REVIEW OF LITERATURE
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HISTORICAL

The fact that some animals of domesticated species fail to carry their young ones for the full period of gestation is known from the earliest time.

Fleming (1871), an English Veterinarian, cited an instance in the Roman Empire: "The Tarentian war was succeeded by a most desolating pestilence, invading both cities and suburbs and carrying off chiefly women and cattle. In B.C. 273, it was known as the Abortus epidemicus and was particularly fatal to pregnant females and cows at Rome". In old Testament times abortions plagued the nomad as implied in the Book of Genesis. Jacob agreed to tend the flocks of his father-in-law, Laban, in payment for the latter's daughter, Rachel and Leah. He apparently was successful at animal husbandry, and also tried his servitude, for he says in Genesis 31:38, "This twenty years have I been with thee, thy ewes and thy she goats have not cast their young, and the rams of thy flock have I not eaten" (cited by Spink, 1956).
The possibility of abortion is again referred to later in the Bible (Exodus XXIII) (cited by Watson, 1962).

According to Huges (1937), a disease pattern compatible with Brucellosis was described by Hippocrates (450 B.C.). A plethora of synonyms for the illness appeared, such as intermittent typhoid, typho-malarial fever, remittent fever, Mediterranean fever, Rock or Gibraltar fever, Malta fever, Neapolitan fever, cyprus fever, and undulant fever.

The first accurate description of Brucellosis as a disease was made by Marston (1861) who wrote in 1860 on "Mediterranean gastric remittent fever", for he was the patient himself. Another faithful account of the disease was made in 1879 by Veale.

Brauer (1873, 1880), Lehnert (1873), Woodhead (1839), Penberthy (1895) realised that the disease is contagious and demonstrated that it is transmitted by intravaginal route. Bang (1897) also experimentally induced abortion in a pregnant ewe by intravaginal injections of a pure culture of Brucella abortus.

Bruce (1887), with the aid of Caruana Scichluna, prepared agar media and succeeded in
culturing a Micrococcus from the spleens of four fatal cases of Malta fever. He had successfully transmitted the disease to monkey and designated the etiological agent as Micrococcus melitensis (Syn. Brucella melitensis) in 1893. The Romans called the Island of Malta "Melita" meaning "honey" - and hence the name "melitensis".

The causative organism was also named as Streptococcus melitensis by Hugos in 1892.

Later, Zammit (1905) of the Malta Board of Health showed that the organism also infected goats. Further work rendered it evident that the goat was in fact the natural host of Brucella melitensis, and that the infection was carried to man by the consumption of raw milk.

Zammit (1905) reported that 5 out of 6 goats which he had examined gave a positive agglutination reaction to Brucella melitensis. From the blood of two of these animals, he succeeded in cultivating the actual organism.

Horrocks (1905) confirmed this, and further demonstrated the frequent presence of Brucella melitensis
in the milk and urine of apparently healthy goats.

While in Africa, Brear et al (1910) also investigated "Mukinyo", a disease of the natives of Uganda and found that it was Brucellosis and that its source was goat.

Nocard (1886), a professor at Alfort, Paris, reviewed the subject of contagious abortions in cattle and concluded that abortions are due to micro-organisms in between the foetal membrane and the uterus; he could not isolate the causal agent.

It was Bang (1897), Danish Veterinarian in Copenhagen with his assistant, Stribolt, who isolated the causal agent and termed it as Bacillus abortus.

The work of Bang was later confirmed by Preiss (1903) in Hungary, Nowak (1908) in Germany, McFadyean and Stockman (1909) in Great Britain, Zwick (1911) in Italy, Ascoli (1913) in Austria.

Zwick and Krage (1913) succeeded in culturing Brucella abortus from cow's milk and infecting goats with the bacillus.
The udder of the cow was discovered as the reservoir of \textit{Brucella abortus} by Schroeder and Cotton (1911) and by Smith and Fabyan (1912).

Mohler and Traum (1911) put forth the masterful presentation on "Infectious Abortion of Cattle" in U.S.A. Besides they recorded the first instance of \textit{Brucella abortus} cultured from the tonsils and adenoids of human sources.

Hutyra (1909) in Hungary and Traum (1914) in U.S.A. isolated the organism causing abortion in cows.

Evans (1913) reported close relationship of \textit{Brucella abortus} and \textit{M. melitensis}. She identified \textit{M. melitensis} as a bacillus and designated it as \textit{B. melitensis}. She concluded also that the agglutination test on human serum could not differentiate the infection due to \textit{Brucella melitensis} from that caused by \textit{Brucella abortus}.

Meyer and Shaw (1920) confirmed her work and suggested the genus \textit{Brucella} in honour of Bruce. Khaled (1921) reported the confirmation of Evan's differentiation.

Bevan in Rhodesia (1921 - 1922) and Keefer in U.S.A. (1926) supported Evan's views.
All members of the genus *Brucella* were believed by Faussier and Meyer (1920) to belong to one serological group and they were supported by Renoux (1952). Only one species, *Brucella brucel* with a number of varieties was suggested by Renoux *et al* (1955). The relationship among the organism isolated from cattle, sheep, pigs and man was under controversy although the naming of the genus was agreed.

It has long been recognised that the differences based on these tests (CO₂ requirement, H₂S production, growth on thionin and basic fuchsin, agglutination in monospecific sera, urease activity, use of methyl violet and pyronin and diethylthiocarbamate (DEDTC), are quantitative rather than qualitative; but where methods are controlled and standardised and cultures in smooth or smooth-intermediate phase are used, the majority of strains in most parts of the world can be identified as belonging to one or other of the three classical species.

Strains of *Brucella* differing in some characteristics have been isolated in many parts of the world.

Huddlestone (1961) proved that mutation among different species of *Brucella* did not occur.
The subcommittee on the taxonomy of Brucella of 8th International Congress for Microbiology, Montreal, August, 1962, concluded that Brucella strains fell into three well defined species: *Brucella melitensis*, *Brucella abortus* and *Brucella suis* with their biotypes (*Br. melitensis* biotypes 3; *Br. abortus* biotypes 9; *Br. suis* biotypes 3).

Three groups of Brucella cultures have not yet been placed in the above taxonomic scheme by the subcommittee: (a) Strains which were isolated from the desert wood rat (*Neotoma lepida*, Thomas) in Utah (U.S.A.) and named as *Brucella neotomae*. (b) The etiological agent of the ram epididymitis was described and named as *Brucella ovis*. (c) Brucella strains isolated from reindeer (*Rangifer tarandus*) in the far north of the U.S.S.R. and named as *Brucella rangiferi tarandus* (F.A.O./W.H.O., 1964).

*Br. canis* has caused outbreaks of disease in dogs mainly beagles (Carmichael and Brumer, 1968).

**INCIDENCE OF BRUCELLOSIS**

**IRAN**

Sabbaghian (1975) reported that 7% of fresh white cheese specimens were found infected with *Br. melitensis* biotype 1.
ITALY

Raffino (1931) showed the evidence of increased number of cases of undulant fever during 1928 - 1930. Rosati (1961) reported that an increase in the incidence of Brucellosis in man in Central Italy is attributed to more intense sheep and goat farming.

ENGLAND

Turton and Peckman (1932) reported the undulant fever in a dairy farmer whose only contact was "to pull the rope". Lawy (1956) reported a genuine fall in the incidence of Brucellosis in human beings. McDiarmid (1960) reported in Oxfordshire that 3 percent cattle were positive to whey tube test and 4.4 percent positive to guinea pig inoculation and in the Isle of Wight the respective percentages were 6.4 and 2.2.

Poole (1975) reported a 6 year survey of human Brucellosis in a rural area.

EGYPT

Zaki (1948) proved that 37.5 percent buffaloes at an abattoir gave positive serological reaction. Hamada et al (1963) reported that the rates of infection were 0% among cattle, 0.46% among buffaloes and 10.29% among camels.
SOUTH AFRICA

Van Drimmelon (1949) mentioned that Brucella abortus infection was first reported in 1913. Positive serum reactions have been found in a limited number of samples from pigs, goats, sheep and equines.

UNITED STATES OF AMERICA

Spink and Anderson (1950) was able to demonstrate Brucella agglutinin in the blood of 19.55 percent apparently healthy donors of blood. Only 1.66% had agglutinin titres of 1 : 160 or higher. There was some evidence that the donor with high titres had contact with the disease in the past. Mingle (1960) stated that 75 per cent in the estimated annual economic losses was caused by Brucellosis. Lewis and Anderson (1973) reported the incidence of Br. canis antibodies in the sera of military recruits. Street et al (1975) reported nine cases of Brucellosis in children. Lovejoy et al (1976) reported 3 out of 302 dogs were positive for Brucellosis. Galphin (1977) recorded 76% stray dogs were positive for Brucellosis at Air Force Base in Mississippi.

