A STUDY ON THE INCIDENCE OF BRUCELLOSIS IN BUFFALOES IN TRICHUR

By

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THESIS

Submitted in partial fulfilment

of the requirement for the degree

MASTER OF VETERINARY SCIENCE

Faculty of Veterinary and Animal Sciences

Kerala Agricultural University

Department of Preventive Medicine

COLLEGE OF VETERINARY & ANIMAL SCIENCES

MANNUTHY — TRICHUR

1978

DECLARATION

I hereby declare that this thesis entitled "A STUDY ON THE INCIDENCE OF BRUCELLOSIS IN BUFFALOES IN TRICHUR" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associato- ship, followship or other similar title of any other University or Society.

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29-7-1978.

CERTIFICATE

Certified that this thesis, entitled "A STUDY ON THE
INCIDENCE OF BRUCELLOSIS IN BUFFALOES IN TRICHUR" is a
record of research work done independently by Smt. K. Baby
under my guidance and supervision and that it has not
previously formed the basis for the award of any degree,
fellowship or associateship to her.

Dr. E.P. Pally

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and Artificial Insemination Centres at Mannuthy and Trichur.

The staff members of the Department of Preventive Medicine for their technical help.

Sri. P.X. Francis, for typing the manuscript.

C O N T E N T S

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INTRODUCTION

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The buffaloes (Bubalus bubalis) are found in wild, semi-wild and domesticated conditions. Domesticated buffaloes are valued for their milk, work and meat.

In India, for many years to come, cattle and buffaloes are likely to continue as the mainstay of agricultural operations, particularly for the small and marginal farmers. Cattle and buffalo raising offers very significant employment and augmented income to the farmers.

During the last quarter of a century, the buffalo population has grown over 300 percent. In 1974, the buffalo population in the world was estimated to be nearly 140 millions. India possesses 57.941 million buffaloes, which constitute nearly 50 per cent of the world population of buffaloes, out of which 4.72 lakhs are in Kerala. (Report of the National Commission on Agriculture, India, 1976, Part VII).

The best milk breeds of buffaloes are located in India. The bulk of milk produced in this country comes from buffaloes. The buffalo milk, with its high fat content, has a good market. The average annual milk production per buffalo in India is estimated at 504 Kilograms as against 157 Kilograms in cows. Indian buffaloes produce 11.24 million tonnes of milk which is equivalent to 54.3 per cent of the total milk production of our country (Report of the National Commission on

Agriculture, India, 1976, Part VII).

It is evident from these facts that buffaloes play an important role in the economy of the country. Various animal husbandry activities aim to evolve high producing healthy livestock population. Freedom from diseases is the most important factor to achieve this goal. Though buffaloes are good dairy animals, they are seasonal breeders having a usually longer calving interval. Hence diseases of the reproductive organs affect their utility more adversely than that of the cows. Among the diseases affecting the genital organs of dairy animals brucellosis occupies an important place.

The disease, brucellosis, in animals and man is caused by members of the genus Brucella. Though extensive knowledge with regard to etiology, epidemiology and preventive measures is available, brucellosis still continues to be a major problem. In cattle, abortion or premature expulsion of the foetus is the most important feature of the disease. The organism is intracellular and forms typical granulomata. These two factors may be responsible for relapses after antibiotic therapy and also for a tendency to assume a chronic form with complications including arthritis, orchitis and involvement of other organs of the body.

As a zoonose, the infection is transmitted to man

through milk, milk products, meat or by contact with infected materials.

Abortion, premature death, decrease in milk yield, sterility and infective nature of the disease cause great economic loss to the farmer and to the nation as a whole. Mathur and Sharma (1974) conducted a study to estimate the economic loss to India due to brucellosis among cattle and buffaloes. According to them the annual economic loss to the country due to reduction in milk yield and loss of calves was estimated to be approximately Rs. 311.47 million. The actual losses would be many times more, if the loss of fertility, prolonged calving intervals among the affected animals, loss of bullock power in the case of work animals, cost of replacement of affected dairy stock and losses suffered by sheep, goats and swine are also taken into account.

The disease is not transmitted, generally, from man to man, directly or indirectly and as such control of brucellosis in animals will result in the prevention of the disease in human beings.

An exact estimate of the overall incidence of brucellosis in buffaloes in this country is not available. A number of local surveys has been made in different parts of the country. Most of these surveys have been carried out in white cattle, sheep and goats.

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In Kerala, the amount of work carried out on the incidence of brucellosis is very scanty and no systematic surveys have been done in buffaloes till date.

Hence it is considered desirable to undertake a study on the incidence of brucellosis in buffaloes. Eradication schemes can be organised on an area basis only if relevant information on the incidence of brucellosis is available.

REVIEW OF LITERATURE

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History

The first accurate description of brucellosis was made by Marston (1863). *Micrococcus melitensis*, the first member of the group, was isolated by Bruce in 1887 from the spleen of patients who had died of malta fever. Bang in 1897 described the second member of the group, *Bacillus abortus*, the causative agent of brucellosis, a disease now known as Bang's disease or infectious abortion of cattle. *Brucella abortus* was later demonstrated in the milk of infected cows (Schroeder and Cotton, 1911).

Micrococcus melitensis of Bruce 1887 and *Bacillus abortus* of Bang 1897 were found to be closely resembling microscopically, serologically and biochemically (Evans,1918). Meyer and Shaw (1920) grouped all the organisms under the genus Brucella in honour of David Bruce. Since the isolation of Brucella organisms the disease has been reported from almost all parts of the world.

Incidence of Brucellosis

Surveys conducted by Polding (1943, 1947 and 1948) revealed that brucellosis occurred as low grade epizootics in many farms and several village-herds in India, producing 1.5 per cent abortion in an year. Numerous workers have

reported the prevalence of brucellosis among the livestock
population, in different states of India (Mathur, 1962).
It was specially pointed out that the incidence in the state
of Orissa was the highest, where, in certain parts, it was as
high as 50 per cent. Raja et al. (1959) recorded brucellosis
in two cows out of seven examined in Trichur Taluk of Kerala
State.

Many workers have recorded the prevalence of the
disease in buffaloes. Panda et al. (1952) examined 30 samples
of buffalo milk and found that seven (23.33%) were positive to
brucellosis. Gentile (1957) demonstrated Brucella abortus
bacteriologically and biologically, in materials collected
from aborted foetuses, foetal membranes and blood samples
collected from buffaloes in which abortion occurred at sixth
to eighth month of pregnancy.

Corner and Connel (1958) reported the incidence of
brucellosis in bison (Bos bison) in Elk Island, National Park,
Alberta, Canada, and they observed 111 samples positive (32.3%)
out of 343 examined.

Studies conducted by Lall and Bakshi (1960) on a herd
of Sahiwal cows and Murrah buffaloes maintained at Chak
Gangeria, for three consecutive years from 1956-1958, revealed
the incidence of brucellosis as 1.1, 5.3 and 4 per cent respect-
ively. Kamel and Fattah (1961) were able to detect seven per cen

positive cases of brucellosis out of 227 samples of buffalo serum examined. Rose et al. (1961) observed positive brucella agglutinin titres in 27 out of 66 buffaloes in Brazil.

While testing the sera samples of East African game animals for brucellosis, Rollinson (1962) discovered 11 reactors (73.33%) out of 15 buffaloes examined. Out of 439 samples of serum from buffaloes examined by Hamada et al. (1963), 0.46 per cent was found positive.

Bhambani and Krishna Murty (1964) were able to isolate Brucella strains from an infected herd of buffaloes maintained at the State Livestock Farm, Madhurikund, Uttar Pradesh. Mohanlingam et al.(1965) detected 6.4 per cent incidence of brucellosis among buffaloes in Madras out of 110 bulk milk samples collected from 1650 animals. Thorpe et al. (1965) were able to detect Brucella abortus agglutinins in the serum sample of bison among the wild life and livestock of West Central Utah (U.S.A.).

