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C E R T I F I C A T E

This is to certify that the thesis entitled "Prevalence of Bovine Brucellosis in Orissa" submitted in partial fulfilment of the requirements for the degree of Master of Veterinary Science in Bacteriology and Virology of the Orissa University of Agriculture and Technology, Bhubaneswar is a faithful record of bonafide research work carried out by Sri Trailokyanath Mohanty, B.V.Sc. & A.H. under my guidance and supervision.

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CHAPTER I
INTRODUCTION

CHAPTER-1

I N T R O D U C T I O N

The importance of bovine brucellosis caused mostly by Brucella abortus, but occasionally by B. melitensis and B. suis, as a disease of major economic importance in most countries of the world, including India, is too well known. Indeed, notwithstanding the discoveries of a large array of bacteria, viruses and even fungi, B. abortus still occupies a preeminent position as the primary cause of decreased milk production by aborting cows. Brucellosis as a disease is well recognised as a common cause of infertility. Other clinical and subclinical effects of the disease include retention of placentas, endometritis and both these conditions seriously interfere with different breeding programmes. Even in advanced countries which claim to have substantially controlled brucellosis among cattle, culling of some valuable cows and calves, even when they react doubtfully to serum tests, has been posing serious economic loss. In countries such as India, where prevalence of the disease is wide-spread in many states, brucellosis is one of the important causes for failure in successfully implementing animal breeding and production programmes. The situation is more distressing on account of paucity of systematic diagnostic and control programmes, although the methods

of control are clearly established and the knowhow and potentialities of vaccine production are not too inadequate to surmount the problem.

Although research on prevalence of bovine brucellosis in India dates back to the early part of this century (Forster, 1906; Lamb and Pai, 1906), it was not until the results of the classical work of Polding (1943; 1947a; 1947b; 1948) that the real significance of this disease *vix-a-vis* animal health and production was appreciated by research workers in the field of animal diseases. On the basis of Polding's observations (*op.cit.*) followed by several reports from different parts of India, the annual economic losses due to bovine brucellosis was estimated by Schwabe (1971) as follows:

I. Losses due to bovine brucellosis:-

Loss of calves	Rs. 50 million
Loss due to lowered milk yield	Rs. 100 million
Loss due to reduced breeding	Rs. 90 million
Total ...	<u>Rs. 240 million</u>

II. Losses in public health:

Rural human population	375 million persons
20% suffer from fever (All causes).	75 -do-
2.4% febrile cases are due to brucellosis	1.5 -do-

Man-days lost @ 21 day per person.	30 million persons man-days.
Loss @ Rs. 8/- per man day	Rs. 240 million
Expenditure for treatment nursing etc.	Unknown

Even if one excludes expenditure for treatment, nursing etc. which has been left as unknown, the financial loss only on account of brucellosis assumes a staggering 240 millions rupees per annum. Almost an equal amount is supposed to be getting lost annually consequent upon public health problems arising out of brucellosis, the total financial burden thus coming to Rs. 480 million. If a conservative amount of Rs. 20 million is added towards expenditure towards treatment and nursing, the overall loss sustained by the country annually on account of brucellosis can be rounded at Rs. 500 million or Rs. 50 crores.

In many western countries which have virtually brought bovine brucellosis under control through such extreme and expensive policy of test and slaughter, it is curious and distressing to note that infection due to B. abortus and perhaps other species of Brucella still continue to pose problems thereby necessitating regular seroexamination of all apparently free herds. In the United States, for example, Amesson (1984) estimated losses from bovine brucellosis to exceed 65 million pounds beef and 35 million pounds milk in 1976.

During the same period, 575 million dollars were spent by state and federal authorities to control the spread of brucellosis. In another advanced country viz., France, 3.12% herds were infected and 2.57% abortions were due to brucellosis leading to the slaughter of 63505 cattle only during one year (Dufore, 1983). These two examples speak for themselves and also highlight the magnitude and complexity of the problem even under ideal situations. Literature from different countries, which will be reviewed in the next chapter, confirm beyond doubt that brucellosis has not been really eradicated from any country although the prevalence rate has been substantially reduced (Aumaj, 1985). Reversely, the prevalence of brucellosis appears to be on the increase in several countries where there appears to be no systematic control programmes. Recent reports from Somali (Andreani, et al., 1983), Libya (Mayer, 1982), Central Africa (Domerech, 1982) and Nigeria (Ukoh, 1980) indicate a high overall positive reactor rate varying between 5-15%. One of the leading countries of the world which earns almost 90% of its revenue from animal and poultry wealth viz., New Zealand had until early eighties 14% infection of brucellosis in cattle — a situation rather unbelievable yet undisputable as reported by Cordee et al. (1979). The economic loss sustained due to brucellosis in cattle herds in such

countries cannot be merely assuaged in terms of money; its impact on animal health is indeed colossal. This creates for the research workers on brucellosis a situation where they are likely to be baffled as to what could be done to keep bovine brucellosis at check in a country like India with its enormous cattle population mostly maintained under primitive conditions and in an unorganised manner, chiefly in rural areas where infectious diseases have no barriers to spread from animal to animal and herd to herd. The early work of Polding (1943; 1947a; 1947b; 1948) brought to light for the first time that Orissa was one of the states which had the dubious distinction of being labelled as an endemic area for brucellosis in the map of the Indian subcontinent and this stigma still continues (Mathur, 1985). That the situation was really so has been repeatedly confirmed.

(Das *et al.*, 1962; Panda and Mishra, 1963; Panda and Das, 1965; Pat and Panigrahi, 1966; Panda and Pat, 1967; Pat and Panda, 1968). During the intervening period i.e. between 1968 and 1985 practically no work was carried out on the prevalence of bovine brucellosis which could well be termed as reprehensible considering the endemicity of the state for this notable disease although an attempt was made to assess the situation in respect of sheep (Acharya, 1984; Acharya and Panda, 1985). The prevalence of brucellosis in the

human population of Orissa has also not been ascertained except for the stray report of Mahakud and Panda (1972) who recorded 3.7% reactors in western Orissa and isolated B.melitensis from cases of Prexial of Unknown Origin (P.U.O). It will thus be appreciated that a survey of bovine brucellosis in Orissa was long overdue, atleast in a limited scale and confined mostly to organised herds. It is with this object in view that the work reported in this thesis was undertaken. Even though the main purpose of this investigation was to assess the prevalence of bovine brucellosis, yet attempts were made to isolate and identify the causative organism(s) wherever possible.

The public health significance of brucellosis as one of the most important zoonotic diseases has drawn attention in many countries and also at international level (Report, 1971; 1982). B.abortus, which is a common infection of bovine animals is transmitted quite frequently to man, usually from cattle (Scott, 1973). In recent years, however, following control of bovine brucellosis, the trend has shifted in favour of B.suis, as in U.S.A. and some other countries (Harrison, 1974). No matter which species of Brucellae cause human infection,

it is now universally recognised that the prevalence of this infection in man is directly traceable to infected animals, which, as far as it is known, are the only source for the disease to occur in man. It is, therefore, not surprising that clinical and/or subclinical cases of human brucellosis have steeply fallen in those countries where large-scale control measures have been undertaken in respect of animal brucellosis. It is in this context that the prevalence of bovine brucellosis has tremendous bearing on human health in a country like India, primarily because a large chunk of the rural population is in intimate contact with animals and secondarily on account of the fact that no systematic attempts have been made so far to prevent the spread of the disease in animals. In India, as in many other countries, human brucellosis happens to be an occupational disease and the causative organism in most cases is B. melitensis. Infectious bovine abortion and other forms of brucellosis in cattle are however known to be caused by B. abortus (Huddleson, 1943; Spink, 1956; Mathur 1985). There are, however, reports of B. melitensis infection occurring in cattle with possibilities of spread to human beings (Stableforth and Galloway, 1959). In view of this, it was considered worthwhile to test the

serum samples of human beings, particularly those working in farms, where the prevalence of bovine brucellosis was studied.

It is earnestly hoped that the results of this investigation would not only throw some light on the magnitude of bovine brucellosis as an animal health problem in Orissa but also help in formulating suitable control measures in the coming years. Needless to emphasize once again that the control of bovine brucellosis would act as a tremendous booster to animal husbandry and at the same time alleviate human suffering from public health point of view.

CHAPTER II
REVIEW OF LITERATURE

CHAPTER-II

REVIEW OF LITERATURE

Even before Bruce (1886) isolated a Gram-negative coccobacillary organism (Brucella melitensis) from the spleen of human beings who had died of Malta fever, Martson (1859) gave a clear account of this clinical entity. Bang (1895) in Denmark isolated a similar organism from contagious bovine abortion which was later classified as B. abortus. B. suis was isolated in 1914 by Traub from cases of abortion in swine in the U.S.A. Suddle and Boyce (1953) discovered a new species of Brucella causing genital disease in Australia and Newzealand which was named B. ovis. Two other species viz., B. neotomae and B. canis were reported in 1957 and 1967 respectively (Stonner, 1957; Lockman and Charmichael, 1967). Although B. abortus, B. melitensis and B. suis have been isolated in India, the occurrence of B. neotomae, B. ovis and B. canis is not yet established here. Brucellosis affects virtually all domesticated animals and occasionally even poultry and is known by different names in different countries such as Malta/Mediterranean fever, undulant fever, goat fever, dust fever, pseudotyphoid, recurrent typhoid, malarial typhoid, new fever etc. The disease in man is often classified under P.U.O. (pyrexia of unknown/uncertain origin) along with febrile conditions whose aetiology it is difficult to establish in the absence of detailed laboratory investigations.

It is impossible in this thesis to review even a fraction of the vast literature on brucellosis which has accumulated since the cause of Malta fever was established. A number of comprehensive text books now available in english literature (Huddleson, 1943; Spink, 1956; Dalrymplechamproys, 1960; Crawford and Madalgo, 1977) bear testimony to the plithora of ~~inf~~ information which continues to accumulate year after year on various aspects of brucellosis. In addition, there are now available numerous monographs and expert Committee reports of brucellosis (Report, 1967; 1971; 1975; 1982). In India, the I.C.A.R. has published a technical bulletin on brucellosis (Gangulee et al. 1960). Keeping in view the title of this thesis and the scope of work envisaged, the literature reviewed in the following pages has been so designed that emphasis has been laid on important aspects of the disease viz., prevalence of bovine brucellosis, serological diagnosis and isolation including identification of the causative agent(s). In addition to relevance, emphasis has been laid on more recent findings and text book information is avoided as far as possible.

1. ANIMAL BRUCELOSIS

A. Position in Foreign Countries:

Argentina, one of the largest exporters of beef, recorded 5-27% prevalence of bovine brucellosis between 1948 and 1958 (Cedro. et al. 1961).

In Egypt, Hamada et al. (1963) accounted a very low reactor rate of 0% to 0.46%.

Although the prevalence of bovine brucellosis in Switzerland was about 5% in early fifties, some ten years later 99.6% cattle were free from brucellosis (Fritsch, 1964)-a remarkable achievement by world standards.

According to Kouba (1964), brucellosis was eradicated in 7 out of 109 regions; but the disease was still present mostly in mountainous regions in Czechoslovakia.

May be because of beef eating habit, only 0.66% reactors were detected in Pakistan among cattle. However, in milk buffaloes the reactor rate was 9.33% (Sheikh et al. 1968).

In Tanzania, the average infection rate of bovine brucellosis was 14.2% as per report of Hoffman and Elsamah (1969). It was further pointed out that variations in infection rate were noticed in different farms; lowest being 3.8% in nature owned herds kept individually and highest being 29.7% in government herds.

Opitz (1969) performed serological survey by employing agglutination and complement fixation tests in 2626 N'Dama cattle belonging to 272 herds in South Africa and reported a positive reactor rate of 10.4%. It was further reported that 13.8% of the females above

2 years of age were positive and out of 272 herds 49% were infected.

On perusal of the above literature from African countries, it is interesting to observe a higher prevalence rate of brucellosis among government dairy herds as compared to private and rural herds. Obviously this may be on account of lack of suitable test, control and follow up measures - a situation comparable to Indian conditions.

In Czechoslovakia, 4,771 outbreaks of brucellosis were recorded between 1952-59 and another 404 in between 1960-64. As a result, the prevalence rate which was 5.5% among farming communities at the beginning of 1960 was slowly reduced to 2.6% by the end of 1964 and further to 1.3% at the end of 1966 (Kouba, 1969).

Out of 500 unvaccinated slaughtered cows tested in Iraq, 2.2% were positive and 1% doubtful to standard tube agglutination test as reported by Ferid and Al-Hashimy (1969).

Turner (1969), by using international tube serum agglutination test with 8,448 random sera samples, reported 4.5% and 3.6% prevalence rate in the Eastern Victoria and Southern Victoria respectively.

Survey was conducted with 105 sera samples in Rathelin island of northern Ireland where cattle were never vaccinated. All the samples were found negative to tube agglutination (S.A.T.) and complement fixation test (CFT).

(Macaughay and Carlisle, 1970). This is one country which appears to be totally free from brucellosis.

Euwoso et al. (1972) performed serological tests to assess the prevalence rate of bovine brucellosis in southern states of Nigeria. Out of six dairy, two beef and one experimental herd tested, positive reactors were detected in five dairy and one beef herds. Thus the prevalence rate was recorded to be 26% in government beef herds and between 2.5% to 14.7% in other herds.

Using SAT and CFT, Tadjekoh and Gate (1972) in Iran recorded prevalence rate of 12% in cattle, one in 36 buffaloes and 12 in 306 human beings.

The prevalence rate of bovine brucellosis in the new territories of Hongkong was reported by Munro (1973) to be 44% by using agglutination test and Rose Bengal plate test (R.B.P.T.).