SPAIN

Elberg (1959) reported that 16 - 29 percent of goats were infected and 2.8 percent human beings
had titres of 160 I.U./ml. or above by serum agglutination test.

MEXICO

Flores Castro and Segura (1976) tested 203 human and 500 dog sera for canine Brucellosis in Mexico city. Positive agglutination titres were found in 13.3% of the human and 28% dog sera.

FRANCE

Gasse et al (1963) reported that the movement of sheep and goats was responsible for the spread, and infection in man was largely by contact (60 - 70 percent) or by ingestion of milk or cheese (within 30 - 40 days after manufacture).

NEWZEALAND

Glass (1964) traced during 1951-60 about 60 percent of human cases due to ingestion of infected cow's milk or milk products, the remainder to direct contact with infected cattle.

RUSSIA

Anishchenko (1964) stated that, in man from 1952 - 1961, 67 percent resulted from contact with animal, while 21.6 percent were of alimentary origin, although the incidence was higher in cattle than sheep.
INDIA

TAMILNADU

During the year 1964-65, 517 specimen materials collected from the field were examined; of them, 160 were positive for Brucellosis (I.C.A.R. Annual Report, 1965).

DELHI

Joshi and Prakash (1971) ascertained the prevalence of Brucella agglutinins in man and animals in Delhi and found 12.3 percent were positive in titres ranging from 20 I.U. - 1,200 I.U./ml. and only 3.9 percent were positive in diagnostic titre (80 I.U./ml. and above). 3 strains each from humans and goats, and one strain from sheep were isolated.

BIHAR

During the year 1960-61, the incidence was 1 percent (I.C.A.R. Annual Report, 1960-61). Of 2,258 blood serum samples, 2,147 were from cattle and buffaloes, 97 from goats and 14 from sheep. Out of these, 30 cattle proved positive and 59 doubtful. The rest was negative. Thus the percentages of positive and doubtful reactors in cattle were 1.39 percent and 2.74 percent respectively (I.C.A.R. Report, 1965).
GUJARAT

Jhatakia (1946), Shukla (1962) and Roy et al (1965) recorded the incidence of Brucellosis in Kathiawar, Baroda, Jamnagar respectively.

HARYANA

Buth and Manchanda (1972) reported 0.96 percent incidence in cattle and 0.54 percent in buffaloes.

HIMACHAL PRADESH

103 serum samples and 80 milk samples were put to agglutination test during the year (1964-65). 13 serum samples and 1 milk sample were positive (I.J.A.R. Report, 1965).

ANDHRA PRADESH

Out of 1,228 animals of Government Livestock Farm, 41 were positive for agglutination test (Annual Report, I.C.A.R., 1965).

UTTAR PRADESH

(a) Animals

<table>
<thead>
<tr>
<th>Source</th>
<th>Year</th>
<th>Animals</th>
<th>No. of animals tested</th>
<th>Positive animals (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 State Farms</td>
<td>1956-57</td>
<td>Buffaloes and Cattle</td>
<td>5433</td>
<td>5.6</td>
</tr>
</tbody>
</table>
### Source, Year, Animals, No. of animals tested, Positive (percentage)

<table>
<thead>
<tr>
<th>Source</th>
<th>Year</th>
<th>Animals</th>
<th>No. of animals tested</th>
<th>Positive (percentage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Govt. Farms</td>
<td>1957-61</td>
<td>Sheep and goats</td>
<td>All sheep and goats</td>
<td>Negative</td>
</tr>
<tr>
<td>17 State Farms</td>
<td>1961-62</td>
<td>- &quot;-&quot;</td>
<td>- &quot;-&quot;</td>
<td>- &quot;-&quot;</td>
</tr>
<tr>
<td>Field</td>
<td>1961-62</td>
<td>Sheep and goats</td>
<td>379 sheep, 1620 goats</td>
<td>31 sheep positive, 11 goats positive</td>
</tr>
<tr>
<td>Slaughter, House, Mathura</td>
<td>1961-62</td>
<td>Sheep and goats</td>
<td>-</td>
<td>0.7 percent serum samples of goats and sheep positive</td>
</tr>
<tr>
<td>Different slaughter houses of the State</td>
<td>1962-63</td>
<td>- &quot;-&quot;</td>
<td>4346 goats, 1235 sheep</td>
<td>21 samples of goats, 14 samples of sheep were positive (0.62 percent)</td>
</tr>
<tr>
<td>Village (with frequent abortions)</td>
<td>1963-64</td>
<td>Sheep and goats</td>
<td>54 sera samples of goats and sheep</td>
<td>6 positive (11 percent) in goats and sheep</td>
</tr>
</tbody>
</table>

(b) Human beings

43 sera samples supplied by American Christian Hospital, Vrindaban, were tested during 1963-64. One serum sample was found positive (Report, Scheme for the investigation of Brucellosis in U.P. for 1956-64). Panjarathinam and Gulrajani (1973) reported 0.4 percent incidence of human Brucellosis in Bareilly district of U.P.
AGGLUTINATION TEST

Wright and Smith (1897) first introduced the agglutination test.

Huddleston and Abell (1928), Huddleston (1932) and Welch and Mickle (1933) recommended the slide method of agglutination for survey work where large number of sera have to be examined.

Montgomerie and Rowlands (1932) carried out a comparative study for the presence of Brucella abortus agglutinins in 615 samples of blood serum and milk from 314 cows of known history and declared that they are not so reliable as blood serum.

Donham and Fitch (1932) pointed out that if rapid tests were held for 8-10 minutes before they are read, the method had no advantage in economy of time over the test tube method for the routine laboratory diagnosis of contagious abortion. Also they found that the titre of a serum in the rapid agglutination test was usually greater than by the tube method.

Fitch and Donham (1933) discussed some fundamental principles of the tube agglutination test and the rapid (plate) agglutination methods. The essential difference
was that the ratio of serum to antigen was very different in the two methods. In the tube method, the ratio of serum to saline was relatively small, whereas in the rapid method there was relatively high ratio of serum to antigen, and this ratio changed markedly in the different dilutions employed.

It has been shown experimentally that the saline solution in the antigen exercised inhibitory influence on the agglutination in the rapid method, possibly on account of its effects in diluting the serum.

The addition of small amount of gelatine increased the sensitivity of the rapid test antigens.

David (1934) observed that the proportion of positive results obtained by the slide method was thus greater, whilst the doubtful results were definitely less and the negative results similar to tube test and concluded that it is, therefore, a suitable method of diagnosis.

Sandner (1934) compared the rapid fresh blood agglutination method and the tube agglutination method and concluded that the rapid method was of no value
for the early diagnosis of infection in guinea pig, because it failed to detect a large percentage of samples which by the tube method were positive. The rapid method detected all positive samples of a titre of 1:400 or over, but failed to detect 1:100 or below.

Galtaneo (1935) concluded that the blood agglutination test was not reliable for the diagnosis of Brucella infection in sheep.

Diernhofer (1935) recommended the more general application of the whole blood for detecting agglutinins in the blood of animals affected with *Brucella abortus*.

Eavaglia (1936) discussed the rapid slide method for the diagnosis of Brucella infection, the examination of the result being done under the microscope. Care was taken to prevent coagulation. The results obtained by this method agreed closely with those obtained by the agglutination test.

Diernhofer (1936) described a rapid agglutination test for *Brucella abortus* infection. One drop of oxalated blood was mixed with one drop of antigen on a glass slide. The mixture was then spread over an area of 2-3 sq. mm. The appearance of bluish white floccules indicated the presence of agglutinins in the blood.
Behnke (1937) suggested that sera may first be submitted to the rapid test and all reacting sera then verified by the tube method.

Diermhofer (1938) looked upon the rapid slide agglutination test as a helpful adjunct to the tube test.

Ratomski and Wisniowski (1955) introduced the modified whole blood agglutination test for use in the field which yielded results that were 95 percent accurate when compared with the tube agglutination test on serum samples. A single drop of blood was added to a drop of 0.25 percent sodium citrate and a drop of antigen stained with crystal violet. The dilution of antigen used was 1:25.

Cedro et al. (1955) described a rapid plate test with whole blood for the diagnosis of Brucellosis in the abattoir, the results obtained were corroborated by the serum agglutination test.

Moreira-Jacob (1955) reported that the rapid slide agglutination test yielded more positive reaction than the tube test in case of both sheep and goat sera.

