EPIDEMIOLOGY OF HUMAN BRUCELLOSIS

IN

OYO STATE OF NIGERIA

BY

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ABSTRACT

Brucellosis is a zoonosis, primarily a disease of domestic animals which, under special circumstances, may be readily transmitted to man. The epidemiological picture of the disease varies from area to area in different parts of the world, and has to be studied carefully in planning control and eradication programmes.

In Nigeria, where livestock management is still largely unscientific and bovine brucellosis is known to be endemic (Esuruoso, 1974b), there are only scanty data on the epidemiology and clinical presentation of human brucellosis. Therefore, the present study, which covered a period of three and half years between September 1973 and February 1977, was undertaken with the objectives of collecting adequate and reliable data on the epidemiology of human brucellosis in Oyo State of Nigeria in order to be able to formulate a realistic control programme. The study also aimed at expanding medical and applied scientific knowledge in the field of brucellosis and occupational medicine in Nigeria.

Over 70 per cent of the total 55 million Nigerians (1963 census Report) live in rural areas and farm settlements. Medical and other social services are grossly inadequate in the whole country and the few amenities are concentrated in the capital cities and other big towns, with the rural agricultural areas virtually neglected.

Agriculture is the mainstay of the Oyo State economy, and approximately 50 per cent of the State's labour force are engaged in agricultural work. Cow meat is the most readily available animal protein to the people of Oyo State. The present epidemiological study was undertaken largely in

several farm settlements scattered all over Oyo State, but the central unit for the study, was based at the Department of Medical Microbiology, University College Hospital, Ibadan, where also the clinical aspects of the study were carried out.

The pilot sero-epidemiological evaluation on human brucella antibodies carried out on 1600 people of various occupation and age groups living in Ibadan, capital of Oyo State, revealed an overall sero-positivity rate of 50.2 per cent. Significantly higher prevalence of infection was found among the occupationally exposed population including herdsmen, abattoir workers and veterinarians, than the general population, including blood donors, pregnant women and school children. The important identifiable sources of human infection include direct transmission from infected animal through abraded skin, nasopharyngeal mucosa and the conjunctivae. Other sources of human infection are by ingestion of infected milk, especially on the farms, or eating of roast meat (barbecue), which is becoming popular at parties among the elites in the urban centres.

Further epidemiological investigations carried out in several livestock farms showed that the important factors determining the rate and level of human brucellosis in Oyo State include: (1) the geographical location of the farm; (2) the number of cattle per head of population and the frequency of contact with infected animals; (3) the rate of active infection in the cattle herd; (4) the system of animal husbandry: whether scientific or traditional methods; and (5) imported bovine infection from neighbouring countries.

An outbreak of active bovine brucellosis with human involvement

which was investigated at Igbo during the study revealed that the hazards caused by Br. abortus infection are of great social and public health importance in Oyo State. The economic loss to the livestock industry due to brucellosis if estimated, would probably run into millions of naira (₦) annually, judging from the high rate of abortion and infertility among heifers in some farms. The public health consequences are also enormous, considering the fact that protein undernutrition is very common in Nigeria (Morley, 1973), especially in the rural areas (Oyenuga 1976) and the ill-health which prevents the farmers from taking proper care of their livestocks. An economically viable livestock industry will be difficult to achieve under the present stage of endemic brucellosis in Oyo State.

The practical problems of controlling brucellosis in a developing economy were highlighted, as the ideal 'slaughter technique' leading to eradication of bovine infection is not feasible because of the present poor state of livestock industry in Oyo State. However, realistic control measures, based on the improvement of the existing agricultural, social, and health systems in the state, are possible. Immediate interim control measures include hygiene on the farms and abattoirs, avoidance of raw milk and introduction of vaccination programmes for beef cattle in settled herds. In addition, every effort should be made to improve the social and medical services in the rural areas of the state.

The long-term control measures should include the establishment of clinical and laboratory services, health education of farmers and abattoir workers, full coordination in the medical and veterinary services, widespread pasteurisation of fresh milk, the establishment

of a state-fi[illegible]nal co-operative Brucellosis Control Progr[illegible], and finally industrial legislation for the improv[illegible]nt of the working conditions of the livestock far[illegible]rs.

The suggested future studies which are directly indicated from the present study include (1) large scale epidemiological survey of Nigeria to determine the overall socio-economic significance of human brucellosis, and therefore, to formulate a National Control Programme, (2) further evaluation of the role played by brucellosis among patients with pyrexia of undetermined origin, pregnant women with second-trimester abortions and patients with neuro-psychiatric disorders and (3) production of safe and effective human brucella vaccine against brucellosis, especially among the occupationally exposed individuals in areas with endemic bovine disease.

ACKNOWLEDGEMENTS

I am indebted to many people who contributed directly or indirectly to the successful execution of the objectives of this study. Many of them will be found mentioned in the body of the thesis and also listed under personal communications. My recommendation is my thanks. If I have inadvertently adopted or adapted where I should have sought permission, I hope it will be excused as oversight.

In particular, I have to acknowledge my indebtedness to Professor O. O. Montefiore, Head of the Department of Medical Microbiology, University of Ibadan for introducing me to the field of Medical Bacteriology and accepting me as a candidate for the Bachelor of Medical Science degree(Bacteriology) in 1966 after my introductory clinical course. I am also deeply grateful to him for placing at my disposal all the facilities of the Department of Medical Microbiology as well as for his valuable advice throughout the period of this study.

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I wish to record my deep gratitude to Dr. A. Awosoyi, the veterinary pathologist at the Mokole Veterinary Centre, Ministry of

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whom I met in 1976 at the East African Medical Research Council Annual Scientific Conference at Nairobi, Kenya; Dr. L. Reinius, the Food Hygienist at the Communicable Diseases Division, World Health Organization, Geneva; and Dr. D. J. H. Payne, Director, Public Health Laboratory, St. Mary's General Hospital, Portsmouth, United Kingdom.

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I wish to thank Messrs. Brightonson Ohonmwinan and Samuel Oniososa for their great assistance and cooperation during the final typing of this thesis.

Finally, I wish to record my gratitude to my wife and children for their patience and understanding during my several trips within and outside Nigeria in pursuance of the objectives of this study.

DECLARATION BY THE AUTHOR

I hereby declare that the materials recorded in this thesis, entitled, "EPIDEMIOLOGY OF HUMAN BRUCELLOSIS IN OYO STATE OF NIGERIA", resulted from research work carried out by me.

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C O N T E N T S

LIST OF FIGURES AND TABLES

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FIGURES

TABLES

LIST OF ABBREVIATIONS USED IN THE TEXT

App.	=	Appendix
A.N.C.	=	Antenatal Clinic
Br.	=	Brucella
C.F.T.	=	Complement Fixation Test
c.m.	=	Centimetre
C.N.S.	=	Central Nervous System
CO_2	=	Carbon dioxide
e.g.	=	For example
F.A.O.	=	Food and Agriculture Organization
Fig.	=	Figure
I.A.A. & T.	=	Institute of Agricultural Research and Training
I.C.D.	=	International Classification of Diseases
I.C.D. 023	=	Brucellosis (according to International Classification of Diseases)
I.T.S.S.	=	Idiopathic Tropical Splenomegaly Syndrome
I.U. or i.u.	=	International Unit
(L)	=	Left side
mg	=	milligram
ml	=	millilitre
No.	=	Number
P.C.V.	=	Packed Cell Volume
P.U.O.	=	Pyrexia of Undetermined Origin
qds.	=	four times daily or six-hourly
R.B.P.T.	=	Rose Bengal Plate Agglutination Test

R.E.S.	=	Reticuloendothelial System
Rev.1	=	Br. melitensis strain Rev.1
R.T.D.	=	Routine Test Dilution
S	=	Schistosoma
S.A.T.	=	Saline Tube Agglutination Test
U	=	micron
U.C.H.	=	University College Hospital, Ibadan
V.D.	=	Venereal Diseases
Vet.	=	Veterinary
Vol.	=	Volume
W.B.C.	=	White blood cell count
W.H.O.	=	World Health Organization
Wt.	=	Weight
2.ME Test	=	Tube Agglutination Test in the presence of 0.05 M 2-mercaptoethanol (as diluent)
%	=	percentage

SECTION ONE

INTRODUCTION

OBJECTIVES OF STUDY

DESCRIPTION OF NIGERIA AND AREAS COVERED BY STUDY

CHAPTER I

INTRODUCTION AND OBJECTIVES

A: ZOONOSES

"Zoonoses are those diseases and infections which are naturally transmitted between vetebrate animals and man" (WHO, 1958).

Since the dawn of history the diseases of man have been compared with those of animals. It was not, however, until the discovery of the pathogenic properties of certain bacteria and other lower organisms that similarities between several communicable diseases of man and animals were properly assessed. Until the beginning of the present century, the animal origin of only a small number of human diseases had been recognized (mainly rabies, cowpox, anthrax, and a few zooparasitic infections). At the present time, far more than 100 zoonoses are known. In fact, it has become evident that the animal world is a reservoir for the agents of numerous human diseases (WHO, 1959), and there is ample reason to believe that most of the present infectious and parasitic diseases of the human race have originated in animals. The pathogenic organisms adapted themselves to the environment of the human body either as parasites or commensals. The effects produced in both man and animals are mainly determined by the invasive capacities of the pathogen and the host's resistance to it.

Several zoonoses are equally harmful to man and to animals (e.g. anthrax, plague, rabies, brucellosis, bovine-type tuberculosis), while others only rarely or slightly impair animal health, but cause serious illness in man (Q-fever, hydatidosis). A third group includes grave epizootics, which seldom affect man (foot and mouth disease, pasteurellosis, pseudorabies). However, the seriousness of a particular zoonosis may differ considerably in various territories and at different times. Zoonoses are among the commonest hazards to man especially so in the tropical and sub-tropical areas where arthropod vectors may play a significant role in their transmission.

A major role in the epidemiology of zoonoses is played by wild or domestic animals, which persistently carry and excrete organisms which are potentially pathogenic to man. Reservoirs of considerable importance are formed by those animals which are partially resistant to the pathogens, and develop a chronic carrier-state, but are not themselves clinically affected.

Apart from nutritional and industrial zoonotic infections, most zoonoses are characterised by their focal distribution within particular geographical territories (the "landscape epidemiology" of Pavlovsky) (Pavlovsky, 1957). Ecosystems in these localities include defined biocenoses (species networks), which are composed of mutually connected animal communities, combined with the local vegetable and microbial world. If an infection transmissible between animals and man appears among the vertebrate animals living in these areas, a zoonotic nidus or focus is created. Frequently, arthropods and other non-vertebrate animals serve as vectors and play an important, or often

necessary, part in the cycles of infection. Climatic condition- produce natural fluctuations in ecosystems because seasonal variations influence hosts, vectors, and even the pathogens in their habits and habitats. The activities of man, brought about by such practices as the reclamation of waste lands, artificial irrigation and dam building, changing crops, killing wild animals, introducing domestic animals or building new industries may change the biocenoses. A parasite may find a new host in human beings who enter an enzootic area temporarily as a hunter or a fisherman, permanently as an agriculturist, but seldom in an industrial worker who engages himself in processing animal products.

It is obvious that most zoonoses occur among idividuals occupationally engaged in the handling of animals, their carcasses and products (veterinary surgeons, slaughter-house personnal, roisers of livestock, milkers, workers employed in factories processing animal products, etc.). Another group of people frequently affected comprises persons who repeatedly come into contact with soil, mud or water, which are readily contaminated by animal excretions (agricultural and sewer workers, fisherman, hunters, bathers). The agents causing alimentary zoonoses (e.g. Salmonella and Helminths) may be present in food of animal origin (milk, meat, and their products).

8: THE PROBLEMS OF HUMAN BRUCELLOSIS IN NIGERIA

In 1968, the World Health Organization concluded that there are over 100 Zoonoses, of which brucellosis "is responsible for more sickness, misery and economic loss than any other Zoonosis" (Spink, 1971). The official statistics available (WHO, 1973b) as well as the literature

indicate that there is virtually no continent in the world at present where at least some cases of brucellosis in man has not been registered. The incidence and the epidemiological pattern of brucellosis among the population of different countries depends on the predominant type of farm animals (vide infra: Epidemiology of Brucellosis).

Within the last two decades, the incidence of human brucellosis in most of the European and American countries has dropped considerably as a result of successful measures for eradicating brucellosis in cattle. In developing and tropical African countries where brucellosis is known to exist (Cox, 1966), little or no efforts are being taken to institute control measures. In many of these tropical countries, including Nigeria, very little or nothing is known about the epidemiology and clinical presentation of human brucellosis, partly because of lack of diagnostic facilities and partly because of a low level of awareness among practising physicians in these countries about this important zoonosis. As far as it can be ascertained from the literature, the only documentation about human brucella infection in Nigeria is that of Collard (1962) who showed that Brucella antibodies appeared in the population who live along the routes that cattle took when driven on foot from the north to the southern parts of the country.

Within the past two decades, observations on nomadic herds of cattle and flocks of sheep and goats in Northern Nigeria and many government-owned settled cattle herds in different parts of the country (Esuruoso, 1965; Adams and Mckoy, 1966; Banerjee and Bhatty, 1970; Esuruoso and Hill, 1971; Esuruoso and Van Blake, 1972; Esuruoso, 1973) have led to the production of a bovine brucellosis Map of Nigeria by

Esuruoso (1974b). The map reveals that bovine brucellosis is endemic in Nigeria but there is a pattern of low and high infection rates in specific areas (Fig. I.1). The infection rate was up to 60 per cent among breeding cows and heifers investigated in Western State of Nigeria (Esuruoso, 1973). But only 4.27 per cent of a total 2,550 goats sampled in different parts of Nigeria had brucella agglutinating antibodies of above 50 I.U. (Falade et al, 1976). Bacteriological investigation of bovine abortions yielded Br. abortus (mainly biotype 2) in some cases, and this appears to be the most frequent species encountered in Nigeria (Esuruoso, Falade and Ojo: personal communication). In the other parts of the country, especially in the northern states, the infection rates in the various herds have been generally low. Zero to 20 per cent of animals examined in different northern herds showed serological evidence of infection. Trade cattle in and from the Northern States, and also those from across the Nigerian northern borders with Chad and Niger showed evidence of infection. Of the 1016 trade cattle tested at slaughter in Ibadan abattoirs between March and June, 1973, 64 (6.3 per cent) showed positive agglutinin titres of 160 I.U. and above (Esuruoso, 1974b). The high incidence of brucellosis among range cattle in the Western State of Nigeria (Esuruoso, 1973; Esuruoso, 1974a and b), with a high abortion and infertility rate (Esuruoso, 1974b), would result in heavy economic loss to the livestock industry in the country. To this end, the Federal Government of Nigeria has recently set up a Committee in the Veterinary Division of the Federal Ministry of Agriculture to look into the problem of brucellosis and other infections as they adversely affect the Agricultural and Livestock

Development plans in the country (Falede: personal communication).

The public health implications of endemic bovine brucellosis in Nigeria would also be enormous, considering the amount of animal protein lost annually to the human population as foodstuffs, in a country where protein undernutrition is still common (Morley, 1973). Also the risk of human infection from direct or indirect transmission of the disease from infected animals to man is a real hazard in Nigeria (Esuruoso, 1974a) with consequent illness, physical incapacity, and loss of manpower (WHO, 1971).

Nigeria is rapidly developing her livestock industry in order to increase food production for the increasing human population. Thus increasing numbers of Nigerians are engaged in rearing and herding, with consequent increase of human contact with livestock; many of those closely exposed to primary source of brucella infection live in the rural areas and they do not have immediate or direct access to effective medical care. Therefore the level of the risk of human infection in Nigeria ought to be investigated (Esuruoso, 1974a).

The gravity of brucellosis in terms of human illness and economic loss remains a matter of major concern in many parts of the World. This realisation led to the formation of the joint FAO/WHO Expert Committee on Brucellosis which meets periodically to discuss and review the current problems about the subject. The last meeting of the Committee was in Geneva from 29 June to 6 July, 1970 (WHO, 1971).

C: OBJECTIVES OF THE PRESENT STUDY

As far as it can be ascertained from the official statistics available as well as published literature, no study such as that

proposed in this thesis has been undertaken in Nigeria. The present study therefore has the following objectives:-

1. Determining the prevalence, incidence and distribution of human brucella abortus infection among the different population groups living in Oyo State of Nigeria over a period of three and half years between September 1973 and February 1977; those at special risk of infection would also be identified for purposes of planning control programme (see also objective No. 5 below).
2. Determining the category of patients, attending the University College Hospital, Ibadan, with brucellosis.
3. Establishing epidemiological, clinical and laboratory criteria for the diagnosis and treatment of human brucellosis in people living in Oyo State.
4. Expanding medical and scientific knowledge in the field of brucellosis and occupational medicine in Nigeria.
5. Evaluating the practicability of the various available control measures against human brucellosis, and formulating in collaboration with the veterinarians, the best applicable control methods possible in Oyo State of Nigeria considering the present economic, social and health systems in the country.

D: APPRAISAL OF THE EPIDEMIOLOGICAL APPROACH ADOPTED IN THE STUDY

Medical research may be divided into four categories, namely:- (a) epidemiological research which seeks to define the nature and determinants of a health problem; (b) applied research which adapts existing

knowledge to solve a given problem; (c) methodological research which aims at developing new tools for solving a specific problem; and (d) basic or fundamental research which seeks new knowledge for its own sake, regardless of its immediate or future application. All types of medical research are probably relevant and necessary in relative proportions, for the solution of Nigeria's medical problems. However, the bulk of medical problems which are of public health significance in Nigeria and many other African countries, consists of preventable communicable, infectious and parasitic diseases. The major difficulty in Nigeria, as in many other developing countries, is the inadequate or lack of reliable and specific data on the nature and determinants of most of the common communicable diseases. In the past, because of inaccurate laboratory data, many efforts aimed at controlling some parasitic diseases, such as malaria and schistosomiasis have not been successful.