In almost all the west African countries, the prevalence of brucellosis varied from 10-16% (Thim and Nauwerck, 1974).

Murru and Dennis (1975) screened cattle herds of northern Nigeria by rapid plate test and tube agglutination test and reported overall prevalence rate of 6%-7.1% in local herds, and 3.6% in government herds. Highest rate of infection was 26.4% in Kwara State. In another survey conducted on 1659 sera samples of slaughtered cattle from three abattoirs, the prevalence

rates were 0.7%, 0.2% and 0.3% being slightly higher in females than in males.

In Australia, serological status against S. abortus infection in bulls frequently changed from positive to negative and vice versa without throwing off infection (Plant et al. 1976).

Award et al. (1977) observed in Egypt that out of 3003 buffaloes and 2492 cows' sera tested by agglutination test, 8 and 7 were found positive respectively and 11 were doubtful.

By conducting ring test in organised dairy farms in Bangladesh, Rahman et al. (1978) reported 4-17% reactors. The same authors in 1983 screened 1531 milk samples from 42 village herds and 461 from four organised farms of Mymensing district. Further, sera samples from 190 dairy and agriculture workers were tested by Rose Bengal plate test. Positive reactor rate was recorded to be 2.35% in village cows, 0% to 16.66% in cows of organised farms (overall reactor rate 3.9%), 15% of 80 dairy workers, 12.85% of 70 workers with animals and 0% of 40 workers with agricultural crops.

Muhammad et al. (1978) tested 902 cattle from farms and 2184 cattle from nomadic herds by employing serum agglutination test in Somalia democratic republic and reported 2.7% and 11.9% prevalence rate respectively.

In a survey of 35,116 cattle blood samples collected in Kenya (10,360), Tanzania (23,017) and Uganda(1739) were tested by Kagombe and Nandokha (1978) and it was found that the incidence was 10% in Kenya, 5.8% in Tanzania and 5% in Uganda.

In a properly designed survey of 786 farms in EL-Salvador, Knoke et al.(1983) observed reactor rates varying between 0.33% and 8.5%.

Madden et al.(1983) have dealt with history of the disease in Great Britain and its eradication in that country which is worth pursuing by all those interested in the control of brucellosis. Tsai et al. (1983) compared the result of serum agglutination, CFT and RBPT by using 36,483 sera samples collected between October, 1979 and March, 1981. In RBPT, 979 (2.7%) were positive whereas in CFT and tube agglutination test 141 (0.4%) were positive.

In southern Sudan livestock disease survey was taken up during 1979-81 in which serum samples from 8000 cattle of the Dinka (Wadai Dinka) and Red Bororo breeds in 400 herds, 800 sheep and goats of 150 herds and 300 people were tested. The prevalence rate was 6.5% in Wadai Dinka and 22.5% in Bororo cattle. The infection was present in 66% and 99% of the herds respectively. Sheep, goats and man were all negative (Bouman, 1984).

Bernudez and Barriola (1984) screened 20,665 serum samples of cattle in Uruguay and recorded 5.5% positive reactors.

Working on the epidemiology of bovine brucellosis in Mexico, Salmeron *et al.* (1984) analysed data from 184 premises and showed that size (area) of farm, local marketing of livestock, dairying as the major activity, swift disposal of positive reactors, vaccination programmes and presence of calving pens in the premises were linked to higher rates of brucellosis.

In an epidemiological study of bovine brucellosis in Benin (Tropical Africa), Akorkpo *et al.* (1985) tested 920 serum samples collected in between 1980 and 1981. 40 were positive to R.B.T. and 76 to CFT. The highest frequency of positive animals (12%) was in those aged 10 years and older.

Blajan and Melendez (1985) have summarised the contribution of the OIE to the control of animal brucellosis around the world. According to them, the situation of brucellosis has deteriorated on a world-wide scale, despite the remarkable result achieved recently in developing countries. According to this report, this deterioration is particularly vexing the developing countries where livestock is imported without minimal sanitary precautions being taken.

In Sri Lanka, Wickram Surya et al. (1985) tested the milk samples collected at milk collection centres in the district of Polonnaruwa, Batticaloa and Ampara by using MRT and observed the prevalence rate to be 7.7% in Polonnaruwa district, 12% and 14.4% in Batticaloa and Ampara districts respectively.

More recently, cross-bred and exotic animals in Bangladesh were found to be more frequently infected with brucellosis (21.3%) as compared to 2% reactors in village cows (Islam et al. 1985).

Sanouel and Onur (1985) examined 3753 cows at 5 dairy stations in Libya and observed a low reactor rate of 0.3 to 1.5% with B. abortus antigen.

Zaghloul and Kamel (1985) examined serum samples of livestock in different areas of Egypt and recorded positive reaction in 2.7 to 8% cattle tested.

Jabloui (1985) examined 753 cattle from six regions of Syria and found 47 (16%) were positive to brucellosis.

In Sicily, Guer-cio et al. (1985) tested 15,000 cattle in 1983 and found a prevalence rate of 3%.

During 1981-82, the ring test for brucellosis was applied to 420 bovine milk samples in Malta and 49 were found positive (Ilacqua et al. 1986).

In a more recent observation from Mexican states, Telcau et al. (1986) tested serum samples from a fairly large number of cattle on 40 ranches and the herd prevalence rates ranged from 0 to 38%.

France appears to have reached the door steps of near total eradication of bovine brucellosis with a reactor rate of 0.22 to 1.68% (Dufour, 1986). During one year i.e. 1984 alone, more than 50,000 cattle were slaughtered, as a part of the eradication campaign.

B. The Indian Picture:-

Contagious abortion in cattle was in existence insiduously in India since a pretty long time (Eyre, 1908). Naik (1932) observed that Brucella agglutinins were present in the sera of the bulls for a much longer period when the animals were infected with European strain of B. abortus than with the strain of Indian origin.

Prevalence of brucella infection in hilly tracts of India was investigated by Viswanathan (1944).

Holding (1943; 1947a; 1947b) made an extensive survey of brucellosis among cattle and buffaloes in Bengal, Assam, Orissa, Tamilnadu, Madhy Pradesh and Andhra Pradesh and reported its prevalence. The infection was less prevalent in buffaloes. The position

in respect of Orissa will be reviewed under a separate heading.

It was reported by Lal (1947) that 2-6% abortion cases in Assam were due to brucellosis.

Dhanda and Ragopalan (1949) summarised the work done on bovine brucellosis in India and recommended standard methods for the serological diagnosis of the disease.

The prevalence of brucellosis among cows and buffaloes in different parts of India was discussed at length in the 10th animal diseases conference held at IVRI in 1962. From large number of papers presented, almost from the entire country, the prevalence of bovine brucellosis was as follows up to that period:-

1. Assam	...	5- 10%
2. Bengal	...	26.0%
3. Bihar	...	23.5%
4. Gujarat	...	6.8%
5. Haryana	...	15.25%
6. Karnataka(Mysore)	...	29.4%
7. Madhya Pradesh	...	1.4%
8. Maharashtra(Bombay)	...	14.3%
9. Gujarat	...	6.8
10. Punjab	...	2.1%
11. Tamil Nadu (Madras)	...	5% - 20%
12. Utter Pradesh	...	0.9%

In a survey of bovine brucellosis, 13,565 animals were tested in the military dairy farms in India and it was reported that 10% adult buffaloes and 13% adult cows were positive. The corresponding figures in the younger stock i.e. six months to calving were 8.9% and 9% and for bulls 6.4% and 14.8% respectively (Anon, 1952).

Mathur, (1968) recorded a prevalence rate of 1.71% on the basis of RRT both among cows and buffaloes in Punjab, Haryana and some parts of Uttar Pradesh.

Sen (1968) tabulated the position regarding the incidence of brucellosis in animals up to 1968 as follows:

TABLE- I

The position regarding the incidence of bovine brucellosis in India in 1966 and 1968.

<u>Locality</u>	<u>1966</u>	<u>1968</u>
Madras villages	3.7%	3.7%
Madras State endemic areas Cattle	20-30%	
Orissa, Balasore (villages)	34.5%	
Orissa, Bhadrak (villages)	24.2%	
Orissa, Cuttack (villages)	24.9%	
Orissa, Bhubaneswar (villages)	18.5%	
Orissa, Puri (villages)	17.2%	
Orissa, Ganjam (villages)	31.3%	
Orissa, Sambalpur (villages)	23.1%	
Orissa, Bolangir (villages)	24.1%	
Orissa (overall)	24.7%	
Andhra Pradesh (State Farms)	6.74%	22.5%
Hydrabad (villages)	3.5%	
Andhra Pradesh (Military Farms)	10.7%	
Uttar Pradesh Government Farms	Nil	
Uttar Pradesh Cowsales	1-5%	
Uttar Pradesh Military Farms	8.8%	
Punjab Military Farms	11.3%	Punjab Haryana Buffalo Himachal 3.8% Pradesh
Punjab (villages)	1.7%	
Assam	8.1%	
Bihar	1.2%	
Bihar Military Farms	8.8%	
West Bengal Military Farms	8.8%	
Himachal Pradesh Military Farms	11.3%	
Madhya Pradesh Military Farms	10.7%	Madhya Cattle-1% Pradesh Buffalo-0.6%
Mysore Military Farms	10.7%	
Koharastra Military Farms	10.7%	

Prakash et al. (1969) tested 1523 buffaloes in Delhi and reported 0.7% seropositive to brucellosis.

In a test of 4,335 cattle and 1189 buffaloes, Kataria and Verma (1969) reported 5% and 3% seropositive cases in Madhya Pradesh.

Prevalence of brucellosis was in low grade enzootic form among buffaloes and cows, much higher in cows in organised dairy farm and rural areas in Andhra Pradesh as reported by Nandgoankar and Rao (1971).

Sethi et al. (1971) recorded 4.4% cattle and 1.3% buffaloes to be positive to brucellosis at Pantnagar in Uttar Pradesh.

By performing milk ring test in the villages around Karnal, Buth and Manchandra (1972) reported 28% cow and 14% buffaloes positive for brucellosis.

Examination of 29,513 animals (cattle and buffaloes) by Sreenivasan (1972) revealed 2.7% prevalence in Tamilnadu.

Kulshrestha et al. (1973) conducted agglutination test on 589 sera samples obtained from cows and 1058 from buffaloes during 1966-71 and found the prevalence of brucellosis to be 13.1%

in buffaloes and 2.3% in cows in Haryana. The same author in 1978 reported a seroprevalence of 8% in cattle and 4.1% in buffaloes. Work of Kapur and Grewal (1974) revealed 2.8% and 3.9% reactors in cows and buffaloes respectively in the same state. It was further revealed that higher prevalence was found in Thompsoner breed cows (9%) as compared to Haryana (4%) and Sahiwal (1.4%).

In Punjab, Randhawa and Dhillon (1974) carried out a serological investigation on brucellosis using standard tube agglutination test on 346 cattle and 65 buffaloes and reported a prevalence rate of 10.4% and 3.1%.

According to Sharma et al. (1979) plate and tube agglutination tests were positive for 6.4% cattle, 4.9% buffaloes in Uttar Pradesh and Delhi.

Work of Baby and Paily (1979) revealed an incidence of 2.24% in buffaloes in Kerala.

Serological studies were conducted by Chakravarty and Kuetra (1980) in Assam, Meghalaya and Nagaland and it was found that 59 of 397 serum samples were positive for brucellosis.

Dubey and Mathur (1980) in Rajasthan reported the incidence of 4.5% in cows and 3.4% in buffaloes.

In a study of 21 serum samples (5 bulls, 5 cows, 11 heifers) in Arunachal Pradesh Gore et al. (1981) found 7 positive (2 cows, 2 bulls and 3 heifers).

Out of 13 aborted fetuses subjected to test for diagnosis of brucella infection, Ramachandra et al. (1981) in Bangalore found 10 positive to brucellosis both by serological and bacteriological methods. The same author during the same year carried out serological investigation in livestock of Andhra Pradesh and detected a prevalence rate of 19.68% and 4.08% in cattle and buffalo respectively.

Bala and Sindhu (1982) found that prevalence of brucellosis was 16.7% in Jersey and Holstein, 25.6% in zebu cattle and 1.5 to 3.2% in buffaloes.

Rapid plate and standard tube agglutination tests were carried out on 194 cows in 13 organised dairy herds and 793 cows in individual holding in West Bengal for brucellosis. Prevalence in organised herds was higher i.e. (17%) as compared to (2.7%) in village cows (De et al. 1982).

Chatterjee et al. (1986) examined 1746 adult cows representing the adult cow population of 933000 in 15 districts of West Bengal and found 62 (3.5%) were positive to the standard agglutination test.

(C) Work done in Orissa

Orissa is one of the endemic states of India in so far as animal brucellosis is concerned (Panda, 1968). It may, however, be recalled that

eporadic testing of cattle was initiated way back in 1939 which indicated that brucellosis was rampantly prevalent in farms as well as village cattle in most districts of the state. Initial studies carried out by disease investigation staff of the state in collaboration with Indian Veterinary Research Institute, Mukteswar clearly indicated the high incidence of brucellosis in indigenous herds (24.6 to 29.8%) as well as in cattle of different breeding farms (17.3% -31.4%) where Ghumusari, Binjherpuri and Redd Sindhi cattle and Murrah buffaloes were being maintained.

Cattle of several parts of Orissa were screened for brucellosis by Polding (1943; 1947a; 1947b; 1948) which is acknowledged as the first major systematic survey of bovine brucellosis in this subcontinent. Polding's work (op.cit.) clearly established that the incidence of brucellosis was alarmingly high in Orissa and its occurrence among cattle continued across the provincial boundary. Thus, at Nawapara (Kalahandi District) 42% reactors were encountered in tests of several village herds. Around Sambalpur, Jharsududa and Angul, the reactor rate was 31%, while in Ganjam the percentage of reactors was as high as 50%, from where two *Brucella* A/M (aberrant type) strains were isolated. In the interiors of Bhanjanagar and Koraput,

however, positive reactor while present were much less frequent than in other parts.