Kobets (1956) stated that the rapid agglutination test for bovine Brucellosis was simple to perform and
in many cases appeared to be more sensitive than the standard agglutination and the complement fixation test.

Khan (1958) was of the opinion that the slide agglutination test gave comparable results to those of the tube agglutination test when high titre sera were tested, but the rapid methods missed a few sera which were weakly positive by the tube agglutination, and the blood slide agglutination test may be used for survey purposes. If it is supplemented with the tube agglutination of negative sera, the method may be used for detection of positive and doubtful cases.

Cedro et al (1960) subjected over 400 goats in herds in 3 provinces to the whole blood plate agglutination test. The results indicated the efficacy of the test. The test was rapid, practical, easy to apply and interpret and can be used for Brucellosis survey. Boguer (1964) compared the plate and the tube agglutination tests for Brucellosis in sheep and goats. The tests agreed on 855 negatives, 33 positives and 18 doubtfuls in 916 sheep and on 1,313 negatives and 7 doubtfuls in 1,331 Angora goats; and the plate test was recommended for screening flocks.
Munford et al. (1975) found that standard agglutination test appears to be most reliable indicator of acute infection.

Buchanan and Faber (1930) observed that the Mercaptoethanol test is superior to the standard agglutination test in determining the adequacy of antibodies.

**COMPLEMENT FIXATION AND AGGLUTINATION TESTS**

McFadyean and Stockman (1909) put into application the complement fixation test (CFT) along with the agglutination test for the diagnosis of bovine Brucellosis. Holth (1909-1910) adopted the same methods and found close agreement between them.

Mohler and Traum (1911) who examined 400 sera by both methods found close correspondence between them; but the results varied only in five instances. Similar results were recorded by Zwick (1910), Wall (1911), Reinhardt and Gauss (1915). Mitchell (1929) advocated both tests for the diagnosis but Lentz (1932) preferred the complement fixation test. Stockmayer (1936) considered the agglutination test to be superior to the complement fixation test for the early and efficient diagnosis of Brucella infection.
Feldt (1936) compared the results of a study of the value of the agglutination, the complement fixation and Weinicke flocculation tests for the diagnosis of Brucella abortus infection. He carried out on 3,634 samples of blood sera. These tests agreed uniformity in the case of 73 percent of the sera.

Lopez (1936) submitted results which showed the close correlation between the tube agglutination and complement fixation methods. Cedro et al (1955) advocated the use of the complement fixation test for the diagnosis of Brucella infection.

Peils (1955) compared the agglutination and complement fixation tests for Brucella melitensis infection on 2,300 bovine blood samples. The complement fixation test was found to be particularly useful for confirming the diagnosis in cases yielding a doubtful reaction to the agglutination test.

Clapp et al (1955) indicated that the complement fixation test could be used to reveal infection in rams with no clinical evidence of epididymitis and from the semen of which the organism could not be isolated and concluded that any sera giving ++++ reaction at a dilution of 1:10 is positive.
Burki (1956) who examined 146 sera from sheep infected with Brucella, confirmed that the agglutination test was only of limited value in the diagnosis and recommended the additional use of the complement fixation test where results with the agglutination test are doubtful.

Schoop and Zettl (1956) compared both tests in diagnosing Brucellosis in sheep and found that out of 17,189 sheep, 81 percent were positive to the agglutination test and 51 percent to the complement fixation test. Hadju (1956) considered the complement fixation test to be more reliable than the agglutination test for the diagnosis of Brucella infection in cattle and pigs.

Krygon (1957), using 13 cows known to be infected with Brucella abortus, carried out the complement fixation reaction, the agglutination test and the ring test every two weeks over a period of 6 months in bovine Brucellosis and observed that the complement fixation test yielded 200 positive, 56 negative and 5 doubtful reactions, whereas the agglutination test gave 127 positive, 93 negative and 1 doubtful.
Gargani and Aleandri (1960) prepared antigens by alternate freezing and thawing from *Brucella abortus* and from *Brucella melitensis* and was able to distinguish between *Brucella abortus* and *Brucella melitensis* infection in experimental animals by using the complement fixation test. In human Brucellosis there were no differences in titre against either antigen, while the reaction was on the whole predominantly positive to abortus antigen in cattle and to melitensis antigen in sheep.

Burki (1960) submitted the whole positive reactors to the slow serum agglutination test in each of 223 herds of cattle to the complement fixation test and noted that 181 were negative, 9 doubtful and 33 positive.

Spink (1956), Stableforth (1959) preferred the agglutination test instead of the complement fixation test in the diagnosis of Brucellosis in human beings and animals respectively. Dalrymple-Champneys (1960) concluded that the complement fixation test was more reliable than the standard agglutination test.

Stockman (1914), Carpenter and Boak (1930), Wise and Graig (1924) and Schoenaers and Kackenbeeck (1953) were of the opinion that the complement
fixation test was a more complicated test and had no distinct advantage over the agglutination test in the diagnosis of human Brucellosis.

Alton (1961) was able to differentiate between infected and vaccinated goats by the complement fixation test using plain antigen (Weybridge). His findings were confirmed by Hill (1958), Burki (1961) and Bertschinger (1961).

Ugorski (1962) used both agglutination and complement fixation tests in 2 flocks of sheep, one known to be infected and the other suspected resulted in rise of positive cases from 16.7 percent (agglutination test only) to 62 percent (where both tests were employed in the infected flock. In the suspected flock there was a 6.2 percent rise.

Jones et al. (1963) examined over 1,400 sera from problem herds by the tube agglutination and the complement fixation tests and concluded that the complement fixation test was a useful supplement test for sera with suspicious agglutination titres. In several recently infected herds, cattle developed the complement fixing antibodies before agglutinins.
Aleandri and Gargani (1963) used the complement fixation test (with frozen-thawed soluble abortus and melitensis antigens) for most of animals and experimental guinea pigs.

Burki (1963) in Switzerland considered that the cattle with agglutination titres of 80 to 160 but negative to the complement fixation test to be free from Brucellosis.

Mackinnon (1963) described the technique of the complement fixation test and compared the results of the complement fixation and agglutination tests in vaccinated and unvaccinated cattle.

Hill (1963) standardised and used the complement fixation test as a routine test for bovine Brucellosis in the Netherlands.

Gargani (1964) prepared and titrated the Brucella melitensis and Brucella abortus antigens for the complement fixation test at the Italian Brucellosis Centre.

Wieniowski (1964) by simultaneous applications of the complement fixation and agglutination tests was able to detect more than 40 percent infected cows
than when agglutination test alone was used; about 9 percent of sera giving a positive complement fixation reaction gave a doubtful agglutination reaction.

Pilet et al (1967) described a cold (4°C) CFT method in which an "acellular antigen (1 unit only) and a small volume of haemolytic system (0.2 ml) were used.

Uniol Williams and Stableforth (1969) made a comparison of 3 serological tests (Coombs' test, CFT and SAT) in detection of Br. melitensis infection in sheep.

Chappel et al (1973) found standard agglutination test (SAT) was generally much less sensitive than CFT when 1337 bovine sera positive for Rose Bengal test (RBT), were tested; besides they reported that the atypical reaction in CFT sometimes caused difficulties in diagnosis.

Roux (1979) recommended CFT for case finding, while a buffered Brucella agglutination is best for mass survey.
INDIRECT BACTERIAL HAEMAGGLUTINATION TEST

Carrere and Roux (1952) first tried the haemagglutination test (HAT) for the diagnosis of Brucellosis in man and sheep. They used "soluble substances" for the sensitization of sheep erythrocytes and found that the haemagglutination test was more sensitive than the tube agglutination test in the diagnosis of infected animals but negative in the sera of vaccinated animals.

Hirschberg and Yarbrough (1952) was able to sensitize the sheep erythrocytes with only three out of many prepared extracts for the haemagglutination test.

Macpherson et al (1953) and Neter et al (1954) reported that large molecules of the polysaccharide complexes could not sensitize properly the erythrocytes, but when it was treated with alkali, the resulting smaller molecules of the complex adsorbed with the erythrocytes.

Boyden (1950), Neter et al (1954) and Fine et al (1955) were able to remove by alkali treatment certain lipids such as cephalin and lecithin present in the bacterial extracts which inhibited the attachment of bacterial antigens to the surface of the erythrocytes.
Landy et al. (1953) found out that phospholipids of lipopolysaccharide complexes inhibited the absorption of the antigens to the surface of the erythrocytes and could be neutralised by alkali and heat treatment.

Mondini (1957) treated the antigen with ammonium sulphate (30 percent and 50 percent) and with heat (70° C for 10 minutes) to eliminate non-specific reactions and found that cattle found doubtful become negative after the treatment.