The incidence of brucellosis in buffaloes was reported by Mathur (1962, 1966, 1968 and 1969); Pat and Panigrahi (1965); Thanappa Pillai (1966); Shivadekar (1967); Soni (1967); Tbrosov (1967); Kapur and Singh (1967); Sadykhov (1968); Staak et al. (1968); Chatterjee and Ganguly (1968) and Panda (1969).

Kataria and Verma (1969) recorded 3.36 per cent as the

total incidence of brucellosis in buffaloes in Madhya Pradesh. The prevalence of brucellosis in buffaloes was observed by Tanwani et al. (1971); Sinha and Pathak (1971, 1973, 1975); Sreemannarayana (1972) and Buth and Manchanda (1972). Sreenivasan (1972) recorded the incidence of brucellosis among cattle and buffaloes in Tamil Nadu to be 2.7 per cent. Dashdamirov (1973) gave an account of brucellosis in buffaloes.

Mamatelashvili (1973) in his review mentioned high percentage (11-54%) of cases of brucellosis in buffaloes during the period 1941-1965.

It was mentioned in the final report of 1976 (unpublished) on the "Project for the Investigation of Microbial Aetiology of Infectious Abortions in livestock in Kerala", that four reactor buffaloes, with low titres below the positive level, were detected out of nine abattoir samples. Besides this, no attempt was seen to have been made to investigate brucellosis in buffaloes in Kerala.

Kulshreshtha et al. (1973a and 1978) reported an incidence of 4.4 per cent in buffaloes in Haryana State. They opined that there seems to be an increasing trend in the prevalence of brucellosis in cattle and buffaloes in that state.

Mathur and Sharma (1974) reported the overall incidence of brucellosis in cattle and buffaloes in India to be 5.21 per cent in organised herds and 6.55 per cent in village stock.

The state-wise incidence of brucellosis as compiled by them
is shown in Tables 1 and 2.

Diagnostic methods

In affected animals the excretion of Brucella through
milk is intermittent and with vaginal discharges occurs only
at a particular time. So a combination of more than one
method is essential for the diagnosis on which control pro-
cedures can be based. Cultural, biological and serological
tests are carried out for this purpose. Generally, some of
the indirect methods are used for the detection of carriers.

Milk, whey, blood, serum and vaginal mucous are the
materials used for conducting laboratory tests. The aggluti-
nation test and the milk ring test are most widely used in
cattle.

Complement fixation test (CFT).

Larson (1912) introduced the CFT for brucellosis. In
India, it is not used commonly. In Sweden and Denmark it is
used as an accessory method for routine examination. Van Der
Schaaf and Jaartsweld (1962) observed that CFT is superior to
the agglutination test as a method of differentiating between
infection with a more virulent strain and a less virulent
strain. Mylrea (1972) stated that the CFT was the most satis-
factory test for identifying infected cows.

Newton et al. (1974) were of the opinion that CFT is an accurate and sensitive means of diagnosis and helped to differentiate residual antibody reactions following vaccination from reactions associated with infection. The test is also valuable in detecting chronically infected herds.

According to Morgan and Richards (1974) most countries employ CFT for use in cases of sera which give inconclusive reactions to the standard tube serum agglutination test. The CFT is approved for use in the diagnostic laboratories engaged in the eradication of brucellosis. It is widely used in the United Kingdom and Australia.

Blocking test.

This test, described by Griffitts (1947), is used when blocking antibodies are present in the serum.

Coomb's test (Antiglobulin test).

Coomb et al. (1945) described this test which brings about agglutination of cellular antigens that have attached to them non-agglutinating antibodies from a previous negative agglutination test. Even in animals nearing parturition, this test had given consistent results. Lewkowicz (1974) and Cunningham (1977) stated that due to its sensitivity, this test is most useful for comparison of total specific immuno-globulins.

Plate or rapid agglutination test (RPT).

Huddleson (1920, 1932 and 1943) discovered this test.
It was widely used for routine examination of cattle in North
and South America giving satisfactory results. Serum, whey
or whole blood can be used for this test. The test can be
standardised with the international serum.

Surface fixation test (SFT).

Castaneda (1950, 1953 and 1954) described a surface
fixation method to test serum on filter paper. Caldas and
Machado (1961) found that whole milk gave false positive
results, but skim milk gave results which agreed with the
results of tube agglutination test. Mathew et al. (1966)
made a modification of this test by using coloured antigen
and positive serum on Whatman filter paper No. I and claimed
that this modified test called MTM test is specific for
brucellosis.

Rapid cup agglutination test.

Jameson (1957) described this test. Concentrated
antigen and serum dilutions are mixed in Perspex plate wells
and incubated at 37°C for 90 minutes. The results are read
by oblique transmitted light against a black background. This
test was found to be very reliable.

Vaginal mucous test.

The vaginal mucous test was described by Kerr (1955).
Marr and Williams (1958) found that a high proportion of the
cows which were positive for vaginal mucous test and milk
ring test were excreting Brucellae in their milk. Boyd and
Reed (1960) found that this test was less affected by extrinsic
factors. A positive reaction in this test is indicative of
virulent field infection. This test would not give a positive
reaction in animals vaccinated with strain 19 vaccine.

Rose Bengal Plate Test (RBPT).

This is a modification of the acid plate test intro-
duced by Rose and Roepke (1957) who noted that the antibody
activity of non-specific agglutinins is destroyed at a low
pH (3.6). The specificity of the specific agglutinins remain-
ed unaffected. The antigen used in RBPT consists of <u>Brucella</u>
<u>abortus</u> strain 19 cells stained with the dye Rose Bengal and
suspended in phenol saline buffered at pH 3.65. This test is
now routinely used for screening bovine sera in Australia and
England. The efficiency of this test was further emphasised
by various workers (Mylrea, 1972; Hunter and Allen, 1972;
Scheibner and Leuchta, 1977; and Kulshreshtha <u>et al</u>., 1978).

Card test

The test satisfies the need for a rapid, sensitive and

accurate one for screening any herd especially in range areas
(Nicoletti, 1967). In this test plasma is quickly produced
through the use of lectins (phyto-haemagglutinins) and an anti-
coagulant by the aid of a microblood plasma separator. The
test uses only one dilution of antigen, plasma or serum and is
read as negative or positive.

Heat Inactivation Test (HIT).

Amerault et al. (1961) developed HIT for differentiat-
ing specific and non-specific agglutination in bovine brucello-
sis. The primary value of this test will be to supplement the
standard tube serum agglutination test and RPT by classifying
the brucellosis status in problem herds. The percentage of
reactors was lesser with HIT when compared to that with STAT
(Kulshreshtha et al., 1973).

Mercaptoethanol test

Rossi and Cantini reported in 1969 that the serum when
treated with mercaptoethanol before conducting a normal STAT,
the non-specific blocking substances in the serum may be
destroyed. In general, this test gives negative results in
vaccinated non-infected herds.

Gel diffusion test

Bruce and Jones, as early as 1958, described this test
in which cultures of Brucella melitensis (but not of Br. abortus

or Br. suis) yielded a diffusible antigen which produced 1 to 3 lines with sera of rabbits, goats or cattle infected with Br. abortus or Br. melitensis. Sinha and Pathak (1971) found that specific precipitin lines developed with heat stable, water soluble antigen of Brucella cells, when the whey of infected cows and buffaloes was used for gel diffusion.

Indirect Haemagglutination Test (IHA).

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

Erythrocytes coupled to either lipopolysaccharide (LPS) or intracellular antigens (IC) of Br. abortus were used by Corbel and Day (1973). IHA test using LPS antigen sensitized erythrocytes, correlated well with STAT and to certain extent with RBPT. The test was found very useful to detect antibodies in the earliest stages of infection, where the sera might fail to react in the standard test. Taran et al. (1977) have also obtained favourable results regarding the sensitivity and speci- ficity with this test in cattle and swine.