The results of survey carried out from 1949 up to 1960 has been presented in Table -II (Panda et al. 1962).

TABLE-II

Results of plate agglutination test on serum samples of cattle of Orissa from 1949-50 to 1960.

Year	Locality	Number of animals tested	Number positive	Number doubtful	Percentage of total reactors
1949-50	Sundargarh) Dairy Farm)	60	10	-	16.6
	Keonjhar) Dairy Farm)	128	4	-	3.1
1950-51	Bhanjanagar Farm	148	16	12	19.3
	Sambalpur private herd	13	-	-	0
	Sambalpur Gossala	8	4	-	50
1952-53	Bhanjanagar Farm	75	29	10	52.7
	Boudh Farm	52	6	3	17.3
	Belangir Farm	94	50	8	61.7
	Angul Farm	62	13	8	35.0
	Baragarh Gossala-9		6	-	66.0
	Sambalpur Jail-14		4	-	28.5
	Balsore Jail 10		5	-	50.0
1959-60	Village cattle Bhanjanagar	115	25	-	21.7
	Village cattle, Bhubaneswar	56	17	-	30.4
	Village cattle Boudh	22	6	-	27.3

Perusal of the above table would show that while certain parts of Keonjhar and Sambalpur districts were relatively less infected, the position in some of the other areas was extremely bad with prevalence rate as high as 61.7%.

From 1960 onwards collaborative studies were initiated on different aspects of brucellosis in animals of Orissa by the research staff of the Animal Husbandry Department and teachers of Veterinary College. Salient results achieved are outlined in the following paragraphs.

Dass and Panda (1960-61) screened 959 animals both in farms and villages and found wide variations in the reactor rate varying between 0-39%, which indicated that endemic areas in state were far and many and they had to be properly located and marked in order to prevent spread of infection among healthy animals and human beings.

A simple method of despatching serum from the field in filter paper strips was developed and standardised with known positive sera from Brucella infected cattle and standard B. abortus tube agglutination antigen to enable field workers to submit dry filter paper strips inside postal envelopes instead of sending serum samples in glass vials (Panda et al. 1961)

Dass et al. (1962) studied the incidence of Brucella reactors in 110 bulls stationed at different

farms and semen collection centres and found that only 5 (4.2%) reacted positively, possibly because of natural resistance of male animals to Brucella infection.

Inapparent form of brucellosis, most probably due to B. abortus, was suspected when 12 poultry birds out of 150 reacted strongly to B. abortus plate (Coloured) antigen (Dass et al. 1963).

Panda and Mishra (1963) studied the efficacy of individual milk ring test for the diagnosis of brucellosis in unvaccinated village cattle vis-a-vis serum agglutination test and found close correlation in 57 out of 59 cows tested.

A common question that use to be asked was, "Did vaccination in cattle with H.S. vaccine affect Brucella agglutination test"? Panda et al. (1963) observed that in several animals there was an increase in serum titre against B. abortus antigen and this continued to remain so up to 123 days although the titre declined after the 22nd day.

In another investigation, Vibrio cholerae was found to cross-agglutinate with B. abortus. However, Salmonella pullorum and Proteus OX19 did not share any common antigen with B. abortus (Mishra and Panda, 1964).

Panda and Dass (1965) recorded their findings of a state-wide survey of bovine brucellosis

involving 862 farm animals and 79 village cattle of Orissa which revealed an alarmingly high percentage of reactors to different diagnostic tests. Except in one livestock farm, the reactor percentage ranged between 10-47, thereby posing serious difficulties in controlling the disease.

In a more elaborate study spread over three years (1965-67), Pat (1967) examined a total of 1948 indigenous milch cows, 35 stud and 2 stray bulls in Orissa for brucellosis from 3 different geographical zones. Of them, 439 cows were found positive by RRT and serum agglutination tests (plate and tube). The percentage of reactors in the costal zone varied between 17.2 to 31.3, in the central zone from 13.7 to 41.1 and in western zone from 0% to 24.1%. The overall reactor rate among cows was 22.5%. Out of 37 bulls, 16 (43.2%) were positive. In infected areas 3.3% to 36.3% of animals had aborted; of them, 79% aborted only once, 12% twice, 5% thrice and 4% four times and bulk of abortion occurred in the second half of gestation.

In the 3rd International Conference on the Global impacts of applied Microbiology, the

entire work done on animal brucellosis in Orissa was presented by Panda (1967). During the intervening period from 1968 till date, animal brucellosis somehow did not attract any attention whatsoever, except for some work on sheep (Acharya, 1984; Acharya and Panda, 1985).

2. HUMAN BRUCELLOSIS:

(A) Work done in Foreign Countries:

While making survey for zoonoses among meat processing workers in Russia, Godlevskay (1968) detected 35.8% positive cases.

Mcdevitt (1970) made survey among Veterinary surgeons in northern Ireland and found that out of 123 subjects, 100 had significant agglutination titre in their sera while 50 showed clinical evidence of infection. Foley et al. (1970) also reported an outbreak in the same year due to heavily infected milk supply.

Brucellosis due to B. abortus has been reported in France by Renoux (1970) in 38% rural workers and he suggested that brucellosis in the human and bovine population was strongly co-related.

In a sero-allergic survey of human brucellosis among people above 10 years of age in Afghanistan, Shinveric et al. (1971) reported 6.5% prevalence in people having animals

Zepatal and Malagah (1971) reported average incidence of 20-46 per 100, 000 in Peru during 1958-67.

In U.S.A., consumption of contaminated cheese caused brucellosis among 6 persons as reported by Young et al.(1975).

In the year 1975, Epidemiology Department of the Directorate of Preventive Medicine and Environmental Health of Spain reported the incidence rate to be 17.9 per 100,000 population.

Chantal et al.(1976) carried out a serological investigation of bovine slaughter house workers in Senegal and found 14.8% prevalence in them.

In Brazil, Tavares et al.(1976) examined 8740 sera samples of healthy blood donors for brucellosis and found that 27 sera agglutinated in dilution of 1:25, 25 in dilution of 1:50 and 25 in dilution of 1:100 or higher against B.abortus.

Fox et al. (1978) reported that the number of cases of brucellosis in human ^{beings} in the U.S.A. in 1965-74 was 2302. The number decreased from 262 in 1965 to 175 in 1973 but increased to 247 in 1974 and the increase was directly related to increase in incidence of bovine brucellosis.

In a sero-epidemiological survey of abattoir workers in Australia during 1977-78, Gilbert et al. (1978) reported 25% sera samples to be positive.

Mautt (1980) detected 7 acute brucellosis cases in Johannesburg (South Africa) in a period of 9 months.

In Nepal, Pyskural et al. (1980) studied the prevalence of B. abortus agglutinin in human sera and reported 2% in town folk to about 5% men in remote mountainous areas where man and animals live in close association.

Alause (1980) studied the serological evidence of exposure to B. abortus in samples from students, staff workers associated with livestock farms. Antibodies to Brucella were found significantly more commonly among those who had worked for two or more years.

In a test of sera samples from practising veterinarians in northern Spain, Cifuentes et al. (1981) found 17 positive cases out 45 Veterinary Surgeons mainly doing large animals practice.

Two patients developed acute brucellosis for the first time in Taiwan which was confirmed by blood culture (Heich et al. 1981).

Young (1981-82) described from Texas (USA) nine active cases of brucellosis.

In Poland, Stroczyński et al. (1981) tested 1,31,220 people occupationally at risk to brucellosis of which 787 cases were declared positive.

The weekly epidemiological record of England (1981) revealed 17 human cases of B. abortus and 5 cases of E. melitensis which were recorded during 1980.

Makram et al. (1982) in Iran tested 232 suspected cases of brucellosis and found 170 (733) patients positive for brucella agglutinins.

Serological tests applied to over 6000 blood samples from veterinary students of Alfort Veterinary School (France) showed a steady fall in the proportion of positive results which averaged 11-13% between 1969-73 and only 5-6% between 1978-81. The proportion invariably increased from the first to 4th year of the Veterinary course throughout the period (Pelet et al. 1984).

In another serological, epidemiological and clinical study of human brucellosis among Veterinary students from the same institution during 1969-77, Vanhoye (1985) tested 3924 serum samples and reported 10% to be positive (ranging by calendar

year from 7.4% to 15.6% and by year of study from 1.95% and 5.6% during the first two years and to 11% and 7.5% during the last two years. In a serological study of human brucellosis in Tunisia, Touel (1984) detected 8 positive cases out of 80 samples tested.

Bettelheim et al. (1984) tested serum samples from healthy blood donors for urban and rural areas of Newzealand between 1977-81 by employing micro-agglutination techniques and detected antibodies against B. abortus.

Russo et al. (1985) in Italy studied 148 hospitalised cases out of 372 reported cases of human brucellosis. 114 of these were male and 34 female. The seasonal incidence showed the main peak around January with a lower peak in June and September. The 20-29 years age groups had the highest incidence. The industrial workers (perhaps relating to animal industries) accounted for 20% of the cases, followed by agricultural workers (15%) and students (13%).

In Sicily, a considerable increase in the number of human cases of brucellosis in 1983 was due to increased consumption of raw cow's milk (B. abortus infection), fresh cheese prepared from the milk of ewes and goats (B. melitensis infection) and the contact with infected animals (Guercio et al. 1985).

Brucellosis, as an occupational disease was studied by Figueiredo (1986). He tested 1183 employees at different meat plants in Belo-Horizonte (Brazil) and found 50 (4.2%) were serologically positive and a further 54 (4.6%) had variable titres.

B. The Indian Picture:-

Reports on human brucellosis in India are very few. Perhaps human cases have been diagnosed but not regularly reported because of sporadic occurrence of the disease and multiplicity of illness. Brucellosis, probably has not been considered as a major illness for diagnosis and a large number of cases have obviously been missed.

In the words of Wallits(1948), "Brucellosis is a disease of mistakes. If we do not think of it, we miss it or we really think of it and test for it, yet, find nothing".

In 1897, Write and Smith were the first workers who diagnosed 10 cases of human brucellosis in India (Polding, 1948).

The position of human brucellosis in India has been reviewed by Mathur (1972) and Sen (1977).

Chaudhury et al.(1943) reported one case of human brucellosis from Bengal.

To detect agglutinins against B.abortus and B.melitensis in human sera samples, Joshi (1944) examined 2,082 sera in Bombay and detected 75 (3.6%) positive against B.abortus and 104 (4.9%) against B.melitensis.

Between 1941 and 1962, there are several stray reports on the occurrence of human brucellosis from different parts of India (Pandalai and Raman, 1941; Soman and Kothari, 1954; Mathur and Sood 1955; Mathur, 1955; Mathur, 1958; Mathur, 1960).

Sukla (1962) examined 150 sera from medical students, antinatal patients, dairy workers in Baroda and detected agglutinins in 92% in titre varying from 1:10 to 1:100.

In a serological examination, Mathur (1964) reported 14% incidence of brucellosis among all cases of PUO in Punjab.

In Tamilnadu, Sivarajan (1965) examined 264 patients and detected Brucella agglutinins in 12 persons; 4 out of these 12 showed high titre of 1:160.

Prakash et al. (1967) tested 3282 human sera samples in and around Delhi and found that 9.5% of the samples contained agglutinin titres 20-160 I.U./ml.

Sen (1968) summarized prevalence of human brucellosis ⁱⁿ different parts of the country as follows:

TABLE- III

<u>Locality</u>	<u>Incidence up to 1968</u>
Delhi and neighbouring areas	... 6.6% (2.2% up to 1966)
Karnal	... 4.18 to 7%
Madras city	... 0.8%
Vellore	... 3%
Kottayam	... 4%
Chingleput	... 3.5%
Trivandrum	... 0.4%
Baroda	... 1%
Cuttack	... 5%
Agra	... 1.3%
Mathura	... 0.9%
New Delhi	... 0.25%
Rohtak	... 5%
Ludhiana	... 0.5%
Calcutta	... 5%

In an epidemiological study of brucellosis among workers of the military farms at Kirkee and Pimpri and workers of butcheries at Kirkee and Poona, 306 blood samples were examined, out of which 20 were found positive (Chatterjee, 1968).

Sen and Das (1971) observed human brucellosis in 2.8% of the population near slaughter houses of Calcutta.

Serological tests were carried out with 191 serum samples from cattle sheep, buffalo and goats and 9 samples from attendants of a commercial dairy farm at Biriapur near Calcutta by Choudhury and Chatterjee (1975) which revealed that brucellosis existed in almost all species of domesticated animals in varying management condition more so in organised herds. Serum sample from one attendant out of 9 samples was also positive which proved the zoonotic importance.

In Madhya Pradesh, Soni (1976) tested 292 human sera samples (male and female) and reported sero-incidence of 19.15% in females.

Sen and Khanna (1978a; 1978b) detected 10 out of 326 human cases of pyrexia to be brucellosis from rural areas in Hooghly district in West Bengal. They also investigated occupational health problems of 46 veterinarians and 10 dairy workers and 6 slaughter house workers for brucellosis in Calcutta and 6 positive cases among veterinarians and one dairy worker were detected. Some of the abattoir workers had detectable titre.

44 human sera samples were subjected to serological tests in Haryana out of which 8 (18.1%) were found positive for brucellosis (Kulashrestha et al. 1978).

Ramana et al. (1982) studied the prevalence of brucellosis from 1976-79 in the rural population of Alwar district (Rajasthan) and the city of Delhi by serum agglutination test. The prevalence of the disease in Alwar population was 4.11% (of 1552 sera) whereas in Delhi it was 0.96% (of 2,575 sera).