Becht (1958) obtained an extract of Brucella abortus after treatment with acetic acid at 100° C followed by alcohol precipitation and found that the haemagglutination test was superior to the agglutination test for the diagnosis of bovine Brucellosis.

Davies et al. (1958) proved that the alkali treated bacterial lipopolysaccharide and "O" somatic antigens absorbed very easily on the erythrocytes. They also showed that heating enhanced sensitizing property and to be less effective than alkali treatment. They concluded that the alkali treatment could remove "O" acetyl residues which prevented the adsorption on erythrocytes.
Cedro et al. (1962) observed that the haemagglutination test gave results similar to those of the serum agglutination test in the diagnosis of caprine Brucellosis.

Ris and Te Punga (1963) used an antigen obtained by ultrasonic disintegration of the cells for the detection of *Brucella ovis* antibodies by the indirect haemagglutination test and they were satisfied with this test, though there was cross reaction with *Brucella abortus* (Strain 19) antibodies.

Khristoforov (1964) prepared polysaccharide antigen from 5 *Brucella abortus*, 5 *Brucella suis* and 2 *Brucella melitensis* by treatment with 95 percent phenol, sodium hydroxide, acetic acid or trichloracetic acid. These antigens were adsorbed on to untreated, tannic acid or trypsin treated sheep erythrocytes. Haemagglutination tests using such sensitized cells and sera from 2,908 pigs, 29 sheep, 37 cattle and 27 horses with Brucellosis were specific, and positive titres were equal to or higher than the corresponding agglutination titres. Polysaccharide antigens from *Brucella abortus* were the most effective. Highest titres were obtained by using antigens prepared by the phenol and alkaline methods, and trypsin treated red blood cells.
Ris (1964) showed that the CFT was nearly always the first test to become positive. Haemagglutination titres were at least 25 times higher than the corresponding CF titres.

Versilova et al (1974) found the diagnostic efficacy of haemagglutination (HA) test was 20-30 percent greater than that of agglutination test, it is more specific and sensitive.

Panjarathinam and Gulrajani (1975) observed that the haemagglutinin titres never agreed with agglutinin titres.

Renoux and Renoux (1973) observed that the coupling of an extract from Br. abortus Str. 99 to sheep red blood cells by chromium chloride allowed cells to develop a highly specific and reliable passive HA test. HA was positive in 99.5 percent of sera from 968 human persons with infection, while the tube agglutination revealed only 95 percent and CF test 90.9 percent positive cases. HA could replace a battery of conventional tests.

Hoffmann et al (1980) obtained optimal results by using bovine erythrocytes with alkali treated antigen at a concentration of 900 µg/ml. in the direct haemolysis test.
GEL DIFFUSION TECHNIQUE

Bechhold (1905) originally observed antigen-antibody reactions in gel media. His observations were dismissed by himself as being examples of Liesegang rings (Liesegang, 1896), a precipitation phenomenon which had been observed when inorganic compounds diffused through certain gel mixtures. Henceforth, the use of this technique remained in abeyance. Later, a great importance was attached to this technique to be used for immunological studies, when Oudin (1946) employed a technique of single diffusion to demonstrate that impure antigens produce a multiplicity of lines, each line representing an antigen-antibody system. His theories were expanded (Oudin, 1947; 1948 a, b; 1949) and still hold good.

Ouchterlony (1943, 1949) and Eleck (1948) evolved independently the double gel diffusion technique which is used today. Ouchterlony (1949 a, b, c, d, e; 1953 a, b; 1959) published numerous papers on this subject. He is acknowledged by the use of the term Ouchterlony technique in reference to double diffusion technique.
Black (1949), Bjorklund (1952), Oakley and Pulthorpe (1953) and Schiott (1953) confirmed the usefulness of the method.

Ouchterlony (1958, 1962) and Crowle (1960, 1961) put forth comprehensive reviews of immunodiffusion methods and their more general application.

Olitzki and Sulitzeanu (1957) reported that sonic extracts of Brucella suis and Brucella melitensis produce 5 and 6 lines respectively with rabbit antisera in gel diffusion method.

Bruce and Jones (1953) reported that cultures of Brucella melitensis yielded a diffusible precipitin antigen which produced lines with sera of rabbits, goats and cattle that had been infected with either Brucella abortus or Brucella melitensis. The precipitin lines varied in number from one to three. However, the supernatant fluid from a heavy suspension of Brucella abortus which had been stored in 0.5 percent phenol saline in the cold for over 6 months produced a line with one of the three sera tested.

Garrere et al (1958) observed a precipitin line of identity between trichloroacetic acid extracts of
Brucella melitensis and Brucella abortus but not with similar preparation of Brucella suis.

Olitski and Sulitzeanu (1953) regularly demonstrated six to nine lines from extracts of the three species, but an additional strong precipitin line designated the "M" antigen, was produced with a sonic extract of Brucella melitensis.

Olitski (1959) reported that Brucella abortus, Brucella melitensis and Brucella suis possess at least 6 soluble antigens which could be demonstrated by the agar gel precipitation technique. These antigens differed in their relative concentration in bacterial extracts of different origins and in their ability to produce antibody titres in immune sera. No antigen specific for a single species was demonstrated among these antigens. Redfearn and Berman (1960) reported that extracts for the gel diffusion technique were produced by heating the Brucella cells in 1 percent phenol at 65° C for 1 hour. Antisera produced in rabbits against Brucella abortus, melitensis and suis gave strong lines of precipitation when tested against an extract prepared from Brucella melitensis 16 M. Sera produced in cattle gave an additional
precipitation line. Of 84 Brucella cultures studied in this way, it was found that cultures that would normally be agglutinated with monospecific melitensis serum, regardless of their other biochemical tests, gave strong precipitation lines against either abortus, melitensis or suis antisera but cultures agglutinated by monospecific abortus sera did not produce a diffusible extract and were negative to gel diffusion test. It was suggested that this method could be used instead of the agglutination test with monospecific sera for the identification of Brucella cultures.

Farnes et al (1961) reported that the antigens of Brucella melitensis 16 M, Suis 1530 and abortus 544 gave 6 precipitation lines with homologous heterologous rabbit antisera, but the atypical melitensis 176 and suis 45 strains gave only 4 and 3 precipitation lines respectively.

Huston et al (1934) found a precipitating substance "S" which was readily extracted from Brucella melitensis but not from Brucella abortus or Brucella suis. Hershey et al (1935) later showed that the "S" substance was combined with protein in the albuminoid fraction of all 3 species, but in addition, was in the free state in extract of Brucella melitensis.
Miles and Pirie (1959) obtained disaggregated antigen from the *Brucella melitensis* antigen by treatment with 80 percent acetic acid.

Paterson et al (1947) proved that all known methods were unsatisfactory in converting the *Brucella abortus* antigen into a water soluble material of relatively low molecular weight.

Annual progress report of the Indian Council of Agricultural Research Scheme for the survey of Brucellosis in Madras State (1964-1965) indicated that a phenol extract of a suspension of *Brucella* organisms was used as an antigen in gel diffusion test and mentioned that all sera samples from vaccinated animals failed to give precipitin lines, whereas only 53 (i.e. 44 percent) out of 132 sera samples from naturally infected animals, i.e. those with titres of 1:80 and above gave positive result; it was also suggested that it may be possible to differentiate by application of this test the agglutination reactions between infected and vaccinated animals.

Fanjarathianam and Gulrajani (1971) advocated the susceptibility of the gel diffusion test for the survey work when the milk ring test can not be performed in dry cows.
Sarre et al (1971) found that lipopolysaccharide and polysaccharide extracts of *Br. melitensis* gave a single line when tested in gel with sera from naturally infected human beings.

Myers et al (1972) observed that the gel diffusion test with saline extract of *Br. ovis* is as sensitive as the CFT for the diagnosis and is more practical.

Corbel (1973) noted that when the reactions with SAT, CFT and HBT were weak, the immuno diffusion test usually gave one or more precipitation lines.

Beh (1974) determined the quantity of specific Brucella antibody by using the single radial immuno diffusion technique.

Diaz et al (1976) observed that a protein antigen of Brucella reacted with 94 percent of the sera in counterimmunoelectrophoresis which appears to be the most promising method in the diagnosis of clinical brucellosis.

Schurg et al (1978) obtained the smooth lipopolysaccharide complex of the outer surface of smooth *Br. abortus* cells and believed to be antigenic
components involved in serological tests routinely used in the diagnosis of Brucellosis.

Moreno et al (1978) purified and characterised the smooth and rough lipopolysaccharide from Br. abortus.