Antibody neutralisation test

Chernysheva and Aslangan (1975) found this test suitable to detect antigen in water, milk or tissues. Normally inactivated rabbit serum, brucellosis immune serum, suspension of sheep erythrocytes sensitized with specific lipopolysaccharide antigen and suspected samples were used for the test. Sadykhov (1977) reported this test was specific and highly sensitive and could be used for testing aborted foetuses and placenta.

It may be mentioned that Obdokov et al. (1977) observed that by treatment with cystine hydrochloride, the IgM fraction in the sera could be inactivated. According to the above workers predominance of IgM characterised the acute phase of the disease, whereas IgG predominance indicated a chronic phase. So agglutination reaction used on samples before and after treatment with cystine hydrochloride could be give an indication of the duration of infection in a herd. The same test would also help to differentiate the immune response in vaccinated animals and in those with latent infection.

The IgM fraction could be selectively removed by rivanol (ethacridine) precipitation as shown by Erdem and Unel (1969).

The inactivation of the IgM fraction by various methods tends to minimise the number of false negative classification

of animals, especially in countries where no vaccination programme is practised (Joint FAO/WHO Expert Committee on Brucellosis, 1971).

Allergic tests

McFadyean and Stockman (1912) studied this test as a means of detecting Brucella infection in cattle. It was found not very reliable in cattle.

Fluorescent antibody method

According to the recommendation of the Joint FAO/WHO Expert panel on Brucellosis fifth report (1971) the fluorescent antibody method provides an accurate means for differential diagnosis. If the laboratory is well equipped and have well trained personnel, this method is recommended for routine diagnosis along with bacteriological examination.

Standard Tube serum agglutination test (STAT, SAT).

Wright and Smith discovered this test in 1897 and Grinsted (1909) was the first to use this test in cattle. According to the Joint FAO/WHO Expert Committee on Brucellosis (1971) this test remains the most commonly used diagnostic tool for bovine brucellosis, though certain limitations were recognised. In all species a positive result is of value.

Huddleson and Smith (1931) followed up for a period of

eight years, the cases of 247 animals which reacted to the test at a titre of 1:25 and above. They found that except four animals, which were permanently negative, all the others remained reactors.

Lamarlier (1938) observed ascending serum titres in animals which later aborted. During the second fortnight after abortion, the highest titre values were observed. Then there was a gradual reduction in the values, which, sometimes disappeared completely and the agglutinin titre again increased when the animals were rebred.

Seminal plasma can be subjected to the STAT. As infected bulls often have high titres in seminal plasma than in serum, the test is of particular value in the surveillance of animals in the artificial insemination centres (Joint FAO/WHO Export panel on Brucellosis fifth report, 1971).

As in the case of many other serological tests for infectious diseases, non-specific reactions were observed in the case of brucellosis which limit the accuracy of the test. Hess and Roepke (1951) demonstrated specific and non-specific agglutinins in bovine serum. The specific agglutinins are relatively heat stable. The non-specific agglutinins are heat labile and inactivated at 70°C.

Kiggins et al. (1955) observed cross agglutination between Vibrio foetus and Br. abortus at low titres, though

it does not interfere with the agglutination test for brucellosis.

Berman (1956) reported that injection of Pasteurella bacterin into cattle, which were vaccinated with strain 19 Br. abortus, in their calf hood, might give rise to anamnestic reaction. Panda et al. (1963) studied the effects of vaccinating cattle with haemorrhagic septicaemia vaccine on Brucella agglutination test in local cattle and observed that in several animals there was an increase in the serum titre against Br. abortus antigen. This response started by about eight days and reached its peak by 15 days. In some cases this level remained stationary for 22 days. The titre declined to the original level by about 123 days. The response due to haemorrhagic septicaemia vaccination increased by one step only. The authors attributed this increase to non-specific stimulus of anamnestic type.

Jaartsveld and Kuringen (1973) noted that following infection of cattle with Yersinia enterocolitica serotype 9, false positive results might be obtained for STAT, CFT, Milk ring test (MRT) etc.

Bakshi and Narain (1964) undertook a study to compare HIT and STAT for differentiation of specific and non-specific agglutinins in the sera of Indian cows and buffaloes, which were showing STAT titres of 1:10 and above. They concluded

that HIT may be conducted as a supplement to STAT, while
Sinha and Pathak (1975) observed high correlation between
STAT and HIT.

Kulshreshtha and Ramachandran (1970) conducted a
study of the correlation of agglutinins, haemagglutinins
and complement fixing antibodies in bovine brucellosis.
They observed that agglutinins appeared much earlier in
vaccinated cattle. The haemagglutinins and complement fix-
ing antibodies disappeared much earlier in them. All the
three types of antibodies persisted longer in infected ani-
mals. According to Hunter and Allen (1972) STAT gave the
best correlation with MRT (93.7%).

Lowkowicz (1973) foundtthat, during pregnancy, the
highest titres were observed in the third month and the lowest
in the fifth month. Nearing parturition the results of the
CFT and STAT were unreliable in some cases.

However, the STAT had been universally adopted and
has been widely employed in the routine detection of infected
cows and as a means of diagnosis, it was found highly satis-
factory for the purpose of separating the infected from the
non-infected animals under a system of control. But there
are circumstances which restrict the use of this test. In
recently infected herds the disease may be spreading rapidly,
the bacilli might multiply in the uterus and even cause

abortions before agglutinins appeared in the serum. In such cases the test should be conducted as often as once a month (Udall, 1972).

The relative value of this diagnostic test for surveying the incidence of brucellosis in bovines was confirmed by the reports of the Joint FAO/WHO Expert Panel on Brucellosis (1951, 1953, 1958, 1964 and 1971). Though Kulshreshtha et al. (1973b) suggested the simultaneous use of CFT to overcome the limitations of agglutination tests, Ergote et al. (1976) considered that STAT was the most sensitive test when compared to CFT and Coomb's test.

Many of the earlier workers recognised that the titre obtained with a given serum was influenced by the test methods. In 1933, in Great Britain, a dried reference-serum was prepared for the standardisation of methods in different laboratories (Stableforth, 1936) and this was adopted by the Office Internationale des Epizooties (OIE) in 1937 following recognition of the wide divergencies in the methods used and in the real significance of the diagnostic criteria of different countries (Stableforth, 1959).

The Joint FAO/WHO Expert Panel on Brucellosis (1951) recommended that international uniformity should be secured and suggested that sensitivity of the test performed should be indicated by expressing 50 per cent agglutination titre with the OIE standard dried serum containing the test antigen and

the method. The test results of STAT should always be stated as international units.

An international standard anti-Brucella abortus serum has been established by the FAO/WHO Expert Committee on Biological Standardisation in 1968. The international serum contains 1000 units per ml of reconstituted serum.

Castaneda (1961) recommended the following formula for converting the 50 per cent agglutination titre to the international units.

Titre of test serum with local antigen x 1000 = International
Titre of standard serum with local antigen   units per ml of test serum.

In India, 50 per cent agglutination at 1:40 is taken as equivalent to 80 international unit (I.U) per ml. The antigen prepared at Indian Veterinary Research Institute (IVRI) shows 50 per cent agglutination at 1/500 final serum dilution with Indian standard anti-Brucella abortus serum having 990 I.U. So to express the results in unit system, double of the serum titre showing 50 per cent agglutination will be the total number of I.U. per ml of serum.

Milk Ring Test (MRT)

Cooledge (1916)was of the opinion that the presence or absence of agglutinins in milk was indicative of the presence or absence of infection in the udder. Gilman (1931)

concluded from his studies on large number of infected
cattle that there was some relationship between the agglu-
tination titre of the whey and the presence of Brucella in
the milk. Doyle (1936) reported isolation of Br. abortus
from the milk of non-reactor animals.

MRT is the same abortus ring test introduced by
Fleishauer (1937) to detect agglutinins in milk. The test
was further studied by Bruhn (1948), de Moulin (1951) and
many others and had been established as a competent screen-
ing test.

Zaki (1948) examined 200 milk samples from reactor
Egyptian buffaloes (Synceres caffer) and found that 30 per
cent of them excreted the organisms in the milk. He isolated
Br. abortus from the milk of buffaloes with a blood titre of
1:20.

