is presented in Table 5. On day zero, except one animal (No:D4), all were negative for antibodies against RB51 antigen. Following seventh day of vaccination, 19.04 per cent were sero converted. On day 14, sero conversion was 23.80 per cent. But on day 21, 19.04 per cent showed significant antibody response. On 28th day of vaccination, animals showed good immune response and found to be 95.24 per cent. By day 60, group I animals achieved 100 per

cent sero conversion. There after a fall in immune response was observed. On 90th day of vaccination only 33.33 per cent of animals maintained the significant antibody response. Only six animals (28.57 per cent) showed significant immune response on day 120. On day 150, 9.52 per cent animals showed good immune response. By day 180, antibody response was found to be vanished completely (Fig. 4).

4.4.1.2 Group II

The percent positivity values of all the animals belonging to group II from day zero to 180th day of vaccination is presented in Table 6. Animals belonging to group II were sero negative for RB51 antibodies prior to and until 14thday of vaccination. On 21st day, 31.57 per cent of animal showed sero conversion and by 28th day, it was increased to 94.74 per cent. All the animals were found to be sero converted on day 60. By 90th day of vaccination, immune response was reduced to 63.15 per cent and on day 120 it became 21.05 % (Plate 4). Two animals were died before the last collection. By day 150 all the animals became sero negative for RB51 antibodies (Fig. 5).

4.4.2 Comparison of percent positivity values between groups

Comparison of percent positivity values between groups were done by paired t-test and presented in Table 7 and Fig. 6 to15 shows comparison of percent positivity values between two groups on each day. A significant difference in percent positivity values ($p<0.05$) between group I and group II were observed on 7th and 14th day. On 60th day and 150th day the difference in percent positivity values between group I and group II were highly significant ($p<0.01$). Comparison of sero conversion between two groups on different days are presented in Fig. 16.

Plate.3 Standard Tube Agglutination Test

A-F - Test Serum Sample diluted from 1:10 to 1:320

G - Positive Control

H - Negative Control

I - Fifty per cent Control

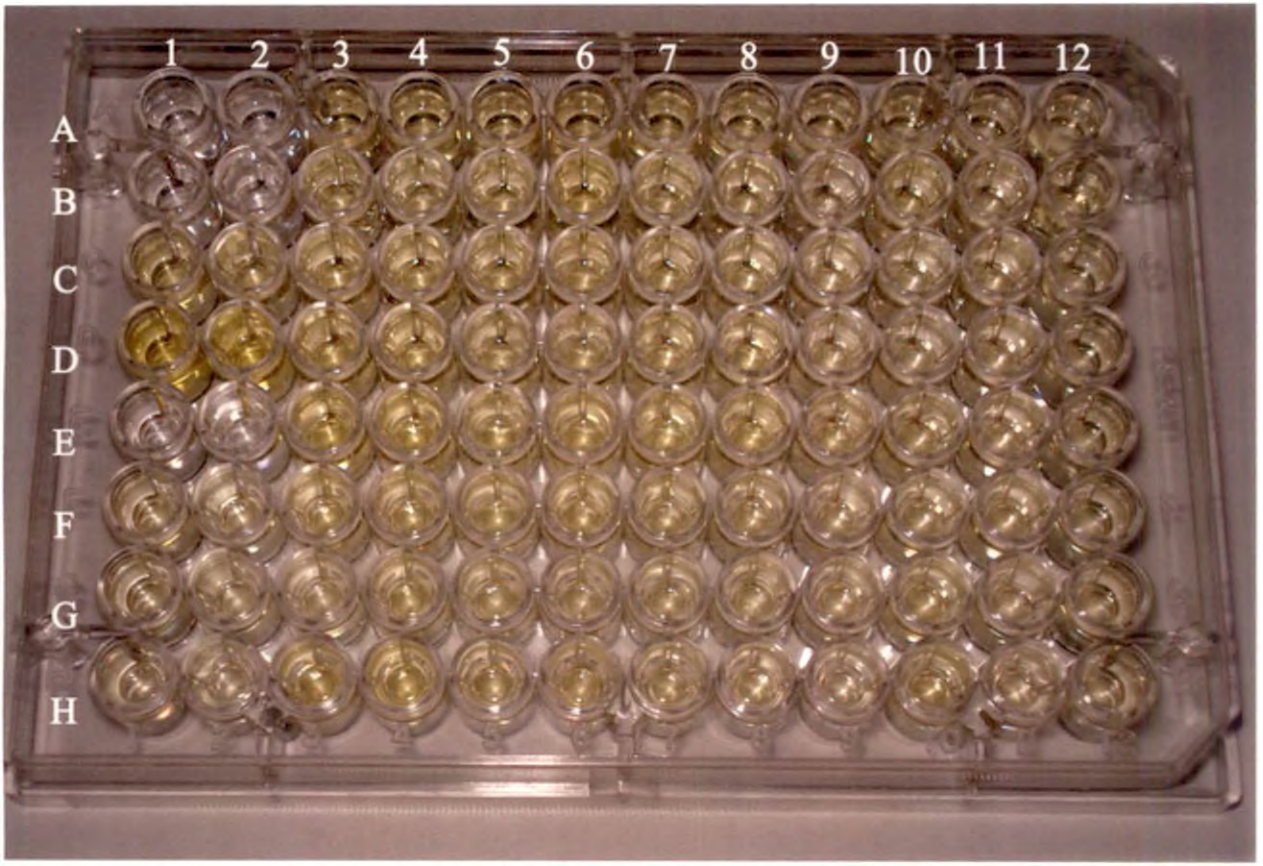


Plate. 4 Indirect Enzyme Linked Immunosorbent Assay- Test Plate

Antigen Negative Control - A_1-A_2

Antibody Negative Control - B_1-B_2

Negative Serum Control - C_1-C_2

Positive Serum Control - D_1-D_2

Test Sera Samples - Column 3 to 12

Table 1. Rectal temperature (^{0} F) of group I

Animal No.	PRID 1		PRID 2		ID		PID 1		PID 2		PID 3		PID 4		PID 5		PID 6		PID 7	
	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
C497	101.2	102.2	101.2	101.8	101.2	102	103.8	102.2	102	104.6	100.2	102.4	100.8	102.2	100.4	102	100.4	102	101.4	102.2
C481	102.2	102.2	101.2	101.8	102	102.2	103.4	104.4	104	104	101.2	102	104	102.2	102	102	102	102	102.2	102.2
C487	101.8	101.6	101.8	101.2	101.8	101.6	104	105	104	105.4	102	102.6	102	101.6	101.4	103.2	101.4	101.4	101.8	101.6
C485	101.4	102.4	102	102	101.4	102.4	103.8	103.4	102	104	101.2	101	100.6	102.4	101.2	101.2	101.2	100.8	101.4	102.4
C498	101.2	102	101.2	101.8	101.2	102	102.2	103.4	103.6	103.6	102.6	103.2	101.6	102	102	102.4	102	102.4	102.2	102
C490	101.8	101.8	102	101.8	101.6	101.8	104	104.4	104.8	104.2	102.6	104.2	101.6	101.8	102.2	102	102.2	101.8	101.8	101.8
C489	101.4	101.8	101.4	101.2	101.4	101.8	102.4	105	104	105.4	102	102.2	102	101.8	101.6	101.8	101.6	101.8	101.4	101.8
C495	101.8	102.2	102	102	101.8	102.2	102.2	103.8	102	103.8	100.4	102.2	101.4	102.2	101.4	102.4	101.4	102.4	101.8	102.2
C493	101.2	101.2	101	102.4	100.2	101.6	104	105	102.2	104	101.2	103	100	101.2	101.2	102.4	101.2	102.4	102	101.2
C491	101.8	101.4	101.8	101.6	101.2	101.4	102.8	104	105	104.2	101.6	102	102	101.4	101.4	103.6	101.4	101.6	101.8	101.4
D1	101.2	102	101.6	102	101.8	102	102	103.2	103	105	100	101.2	100.2	102	101.2	101.6	101.2	102.4	101.2	101.8
D2	101.6	101.2	102	101.8	101.8	102.2	104	103.2	102.8	105.4	102	101.2	101.6	101.2	100.8	102	100.8	102	101.6	101.2
D4	101.2	101.8	102	101.2	101.2	101.8	103.4	101	102.2	104.6	102.4	102.2	100.4	101.8	101.8	101.8	101.8	101.8	101.2	101.8
D5	101.4	101.8	102.2	102.2	101.4	101.8	104	105	105.8	104.8	101.4	101.8	101.4	101.8	101.4	101.8	101.4	101.8	101.4	102
D6	101.2	101.2	101.2	101.8	101.2	101.4	104	104	101	104.4	102.2	102.8	102	101.2	100.6	102	100.6	102	101.2	101.2
D8	101.4	101.6	102	101.6	101.4	101.6	103	103.8	104	104.2	102.4	104	101.6	101.6	102.2	102.2	102.2	102.4	101.4	101.6
D9	101.6	101.4	101.8	102	102	101.4	104	102	104.4	106	102.2	103.8	102.8	101.4	101.4	101.8	101.4	101.8	101.6	101.4
D10	102.4	101.2	101.2	102.4	102.4	102.2	103.2	103	105	104.6	104.4	104.4	104.4	101.2	101.6	102	101.6	100	102.4	101.2
D12	101.2	101.2	101.4	101.6	102.2	101.4	102.2	105	104	104.4	102	105.8	102.4	101.2	101.8	101.6	101.8	104	101.2	101.2
D15	101.4	101.6	101.8	101.8	101.4	101.6	104.4	101.4	105.2	102	101.6	105	101.8	101.6	102.2	102.4	102.2	102.4	101.4	101.6
C483	101.4	102	101.4	102.2	101.6	102	102.8	105	104.6	106	101.4	103.8	100	102	101.8	102.4	101.8	102.4	101.4	102
mean± SE	101.5± 0.07	101.7± 0.08	101.6± 0.08	101.8± 0.07	101.5± 0.10	101.8± 0.06	103.3± 0.17	103.7± 0.27	103.6± 0.29	104.5± 0.19	101.8± 0.20	102.9± 0.28	101.6± 0.25	101.7± 0.08	101.5± 0.11	102.1± 0.12	101.5± 0.11	101.9± 0.17	101.6± 0.07	101.7± 0.08

PRID : Pre inoculation day ID : Inoculation day PID : Post inoculation day M: Morning E: Evening