McMahon et al (1979) made a comparison of the results obtained with a Brucella agar gel immuno diffusion test and the standard agglutination test on 612 human sera. An agreement between the tests was 97 percent when the titre was 1:160 or higher.
Milk Ring Test

Fleischhauer (1937) first described the ring test (MRT) and concluded that in the testing of herd samples, only reactions which occur within twenty minutes following incubation should be considered as positive. Hermann (1937) proved the test to be as accurate as the tube method.

Smitmanns (1933) concluded that reactions on herd samples should be considered positive if the reactions started within twenty-five minutes following incubation and there were a 2-4 mm. wide blue violet ring sharply defined from the underlying white milk after two hours' incubation. Fleischhauer and Hermann (1933) compared the ring agglutination test with the standard tube and slide methods for the diagnosis of Brucellosis and concluded that such tests merely indicated that the cows producing the milk are strong positive reactors to the agglutination test and that it is probable that a proportion of them may be infected with Brucella abortus in the udder. Meyer and Huddleston (1938) found out that Brucella abortus and agglutinins may occasionally be present in the udder in the absence of agglutinins in the blood.
Norell and Olson (1943) proved the ring test to be far more sensitive on herd samples than did the slow whey agglutination test. When results obtained from the herd samples of milk were compared with blood tests of individual animals, there was agreement in 94.6 percent of the cases.

When the results with the herd milk ring tests were compared with the blood tests of individual animals, Seif and Jorgensen (1944) found only a 3.9 percent disagreement.

Bruhn (1948) reported that by means of the ring test he was able to detect 97 of 121 (80.2 percent) cows which were blood reactors at 1:20 or higher. There were 24 instances of positive blood and negative ring tests. The ring test failed in 3 instances, in 1 with the blood agglutination titre 1:100 and in 2 cases with the titre 1:200. Bruhn concluded that these negative ring test reactions in samples from individual cows which are strong reactors are usually due to the lack of cream rising capacity of the milk. When these samples are mixed with ring test negative pooled milk, positive reactions are obtained.

Christiansen (1948) reported results on 6,266 herds that had been blood tested at the time of
performing the milk ring test. There was a complete agreement between the two tests in 93.2 percent of the tested herds, partial disagreement in 2.4 percent, and complete disagreement in 4.4 percent. Ring tests carried out simultaneously with blood tests on 3,952 individual animals showed an 84.3 percent agreement, partial disagreement in 4.1 percent and total disagreement in 11.6 percent of the instances.

Van Drimmelon (1949) developed a modification of the "ABR" test which can be used for milk from individual cow and which is claimed to distinguish between vaccinated and naturally infected cattle. Three tubes were used. They contained undiluted milk, and dilution of 1:2 and 1:4 with non reacting milk. Naturally infected animals give positive reaction in all 3 tubes, whereas vaccinated animals gave a slight positive reaction in the first tube but there was no reaction in other two tubes. The blood titre remained for months or sometimes for years, but the ABR dilution test gave a negative reaction before 10 weeks.

Koepeke et al (1950) found an agreement between the ring test and the blood serum agglutination test on 96.2 percent of 8,469 herds tested. The ring
test was positive for 63 percent of 335 herds considered infected on the basis of blood tests.

Gilman (1950) concluded that the ring test is of value in detecting infected herds but it must be used in conjunction with the blood test.

Horse et al. (1952) considered that the milk ring test was slightly more accurate in detecting infected herds than the capillary tube test described by King (1951).

D’Alo (1952) found considerable agreement between the Brucella abortus ring test and the blood serum agglutination test in infected sheep.

Alivisatos and Edipides (1953) performed the ring test and the tube agglutination test with blood sera from 206 goats. In most cases the reactions with the two tests agreed.

Ferguson and Robertson (1954) found that on analysis of 436 simultaneous blood serum agglutination and milk ring tests, the ring test to be correct in 93 percent of cases in detecting cows whose blood serum is positive to the Brucella agglutination test.
Moran and Maubacin (1955) considered that the ring test can be complementary to serum agglutination for bulk tests in epidemiological survey.

Feja (1956) observed that out of 2,000 cows tested for Brucellosis, 419 were positive and 1,363 negative to both tests; 119 were positive and 41 doubtful only to the blood test and 53 were positive only to the milk test. Hence he recommended that both tests can be used for diagnosis.

Pauluzzi and Gimenti (1956) considered the diagnostic tests (the haemagglutination, ring and milk plate agglutination tests) to be most efficient in the order given.

Thorold and Holmes (1957) tested over the past 3 years 2,073 animals on 93 farms in Kenya by the ring test; 20 percent were positive revealing 33 percent of the farm infected.

Owejero et al (1958) observed that the test failed to give results with milk from infected sheep and goats, but it became reliable if the milk was mixed in a proportion of 1:10-1:30 (sheep) or 1:50-1:60 (goats) with milk from infected cows. Results were improved when fat was removed by treatment of the milk with chloroform.
Lostia (1959) tested 1,048 individual milk samples, of which 213 were positive, 252 doubtful and 577 negative. Most of 56 bulk milk samples were positive.

Oneremalin (1960) compared the results of ring tests on milk from 703 ewes in an infected flock and from 383 ewes inoculated 1-3 times with strain 19 vaccine with those of agglutination and complement fixation tests. He concluded that the test was sensitive and specific. The test was positive 20 days after vaccination but negative after 6-12 months.

Hubrig (1961) stated that cows inoculated with killed vaccine became negative to the ring test 4-5 weeks afterwards. Positive tests obtained later than that were indicative of true (possible mammary) infection.

Pargaonker and Raj (1962) reported that 8 were positive out of 226 samples of pooled milk and the high fat content of buffalo milk did not interfere with the test.

Brus and Jaarsveldt (1963) observed that the results of the ring and the blood tests of 7,142 animals corresponded in only about 60 percent.
A M test was compared with serum agglutination test and it was observed that the accuracy with milk was 60-70 percent (Annual Report, 1964-65, Himachal Pradesh, I.C.A.R. Scheme).

Hill (1964) recommended that the ABR test, plate, tube agglutination tests and Coombs' test can be used as combined method to detect the infection.

Jaartsveld and Jilesen (1967) noted that ABR titration in milk is a valuable aid in the diagnosis of Brucellosis.

Holmes (1967) reported a modified MRT for the recognition of Brucellosis in individual lactating cows.

Hunter and Allen (1972) compared between 2 milk tests, MRT and CFT on whole milk and 3 blood tests, SAT, CFT and RBT to diagnose Brucellosis and found that no single test correctly identified all infected animals. RBT detected the highest proportion of infected animals and was simple and rapid.

Mylrea (1972) claimed the RBT, the crystal violet test, the serum agglutination test and MRT were
efficient in detecting culturally positive cows and those giving positive reactions to the CFT.

Panjarathinam and Gulrajani (1975) observed that the Milk Ring Test (MRT) serum plate agglutination, tube agglutination and gel diffusion tests agreed very closely.

**THE ANTIGLOBULIN (COOMBS') TEST IN BRUCELLOSIS**

Jones and Wilson (1951) published a preliminary note on the first few cases.

Sohhardt et al (1951) described the use of the blocking antibody test and of the developing (modified Coombs') test in detecting antibodies to Brucella.

Wilson and Merrifield (1951) applied this test for the detection of Brucella agglutinins. The test showed a significant titre in 17 cases of possible Brucellosis in which the conventional test was negative.

Jentzsch (1964) described the "fluorescence Coombs' test" to demonstrate incomplete antibodies.

Kuhlmann (1965) demonstrated that the commercially available precipitating sera can be used as Coombs' sera.
Schassan (1967) reported that because of the occurrence of incomplete antibodies inhibiting the agglutination, it is necessary to perform the Coombs' test in addition to SAT, and CFT. A titre of 1:20 in Coombs' test enables to detect the infection.

Foa (1969) described a technique (Coombs' test) for the evaluation of incomplete antibodies in the serum.

Henderson et al (1975) found saline agglutination test and CF test are reasonably satisfactory. The mercaptoethanol and Coombs' tests have no advantage over the other two and could be dropped.

Diaz et al (1976) observed that the results obtained with rapid plate agglutination test, the Coombs' test were inferior. IgM was not active in Coombs' test, but IgG and IgA were active.