The first prerequisite of any control project is accurate epidemiological data - the distribution, incidence, prevalence, morbidity and mortality of the disease to be controlled. Such epidemiological studies must have laboratory support because decisions based on clinical grounds alone might lead to serious mistakes in national health planning, especially in communicable infectious diseases control programmes (WHO, 1972). Generalisations based on small, scattered prevalence surveys, and official statistics from hospital records are known to be incomplete and inaccurate. Furthermore, hospitals see only the tip of the iceberg, that is the few among the infected many in whom the infection does not reach the severity of a disease requiring hospitalisation: the so-called iceberg phenomenon of laboratory services in developing countries (Fig. I.2).

Epidemiological research which provides accurate data on an infection in a given community is vital to the planner and public health workers, including those in the veterinary services.

It is hoped that the results obtained from the present study and similar investigations in other parts of the country would eventually provide reliable data on which to plan a National Programme for Brucellosis Control, which is being contemplated by the Nigerian government. Therefore, the evaluation of the problems of human brucellosis should be regarded as a timely and justified project at this period of socio-economic development of the country (Oluwasanmi: personal communication).

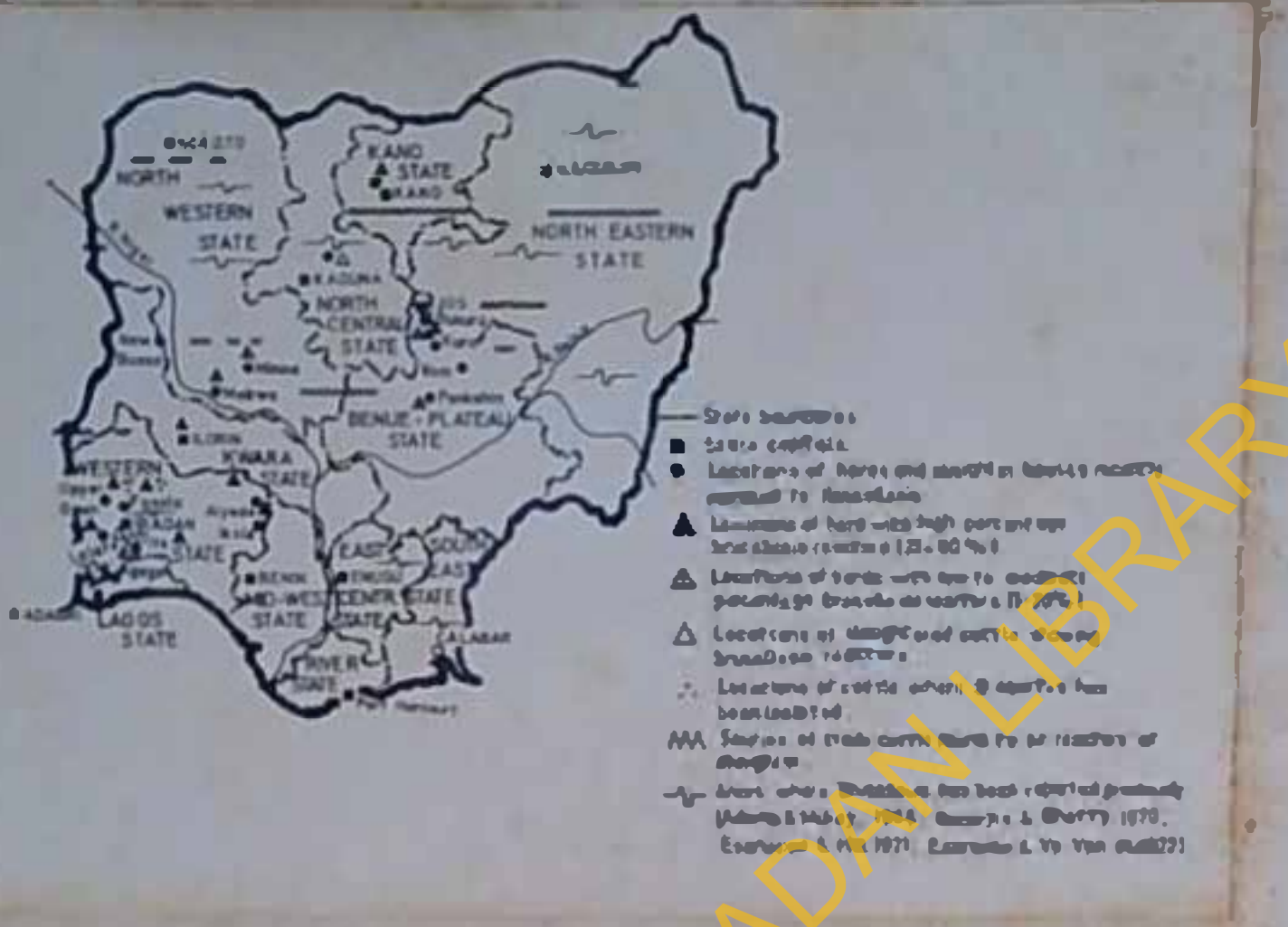

Fig.1.1: Distribution of Bovine Brucellosis in Nigeria (After Esuruoso, 1974b)

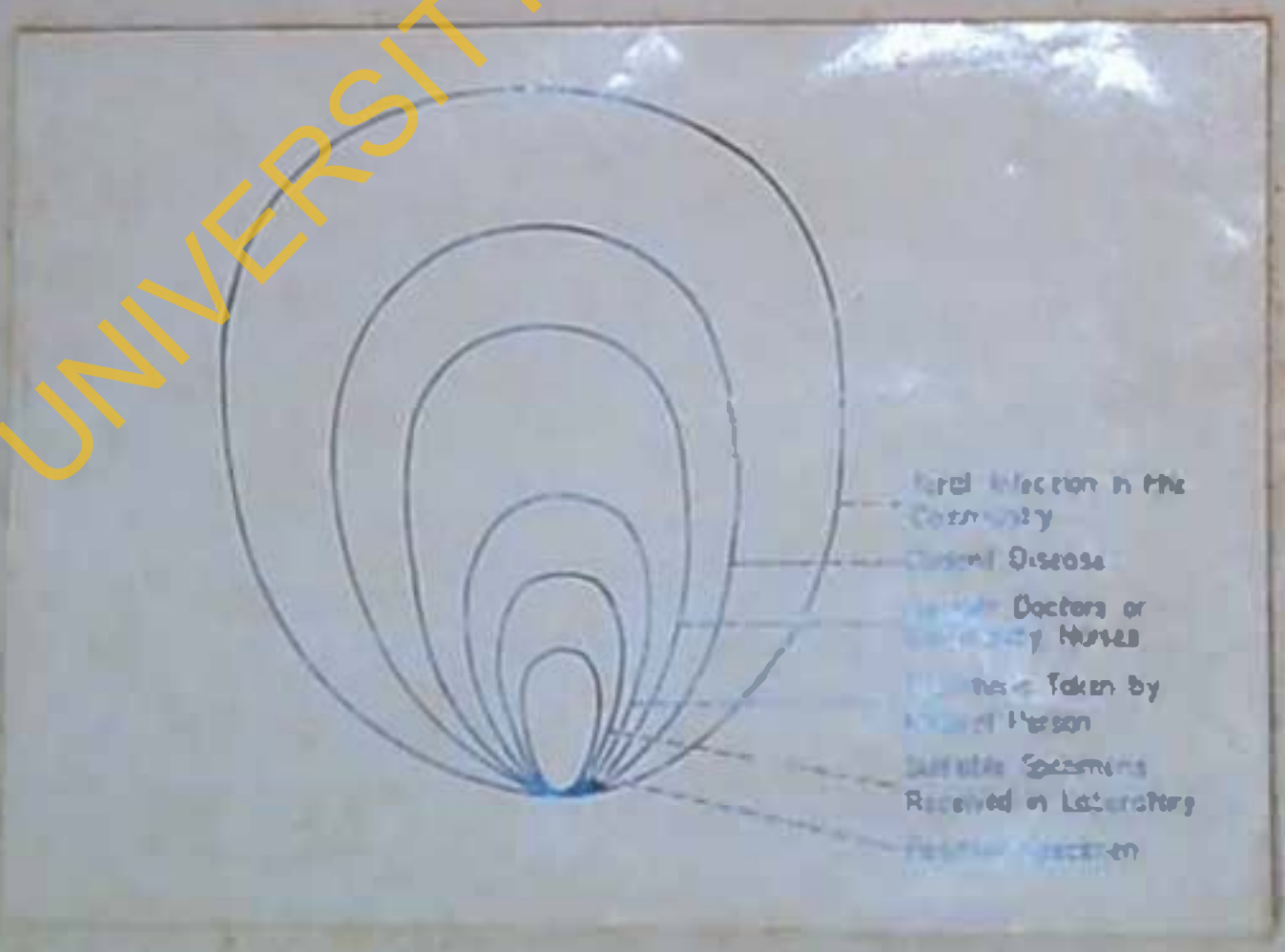

Fig.1.2: Iceberg Phenomenon of Microbiological Laboratory Services in Developing Countries.

CHAPTER II

DESCRIPTION OF NIGERIA AND AREAS COVERED

A: GEOGRAPHICAL DESCRIPTION OF NIGERIA

Nigeria is the largest single geographic unit along the west coast of Africa. It lies between latitude $4^{o}20'$ and 14^{o} north of the equator and between longitude $2^{o}20'$ and $14^{o}30'$; thus it is situated entirely within the tropical zone. It occupies an area over 92 million hectares extending from the southern coastline northward for over 1060 kilometres, and from the western border to the east there is a distance of 1120 kilometres at the widest part. Its population of over 55 million, according to the official 1963 census, is by far the largest in Africa: approximately 70 per cent of the population live in rural areas.

Nigeria is bounded on the north by the Federal Republic of Nigor, on the west by the Republic of Benin (formerly Dahomey), and on the east by the Federal Republic of Cameroun and the Chad Republic. The Atlantic Ocean, known variously along the west coast as the Gulf of

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Nigeria is bounded on the north by the Federal Republic of Niger, on the west by the Republic of Benin (formerly Dahomey), and on the east by the Federal Republic of Cameroun and the Chad Republic. The Atlantic Ocean, known variously along the west coast as the Gulf of

Guinea, the Bight of Benin and the Bight of Biafra, washes the coastline in the south for some 800 kilometres (Fig. II.1).

Nigeria is naturally divided into three major and unequal geographical sectors by the River Niger and its main tributary, the River Benue. Almost unbroken sandy beaches stretch along the coast. Inland from the coastline the tropical rain forest takes over to a depth of between 100 and 160 kilometres northward. The oil palm is found in profusion but there is much valuable commercial timber and excellent cocoa growing areas. Nowhere in this area is there any high ground until the northern limits of the forest are reached and the vegtation takes on the character of Guinea Savannah with high forest in the river valleys. Low hills occur in the west reaching 600 metres at the highest point, between the forest and the River Niger valley.

Beyond the valleys of the Niger and Benue rivers park-like savannah predominates until it merges into Sudan Savannah over the northern border and into the Sahara desert. A conspicuous feature of the northern part of the country is the great plateau which rises as a steep escarpment from the riverain plains of the Niger-Benue to an average height of 600 metres with ranges of hills between 1500 metres and 1800 metres around Jos.

The climate is tropical with some variation mainly due to differences in latitude; the south is hot and wet while the north is hot and dry. In general there are two seasons: a wet season from late April to October (starting later and finishing earlier in the north) when the prevailing monsoon winds blow from the south-west; and a dry season from November to March, when the harmattan blows from the north-east. The

southern states have a warm climate with relatively high humidity for most of the year. Most of the northern states have a hot, dry climate, although temperatures drop during January and February due to the cooling effects of the harmattan: the plateau area is cooler throughout the year than the rest of the Northern States.

Temperatures at the coast vary from 21° to 32°C and humidity ranges between 80 per cent during the day and 100 per cent at night. In the north, the climate is drier and extremes of temperature are more common from October to April - sometimes reaching as high as 43°C, and falling to 10°C especially at night.

8: POLITICAL ADMINISTRATION OF NIGERIA

Nigeria gained her independence from Britain in October 1, 1960, and she adopted a federal system of government, comprising a Federal Parliament in Lagos and three Regional Governments (the Western, the Eastern and the Northern Regions). A fourth region, the Mid-West Region, was carved out of the existing Western Region in 1962. On January 16, 1966 the Armed Forces, following a coup detat, came into power, and legislative and executive powers were vested in the Federal Military Government.

In May 1967, twelve states were created out of the four existing Regions (Fig.II.2). The country was further divided into nineteen states in January, 1976 (Fig. II.3); the states are subdivided into urban and district local councils.

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Under the Nigerian Constitution, there is an "exclusive list" whereby the Federal Government retain sole power in a number of fields

including external affairs, aviation, banks, census, maritime shipping, mines and minerals, defence, posts and telegraphs, trunk roads and railways; and a "concurrent list" of matters (which includes education, health and agricultural matters) falling within the provinces of both the Federal and state governments. Some subjects are the sole responsibility of state governments. Federal law is superior in case of dispute.

Lagos is the political and administrative capital of the Federal Republic of Nigeria. It is also the financial, commercial and industrial centre, as well as the main airport and seaport of the country. The population is over 700,000, according to the 1963 census. It is a rapidly developing cosmopolitan city. The flow of people and traffic in Lagos, due to influx from the other parts of the country, is ceaseless.

C: SOCIO-ECONOMIC DESCRIPTION OF NIGERIA

All the governments of the Federation of Nigeria lay great emphasis on the development of industrial enterprises, but fundamentally recognise that agriculture is the basis of the country's wealth and the root source of the implementation of majority of future development plans. It is estimated that more than 80 per cent of the working male population is engaged in agricultural work. The exports of Nigeria are crude petroleum, cocoa, palm kernels and oil, groundnut, cotton, rubber, timber, hides and skins, tin, coal, asphalt and columbite.

Cattle, sheep and goats form a very considerable part of the internal economy of the Northern states and the federation. Some 600,000 cattle pass annually by marked cattle tracks and by roads and

rail from the north to feed the great centres of population in the more southerly centres of Nigeria. The hides and skins from these animals represent one of the major exports by value from Nigeria bringing in about N20 million each year.

An important social problem in Nigeria is the low literacy rate (the highest literacy rate being 25 per cent in Lagos and parts of the Eastern States). Free and universal primary education was only recently introduced, from October 1976, and it is financed by the Federal government. Secondary schools which are provided by the various state governments are grossly inadequate and only those parents who are rich can afford the high tuition fees. There is therefore an increasing gap between the number of children leaving the primary schools and those entering the secondary schools. Most of the less educated and illiterates live in rural areas, where they are engaged in agricultural work, although there is an increasing trend for young people to migrate to the towns in the usually vain hopes of a better standard of living.

Post-secondary educational institutions are being rapidly expanded by the Federal government. Tuition and boarding fees are free for students in these higher institutions. The annual output of technicians from Technical and Vocational Training institutes is over 5,000; out of these over 600 trained agricultural assistants and superintendents are produced each year. Over 30,000 students are enrolled in all the Nigerian Universities, and the governments still award scholarships for university education overseas. Facilities are being increased for science, agriculture, medicine and economics in all the universities in the country.

Medical and health services in Nigeria began several decades ago. At the outset, these services were provided mainly for the benefit of the colonial civil servants. Later, the services were extended to the indigenous civil servants and eventually to the citizens at large. However, medical and health services are mainly hospital based in urban areas with more emphasis on curative than preventive medical services.

The medical institutions in Nigeria are owned and administered by the Federal government (Teaching and Specialist Hospitals), state governments, local government authorities, the voluntary agencies (which include missions), private individuals and commercial enterprises. The provision of primary health care delivery service to students of all categories civil servants (and their dependants) are the responsibility of the government and it is free of charge. Health services to the rest of the population vary from state to state.

In general better health facilities and services are available in the south than in the north of the country. Medical personnel are generally in short supply and the few that are available prefer to work in urban centres which have basic social amenities; the remote agricultural rural areas are worst affected in the provision of medical services. In 1972, the total number of physicians in Nigeria was 2,271, with a doctor/population ratio of about 1 in 25,550. Only 670 doctors were working in government hospitals. During the same year, there were only 107 veterinarians working in the whole country (WHO, 1975). There are no proper health statistics in Nigeria, as registration of births and deaths is not compulsory. It is therefore difficult to estimate the actual yearly natural increase of the population: however, the

estimated natural increase is usually based on 2.5 per cent.

As in other developing countries, the major health problems in Nigeria are nutritional deficiency, communicable infectious and parasitic diseases, poor maternal and child welfare services, and road traffic accidents. Moreover, health laboratory services are poorly organized, and laboratory facilities and personnel are less available than are clinical services. There is no reliable data showing the number and type of communicable diseases diagnosed in Nigeria. Very often many epidemiological studies and hospital services are provided without laboratory support. Except in some of the medical schools with teaching hospitals and a few big medical centres in large cities, aetiological diagnosis of the common infections are not carried out (Alausa and Osoba, 1974). Thus most patients having fever, from whatever cause, are usually treated with antimalarials and antimicrobial agents. There is therefore widespread use and misuse of antibiotics and other anti-microbial chemotherapeutic agents in Nigeria (Alausa et al, 1975).

Medical research in Nigeria often relates to patients seen in hospitals; research on community health problems based on a defined population, as carried out in the present study, is still uncommon in the country.