Table 2. Rectal temperature (^{0}F) of group II animals

Animal No.	PRID 1		PRID 2		ID		PID 1		PID 2		PID 3		PID 4		PID 5		PID 6		PID 7	
	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
C463	101.8	101.8	101.2	102	101.2	101.8	104	104.6	102.8	103.6	101.4	102.8	102.4	101.8	101.8	101.8	101.8	101.8	101.2	101.8
C173	101.6	102	101.6	102.2	101.6	102	102	103.6	102	103.4	101.8	101	102.4	102.6	101.6	102	101.6	102	101.6	102.2
C474	101.6	102.2	101.4	102.2	101.4	102.2	102.4	105.2	104	105.6	104.2	102.8	102.2	102.6	101.6	102.2	101.4	102.2	101.4	102.2
C486	101.2	101.8	101.8	102.4	101.2	102	103.8	104.4	102	103.6	101.8	102.2	101.2	102.4	101.4	101.8	101.2	101.8	101.8	102.4
C496	101.2	102.4	102	101.8	101.6	102.4	105.2	104.4	103.6	104	102.4	103.4	101	102.4	101.6	102.4	101.2	102.2	102	101.8
C484	101.4	101.8	102	101	101.4	101.8	103.4	105.4	104	104.6	102.4	103.6	102.6	103.4	101.2	101.8	101.4	101.8	102	101.6
C471	101.8	102.2	102.4	102	101.8	102.2	102	105.8	103	105.4	101.4	103.6	103.4	105	101.4	102.2	101.8	102.2	102.4	102
C482	102	102.4	101.2	102.4	102	102.4	103	105.6	104	104.8	102.4	103.6	100.2	104.2	102	102.4	102	101.8	101.2	101.4
C470	101.8	101.2	102	101.8	102	101.2	102.4	104.8	104	104.2	102	103.6	102.2	104.6	101.8	101.2	101.8	101.2	102	101.8
C479	101.8	101	101.8	102	101.8	102.4	103.2	105	103.6	102.8	102.4	103.6	101.2	102.4	101.8	102.4	101.8	101.4	101.8	102
C488	101.4	101.8	101.6	101.8	101.4	101.8	104	105	103	105.8	102.4	103.6	102.4	104.8	101.4	101.8	101.4	101.8	101.6	101.8
C480	102	102	101.2	102.2	102	102	102.6	105	103.2	103.8	101.6	103.6	100.8	102.8	102	102	102	102	101.2	102.2
C477	101.2	102.4	101	101.8	101.4	102.4	103	104	103.2	103.6	102.2	103.6	101.4	103.6	101.2	102.4	101.2	102.4	101	101.8
C476	102.2	101.6	101.4	101.6	102.2	101.6	102.8	105.2	103	104.8	101.4	103.6	102	104	102.2	101.6	102.2	101.6	101.4	101.6
C252	101	101.6	101.6	101.4	101	102	101.8	103	102.4	102.8	101.6	103.6	101	101.4	102	101.6	101.2	101.6	101.6	101.4
C454	101.8	102.4	102	102	101.8	102.4	103.4	102.4	103	105	101.4	103.6	100.6	101.6	101.8	102.4	101.8	102.4	101.8	101.6
C450	101.6	102.6	101.8	102.8	101.8	102.6	104	103.2	102	105.2	99.6	103.6	101.6	101	101.6	102.6	101.6	102.2	101.8	101.2
C446	101.6	101.4	101.6	102.4	101.6	101.6	103.4	104.6	103.8	104.8	102.4	103.6	103	103.8	101.6	101.4	101.6	101.4	101.6	102.4
C469	102	101.8	101.8	101.8	101.4	101.8	102.8	103.4	102.6	103.6	102.4	103.6	102	103.6	102	101.6	102	101.8	101.8	101.8
mean± SE	101.6± 0.08	101.9± 0.10	101.6± 0.08	101.8± 0.07	101.6± 0.07	102± 0.08	103.1± 0.19	104.4± 0.22	103.1± 0.16	104.2± 0.21	101.9± 0.20	103.6± 0.16	101.7± 0.20	103± 0.27	101.6± 0.07	102± 0.91	101.6± 0.07	101.8± 0.08	101.6± 0.08	101.8± 0.08

PRID : Pre inoculation day ID : Inoculation day PID : Post inoculation day M: Morning E: Evening

Table 3. Rectal temperature (^{0}F) of group III animals

Animal No.	PRID 1		PRID 2		ID		PID 1		PID 2		PID 3		PID 4		PID 5		PID 6		PID 7	
	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E
C464	101.4	102	101.8	102.2	101.4	102	104	105.8	105.8	102.6	102.4	104.2	102.4	103	101.6	102	101.4	102	101.8	102
C449	101.2	102.2	101.6	102.4	101.6	102.2	104.2	104.4	104	102.6	102.2	102.8	101	102	101.6	102.4	101.2	101.6	101.6	102.4
C466	101	101.8	101.2	102	101.2	101.8	103.2	102	104.6	102	102.4	103.6	103	102.2	101.4	101.8	101.2	101.8	101.2	101.4
C410	101.6	102	101.6	102.4	101.6	102.2	103	103.2	102.4	101	102.2	104.2	103.2	102.4	101.6	102.4	101.4	102	101.6	102.4
C1124	101.2	102.4	101	101.8	101.2	102.4	102	102	102	103	101.2	104	103.6	102.8	101.2	101.8	101.2	102.4	101.2	101.8
C465	101.8	102.6	101.4	101.8	102	102.8	103.2	104	105.6	102	101.8	104.2	104.4	100	101.4	101.6	101.2	101.6	101.4	101.6
mean± SE	101.4± 0.12	102.2± 0.16	101.4 0.12	102.1± 0.11	101.5 ±0.12	102.2± 0.14	103.3± 0.32	103.6± 0.61	104± 0.65	103.8± 0.29	102.9± 0.19	102.2± 0.23	102± 0.47	102± 0.44	101.5± 0.07	102± 0.14	101.2± 0.04	101.9± 0.12	101.4± 0.08	101.9± 0.17

PRID : Pre inoculation day ID: Inoculation day PID : Post inoculation day M: Morning E: Evening

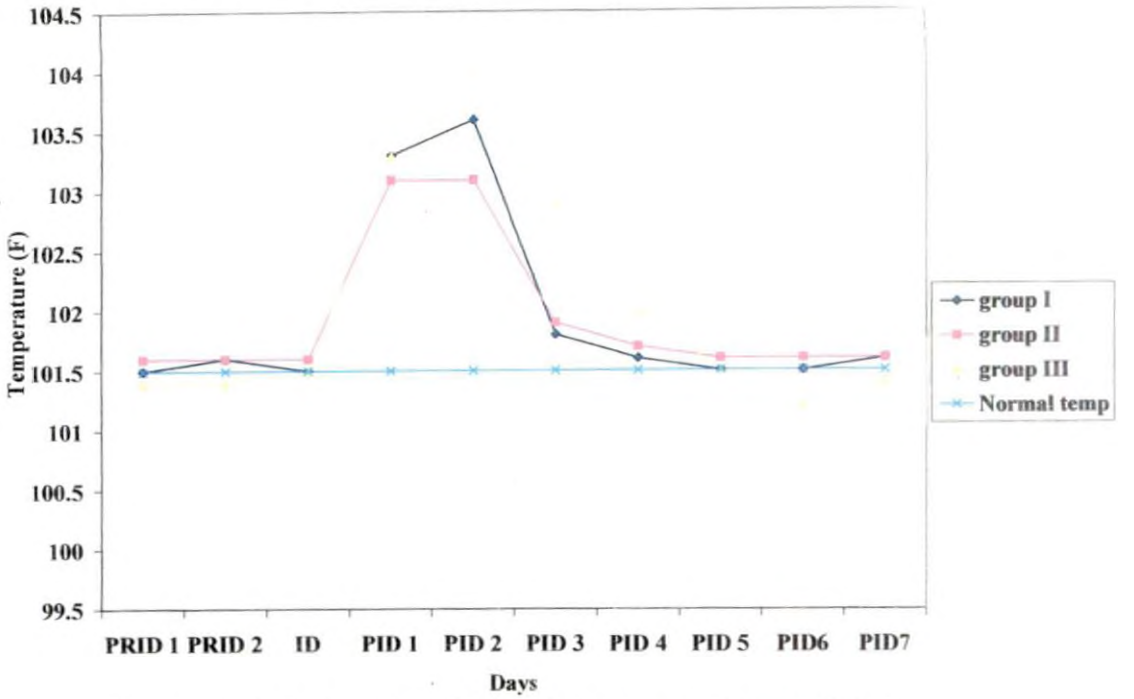


Fig. 1 Comparison of mean morning rectal temperature of group I, II & III

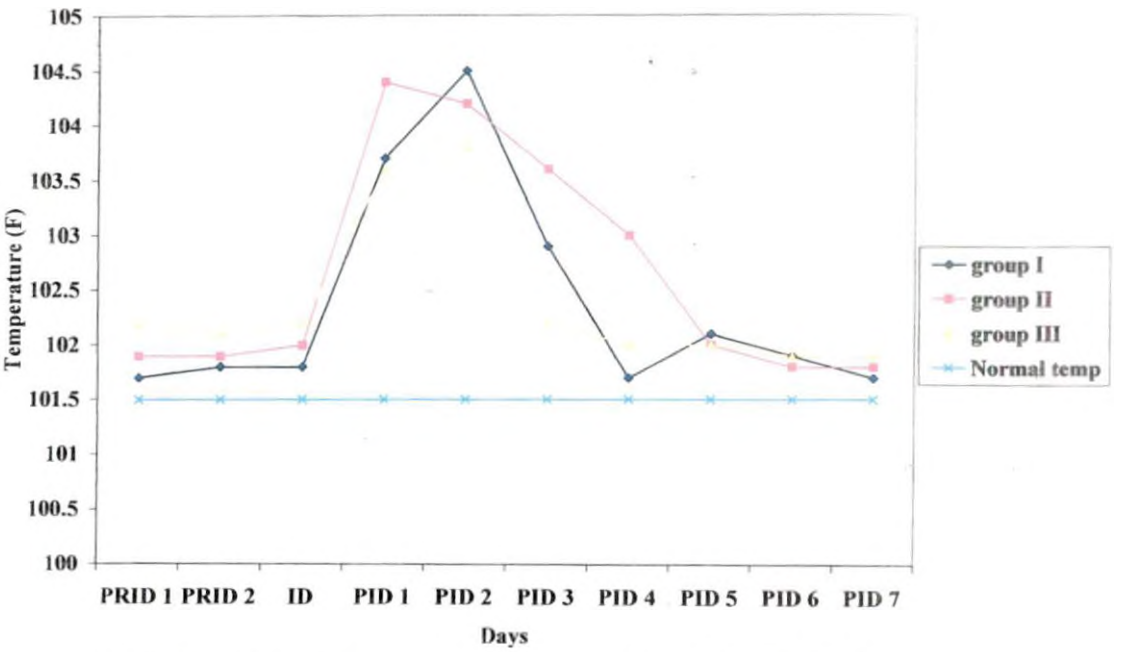


Fig. 2 Comparison of mean evening rectal temperature of group I, II & III

PRID : Pre inoculation day
ID : Inoculation day

PID : Post inoculation day

Table 4. Antibody titres of group III animals in Standard tube agglutination test