Schoutens et al (1973) noted that Coombs', passive haemagglutination and immuno diffusion tests tended to confirm the results of agglutination test.
Since the diagnostic utility of Rose Bengal test (RBET) and counterimmunoelectrophoresis (CIEP) test in Brucellosis was well established by Diaz et al (1976); Diaz et al (1978) were interested to find out whether these tests could be used to detect Brucella antibodies in Cerebrospinal fluid (CSF) of patients with Brucella meningitis, to establish a rapid diagnosis of this complication. To their knowledge, no report has been made on the behaviour of Immunoglobulin G (IgG) in C.S.F. of patients with Brucella meningitis. In their study they have also investigated the concentration of IgG in CSF from such patients.

The CSF studied was obtained from 5 patients with Brucellosis confirmed by sero agglutination, Coombs', CFT, RB, CIEP tests. All these patients had, in addition, clinical evidence of meningitis. Control CSF was selected from 7 patients with Brucellosis but not showing any symptoms of meningitis and was used both in serological test and for the determination of concentration of IgG. The results indicated that the determination of IgG concentration of CSF could be of value in the diagnosis of Brucella meningitis.
Morgan et al. (1969) made use of the Rose Bengal plate agglutination test for the diagnosis of bovine Brucellosis.

Davies (1971) reported that RBT detects infection earlier than SAT and is more efficient.

Gower and Wright (1974) described an automated Rose Bengal agglutination test for Brucellosis. Its sensitivity in relation to serum agglutination and CF tests is 92 percent and is better than the manual test. The specificity is 59 percent rather less than that of the manual test. The machine can handle up to 200 samples per hour.

Oomen and Waghela (1974) noted that the percentage of false positive findings on RBT was 0.22 percent; when compared with SAT; 0.44 percent with CFT. The RBT may be considered as a highly reliable test in the diagnosis of human Brucellosis.

Strohl (1974) found RBT is far more specific than standard agglutination test.

Dias et al. (1976) observed that the results obtained with RBT correlated very well with those of the standard tube agglutination test, whereas the results with rapid plate test and Coombs' test were inferior.
Rey (1977) indicated that RBT proves effective for the diagnosis of acute, latent infection and more often positive than standard agglutination test and can be carried out by a non-specialised personnel.

Russell et al (1973) noted the value of the card test in humans appears to be low.

Andriambololona (1973) suggested that HBT is useful and simple technique giving immediate result. It can be indicated for large scale epidemiological investigation.

George and Carmichael (1973) reported that RBT is a useful screening procedure for the detection of canine Brucellosis.

C-REACTIVE PROTEIN DETERMINATION

C-reactive protein determination has also been employed in the Brucellosis laboratory at the University of Minnesota (Stollerman et al. 1953). This test is based upon the presence of an abnormal protein found in the serum of patients with inflammatory conditions. Precipitate is formed when such serum is added to C-reactive protein rabbit antiserum. These preliminary studies indicate
that the test is positive in the bacterimic patients with Brucellosis and it may very well provide further aid in detecting the presence of active disease (Spink, 1956).

Bezborodko (1963) demonstrated C-reactive protein in latent rheumatism.

Elliott and Horner (1964) noted false positive C-reactive proteins due to serum lipoproteins.

Vedros and Fischel (1964) showed the presence of C-reactive protein in fractionated normal human sera.

Starzyk and Przesmycki (1964) observed C-reactive protein in the sera of patients in the acute phase of primary syphilis.
ISOLATION OF BRUCELLA ORGANISMS

ANIMALS

Cow

Schroeder and Cotton (1911) first demonstrated that the Brucella organisms are excreted in the milk from the udder.

Evans (1918) isolated Brucella abortus from 45 fresh cows' milk samples.

Sheather (1923) observed that 34 percent of cows giving a positive blood serum agglutination reaction were yielding infected milk. The organisms may appear in the milk within 1 or 2 days of abortion and are usually demonstrable within a week, though in some animals as long as 5 months may elapse before excretion commences (Wall, 1930).

Karsten (1932) found that the proportion of aborting cows in which the milk was subsequently shown to be infected varied on different farms from 24 to 70 percent, and that in infected herds 19-33 percent of cows going to full term excreted the organism in their milk. Beattie (1932) isolated 36 strains of Brucella abortus from milk, all were of bovine type.
Doyle and Beckett (1936) isolated *Brucella abortus* from the milk of two cows with negative blood reactors to the agglutination test. Lerche (1937) demonstrated *Brucella abortus* in milk.

Lisbonne (1939) isolated *Brucella melitensis* and *Brucella abortus* strains from four cows' milk. Fitch et al. (1939) showed the presence of *Brucella abortus* from the milk samples with a titre of 1:25 and in some samples showing no agglutination at this dilution.

Webb and Webb (1943) first recovered a strain of *Brucella abortus* from a foetus aborted by a cow in Mauritius and hinted at the possible occurrence of undulant fever in man.

Aum et al. (1950) brought into evidence *Brucella abortus* infection by the results of agglutination tests and isolated *Brucella abortus* from the aborted foetus in Singapore.

Huddleston and White (1954) isolated *Brucella abortus* type II (Wilson) for the first time from the milk of infected dairy cows in Michigan.
Parnas and Chodkowski (1956) carried out a survey of human and animal Brucellosis and isolated 160 strains of Brucella.

Nguyen-Van-Ai and Nguyen-Ngoc-Dai (1957) was able to isolate *Brucella abortus* from the amniotic fluid of the aborted foetus of a cow. Korotich (1957) stated that out of 236 strains isolated from cattle, 219 were *Brucella abortus*, 21 *Brucella melitensis* and 42 *Brucella suis*.

Burki (1959) showed Brucella in 112 of 1,139 placenta from cattle by Koster stain. Culture in "W" medium revealed the organism in a further 24 placenta, 23 of which (yielding 22 strains of *Brucella abortus* and *Brucella intermedia*) were virulent to guinea pigs.

Bouvier (1960) isolated Brucella from 125 of 140 bovine placentas in which Brucella was seen microscopically and from 4 of 250 microscopically negative placentas.

Gulasekharen and Cockburn (1961) isolated *Brucella abortus* strain from a milk sample in Ceylon.
Sheep and goats

Horrocks (1905) demonstrated the frequent presence of *Brucella melitensis* in the milk and urine of apparently healthy goats.

Zammit (1906) was able to isolate *Brucella melitensis* from every milk giving a strong reaction.

Nilakantan and Pandé (1948) isolated *Brucella abortus* from goat milk in Punjab.

Stoenner (1951) stated that he was able to isolate a strain of *Brucella abortus* from a batch which contained milk from 10 ewes.

Levi *et al.* (1952) reported to have isolated a strain of Brucella from an outbreak of abortion among cows and goats on one farm in Israel.

Renoux and Suire (1963) proved that the acute Brucellosis can produce infertility in goats and about 1/3 of the new-born kids may act as carriers.

Singh (1965) isolated 4 strains of *Brucella abortus* out of 431 milk samples, and many were positive to ABR test.
Panjarathinam and Gulrajani (1974) isolated four strains of *Brucella abortus* biotype 1 from milk of cows.

Sabbaghian and Nadim (1974) reported that *Brucella melitensis* is highly prevalent in Isafahan province in man and animals.

Essurutso (1974) isolated *Brucella abortus* from aborted cow in Nigeria.

Blankenship and Sandford (1975) reported *Brucella canis* as a cause of undulant fever.

Sabbaghian (1975) recorded that 7 percent white cheese specimens were found infected with *Brucella melitensis* biotype 1.

Verger et al (1975) isolated *Brucella canis* from a bitch at Madagascar it was similar to *Brucella suis* biotype 5.

Young and Suwannoparrot (1975) isolated *Brucella melitensis* in six cases due to ingestion of raw goat cheese.

Flores Castro and Segura (1976) isolated *Brucella canis* from the blood of 3 dogs.

Oomen (1976) isolated *Brucella melitensis* from patients suffering from Brucellosis in Kenya.
ISOLATION OF BRUCELLA FROM HUMAN ABORTED CASE

The pathogenicity of certain species of *Br. abortus* for man has been demonstrated by the comparatively large number of cases of undulant fever reported in the medical literature. Because abortion is the chief manifestation of the disease in those species of domestic animals that are most susceptible to this infection, it has been suspected for a long time that *Brucella abortus* may be responsible for certain cases of abortion in women.

In the human family, *Br. abortus* has been isolated from the blood, urine, stool, joints, tonsils, ovaries and once each from an oviduct and from an epididymis.

The cause of a large percentage of abortion in women living in rural districts where abortion in cattle is prevalent has not been determined, although reports of miscarriages among farmers' wives at a time when infectious abortion existed in the herd were often obtained (Carpenter and Boak, 1931).