D: GENERAL INFORMATION ABOUT THE TYPES OF LIVESTOCK ACTIVITIES AND THE MARKETING OF ANIMALS AND ANIMAL PRODUCTS IN NIGERIA

The number of cattle per population is highest in the northern states of Nigeria (Buchanan and Pugh, 1955). About 90 per cent of the estimated 8.5 million heads of cattle in Nigeria in 1974 belonged to the Fulanis of the Northern States (Sanwo, 1975). The majority of these Ful

are nomadic herdsmen and they manage their herds by means of traditional and extensive system. Nigeria imported some 287,000 head of cattle in 1974 from neighbouring northern countries, notably Niger and Chad Republics: imported cattle usually arrive into Nigeria through marked routes between the Nigerian northern borders and these neighbouring countries without any international health regulations. As incomes continue to rise in Nigeria, the demand for meat, and therefore for livestock production, increases.

Livestock management is still therefore largely unscientific in Nigeria. Under the Fulanis' indigenous system of animal husbandry, the growth rate and productivity of breeding animals are low due mainly to diseases of reproductive system and other unfavourable herding conditions. The country's herd population growth rate is estimated at about 1.5 per cent per year, while the human population is believed to be growing at the rate of about 2.5 per cent a year (Sanwo, 1975). In the past, the country was dependent to some extent on imported animals from neighbouring countries to overcome the shortage of meat for the increasing population. But when the ban on the exportation of cattle from Niger and Chad Republics was imposed in April, 1975, following the abolition of "jangali" (cattle tax) by the Nigerian government, there had been a noticeable shortage of meat available in the markets. Therefore, the Federal government resorted to importation of frozen beaf and chicken from many European countries to supplement the available meat in the country. The cost of imported meat is beyond the means of most Nigerians (Bunmi Sofola, 1976), and there is the added difficulty of proper storage owing to frequent electricity supply failure in the country.

The majority of the settled herds in Nigeria are privately owned and without veterinary supervision at all. The few government-owned herds lack adequate livestock management and they have been found to have a high prevalence of bovine brucellosis (Esuruoso, 1965, 1973, 1974a, 1974b; Adams and Mckay, 1966; Benerjee and Bhatty, 1970; Esuruoso and Van Blake, 1972). The highest prevalence of infection was found in Western State (now divided into three new states: Oyo, Ogun and Ondo States). Cattle imported from neighbouring Niger, Chad and Guinea Republics have been shown to be serologically positive to Br. abortus (Esuruoso, 1974b).

There are also very few meat-packing factories in the country and the surroundings of most of the local council abattoirs that exist are dirty and not conducive to the slaughter of cattle for human consumption. Also, private slaughtering of animals is done on a large scale in Nigeria, particularly during ceremonies and religious festivals. Retail distribution of meat products is done under the most deplorable sanitary conditions in the public markets and very often no previous inspection is conducted before meat is sold to the public. These situations obviously encourage direct spread of Brucella organisms from infected animals to abattoir workers, meat sellers and the consumers.

E: THE WESTERN STATE OF NIGERIA

The Western State was one of the twelve states created by the Federal Military Government of Nigeria on 27th May, 1967. The population of the State according to the November, 1973 census is approximately 9.5 million, with an area of 7. million hectares. The state is

inhabited by the Yorubas, a group of people from different provinces but with very related socio-cultural background. There are also many Nigerians from other parts of the country and foreigners living in the state, particularly in the capital city of Ibadan. Table II.1 shows the employed population of the Western State grouped according to sex and type of occupation in different component provinces.

Ibadan is the capital of the Western State with a population of 1.3 million (according to the 1963 census reports). It is Nigeria's leading university town, and the largest indigenous African city covering over 26,000 hectares.

Ibadan is 140 kilometres north of Lagos by road and 193 kilometres by rail; it has an aerodrome for domestic flights only. The fact that it is linked to all parts of the Western State by roads accounts for the dominant position in distributive trade.

Agriculture, including livestock, is the mainstay of the Western State economy: the state has no productive oil fields. Agriculture alone employs approximately 50 per cent of the state's labour force (Table II.2), and it also accounts for about 60 per cent of the state's gross domestic product. The cash crops are mainly cocoa and oil palm, but other products such as rubber, cotton, coffee, tobacco and grapefruits are also exported in commercial quantities.

Livestock Activities in Western State

Cow meat is the most readily available animal protein to the people of the state (Table II.3). Most of the livestock and other animal products are derived from the northern states of the country (Table II.4) and some are imported from neighbouring African countries.

There are many different types of herds in the state, including government-owned settled herds, settled Fulani herds and nomadic herds. The indigenous cattle of Southern Nigeria (including the Western State) are the humpless dwarf Muturu and Keteku, while the humped Zebu are herded down from the Northern States. The N'Dama, which are also humpless and were originally imported from Guinea, Sierra Leone and Congo (now Zaire) have great resistance to trypanosomiasis and therefore have become the cattle of choice for range management in Southern Nigeria. There are many large government-owned breeding centres in various parts of Western State with N'Dama heifers and steers. In addition, many farmers buy this breed of cattle to add to their indigenous Keteku cattle to start their own herds, and thus the development of beef herds has become popular among farmers and co-operative societies in the Western State. However, most of the N'Dama breeding centres were found during investigations by Esuruoso to be heavily infected with brucellosis and there is evidence that the disease was transferred to new centres wherever the animals were sent especially to small-scale farmers (Esuruoso, 1974a, 1974b). Therefore it is probable that apart from being an occupational hazard to farmers, veterinarians, cattle-herders and meat sellers, brucellosis may in certain circumstances infect a limited number of people in whom it is not likely to be suspected. An investigation into the human public health aspect of brucellosis should therefore be considered as useful and timely.

Medical Services in Western State

The medical services provided by the state government are grossly inadequate and unevenly distributed: in addition the few medical

personnel in the overcrowded government-owned hospitals are usually overworked. The preventive aspect of medical and health services is carried out by the medical personnel assigned by the state government to the various district council areas. Complementary specialist medical services are provided by the two Federal government-owned teaching hospitals in the state.

The University College Hospital, Ibadan is the first medical teaching institution in Nigeria and it is financed by the Federal government. It is situated on the north end of Ibadan city. It is a 550-bed modern teaching hospital, providing general and specialist medical treatment to many Nigerians. The yearly admissions range between 8,500 and 10,000. Over 80 per cent of the patients that attend the hospital are from Ibadan and Oyo Provinces of Western Nigeria. However, patients are referred from other parts of the country, and sometimes from neighbouring West African countries, to the hospital. The medical staff of the hospital are encouraged and provided with facilities to carry out clinical and epidemiological researches into various common diseases encountered in Nigeria. Apart from this hospital, there is no other medical institution in Western State where laboratory diagnosis of human brucellosis is carried out.

F: AREAS COVERED BY THE STUDY

The present study was carried out in the Oyo and Ibadan provinces of Western State of Nigeria: these two provinces now constitute the Oyo State of Nigeria with the capital in Ibadan, following the establishment of the nineteen-state structure in January, 1976 (Fig. II.4).

The reasons for choosing Oyo and Ibadan provinces were based on ecological, geographical, epidemiological and clinical considerations:

(a) 4,047 (90.1 per cent) out of the total 4,491 livestock farmers in Western State are found in these two provinces, according to the 1963 census reports (Table P.2). Also over three quarters of government-owned cattle breeding centres are in these two provinces.

(b) Majority of the cattle herded down from the north pass through these two provinces before being distributed to other parts of Western State.

(c) The two provinces constitute a homogeneous group with very close cultural and historical background. The population, according to the 1963 census reports, was 4.2 million or approximately 50 per cent of the whole Western State.

(d) Majority of the patients seen at the University College Hospital, Ibadan, come from the two provinces (Shoge: personal communication).

(e) The central unit for the study is based at the Department of Medical Microbiology, University College Hospital, Ibadan. This unit is responsible for planning and co-ordinating the various aspects of the study, collecting specimens from the fields, carrying out the laboratory tests, analysing the data which will be used to ascertain the results of the study and evaluating the progress of the research programme.

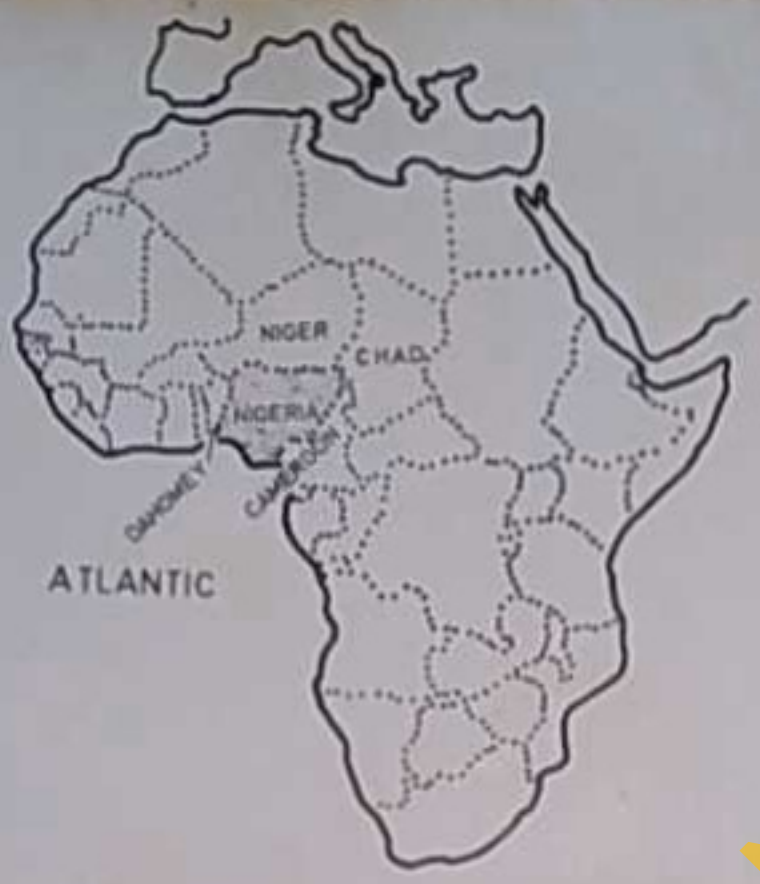

Fig.II.1: Map of Africa - showing the Geographical Boundaries of Nigeria.

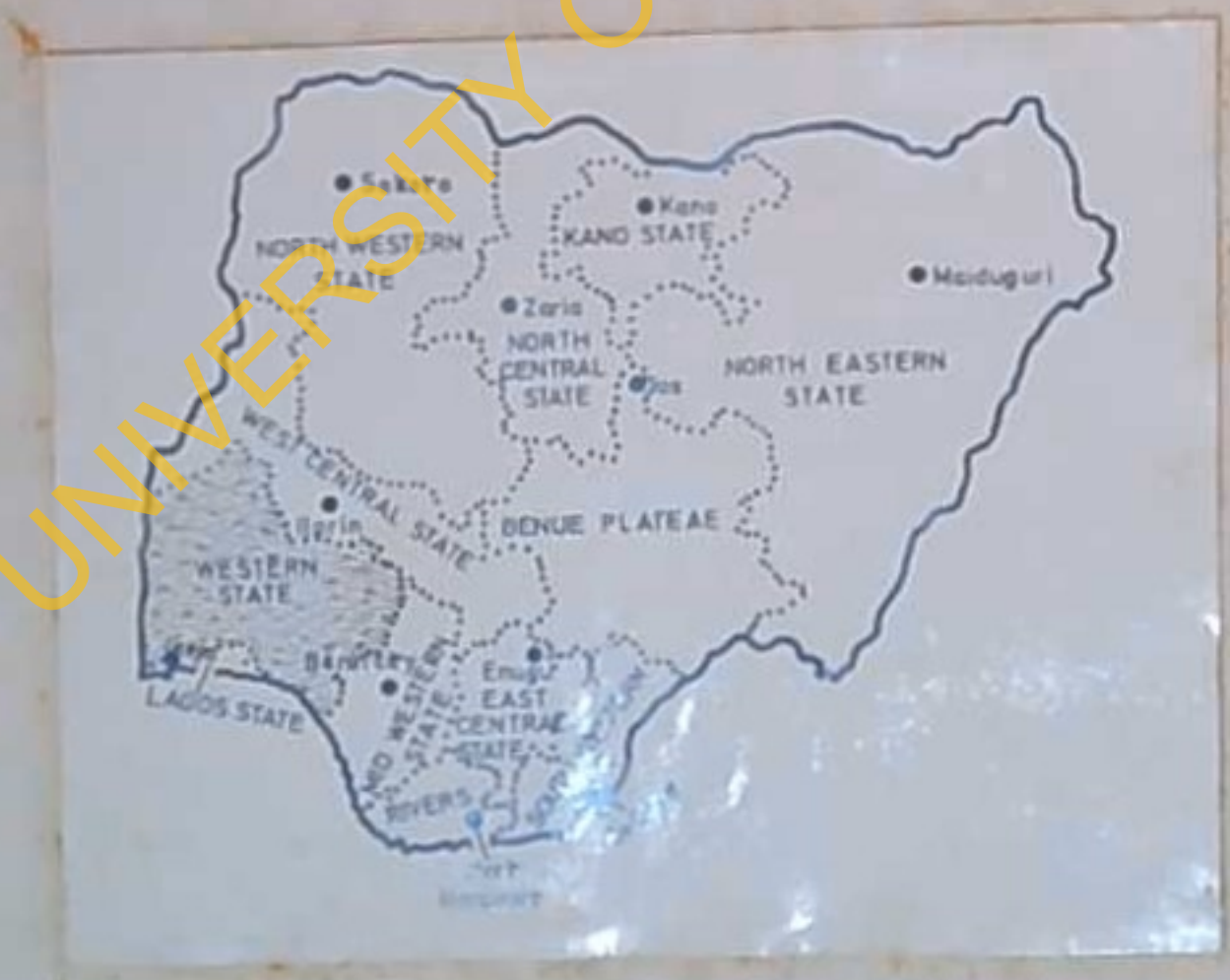

Fig.II.2: Map of Nigeria: 12-state structure, showing the Western State.

TABLE II.1

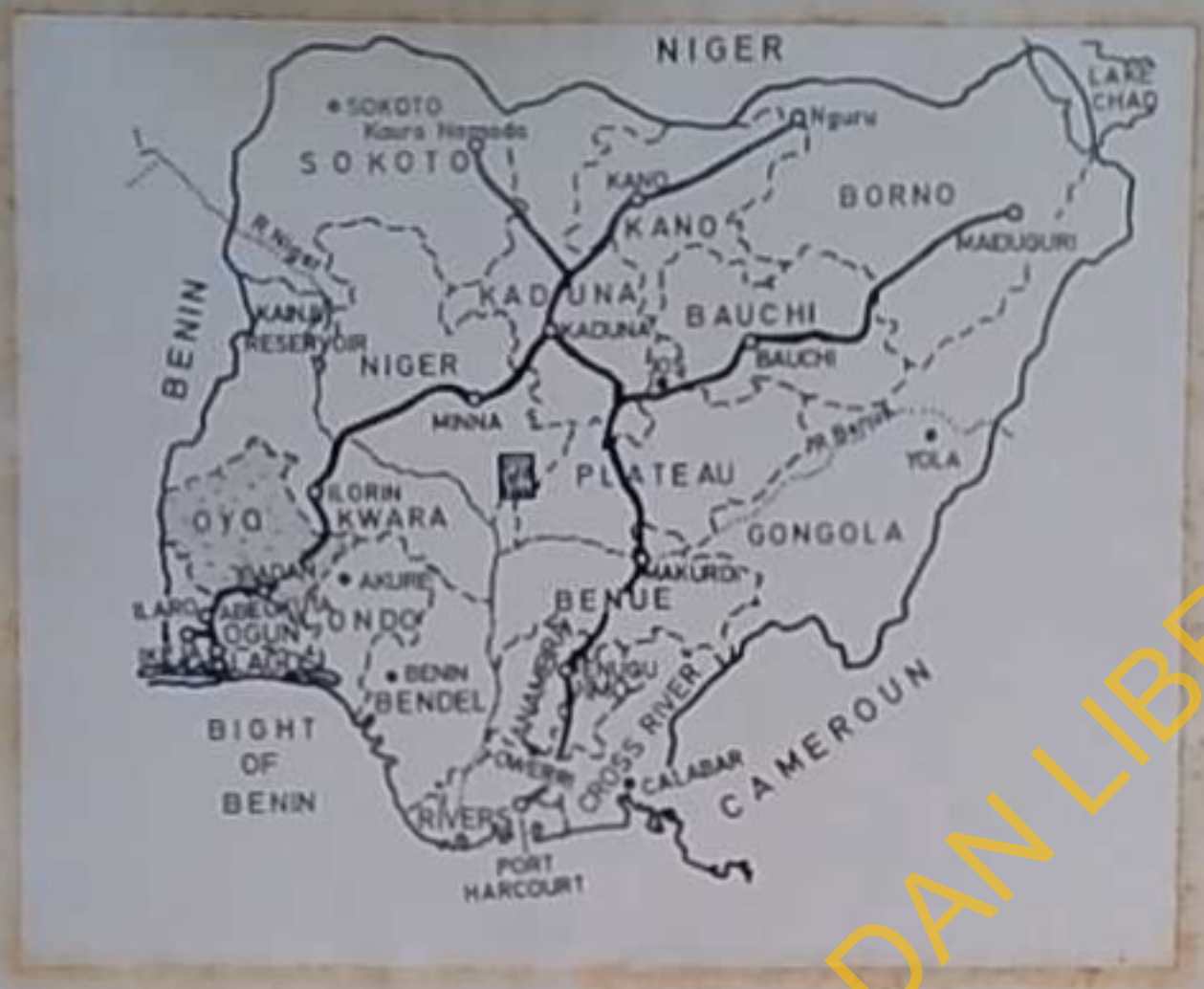

Fig.II.3: Map of Nigeria: 19-state structure, showing the Oyo State.