SI N0:	Animal No:	Day0	Day7	Day14	Day21	Day28	Day60	Day90	Day120	Day150	Day180
1	C464	-	80	160	160	80	20	-	-	-	-
2	C465	-	320	640	320	160	20	20	-	-	-
3	C466	-	160	160	320	80	40	20	20	20	20
4	C449	-	160	1280	320	320	40	20	20	20	20
5	C410	-	40	80	160	40	20	-	-	-	-
6	C1124	-	80	320	80	80	40	20		-	-
7	Mean± SE	-	140 ± 41.147	440 ± 187.488	226.67± 43.579	126.67± 42.009	30 ± 4.489	13.33± 4.232	13.33± 4.232	13.33± 4.232	13.33± 4.232

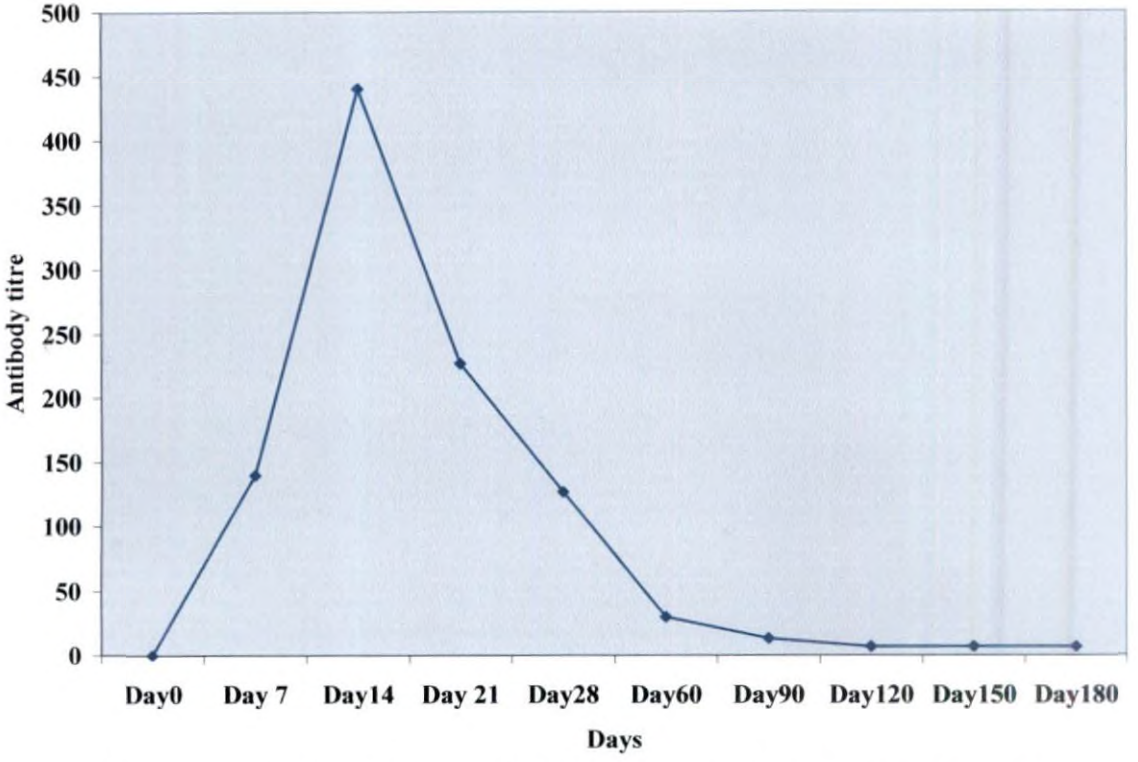


Fig.3 Antibody titres of group III animals in Standard tube agglutination test

Table 5. Percent positivity values of Group I animals to *Brucella abortus* SRB51 vaccine

* sero converted animal

Animal No:	Days									
	0	7	14	21	28	60	90	120	150	180
D15	7.352	8.564	1.916	3.938	9.585	31.213*	4.225	14.215	12.238	7.291
C495	20.882	71.064*	30.834*	68.380*	57.118*	37.475*	5.569	23.655*	9.261	8.906
C491	10	43.055	25.535	27.461	32.343*	25.097*	4.481	11.306	8.710	7.708
C489	16.764	32.407	12.795	11.269	19.338*	21.941*	0.768	9.769	15.325	7.708
D2	55.588	41.203	16.459	16.739	29.820*	22.475*	0.768	4.006	8.930	1.354
C481	30.882	67.129	22.040	30.306*	23.991*	24.320*	12.483*	22.996*	29.327*	8.75
D10	17.647	68.518	15.276	20.568	23.150*	22.621*	2.880	9.275	11.466	3.281
D4	73.823*	105.787*	61.386*	26.914*	47.701*	20.873*	7.362*	5.049	10.915	4.739
D8	1.764	11.111	1.1273	1.750	14.854*	18.203*	-0.192	6.970	25.082	8.541
C497	4.117	18.055	5.918	2.625	27.186*	26.116*	2.368	6.915	20.727	9.427
D9	5.588	19.675	29.425*	14.223	15.695*	20.388*	0.640	5.488	9.426	3.75
C490	10.588	43.287	14.712	1.4223	29.147*	22.475*	1.216	24.313*	10.915	10.208
D1	33.823	59.490	24.915	6.892	48.038*	20.145*	13.636*	21.075*	16.207	8.854
D5	53.529	175.925*	30.552*	28.555*	23.766*	21.941*	6.978*	13.007	19.955	12.552
D12	36.764	47.685	20.969	12.363	21.973*	25.679*	3.841	8.891	20.066	12.395
D6	30.882	43.75	6.3134	8.643	37.163*	20.145*	-1.152	8.068	24.090	4.479
C485	25.882	44.444	8.342	13.019	17.600*	21.456*	23.687*	26.015*	25.358	4.635
C487	49.705	44.444	7.722	10.175	35.201*	21.699*	19.142*	21.734*	19.735	17.031
C498	10.294	43.75	29.537*	13.676	23.094*	19.466*	6.978*	15.093	31.973*	5.781
C483	40.882	91.203*	15.558	18.927	28.307*	19.902*	3.329	9.604	10.584	13.802
C493	44.117	66.203	7.666	9.080	37.163*	19.029*	2.240	13.007	15.435	7.968
Mean± SE	27.661± 4.351	54.607± 8.084	18.524± 2.999	16.520± 3.234	28.678± 2.598	22.984± 0.970	5.774± 1.413	13.355± 1.550	16.939± 1.563	8.055± 0.829

Table 6. Percent positivity values of Group II animals to *Brucella abortus* SRB51 vaccine

* sero converted animal

Animal No:	Days									
	0	7	14	21	28	60	90	120	150	180
C470	30.588	28.240	9.188	21.115	19.450*	37.184*	9.923*	9.659	10.639	7.395
C488	11.470	33.564	10.879	15.317	26.345*	29.611*	4.545	6.805	7.717	9.895
C486	12.647	20.601	12.232	45.623*	28.979*	37.135*	5.633	9.549	6.615	-
C482	12.352	21.527	5.355	14.004	18.105*	39.708*	7.106*	15.257	7.607	17.343
C479	13.235	16.203	11.104	6.783	45.123*	52.281*	18.245*	8.232	8.654	-
C474	17.941	26.388	12.232	15.317	16.984*	31.310*	2.944	13.391	10.088	10.416
C446	31.176	33.79	10.315	5.579	16.647*	25.388*	1.664	4.829	14.388	11.875
C476	34.411	41.666	6.651	5.032	29.652*	40.388*	9.154*	13.172	11.742	18.593
C463	31.470	52.083	17.981	44.310*	34.080*	33.106*	7.170*	10.922	4.244	0.260
C454	38.823	31.481	10.597	8.533	14.966*	32.961*	3.905	17.727	12.403	32.708
C173	13.823	23.842	21.307	28.118*	34.360*	33.543*	7.362*	14.873	7.276	10.781
C450	20	43.981	8.511	9.737	14.069*	28.106*	7.106*	13.227	11.521	5.885
C471	17.647	19.675	0.958	0.765	6.334	35.728*	22.407*	41.986*	23.594	19.375
C484	33.235	38.888	3.945	5.361	15.302*	31.019*	8.898*	26.619*	9.316	29.843
C480	1.7647	55.787	21.307	11.050	28.531*	23.106*	9.218*	21.130*	13.726	12.031
C496	22.352	54.166	8.624	30.525*	14.630*	27.135*	4.801	9.440	18.687	13.593
C252	58.823	56.481	19.052	33.041*	21.076*	29.951*	11.203*	26.180*	20.727	11.458
C477	19.117	28.472	8.793	33.588*	25.896*	27.233*	4.545	14.489	7.276	9.062
C469	30	16.203	2.593	9.299	20.291*	28.398*	19.334*	6.476	17.530	4.531
Mean± SE	23.730± 2.994	33.845± 3.106	10.612± 1.345	18.058± 3.158	22.675± 2.125	32.805± 1.538	8.693± 1.295	14.946± 2.050	11.776± 1.196	13.825± 4.747

Table 7. Comparison of the mean (mean±SE) percent positivity values of group I and II

Groups	Days									
	0	7	14	21	28	60	90	120	150	180
GroupI	27.661± 4.351	54.607± 8.084	18.524± 2.999	16.520± 3.234	28.678± 2.598	22.984± 0.970	5.774± 1.413	13.355± 1.550	16.939± 1.563	8.055± 0.829
GroupII	23.730± 2.994	33.845± 3.106	10.612± 1.345	18.058± 3.158	22.675± 2.125	32.805± 1.538	8.693± 1.295	14.946± 2.050	11.776± 1.196	17.025± 4.747
	NS	*	*	NS	NS	**	NS	NS	**	NS