De Forest (1917) reported eleven abortions in women the history of which suggested that the source of infection causing the interruption of
the pregnancy came from cattle that were infected with *Br. abortus*. In most of the cases, these women had lived on farms at a time where the cattle were suffering from infectious abortion or had spent their vacation on farm where the disease existed. A bacteriological study of human foetuses and placentas from women who had aborted was begun in 1926 by Carpenter and Boak; only specimens from cases of spontaneous abortion and those whose Wassermann test was negative were examined.

Since the isolation of *Br. abortus* from a human foetus in 1927, other reports of the infection in the genital tract of women have been recorded in the literature.

Eyre (1912) has reported amenorrhea as well as abortion to occur in patients suffering from Malta fever. Amoss and Poston (1929) reported the isolation of a number of the genus *Brucella* from the right ovary and tube surgically removed from a patient who was convalescing from undulant fever of long duration.
AGGLUTINATION TEST IN ABORTED CASES

Cornell and De Young (1929) failed to find any serum from 1,015 pregnant women in Chicago that showed definite agglutination. Only one of the 23 sera from aborting women was positive to the agglutination test in a dilution of 1:30.

Simpson and Frrazier (1929) observed several women with negative Wassermann reactions who had aborted repeatedly and gave histories of undiagnosed fevers. Their titres ranged from 1:80 to 1:320 with an abortus antigen.

Poole et al (1972) reported a case of abortion due to Br. abortus biotype 2.

Randhawa et al (1974) observed 2 cases, out of 43 aborted women, to be serologically positive for Brucellosis in India, and did not attempt to isolate Brucella organisms.

Porreco and Haverkamp (1974) discussed the controversy over whether the Brucellae play any unique role in human abortion, with special attention to the significance of erythritol in determining the susceptibility to the acute
placentitis and reproductive failure in some domestic animals.

Sarram et al (1974) reported that, in the endemic area of Isfahan, 6 out of 51 women with second trimester showed clinical signs and laboratory findings of Brucellosis. Placental and foetal material obtained from 6 patients was cultured for Brucella. In 5 cases, positive cultures were obtained. The relationship between maternal Brucellosis and second trimester abortion was discussed.

WORK DONE IN INDIA

ANIMALS

Contagious abortion in cattle was reported from some of the military dairies during the year 1917 and 1918, but at that time attempts to isolate the causal organism of the disease did not meet with success (Rep. I.V.R.I. year ending 31st March, 1917 and 1918).

Agglutination tests carried out during 1925 on the blood serum of animals at some of the Govt. military dairies revealed that the incidence of the disease was very heavy and about 50 percent of the cattle was infected and that about 20 percent aborted, usually repeatedly. Similar tests carried out on the "Grantee" farms in Punjab, however, showed that under natural conditions of livestock raising in India, about 10 percent cattle were infected and the incidence of clinical cases resulting in abortion was rare. Efforts to vaccinate the animals at the military farms with massive doses of living cultures, whether of low or high virulence, were rather discouraging (Rep. I.V.R.I. year ending 31st March, 1925). Edwards (1926) made this important
disease the subject of his special study, and investigated into different types of causal organisms isolated from numerous herds in India.

Strains of causal organisms of bovine abortion isolated from various herds in India, on serological test (cross-agglutination and agglutination absorption) were found to vary in type, and strains corresponding to each of types differentiated by Meyer among Brucella abortus-malitensia strains were discovered. Consequently, the vaccine issued from the laboratory for the controls of the disease was made by incorporating strains of all types, which had lost their virulence by repeated transplantations on artificial media (Rep. I.V.R.I. year ending 31st March, 1927).

Vaccination was recommended for the control of the disease on farms. Experimental evidence was put forth indicating that at one farm vaccination might possibly have been responsible for increasing the fertility of cows (loc. cit., year ending 31st March, 1930).

Work was continued by Naik (1931) as in previous years consisting of the carrying out of agglutination tests and the collection of statistics.
In addition, an attempt was made to study the relationship between sterility in cows and organisms of the Brucella group. He isolated an organism almost identical with *Bacillus viscosus equi* from uterine washing of sterile cow (Ann. Rep. 1931).

Nair showed that the blood serum of a bull infected with *Brucella abortus* (European Strain) gave a positive agglutination reaction for a much longer period than with strains of Indian origin. Also he recorded in 1932 the abortion in goats in Missar was due to *Brucella melitensis* (I.V.R.I. Rep. 1931).

Haddow continued the work and found out that the incidence of actual abortions in known infected herds has been comparatively low during the last four years (Ann. Rep. Vet. Res. Inst. Mukteswar 1932-33).

In 1939, the Indian Council of Agricultural Research implemented a scheme for the investigation of bovine abortion and the survey work was carried out in some States of India. The report indicated that the incidence in Uttar Pradesh and Punjab was 1 percent in indigenous cattle and 2.5 to 5 percent in European and cross bred cattle.
A survey of Brucellosis was conducted by Viswanathan (1943) in ten districts of Madras State and the wide prevalence of this disease was encountered mostly in the hilly areas and jungles. Three strains of Brucella were isolated by him.

Folding (1943) observed that Brucellosis was by far the most common cause of contagious abortion in India. Of the 46 strains isolated by him, 22 were typed as pure *Brucella abortus*, 13 as intermediate between *Brucella abortus* and *Brucella melitensis*, and three as *Brucella melitensis*. The remaining three were not typed. All village strains were typed as *Brucella abortus-melitensis*, thereby suggesting that the indigenous Indian Brucella strain is of this kind. Of the three *Brucella melitensis* strains typed, one was from a human case, one from a goat and the third from a cow. According to this author, European cross-bred cows were the most susceptible to abortion, country bred cows and buffaloes being much less so. The disease was found to be prevalent in all organised farms, the incidence being the highest and loss of calves about 3 percent in farms where European blood was present. In village cattle, Brucellosis was probably common in Bengal and Assam, it was certainly
common in Orissa, eastern and central part of C.P. (now Madhya Pradesh) and in the South-Eastern parts of Hyderabad. Surveys had not been made of villages north of the line from Bihar to Bombay. Among conditions responsible for the spread of the disease in India were the magnitude of existing reservoirs, the prevailing conditions of animal husbandry, the climate and the phasic susceptibility of animals.

Bolding (1947 a) analysed the results of surveys of Brucellosis and reported that amongst the farms the spread of infection was uniform but in village cattle, it occurred throughout the rainfall areas only. The incidence of infection in farms was high where the rainfall is very high. In villages, the infection was negligible in some desert areas, but quite high in humid coastal areas. He concluded that the humidity, rainfall and sunlessness were conducive for the spread and the continuous sunlight checked the dissemination of infection. According to him, unhygienic conditions and congestion of animals were important in the spread of infection.

Bolding (1947 b) examined three more strains of Brucella besides the 46 strains reported by him earlier and came to a conclusion that the strains from indigenous village cattle were often intermediate type.
Mohan (1947) examined 739 animals in 13 breeds in Bengal and found out that the infection was established in eight of them, and also one of the herds showed over 30 percent reaction. The incidence of abortions was higher in commercial and other farms in which new animals were added, but it was low in many small self-contained herds.

Folding (1948) dealt under the Indian Council of Agricultural Research Scheme (1939, 1941) with the various aspects of the disease, e.g. epidemiology, bacteriology, diagnosis, control, human infection etc. Some of the additive features of the disease were that (I) the principal reservoirs were in congested cities stables in Calcutta, Bombay etc. (II) in South Indian villages the buffalo farms under military control were additional sources and (III) field trials of vaccination could not give any conclusion as regards its utility in the control of the disease. In private farms where "Strain 19 vaccine" was used, a definite decline in abortion was observed, but in military farms the formation of a definite opinion was not possible.

Nilakantan and Pande (1943) investigated into Brucellosis among goats at Government Livestock Farm, Hisar
and isolated *Brucella abortus* by inoculation of milk sample into guinea pigs.

Dhanda and Rajagopalan (1949) suggested a programme for the control of Brucellosis in cattle in India in organised farms and in village herds.

It was reported that the susceptibility of Indian sheep to Brucellosis is very slight (Rep. I.V.R.I. 1949-50).

Forty serum samples from sheep were subjected to agglutination. Of these, 36 proved negative and the remaining four showed a positive reaction, the end titre being 1:40, in one case, 1:80 in two cases and 1:160 in the last. Six cases of abortion in sheep were reported locally, but on cultural and biological examinations all proved negative to Brucellosis (Ann. Rep., I.V.R.I. 1950-51).