Although the nature of the ring test reaction was not
fully understood, it was presumed by Van Drimelon (1951) that
the rising cream layer acted as a filter or sieve, preventing
the clumped bacilli from sinking and carrying them up into the
cream layer. He found that the MRT remained positive in natu-
rally infected animals as long as Brucella organisms were
being shed, eventhough serum agglutination titre had declined.

Roepke et al. (1957) noticed marked variations between
quarter milk titres and also in the relative levels of aggluti-
nins in the blood. These variations suggested that specific

Brucella agglutinins in the milk didnot result from blood agglutinins, but from udder infection and also indicated the presence of a barrier which prevented the free movement of the agglutinins. Marr and Williams (1958) observed that a consistently positive MRT in herd milk samples reflected infection in that herd.

McDiarmid et al. (1958) concluded that eventhough the ring test (MRT) was very sensitive, it was of no great value in determining the state of immunity of cattle vaccinated with Strain 19 vaccine at calf-hood and subsequently exposed to infection.

Janney et al. (1958) tested 3615 herd samples of milk and concluded that milk ring testing every six months in conjunction with STAT on milk ring suspicious herds appeared to be a highly effective procedure for reducing the incidence of bovine brucellosis.

Kerr et al. (1959) stated that the udder may become the site of primary invasion and so testing the herd under an eradication programme should include the milk test and the serological tests.

Pargaonkar and Premraj (1962) found that the high fat percentage of buffalo milk did not interfere with the result of MRT. They observed that a distinct cream layer was helpful for the interpretation of the results.

TMulsiram and Sharma (1962) reported that there was no correlation between the results of STAT and MRT.

Panda and Mishra (1963) investigated the efficiency of individual MRT for the diagnosis of brucellosis in cows. The results showed that MRT could be employed as a routine test for the survey of brucellosis with as much accuracy as the serum test.

Pat and Panigrahi (1965) presumed that ABR (IRT) test might show positive reactions before seroagglutination test became positive. The correlation between STAT and MRT was 71 and 49 per cent in cows and buffaloes respectively.

Mohanlingam et al. (1965) used MRT as a screening measure in the Brucella survey work in organised units of a dairy colony in Madras and found 100 per cent correlation with STAT. They also found that the normal range of fat content in the milk did not interfere with MRT results. They were of the opinion that MRT in conjunction with serum agglutination test in five minutes was reliable to screen the sub-clinical cases of brucellosis.

Thanappa Pillai (1966) found that although all STAT positives were positive to MRT, all MRT positives were not positive to STAT. He recommended MRT for a primary survey to identify the reactors. Kapur and Singh (1967) recommended ABR as a screening test for Brucella infection in individual

animals. They found 86 per cent correlation in buffaloes between STAT and MRT. Cent per cent of buffaloes reacting positively to STAT were also positive to MRT. Soni et al. (1968) concluded that milk with a high fat content can be used for MRT. Chatterjee and Ganguly (1968), while testing 500 milk samples from cows and buffaloes, found 100 per cent correlation between STAT and MRT.

Roepke and Stiles (1970) concluded that a single milk ring test would have a 65 per cent probability of detecting one reactor cow in more than 95 per cent of the herds, if the test was conducted on bulk tank samples. Nandagoankar and Narayana Rao (1971) observed close agreement between MRT and STAT results. They found that MRT was quite sensitive for testing pooled buffalo milk can samples.

Cunningham (1971) tried to find out the relationship of the presence of agglutinating, complement fixing and incomplete antibodies to Br. abortus in milk to the MRT. They noticed that the intensity of the MRT reaction was proportional to the total antibody content of the milk. The agglutinins caused a MRT reaction within one hour. After incubation for 18 hours at room temperature, the MRT titre was positively related to the total antibody content, whether agglutinins were present or not.

The nature of reaction in MRT, ie., ring or sediment,

is dependent on the nature of the fat globule membrane. Milk samples containing fat globules give ring reaction, but those without fat globules give sediment reaction (Tanwani and Pathak, 1971).

According to McCaughey (1972) MRT results were dependent on seasonal variations. He observed an overall MRT efficiency of 73.4 per cent, while conducting examinations on 3,75,000 churin's and 737 herd's samples.

Buth and Manchanda (1972) carried out MRT on pooled milk samples from lactating cows and buffaloes in 24 villages around Karnal. He tested 310 samples from 1113 cows and 183 samples from 1271 buffaloes. The positive results were confirmed by tube and plate serum agglutination test.

Sinha and Pathak (1975) collected the individual serum and quarter milk samples of 40 lactating cows and buffaloes, which were ascertained to be Brucella positive. Samples from known non-reactor animals were also taken. They observed a wide variation in the MRT titre given by each quarter. High serum titres compared well in the STAT and HIT. The corresponding MRT and whey tube agglutination test (WTT) titres showed correlation both in cattle and buffaloes. But the authors doubted the usefulness of MRT titres for the appraisal of the Brucella status of an animal in the field conditions.

Nicolas (1977) claimed that tho ring test on herd milk samples detected brucellosis in the third month of infection in 65 per cent ofcases and in the fifth month in 95 per cent of cases. Serodiagnosis detected only 52 per cent of cases in the third month and ten per cent of cases remain undetected even in the nineth month.

Patterson and Deyoe (1977) studied the physical properties of fat globules from milk samples and found that the size or dispanty of globule size could not be correlated with MRT sensitivity. Inhibitory and enhancing creaming factors could be transferred from the cream to the skim milk. Those factors could be reacted with other cream and changes in the sensitivity could be shown. The proportion of clustered milk fat globules directly determined the quantity of agglutinins detected by the test. An insufficient amount of adsorbed fat globulos might be the cause to the failure of some brucella positive milks to react in the MRT.

Kerr (1960) observed that milk tests wore reliable only during the period of normal lactation. During the last 6 to 8 weeks of the drying off and in colostrum, seroglobulins bearing the seroagglutinins were present, in the milk, in a higher concentration.

According to the Joint FAO/WHO Expert Committee on Brucellosis (1971) the MRT is especially valuable for locating infected herds with a minimum of effort and expense, thereby

eliminating the need for extensive blood testing in herds
that are free from brucellosis.

Control and Eradication

Conditions in different countries throughout the world
vary much. A single universal programme for the control and
eradication of brucellosis is not possible. Factors like
prevalence of the disease, economic, social, cultural and
religious considerations may influence the methods of control.

Norway and Japan adopted successfully the method of
test and slaughter of the affected animals with payment of
compensation to the cattle owners. Majority of the countries
found that test and segregation before final elimination of
the affected animals with simultaneous calf-hood vaccination
with or without vaccination or non-reactor adult animals
with strain 19 vaccine was an effective method. The United
States of America, Canada, Yugoslavia, U.S.S.R., Australia,
Poland, Bulgaria, New Zealand, South Africa and Venezuela,
used this method for the control of brucellosis (Stableforth,
1959).

In Great Britain, the incidence of abortion was con-
siderably reduced after using strain 19 vaccine both in calves
and adult animals. But due to indiscriminate vaccination and
imperfect marking of the vaccinated adult animals complete
eradication of the disease has not yet been achieved (Govaerts,

The Joint FAO/WHO Expert Committee on Brucellosis recommended the following type of programme for the eradication of the disease.

>   1. Surveillance procedure for detecting infected herds. The milk ring test is very valuable for locating infected dairy herds. The test may be conducted, at least 3 to 4 times an year. In herds or areas where milk ring test is not possible (e.g. beef herds), market cattle testing for at least five per cent of the breeding cows per year is advisable. An efficient identification system is essential for the success of the market cattle testing. Blood tests of breeding cows, in herds not covered by these two tests, should be conducted at least every three years.

>   2. Control procedure for potentially infected herds detected by surveillance procedure is based on the principle of elimination of reactors with or without vaccination of heifer calves and possibly of non-reactor adult animals.