In Karnataka, Nagalotimath (1979) studied 205 suspected cases of P.U.O. in human beings and found 95 cases had diagnostic titre of 80 I.U. and above against brucellosis.

Tube agglutination test was conducted with 2256 human sera samples in Punjab by Randhwa et al. (1972) and 10.7% sera were found positive for brucellosis.

Rameshchandra et al. (1981b) screened out 26 sera samples of man in Andhra Pradesh for brucellosis of which 4 (15.38%) were found positive reactors.

In a study of incidence of brucellosis in professional blood donors in Clara-Swain Mission Hospital, Barrilly (U.P.), 180 blood samples were tested by using tube agglutination test. The incidence

was highest (10.5%) at 20 I.U/ml and moderate (27.7% and 4.4%) at 40 and 80 I.U/ml respectively (Panjarathinam and Gulrajani, 1983). This agreed with the opinion of Wood (1955) who reported that blood donors were responsible for the transmission of brucellosis directly from man to man. Because professional blood donors were leading unhygienic life by sleeping on the foot path or on the grazing land in close association with the animals, the infection in animals was easily transmitted to them.

A sero epidemiological study on brucellosis was conducted by Rana et al. (1985) among workers of veterinary hospitals and slaughter houses of union territories of Delhi in which 148 sera samples from veterinarians from June '83 to March '84 were examined. The result revealed that 41 (27.7%) showed a titre of 80 I.U/ml; 9 positive individuals exhibited clinical signs. Most positive cases occurred in veterinary dispensaries (51.4%), followed by assistant veterinary surgeons (40%). Most (37%) were aged 31 to 40 years and fewest (11.6%) were aged 21-30 years. Prevalence was higher in rural than urban districts.

Patel et al. (1986) reported prevalence of infection in 3% of the 100 serum samples of hospital patients examined in Raich district of Gujarat. They suggested the cause might be due to consumption of milk. More incidence was among males and rural population.

In Orissa, Mahakur and Panda (1972) tested 215 cases of pyrexia of long duration for brucellosis out of which 8 (3.7%) cases were found positive. B. melitensis was isolated in 5 cases and 3 cases were diagnosed by the agglutination test.

Isolation and identification of the causal organism(s):

In dealing with the literature with regard to isolation and identification of the members of the genus *Brucella*, research workers in India are up to the present time concerned with B. melitensis, B. abortus and B. suis. The other three recognised species viz., B. neotomae, B. ovis and B. canis have not so far been reported in India. B. melitensis typically causes brucellosis in sheep and goats and is an important zoonosis in man.

According to Alton et al. (1975) B. melitensis causes bovine infections. In addition to contagious abortion in cattle, B. abortus is responsible for the cause of troublesome human infection which occurs quite frequently although this species rarely causes active infection in animals other than cattle (Report, 1975). B. suis is primarily a pathogen of pigs but at the same time highly virulent for man (Spink, 1974).

B. melitensis has three biotypes which differ from one another only in their behaviour with monospecific sera; B. abortus has nine biotypes on the basis of biochemical and serological differences whereas four biotypes of B. suis have been recognised so far.

According to Wilson and Miles (1975), most cases of undulant fever in man in U.K. are caused by B. abortus biotype 1, but occasional cases by biotype 5 (the so called British Melitensis Type) are on record. Among veterinarians, infection with attenuated vaccinal strain S19 is not uncommon (Hagen and Bruner, 1981).

In recent years, with the development of laboratory facilities, mostly through the aegis of FAO and WHO, complete typing of Brucella organisms has received increasing importance in developed as well as developing countries.

In Nigeria, Eze (1979) isolated B. abortus biotypes 1 and 2 from the milk of infected cattle. Subsequently, Ball and Kumi-Diaka (1980) reported isolation of biotypes 1,3 and 4.

In U.S.A., B. abortus biotype 1 as well as S19 were isolated from a herd of cattle in Colorado (Ewact et al. 1979).

Jones et al. (1983) recorded the occurrence of B. abortus biotype 1 and 2 from tissues of a naturally infected cow in Columbia.

In Malaysia, out of 168 cultures of B. abortus isolated from cattle, 86.9% were biotype 2, 10% biotype 1, 2.3% biotype 9 and 0.8% biotype 6 (Joseph et al. 1981).

Brazilian cattle were found to be infected with B. abortus biotype 1 and the same biotype was also prevalent in swine (Poester, 1961).

xxx In a test of 1500 cattle in Texas, 153 reactor were found and B. abortus biotype 1 was isolated from 63 reactors. (Crowford et al. 1983).

Mitos et al. (1984) isolated B. abortus biotypes 1,2,4,7,9 from the milk of adult Holstein cows in Italy.

In Greece, Giantzie et al. (1985) isolated 5 strains of Brucella from cattle two of which were B.melitensis (biotypes 1 and 3) and the rest B.abortus (biotypes 1 and 3).

The occurrence of B. abortus biotypes 1 and 2 has been recorded by Tsai (1985; 1986) in Taiwan.

Andreani et al. (1986) in Poland isolated B. abortus biotype 1 from the spleen, udder and lymph nodes of an arthritic cow which was serologically positive.

In an investigation of Brucella infection in Iran among cattle, Zoughi and Ebadi (1986) isolated B.melitensis from 100 animals out of 21,196 animals screened.

In India, large number of Brucella strains isolated by Palding (1943; 1947a; 1947b; 1948) were typed as B. abortus, B.melitensis and the intermediate/aberrant (A R) types. It may be remembered that the system of biotyping had not been developed by that time.

The isolation of B. abortus biotype 1 in U.P was reported by Murty and Kausik (1983).

In Tamilnadu, 28 strains of B. abortus biotype 3 were isolated by Srinivasan (1968) mostly from village cattle.

In Punjab, Mathur (1968) isolated one strain of B. abortus biotype 3 from cows of an organised farm.

The distribution of different biotypes of B. abortus in cattle was summarised by Sen (1968) on the basis of typing results at IVRI which has been summarised in table-4.

TABLE-IV

Distribution of Brucella biotypes in India up to 1968

<u>State</u>	<u>Host</u>	<u>Brucella biotypes</u>
<u>Orissa</u>		
Cuttack	Cattle	<u>B. abortus</u> type 1
Bhubaneswar	-do-	<u>B. abortus</u> types 9 & 3
<u>Bihar</u>		
Patna	-do-	<u>B. abortus</u> type 1
<u>Uttar Pradesh</u>		
Mathura	-do-	<u>B. abortus</u> type 1
Izatnagar	-do-	<u>B. abortus</u> type 3
Patnagarh	-do-	<u>B. abortus</u> type 1

<u>State</u>	<u>Host</u>	<u>Brucella biotypes</u>
<u>Punjab & Haryana</u>	Buffalo	<u>B. abortus</u> type 1
Karnal	Cattle	<u>B. abortus</u> type 5 <u>B. melitensis</u> type 1
Hissar	Cattle	<u>B. abortus</u> types 1 and 3.
<u>Tamilnadu</u>		
Ranipet	Cattle	<u>B. abortus</u> types 1, 3, 4, 6 <u>B. melitensis</u> type 2.
<u>Andhra Pradesh</u>		
Kurnool	Cattle	<u>B. melitensis</u> type 1

According to Mathur (1985), cows and buffaloes of organised farms all over India harbour mostly B. abortus biotype 1 infection and exceptionally biotype 3.

Halder and Sen (1986) reported isolation of B. abortus biotype 1 in Calcutta by plating milk cream sediment mixture on 1% tryptose agar plate containing crystal violet and brilliant green.

CHAPTER III
MATERIALS AND METHODS

CHAPTER-III

MATERIALS AND METHODS

a) General:

The present investigation centred around organised dairy herds of Orissa located in different districts. The entire lot of animals included in the study belonged to government dairy/cattle breeding farms managed by the state government and the government of India. At present, there are no private dairy herds in Orissa with sizeable number and, further, the testing of village herds was beyond the scope of this limited work.

The management and feeding in all the farms included in this work are almost identical although the two government of India farms, which have come into being in recent year, are better planned and managed as compared to most of the state government farms which are in existence even before the formation of the state of Orissa in 1936.

Artificial insemination is practised in all these farms either with liquid semen or, of late, with frozen semen. The names of the farms, location and the zone of the state to which they belong have been presented in table-V and the situation of these farms has been reflected in the map appended.

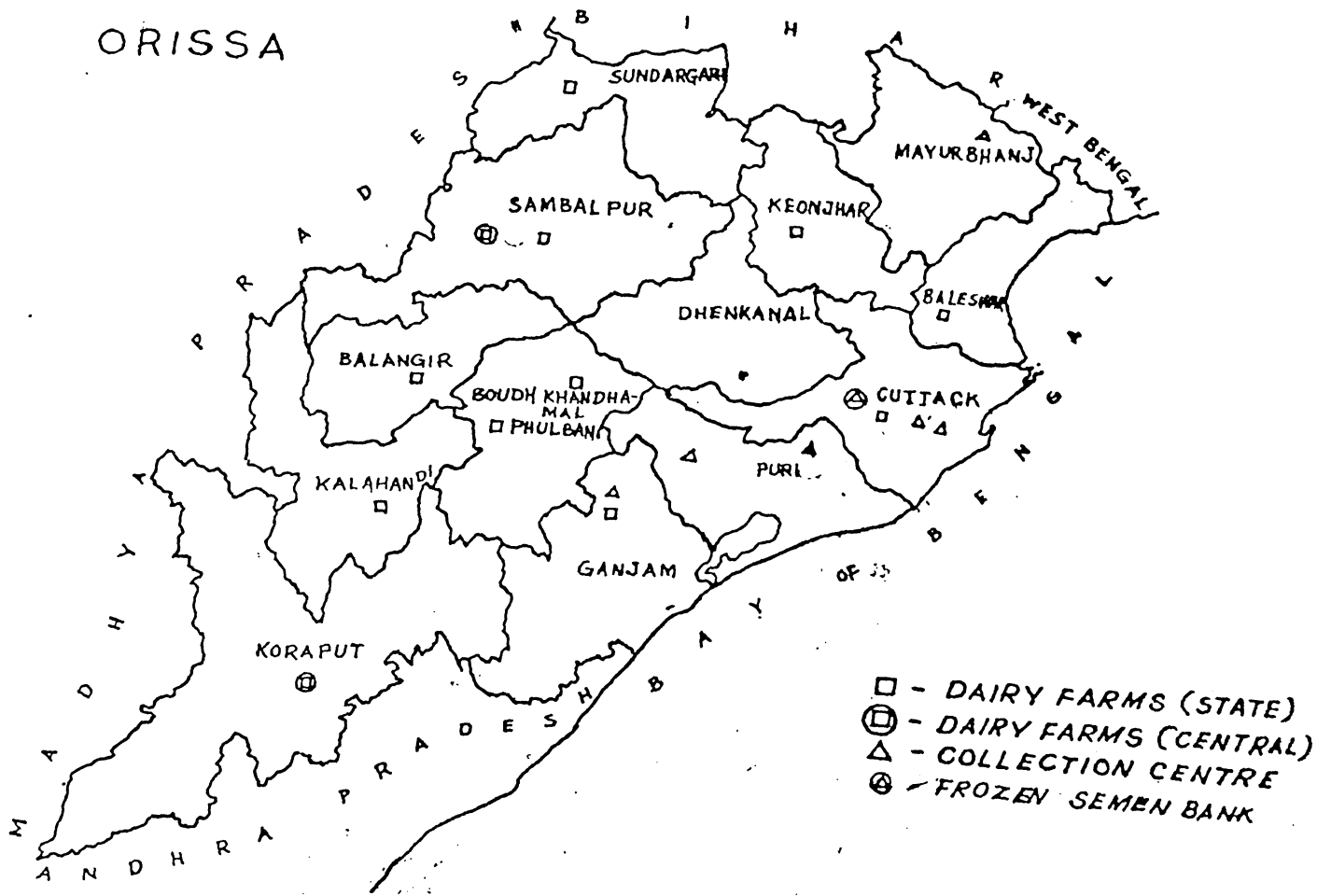
TABLE-V

Details of dairy/cattle breeding farm surveyed

Name of the farm	Location	District	Geographical zone	Total no. of live-stock available.
1. Bhawanipatna	Bhawanipatna	Kalahandi	Western zone	87
2. Remuna	Remuna	Balasore	Eastern zone	105
3. Sundargarh	Sundargarh	Sundargarh	Western zone	109
4. Kuanrmunda	Kuanrmunda	Sundargarh	Western zone	63
5. Bolangir	Bolangir	Bolangir	Western zone	105
6. Khepuria	Khepuria	Cuttack	Eastern zone	99
7. Chipilima	Chipilima	Sambalpur	Western zone	129
8. Boudh	Boudh	Phulbani	Southern zone	53
9. Bhanjanagar	Bhanjanagar	Ganjam	Southern zone	65
10. Phulbani (UGS farm)	Phulbani	Phulbani	Southern zone	28
11. Central farm Sunaboda	Sunaboda	Koraput	Southern zone	615
12. Central farm Chipilima	Chipilima	Sambalpur	Western zone	276
13. Gosadan (Babandh)	Babandh	Dhenkanal	Eastern zone	37
14. Keonjhar farm	Keonjhar	Keonjhar	Western zone	61
15. S.C.C. Kendrapara	Kendrapara	Cuttack	Eastern zone	11
16. S.C.C. Salipur	Salipur	Cuttack	Eastern zone	9
17. S.C.C. Nayagarh	Nayagarh	Puri	Eastern zone	7
18. S.C.C. Bhubaneswar	Bhubaneswar	Puri	Eastern zone	8
19. S.C.C. Baripada	Baripada	Mayurbhanj	Western zone	7
20. Miscellaneous	-	-	74 (Venusin Pvt. farm and serum samples from different sources for test.)	