- *significance at 5% level
- ** significance at 1% level
- NS- nonsignificant

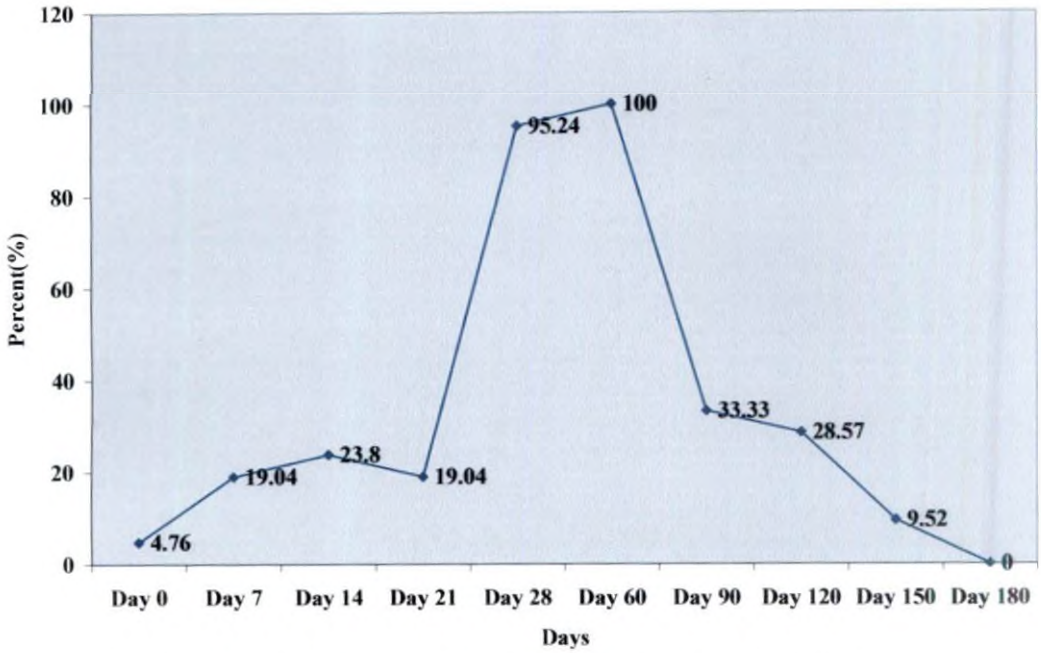


Fig.4. Sero conversion of group I to *Brucella abortus* SRB51 vaccine

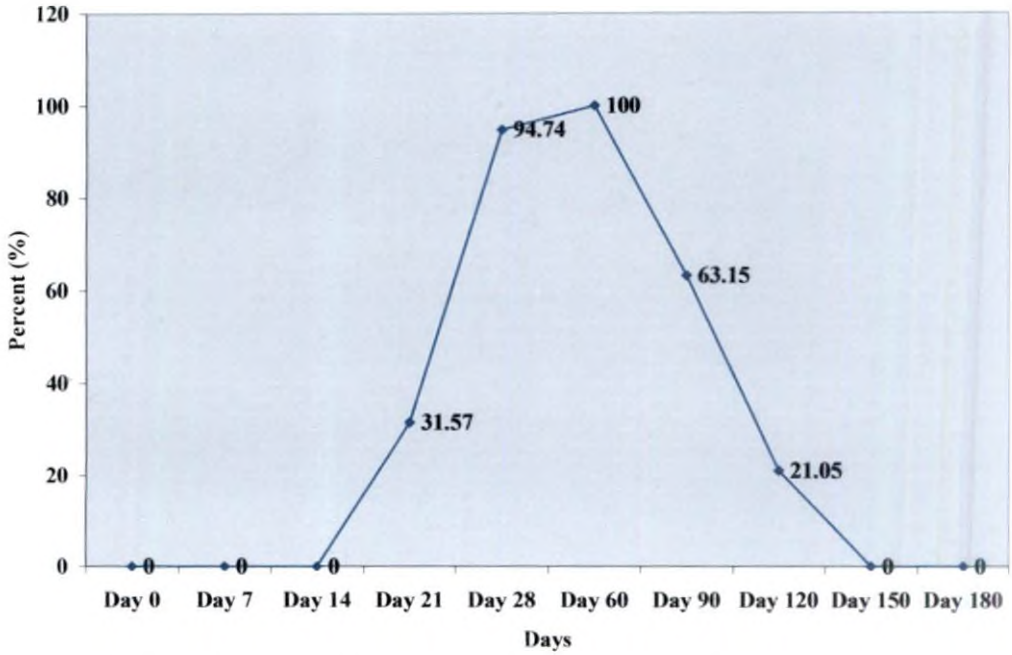


Fig.5. Sero conversion of group II to *Brucella abortus* SRB51 vaccine

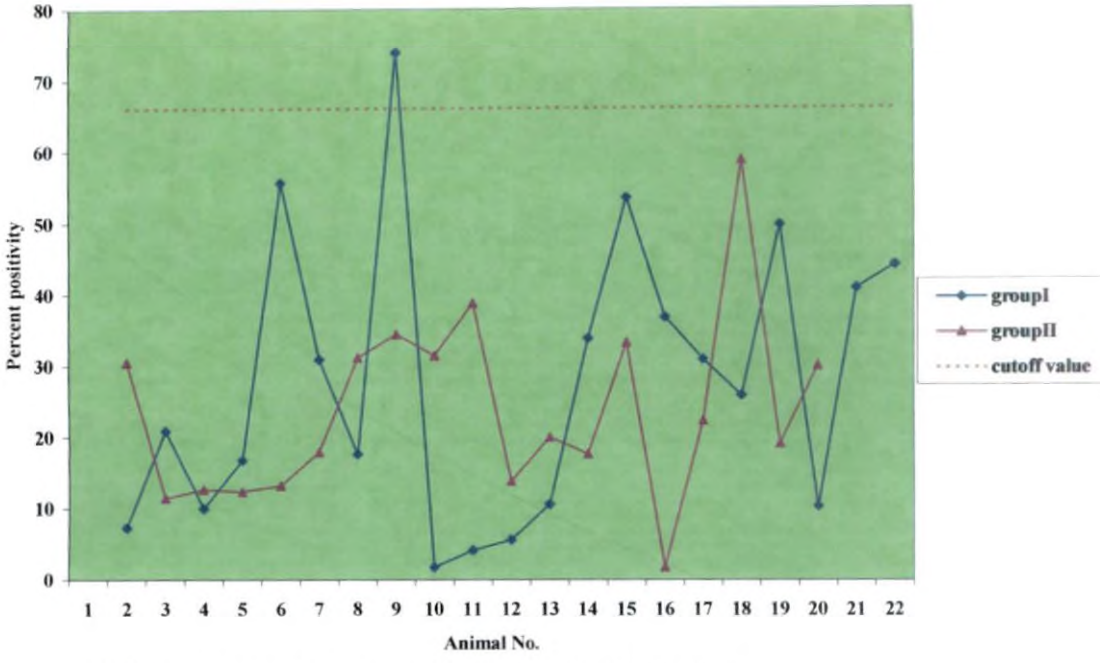


Fig.6. Sero conversion to *Brucella abortus* SRB51 on day 0

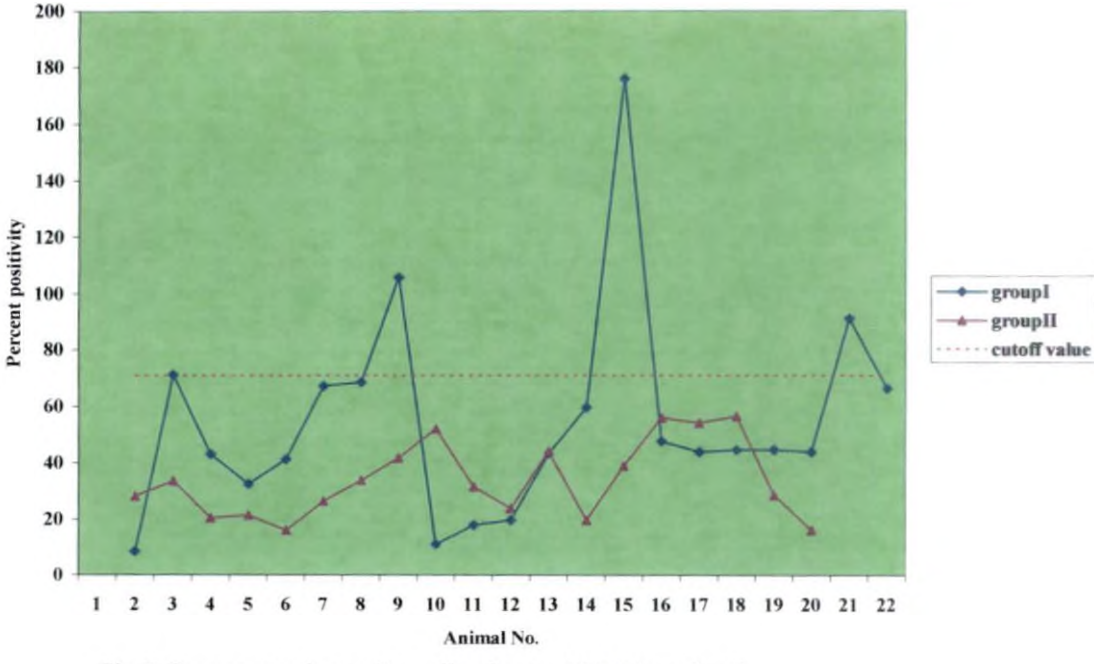


Fig.7. Sero conversion to *Brucella abortus* SRB51 on day 7

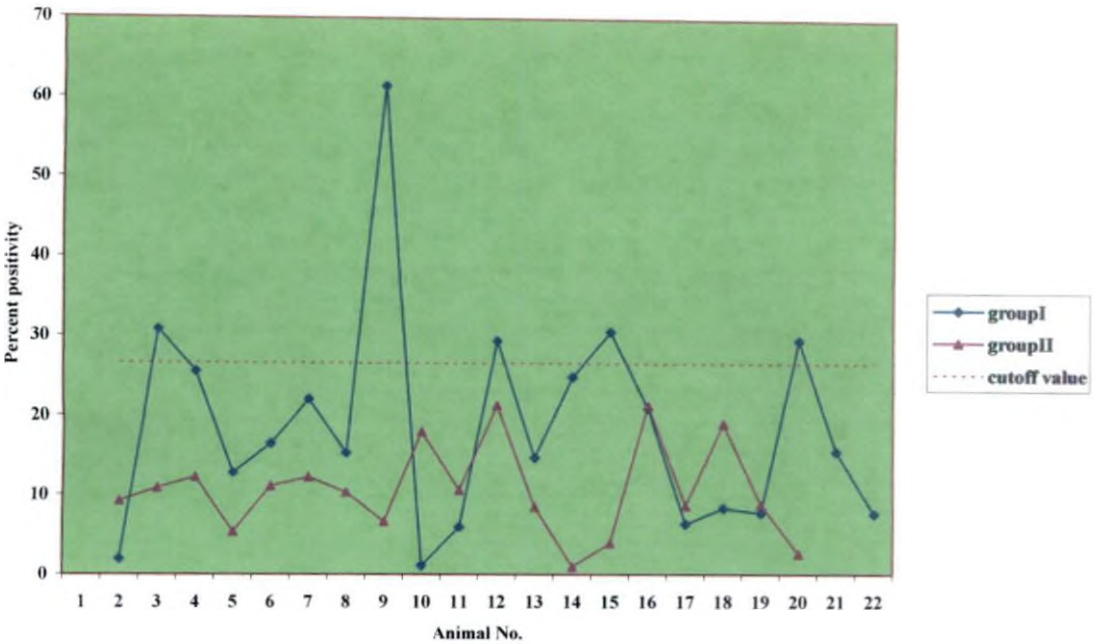


Fig.8. Sero conversion to *Brucella abortus* SRB51 on day 14

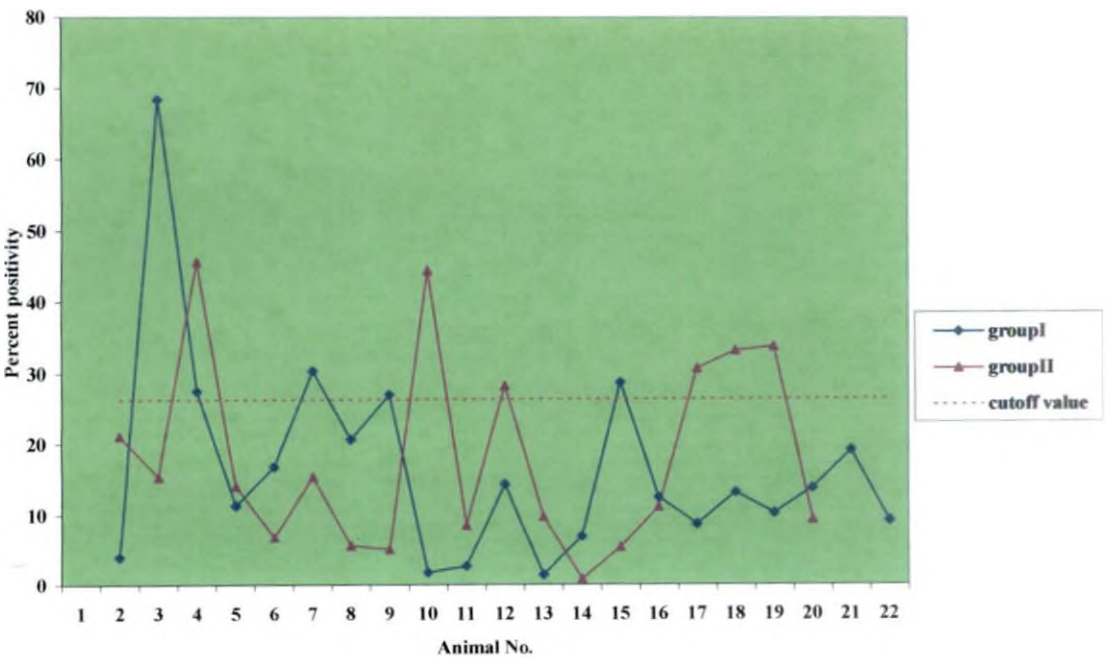


Fig.9. Sero conversion to *Brucella abortus* SRB51 on day 21