In areas, where the above procedures are not feasible economically, a modified control programme may be adopted.

Surveillance and testing procedures are the same as mentioned above. But the reactors are eliminated only in herds

with low rates of infection. The heifer calves and non-reactor adult animals may or may not be vaccinated. In herds with high rates of infection, testing with retention of reactors which are permanently identified and segregated from other animals, is recommended. Vaccination of heifer calves and non-reactor adult animals with approved vaccines may also be undertaken, because of the potential exposure to infection. In some areas neither of the above programmes may be feasible due to a high prevalence of the disease. In such cases, the heifer calves and adult cattle are to be vaccinated, with approved vaccines, putting permanent identification marks on them. Surveillance procedures or testing need not be undertaken until such time as both these procedures can be initiated.

MATERIALS AND METHODS

MATERIALS AND METHODS

This investigation was undertaken to study the
incidence of brucellosis in buffaloes in Kerala, especially
in the area surrounding Trichur. The work was carried out
in three groups.

> (1) Buffaloes, both males and females brought
> to the hospitals and artificial insemination
> centres at Mannuthy and Trichur and buffaloes
> kept by private individuals for milk, meat or
> work purposes.

> (2) Buffaloes maintained in three organised farms
> in the State, located at Kodappanakkunnu,
> Mannuthy and Thiruvazhamkunnu.

> (3) Buffaloes brought for slaughter for meat at
> the abattoir, Kuriachira, Trichur.

A total number of 1026 animals was examined during
the present study. STAT and MRT were employed as diagnostic
tests.

In the first group, history of previous abortions,
breeding history and sexual health of each animal were
ascertained, whenever possible and recorded. The samples
of blood collected from these animals were subjected to STAT

to detect Brucella reactors. In this group, 590 animals were tested. Milk was collected from 73 dairy animals for conducting the MRT.

The second group of animals included male and female buffaloes maintained under scientific managemental practices. STAT was conducted on 146 animals and MRT on 41 samples.

The third group consisted of 290 animals brought to the abattoir. There were 213 males and 77 females in this group. Blood was collected from this group of animals and tested.

Collection of blood

Blood was collected under aseptic conditions from the jugular vein or ear vein. Approximately three ml of blood was collected from each animal. The test tubes, after collection of blood, were allowed to stand for some time in the slanting position and the blood was allowed to clot. These tubes were then kept overnight in a vertical position in a refrigerator. The next day the tubes were centrifuged at 2000 R.P.M. for five minutes. The supernatent serum was drawn with pasteur pipettes and used for the agglutination test.

Antigens

The Br. abortus coloured antigen used for conducting

the milk ring test was obtained from the Indian Veterinary
Research Institute, Izatnagar and the Br. abortus plain
antigen which is used for the tube agglutination test, from
the same source and the Institute of Veterinary Preventive
Medicine, Ranipet. The antigens were kept in the refrigera-
tor at 4°C until use.

Collection of milk

For collection of milk samples the udder and teats
of each animal were washed thoroughly with water and dried
with a clean towel. The milk samples were collected from all
the four quarters after discarding the foremilk. About five
ml of milk was drawn into a sterile vial. The samples were
subjected to the milk ring test within two hours after collect-
ion. In the case of samples collected at Kodappanakunnu and
Thiruvazhamkunnu, one drop of 1:2 dilution of formalin (37%
to 40% solution of formaldehyde) was added to each sample at
the time of collection. The samples were kept in the refri-
gerator at 4°C for two days before testing.

Test procedures

Methods adopted in this work for conducting blood
serum agglutination tests were those developed and used at
the Central Veterinary Laboratory, Weybridge, England and
recommended by Alton and Jones (1963). The blood serum
samples were tested against standard Brucella abortus antigen

in the two fold dilutions of 1 in 10 to 1 in 160 in five
tubes to prevent the occurrence of 'prozone phenomenon'.
Samples which gave more than 50 per cent agglutination at
1:160 dilutions were re-tested with higher dilutions to
find out the exact titre at which they showed 50 per cent
agglutination. Normal saline containing 0.5 per cent phenol
was used as a diluent. The diluent was placed in each tube,
0.8 ml in the first tube and 0.5 ml in each succeeding tubes.
The saline in the first tube was mixed thoroughly with 0.2 ml
of the serum to be tested, and 0.5 ml of the mixture was
transferred to the second tube and mixed well. From this
tube 0.5 ml was carried over to the third tube. This process
was continued until the last tube, where after mixing, 0.5 ml
of the serum dilution was discarded. 0.5 ml of the standard-
ised Br. abortus plain antigen was added to each tube and
mixed well by rolling the tubes in between the palms. The
final dilution of the serum in the tubes was thus made to
1/10, 1/20, 1/40, 1/80 and 1/160. The tubes were then incu-
bated at $37^{\circ}C$ for 24 hours before the results were read. For
each day's work a set of antigen control tubes, as shown in
protocol below, was prepared to compare the results of the
test samples.

Protocol for the tube agglutination test

Tube No	Test serum					Antigen control				
	1	2	3	4	5	6	7	8	9	10
0.5 per cent phenol saline (ml)	0.8	0.5	0.5	0.5	0.5	1.0	1.25	1.50	1.75	2.0
Serum	0.2	0.5	0.5	0.5	0.5					
	Mixed thoroughly and transferred and discarded 0.5 ml from tube No. 5									
Standardized antigen (ml)	0.5	0.5	0.5	0.5	0.5	1.0	0.75	0.50	0.25	-

The antigen control tubes were also incubated at 37°C for 24 hours along with the samples under test.

The tubes were kept for one hour at room temperature before recording the results. The degree of agglutination in each tube was judged by the opacity of the supernatent fluid. The highest serum dilution showing 50 percent agglutination or 50 per cent clearing was taken as the titre of the serum.

The following key was used for comparing the results with control tubes.

```
Positive   ++++  (Comparable with tube 10) = 100% agglutination
           +++   (              "         9) =  75%        "
           ++    (              "         8) =  50%        "
           +     (              "         7) =  25%        "
Negative   -     (              "         6) =   0%        "
```

The titre of the serum was converted into internat-
ional units per ml of serum by doubling the serum titre show-
ing 50 per cent agglutination. The results were interpreted
according to the standard given by Gangulee et al., 1963, as
shown below.

International units	80 I.U. and above	40 I.U. and above	Less than 40 I.U.
Human beings	positive	doubtful	negative
Cattle and buffaloes	positive	doubtful	negative

The milk ring test was conducted as recommended in
the Standard methods for the examination of the dairy products
(1953).

Wasserman type clean test tubes were used fo· the above
purpose. Two ml of the whole milk was measured into a clean
test tube and two drops of the coloured antigen were added to
the milk with the help of a 1 ml pipette. The tube was gently
inverted several times so that the antigen was mixed with the
milk uniformly. The tube was incubated at 37°C for one hour.
Then the tube was allowed to stand at room temperature for 90
minutes before the results were observed. The formation of a
cherry coloured ring at the cream layer was taken as positive
reaction. The results were interpreted as follows:

Positive	+++	An intensely cherry coloured ring at the cream layer and absence of colour in tho milk column.
"	++	Ring formation at tho top and incomplete decolourisation of milk column.
"	+	No discolouration of milk, but ring formation.
Negative	-	Milk column coloured and white ring of cream.

RESULTS

Group I

In this group a total number of 590 animals were examined by STAT. The number of positive reactors in this group was 15, out of which 14 were adult she-buffaloes and one was a heifer. Among these reactors, 11 animals gave a titre of 80 I.U., three 160 I.U., and one 320 I.U. per ml of the serum. A titre of 40 I.U. per ml of the serum was shown by 57 animals and hence they were classified as suspicious reactors. After a period of three weeks, on subsequent examination, 22 animals among the suspicious reactors gave a descending titre and these animals were declared as negative reactors. The same titre was maintained by four animals. They were classified as chronic carriers. Only one animal showed an ascending titre of 80 I.U. per ml of serum. This animal was classified as an actively infected one. The rest of the animals in the suspicious group could not be re-examined, as they were disposed off by the owners. All the 22 male buffaloes examined in this group were negative to STAT. Most of them were castrated males used for work.