Grand total:

1948



MAP SHOWING THE LOCATION OF DIFFERENT DAIRY FARMS WHERE BRUCELLOSIS TEST WAS DONE.

b) Selection of animals:

In selecting animals for serum agglutination tests, animals below six months of age were excluded. There was no history of vaccination with Brucella abortus strain 19 during the past 10 years and there were no records available as to the infection status in these herds and tests done, if any, against brucellosis barring published papers in the early sixties. None of the farms had buffaloes, and there were no other domesticated animals such as sheep and goats inside the farm premises. The animals were exclusively stallfed and watered inside the premises except on rare occasions when they were being taken to nearby ponds for cleaning and bathing. Abortions, though not frequent, were known to occur in all these farms; but actual figures were not available.

c) Collection of serum samples:

Blood samples, in quantities of 5-10ml were collected individually from the jugular vein, mostly in the early hours of the morning. After collection, samples were transferred to test tubes with cotton plug or screw cap vials and kept slanting until the coagling of serum. During the hot days, these

slants were left at room temperature for 2-3 hours and then stored inside a refrigerator ($\pm 4^{\circ}\text{C}$). In any case, except under most adverse conditions, the plate test was performed and completed before sunset. For the tube agglutination test, either the slants or serum samples separated in small vials were brought to the college laboratory either the same day or next day by keeping them inside thermoflasks containing ice. Before the day's work, syringes and needles were thoroughly boiled and rinsed with sterilized normal saline solution (NSS) atleast five times, before bleeding the next animal. All the test tubes, serum cap vials, Pasteur pipettes and other glasswares were sterilized in the hot air oven, properly plugged and wrapped, at 160°C for 30 minutes or 150°C for one hour. For performing plate agglutination test on the spot, there was no necessity, in most cases, to centrifuge the blood for separating the serum. For the tube agglutination test, however, blood clots were centrifused at 3000 r.p.m. for 15-30 minutes and only clear serum samples were used. In the event of some delay, serum vials, properly labelled were left inside a deep freezer (-10 to -15°C) or freezing chamber of the refrigerator (-4°C). No preservative was added and all attempts were

made to complete the serological tests in 2-3 days time.

d) Plate and tube agglutination tests:

Antigens for these two tests were obtained either from the Institute of Veterinary Preventive Medicine, Ranipet or Indian Veterinary Research Institute, Izatnagar and stored constantly inside refrigerator at $\pm 4^{\circ}\text{C}$. The plate test was performed on the lines of Dhanda and Rajgopalan (1949). On a ruled glass plate, 3 drops of serum, 2 drops (0.08ml) in the first square and one drop (0.04ml) in the next square, were placed and to these, one drop of the coloured antigen was added separately and mixed with a toothpick. After mixing of the serum with antigen, the plate was held over a white surface reflecting refracted light and rocked gently from side to side for 4-5 minutes. In strongly positive cases, visible agglutination could be observed during the first 30 seconds in both the squares and such a reaction was graded +++. The reaction was graded ++ when reaction was observed after 2 minutes but before 3 minutes. One plus reaction was characterised by fine powdery particles appearing between 4-5 minutes. A sample was declared negative if no visible agglutination could be observed even with the help of a hand magnifying glass up to 5 minutes.

a) Tube agglutination test:-

Five sugar tubes or Kahn's agglutination tubes were placed in a rack and 0.8ml of carbosaline was put in the first tube followed by 0.5ml in the other tubes. 0.2ml of serum under test was added to the first tube, mixed well with a pipette or tuberculin syringe and 0.5ml from this tube was transferred to the second and mixed thoroughly. This process was continued up to the last tube from which 0.5ml of serum saline mixture was discarded. Standardised B. abortus plain antigen was added @ 0.5ml to each tube, mixed by rolling or slight shaking. The rack was left at 37°C for 24 hours.

In order to determine 50% agglutination, which is accepted internationally as the end titre, a set of antigen control tubes was put for each day's work for comparing the results (Sen, 1984). The pattern of setting the test has been provided below. The control tubes were always incubated along with the test samples in the same incubator or water bath and results of the test were recorded only if control tubes were found to be in satisfactory state.

Protocol of the antigen control test:-

<u>Antigen control tubes.</u>	<u>0.5% Carbol saline</u>	<u>Antigen</u>	<u>Degree of agglutination.</u>
Tube-I	1.0 ml	1.0ml	No agglutination
Tube-II	1.25	0.75	25% agglutination
Tube-III	1.50	0.50	50% agglutination
Tube-IV	1.75	0.25	75% agglutination
Table-V	2.0	Nil	100% agglutination

The degree of agglutination was judged by opacity of the supernatant fluid.

- (i) ++++ (Comparable with tube V of the antigen control series).
- (ii)+++ (Comparable with tube IV of the antigen control series).
- (iii)++ (Comparable with tube III of the antigen control series).
- (iv) + (Comparable with tube II of the antigen control series).
- (v) No agglutination comparable with tube I of the antigen control series.

50% agglutination was considered as the end point. To express in unit system, double of the serum titre showing 50% agglutination was the total number of International Units (I.U) per ml of serum. In females, an end titre of 80 I.U. and above was accepted as positive, 20-40 I.U. as doubtful and less than 20 I.U. as negative. In male animals, however, a serum titre of 40 I.U. was

diagnostic, 20 I.U. doubtful and less than 20 I.U. was negative.

d) Milk ring (ABR) test:

This test was performed as outlined by Alton et al.(1975). Milk samples were collected from individual animals in 14x100mm test tubes and mixed thoroughly for even dispersal of the cream. One ml quantities of milk were transferred to sugar tubes and one drop (0.03ml) of the antigen shaken well after taking out of the refrigerator was added to each tube. Milk and antigen were mixed thoroughly by gently shaking and inverting the tubes several times. The racks with tubes were left inside water bath/incubator at 37°C and results recorded on the lines of Pande and Mishra (1963) and samples showing ± reaction and above were graded as positive. Antigen for this test was obtained from Ranipet and stored inside the refrigerator.

e) Testing of human sera samples:-

Although it was not the purpose of this investigation to attempt a survey of human brucellosis, yet at several places human beings, mostly working in dairy farms, volunteered to get their serum samples tested against brucellosis. Using the same methods, as described above for cattle, a total of 175 samples could be tested as per details given below in table-6.

TABLE-VI

Details of human sera samples tested against brucellosis

Locality	No. of samples tested.	Brief particulars of persons
Bhawanipatna	13	Vets, paravets and animal attendants
Bhanjanagar	9	-do-
Bhubaneswar	6	-do-
Bolangir	14	-do-
Boudh	9	-do-
Chipilima(State farm)	17	-do-
Chipilima(Central farm)	14	-do-
Cosoda(Sabandh, Dhenkanal)	2	Paravets and animal attendants
Khapuria	13	Vets, paravets and animal attendants
Kendrapara	16	-do-
Kuanrunda(Rourkella)	17	-do-
Nayagarh	4	Vets, paravets and animal attendants
Phulbani	3	-do-
Remuna	5	-do-
Salipur	9	-do-
Sundargarh	10	-do-
Sunabada	9	-do-
Miscellaneous	20	Samples from hospital patients with history of P.U.D. submitted by medical officers P.H.C. and rural health centres for Brucella test.
Total	190	

Isolation and identification:-

The established practice is to confirm positive serological findings by isolation of the causative organism(s) and accordingly attempts were made to isolate and identify, to the extent possible, Brucella organisms prevailing in infected animals/herds of Orissa. Initial attempts to obtain aborted materials such as placentae, uterine discharge and aborted calves in a fresh state, fit for isolation studies, were not successful, in as much as such materials were grossly contaminated either on account of unhealthy surroundings or were partially putrefied. It was, therefore, decided to attempt isolation from milk samples of cows with significantly high serum titre (640-1280 I.U./ml) and showing +++ reaction to the ABR test.

The criteria for selection of milk samples and their processing were on the lines of Stableforth and Galloway (1959) and Alton *et al.* (1975). After stripping for 4-5 times, pooled milk samples from individual cows were drawn into large sterilised test tubes (25x150mm) almost up to half, from all four quarters and stored after labelling cow-wise in the refrigerator (+4°C) or inside thermoflask containing ice until they were brought to the laboratory for processing. In every case, samples were collected in the evening and brought to the laboratory as quickly as possible but latest by next morning even from long

distances by availing superfast night bus services. The udder and teat of the animals were cleaned and disinfected as is usually done for the collection of mastitic milk.

In the laboratory, milk samples were thoroughly homogenised by shaking and 20ml quantities were pipetted into a set of sterilized test tubes using separate sterilized pipettes, and centrifuged in an IEC (made in U.S.A.) bottle centrifuge at 5000 r.p.m. for 30 minutes. The sediment and cream inside the tubes were mixed after pipetting out the milk column in between these two layers and reconstituted by adding sufficient sterilised and chilled N.S.S. to make 2ml volumes in each tube.

Adult guineapigs of either sex were obtained through the kind courtesy of the Joint Director, Orissa Biological Products Institute, Bhubaneswar and Pathologist and Bacteriologist, Government of Orissa, Cuttack. The animals were maintained and observed in the laboratory for 4-5 days at the end of which they were bled from the heart and the serum samples tested for the presence of Brucella agglutinins with B. abortus plate (quick) antigen. None of the guineapigs had observable agglutinins against B. abortus. One ml quantities of the cream cum sediment mixture were inoculated subcutaneously into sets of two guineapigs with each sample. The experimental animals were fed

and watered on standard lines and observed until they were sacrificed. Such of the animals which died within the first week were ignored for the purpose of isolation of Brucella.

Out of the two guineapigs inoculated with milk of one cow, one was bled from the heart on the 21st day and sacrificed by cutting the throat. The other guineapig was maintained up to the 42nd day and dealt similarly as in the case of the first guineapig. Serum samples from heart blood were screened for the presence of Brucella agglutinins as described earlier. Irrespective of the results, small pieces of the following tissues, obtained at postmortem were removed by sterile forceps and scissors, and inoculated into duplicate tubes of tryptose broth (pH 6.8).

1. Liver
2. Spleen
3. Kidneys

One set of culture tubes was incubated aerobically and the other under 10% CO₂ inside a Macdintosh and Field's anaerobic jar (4 litre capacity) at 37°C. The method of generating 10% CO₂ was on the lines of Alton et al. (1975). One gram of NaHCO₃ was kept inside a test tube to which 1ml of concentrated HCl was added. CO₂ was liberated with visible effervescence inside the tube. An indicator solution (0.1% Na₂HCO₃ with 1 drop of 0.5%

bromothymol blue aqueous solution) was also kept inside the anaerobic jar. Approximately 10% CO₂ tension could be constantly maintained by this method inside the jar as indicated by the reduction of the colour of the indicator from blue to green. Growth inside the culture tubes, if any, was observed daily and such of the tubes which revealed turbidity were taken out for plating.

Tryptose agar (pH 6.8) was used for plating from broth cultures showing visible growth as recommended by Spink (1956). After thorough shaking, one 4mm loopful culture from tryptose broth was plated on two sets of tryptose agar in 8 or 10cm petriplates and incubated aerobically and under 10% CO₂ tension as described above. In most positive cases, there was visible growth in the broth tubes by the end of 48 hours. The tryptose agar plates were observed daily and in positive samples colonies were visible also at the end of 48 hours. Isolated colonies suggestive of Brucella were transferred to tryptose agar slants and incubated as before.

Such of the tryptose agar slants which revealed confluent growth and did not possess Gram negative coccobacillary organisms on staining were discarded. The rest of the cultures were subjected to preliminary serological tests by mixing small quantities of the growth, smeared with a platinum loop with one

loopful of Brucella abortus positive serum obtained from Ranipet. Such of the cultures which agglutinated within 30 seconds were tentatively considered as Brucella and those which reacted late or negatively were discarded. Altogether 26 milk samples from reactor cows were induced in the isolation trials.

All the probable cultures were subjected to the following tests as described by Alton et al. (1975):-

1. CO₂ requirement
2. H₂S production
3. Urease activity
4. Growth in the presence of dyes (thionin and basic fuchsin)
5. Agglutination in monospecific sera (Abortus and Melitensis).

1. CO₂ requirement:- The method employed has since been described earlier.
2. H₂S production:- The cultures to be tested were inoculated on liver infusion agar slopes and dried lead acetate filter paper strips inserted between cotton plug and mouth of the tube. Production of H₂S was indicated by browning or blackening of the tip of the strips. If there was no change up to the 7th day, the test was considered negative.
3. Urease test:- The method of Christensen (1946) was followed. The urea agar slants were inoculated on the surface with a loopful of culture from tryptose agar kept at room temperature and observed every hour and finally after 24 hours. Urease production

was indicated by change of the colour of the medium to purple - pink from pale yellow.

4. Growth in the presence of dyes:- Tryptose agar was the basic medium used to which thionin and basic fuchsin were added at the following final concentrations:

(i) Thionin | a) 1: 25,000
 | b) 1: 50,000
 | c) 1: 100,000

(ii) Basic fuchsin | a) 1: 50,000
 | b) 1: 100,000

On 10 cm. Petri plates, 5 to 6 cultures were inoculated in single straight lines from tryptose agar slants. These were properly labelled and incubated in the usual manner. Development of growth was observed daily up to 7 days.

All culture media and dyes used were of Himdia Laboratories, Bombay.

5. Agglutination in monospecific sera:-

Monospecific sera, both Abortus and Melitensis, were kindly supplied by a) Central Veterinary Laboratory, Weybridge, England and b) National Animal Diseases Laboratory, Iowa, U.S.A.