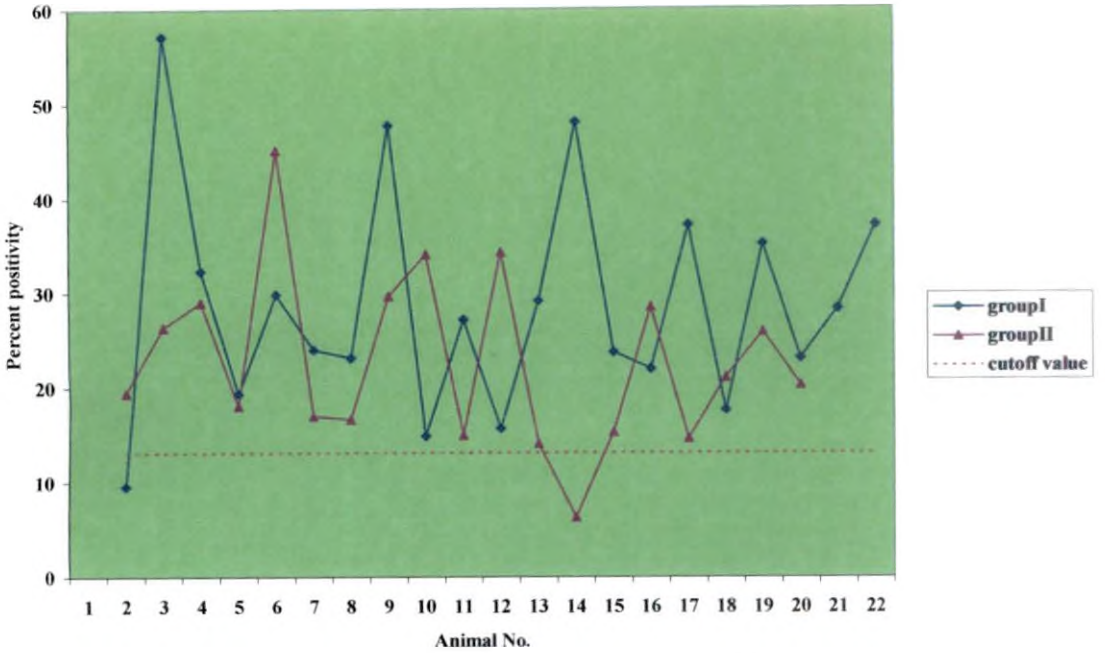


Fig.10. Sero conversion to *Brucella abortus* SRB51 on day 28

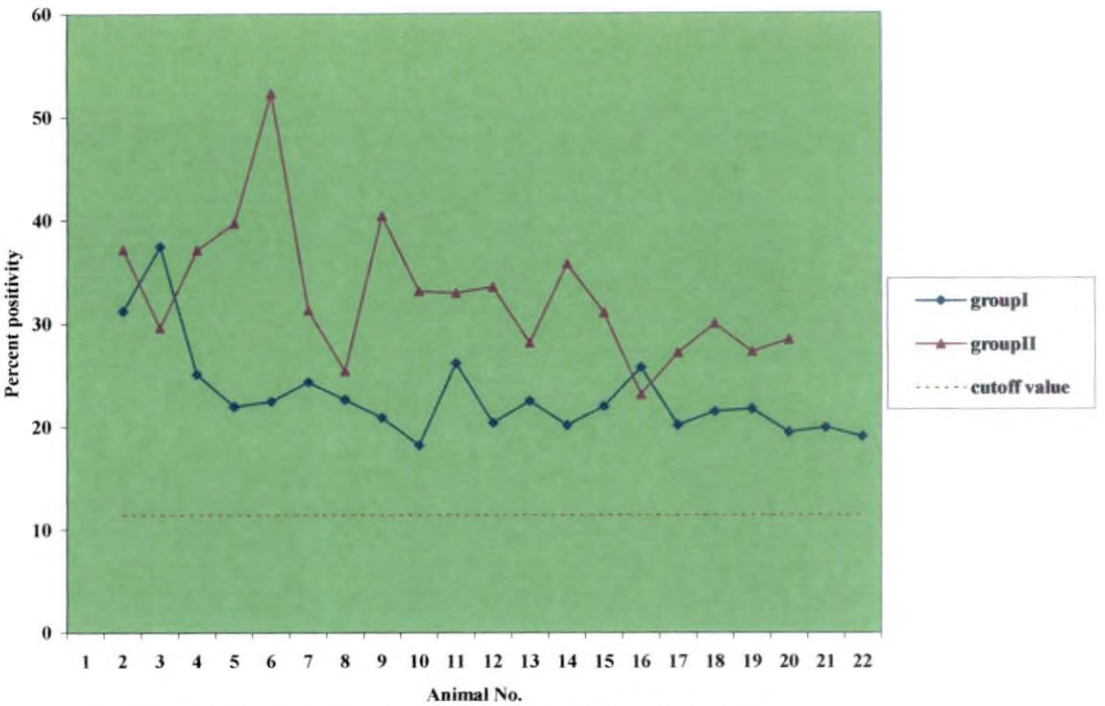


Fig.11. Sero conversion to *Brucella abortus* SRB51 on day 60

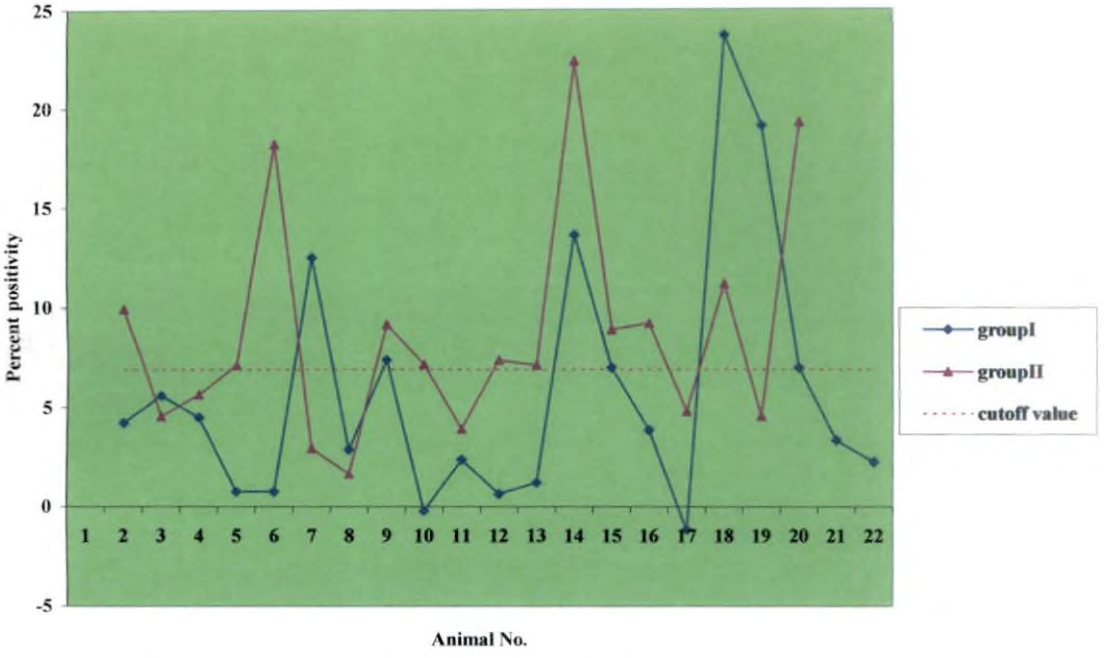


Fig.12. Sero conversion to *Brucella abortus* SRB51 on day 90

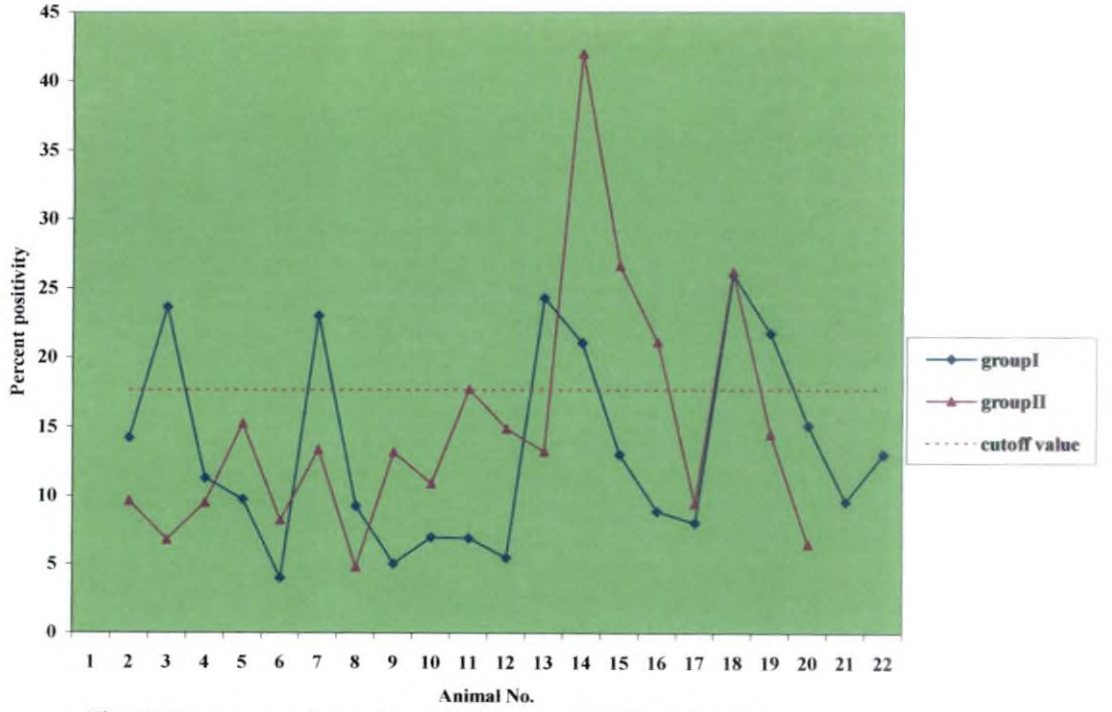


Fig.13. Sero conversion to *Brucella abortus* SRB51 on day 120

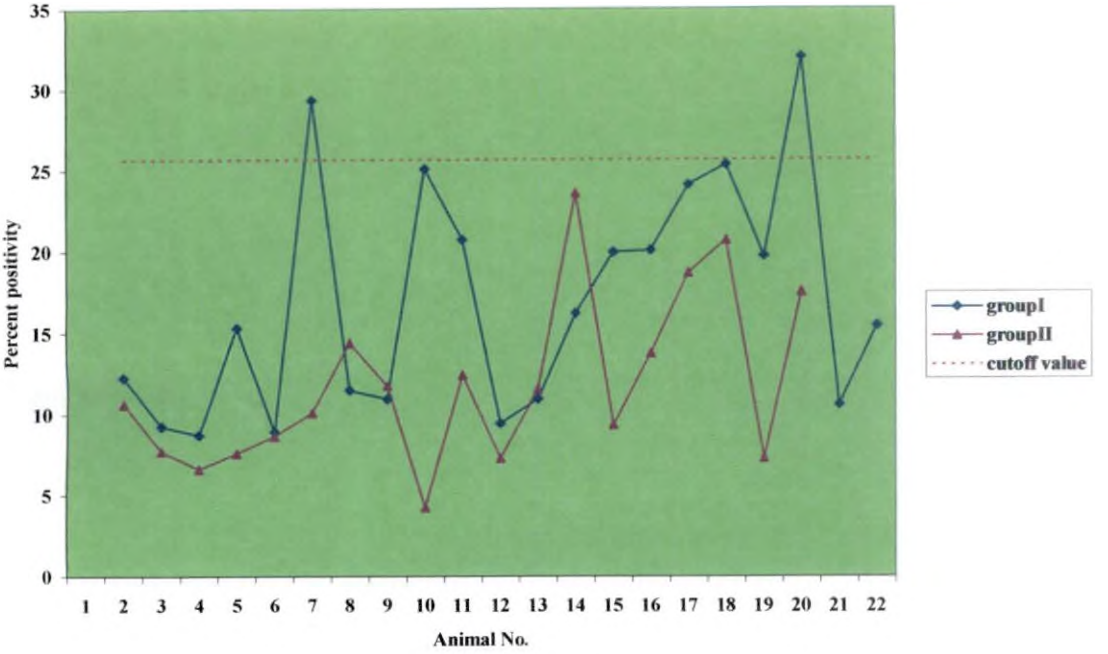


Fig.14. Sero conversion to *Brucella abortus* SRB51 on day 150

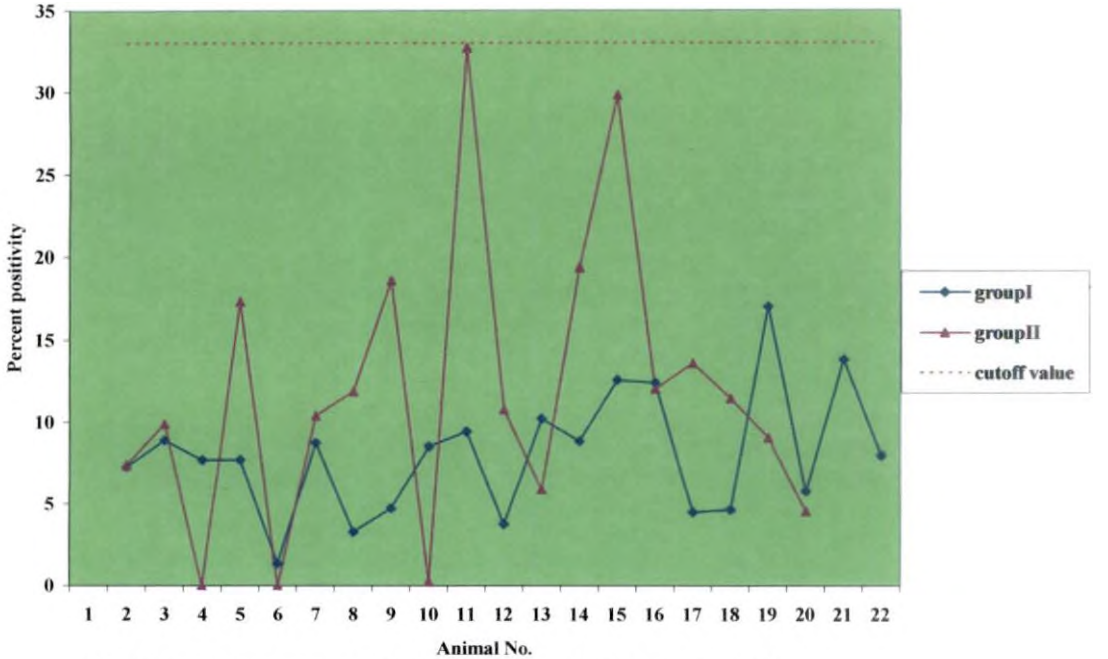


Fig.15. Sero conversion to *Brucella abortus* SRB51 on day 180

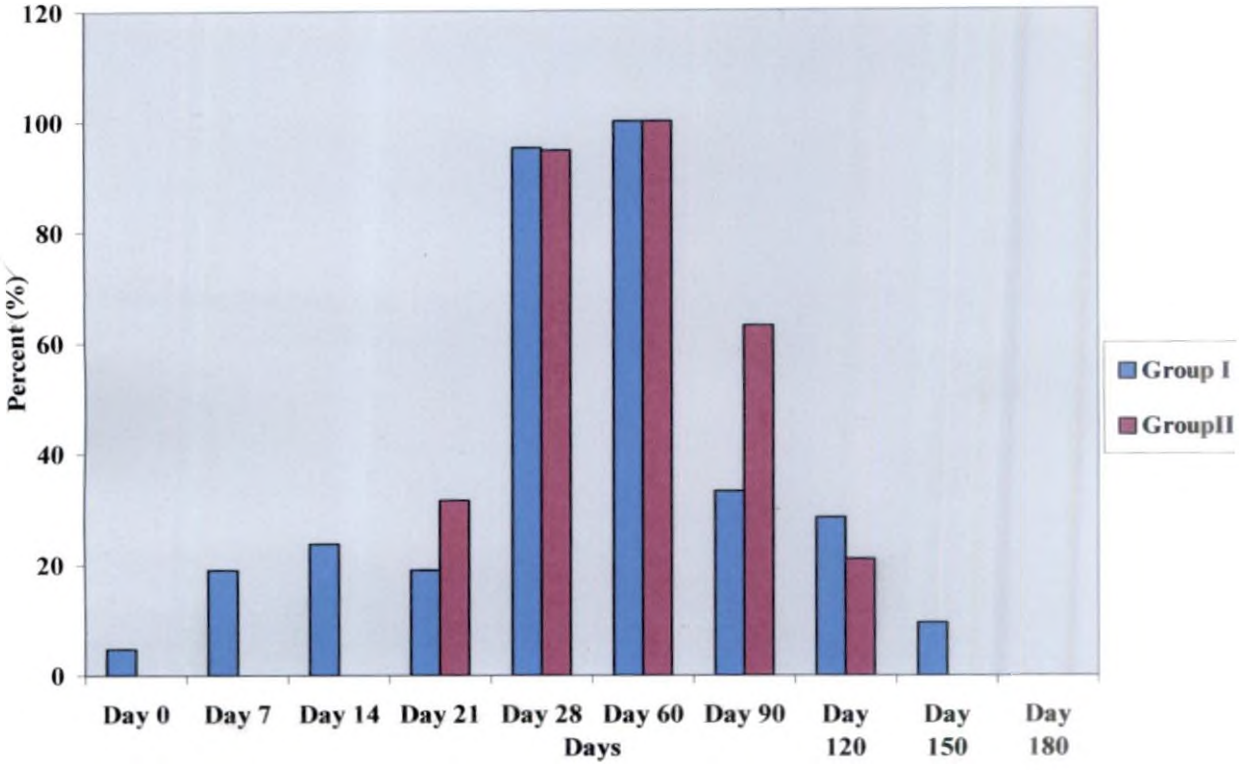


Fig. 16 Comparison of percent of sero conversion to *Brucella abortus* SRB51 between group I & II

Discussion

5. DISCUSSION

In the present study, the sero conversion to two doses of *Brucella abortus* strain RB51 vaccine was assessed in cattle. Interference of *Brucella abortus* strain RB51 with routine diagnostic tests for brucellosis was also compared with *Brucella abortus* strain 19 vaccine. Immune responses were assessed at weekly interval during the first month and there after at monthly interval for a period of six months employing indirect Enzyme Linked Immunosorbent Assay (ELISA). Clinical observations of all animals were also made two days before and one week after vaccination.