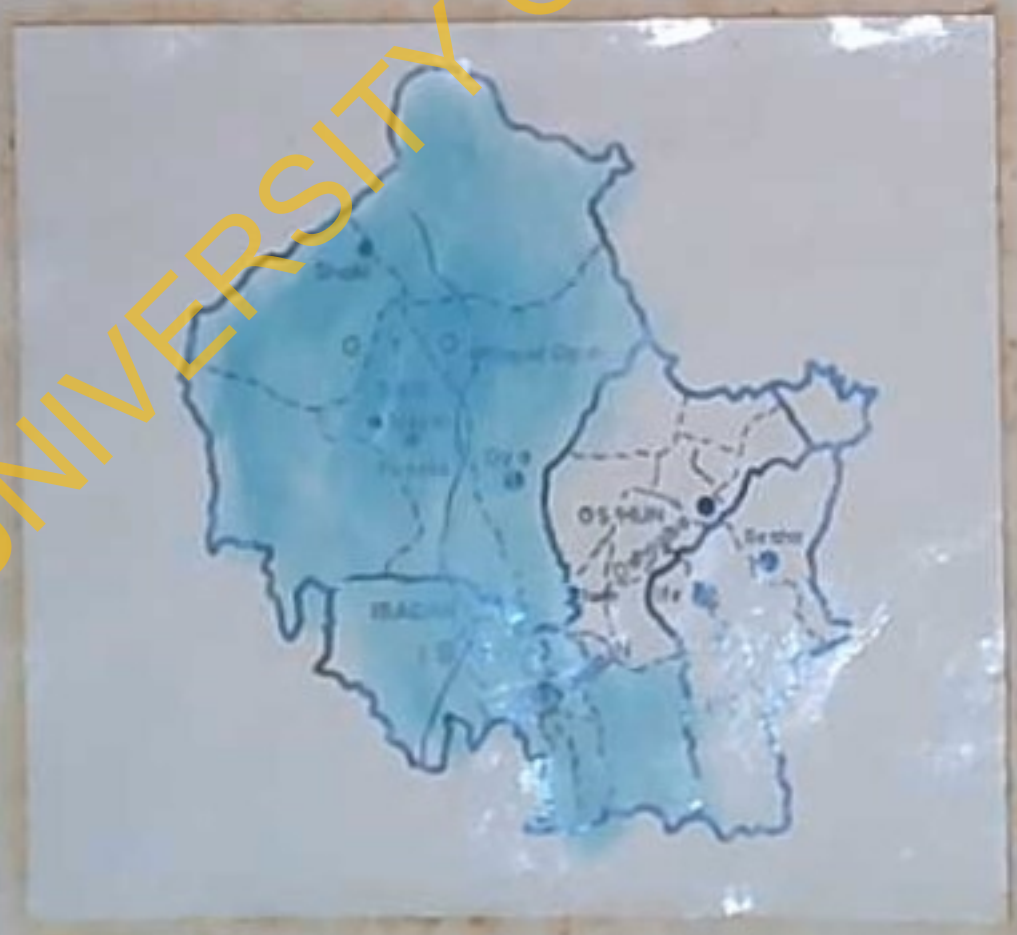

Fig.II.4: Map of Oyo State of Nigeria: showing the Provinces, Divisions, Districts and Areas Investigated (shaded).

TABLE II.1

TABLE II.1

EMPLOYED PERSONS IN THE WESTERN STATE BY MAJOR OCCUPATION, SEX AND PROVINCE, 1963

O C C U P A T I O N	SEX	P R O V I N C E					TOTAL
		IBADAN	OYO	ABEOKUTA	IJEBU	ONDO	
Professional, Technical and Related Workers	Male	37,236	16,972	10,718	7,601	26,006	98,533
	Female	9,111	4,175	2,566	2,611	7,954	26,417
Administrative, Executive and Managerial Workers	Male	4,782	2,741	827	632	1,718	10,700
	Female	685	392	88	108	109	1,382
Clerical Workers	Male	33,064	11,670	5,483	5,648	13,089	68,954
	Female	4,693	1,399	468	872	1,506	8,938
Sales Workers	Male	120,775	53,854	21,208	14,156	44,224	254,217
	Female	416,632	218,073	146,016	79,437	176,882	1,037,040
Farmers, Fishermen, Hunters, Diggers and Related Workers	Male	481,658	307,424	157,767	69,583	408,283	1,424,415
	Female	19,154	9,202	11,695	11,655	53,202	104,908
Miners, Quarrymen and Related Workers	Male	210	410	193	50	443	1,306
	Female	15	14	3	3	5	40
Transport and Communication Workers	Male	46,585	14,492	11,336	6,191	17,526	96,130
	Female	687	196	119	102	349	1,453
Craftsmen, Production - Process Workers and Labourers	Male	207,011	87,170	45,236	37,245	121,345	498,007
	Female	30,763	34,600	10,879	6,907	102,103	185,252
Service, Sports and Recreation Workers	Male	34,817	14,006	7,287	5,575	18,141	79,826
	Female	19,053	10,985	2,514	1,828	12,506	46,886
Unspecified Workers	Male	7,976	4,429	1,057	1,207	2,416	17,085
	Female	164,393	118,225	161	47,281	75,153	405,213
TOTAL EMPLOYED PERSONS	Male	973,814	513,168	261,112	147,888	653,191	2,549,173
	Female	665,186	397,261	174,509	150,804	429,769	1,817,529

SOURCE: 1963 Population Census Reports.

TABLE II.2

NUMBER OF PERSONS ENGAGED IN AGRICULTURAL ACTIVITIES

BY CATEGORY AND PROVINCE 1963

CATEGORY	PROVINCES					TOTAL
	IBADAN	OYO	ABEOKUTA	IJEBU	IJEBU ODO	
CROP FARMERS	456,428	281,559	142,785	65,784	383,991	1,330,587
LIVESTOCK FARMERS	597	3,450	159	65	220	4,491
MARKET GARDENERS	28,583	12,093	9,646	4,943	8,690	63,955
CATTLE HANDS	214	179	71	19	87	570
OTHER FARM WORKERS*	7,692	9,232	12,795	5,601	37,128	72,448
TOTAL	493,514	306,553	165,456	76,412	430,116	1,472,051

NOTE:- *THESE INCLUDE FARM EQUIPMENT OPERATORS, GARDENERS AND OTHER FARM WORKERS NOT SEPARATELY LISTED ABOVE.

SOURCE:- 1963 CENSUS REPORT Vol.II.

TABLE II.3

NUMBERS OF LIVESTOCK SLAUGHTERED IN THE WESTERN STATE OF NIGERIA 1964 - 1973

ANIMAL	Y E A R									
	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973
CATTLE	133,640	112,469	85,028	89,138	116,854	101,489	134,577	135,356	128,287	135,797
SHEEP AND GOATS	10,478	9,122	5,454	7,046	11,242	10,068	12,060	16,272	17,424	20,772
PIGS	5,432	4,323	3,911	3,850	4,059	3,059	3,573	4,517	4,924	6,799

SOURCE:- Veterinary Division, Ministry of Agriculture and Natural Resources, Ibadan.

TABLE II.4

LIVESTOCK "IMPORTED" FROM THE NORTHERN STATES TO WESTERN STATE 1960 - 1973

YEAR	CATTLE*		SHEEP		GOATS*	
	RAIL	ROAD	RAIL	ROAD	RAIL	ROAD
1960	14,327	113,767	5,010	9,954	[illegible]	2,751
1969	11,020	155,403	11,554	27,[illegible]	5,834	19,493
1970	17,960	140,856	3,8[illegible]	19,837	3,913	3,633
1971	9,537	152,640	2[illegible],803	31,625	38,961	60,591
1972	11,212	[illegible]	2,931	17,201	23,382	332,177
1973	4,[illegible]	289,695	2,344	30,408	8,905	733,328

NOTE:- *In absolute number

SOURCE:- Veterinary Division, Ministry of Agriculture and Natural Resources, Ibadan.

SECTION TWO

REVIEW OF THE RELEVANT LITERATURE

CHAPTER III

HISTORICAL BACKGROUND AND BACTERIOLOGY

A: HISTORICAL BACKGROUND

Brucellosis is one of the most widespread and economically the most ravaging of more than 100 zoonoses which are recognized. It is primarily an infectious disease of domestic animals, particularly herbivores and swine, which are the main sources of human infection under special circumstances.

Brucellosis has been present in hoofed animals since ancient times, The fossilized remains of animals in certain regions show bone changes consistent with those observed in brucellosis. The European bison, the Persian hillgoat, and the Maltese goat have been suffering from brucellosis for a long time (Van der Hoeden, 1964). The first clinical report on the disease in man is ascribed to Hippocrates, as early as the 5th century A.D. Later, diseases resembling brucellosis were frequently reported in the mediterranean area, their designation being usually based on their analogy to other well-known maladies, according to the most characteristic symptoms or the locality in which they prevailed. Historical names, some still occasionally used, include

pseudotyphus
intermittent typhoid fever
febris typho-malarine
fievre sudorale
undulent fever or intermittent fever or goat fever

bovine contagious-, infectious- or enzootic abortion
Bang's disease
slinking of the calf
melitococcia
Mediterranean (gastric remittent) fever
Gibralter-Rock fever
Cyprus fever
Malta fever
Neapolitan fever

Brucellosis reached Spain in the 15th century and was brought by the Spaniards to America. Malta acquired importance as a focus for the spread of brucellosis during the Crimean war, when the disease was differentiated as a special one, distinct from other febrile ailments in the area.

The history of the discovery of epidemiology of brucellosis and subsequent discovery of the causative agent is fascinating and instructive. In 1884, David Bruce, a medical officer, was sent to Valetta, Malta, where a large contingent of the British army and navy was stationed. During his stay in Malta he treated 400 patients with Malta fever. He gave a detailed description of symptoms and signs in 37 patients and ruled out typhoid fever as a cause of Malta fever. In 1886, two years after his arrival at the naval base, he discovered the actiologic agent of Malta fever when he was working in Sims Woodhead laboratory, with the assistance of his wife. He isolated the causative organism from the spleen of four soldiers who had died of the disease in Malta. He regarded the microorganism as a coccus and named it Micrococcus melitensis (after Malita - a Roman name for the island of Malta). Bruce successfully transmitted the disease to monkeys who contracted a somewhat similar disease and he recovered the organism in

pure culture from their livers and spleens (Bruce, 1887). Hughes (1897), a medical officer stationed at Malta, published a classical monograph about Malta fever which he baptised undulant fever.

In 1895, Bang, a Danish veterinarian, isolated a gram-negative bacillus from a gelatinous exudate between the uterine wall and the foetal membranes of an aborting cow and he named the organism Bacillus abortus (Bang, 1897). Injection of these organisms (Bacillus abortus or Bacterium abortus infectiosi) into pregnant cows resulted in abortion.

It was, however, not until 1918 that the close morphological, cultural and antigenic resemblance of the "micrococcus" of Bruce and the "bacterium" of Bang was demonstrated by Alice Evans (1918), an American bacteriologist. Since 1920, both microorganisms have been designated by the generic name Brucella (Meyer and Shaw, 1920).

A strain which Traum had isolated from stomach and kidneys of a premature pig from a farm in Indiana, U.S.A., where some were aborting, showed differences from Br. melitensis and Br. abortus, This strain was named Br. suis and it was the third species of Brucella discovered (Traum, 1914). Thomsen (1934) cultivated from pigs in Denmark, brucellae which in some respects differed from the American strain of porcine origin. Afterwards, the Danish variety was also found in hares. Some organisms have been tentatively classified as members of the genus Brucella: Br. ovis has been isolated in Australia by Buddle and Boyes (1953), and later in New Zealand and California, from sheep with genital lesions especially causing ram epididymitis. Br. neotomae has been isolated from the desert wood rat, Neotoma lepida, in Utah, U.S.A. by Stoenner and Lackman (1957), but appears

to be of no importance to man. Br. rangiferi tarandi has been isolated from rander (Rangifer tarandi) in Russia (Davgdov, 1961) and similar strains have been isolated from Eskimos and from the Caribou (Rangifer arcticus) in Canada and Alaska. Br. canis has been identified as a cause of abortions in dogs, particularly beagles (Carmichael and Kenny, 1966). The existence of these other types of Brucellae, apart from the three classical species, may suggest the possibility of other reservoirs of infections in nature.

The relationship of the disease in animals to human infection was not well understood until comparatively recently. Full light was thrown on the epidemiology of the disease by the research of the British Royal Commission on Mediterranean Fever under Sir David Bruce's direction. A Maltese physician, Themistocles Zammit (1905), a member of the British Mediterranean Fever Commission, found accidentally that Maltese goats, used for scientific experiments had positive agglutination titres to the Micrococcus melitensis. Subsequently, this organism was cultured from blood and milk of goats. Zammit therefore concluded that man acquired Malta fever from drinking infected goat's milk and first used the agglutination test to detect animals discharging Brucella in milk. Zammit's observation was outstanding and one of the most significant in the epidemiology of brucellosis. In 1906, following the researches made by Zammit, troops stationed in Malta were forbidden to drink unboiled goats' milk, and immediately there was remarkable drop in the cases and deaths from undulant fever among the military population in contrast to the still frequent incidence amongst the civilian population, which was not subjected to the prohibitive law (Eyre, 1908).

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The pathogenicity of Br. abortus to man was also suggested by Bevan (1921), who observed in Rhodesia several cases of a disease which resembled Malta fever in persons working on farms on which cattle suffered from contagious abortion. Brucellae were isolated from one of these patients, a man employed as a butcher. It was soon evident that sources of infection other than milk and dairy products were responsible for human infection. More recently, detailed accounts of the nature of brucellosis have been given by Spink (1956) and Dalrymple-Champneys (1960).

B: BACTERIOLOGY

Aetiology: Three closely related species of the genus Brucella are recognized as causing brucellosis in man:

(i) Br. melitensis (Bruce 1887), with goat and sheep as natural hosts. It is the type species of the genus, Brucella;

(ii) Br. abortus (Bang 1897), with cow and, in 5 to 15 per cent of cases, the horse as natural hosts. In cattle, the brucellae are restricted primarily to the pregnant uterus, and secondarily to the udder ducts;

(iii) Br. suis (Traum, 1914), with the pig as natural host in the United States, hares have also been found to be infected naturally with the Danish strain of this organism (Witt, 1941).

Terminology, Classification and Specialization

The changing and confusing nomenclature of the first two species of Brucella discovered was noted by Hull (1963):

Brucella abortus

Bacillus abortus (Bang, 1897)
Corynebacterium abortus (Preisz, 1903)
Bacterium abortus (Society of American Bacteriologists, 1918)
Alcaligenes abortus (Castellani and Chalmers, 1919)
Brucella abortus (Meyer and Shaw, 1920)

Brucella Melitensis

Micrococcus melitensis (Bruce, 1887)
Bacillus melitensis (Jurdan, 1912)
Bacteruim melitensis (Society of American Bacteriologists, 1918)
Alcaligenes melitensis (Castellani and Chalmers, 1919)
Brucella melitensis, Meyer and Shaw, 1920)

The difficulties encountered by the bacteriologist in studying Brucella have been concerned with isolation, varying morphology, differentiation, and classification of strains isolated from human and animal sources. The changing morphology of the brucella, however, is better understood as a result of extensive studies of bacterial dissociation. Variation among bacteria is now expected when formerly it was only suspected. Differentiation of the various species of the genus Brucella (Table III.1) is based upon rather subtle laboratory methods which were, for the greater part, first described by Huddleson in 1928 (Huddleson, 1943). Outstanding work on colonial dissociation of brucella was carried out by Henry in 1933 who observed several factors which brought it about. On artificial media, Brucella tends to a rapid dissociation from "smooth" to "rough" growth, with intermediate colonial types. A change from smooth ("S") to rough ("R") variation is also associated with a change in ability to agglutinate. This interferes with the serological differentiation of such strains. Br. ovis is only known in the "R" state and cannot, therefore, be satisfactorily classified on the basis of its antigenic structure.

The conventional methods for species differentiation in the genus Brucella have been based on the following properties (Joint FAO/WHO, 1954a):

(a) the need for added carbon-dioxide for growth, especially on primary isolation;

(b) the production of H_2S;

(c) agglutination in monospecific sera.

Other tests, such as urease activity, sensitivity to thionin and basic fuchsin, and use of other dyes (methyl violet and pyronin) and diethyldithiocarbamate (DEDTC) tests are used by some workers.

It has long been recognized that the differences based on these tests are quantitative rather than qualitative but that, when methods are controlled and standardized and cultures in the smooth or smooth-intermediate phase are used, the majority of strains in most parts of the world can be identified as belonging to one or other of the three classical species as shown in Table III.2.

Since 1967, research has progressed on speciation and two methods have been developed which help to place strains of brucella into one of the three fairly well-defined species. These methods are the oxidative metabolic tests and the use of brucella phage: these two methods are recommended by the Joint FAO/WHO Expt. Comm. on Brucellosis (1971) as the principal basis for speciation.

The rates of oxygen uptake of a large number of brucella cultures on eight amino-acid and four carbohydrate substrates have been determined. Studies with cultures which by conventional typing methods are typical Br. abortus, Br. suis or Br. melitensis have shown that a characteristic metabolic pattern can be associated with each species.

Stable brucella phage was first isolated in the USSR and has been found to be lytic for smooth Br. abortus, but generally not for smooth Br. suis or Br. melitensis. The use of oxidative metabolic and phage susceptibility tests on the same cultures has shown so far that only those cultures with the metabolic pattern of Br. abortus, irrespective

of their properties by conventional tests, are susceptible to lysis by this phage.