Smooth colonies from tryptose agar plates were grown on tryptose agar slants and dense

suspensions prepared by harvesting the growth in 2ml saline. The suspensions were heated at 65°C in a waterbath for one hour (heat-killed antigen) and both plate and tube agglutination test were conducted as described by Altom et al. (1975) using monospecific sera. Each antigen lot was tested in two separate rows, one with 'Abortus' and the other with 'Melitensis' serum. The tubes were incubated and results recorded as per standard Brucella tube agglutination test.

CHAPTER IV
RESULTS

CHAPTER-4

R E S U L T S

In order to present the results, the state of Orissa was divided into four agroclimatic zones as detailed below:

Eastern (Coastal) Zone:

This zone includes Cuttack, Balasore, Puri and Dhenkanal districts of Orissa, the first/^{three} of which are situated close to the bay of Bengal. The last named district viz., Dhenkanal is about 100 Kms from the sea shore, but is adjacent to the other 3 coastal districts mentioned earlier.

Western Zone:

Included in this zone are the districts of Sambalpur, Sundergarh, Bolangir and Kalahandi which have a humid climate with plenty of forests and hilly tracts.

Northern Zone:-

Only two districts are included in this zone viz., Mayurbhanj and Keonjhar which climatically bear close resemblance with the districts of the western zone.

Southern Zone:-

Three districts viz., Ganjam, Phulbani and Koraput are included in this zone. The district of Ganjam is located in the coastal belt of Orissa and is

climatically more akin to the districts of eastern zone. Kalahandi and Koraput districts, On the other hand, are hilly tracts and their climate bears close resemblance to the districts of northern and western Orissa.

There is no significant difference in rain fall in the four zones, although the coastal districts of the eastern and southern zones receive a slightly higher and comparatively regular rain fall, under the influence of bay of Bengal.

Serum agglutination tests:-

Although plate and tube agglutination tests were conducted separately, the final results which emerged after performing both the tests have been summarised in table VII.

// For table VII please see page 66 //

TABLE-VII

Results of Brucella serum agglutination tests in cows and female livestock in different farms of Orissa

Location	No. of cows and other female livestock available	Breed	Number actually tested	Number positive with percentage.	Number doubtful with percentage	Number negative
<u>Eastern zone</u>						
Khapuria	62	Mixed	53	1(1.8)	0	52
Remuna	81	Mixed	64	0(0)	0	64
Miscellaneous	-	Mixed	78	3(3.8)	2(2.5)	73
<u>Western zone</u>						
Bhawanipatna	69	Jersey	60	34(56.6)	6(10)	20
Bolangir	94	Mixed	94	2(2.1)	0	92
Chipilima (State Farm)	78	Mixed	78	21(26.9)	0	57
Chipilima (Central Farm)	109	Red sindhi & Jersey	29	0(0)	0	29
Kuanraunda (Rourkela)	59	Exotic	59	0(0)	0	59
Sundargarh	103	Exotic	103	0(0)	0	103
<u>Northern zone</u>						
Keonjhar	47	Mixed	19	0(0)	0	19
<u>Southern zone</u>						
Bhanjanagar	43	Mixed	26	0(0)	0	26
Boudh	37	Mixed	24	0(0)	0	24
Phulbani	21	Mixed	21	0(0)	0	21
Sunabada (Central Farm)	436	Jersey	69	0(0)	0	69
Total:	1239		777	61	8	708

As may be seen from the above table, two cattle farms viz., Bhawanipatna and Chipilima, both located in western zone, had significantly high percentage of positive reactors to the tune of 56.6 and 26.9 respectively. In addition, there were quite a few doubtful reactors also, 10% in Bhawanipatna and 5% in Chipilima. Barring these two farms, the overall picture which emerged out of serum agglutination tests was rather a happy one, in as much as animals tested in the entire southern and northern zones, 3 out of 6 farms of western zone (Chipilima Central, Kuanrunda and Sundargarh) and the rural dairy at Remuna in eastern zone were found to be totally free from reactors. In rest of the places, where reactors were detected, the percentage was rather low, maximum being 3.8 and 2.5 doubtful. It is worthwhile mentioning that the entire lot of animals in the two badly affected farms at Bhawanipatna and Chipilima (State) were either pure or cross Jersey animals.

Table VIII summarizes results of present investigation ^{as compared} with those of earlier studies of Orissa among female farm animals.

TABLE-VIII

Comparison of the prevalence rate of Brucella reactors among cows and other female stock in different farms with figures of past investigations in Orissa

Location	Earlier figures in %		Present rate(%)
	Panda et al. (1962)	Panda and Dass (1965)	
Bhanjanagar	19.3	41.7	0
Bhawanipatna	-	-	56.6
Bolangir	61.7	8.7	2.1
Boudh	17.3	47.0	0
Chipilima (State Farm)	-	-	26.9
Chipilima (Central Farm)	-	-	0
Keonjhar	3.1	4.5	0
Khepuria farm	-	10.8	1.8
Remuna	-	-	0
Sundergarh	16.6	-	0

Milk ring (ADR) test:-

The results of milk ring test, in respect of individual cows, performed in different farms and tabulated in table IX revealed 100% reactors in Bhuvanipatna where the serum tests had already proved the existence of brucellosis in an endemic form (Vide Table-VI). Similarly, cows of Chipilima state farm also had high reactor rate (36%) on the basis of results of ABR test. This farm, incidentally, had 26.9% positive reactors among female stock on the basis of serum agglutination tests (SAT). The only farm where 100% positive reactors were observed is located in a remote area of Dhenkanal district which maintains animals reacting positively to the important infectious diseases in isolation. As such, all the 7 cows tested were positive to ABR and this confirmed their infection status. Thus, the results of the milk ring test were in agreement with those of serum agglutination test in all the 4 zones of the state. No separate attempt has been made to tabulate the comparative data in respect of serum and milk tests in as much as the results were in close agreement with each other.

Table X summarizes the results of serum tests in bulls and other male animals in different places of Orissa.

TABLE-IX

Results of milk ring (ABR) test in milch cows of different farms in Orissa

Location	No. of animals available for test	No. actually tested	No. found positive with percentage	No. found negative
<u>Eastern zone</u>				
Dhenkanal (Babondh)	7	7	7(100)	0
Khapuria	48	48	1(2.0)	47
Remuna	45	45	0(0)	45
Miscellaneous	38	38	4(10.5)	34
<u>Western zone</u>				
Bhawanipatna	26	26	26(100)	0
Bolangir	24	24	2(8.3)	22
Chipilima (State)	72	72	26(36)	46
Chipilima (Central)	95	95	0	95
Kuanrunda	21	21	0	21
Sundargarh	52	52	0	52
<u>Northern zone</u>				
Keonjhar	26	26	0	26
<u>Southern zone</u>				
Bhanjanagar	26	26	0	26
Boudh	24	24	0	24
Phulbani	8	8	0	8
Sunabada	197	197	0	197
Total	709	709	66	643

TABLE-X

Results of serum tests in bulls and other male animals in different places of Orissa

Location	No. of animals available	No. of animals tested	No. found positive with percentage	No. of doubtful reactors	No. found negative
<u>Eastern zone</u>					
Khapuria dairy farm	19	14	0	0	14
Khapuria F.S.B.	18	15	2(13.3)	0	13
Bhubaneswar S.C.C.	8	8	1(12.5)	0	7
Nayagarh S.C.C.	7	7	2(28.5)	0	5
Kendrapara S.C.C.	11	11	0	0	11
Remuna Dairy farm	24	16	0	0	16
Salepur	9	9	0	0	9
<u>Western zone</u>					
Bhuvanipatna	18	16	Nil	0	16
Bolangir	11	7	0	0	7
Chipilima State Farm	51	39	0	0	39
Chipilima Central Farm	167	96	0	0	96
Kumarmunda	4	4	0	0	4
Sundergarh	6	6	0	0	6
<u>Northern zone</u>					
Keonjhar	14	6	0	0	6
Baripada S.C.C.	7	7	1(14.2)	1	5
<u>Southern zone</u>					
Boudh	16	14	0	0	14
Bhanjanagar dairy farm	14	14	0	0	14
Bhanjanagar S.C.C.	8	8	0	0	8
Phulbani	7	4	0	0	4
Sunabeda	179	84	0	0	84
Total:	598	385	6	1	378

TABLE-XI

Comparison of the prevalence rate of Brucella reactors among bulls and other male livestock in different farms with figures of past investigations in different places of Orissa

Location	Earlier figures Panda and Das(1965) (%)	Present rate (%)
Khapuria	(6.1)	0
Khapuria(Frozen Semen Bank)	-	13
Bhubaneswar (Semen Collection Centre- S.C.C.)	-	12.5
Nayagarh	-	28.5
Kendrapara	-	0
Salipur	-	0
Bhawanipatna	-	0
Bolangir	3	0
Chipilima (State Farm)	-	0
Chipilima (Central Farm)	-	0
Kuanrunda	-	0
Sundergarh	-	0
Keonjhar	0	0
Baripada	0	14.2
Boudh	16.6	0
Bhanjnegar Dairy Farm	10	0
Bhanjnegar S.C.C.	-	0
Phulbani Farm	-	0
Sunabeda	-	0
Buxibazar (Regional A.I. Centre)	0	-
Patha (Cutteak)	0	-

Perusal of this table would show that the largest concentration of male animals was existent in three organised herds, which may be termed as breeding centres. Two of them, one at Sunabeda and the other at Chipilima, belong to the Government of India and both these were found totally free from brucellosis. The male animals of Chipilima state farm were also free from brucellosis, although the female stock were definitely infected as recorded earlier. The reactor rate of 28.5% at Nayagerh, 13% at Khapuris, 12.5% at Bhubaneswar and 14.2% at Baripada may appear alarming; but the figures do not appear statistically significant when one looks at the very small number of animals tested and one or two reactors detected in these places. Notwithstanding this observation, the significance of even small number of bulls reacting positively to Brucella agglutination test will be discussed at length in the next chapter.

Once again, the prevalence of brucellosis in male stock, presently detected, has been compared with that of 1962 and 1965 in table- XI.

Brucellosis in human beings:-

Results of this small-scale investigation have been reflected in table XII. Here again, attention is bound to be drawn towards Bhawanipatna and Chipilima

state farms where the percentage of reactors in animals was high and naturally the workers of these farms stood exposed to Brucella infection as an occupational hazard. In both these places, positive reactors could be detected (3 out of 13 at Bhawanipatna and 2 out of 17 at Chipilima). In Bolangir farm, there were 2 reactors (1 positive and the other doubtful), out of 14 samples tested. Isolated cases (1 each) were detected at Kuarmunda (Rourkela), Kendrapara and Salipur (Cuttack district). The figures under "Miscellaneous" head represent those serum samples which were referred by the medical college at Cuttack primary health centres, hospitals and dispensaries located in and around Shubaneswar. Interestingly, 4 out of 20 samples reacted positively and another 2 doubtfully to the Brucella tube agglutination test. It was not possible to attempt isolation of causative organism(s) from human reactors of the farm; but these reactors had the history of intermittent fever and, in addition, some of them complained of orchitis, arthritis and neuralgia. In a couple of cases, the intermittent fever was serious enough and warranted their transfer from the farm to local veterinary dispensaries with a view to keeping them away from infected surroundings.

TABLE-XII

Prevalence of Brucella reactors among human beings

Location	No. of samples tested	No. of samples found positive with percentage	No. of samples found doubtful with percentage	No. of samples found negative
Bhawanipatna	13	3(23.0)	1(7.6)	9
Bhanjanagar	9	0(0)	0	9
Bhubaneswar	6	0	0	6
Bolangir	14	1(7.1)	1(7.1)	12
Boudh	9	0	0	9
Chipilima State Farm	17	2(11.7)	0	15
Chipilima Central Farm	14	0	0	14
Gosadan(Babandh) (Dhenkanal)	2	0	0	2
Kuanrunda	17	1(5.8)	0	16
Khapuria	13	0	0	13
Nayagarh	4	0	0	4
Phulbani	3	0	0	3
Remuna	5	0	0	5
Salipur	9	1(11.1)	0	8
Sundargarh	10	0	0	10
Sunabeda	9	0	0	9
MISCELLANEOUS	20	4	2	14
Total:	190	13	4	173

Isolation and Identification:-

Barring 3 instances, the guineapigs inoculated with centrifuged specimens of milk survived up to the desired period of time i.e. 21 and 42 days post-inoculation. Except in the case of two, from which Brucella organisms could be successfully isolated, the serum samples of the other guineapigs agglutinated B.abortus coloured antigen within 30 seconds when tested on the 21st day. However, most of the animals reacted negatively or doubtfully when tested on the 42nd day. Even then, organisms could be isolated from such negative animals without much difficulty by the isolation procedures adopted. Tissue wise, the spleen yielded 100% isolations, whereas liver and kidney were positive in approximately 65% and 43% of infected guineapigs. Since the total number of samples were only 26, no statistical analysis was possible.

The differential characteristics of all the 26 Brucella strains isolated from milk of infected cows has been presented in table-XIII.