5.1 CLINICAL OBSERVATIONS

All the vaccinated animals showed febrile reaction during the first 24 to 48hours post inoculation. Elevated temperatures ranged from 103.1^0 F to104.5^0 F (normal range 101.5 ± 0.5^0 F). But returned to normal temperature by 72 hours post inoculation. A similar observation was recorded by Palmer *et al.* (1996) where the elevated temperature ranged from 102.4^0 F to 105.9^0 F during 24 to 48 hours post inoculation.

Eighty two per cent of the vaccinated animals developed swelling at the inoculation site 48 hours after vaccination but found to be reduced by 96 hours and became normal after one week. Animals were quite active and with good appetite throughout the observation period.

5.2 INTERFERENCE WITH SEROLOGICAL TESTS

5.2.1 Rose Bengal Plate Test (RBPT)

Animals belonging to group I and group II, which were vaccinated with *Brucella abortus* strain RB51 (SRB51), gave negative results in the RBPT throughout the study period. This agrees with the findings of previous workers

(Schurig *et al.*, 1991; Adone and Ciuchini, 1999; Uzal *et al.*, 2000), who also observed negative results for RBPT. As *Brucella abortus* strain RB51 is lacking the lipopolysaccharide O-side chain, the lack of specific antibody is responsible for this. Though the group I and group II differs in the dosage of SRB51, both groups did not give any reaction with RBPT. This agrees with the finding of Uzal *et al.* (2000) that vaccination with one, two or three of the recommended adult doses of SRB51 did not interfere with the conventional serological techniques. This confirms previous studies with one or two immunizations with SRB51 (Cheville *et al.*, 1993; Stevens *et al.*, 1994; 1996; Lord *et al.*,1998).

Brucella abortus strain RB51 is a lipopolysacchride (LPS) O-antigen deficient stable, attenuated, rough mutant of the virulent strain 2308 of *Brucella abortus* therefore, vaccination with RB51 does not induce antibodies to the O-polysacchride of *Brucella* species that are detected by use of the standard sero-diagnostic test for brucellosis (Cheville *et al.*, 1992; 1993; 1996). The vaccine *Brucella abortus* strain RB51 does not induce false positive reactions on the standard brucellosis serologic tests (Stevens *et al.*, 1994) and it is less pathogenic than strain 19 vaccine. Additionally strain RB51 protected cattle against experimental challenge with virulent *Brucella abortus* (Cheville *et al.*, 1993). Strain RB51 is being evaluated as an alternative to *B. abortus* strain19 as a vaccine for preventing brucellosis and abortions in cattle because, unlike S19, it does not induce antibodies to the *Brucella* LPS O antigens that are detected by serodiagnostic test for brucellosis (Stevens *et al.*, 1995). Consequently, the replacement of S19 with SRB51 for use as a vaccine may facilitate the identification of cattle with brucellosis and their removal from vaccinated herds.

Lord *et al.* (1998) conducted field study in which female cattle of 3-8 or 10-12 months age were vaccinated with strain RB51 once or twice, did not induce seropositivity in the conventional serologic tests, including AGID. It also indicated

that vaccination with 5×10^{10} CFU of strain RB51 was highly effective in protecting cattle against abortion under field conditions involving herds with high or low brucellosis prevalence. This protection, tested at least one year after vaccination, was superior to the protection afforded by vaccination with 5×10^{10} CFU of strain 19. Strain RB51 is less virulent than other brucella vaccine strains, including S19, when administered intravenously (Mingle *et al.*, 1941; Smith *et al.,* 1961). This decreased virulence in pregnant cattle contributes to the attractiveness of SRB51 as a vaccine for the prevention of bovine brucellosis. Moreover, lack of antibodies that are detected by brucellosis surveillance tests, allows differentiation of SRB51 vaccinated animals from naturally infected animals (Stevens *et al.*, 1994). Such differentiation is not possible following S19 vaccination. Strain RB51 is now the official vaccine being employed in the United States, replacing S19. In the United States, the official recommendation is calfhood vaccination with $1\text{-}3.4\times10^{10}$ CFU per dose.

Group III animals, which were vaccinated with *Brucella abortus* strain 19 showed positive response in RBPT up to 60 days. Similar observation was recorded by Venkatesha and Upadhye (1987) in which sera from calves after primary vaccination with S19 showed positive response in RBPT up to two months.

Persistence of post vaccinal serologic titres that interfere with serological test interpretation is one of the most important disadvantages of the vaccinal strain 19 of *Brucella abortus* (Stevens *et al.*, 1994; Lord *et al.*, 1998). In order to avoid this problem to some degree, calf-hood vaccination before ten months of age was recommended, although persistence of antibodies may also be observed in adults vaccinated as calves (Nagy *et al.*, 1967). This problem became more persistent as the age of vaccination increases and a reduced dose of S19 must be used for vaccination of adult cows to reduce the serological problem. Vaccination of pregnant animals must be avoided as this vaccine could induce abortions (Nicoletti, 1990). The presence of LPS-O side chain in S19, which is highly immunogenic, produces

specific antibodies which are detected by conventional test like RBPT. This can make it difficult to distinguish some vaccinated animals from those infected with virulent field strains of the organism. This factor often makes the brucella control programme difficult.

5.2.2 Standard Tube Agglutination Test (STAT)

Brucella abortus SRB51 vaccinates (group I and group II) did not develop serum antibody titre that was detected by STAT throughout the study period. Several workers (Schurig *et al.*,1991; Cheville *et al.*, 1992; 1996; Palmer *et al.*, 1997) had observed the same finding. *Brucella abortus* strain RB51 lacks O-side chain found in strain 19 and virulent strains, it is this side chain which causes serologic reactions on the standard tests (Schurig *et al.*, 1991).

Stevens *et al.* (1994) evaluated the serological responses in the particle concentration fluorescence immuno assay, the card agglutination, complement fixation, and the agglutination tests for ten weeks after vaccination of cattle with either *Brucella abortus* S19 or strain RB51. The responses of strain 19 vaccinated cattle were positive, whereas those of strain RB51 vaccinated cattle were negative, in all of the tests. These results indicated that cattle vaccinated with strain RB51 failed to produce antibodies that could be detected by conventional serological tests that are used to diagnose bovine brucellosis. Antibodies detected by STAT are directed to the O-side chain, therefore, negative antibody responses in SRB51 vaccinates is likely attributable to the lack of O-side chain on SRB51. Palmer *et al.*(1997) also stated the specificity of rough mutant of *Brucella abortus* SRB51 and its lack of LPS-O side chain.

In group III animals, out of six, five exhibited highest serum antibody titres (440 I U) at 14th day of vaccination in STAT , which correlated with the findings of Stevens *et al.*(1995) who got maximum titre (850 I U) in the STAT at two weeks after vaccination. Misra *et al.* (1982) reported that the peak titre was obtained on 15th day following S19 vaccination in cross bred calves. A similar finding was also observed by Das and Mulbagal (1983) in which cross bred calves attained maximum titre (416.66 I U) on 15th day of vaccination.

In the present study, agglutinating antibody titres were detectable up to 120th day of vaccination except in case of two animals, which showed antibody response till 180th day. Venkatesha and Upadhye (1987) observed that agglutinating antibodies became negative by third month itself, when calves of 3-5 weeks old were vaccinated with S19 vaccine. Stevens *et al.* (1995) detected measurable antibody titre up to 10 weeks post inoculation. Group III animals, which were vaccinated with *Brucella abortus* strain 19 showed positive response in RBPT up to 60 days. Similar observation was recorded by Venkatesha and Upadhye (1987) in which sera from calves after primary vaccination with S19 showed positive response in RBPT up to two months.

Misra *et al.* (1982) observed that calves became serologically negative for Brucella agglutinins in ten months, whereas Red dane cows agglutinins persisted throughout the study period i.e. two years and eight months. Rapidity of sero conversion to negative status following S19 vaccination is inversely related to age at the time of vaccination (Barton and Lomme, 1980). Plackett *et al.* (1980) reported that S19 vaccination at an early age with reduced dose will minimize the production of serum agglutinations.

Present study confirmed the advantages of non interference of SRB51 antibody in the conventional test results, comparing S19 where false positive

reactions are obtained. Hence, SRB51 vaccine can be used as a Brucella vaccine strain in areas where Brucella control is done by both vaccination and test and slaughter policy.

5.3 SERO CONVERSION OF *BUCELLA ABORTUS* STRAIN RB51 VACCINE

5. 3.1 Group I

All the animals of group I were vaccinated subcutaneously with 2 ml (1.8×10^{10} CFU) of *Brucella abortus* strain RB51 vaccine. Except one animal all were negative for antibodies against RB51 antigen in the indirect ELISA, which may be due to non specific interference (Selby, 1999)

In the present study sero conversion started seventh day onwards and 19.04 per cent animals showed sero conversion on day seven. This is in agreement with the findings of Adone and Ciuchini (1999) who found that anti-RB51 antibodies were produced in cattle seven days post vaccination.

In the present study maximum (100 per cent) sero conversion was obtained at day 60. This is not in agreement with Stevens *et al.* (1995) who observed peak titre at fourth week of vaccination. Olsen *et al.* (1997) also recorded peak titre at fourth week in the dot blot assay, following vaccination of cattle with SRB51 ($0.85-1.22\times10^{10}$ CFU) subcutaneously.

A fall in immune response was noticed on 90th day of vaccination (33.33 per cent) and vanished completely by day 180. This agrees with the observation of Olsen *et al.* (1997), where a decrease in immune response was noticed at 12th week of vaccination, following inoculation of cattle with $0.85-1.6\times10^{10}$ CFU of SRB51. But Stevens *et al.* (1995) noticed a decrease in immune response in cattle inoculated with $1-1.4\times10^{10}$ CFU, at 10 weeks after vaccination.