At the 8th International Congress for Microbiology in 1962, the subcommittee on Taxonomy of Brucella reported on the definition and speciation of the genus, Brucella, as follows:

Definition of the Genus:

"Coccobacilli or short rods, 0.5.- 0.7 by 0.6 - 1.5 μ, arranged singly, more rarely in short chains. No capsules. Non-motile. Do not form endospores. Gram-negative. Do not show bipolar staining. Chemo-organotrophs; metabolism respiratory.

The following vitamins are required for growth: Thiamine, niacin, and biotin; calcium pantothenate often stimulates growth; haemin (X factor) and coenzyne 1 (V factor) are not required.

Catalase-positive; oxidase-usually positive, but Br. neotomæ and Br. ovis are oxidase-negative. Urea hydrolysed to a variable extent. Nitrates reduced to nitrites (except for Br. ovis). Citrate not utilized; indole not produced; methyl red test and Voges-Proskauer reaction negative. Litmus milk - no change. Strict aerobes; some require 5-10% of added CO_2 for growth, especially on initial isolation.

Temperature range 20 - 40°C, optimum 37°; optimal pH 6.6 - 7.4. Mammalian parasites and pathogens; facultatively intracellular with a relatively wide host-range.

The guanine-plus-cytosine content of the DNA ranges from 55 - 58 moles per cent (buoyant density); members of the genus comprise a closely knit and sharply demarcated genetic group as defined by DNA hybridization studies."

SPECIES DIFFERENTIATION

1. Brucella melitensis "Aerobic. Produces no H_2S, or not more than a trace, on peptone media. Usually grows in the presence of basic fuchsin and thionin. Usually M antigen predominant. Usually pathogenic for goats and sheep and can also affect other species including cattle and man.

FAO/WHO Reference neotype strain - Br. melitensis strain 16M

Three biotypes are recognized" (Table III.1)

2. Brucella abortus "Usually requires added CO_2 (5%) for growth, especially on primary isolation. Usually produces moderate amount of H_2S but may be negative. Usually grows in presence of basic fuchsin but inhibited by thionin. Usually has A antigen predominant. Cultures in the smooth or smooth-intermediate phase are lysed by brucella phage Tb at routine test dilution (RTD). Usually pathogenic for cattle causing abortion, can also affect other species including man.

FAO/WHO Reference neotype strain - Br. abortus 544.

Nine biotypes are recognized" (Table III.1).

3. Brucella suis "Produces large amount of H_2S or none at all. Grows in the presence of thionin but usually inhibited by basic fuchsin. Usually has A antigen predominant. Not lysed by brucella phage Tb at RTD. Usually pathogenic for pigs, but can also affect hares and other species including man.

FAO/WHO Reference neotype strain - Br. suis 1330.

Four biotypes are recognized." (Table III.1).

Typing of Brucella

In the majority of cases, the phage and conventional tests are

adequate in identifying cultures encountered in diagnosis and surveys. The use of the conventional tests to define biotypes has been invaluable in epidemiological studies, as it is possible to identify strains that could not be differentiated with phage and oxidative tests. Many laboratories find it useful to employ additonal simple tests in order to characterize other strains endemic in their geographical area. Whether minor differences justify the establishment of a new biotype or species will depend on how widespread and how epidemiologically significant it is.

FAO/WHO recommended methods for identifying brucella species which have been summarized by Alton and Jones (1974b, 1975). The reference strains of the three species should always be examined at the same time as the unknown strains. Reference strains for the species and their biotypes are available from the FAO/WHO Brucellosis Centre, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, Weybridge, England.

Resistance

Brucellae are killed by heating at 60°C for 10 minutes (pasteurization) and by exposure to one per cent phenol for 15 minutes. However, in nature, Brucellae are rather resistant to various environmental conditions and can survive for long periods. In direct sunlight they are often killed in a few hours, but in damp shade they may persist for months. In milk they survive for several days, or till the milk turns sour, when they are killed by acidity. In fresh cheese made from goats or sheep's milk they flourish enormously, and such cheeses have been frequent sources of infection (Galbraith et al, 1969). Hard fermented

cheese is safe, though how long the cheese must age to ensure this safety is not clear (Spink, 1956; Dalrymple-Champneys, 1960). Cheese made from pasteurised milk is always safe. Butter is usually regarded as safe, as the souring of the milk kills the brucellae (Smith, 1934; Pullinger, 1935). But, in one investigation, brucellae were isolated from butter kept for 142 days at 46°F and from cheese after two months (King, 1957).

In tap water, brucellae may remain alive for 57 days at 8°C and for 10 days at 25°C (Horning, 1936). In human urine they remain alive for at least one week and in animal faeces the organisms have survived in the open for 100 days, at 8°C for over a year (Horning, 1936; King, 1957). From the walls and floors of cowsheds the organisms have been isolated for as long as four months, and from the dung-contaminated ground outside for five weeks (Beattie and Rice, 1934).

A very large number of observations have been made on the survival of brucellae outside their host. Figures vary under different environmental conditions, but it is clear that once an environment is contaminated, it may remain so for a very long time, a fact of great importance in the epidemiology of brucellosis.

CULTIVATION, ISOLATION AND PRESERVATION OF BRUCELLA

The only absolute proof of a Brucella infection is the isolation of Brucella organisms from the diseased person or animal. Good techniques in the collection and preparation of specimens, the inoculation of media, and the injection of laboratory animals are a prerequisite for the successful isolation of brucella. All operations should be done under aseptic condition. Isolation of Brucella organisms, however, is

not always easily accomplished (Spink et al, 1952). Br. suis and Br. melitensis are more readily cultivated than Br. abortus, many strains of which require an atmosphere of from 10 - 20 per cent CO_2.

Direct inoculation of the specimen onto appropriate solid media is preferred to broth methods of isolating brucellae. The use of solid media limits the establishment of non-smooth mutants and facilitates recognition and isolation of growing colonies. Liquid media are widely used for primary isolation from blood and other body fluids; it is essential to make subcultures from liquid onto solid media early, in order to detect growth and to limit dissociation of the organisms which occurs with continued growth in liquid media. Although most recoveries are made within 7 to 14 days, the inoculated media should not be discarded as negative until at least 35 days. With foods or other heavily contaminated material, guinea-pig inoculation may be necessary.

Specimens should be cultured on basal (non-selective) enriched media (by the addition of 5 per cent serum), as well as on selective media. Inasmuch as the species of Brucella to be isolated cannot be predicted, duplicate plates should also be made and incubated both under atmospheric conditions and in the presence of added CO_2 (10%). Better results are obtained when several suitable media are employed.

Basal Media

The choice of basal medium frequently depends on the considerations of cost and availability. The following basal media are recommended as having been found satisfactory (Alton and Jones, 1967, 1975):

(a) Serum-dextrose agar

(b) Serum-potato-infusion agar

(c) Commercial media: trypticase soy, tryptose agar, and brucella agar containing serum

(d) Blood agar (5 per cent sheep blood in a blood-agar basal medium).

Liquid Media

(a) Trypticase soy broth

(b) Tryptose broth

(c) Brucella broth (Albimi)

For the isolation of brucella from blood or other body fluids, Castaneda (1947) recommends the combined use of agar and broth in small bottles or flasks.

Selective Media

The selective agents consist of various antibiotics with or without dyes (ethyl violet). They allow growth of Brucella while suppressing growth of contaminants. The antibiotics commonly used are bacitracin, polymyxin B, and cycloheximide. Amphotericin may replace cycloheximide or be used in addition to it. The addition of ethyl violet dye aids in suppressing contaminants but inhibits growth of dye-sensitive Brucella biotypes, notably Br. abortus biotype 2. The choice of selective media to be used will depend, therefore, upon the nature and purpose of the work.

Identification of Brucella may be made tentatively on the appearance of the smooth type of colony which is usually small, convex, moist, and glistening. It has a greyish-blue appearance, is not sticky and does not adhere firmly to the medium. The colony usually becomes visible on the third day and gradually becomes larger till the seventh day of incu-

lation. After a week or ten days, it is more opaque and acquires a light brown colour, particularly the centre which is elevated. Confirmation is dependent upon agglutination of the suspected organism by monospecific Brucella antiserum. This is true only for "S" culture. Dissociated cultures agglutinate poorly or not all, while rough cultures agglutinate spontaneously.

Dissociation of cultures occurs easily (Henry, 1933). Changes in colonial morphology go together with changes in infectivity and antigenicity. Smooth cultures are pathogenic, highly infective and antigenic; rough ("R") cultures are not. The different colonies can be identified as to their dissociation by way of morphology. Examination of colonies under obliquely-transmitted light affords a dependable method for distinguishing smooth from non-smooth cultures (Henry, 1933): smooth cultures are glistening, while the rough ones have a dry, granular appearance; mucoid colonies contain some slimy material, and intermediate forms are difficult to classify. Differentiation with the use of the acriflavine test of Braun and Bonestell (1947) and by the 2, 3, 5, triphenyl tetrazolium chloride test of Huddleson and Baltzer (1950) is easier than by morphology: smooth cultures, when suspended in a drop of acriflavine remain in suspension, rough forms will agglutinate, mucoid forms will form threads.

To prevent dissociation of colonies the period of incubation and the number of transfers to subcultures should be kept short and low respectively. When not needed, cultures should not be kept at room temperature but refrigerated at $4^{o}C$. Reference and vaccine strains and vaccines, which consist of living cells, must be preserved by drying

(Alton and Jones, 1967; Alton et al, 1975), freeze-drying, or storage at -65°C or lower temperatures, e.g., in liquid nitrogen (Morgan, Boyce and Casey, 1970).

Large-scale Cultivation

Control and eradication campaigns against brucellosis have led to demands for large volumes of Brucella cells, both for vaccine production and for diagnostic antigens for use in an increasing array of serological tests.

The two main methods in use are culture on a solid medium in Roux flasks (Alton and Jones, 1967; Alton et al, 1975) and culture in a liquid medium with or without the use of dialysis membranes (Hulse and Carnagnan, 1970; Sterne, 1958).

Nowadays, the following vaccines are in use for veterinary medicine: Br. abortus strain 19, Br. melitensis strain Rev. 1, and killed Br. melitensis strain H 38 in adjuvant (Alton and Jones, 1967; Alton et al, 1975).

Guinea Pig Inoculation

The inoculation of guinea pigs is a valuable method for the isolation of Brucella from potentially contaminated specimens (such as milk, urine and genital discharges), although direct cultural techniques are superior for uncontaminated material. Inoculations are made subcutaneously and two guinea pigs are used per sample. One guinea pig is killed three weeks and the second six to eight weeks after inoculation. Blood is collected for the serum agglutination test, macroscopic lesions are recorded, and the spleen and other tissues are cultured. Tests for brucellar allergy can also be made before autopsy.

It is important to note that some strains of Br. abortus are not highly virulent for the guinea-pig: they may be able to cause a temporary infection but unable to cause progressive disease. If the animal is killed at three weeks, the organisms may be isolated from the organs and serological evidence of the infection may be obtained, but in a guinea pig killed at six weeks, such evidence of infection may have disappeared (Wilson and Miles, 1964).

Precautions During Cultivation of Brucella

Since brucellosis is an easily acquired laboratory infection, great care should always be taken in handling Brucella cultures, especially in large volumes. Precautions should always be taken when inoculating, harvesting, bottling and freeze-drying brucellae. Periodic serological and health checks should be made on personnel involved in brucella work to detect those infected as early as possible.

						II	III	I	II	III	A	M	Ac	Se	
Br.melitensis	1	–	–	–	–	+	+	–	+	+	–	+	–		Sheep, goats
	2	–	–	–	–	+	+	–	+	+	+	–	–		"
	3	–	–	–	–	+	+	–	+	+	+	+	–		"
Br. abortus	1	+	+	+	+	+	+	–	–	–	+	–	–		Cattle
	2	+	+	+	+	–	–	–	–	–	+	–	–		"
	3	+	+	+	+	+	+	+	+	+	+	–	–		"
	4	+	+	±	+	+	+	–	–	–	–	+	–		"
	5	+	+	–	–	+	+	–	+	+	–	+	–		"
	6	+	+	–	+	+	+	–	+	+	+	–	–		"
	7	+	+	–	+	+	–	–	+	+	+	–	–		"
	8	+	+	+	–	+	+	–	+	+	–	+	–		"
	9	+	+	±	+	+	+	–	+	+	–	+	–		"
Br. suis	1	–	+	–	+	–	–	+	+	+	+	–	–		Pigs
	2	–	+	–	–	–	–	–	+	+	+	–	–		Pigs,hares
	3	–	+	–	–	+	+	+	+	+	+	–	–		Pigs
	4	–	+	–	–	+	+	+	+	+	+	+	–		Reindeer
Br. neotomae		–	+	–	+	–	–	+	+	+	+	–	–		Wood rat
Br. ovis		–	–	+	–	+	+	+	+	+	–	–	+		Sheep (rams)
Br. canis		–	–	–	–	–	–	+	+	+	–		+		Dogs

(a) Species differentiation is obtained on Albimi or tryptose agar with the following graded concentrations of dyes: 1:25,000 (I), 1:50,000 (II), 1:100,000 (III). Other concentrations may be preferable with other growth media. Interpretation of results should be controlled with the reference strains of each species.

(b) A = abortus; M = melitensis.

... (1971). Fifth Report. Techn. Rep. Ser. No. 464.

TABLE III.2

DISTINGUISHING PROPERTIES OF
THE THREE CLASSICAL SPECIES OF BRUCELLA

SPECIES	CO_2 Reqt.	H_2S Production	Growth on:		Agglutination in monospecific sera	
			Thionin	Basic/ fuchsin	abortus	melitensis
Br. melitensis	-	-	+	+	-	+
Br. abortus	+	+ 4 days	-	+	+	-
Br. suis	-	++ 5 days	+	-	+	-

CHAPTER IV

IMMUNOLOGY, PATHOGENESIS AND PATHOLOGY

A: ANTIGENIC STRUCTURE OF BRUCELLA ORGANISMS

The antigenic structure of the Brucella organisms is complex. Major antigenic changes occur when brucellae undergo the smooth-rough variation. These changes appear to be due to loss of the specific S antigen, and may be accompanied by colonial alterations and reduction in virulence for laboratory animals.

The use of the quantitative agglutinin-absorption test with mono-specific agglutinating sera allows differentiation between the smooth phase antigens of Br. melitensis (M antigen) on the one hand and of Br. abortus and Br. suis (A antigen) on the other (Wilson and Miles, 1932). In Br. abortus and Br. suis, A antigenic determinant is in excess (A/m = 20/1), while M antigenic determinant is present in excess in Br. melitensis (M/a = 20/1). Barbar et al. (1961) extracted the M antigen with 90% phenol and identified it as a polysaccharide containing a nitrogenous substance.

The Brucella organisms have common surface insoluble agglutinating antigenic components with Vibrio, Pasteurella, Yersinia and Salmonella organisms. Positive cross-agglutinations are described following infec-

tion or vaccination with Vibrio cholerae (Eisele, et al, 1947). Incubation at 56°C for 16 hours inactivates these cross-agglutinating antibodies in some cases (Feinberg and Wright, 1951). On the other hand, the internal soluble precipitin antigens are distinctive for the genus, and they show no cross-reaction with other genera, such as Pasteurella, Yersinia, Salmonella and Vibrio.

Little insight has been obtained in the relation of antigenic structure and the problems of host preference and pathogenicity. We know that a specific host preference exists: Br. abortus prefers cattle, Br. melitensis sheep and goat, Br. suis pigs, but we fail to understand the relation between this preference and the antigenic structure of the organisms. Br. abortus has a high degree of infectivity, but low level of pathogenicity in man, whereas Br. melitensis often causes acute incapacitating human infection (Christie, 1974).

B: ANTI-BRUCELLA IMMUNOGLOBULINS

Considerable advances have been made in the study of immunoglobulins (Ig's), including their nature, structure, formation, and factors affecting their production and persistence. World Health Organization meetings on the Nomenclature of Human Immunoglobulins have led to an agreed method of definition and nomenclature (WHO, 1964b; WHO, 1968a). In general, high molecular weight antibodies, sedimenting close to 19S in the ultracentrifuge and sensitive to treatment by 2-mercaptoethanol, appear analogous to human brucella agglutinin IgM. Antibodies sedimenting close to 7S in the ultracentrifuge and not usually sensitive to 2-mercaptoethanol appear analogous to human brucella agglutinin IgG (Anderson et al, 1964; Feinin, et al, 1965).

C: IMMUNOLOGICAL RESPONSE IN BRUCELLOSIS

Introduction of Brucella antigen stimulates humoral and cellular responses by the host. Only a limited number of antigens stimulate antibody production, others might produce hypersensitivity or relative immunity (Rasooly, et al, 1965). The immunological response of the host has been studied along two general lines. First, the humoral response after vaccination and infection with brucellas have been studied in man (Reddin et al, 1965) and in animals (Knyazeva et al, 1974). Second, the nature of the characteristic granulomatous reaction, the dermal response to Brucella antigens, and the basic role of macrophages or mononuclear cells have been identified (Knyazeva et al, 1974; Braude, 1951).