Among the positive reactors, one animal had a previous history of abortion, one had a history of retained placenta and another had propartum prolapse of the uterus. The general

practice among the people who keep buffaloes as dairy animals, seems to be to sell the animal when it goes dry and to purchase newly calved ones. So, many of the owners were unable to give a correct breeding history of the animal. An animal which had aborted thrice and another with a previous history of abortion, were negative to STAT. Another animal which had a history of abortion and one with a history of retention of placenta gave a titre of 40 I.U. per ml of the serum. The former, when re-tested after four months, gave a stationery titre. The animal with the history of retention of placenta showed a negative result with STAT. The results are tabulated in Tables 3 and 9. The percentage of positive reactors in this group was 2.54.

Individual milk samples from 73 animals belonging to this group were subjected to MRT. The animals were selected at random. Positive results were observed in two samples which were collected within 20 days after calving. One animal which gave a positive reaction to MRT was negative to STAT. The other one showed a titre of 80 I.U. per ml of serum. The results are given in Table 7.

Group II

In this group there were 146 animals out of which 101 were females and 45 males.

None of the animals showed a positive reaction to STAT. Two male calves at Mannuthy and two she-buffaloes at Kodappa-nakunnu were seen to be doubtful reactors as their sera gave titres of 40 I.U. per ml. These doubtful reactors were re-tested after a month and they revealed descending serum titres. There were no positive reactors in this group. The results are presented in Table 4.

Individual MRT was conducted on 42 milk samples collected from the animals of this group. One sample which showed a +++ reaction to MRT was negative to STAT. All other samples were negative to MRT. The results are presented in Table 8.

Group III

In this group, 213 males and 77 females brought to the abattoir were tested by STAT alone. Six males and two females were found to be positive reactors, while 32 males and 12 females gave doubtful reactions. Comparatively greater number (44) of doubtful reactors was found in this group and re-testing could not be conducted since the samples were collected from the slaughter house. The results are presented in Tables 5 and 10. The incidence of brucellosis in this group was 2.76 per cent.

The particulars of results obtained by conducting STAT in the random buffalo population under investigation in the

present study are given in Tables 6 and 11. The overall percentage of incidence of brucellosis of these three groups comprising of 1026 buffaloes was 2.24.

Table 1. Incidence of brucellosis in cattle and
buffaloes in organised farms.

Sl. No.	State	No. of animals tested	No. of found positive	% of positive animals	Source
1	Andhra Pradesh	5192	63	1.20 (8-17 in three farms)	Rao (1972)
2	Assam	n.a	n.a	20.00 (4 farms)	Polding (1948)
3	Bihar	13098	133	1.0 (0.56-2.6)	ICAR (1959-65)
4	Delhi	n.a	n.a	n.a	
5	Gujarat	200	0	0	ICAR (1956-57)
6	Haryana	4197	120	2.80 9.14	ICAR (1965-68) Mathur (1965)
7	Himachal Pradesh	423	0	0	ICAR (1959-66)
8	Jammu & Kashmir	-	-	-	ICAR (1944-55)
9	Karnataka	3500	210	6.0	ICAR (1970-71)
10	Kerala	206	4	2.0	ICAR (1954-55)
11	Maharashtra	4153	958	18.0	ICAR (1932-48)
12	Madhya Pradesh	5486	65	1.2	ICAR (1963-68)
13	Nagaland	50	0	0	ICAR (1969)
14	Orissa	n.a	n.a	1.7 to 8.3	Pal (1968)
15	Punjab	455	6	1.84	(ICAR 1965-68)
16	Rajasthan	-	-	-	-
17	Tamil Nadu	8688	317	3.6	ICAR (1957-68)
18	Utter Pradesh	3813	21	0.55 to 2.3	ICAR (1971-72)
19	West Bengal	1101	169	15.3	Romvary (1972)

All India average, 5.21%. n.a; not available.
Mathur and Sharma (1974)

Table 2.   Incidence of brucellosis in cattle and buffaloes
in village stock.

Sl.No.	State	No. of animals tested	Number found positive	% of positive animals	Source
1	Andhra Pradesh	14193	195	1.37	Rao (1972)
2	Assam	-	-	-	-
3	Bihar	-	-	-	-
4	Delhi	1523	386	25.3	Satya Prakash et al. (1967)
5	Gujarat	-	-	-	-
6	Haryana	755	45	6.0	Singh (1968)
7	Himachal Pradesh	238	5	2.0	Sharma (1965) ICAR (1959-66)
8	Jammu & Kashmir	-	-	-	-
9	Karnataka	3500	210	6.0	ICAR (1970-71)
10	Kerala	126	0	0	ICAR (1954-55)
11	Maharashtra	126	7	5.5	Polding (1948)
12	Madhya Pradesh	516	20	4.6	Pathak (1968)
13	Nagaland	-	-	-	-
14	Orissa	1948	439	22.5	Pat (1968)
15	Punjab	921	19	2.1	ICAR (1965-68) Sharma (1965)
16	Rajasthan	-	-	-	ICAR (1945-59)
17	Tamil Nadu	41207	1417	3.4 (20-30% in some areas)	Report (1957-68)
18	Uttar Pradesh	1006	67	7.0	ICAR (1971-72)
19	West Bengal	119	0	0	Sen and Joshi (1968)

All India average, 6.55%.
Mathur and Sharma (1974).

Tablo 3. Positive and suspected reactors detected by standard tube
serum agglutination test.

<u>Group I</u>

S. No.	Particulars of the animals tested	Number of animals tested	Number of positive reactors 80 I.U. and above	Number of suspected reactors 40 I.U.	Percentage of positive reactors	Percentage of suspected reactors
1	Adult females	471	14	49	2.97	10.40
2	Heifers	97	1	8	1.03	8.25
3	Males	22	-	-	-	-
	Total	590	15	57	2.54	9.66

Table 4. Positive and suspected reactors detected by STAT.

Group II

Sl. No.	Particulars of the animals tested	Number of of animals tested	Number of positive reactors 80 I.U. and above	Number of suspected reactors 40 I.U.	Percentage of positive reactors	Percentage of suspected reactors
1	Adult females	62	-	2	-	3.23
2	Heifers	37	-	-	-	-
3	Males	47	-	2	-	4.26
	Total	146	-	4	-	2.74

44

Table 5. Positive and suspected reactors detected by STAT.

Group III

Sl. No.	Particulars of tho animals tested	Number of animals tested	Number of positivo reactors 80 I.U. and above	Number of suspected reactors 40 I.U.	Percentage of positivo reactors	Percentage of suspected reactors
1	Male	213	6	32	2.82	15.02
2	Female	77	2	12	2.60	15.58
	Total	290	8	44	2.76	15.17

Table 6. Brucellosis reactors detected by STAT in the buffalo population under investigation

Sl. No.	Sex	Category	Number of reactors detected in Group I			Number of reactors detected in Group II									Number of reactors detected in Group III		
						Kodappanakunnu			Mannuthy			Thiruvazhamkunnu					
			P	S	N	P	S	N	P	S	N	P	S	N	P	S	N
1	Males	Adults	-	-	16	-	-	3	-	-	-	-	-	6	6	32	175
		Calves	-	-	6	-	-	7	-	2	25	-	-	4	-	-	-
2	Females	Adults	14	49	408	-	2	34	-	-	1	-	-	25	2	12	63
		Calves	1	8	88	-	-	10	-	-	4	-	-	23	-	-	-

P., positive; S., suspecious; N., negative.

Table 7. Results of MRT - Group I

No. of milk samples examined	Results of MRT		Particulars of the reactors	Remarks
	Negative	Positive		
73	71	2	Serial number 478 calved 20 days prior to the collection of samples. No history of abortion.	Positive to bruce- llosis by STAT with a titre of 80 I.U. per ml of serum
			Serial number 347 calved ten days prior to the date of colle- ction of samples. The placenta was retained. No history of abortion.	Negative by STAT.