TABLE-XIII

Differential characteristics on 26 Brucella strain isolated from milk of infected cows

Laboratory number of isolates	CO ₂ requirement	H ₂ S production	Urease activity	Growth in presence of dyes:					Agglutination in monospecific sera		Species and biotype (biovar)
				Thionin			Basic Fuchsin		A	M	
				1: 25,000	1: 50,000	1: 100,000	1: 50,000	1: 100,000			
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)
P ₁ T ₁	+	+	1-2 hours	+	+	+	+	+	+	-	<u>Brucella abortus</u> Biotype-3
P ₁ T ₂	+	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₂ T ₁	-	+	1-2 hours	-	+	+	+	+	-	+	<u>B. abortus</u> Biotype-9
P ₂ T ₁	-	+	1-2 hours	-	+	+	+	+	-	+	<u>B. abortus</u> Biotype-9
P ₃ T ₂	+	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-1
P ₃ T ₂	+	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-1
P ₄ T ₂	+	+	1-2 hours	-	-	-	+	+	+	-	<u>B. abortus</u> Biotype-1
P ₄ T ₁	+	+	1-2 hours	-	-	-	+	+	+	-	<u>B. abortus</u> Biotype-1
P ₅ T ₁	+	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₅ T ₂	+	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₆ T ₁	-	+	1-2 hours	-	+	+	+	+	+	-	<u>B. abortus</u> Biotype-6
P ₆ T ₂	-	+	1-2 hours	-	+	+	+	+	+	-	<u>B. abortus</u> Biotype-6
P ₇ T ₁	-	+	1-2 hours	-	+	+	+	+	-	+	<u>B. abortus</u> Biotype-9
P ₇ T ₂	-	+	1-2 hours	-	+	+	+	+	-	+	<u>B. abortus</u> Biotype-9
P ₈ T ₁	-	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-9
P ₈ T ₂	-	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₉ T ₁	-	+	1-2 hours	-	+	+	+	+	+	-	<u>B. abortus</u> Biotype-6
P ₉ T ₂	-	+	1-2 hours	-	+	+	+	+	+	-	<u>B. abortus</u> Biotype-6
P ₁₀ T ₁	-	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₁₀ T ₂	-	+	1-2 hours	-	-	-	+	+	+	-	<u>B. abortus</u> Biotype-1
P ₁₁ T ₁	-	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₁₁ T ₂	-	+	1-2 hours	+	+	+	+	+	+	-	<u>B. abortus</u> Biotype-3
P ₁₂ T ₁	-	+	1-2 hours	-	+	+	+	+	+	+	<u>B. abortus</u> Biotype-7
P ₁₂ T ₂	-	+	1-2 hours	-	+	+	+	+	+	+	<u>B. abortus</u> Biotype-7
P ₁₃ T ₁	-	+	1-2 hours	-	+	+	+	+	+	+	<u>B. abortus</u> Biotype-7
P ₁₃ T ₂	-	+	1-2 hours	-	+	+	+	+	+	+	<u>B. abortus</u> Biotype-7

A B S T R A C T

<u>Biovar</u>	<u>No. of strains isolated</u>
<u>B. abortus</u> Biovar- 1:	3
<u>B. abortus</u> Biovar- 3:	11
<u>B. abortus</u> Biovar- 6:	4
<u>B. abortus</u> Biovar- 7:	4
<u>B. abortus</u> Biovar- 9:	4

Total: 26

It may be observed that altogether 5 different biotypes of B.abortus were isolated from 26 samples and their distribution, zone-wise, was as follows:-

	<u>B.abortus</u>		<u>No. of strains isolated</u>
<u>Western zone:-</u>	Biotype-1	3
	Biotype-3	9
	Biotype-6	4
	Biotype-7	4
	Biotype-9	2
<u>Eastern zone:-</u>	Biotype-3	2
	Biotype-9	2

As regards southern and northern zones, suitable clinical materials could not be procured from the stray reactors and as such no isolations could be possible.

On the basis of various differential tests deployed, all the 26 isolates were found to be B.abortus. Among the prevailing biotypes in Orissa type-3, accounted for the largest number i.e. 11, followed by biotypes 6, 7 and 9 numbering 4 each and the other three belonged to biotype 1.

Coloured photographs of the dye inhibitin test performed with 12 isolates have been appended and illustrated vide plate 1. Representative photographs of H₂S and urease test, performed have also been shown in plates 2 and 3.

Plate - 1

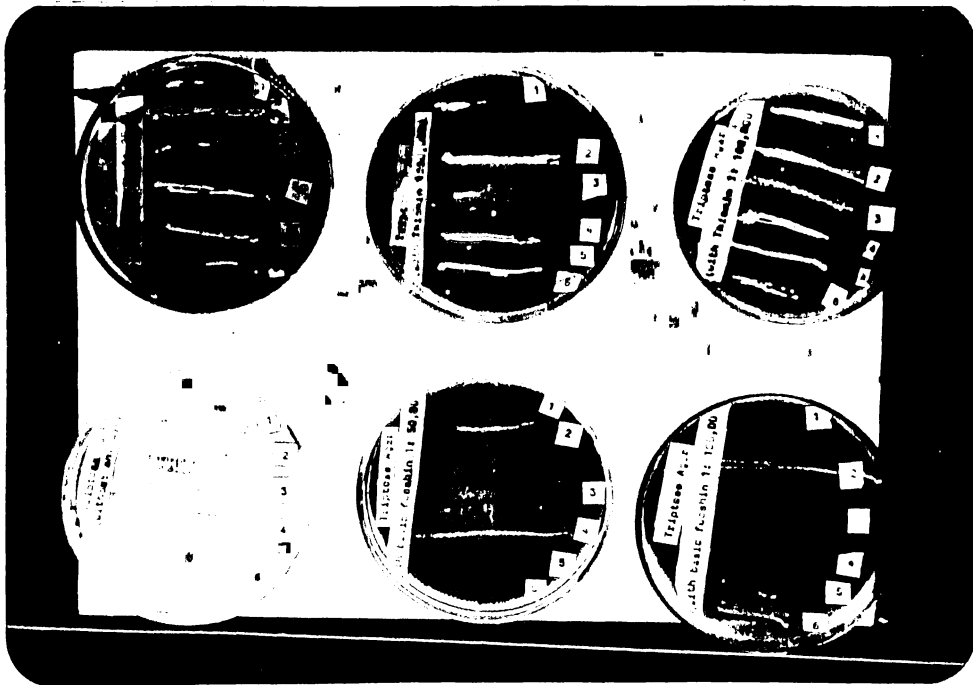


Fig. 1.

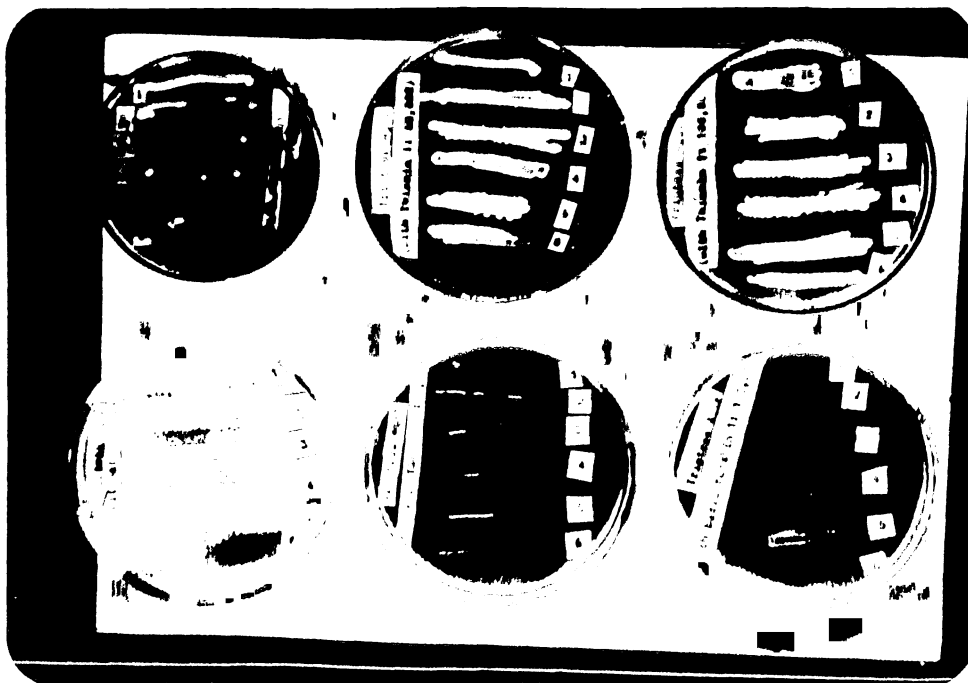


Fig. 2



Plate-2

Fig. 1



Plate-3

Fig. 1

CHAPTER V
DISCUSSION

CHAPTER-5

D I S C U S S I O N

The object of this investigation was to reassess the prevalence of bovine brucellosis in Orissa which, as may be appreciated, is a tremendous task considering that the state of Orissa has a bovine population ^{of} nearly 13 million, mostly living in the vast rural set up under varied agroclimatic and animal husbandry conditions. It was, as such, felt appropriate that the work should be carried out in stages, to start with in organised dairy herds, commonly known as farms, and subsequently in village herds. Brucellosis, as a disease of cattle, has all along been a heavy burden on the agricultural economy of various countries, but of late has been virtually brought under control in many countries through years of sustained, painstaking and costly preventive and control measures. In recent years, more and more countries are slowly reaching the goal of near elimination of this disease, an achievement which will not only bring laurels to the veterinary activities of these countries but also be a major contribution to the science of Veterinary Public Health.

Lack of organised control measures in respect of animal diseases has made the countries of the third world highly vulnerable to the vagaries of several cattle diseases of which brucellosis can justifiably be ranked as one of the most important infections. Notwithstanding

its wide prevalence practically throughout India, it is sad that even with a huge net work of veterinary organisation, practically nothing is being done to keep the disease under control. More unfortunate is the way money and man power are being diverted towards the study of other bacterial and viral agents such as leptospira, Mycoplasma, Chlamydia, IBR, P1₃, etc. which are allegedly responsible for reproductive disorders. While research on these emerging entities cannot be altogether called unimportant, the basic fact that has to be realised is that even rich countries had to decide on priorities while dealing with infectious agents, both bacterial and viral, responsible for reproductive disorders. In doing so, they first decided to control brucellosis and, having done that, are now actively engaged in tackling the other problems. Unfortunately, the situation, as obtaining at present in India, is just the reverse with major diseases like brucellosis having been relegated in preference to several minor bacterial and viral entities quite disproportionately to the economic loss they possibly inflict as compared to brucellosis. A country which has embarked upon "white revolution" and "operatio flood" can ill-afford priorities if such programmes have to make satisfactory headway.

It is difficult to assess the contention of Polding (1948) that brucellosis chiefly occurred in animals of humid, Indian peninsula and that there were

two forms of the disease viz., (i) an imported form which occurred in organised farms and (ii) an indigenous form confined to village herds. As far as Orissa is concerned, brucellosis has been known to occur in dairy farms as well as among village cattle in almost all the districts, although the survey carried out by Folding (1943; 1947; 1948) clearly established that its prevalence was higher across the provincial boundary. The geography of the state has completely changed with the addition of seven new districts to the then existing six since the year 1948 and this necessitated a fresh over view of the occurrence of brucellosis in the reorganised state of Orissa. The position up to 1965 was that none of the organised farms of Orissa was free from brucellosis although the reactor rate varied between 4.5% to 41.7% (Panda and Dass, 1965). It is surprising that even in face of such clear cut evidence, supported by isolation and published papers (Panda et al. 1962; and Panda and Dass, 1965; Pat and Panda 1967), further work was virtually discontinued and no body knew anything of the disease although it is well known that infection could spread, not only within a herd but also from farm to the villages and vice versa through various agencies. It is in this context that the present investigation was considered essential and relevant in the larger interest of Orissa.

On the lines of work carried out earlier (Panda et al. 1962; Panda and Dass, 1965) all the

organised dairy farms were brought under the ambit of the present survey although some new centres covered at present were non-existent in 1965. It is gratifying to note that except in two farms viz., District Livestock Breeding Farm, Bhawanipatna and Chipilima State Farm, there is a perceptible decline in the reactor rate among the female stock of the other eight organised dairy herds of the state. Indeed, in six out of the eight dairy farms of the second category, the reactor rate was zero. In the other two farms, the reactor rate of 1.8% and 2.1% is such that with the elimination of a few animals, the entire herd can be made Brucella-free. It must, however, be noted that the above contention is based entirely on a single serum and/or milk test which is not conclusive in view of the findings of Halder and Sen (1986) that the agglutinin titres of even infected animals vary from time to time. This leads to the conclusion that at least 2 to 3 tests must be carried out at an interval of 3 to 6 months before declaring a herd as totally free from brucellosis. Needless to emphasize that with minimal efforts these ten farms, which have either low reactor rate or no reactors at all, can continue to enjoy Brucella-free status which will be no mean achievement under the existing circumstances.

The detection of a very high rate of reactors in the state's two prestigious farms viz., Bhawanipatna and Chipilima (State Farm) is a distressing feature. Both these farms maintain exotic animals which according to some workers (Polding, 1943) are more susceptible to Asian strains of Brucella although there is no conclusive evidence to support such a hypothesis. Indeed, the Central Livestock Breeding Farm, Sunabada which maintains over 400 pure Jersey cows did not have a single reactor when representative animals were tested in the course of the present investigation. It is, as such, evident that infection, once it enters into a farm, can spread quite swiftly, as has been observed at Bhawanipatna and Chipilima.

Normally, the milk ring (ABR) test is performed on 8 to 10 pooled milk samples, mostly for assessing the infection status on herd basis. However, the efficacy of this test in locating individual infected animals has been repeatedly confirmed (Panda and Pat, 1968). In the course of the present investigation, there was very close agreement between the serum agglutination and milk ring test. This is in consonance with the earlier findings of (Panda and Mishra, 1963).