1. Immunoglubulin Response in Brucellosis

Following intracutaneous introduction of living organisms into human subjects, the most commonly recognised immune response is the appearance of Brucella agglutinins at the end of the first week. The highest titres occur between the second and fourth week with a gradual and continuing decline over 12 to 24 weeks. The total concentration of agglutinins represents a mixture of at least two components: 19S (IgM) appearing first and 7S (IgG) being detected later (Reddin et al, 1965), but with 19S always in predominant amount. The 19S macroglobulins develop in response to a much smaller antigenic stimulus than the 7S microglobulins (Christie, 1974). Extensive studies in human patients with either acute or chronic illness have indicated that after recovery only 19S immunoglobulin is usually demonstrated and that the presence of 7S

agglutinins is indicative of active disease (Reddin et al, 1965; Kerr et al, 1969). The 7S agglutinins have been particularly helpful in the diagnosis of chronic disease in instances where bacteriological data are lacking and the Brucella titre is very low (50 or 100 i.u.). Furthermore, it appears that 7S antibody fixes complement (Anderson et al, 1964) which makes the complement-fixation reaction a more specific test for the detection of active brucellosis. These immunological reactions have also been valuable in the detection of active bovine brucellosis, particularly in members of a presumably healthy herd (Anderson et al, 1964).

Similar findings are reported in veterinary medicine. Morgan (1969) established that antibody response to either infection or vaccination (Br. abortus strain 19) consists of an initial IgM production followed by IgG. Vaccination of cattle produces 19S antibodies as early as the fourth day with a maximum titre on the 13th day; 7S antibodies appear on the seventh day with a maximum titre on the 28th day. The IgG antibodies disappear after six months while the IgM antibodies will persist longer. In case of chronic infection the IgG antibodies will persist as long as the disease in active, whilst the IgM antibodies may disappear completely or decrease to a low level.

The duration and seriousness of the disease in fact determines the type and quantity of the produced immunoglobulins (Reddin et al, 1965). The presence of IgG antibodies reflects the activity of the infection (MacDonald and Elsmie 1967; Coghlan and Weir, 1967). The presence of IgM in lower titres does not necessarily indicate present infection. In endemic areas low titres might be common, and patients with a previous, cured

brucellosis may have raised IgM titres for many years.

The distinguishing characteristics of the brucella-antibody types can be explained in part by the observations of Svehag and Mandel (1964) in which the antigenic stimulus necessary to produce 7S poliovirus antibody was fifty-fold greater than that required to stimulate a macroglobulin poliovirus antibody. In brucellosis, it is possible that only a small residue of antigen is present in tissues of convalescing patients or in those just recently exposed to the disease, and as a consequence only macroglobulin antibody is demonstrated. However, during the active and acute phase of brucellosis or in a chronic suppurating stage, sufficient antigen is present to provoke 7S antibody.

Blocking Antibodies and Coombs Test

As the chronic stage of human brucellosis is reached, usually in three to six months, blocking antibodies can appear and the prozone phenomenon is seen in the agglutination reaction. Olitzki (1928) was the first to report the prozone phenomenon in the tube agglutination reaction; agglutination, inhibited in the lower dilutions of immune serum, was present in the higher dilutions.

The blocking antibodies (= non-agglutinating antibodies) are more avid than the agglutinating antibodies which are also present in the reacting system. These blocking antibodies combine with the antigen at low dilution of sera but are unable to form a complex with the neighbouring antigen. At high sera dilution, where relatively more antigen is present, agglutination will be formed. Addition of antigen has been suggested as a way to overcome the blocking phenomenon (Glenchur et al, 1961; Zinneman et al, 1964). The most reliable way to estimate

brucellosis may have raised IgM titres for many years.

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non-agglutinating antibodies is the antiglobulin test of Coombs (Coombs et al, 1945; Wilson and Merrifield, 1951). Kerr et al (1968) pointed to the presence of non-agglutinating antibodies of the IgG and IgA class in 15 cases of chronic brucellosis. A Coombs test or complement fixation test was necessary to establish the diagnosis. The blocking antibodies have been found to persist longer than the agglutinating and complement-fixing antibodies after vaccination with strain 19 or strain 45/20, and after infection in cattle (FAO/WHO, 1971). The IgA portion of the non-agglutinating antibodies is skin-sensitising antibody and is associated with skin rashes so frequently encountered in veterinary surgeons (Kerr et al, 1968).

The various globulins in different animal species appear to differ in their ability to fix complement and to cause agglutination. In human brucellosis IgM is said to be the main agglutinating antibody, whereas the IgG fixes complement but frequently does not cause agglutination. In cattle, however, both IgM and IgG are able to fix complement and to cause agglutination. The temperature of fixation, the structure of the antigen, the species of animal serum, and the class of antibody have been reported to affect the relative complement fixing properties of immunoglobulins (FAO/WHO, 1971).

2. Cellular Immunological Mechanisms in Brucellosis

Brucellosis can be designated a disease of intracellular parasitism. Certain aspects of this parasitism are important in the pathogenesis of the disease and in recovery. The first line of cellular defense against brucellae is opsonization and subsequent phagocytosis by the circulating polymorphonuclear neutrophil. Phagocytosed brucellae are carried in the

neutrophils and later localised in the liver, spleen, and lymph nodes where both neutrophils and brucellae are ingested by the major defence cells, or macrophages, in the reticuloendothelial system. Braude (1951) followed the tissue changes serially in experimentally infected animals. In a few days large numbers of brucellae were detected in the cytoplasm of large macrophages, surrounded by lymphocytes and occasional polymorphonuclear neutrophils. In a week or ten days, sites of Brucella localisation showed an abrupt change in cellular morphology. The disappearance of brucellae coincided with the appearance of solid granulomas composed of epithelioid and giant cells. It is not unlikely that the latter cells originated from the macrophages or mononuclear cells. Such granulomas have been detected in human tissues early in the course of brucellosis (Spink, et al, 1949; Sundberg and Spink, 1947), and represent an excellent defence mechanism. The proliferation of brucellae in localised sites is associated with accumulations of polymorphonuclear neutrophils, necrosis, and caseation, especially in infections due to Br. suis.

On the other hand, intracellular localization of brucellae could cause damage to the host tissue. Shaffer, Kucera and Spink (1953) observed that, in vitro, intracellular brucellae were protected against the anti-brucella action of human serum and antibiotics employed for the treatment of brucellosis. When groups of mice were infected with relatively small numbers of Br. melitensis and treated intermittently or continuously for several months with tetracycline, serial studies revealed the persistence of brucellae in splenic cultures after the completion of treatment (Spink and Bradley, 1960). It is therefore not unlikely that presumably healthy persons who recovered from brucellosis have

parasitized tissues, which may account for the persistence of a low titre of Brucella agglutinins and the continued exhibition of brucella hypersensitivity. Clinicians consider that this hypersensitivity contributes to chronic illness.

D: RELATION OF HYPERSENSITIVITY TO ILLNESS

The contribution of acquired Brucella hypersensitivity to the symptoms and signs of active brucellosis has been studied in groups of individuals, including veterinarians, whose occupations bring them into contact with the organisms or antigens. The relation between hypersensitivity and illness has been most significant in veterinarians known to have had brucellosis in the past and who have accidentally infected themselves with the vaccine, Br. abortus strain 19, while immunizing cattle (Spink, 1957). In these circumstances, following introduction of brucellae into the skin, severe local swelling and redness have occurred within four to six hours, associated with chills and fever. After appropriate antibiotic therapy, the localised swelling and systemic manifestations have usually subsided within 24 to 48 hours. However, if a similar mishap occurs in a veterinarian who has not had a previous Brucella infection, no immediate local reaction follows the accident. After a week or more, such an individual may complain of an influenza-like illness. At this time, agglutinins in the serum can be demonstrated and the skin reaction is usually positive. In addition, Br. abortus strain 19 organisms can be isolated from the blood.

The role of Brucella antigen in the pathogenesis of brucellosis on the basis of hypersensitivity has been further explored with Brucella endotoxin (Abernathy and Spink, 1960). The injection of endotoxin into

parasitized tissues, which may account for the persistence of a low titre of Brucella agglutinins and the continued exhibition of brucella hypersensitivity. Clinicians consider that this hypersensitivity contributes to chronic illness.

D: RELATION OF HYPERSENSITIVITY TO ILLNESS

The contribution of acquired Brucella hypersensitivity to the symptoms and signs of active brucellosis has been studied in groups of individuals, including veterinarians, whose occupations bring them into contact with the organisms or antigens. The relation between hypersensitivity and illness has been most significant in veterinarians known to have had brucellosis in the past and who have accidentally infected themselves with the vaccine, Br. abortus strain 19, while immunizing cattle (Spink, 1957). In these circumstances, following introduction of brucellae into the skin, severe local swelling and redness have occurred within four to six hours, associated with chills and fever. After appropriate antibiotic therapy, the localised swelling and systemic manifestations have usually subsided within 24 to 48 hours. However, if a similar mishap occurs in a veterinarian who has not had a previous Brucella infection, no immediate local reaction follows the accident. After a week or more, such an individual may complain of an influenza-like illness. At this time, agglutinins in the serum can be demonstrated and the skin reaction is usually positive. In addition, Br. abortus strain 19 organisms can be isolated from the blood.

The role of Brucella antigen in the pathogenesis of brucellosis on the basis of hypersensitivity has been further explored with Brucella endotoxin (Abernathy and Spink, 1958). The injection of endotoxin into

patients who had recovered from brucellosis caused fever, chills, sweating, myalgia, headache, and malaise. The severity of the reaction was directly related to the local intensity of the Brucella skin reaction. These systemic manifestations could be suppressed or greatly ameliorated by pre-treatment with corticosteroids. Furthermore, patients with severe acute brucellosis occurring under natural conditions promptly improve following treatment with corticosteroids (Spink and Hall, 1952). These experimental and clinical observations suggest that the action of Brucella endotoxin involves an antigen-antibody interaction of the delayed type.

E: BRUCELLIN DERMAL SENSITIVITY TEST

The brucellin skin test measures specific sensitization of the host to Brucella. Numerous antigens and techniques have been proposed for determining the dermal sensitivity of man for Brucella. Like the tuberculin reaction, a positive reaction does not indicate the presence of active infection. The incidence of dermal sensitivity in a population is directly proportional to the amount of infection that is present among domestic animals in an area (Spink et al, 1952). In different parts of the United States from 10 to 25 per cent of healthy adults give a positive reaction to this test. In the majority of such persons a history of disease resembling brucellosis cannot be elicited at any time in the past. In known infected persons there is no correlation between the severity of the infection and the degree of sensitivity. Also a small proportion of persons do not develop dermal hypersensitivity when exposed or infected.

A careful appraisal of the Brucellin skin test was recently conducted in a survey of healthy persons in the United Kingdom (Robertson et al, 1972). Among the urban inhabitants the frequency of sensitivity to Brucellin was six per cent. In rural inhabitants of similar age it was eleven per cent, increasing to 38 per cent among those aged 50 to 59 years. Antibody was detected in serum from 25 per cent of persons giving negative skin reactions. The Brucellin test itself stimulated as much as a four-fold rise in antibody titre, in subjects with pre-existing detectable serum antibody.

The skin test is therefore not very reliable and it does not diagnose Brucella infection. It is not a definitive aid in diagnosis of brucellosis either on its own or in conjunction with the examination of paired sera, and performing such a test serves little useful diagnostic purpose. However, since the dermal test is specific for Brucella, the test does have some merit as a tool for epidemiological surveys to detect the degree of exposure of a population to brucellosis.

F: HYPERSENSITIVITY AND THERAPY

In patients with presumed chronic brucellosis, the diagnosis of which has been substantiated by correlating non-specific symptomatology with a positive intradermal Brucella reaction, it was common practice to attempt desensitization of the patient with one of several Brucella antigens. Specific antigen therapy seems now to be heading into disrepute, even in areas where it was once most strongly advocated. In the Stavropal Scientific Conference on Brucellosis in 1971, Kasatkina and Nedlin reported that this form of therapy quite often leads to a progression of the lesions in bones and joints which they attribute to

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intensification of autoallergic reactions (Versilova: personal communication). Patients under such therapy are more likely to exhibit autoantibodies to brain, liver, spleen and joint tissue, and most experts do not subscribe to the treatment of brucellosis by desensitization with Brucella antigens (Spink et al, 1952).

G: PATHOGENESIS AND PATHOLOGY OF BRUCELLOSIS

Meyer (1964) revealed in an examination of 550 strains of Brucella that each species of Brucella had a decided host preference: Br. abortus for cattle, Br. melitensis for sheep and goat, Br. suis for swine. Susceptibility of the host and the virulence of the organism determine both the preference of a certain organism for a certain animal host and the outcome of first contact. Cruickshank (1968) defines pathogenicity as the capacity to invade tissues, multiply therein and produce toxic effects.

Whether the Brucella organisms enter the body via the oral or respiratory route or via the skin or the conjunctiva, they appear first to be conveyed to the regional lymph nodes. Depending on the invasiveness of the organisms and the susceptibility of the host, they may be held up and destroyed, or they may multiply in spite of phagocytosis in the lymphoid tissue and escape into the bloodstream (McCullough, 1970). They are then carried around the body and localize mainly in tissues having an abundance of reticulo-endothelial cells: liver, spleen, bone marrow, lymph nodes and kidney. In domestic animals (sheep, goats, cattle, dogs), the organisms are localised in the female genitalia (pregnant uterus).

Intracellular localisation is a very important aspect in the pathogenesis of brucellosis and in recovery. Goodpasture and Anderson (1937) observed that in a chicken embryo brucella organisms preferred to multiply intracellularly. Meyer observed in 1943 for the first time the intracellular presence of brucella in a case of human brucellosis. Castaneda (1947) discovered in guinea-pigs the presence of brucella in phagocytic cells. He also noticed intracellular multiplication which finally caused rupture of the cell, after which the liberated organisms were re-exposed to phagocytosis. As a result of this intracellular localisation of the brucellae, humoral antibodies, complement, and antibiotics fail to have any real impact on the bacilli as in the similar case of intracellular Salm. typhi. Their spread to different organs is enhanced by their presence in free macrophages. Their persistence adds to the effect of a maintenance of hypersensitivity, which is a peculiar characteristic of brucellosis and doubtless in part contributes to the chronicity of the disease. It is also possible that L-forms develop inside the cells and these, escaping into the bloodstream, may be even harder to isolate in culture than the normal forms (Hatten and Sulkin, 1966).

Erythritol, a simple carbohydrate, has been found to play some role in the localisation of brucellae in host tissues. In a series of investigations in Great Britain (Williams et al, 1962; Keppie et al, 1965), bovine fetal membranes of aborted material were shown to contain an abundance of erythritol. This substance had been known to be an important in vitro growth stimulus for brucellae (Keppie et al, 1965). The tissues of animal species highly susceptible to infection by brucellae contain large amount of erythritol, whereas tissues of highly

resistant species including man contain little or none of this carbohydrate. These investigations established a basic cause for the proliferation of brucellae in selected tissues of certain animal species, a biochemical finding that probably has wide implications for other infections. This finding is also used as an argument against the probability of transmission of brucellosis in man by sexual intercourse and against abortion being a manifestation of human brucellosis. Although erythritol stimulates the growth of virulent bacilli, strain virulence and erythritol concentration are of an independent nature (Meyer, 1966).

Braude (1951) studied the histopathological changes in guinea pigs and mice infected with the three different species of Brucella. In mice infected with brucella organisms, it was observed that with the formation of granulomata, the number of invading bacilli decrease dramatically. Hence granuloma formation is a characteristic and efficient defence mechanism of a host against a brucella invasion by which many organisms are destroyed. The solid granuloma without necrosis probably reflects a good defence against a species of low virulence, while necrosis in multiple abscesses indicates a weak host reaction to a virulent organism. In man pathological changes could not be studied on autopsy materials (brucellosis in man has a low mortality) but biopsy studies from bone marrow, liver and spleen showed the typical lesion consisting of giant cell surrounded by epithelioid cells near the centre and lymphocytes more peripherally (Spink, 1956).

Most Gram negative bacilli produce endotoxin, a lipo-polysaccharide present in the cell wall of these organisms. Spink and his

co-workers (1956) emphasised the probable role of endotoxin in the production of the symptomatology of brucellosis in general and of nervous diseases in particular. Prior exposure to a Brucella organism or its endotoxin is necessary for elicitation of a reaction. Patients suffering from brucellosis reacted strongly to an injection of endotoxin. A small amount of endotoxin caused shock and could initiate the whole disease pattern as if reinfection occurred.

The activity and role of endotoxin in the pathogenesis of brucellosis has been studied by Abernathy and others (1955, 1958), Mice infected with brucellae showed an increased susceptibility to a challenging dose of endotoxin. The increased mortality could not be explained by a more intensive infection as the bacterial count in the spleen remained the same. The increased susceptibility triggered off a shock syndrome which was considered to be a hypersensitivity of the anaphylatic type (Type 1 Reaction) to bacterial endotoxin. There are also strong arguments that the action of endotoxin materialises through a specific cellular type of hypersensitivity. Kessel et al (1966) observed that cytotoxicity of brucella endotoxin for macrophages depended on prior sensitisation and that a cell-bound antibody was a prerequisite for that reaction.

Symptoms in brucellosis may be caused as organisms escape from the reticulo-endothelial cells into the bloodstream, or as the organisms disintegrate within the cells and release toxic substances (Spink, 1956; Ganado, 1965; McCullough, 1970). Ganado considers that this second mechanism explains the pyrexia of brucellosis. The same mechanism operates, in his view, when the Herxheimer-type of reaction follows the

administration of an antibiotic, due to sudden release of endotoxin from the killed brucellae, or when vaccine is injected into a hypersensitive subject. The presence of the organisms within the host cells may induce little change in the host tissues other than the granulomatous reaction, but, in some cases, hypersensitivity develops, as in tuberculosis, and this may greatly affect the reaction of the body. The patient suffering from recurrent bouts of fever may have been sensitized during the incubation period of the disease and reacts with fever each time endotoxin is released (Ganado, 1965; Zammit, 1969).