48

Table 8. The results of MRT - Group II

No. of milk samples examined	Results of MRT		Particulars of reactors	Remarks
	Negative	Positive		
42	41	1	Calved 15 months ago	Negative by STAT

Table 9. Titre of positive reactors to STAT - Group I

Sl.No.	Sample no. of reactors	Titre recorded I.U. per ml	Remarks
1	14	160	Aborted two years back
2	15	160	No history of abortion
3	16	320	Had a history of retention of placenta
4	38	80	No history of abortion
5	135	80	History not known
6	136	80	"
7	137	160	"
8	223	80	No history of abortion
9	231	80	"
10	264	80	Heifer
11	439	80	No history of abortion
12	478	80	"
13	529	80	"
14	530	80	"
15	576	80	"

Table 10.   Titro of positive reactors to STAT - Group III.

Sl. No.	Date of collection of the sample	Particulars of the animals tested	Titre recorded I.U. per ml
1	26-11-77	Male	80
2	17-12-77	Female	160
3	7-1-78	Female	160
4	28-1-78	Male	80
5	11-2-78	Male	80
6	18-2-78	Male	80
7	15-4-78	Male	80
8	22-4-78	Male	160

Table 11. Number and titre of reactors to STAT in the
buffalo population under investigation.

Particulars	Results of STAT (Titres in I.U.)						Total number of animals examined
	Negative	20	40	80	160	320	
Number of animals	691	207	105	16	6	1	1026

FIG. 1. DISTRIBUTION OF REACTORS IN DIFFERENT TITRES OF STAT
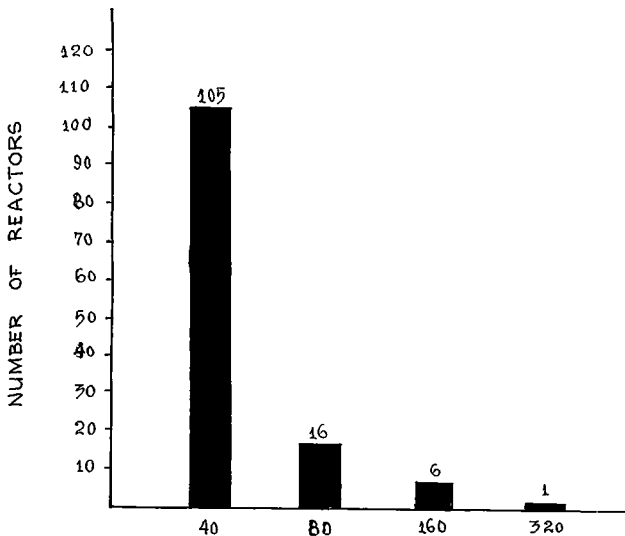
40, 80, 160 & 320 REPRESENTS I.U. PER ML OF SERUM

DISCUSSION

DISCUSSION

There are a number of schemes operating in the State of
Kerala, which aim at improving the milch quality of local
animals by upgrading them by cross breeding. The offsprings
resulting from cross breeding give higher milk yield. There
are provisions to help the small farmers by incentives in the
form of loans for the purchase of milch animals. Many farmers
have purchased milch animals through the help of the Small
Farmers' Development Agency (SFDA). Because of this incentive,
a large number of buffaloes are brought to our state along with
cows and goats. The chief source of these buffaloes is the
neighbouring state of Tamil Nadu, where brucellosis seems to
be endemic. With good transportation facilities, there has
been an increase in the cattle movement between states.
Polding (1943) observed that the exotic breeds and their crosses
are comparatively more susceptible to brucellosis than the
indigenous breeds. The increase in the number of crossbred
animals has enhanced the possibility of susceptibility of
animals in this state to brucellosis. Since cows and buffa-
loes mingle freely during transport, the buffaloes are equally
exposed to infection. This is further enhanced by the exist-
ing animal husbandry practices in the state where different
species of livestock are housed together.

In this study 1026 buffaloes were tested. There were

no positive reactors among the animals maintained in the organised farms. The overall incidence of brucellosis among the population tested was 2.24 per cent.

On examining the incidence of brucellosis (positive cases) with reference to Group III, and Group I it was found to be statistically significant. The normal deviation values (u) in these two cases turned out to be 2.87 and 3.92 respectively.

On comparing the incidence of brucellosis in terms of positive cases between Group III and Group I, no significant difference between the proportions could be found (u = 0.43).

No significant difference was observed in the *proportion* of positive cases between males and females (u = 0.15).

On examining the incidence of brucellosis (suspected cases) with reference to Group III samples and Group I samples, it was found to be statistically significant. The normal deviation values in the two cases were 7.20 and 7.94 respectively.

The suspected cases were also found to be significant in organised farms with normal deviation value 2.03. The three groups were compared with regard to the suspected cases. It was found that the suspected cases were proportionately more in Group III than in Group I and their difference was significant (u = 2.46). Similar differences between Group III

and Group II were also found to be significantly different.
The respective normal deviations were 3.91 and 2.86. Pro-
portionately the incidence in Group III animals exceeded the
same in both Group II and Group I. However, the incidence in
Group II was found to be proportionately less than the same
in Group I.

On further analysing the suspected cases, it was found
that there was no significant difference in their proportions
between male and female (u = 1.44). However, significant
difference was observed in their proportions between adults
and young (u = 2.72).

The brucella free status of the organised farms may be
due to efficient management practices and good sanitary con-
ditions. According to Polding (1948) lack of sunlight and
high humidity favour the spread of brucella infection. The
animals in the organised farms are not exposed to infection
from external sources, as they are seldom taken out of the
farms in which they are kept. The suspected cases in the
organised farms were statistically significant on the first
(occasion of) testing, but on re-testing, descending titres
were observed, indicating that the animals were negative to
brucellosis. The policy of artificial insemination adopted
in the farms for breeding, perhaps, may be, one of the factors
which contribute towards the non-occurrence of the disease in
Group II animals.

Milk ring test was done in individual animals to identify the reactors. In the present study, 73 animals from Group I and all the 42 milch animals in Group II were tested by individual MRT. In the first group, one animal gave a +++ MRT, but it was negative to STAT. The samples were taken ten days after calving. Another animal of the same group from which the samples were taken 20 days after calving, showed a + MRT reaction and a STAT titre of 80 I.U. per ml. One she-buffalo, from Group II, which gave a +++ MRT reaction was negative to STAT. All the other milk samples collected from the two groups were negative to MRT.

The animals in Group I which showed MRT positive and STAT negative reactions were in the early stage of lactation. The MRT positive reaction may be due to the occurrence of agglutinins in the milk earlier than they appear in the blood as suggested by Wall (1930). Since the animal was sold out further studies could not be taken up. Another sample of the same group gave a positive reaction to both STAT and MRT, because the agglutinins appeared simultaneously in milk and blood as the sample was taken on the 20th day after calving. The agglutinins normally make the appearance in the blood within 2 to 20 weeks after calving or abortion as stated by Stableforth (1959). Hence the finding is in accordance with the observations made by Mohanlingam et al. (1965), Kapur and Singh (1967) and Chatterjee and Ganguly (1968), wherein 100 per cent correlation was observed between MRT and STAT.

The animal, from Group II, which showed a +++ MRT
and a negative STAT reaction was in the late stage of lacta-
tion. This may be a false positive reaction and night have
been due to the presence of seroglobulins bearing the sero-
agglutinins which make an increasing appearance in the last
6 to 8 weeks of drying off period (Kerr, 1960). Similar
false positive results may be also occur due to the presence
of non-specific agglutinins.

The limited number of suspected cases which were re-
tested showed that the majority of them were actually negative
to brucellosis. Haemorrhagic septicaemia vaccine is regularly
administered to cattle in Kerala State. This vaccination may
cause an increase in the serum titre against the Br. abortus
antigen by one step (Pandaet al., 1963). The occurrence of
a large number of suspected cases may be attributed to this
cause.