Positive milk samples preserved with 1 percent boric acid were transported to Mukteswar in hot weather and tested with ABE antigen. They gave positive reaction showing thereby that exposure to heat did not destroy the agglutination in milk (Rep. I.V.R.I. 1953-54).
Venkataraman (1953) in Coimbatore district of Madras State was able to report by agglutination test and clinical symptoms in cattle the high incidence of the disease mostly in hilly tracts. Mathur (1959) reported the human Brucellosis in Punjab and its importance to the veterinarians.

Raja et al. (1959) put forth the first confirmatory record of the incidence of Brucellosis in a privately managed farm in Trichur District in Kerala and confirmed the clinical diagnosis of a cow which aborted twice by serum agglutination test.

Ball and Bakshi (1960) reported that a fairly large number of animals at State Livestock Farm, Chakganjaria, Lucknow with a past history of abortion had failed to show sero agglutination titre against Brucella abortus antigen. Microscopical, cultural and serological examinations conducted at the time of abortion revealed that most of the abortions were due to Brucella infection.

Mukerjee (1960) reported that bull acts as spreader of contagious abortion and recorded the result of the tube agglutination test with sera from 70 stud bulls conducted at Bengal Veterinary College, Calcutta. Narayana et al. (1961) reported a case of Brucella orchitis in a bull.
Lall and Bakshi (1961) recorded the excretion of *Brucella abortus* in the milk of reaction animals and aborted animals.

Das *et al.* (1961) investigated into the incidence of *Brucella* reactors among goats and sheep in Orissa by using rapid plate and tube agglutination tests.

Army Remount and Veterinary Services (1962) found out in a survey the incidence of *Brucellosis* in Military farms by using single serological test; it revealed that adult cattle have high percentage of reactors than adult buffaloes and there are more reactors in the state of Punjab and Himachal Pradesh.

Mathur (1962) presented a data on the incidence of *Brucellosis* in the different States of India among human beings, cattle, sheep and goats. The need to study the incidence among pigs and in goats and sheep in certain areas had been pointed out.

Pargaoiker and Raj (1962) examined by the ABR test (Abortus Bang's Ringprobe test) pooled samples of milk from various localities of the cities of Hyderabad and Secunderabad (Andhra Pradesh) and also from few surrounding villages. The percentage of positive samples was 3.5. The test was found to be useful for conducting survey of *Brucellosis*.
Mathur (1963) proved the presence of Brucella organisms in the milk supply of Karnal town and the surrounding villages, and their pathogenicity for human beings and animals.

Mathur (1963) isolated 20 strains and 2 strains of *Brucella abortus* from cows and buffaloes respectively and they were reported to be lysed by phage Tbilisi (Tb.). One strain of *Brucella melitensis* was isolated from goats' milk and all the 12 human strains were of *Brucella melitensis*.

Krishnamurthy and Kaushik (1963) used a rapid whole blood agglutination test for survey of Brucellosis in rural areas.

Panda *et al* (1963) studied the effect of haemorrhagic septicaemia vaccination on Brucella agglutination test in cattle. It was observed that in several animals there was an increase in the serum titre against standardised *Brucella abortus* antigen. A sample of haemorrhagic septicaemia hyperimmune serum did not give any significant titre in the tube test.

Mathur (1964) isolated 42 strains of *Brucella* from cows, buffaloes, goats and human beings at
Karnal and concluded that the prevalence of the disease among goats reflected on its incidence among human beings of that area.

Misra and Panda (1964) investigated into the possibility of cross reactions occurring between Brucella abortus, Salmonella pullorum, Vibrio cholerae and Proteus OX 19. No significant cross reactions occurred in other organisms except Brucella abortus and Vibrio cholerae. He suggested that fowls could be excellent substitutes for rabbits in the production of high titre Brucella serum.

Bakshi and Narain (1964) carried out the comparative study on standard tube test and heat inactivation test in the detection of specific and non-specific Brucella agglutinins in the sera of cows and buffaloes and advised the regular heat inactivation test as a supplement to standard tube test in Brucellosis control programme.

Panda and Das (1965) carried out a survey of the incidence of bovine Brucellosis in the State of Orissa.

Pat and Panigrahi (1965) put forth a preliminary report on comparative study of ABR test and serum
agglutination test in cows and buffaloes. Correlation between ABR and serum agglutination tests were obtained in 71 percent of cows and 49 percent of buffaloes.

Misra and Panda (1965) found out that there was no observable cross reaction, if variant strains of Salmonella gallinarum pullorum were used with Brucella in the plate agglutination test.

Kulahreshta et al. (1973) reported the prevalence of Brucellosis among cattle and buffaloes in Haryana State.

Sen et al. (1975) made a serological study of Brucellosis among horses in India.

Dadhich (1978) carried out a survey of incidence of Brucellosis in 1378 lactating animals in Punjab by employing Milk Ring Test and tube agglutination test.

Stephen et al. (1976) conducted a serological survey of Brucellosis on 11 dogs, 92 sheep, 125 goats and 50 cattle in coastal Karnataka.
In the year 1897, Wright and Smith first diagnosed ten cases of Malta fever in human beings in India by sedimentation reaction of the serum with *Micrococcus melitensis* (syn. *Brucella melitensis*).

In Madras State, Viswanathan (1943) put into record six cases of Brucellosis. Mody (1946) diagnosed a case of Brucellosis in the name of 'Abortus fever' in Sholapur on the basis of agglutination reaction and clinical symptoms, but source of infection was not traced. Jhatakia (1946) recorded the undulant fever in Kathiawar (Saurashtra) by agglutination test.

Bhuyan and Berua (1947) recorded another case of infection in a 10½ year old girl due to *Brucella abortus*. Manchanda (1953) diagnosed and gave treatment for nine cases of Brucellosis in man.

Soman and Kothari (1954) in Bombay examined sera from human patients belonging to different professional group dealing with cattle, such as veterinarians, cattle attendants, butchers etc., and found that the disease was quite prevalent among people closely associated with cattle.
The prevalence of Brucellosis in this country had been reported by Mathur (1955, 1955a, 1959, 1963, 1964), in Punjab. Besides the record of Brucellosis in a family, he also isolated strains of *Brucella melitensis* at Karnal. In the year 1964, he emphasized the importance of the problem of Brucellosis in Punjab.

Meenakshi and Natarajan (1961) reported that a veterinarian contracted Brucellosis from his close contact with sick animals at Bodinayakanur (an endemic area of Brucellosis), Madras. The titre was 1:800 and it is the first report from Government Erskine Hospital Madurai, Madras State. Both *Brucella abortus* and *Brucella melitensis* were isolated from the individual.

Shukla (1962) tested 150 normal individuals and 25 cases of pyrexia of unknown etiology in Baroda and found out an 1/10 agglutinin titre in normal individuals. He isolated 4 strains of *Brucella melitensis*.

Jogarao (1965) reported the prevalence of human Brucellosis in Andhra Pradesh.
Rao et al (1964) described a first case of human Brucellosis from Thanjavur, Madras State. The subject was a 50 year old agricultural labourer showing clinical symptoms of Brucellosis, whose serum showed a higher titre 1:1600 to both Brucella melitensis and abortus antigens. Brucella melitensis was isolated from him. The disease was controlled by antibiotics treatment and his titre came down to 1:100.

Balbir Singh and Saxena (1964) screened 2,020 human sera samples from cases of pyrexia of unknown origin in Delhi and found agglutination against Brucella in 6 and from 696 patients suspected for undulant fever, 46 showed agglutination against Brucella. In seven out of twenty six cases Brucella melitensis was isolated by blood culture.

Dhamdhere et al (1964) recorded Brucellosis in a family.

Roy et al (1965) carried out the serological study of Brucellosis in man and cattle in Jamnagar, indicated the prevalence of Brucella abortus infection in Jamnagar area and suggested the measures to control the infection in human being.
Mathur (1965) gave a clear picture of Brucellosis in Punjab and other States in India.

Koshi and Meyers (1969) made a note on cases of human Brucellosis encountered in the Christian Medical College Hospital, Vellore since 1965.

Manjit and Madhusudhan Das (1970) recorded Brucellosis in and around Calcutta.


Kulshreshta et al (1971) studied the immuno-electrophoretic characterisation of Brucella antigen.

Phadke and Phadke (1974) reported the prevalence of Brucella agglutinins in sera received for serological test for syphilis and widal test.

Randhawa et al (1974) recorded some sero-epidemiological observations on Brucellosis in humans and animals.

Stephen et al (1978) conducted a serological survey of Brucellosis in coastal Karnataka by employing Brucella abortus plain antigen in standard tube agglutination test. 406 humans were studied, and the prevalence of Brucella agglutinin was 11.33 percent. Attempts to isolate the organisms was unsuccessful presumably because of the antibiotic treatment given to the patients.