CHAPTER V

EPIDEMIOLOGY OF BRUCELLOSIS

The epidemiology of brucellosis presents one of the most fascinating chapters in medical annals. The Brucellae infect an extensive domestic host range including man, cattle, goats, sheep, swine, dogs, horses, mules, cats, and chickens. Brucellae have also been recovered from a number of wildlife species, including deer, buffalo, European hares, jack rabbits, and the desert wood rat, and there is evidence that wildlife plays a significant role in the epidemiology of brucellosis (Bendtsen et al, 1954; Edwards, 1959). Thus, a vast animal reservoir of infection is available to infect man. Experimentally, the susceptible animals include monkeys, rabbits, guinea pigs, rats, hamsters, mice and chickens.

Brucellosis in man and animals may be caused by any one of the main three species of Brucellae. However, brucellosis is primarily a disease of animals and they serve as sources of human infection. Spread of infection from man to man very rarely occurs. By contrast, infection of animals is an important factor in the ecology of brucellae, because the infected animals readily infect one another. Man's role, in the spread of infection among animals, is still an important one, since by domesticating the animals and herding them together he provides condition

which, though age-old, are quite unnatural and greatly increase the ease with which brucellae survive both inside and outside their hosts.

The animals that are commonly known to serve as sources of human infection are goats, sheep, cattle and swine. Infection of reindeer, caribou, camels, and yaks is of epidemiological importance in some parts of the world. Ecologic investigation of the disease in wildlife also points to their role both in transmission of Brucellae infection directly to man and as reservoirs of infection for domestic animals (Edwards, 1959). The modes of transmission to man are ingestion, contact, inhalation, and accidental inoculation (WHO, 1971).

Epidemiological studies in a few endemic areas of the world have shown that man's risk of infection is closely related to his methods of animal husbandry, food habits, standards of hygiene, and economic activities (Abdussalam and Fein, 1975). Contact with aborting and infected animals, consumption of infected milk, milk products and meat, exposure to aerosols of infective dust, and handling of wool or infected animals in the slaughter-house all increase human risk. The geographical and epidemiological picture therefore varies from area to area in different parts of the world, and has to be studied carefully in planning control and eradication programmes.

A: GEOGRAPHICAL DISTRIBUTION

The distribution of brucellosis in man and animals is practically world-wide (Table V.1) and prevails wherever domestic animals and man cohabit (Mediterranean Fever Commission, 1905-07; Spink, 1956; Dalrymple-Champneys, 1960; WHO, 1964a; Roy et al, 1965; Cox, 1966; WHO, 1973b). Bovine infection has the widest distribution: the United States,

British Isles, Central Europe, Canada, Northern Japan, Mexico and South Africa are the areas of highest bovine infection with Br. abortus (WHO, 1971).

Bovine brucellosis caused by the abortus species has occurred in nearly every country. Stableforth (1959) claimed an infection rate of 10 to 30 per cent in most European and South American regions. Herd infection in Great Britain amounted to 10 to 15 per cent. Serological evidence of brucellosis was detected in 25 per cent of the dairy herds of northern Scotland between 1954 and 1956. In West Germany an overall infection rate of 20 per cent was estimated (Götze, 1954) but significant differences of incidence (0.3 to 62 per cent) were recorded in various localities. In the different provinces of the Netherlands, herd infection varied between 36 and 82 per cent in 1956 with an infection rate in individual adult cows of between five and ten per cent. In Nigeria, Esuruoso (1973) recorded a bovine brucellosis prevalence rate of 60 per cent in the Southern part of the country, but a much lower rate in the northern states of the country.

Brucellosis due to Br. melitensis is confined chiefly to the countries in which goat-raising is extensively practiced. The caprine type of the disease thrives in a subtropical climate usually where the standards of living are low and poor sanitation exists. The infection is common throughout the Indo-Pakistan sub-continent and is responsible for a large proportion of human cases of brucellosis. The infection is also endemic in Southern Europe south of the 46^{o} latitude, particularly in the Mediterranean littoral. It prevails in the Asian parts of the Soviet Union (Versilova and Aslanjan, 1974), South and Central America,

North Africa, Persia, and Mexico (Abdussalam and Fein, 1975). The overall prevalence rate of caprine brucellosis in Nigeria was recently found to be about 4.3 per cent (Falade et al, 1976).

Swine brucellosis is largely a problem of the Americas, although a few foci have recently been discovered in other parts of the world, notably in Central Europe and Southern Asia (Abdussalam and Fein, 1975). In the United States, it is endemic in the great Midwestern pig-raising states and on the West Coast (Center for Disease Control, 1972).

Human infection is widespread and its epidemicity depends upon the prevalence of the disease in animals and the customs of the people in those areas (Thomas et al, 1974). Table V.1 summarise the officially reported cases of human brucellosis in certain countries (WHO, 1973b). The officially reported data are generally incomplete and the actual incidence may in most cases be much higher than indicated.

8: NOMADIC HERDS AND FLOCKS

Recent observations on nomadic herds of cattle and flocks of sheep and goats in Northern Nigeria have shown that these animals are frequently infected with Br. abortus and Br. melitensis respectively (Bannister and Bhatty, 1970). The fact that these animals roam over wide areas and live on open meadows in the Savanna heat probably in some way imposes a natural limit on the rate of brucella infection (Esuruoso, 1974b) since beef in settled herds in Oyo State of Nigeria have been found to be more heavily infected than those in the nomadic herds of Northern States. However, the nomadic animals do come together in relatively small areas at certain seasons when watering places and

grazing areas are restricted and herds and flocks have to converge on them for survival. Human infection in areas of nomadic animal husbandry is common (Abdussalam and Fein, 1975): Most cases occur among young adults tending the animals and follow the lambing or calving season.

In parts of Iran where sheep and goat husbandry is of a semi-nomadic type, infection contracted through consumption of dairy products, especially cheese, comes into prominence (Sabbaghian and Nadim, 1974). This type of infection is also common in younger age groups because of their greater consumption of dairy products.

In tropical Africa brucellosis is common among domesticated ruminants and people coming into contact with them, the type of human infection depending on the species and breeds of animals which are raised (Abdussalam and Fein, 1975). There is some evidence that African wild animals may also serve directly as sources of human infection (Thimm, 1972).

Another type of brucellosis in nomadic herds is the infection caused by Br. suis biotype 4 in reindeer and caribou (Rangifer tarandus) in Siberia, Alaska and Canada. The infection is frequently transmitted to man and also to dogs and wolves which feed on reindeer meat (Neiland, 1970).

C: SEASONAL PREVALENCE AND INCIDENCE OF BRUCELLOSIS

The seasonal prevalence of abortion in cattle occurs at that time of the year when the majority of cows are pregnant. Many human infections are acquired as the result of cows grazing in pastures contaminated with infected exudates from abortion. The same is true of porcine brucellosis. However, observers often fail to recognize that most

animals remain infected for many months. Thus, the chronicity and latency of the disease, in addition to the lack of dependable methods for detecting the infection, make epidemiological studies difficult.

The incidence and prevalence of brucellosis in man and animal is difficult to determine in any particular locality and is therefore not accurately known in many parts of the world. As in tuberculosis, the reported cases may bear very little relation to the real incidence or prevalence of infection (Wilson and Miles, 1964). The disease is unfamiliar to many practitioners in many countries. Not all countries require physicians to report cases of undulant fever, and Veterinarians do not report cases of infectious abortion to public health authorities. Many farmers where control programmes are in progress make every effort to conceal abortion in their animals. Also there is often lack of agreement concerning an agglutinin titre diagnostic of brucellosis in man, with marked variation in results of serological tests obtained in different laboratories. Figures may therefore be deceptive, for in developed and economically advanced countries where investigations are actually carried out and cases are reported to the public health authorities, the incidence/prevalence may appear high, whereas in poor and developing countries few cases may be reported though infection may be rife.

D: AGE AND SEX PREVALENCE

The disease is more prevalent among males than females, the ratio being approximately 2 : 1 (Spink, 1956, Dalrymple-Champneys, 1960). Males are undoubtedly exposed to the infection more frequently than females.

More cases of brucellosis occur among young adult males from fifteen to forty-five years than in any other age group. Veterinarians and dairy husbandmen who come in contact with infected animals develop the infection most frequently. Many cases are detected among employees in abattoirs and in the meat packing industry who handle uncooked meat (Boycott, 1964; Glass, 1964; Damon and Parker, 1950; Derrick and Brown, 1950; Versilova, 1965; Taylor et al, 1938). Laboratory personnel who work with cultures of brucella, with infected experimental animals and other animal tissues often acquire the disease (Spink, 1956; Dalrymple-Champneys, 1960; Meyer and Eddie, 1941; Hare et al, 1947). A considerable number of cases of brucellosis have been described among individuals who drink unpasteurized milk or consume dairy products prepared from unpasteurized milk. Several Veterinarians who have accidentally inoculated themselves with Br. abortus vaccine, strain 19, have developed acute brucellosis (Joffe and Diamond, 1966; Pivnik et al, 1966). Gilman (1944) describes a case of undulant fever in a man who, while using a defective syringe, accidentally sprayed some of the vaccine into his eyes.

E: BRUCELLOSIS IN CHILDREN

The theory that gastric hydrochloric acid may protect against milk-borne brucellosis (Garrod, 1937) has fostered the belief that children are immune from brucellosis. However, children on farms play with animals and implements and are therefore exposed in other ways. In U.S.S.R., there has been a tendency for an increase in the number of brucellosis among children under 14 years of age: cases among children rose from 7.8 per cent of total morbidity in 1967 to 9.3 per cent in

1971 (WHO, 1973a; Elberg, 1973). The trend towards non-occupational contraction of the disease and the rise in morbidity among children are due to the relative increase in the importance of cattle and in particular personally-owned cattle, as a source of infection. Brucellosis should therefore be included more often in the differential diagnosis of childhood fevers (WHO, 1973a).

F: PORTAL OF ENTRY OF BRUCELLAE IN MAN (MODE OF INFECTION)

1. Infection by ingestion may occur via the gastro-intestinal tract or by penetration of the mucous membrane of the throat. The usual vehicles of infection for man are:

   (a) untreated food products of raw milk origin from infected animals;

   (b) raw vegetable contaminated by infected animals urine or faeces;

   (c) viscera, bone marrow, and lymph nodes andmuscle tissue from infected carcasses, which may retain viable brucellae for more than a month after slaughter, and for much longer in the case of frozen or chilled meats;

   (d) water supplies, such as wells, contaminated by infected animal excreta.

   As knowledge accumulates concerning the dietary habits of various peoples, new modesof transmission to man are revealed. For example, a strain of Br. suis biotype 4 was recovered in blood cultures from an Alaskan Eskimo girl who apparently was infected from ingesting raw caribou bone marrow (Edwards, 1959).

2. Contact with brucellae in vaginal discharges, foetuses, placentae, urine, manure, carcasses, and salvaged animals causes a large proportion of human cases (WHO, 1973). The skin and mucous membranes,

including the conjunctivae, provide the portals of entry (WHO, 1971). The contact route is an important occupational hazard among veterinarians, farmers, rendering-plant employees, packing-house workers, animal handlers and laboratory workers engaged in cultivation of brucellae. Infections by contact play an important role among those who because of climatic conditions, bring their animals very close to human habitation, where transmission of brucellae from animals to humans occurs, especially among children who use the animals as pets (WHO, 1971; Elberg, 1973).

3. Infection occurs when man inhales infected dried materials of animal origin, such as the dust from sheep wool, railway trucks and lorries that have transported infected animals, abattoirs, infected farm premises, and brucella laboratories. Nomadic herdsmen who live in dusty compounds or huts contaminated with brucellae may also be infected by inhalation.

4. Infection by accidental inoculation is not infrequent among veterinarians and laboratory workers.

Those involved in the large-scale production of brucella vaccines and diagnostic antigens are also at special risk of infection and/or sensitization reactions.

Brucellosis of wild-animal origin is mostly transmitted to man indirectly through domesticated animals, but a few cases of direct transmission through ingestion, contact, or blood-sucking arthropod insects are known (WHO, 1971).

In any given population one of the modes of transmission of infection to man will be preponderant depending on the type of the invading

Brucella species, occupation of the population, local customs and standard of hygiene. In U.S.A., France, Germany and England, where Br. abortus is prevalent and hygienic conditions are good, the disease is occupational and mainly found among adult males, and it is caused by direct skin contact with the organism and not by drinking milk. The epidemiology of Br. melitensis infection is different. This species is more invasive and infections in childhood, caused by drinking infected milk, are rather common. In Malta, where Br. melitensis infection is preponderant, brucellosis is common in children under five years of age. Huddleson (1947) states that infection in younger children might be even more common as the disease in this group runs a subclinical course and brucellosis remains unnoticed.

TABLE V.1

GEOGRAPHICAL DISTRIBUTION: BRUCELLOSIS IN MAN[1]

Country	Y E A R S					
	1967	1968	1969	1970	1971	1972
AFRICA						
Chad	1	1	3	15	1	8
French Terr. of the Afars and the Issas	-	3	-	-	+	+
Kenya	71	81	98	65	+	+
Mozambique	1	-	-	-	-	-
Rwanda	7	-	6	2	4	5
South Africa	3	3	1	1	+	+
Swaziland	1	3	3	-	16	+
Uganda	212	133	201	141	+	+
United Rep. of Tanzania : Tanganyika	256	281	...	209	+	+
AMERICA						
Argentina	1,601	1,442	1,202	909	1,119	+
Canada	43	19	16	31	8	17
Chile	5	3	5	1	+	+
Colombia	84	42	23	35	42	+
Cuba	5	33	14	11	21	+
Honduras	-	3	9	5	2	2
Mexico	1,513	1,098	672	612	777	+
Panama	-	-	-	1	-	-
Paraguay	-	4	-	1	2	-
Peru	2,456	1,719	1,454	1,118	1,284	+
United States	265	218	235	213	183	188
Uruguay	10	3	2	-	3	+
Venezuela	12	6	3	7	4	-
ASIA						
Iran	2,142	4,696	3,879	2,371	+	+
Isreal	23	30	15	6	*8	*36
Japan	...	...	...	...	...	...
Kuwait	-	-	1	1	+	+
Laos	-	-	6	-	-	-
- .	71	63	42	37	70	+

CHAPTER VI

CLINICAL MANIFESTATIONS, DIAGNOSIS AND TREATMENT OF BRUCELLOSIS

A: CLINICAL FEATURES

The symptomatology of brucellosis is notoriously protean, and there are few diseases with a greater variety of clinical manifestations. No one organ is to be found which has not been described as having been affected by a Brucella organism. Also the symptoms and signs as described by various authors differ considerably in various countries. The symptomatology depends on several factors:-

(a) The virulence of the attacking organism: Br. melitensis has the highest virulence with a preference for nervous tissue.

(b) The susceptibility and resistance of the host: in developing countries, this might be related to the state of nutrition and intercurrent infections.

(c) The onset and duration of infection determining the stage of hypersensitivity and/or immunity.

Table VI.1 gives a list of symptoms and signs of various groups of patients suffering from brucellosis from different countries (Spink, 1956; Huddleson, 1947; Dalrymple-Champneys, 1960).

Cough, nervous symptoms and enlargement of various organs of the reticuloendethelial system (RES) are much less common in Dalrymple-Champneys' series than in those of Spink and Huddleson. Undulant fever was not found in thegroup of Spink.

Description of the Clinical Picture of Brucellosis

Brucellosis is a septicaemia and it presents as an acute or a chronic illness. Localisation of organisms in various organs of the body is a common feature of this disease.

Acute Brucellosis

The frequency with which a period of ill-health precedes the onset of fever makes it difficult to estimate the incubation period. Most authorities agree that the incubation period is short, usually one to three weeks after the ingestion of, or contact with, the organism, but it could sometimes be as long as six or seven months (Christie, 1974).

Illnesses caused by Br. melitensis and Br. suis are more often of sudden onset with fulminating clinical course, while illnesses due to Br. abortus are usually more of insiduous onset. However, life-threatening fulminating infection with Br. abortus can occur when resistance is low, owing to malnutrition, immunodeficiency diseases, malignancy or after major surgery (Christie, 1974).

Dalrymple-Champneys (1960) describes the following clinical varieties of brucellosis in England and Wales:

1. Ambulant Type

The patients complain of increasing fatigue and malaise but not severe enough to compel them to stop work. Most of them subsequently develop the full clinical picture while some remain ambulant for a long period.

2. Undulant Type

This is the commonest clinical type. The temperature runs the typical "undulant fever" course. The daily rise usually occurs in the afternoon while the fall comes during the night or early morning and is accompanied by drenching and often foul-smelling sweats. In long continued cases, exhaustion and depression ensue. Anorexia, aching of limbs and back and sometimes arthralgia and sore throat become more pronounced as the disease progresses. Apyrexial periods of varying length may intervene between febrile phases.

3. Moderate Continuous or Daily Remittent Type

In addition to the symptoms described under "ambulant type", these patients suffer from constipation, anorexia, pains in the limbs, joints or back and occasional rigors.

4. Moderate Relapsing Type

There is a more regular fever with febrile intervals of several days or weeks followed by moderate exacerbation for a limited period. Enlargement of liver and spleen and arthralgia or arthritis are prominent features.

5. Nervous and Toxic Type

There is usually a sudden onset with brisk fever, delirium and suicidal tendencies which seem to be attributable to toxaemia rather than actual invasion of the central nervous system.

6. Malignant, Fulminating and Haemorrhagic Type

The onset is sudden with severe headache and generalized aches and pains. The tongue is swollen and heavily furred and the breath offensive. There may be abdominal tenderness, nausea, vomiting,

thirst and anorexia.