The higher incidence of suspected cases of brucellosis
among animals of Group III may be due to inapparent brucella
infection. Free trade of livestock exists between the
adjascent states of Tamil Nadu and Kerala. The majority of
the animals sent for slaughter at Trichur, are brought from
Tamil Nadu, an endemic area for brucellosis. These factors
may contribute for the higher incidence of suspected reactors
among the animals brought to the abattoir. Stableforth (1959)
stated that "it is not possible to reach any satisfactory

conclusion regarding relative sex susceptibility in bovines
because males and females are kept under such different con-
ditions". In the present study, the overall incidence among
males and females was more or less the same. So it is inferred
that males and females are equally susceptible to brucellosis.
This finding is in disagreement with the inference of Kataria
et al. (1969) who noted that the buffalo bulls were affected
with brucellosis to the extent of 15.71 per cent when the
corresponding figure for she-buffaloes was only 3.74 per cent
in Madhya Pradesh.

It was observed in this study that adult animals are
more susceptible to brucellosis than young ones, which is in
accordance with the findings of a survey of brucellosis among
animals of military farms (Anon, 1962). According to
Stableforth (1959), calves are relatively insusceptible upto
the breeding age.

Methods of control

Control measures are normally based on hygiene, vacci-
nation, testing and disposal of affected animals. Complete
co-operation from the stock owners is the most important re-
quirement to tackle this problem. Eradication by test and
slaughter is the best policy when the incidence is low. The
Joint FAO/WHO Expert Panel on Brucellosis (1951) recommended

testing and elimination of reactors in places where the prevalence of brucellosis is in less than three per cent individual animals.

Under the existing socio-economic conditions in the country, it is difficult to advocate test and slaughter method in India. The policy of test and segregation is the only method which is practical and feasible under Indian conditions. Eventhough this method is slow and laborious, it has been effectively used in establishing clean herds. In the majority of the states, the reactors are left either mixed with healthy animals or segregated in the same farm premises, the attendants being common for both the herds.

The test and segregation method has been adopted with success in Uttar Pradesh, where the incidence was brought down from 5.6 per cent to 1.42 per cent within a period of eight years. The positive reactors were transferred to a separate farm in Uttar Pradesh. In Haryana, the method was adopted by segregating the positive reactors in the same farm, but far away from the testing herd. The percentage of positive reactors was brought down from five to nil within five years. Madhya Pradesh also adopted the test and segregation method. Since new farms were started, the final results are yet to be ascertained (Mathur and Sharma, 1974). In the neighbouring state of Tamil Nadu, a certified herd scheme is in operation, wherein units of ten animals and more

can be registered at nominal charges. Periodical testing is
carried out to detect and eliminate the positive animals.
The implementation of the scheme will reduce the incidence
of brucellosis gradually loading to the formation of brucella
free units, which, in turn will load to the natural exit of
the disease.

An estimate of the incidence of brucellosis, in other
species of animals like white cattle, sheep, goats and swine,
in Kerala was not available. Local surveys on the incidence
of brucellosis in above species of animals have to bo under-
taken to arrive at definite conclusions. The following pro-
cedures are to be adopted for control and eradication of the
disease. If the overall incidence is below 3 per cent the
test and slaughter policy is the best. Test and slaughter
method if not feasible in Kerala due to economic reasons,
the test and segregation of the affected animals is recom-
mended.

Milk ring test can be used to detect the infected herds
Since there are co-operative milk societies in most of the
villages, collection of pooled samples of milk will be easy.
The test should bo conducted at least twice an year in each
locality. Standard tube serum agglutination test should bo
conducted on all animals in the infected herd. The reactors
must be branded and separated. As soon as possible, these
animals should be eliminated.

A programme to vaccinate all calves at the age of
4 to 6 months must be undertaken with immediate effect,
preferably with strain 19 vaccine.

Effective information compaigns are necessary to
make the cattle owners understand that it is not economical
to keep Brucella infected animals. The public may also be
made aware of the hazards of using infected milk and its
products. The cattle owners should be made to realise the
benefits derived from establishing an accredited herd for
brucellosis. To encourage the formation of accredited herds,
the cattle owners should be given an incentive. Extension
publications, press releases, films and propaganda works can
be made use for this purpose.

Infected materials should be disposed off or disin-
fected properly, so that the spread of the disease through
them is prevented.

It should be made legally obligatory that cattle
owners should possess a licence which is issued on the basis
of a certificate showing the brucella free status of the
animals. The licence may be renewed annually. The issue of
the certificate should be on the basis of blood tests.

It is desirable that the Veterinary Institutions in
the State should be well equipped to screen the blood samples
so that the cattle owners can renew their licence without delay

Tho authoritics should insist that animals arc permit-
tod to entor tho state only on the production of a cortificate
to the effect that thoy arc frco from tho diseaso or with a
valid vaccination cortificato.

The public health authoritics and the staff engaged in
tho animal husbandry activitics in tho state should follow
uniform rulos and rogulations with respect to the issue of
cortificatcs, cattle movemonts, periodical blood tosting and
logislation for tho control and cradication of the disoaso.

S U M M A R Y

SUMMARY

A field investigation was carried out to assess tho
incidence of brucellosis in buffaloes in and around Trichur
and in three organized dairy farms in different parts of
the State. During this investigation 1026 buffaloes were
tested. These animals were divided into three groups.

The first group comprised of 590 animals, in and around
Trichur, including those which were brought to the Veterinary
hospitals and artificial insemination centres, attached to
the Kerala Agricultural University, at Mannuthy and Trichur.
There were 568 females and 22 males in this group. All these
animals were tested by the standard tube serum agglutination
test (STAT) and 15 animals were found to be positive to bru-
cellosis. All the males were negative to STAT. The percent-
age of positive reactors in this group was 2.54. Individual
milk ring test (MRT) was conducted on 73 milk samples, collect-
ed at random, from these animals. Out of the two animals which
were positive to MRT, one was positive to STAT also.

Among the STAT positive animals, one had previous
history of abortion two years back and the other had retention
of placenta.

In the second group, a total number of 146 animals
located in the three organised farms in the state were examined.

There were 101 females and 45 males in this group. All the animals were negative to brucellosis by STAT. Individual milk ring samples from 42 she-buffaloes from this group were subjected to MRT. One animal in the late lactation gave a positive MRT reaction which was considered to be a false positive reaction.

The third group included 213 males and 77 females brought to the abattoir at Kuriachira, Trichur. Positive reactions to brucellosis by the STAT were shown by six males and two females. The percentage of incidence, in this group, was 2.76.

The overall incidence of brucellosis among the buffalo population under study was 2.24 per cent. However, local surveys on the incidence of the disease in other species of animals have to be conducted to arrive at definite conclusions. If the overall incidence is below three per cent the best method of control and eradication is the test and slaughter policy. If this method is not feasible due to economic reasons, test and segregation as recommended by the Joint FAO/WHO Expert Panel on Brucellosis (1971) with vaccination is recommended.

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A STUDY ON THE INCIDENCE OF BRUCELLOSIS
IN BUFFALOES IN TRICHUR

By

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ABSTRACT OF A THESIS
Submitted in partial fulfilment of
the requirements for the degree

MASTER OF VETERINARY SCIENCE

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1978

ABSTRACT

The result of an investigation, carried out on 1026 buffaloes in and around Trichur, to assess the incidence of brucellosis in buffaloes was recorded. Standard serum tube agglutination test was carried out on the blood samples collected from 590 animals in and around Trichur, 146 animals maintained in the three organised farms in the State and 290 animals slaughtered at the abattoir, Kuriachira, Trichur. Milk ring test was conducted on 115 individual samples of milk. The overall incidence of brucellosis in the buffalo population tested was 2.24 per cent. All the animals maintained in the organised farms gave a negative result to brucellosis. The possible methods of control and eradication of brucellosis in Kerala State were discussed.