5.3.2 Group II

Animals of this group II were vaccinated with 1×10^{10} CFU SRB51 vaccine subcutaneously. They did not show sero conversion until 14th day of vaccination. Similar observation was made by Cook *et al.* (2000) where male elk calves inoculated with 1×10^{10} CFU SRB51 intramuscularly showed positive immune response only at fourth weeks on wards in the indirect ELISA. But this is not in agreement with findings of Stevens *et al.* (1995) who observed SRB51 vaccine induced antibodies two weeks after vaccination, when assessed by dot ELISA. Stevens *et al.* (1996) recorded a similar observation in mice vaccinated intra peritoneally with SRB51, where as SRB51 antibodies did not appear until 4-8 weeks in mice vaccinated orally with SRB51.

Adone *et al.* (2001) reported that there was 50 per cent sero conversion at eight post inoculation week, following vaccination of cattle with 10^9 CFU of SRB51. But in the present study, animals attained 100 per cent sero conversion at eight post inoculation week. This is not in agreement with findings of Adone and Ciuchini (1999) who found that highest titres occurred at 15-30 days after vaccination of cattle with 1×10^{10} CFU of SRB51 vaccine. They also reported that sheep developed peak antibody titres at 15 days post vaccination. Elk vaccinated with 1×10^{10} CFU showed peak antibody titres at 4th week (Olsen *et al.*, 2002).

In the present study immune response was reduced to 63.15 per cent by 90th day and it became 21.05 per cent on day 120. By day 150 animals became sero negative. These findings correlates with the observation of Adone and Ciuchini (2001) who suggested that cattle vaccinated with 10^{10} CFU of SRB51 developed antibodies detectable by RB51 based Complement Fixation Test (CFT) until 15 weeks post inoculation. In sheep also, antibodies were present till 110 post vaccination days. Similar observation was recorded by Cook *et al.* (2000) who found

that 80 per cent of elk calves inoculated with 10^9 CFU of SRB51 were still present at 18 post inoculation week. Heifers inoculated with 10^9 CFU of SRB51 showed peak antibody titre on day 29 and decreased at day 97 (Uzal *et al.*, 2000). Palmer *et al.* (1996) reported that pregnant heifers inoculated intravenously with 1×10^{10} CFU in the sixth month of gestation, showed persistence of infection up to 14th week, whereas Cheville *et al.* (1992) found that SRB51 was cleared by six weeks post inoculation in subcutaneously vaccinated heifers.

Mice vaccinated intraperitoneally with SRB51 had higher serum antibody titres to the surface antigens of intact SRB51 bacteria from week two to twelve than did mice vaccinated orally with SRB51. Previous studies had noted that intraperitoneal or subcutaneous vaccination of SRB51 effectively increased the resistance of mice to strain 2308 infection (Schurig *et al.*, 1991; Jimenez de Bagues *et al.*, 1994). Oral vaccination would be less effective than parenteral vaccination with SRB51 in preventing brucellosis in ruminants (Stevens *et al.*, 1996).

Calfhood vaccination of bison heifers with 10^{10} CFU of SRB51 reduced the incidence of abortion, fetal infection, or maternal mammary gland infection when compared to non vaccinated bison (Olsen *et al.*, 2003). Vaccination with RB51 did not cause any apparent lesions or disease in cattle, swine, goats or mice (Cheville *et al.*, 1992; 1993;1996)

Typically SRB51 was only present in sexually mature cattle for 6-8 weeks after vaccination (Elzer *et al.*, 1998). The lack of persistence of SRB51 in blood sample was possibly due to increased phagocytosis and killing of rough strains as compared to smooth strains (Harmon *et al.*, 1988; Price *et al.*, 1990). Strain RB51 is more sensitive to the bactericidal effect of complement when compared to smooth strain (Corbeil *et al.*, 1988). Interferon-γ had been shown to be important in the

control of intracellular growth of SRB51 in murine macrophages (Jiang and Baldwin, 1993).

From this study, it was observed that animals vaccinated with higher dose (1.8×10^{10} CFU)of SRB51 produced significant immune response with in seven days, when compared to animals vaccinated with lower dose(1×10^{10} CFU), which showed significant immune response only on 21st day of vaccination. Both groups achieved maximum immune response (100 per cent) on 60th day of vaccination. Among the animals with higher dose 9.52 per cent maintained significant antibody level till day 150, whereas the other group (21.05 per cent) showed significant antibody response only up to 120 days.

None of the *Brucella abortus* SRB51 vaccinated animals, both with higher or lower dose, produced detectable antibody response in the conventional serological tests *viz.*, RBPT and STAT throughout the study period, whereas animals vaccinated with *Brucella abortus* S19 produced detectable antibody responses in RBPT and STAT till 90th day, except two animals which produced antibody responses till day 180 in STAT. In STAT, peak titre was achieved on 14th day except in case of two animals, which showed highest titre on 21st day.

From this study it was concluded that

1. The new vaccine strain *Brucella abortus* SRB51, unlike the traditional brucella vaccine strain *Brucella abortus* S19, does not induce O-polysaccharide specific antibodies that interfere with standard serodiagnostic tests for brucellosis in cattle. So it is an ideal vaccine candidate. It is anticipated that replacement of S19 with SRB51 for calfhood vaccination will facilitate the identification and removal of cattle infected with brucellosis from vaccinated herds.
2. Cattle vaccinated with higher dose of *Brucella abortus* SRB51 produced significant antibody level earlier than those with lower dose and persisted

longer, when compared to the second group. But both groups showed maximum immune response on the same observation period i.e on 60th day of vaccination. The proportion of animals with significant immune responses were also higher in animals vaccinated with higher dose than those with lower dose except on day 21 and day 90.

Summary

6. SUMMARY

The sero conversion to two doses of *Brucella abortus* strain RB51 vaccine was assessed in cattle. Interference of *Brucella abortus* strain RB51 with routine diagnostic tests was also compared with *Brucella abortus* strain 19 vaccine. Vaccinations were done in sero negative calves of four months and above age. The study animals were grouped into three. Animals of group I and II were subcutaneously vaccinated with 1.8×10^{10} CFU and 1×10^{10} CFU of *Brucella abortus* strain RB51 vaccine respectively, whereas group III animals were vaccinated with 4×10^{10} CFU of *Brucella abortus* strain 19 subcutaneously. Immune responses were assessed at weekly intervals during the first month and there after at monthly intervals up to six months employing indirect ELISA. Clinical observations of all animals were also made two days before and one week after vaccination.

All the vaccinated animals showed febrile reaction during the first 24 to 48 hours post inoculation. Elevated temperatures ranged from 103.1^0 F to 104.5^0 F. But returned to normal temperature by 72 to 96 hours post inoculation. Eighty two per cent of the vaccinated animals developed swelling at the inoculation site 48 hours after vaccination but found to be reduced by 96 hours and became normal after one week. Animals were quite active and with good appetite throughout the observation period.

None of the *Brucella abortus* SRB51 vaccinated animals, both with higher or lower dose, produced detectable antibody response in the conventional serological tests *viz.*, RBPT and STAT throughout the study period. Whereas animals vaccinated with *Brucella abortus* S19 produced detectable antibody responses in RBPT and STAT from seventh day to 90^{th} day, except two animals which produced antibody responses till day 180 in STAT. In STAT peak titre was achieved on 14^{th} day except in case of two animals, which showed highest titre on 21^{st} day.

On day seven, 19.04 per cent of group I animals showed significant antibody response, where as group II animals showed sero conversion only on 21st day (31.57 per cent). Maximum immune response (100 per cent) was achieved on day 60 by both groups. Out of 21, two animals (9.52 percent)of group I maintained significant antibody response till 150th day, where as group II animals (21.05 per cent) showed significant immune response only up to 120th day.

Significant difference in percent positivity values between group I and group II were observed on day 7, 14, 60 and 120 following vaccination.

From this study it was concluded that

1. The new vaccine strain *Brucella abortus* SRB51, unlike the traditional brucella vaccine strain *Brucella abortus* S19, does not induce O-polysaccharide specific antibodies that interfere with standard serodiagnostic tests for brucellosis in cattle. So it is an ideal vaccine candidate. It is anticipated that replacement of S19 with SRB51 for calfhood vaccination will facilitate the identification and removal of cattle infected with brucellosis from vaccinated herds.
2. Cattle vaccinated with higher dose of *Brucella abortus* SRB51 produced significant antibody level earlier than those with lower dose and persisted longer, when compared to the second group. But both groups showed maximum immune response on the same observation period i.e on 60th day of vaccination. The proportion of animals with significant immune responses were also higher in animals vaccinated with higher dose.

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SERO CONVERSION STUDIES OF *BRUCELLA ABORTUS* STRAIN RB51 VACCINE IN CATTLE

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ABSTRACT

In the present study, the sero conversion to two doses of *Brucella abortus* strain RB51 vaccine was assessed in cattle. Interference of *Brucella abortus* strain RB51 with routine diagnostic tests for brucellosis was also compared with *Brucella abortus* strain 19 vaccine. Vaccinations were done in sero negative calves of four months and above age. The study animals were grouped into three. Animals of group I and II were subcutaneously vaccinated with 1.8×10^{10} CFU and 1×10^{10} CFU of *Brucella abortus* strain RB51 vaccine respectively. Whereas group III animals were vaccinated with 4×10^{10} CFU of *Brucella abortus* strain 19 subcutaneously. Immune responses were assessed at weekly interval during the first month and there after at monthly interval for a period of six months employing indirect Enzyme Linked Immunosorbent Assay (ELISA). Clinical observations of all animals were also made two days before and one week after vaccination.

All the vaccinated animals showed febrile reaction during the first 24 to 48hours post inoculation. But returned to normal temperature by 72 hours post inoculation. Eighty two per cent of the vaccinated animals developed swelling at the inoculation site 48 hours after vaccination but found to be reduced by 96 hours and became normal after one week. Animals were quite active and with good appetite throughout the observation period.

None of the *Brucella abortus* SRB51 vaccinated animals, both with higher or lower dose, produced detectable antibody response in the conventional serological tests *viz.*, RBPT and STAT throughout the study period. Whereas animals vaccinated with *Brucella abortus* S19 produced detectable antibody responses in RBPT and STAT till 90^{th} day.

Cattle vaccinated with higher dose of *Brucella abortus* SRB51 produced significant antibody level earlier (seventh day) than those with lower dose (21st day) and persisted longer (upto150 days), when compared to the group II (only upto 120 days). But both groups showed maximum immune response on the same observation period i.e on 60th day of vaccination. The proportion of animals with significant immune responses were also higher in animals vaccinated with higher dose than those with lower dose except on day 21 and day 90.

From the serologic point of view, it is concluded that *Brucella abortus* strain RB51 is an ideal vaccine candidate than *Brucella abortus* strain 19 and animals vaccinated with higher dose produced better immune response to *Brucella abortus* SRB51, when compared to those vaccinated with lower dose.