In so far as the prevalence of brucellosis among bulls and other male livestock is concerned, the situation is not easily comparable with that of cows. Besides, the figures of Panda and Das (1963) as well as those obtained now are based on the testing of a relatively

small number of animals as against larger number of cows tested. There cannot be two opinions that all male animals used as bulls should be totally free and even the semen of bulls negative to serum tests but with some symptoms suggestive of brucellosis such as hygroma and/or orchitis should not be used for insemination. In advanced countries, such animals are destroyed and in India they are to ~~be~~ be castrated and used as bullocks. Unfortunately, in Orissa, even bulls have not been tested regularly; otherwise, positive reactors, even in small numbers, could not have been detected in 4 out of 19 semen collection centres. The role of bulls in the spread of brucellosis^{is} too well known and it is criminal if one uses the semen of such bulls in any breeding programme.

It is pertinent at this stage to examine as to how the overall prevalence of bovine brucellosis has shown a downward trend in most farms of the state, contrary to the general apprehension that in the absence of rigorous control measures brucellosis, like other chronic bacterial disease, is likely to engulf an entire farm even if one or more infected animal(s) are allowed to remain inside the herd. In addition to other factors which influence the spread of bacterial infections such as sanitation, disposal of infected animals, introduction of disease-free animals and disposal of old and infected livestock, which has been in practice for many years, is the use of frozen semen in place of natural service

and artificial insemination with chilled semen. The former, in all probability, was and still continues to be the signal factor which has prevented the occurrence of fresh cases which was very likely in the early sixties when artificial insemination was dependant upon chilled semen, supplied by various semen collection centres. The work of Dass et al. (1962) did clearly reveal that bulls of several semen collection centres at that time were certainly infected with Brucella; yet, such animals used to donate semen for A.I. work. This hopeless situation appears to have improved and the retrieval to the present position is perhaps largely on account of the centralised semen collection at Cuttack and the introduction of frozen semen technology from 1978 onwards. The state of Orissa is now in a position to embark upon a control programme against bovine brucellosis and all efforts should be made to take full advantage of the present favourable situation of low prevalence in most farms. Removal of the positive reactors as early as possible, periodical blood and milk tests and other preventive measures should make Orissa farms free of Brucellosis in the coming years.

In so far as human brucellosis is concerned, it is at best possible to discuss briefly the significance of this disease as one of the important causes of P.U.D. and its observed complications which

include pleurisy, pleural effusion, lung abscess, millet-seed pulmonary calcification, empyema, pneumonia, chronic pulmonary granuloma, spondylitis, suppurative arthritis, osteomyelitis, hydroarthrosis, epididymitis, orchitis, cystitis, pyelitis, nephritis, optic neuritis, keratitis, uveitis, retinopathy, meningitis, encephalitis, neuritis, haemolytic anaemia, thrombocytopenia, cholecystitis, subdiaphragmatic abscess, chronic cutaneous ulcers, endocarditis, myocarditis, thrombophlebitis, pulmonary embolisation and cardiac rupture. According to Beeson *et al.* (1979) many diseases more common than brucellosis have signs and symptoms that partially or almost totally mimic brucellosis. No wonder, cases of human brucellosis are very often missed and mistaken for more common diseases such as malaria, typhoid, paratyphoid, influenza, etc. which are rampant in countries such as India. It is in this context that the findings of small-scale surveys conducted in different parts of India gain special significance. An important picture that emerges out of the available literature is that wherever any survey has been conducted, cases of human brucellosis have been invariably detected, although the reactor rate is not as high as one would have expected (Godlevskiy, 1968; Mathur, 1985). Different workers (Mathur, 1972; Randhawa and Dhillon, 1974; Kulkarni *et al.* 1978; Sharma *et al.* 1979; Nagelotnath 1979;

Ramachandra et al. 1981b; Panjarathiram and Gulrajani, 1983; Patel et al. 1983; Patel et al. 1986) have recorded the prevalence rate of human brucellosis varying between 0.9% to 18.1% on the basis of serum agglutination tests conducted in different parts of India. The maiden study of Mohakur and Panda (1972) in Orissa revealed 3.7% positive reactors among human beings in and around Burla (a town in western Orissa). In the same area, 6% sheep and goat sera had agglutinin titres varying between 40 to 160 I.U. which is in agreement with the earlier observations of Das et al. (1961) and the recent findings of Acharya and Panda (1985). In the course of the present investigation, 13 out of 190 i.e. 6.8% human serum samples were clearly positive to the Brucella tube agglutination test (80.I.U./ml and above). The present reactor rate of 6.8% is almost double as compared to the figures of Mohakur and Panda (1972) although in both the cases the test was performed in highly vulnerable population viz., febrile patients in the former case and farm workers in the latter. It appears probable that the infection among dairy farm workers is higher as compared to the average rural febrile patients ostensibly because only a few may be coming in close contact with infected animal and animal products. This situation is in agreement with the latest trend in U.S.A. where workers in pig abattoirs are found frequently infected with B. suis (Spink, 1974).

Further, the reactor rate as detected in Orissa now falls in line with the figures obtained in different states of India (see Mathur, 1985). There is scope and pressing necessity to extend this type of investigation to larger representative population spread over the entire state, preferably through collaborative efforts of veterinary as well as medical scientists.

In attempting isolation of Brucella from cases of P.U.O. in human beings, the medical bacteriologist is always in an advantageous position in as much as blood drawn from a vein under strict aseptic precautions is directly inoculated into suitable liquid and/or solid media, thereby avoiding chances of contamination from extraneous sources (Scott, 1973). The veterinary bacteriologist, on the otherhand, is faced with the arduous task of isolating Brucella from grossly contaminated and semiputrefied specimen such as placentas, cotyledons, uterine / vaginal discharge and milk. Although the stomach contents of an aborted foetus is known to store B. abortus in pure form, it is not always possible to procure this material particularly under village conditions. Most cattle farms in the state are distantly located from the Veterinary College which, incidentally, is the only place where isolation of Brucella can be attempted. It was under such circumstances that milk samples from serologically positive cows were the only materials considered fit

and it is gratifying that all the 26 samples on culture, resulted in positive isolation of B. abortus. Although milk samples were collected under aseptic precautions, direct plating of the cream-cum-sediment mixture on tryptose agar containing inhibitory dyes (gentian violet or malachite green) posed serious difficulties in fishing out suspected Brucella colonies on account of contaminants. The guineapig inoculation method was the only other alternative left and this approach yielded rich dividends. It was, however, surprising that nearly half of the guineapigs did not reveal agglutinins against B. abortus coloured antigen when bled on the 21st and 42nd days post-inoculation. However, the spleen, liver and kidney tissues of such animals on culture were found to harbour B. abortus as judged from the results of isolation studies. It is difficult to offer suitable explanation to this baffling situation. However, Mohakur and Panda (1972) have reported that they could isolate two strains of B. melitensis from febrile patients who were serologically negative and felt that Brucella organisms might behave erratically inside the animal body even when they are virulent and fully antigenic. A similar picture has also been observed in infected cattle by Halder and Sen (1986). Further if the samples contain very small number of organisms (5 to 50), no agglutination is likely to occur when the guineapigs are bled earlier than at four weeks, even though the

spleen will contain enough bacteria to enable isolation (Stableforth and Gallauey, 1959).

The usual practice in most laboratories is either not to attempt isolation, in view of the proven danger posed by Brucella to laboratory workers, or to submit cultures for confirmation and typing to a national or international centre approved by FAO/WHO. However, in recent years several laboratories, both veterinary and medical, have developed facilities for typing of Brucella as evidenced from published papers. Even in face of real public health hazard, it was decided to type the isolates locally, to the extent possible, mainly to prevent inordinate delay in obtaining results from a central laboratory. Even then, two tests viz., susceptibility to Brucella phage and oxidative metabolic test had to be avoided in the light of the following warning (Alton et al. 1975):

".....and there is a serious risk of personnel becoming infected with Brucellae; thus they do not lend themselves to the routine typing of large number of cultures. It is therefore suggested that, if conventional tests and phage typing do not permit a culture to be typed satisfactorily, it should be sent for examination to one of the following laboratories:

US Department of Agriculture, veterinary services laboratories, post office Box 70, Ames, IOWA, 50010, U.S.A.; FAO/WHO collaborative centre for Brucellosis, Central Veterinary Laboratory, Weybridge, England; Pan American Zoonoses Centre, Casilla 23, Ramos Mejia, Buenos Aires, Argentina. Permission for shipment should first be obtained by writing to the laboratory. Procedure to be followed in the shipment of cultures across national boundaries will then be provided by the laboratory. Information on its source and history should accompany the culture. The detailed procedures are presented for those who wish to perform the oxidative metabolic tests."

All the 26 isolates were, therefore, subjected to the other mandatory tests viz., CO₂ requirement, H₂S production, urease activity, growth on thionin and basic fuchsin and agglutination in monospecific sera.

On the basis of results obtained eleven isolates belonged to type 3, four each to 6, 7 and 9 and three to biotype 1. Biotype 1, 3 and 9 were previously isolated from cattle in Orissa (Pat and Panigrahi 1967; Sen, 1968) and this is in agreement with the present findings. Biotype 9 does not require CO₂ and agglutinates in Melitensis monospecific serum and appears to be prevalent only in this part of India.

CHAPTER VI
SUMMARY

CHAPTER-6

S U M M A R Y

Brucellosis among cattle is already known to be endemic in most parts of Orissa for many years. On the basis of work done from early forties up to late nineteen hundred sixties, the prevalence of brucellosis in farm as well as village cattle was found to vary between 0% to 50%. Such a situation in organised dairy herds, as distinct from village cattle, continued to pose serious challenge to the veterinary activities of the state. The position during the intervening period of about 18 to 19 years i.e. from 1968 to 1986 has remained in dark, as neither any systematic survey was undertaken nor worthwhile control measures adopted during this long interval of time. Meanwhile, the breeding policy in the state swunged in favour of cross breeding with exotic animals and use of frozen semen from such animals in place of chilled semen of Haryana and Red Sindhi bulls. Against this back-drop, the current prevalence of bovine brucellosis was reassessed through examination of almost the entire farm animals and bulls stationed in different parts of the state, using internationally approved serological techniques such as plate and tube agglutination as well as milk ring (ABR) tests. Attempts were also made to confirm the results of serological findings by isolating, identifying and biotyping such isolates to the extent possible with available laboratory facilities. Results of these

investigations have been summarised in the following pages.

A total of 13 livestock farm were surveyed out of which two farms in western Orissa viz., District Farm at Bhawanipatna and State Livestock Breeding (SLB) Farm, Chipilima had very high percentage of reactors among cows, 56.6 and 26.9 respectively. In the other nine state-owned farms, reactors in very small numbers could be detected in two of them viz., Bolangir (2.1%) and Khapuris (1.8%) whereas the other seven had no reactors at all. The blood samples of 78 cows originating from miscellaneous sources such as private cow owners, clinics etc. were tested and 3.8% reactors were detected.

Both the farms maintained by Government of India at Chipilima and Sunabeda were found free from brucellosis on the basis of representative samples of serum and milk tested.

In the milk ring (ABR) test, 100% of cows at Bhawanipatna and 36% of SLB farm, Chipilima reacted positively. Almost 100% of milch cows which had high serum titre were also positive to milk test. Seven cows, which were rejected having been tested earlier and kept isolated in Dhenkanal, were all positive to the ABR test. The position in other farms was in close agreement with the results of serum test, thereby confirming either very low or zero % reactor rate.

The overall picture that emerged from the results of serum agglutination as well as milk ring (ABR) tests was that barring two state-level farms at Bhawanipatna and Chipilima, the prevalence of bovine brucellosis in the female stock, now being maintained in the other farms, was so low that with the disposal of all positive reactors, numbering a mere one to three, these farms could be made totally free from brucellosis. The reason for such a steep fall in the reactor rate, as observed in most of the farms as compared to the figures of 1968, could well be due to the use of frozen semen, improved sanitation and animal husbandry practices during the past few years.

As regards bulls and other male animals, 15 out of 19 centres were totally free whereas in the other four centres one to two bulls out of 7 to 15 reacted positively. Such a situation was also prevalent when the last survey work was carried out. However, with the removal of the few positive reactors, the entire stock of breeding bulls could be considered free from brucellosis.

Out of 190 serum samples of vets, paravets, farm attendants and persons with pyrexia of uncertain origin (PUO) tested, 13(6.8%) reacted positively and another 4 (2.2%) doubtfully to the tube agglutination test. Most of the positive reactors had history of

recent or past fever, orchitis, arthritis or myalgia; but the presence of active infection could not be confirmed as no blood culture could be done.

From the milk samples of reactor cows, 26 strains of *Brucella* could be isolated and all these were identified as *B. abortus*. On the basis of CO₂ requirement, H₂S production, urease activity, growth in the presence of different concentrations of thionin as well as basic fuchsin and agglutination with monospecific sera (*Abortus* and *Melitensis*), eleven belonged to biotype (bioavar 3), four each to biovars 6,7 and 9 while the rest three were typed as biovar 1.

Inoculation of guineapigs with milk samples was 100% successful in isolating *B. abortus*, using tryptose broth and agar as media for isolation. This method obviated the addition of inhibitory dyes and antibiotics although it was more time taking (4-5 weeks) as compared to direct plating of suspected material(s). At times, even infected guineapigs reacted negatively to the serum agglutination test which was somewhat surprising. Biovars 1,3 and 9 were isolated earlier in the state but two other biovars viz., 6 and 7 are new entries to the state. The A/M strains isolated earlier in the state were in all probability biovar 9 of *B. abortus* which does not need carbon dioxide for its growth and agglutinates only with 'Melitensis' monospecific serum. This particular biovar appears to be

more prevalent in Orissa since this has been isolated very occasionally other states.

The literature on brucellosis, particularly of recent years, has been reviewed, the current prevalence of bovine brucellosis among farm cattle in Orissa has been surveyed, different biovars of B. abortus isolated and the overall significance of these findings in relation to possible control of the disease in animals including its impact on human health has been discussed.

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