SYMPTOMS AND SIGNS IN BRUCELLOSIS

A. Septicaemic Forms

(a) Fever: Classically described as undulant by Hughes (1897): "it need not be held to mean a definite quantity or amount of intensity, but as in nature undulations vary from ripple or broader swell to the chopping wave or overwhelming breaker, so may the expression be applied to the various pyrexial waves of this fever however much they vary length and magnitude." The temperature may be continuous but as well remittent or irregular. Nowadays, under the influence of antibiotics, the undulant type of fever is less frequently encountered.

(b) Sweating: Abundant sweating, persistent during apyrexial periods, is the commonest of all symptoms. It occurs mainly in the later part of the night and it may be of the typical foul-smelling, drenching type or just the moderate variety that may occur with any fever. The drenching perspiration and high fever, which are common in Malta fever and in suis infection, are usually absent in abortus infections, or if they do occur are relatively mild.

(c) Pain: It occurs in about 50 per cent of the cases and presents as a general bodyache, headache, myalgia or arthralgia. The pain may have a diffuse character, it may be localized or it may migrate from one place to another.

(d) Skin Rash: Various forms of skin rash have been described: they may be morbilliform, scarlatiniform, rubelliform, or, in severe

cases, purpuric.

(e) R.E.S. and Lymph Glands Enlargements: During the bacteremic phase, when the organisms are multiplying in the blood and R.E.S., and mechanisms of humoral and cellular resistance are activated, enlargement of the spleen and liver are outstanding signs. Generalized lymphadenopathy, however, is inconstant. Local lymphadenopathy is encountered and connected to the portal of entry.. Fistulae of glands are unusual.

B. Localised Forms and Complications of Brucellosis

Depending on the virulence of the invading organisms and the resistance of the host, the disease may be self-limiting, run a subacute course or become chronic. When the course is protracted bacteria tend to be localised in one or more organs from which they may be cultured. The septicaemic features of the disease recede and the signs of a local infection become more apparent. Localisation of the disease process follows a preferential pattern: R.E.S. (Liver, spleen, lymph glands), osteoarticular tissue and nervous tissue. But no organ (lung, heart, gastrointestinal tract, kidney) is exempt and this accounts for the protean symptomatology of the disease.

(a) Liver: Jambon and Bertrand (1965) made a thorough investigation of the liver pathology in brucellosis. According to their findings 36 per cent of Brucella-infected patients do have liver abnormalities in the sense of a painful enlarged liver with slightly impaired liver functions and sometimes mild jaundice. The hepatitis can be followed by a cirrhosis and is clearly demonstrated by McCullough and Eisele (1951). Fortunately, these conditions are

rare. Underlying liver damage caused by malnutrition, parasitic infections and alcoholism are of importance in the manifestation of this rare complication.

(b) Spleen: Splenomegaly is associated with an increase in cellular activity and the formation of focal granulomata. The spleen protects the liver against an invasion of organisms, as could be demonstrated on splenectomised animals in which the number of hepatic foci were more numerous. According to Spink (1956) the activity of the disease is clearly correlated to the presence of an enlarged spleen: firstly it is more easy to obtain positive blood cultures from patients with splenomegaly and secondly the spleen does not reduce in size in patients with a chronic disease. In patients with a chronic localised infection the spleen is usually much enlarged; in these patients blood cultures are negative and serology reaches only a low titre level. Br. suis is the common agent in this type of lesion.

(c) Osteo-articular Localization: This is the most favourite place of abode for Brucella organisms. Spink (1956) found brucella spondylitis a frequent complication affecting chiefly the lumbar spine. The major joints, especially the hip-joints, are also commonly affected (Kelly et al, 1960; Jones, 1955; Serre et al, 1966; Adam et al, 1967; Makin et al, 1957). The joint contains a small amount of exudate with inspissated fibrin, so called "rice bodies", causing swelling, warmth and loss of function. A positive culture of the organism is more often obtained from the granulomatous synovia than from the synovial fluid. Suppurative arthritis may also occur in

knee and shoulders while osteomyelitis of bones other than the vertebrae have been reported for example, femur, tibia, ribs, humerus and wrist bones.

In spinal column involvement the focal lesion is found in the epiphysis of the vertebral body. Muscle spasms are severe, pain radiates into the legs and is sometimes so severe that turning over in bed becomes impossible for days (Bishop, 1939). The X-ray shows a narrow irregular disc with involvement of the superior-anterior aspect of the vertebral body and much new bone formation.

(d) Nervous Tissue: Marked fatigue, weakness and debility are all indications of the involvement of the central nervous system. All the manifestations in the neurological and psychiatric fields are classified by the French School under the name of "meningo-neurobrucellose" (Roger and Poursines, 1938).

The neuro-psychiatric complications are differently appreciated by various authors. Spink (1956) states an involvement of ten per cent in Br. abortus but Br. melitensis is still more devastating to the nervous tissue. The late appearance of the manifestations of neurobrucellosis in the course of the disease is probably related to the phenomenon of hypersensitivity to Brucella antigen. Hughes (1897) reported the first case of meningo-myelitis. This is not an unusual complication of brucellosis, occurring even at first onset of the disease (Sahadevan et al, 1968). Polyradiculitis and peripheral neuritis have been reported (Mathur, 1969). These conditions are explained either by an immunological reaction in nervous tissue to the presence of antigen or by compression of

peripheral nerves at their passage through intervertebral foramina. Brucellosis might even be one of the causes of the Guillain Barre Syndrome (Mathur, 1969). The mental symptoms including misery, dejection and suicidal depression may become more prominent than any physical symptoms and signs in some patients with brucellosis (Evans, 1934).

(e) Respiratory Tract: Coughing occurs frequently and there is evidence of mild bronchitis in about three-quarters of all cases. Broncho-pneumonia is a rare complication. Pleural effusion and empyema are very uncommon but serious complications (Spink, 1956). In patients treated early with antibiotics, pulmonary complications are rare.

(f) Cardiovascular System: Tachycardia is the main sign, but myocarditis is not infrequent. Endocarditis has been reported in a few cases; it is considered to be the most dangerous complication of brucellosis. It is one of the rare causes of death from brucellosis; it occurred in four of Spink's 244 patients and in five of Dalrymple-Champneys' 1500 patients (Spink, 1956; Dalrymple-Champneys, 1960).

Thrombophlebitis may occur in any febrile condition which keeps the patients in bed, but specific granulomatous changes of brucellosis may occur in vessel walls and cause thrombosis in deep veins (Barrett, 1954). Pulmonary embolism may follow the thrombosis (Spink, 1956; Barrett, 1954).

(g) Reproductive System: Epididymo-orchitis occurs in between at least two and five per cent of adult males. A rate as high as 14 per cent has been recorded (Castro, 1946). The incidence may vary in

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(g) Reproductive System: Epididymo-orchitis occurs in between at least two and five per cent of adult males. A rate as high as 14 per cent has been recorded (Castro, 1946). The incidence may vary in

different parts of the world, and, even in the same area, different rates may be reported. In Malta, for example, one author regards it as uncommon (Castro, 1946), another as common (Ganado, 1965). Orchitis is more commonly seen than epididymitis (Ganado, 1965). The acute inflamation usually subsides within a week, though the swelling may last several weeks longer. Whenever inflamation of the male genital organs occurs the question of subsequent sterility is raised, but is seems that in brucellosis, as in mumps, the risk is slight (Christie, 1974).

Abortion being a leading manifestation of brucellosis in animals, its occurrence in women would obviously be a matter of great concern. There is no evidence that infection of the uterus or other parts of the genital tract occurs with anything like the frequency seen in animals, and abortion does not occur more frequently than in other acute septicaemic illnesses (Spink, 1956). Brucellae have been isolated from the ovary (Harris, 1937), and from an aborted human foetus (Carpenter and Boak, 1931; Williams, 1973). Disturbances of the menstrual cycle are common in any form of brucellosis, but not more so than in other acute or chronic disease (Christie, 1974).

(h) Urinary Tract: Brucellae are shed freely in the urine of patients (Ganado, 1965). During the bacteraemic phase a transient urinary infection (acute cystitis, pyelonephritis) may occur with rapid recovery. At a later stage the infection may become localised in the urinary tract with formation of granulomata in the kidney, ureter and bladder. Calcification sometimes occurs, and the resemblance to tuberculosis of the kidney may be very close. The bladder wall

may be invaded with evidence of chronic cystitis. Haematuria, dysuria, frequency and nocturia have been reported in such patients (Ganado, 1965; Barrett, 1954; Barrett and Richards, 1953; Zinneman, Glencher and Hall, 1961). Nephrectomy has been required in several patients with renal brucellosis (Zinneman, Glencher and Hall, 1961; Abornathy, Price and Spink, 1955).

Chronic pyuria requires investigation for brucellosis (Dunea et al, 1969; Greene et al, 1952) and urinary complications should be suspected in any patient with possible chronic brucellosis (Ganado, 1965).

(i) Eyes: The eyes may be infected directly or via the blood stream. Eye complications of some diversity have been reported in brucellosis, especially in cases due to Br. melitensis (Spink, 1956; Dalrymple-Champneys, 1960; Foggitt, 1954) and it may affect any structure of the eye - the conjunctiva, cornea, iris, choroid, the chambers, retina or optic nerve. The clinical picture may vary from temporary loss of visual acuity, which is common, to glaucoma, which is very rare and when it occurs, possibly coincidental. The frequency of eye complication encountered by different authors appears to vary with the interest of the clinician in eye examination. In Br. abortus infections, temporary visual disturbances are common, while the more severe eye complications are rare.

(j) Gastrointestinal Tract: Furring of the tongue is the rule and improvement in this is one of the few reliable indications that recovery is at hand. Some degree of stomatitis and gastritis is

not unusual as shown by the frequent occurrence of anorexia, dyspepsia, foul taste in the mouth, epigastric tenderness, nausea and vomiting. Progressive anaemia, mild leucopenia and relative lymphocytosis may be found, particularly in chronic cases.

A tendency to bleeding is a well-known feature of the disease: bleeding from the nose is the most common but it can also occur from the intestine, lungs, stomach, uterus, tongue, lips, mouth and cheeks (Dalrymple-Champneys, 1960).

Chronic Brucellosis

The onset of chronic brucellosis may be insidious or it may follow an acute attack, untreated or inadequately treated (Spink, 1956; Ganado, 1965). It may also take the form of an acute relapse long after an assumed cure, and precipitated sometimes by another infection or, interestingly, by trauma (Williams, 1973).

Commonly, four symptoms are almost invariably present: weakness, headache, pain and sweats. Mild weakness, allowing work and often described as breathlessness, may force the patient to take some rest from time to time, especially after heavy tasks. Often, he is compelled to sleep after lunch. Headache is frontal or retro-orbital, and it is sometimes wrongly attributed to chronic sinus disease. Low back pain is common, unassociated with radiological evidence of spondylitis. Sweats occur especially after effort and also at night, their frequency reflecting the severity of the disease. When the diagnosis seems certain but the patient denies nocturnal sweats his wife must be asked, for it may happen that she also is disturbed (Williams, 1973).

Other rare symptoms that may be present include lack of appetite, depression and irritability.

Weight loss is not invariable and the patient may look deceptively well, though usually his appearance points to the presence of serious disease.

As in acute brucellosis, moderate splenomegaly is the only noteworthy sign. It is present in about ten per cent of patients. When the spleen is very large - more than four finger breadths below the costal margin - coincidental disease such as cirrhosis, a reticulosis, or leukaemia must be sought (Williams, 1973).

In the absence of any occupational contact with animals it may be some time before the possibility of chronic brucellosis suggests itself to the attending physician. When it does, examination of the patient's blood for brucella antibodies may lead to the diagnosis. If the agglutination test, the mercapto-ethanol test, complement fixation, and anti-human globulin tests are all positive at significant titre (vide infra: Diagnosis), the diagnosis of chronic brucellosis can be made with some assurance even if there is no occupational exposure. If these tests are all negative such a diagnosis could be reasonably excluded. Positive serological findings may not however always make the diagnosis of chronic brucellosis any easier, especially in patients whose occupation exposes them to infection. Therefore, clinical, epidemiological, and serological findings must be carefully and critically assessed in each patient before the diagnosis is made. A therapeutic test may help to confirm the diagnosis, especially if along with clinical improvement there is also a fall in antibody titre. But in some cases when the effect of treatment is not

dramatic the diagnosis may remain in doubt (Editorial, 1974), particularly in cases due to Br. abortus infection in which positive blood cultures are rare, except occasionally in acute cases (Payne: personal communication).

The tragic results of misdiagnosis of chronic brucellosis were well illustrated in the case of Dr. Alice Evans who first showed that contagious abortion in cattle and undulant fever in man were caused by almost identical organisms. Dr. Evans infected herself while working with cultures of Br. melitensis. The disease took a mild course for nine months and a diagnosis of neurasthenia was made. Five years of ill-health followed but the illness was still labelled "neurasthenia". The true nature of the condition would not have been revealed had it not been that, after the development of an acute emergency requiring surgery, Br. melitensis was isolated. To quote her own words "At last, accidentally, came relief from the misunderstandings which inevitably arose when a patient is said to be suffering from imaginary ills" (Evans, 1934).

Chronic brucellosis must be distinguished from hypersensitivity to brucellar antigen. Such a patient, in spite of high antibody titres and a positive reaction to the brucellin dermal test, need not be suffering from chronic brucellosis, though some patients with chronic brucellosis may also be hypersensitive to brucellar antigen (Christie, 1974).

Causes of Death in Brucellosis

Death from brucellosis is very rare. Some of the complications of brucellosis which could lead to death include endocarditis (if untreated), pulmonary embolism, hepatic cirrhosis and suicidal depression (Williams, 1973). Furtunately, these complications are not common (Spink, 1956;

Dalrymple-Champneys, 1960).

B: DIFFERENTIAL CLINICAL DIAGNOSIS OF BRUCELLOSIS

It is obvious that the differential diagnosis of brucellosis covers a very wide range of conditions, and it may simulate:

Influenza

Typhoid fever

Pulmonary tuberculosis

Hepatic cirrhosis from other causes

Infectious mononucleosis

Hodgkins Disease

Sarcoidosis

Rheumatic fever

Pyelitis

Subacute bacterial endocarditis

Appendicitis

Psychoneurosis

Brucella infection should therefore be considered in every case of "pyrexia of undetermined origin."

Acute brucellosis may at first be indistinguishable from influenza, especially when salicylates can be blamed for the sweats.

When symptoms are typical and there is an emotional overlay the diagnosis is more likely to be missed and in a few patients psychoneurosis cannot be excluded. It is better to admit uncertainty, even to the patients, than to make the conveniently equivocal diagnosis of "neurobrucellosis" (Williams, 1973).

C: LABORATORY DIAGNOSIS OF BRUCELLOSIS

The symptomatology of human brucellosis simulates many other illnesses, hence a definitive diagnosis of the disease relies heavily on laboratory investigations. Accurate diagnosis is important for confirming a clinical illness due to Brucella infection in man, and also for all control and elimination programmes in animal husbandry.

Generally, human brucellosis presents in three main clinical forms - acute brucellosis, chronic brucellosis following an acute attack (sometimes called subacute brucellosis), and chronic brucellosis of insidious onset. In the acute stage very few abnormal physical signs may be present, but laboratory investigations are likely to be most helpful. The subacute and chronic human infection present a difficult problem to the clinician and laboratory worker: laboratory results become more difficult to interpret, and thorough clinical investigation is therefore essential in addition. Many authors are agreed that large numbers of cases of brucellosis are missed because of the peculiar difficulty in making a clinical diagnosis (Wilson and Miles, 1964).

Laboratory Investigations

1. Isolation of Brucellae: The only absolute confirmation of the diagnosis of brucellosis is the isolation of Brucella organisms from the patient. Therefore, cultural studies should be carried out, whenever possible, in every patient in whom a presumptive diagnosis has been made.

Blood Culture should be repeated several times, preferably during febrile episodes: in the average case of brucellosis the number of organisms per unit of circulating blood is not very high and for this reason, single specimens of blood may not contain Brucella.

Since Brucella organisms tend to localize in sites having a large amount of reticuloendothelial tissue, aspirated sternal bone marrow culture is sometimes, but not always, more successful (Lal, et al, 1970). Brucella organisms may be cultured from excised lymph nodes, particularly in patients with long-standing disease: this procedure should be attempted in persons whose marrow and venous blood culture have remained sterile (Spink et al, 1952). Organisms are more likely to be obtained from cervical nodes that enlarge during febrile relapses.

Urine should be cultured several times in every case particularly in patients with chronic pyelonephritis, and if a lumbar puncture is performed, the C.S.F. should be cultured.

Cultural studies are indicated for any biopsy material of liver (Lal et al, 1970). Brucellosis is occasionally associated with chronic cholecystitis, and bile obtained by duodenal drainage should be cultured. Granulomatous tissue of unknown aetiology excised from bone or lung should be cultured for Brucella (Spink et al, 1952).

Brucellae have been isolated occasionally from sputum, placenta, breast milk, vaginal discharge and seminal fluid (WHO, 1964a), and these sources should be kept in mind. When animal milk is suspected as the source of infection this should, of course, be examined.

Special Notes on Isolation of Brucellae

Isolation of the organisms is difficult if optimal culture media and proper techniques are not used.

The melitensis and suis organisms are easier to grow from blood culture than is abortus (Ramsay and Emond, 1967). Attempts to isolate Br. abortus, even in acute illness, are more likely to fail than to

succeed (Williams, 1973): the rate of isolation is not more than 10 - 20 per cent, even under the best cultural conditions (Wilson and Miles, 1964). Furthermore, the results may not be known for several weeks, by which time treatment should already have been prescribed: growth of the organisms is slow, hence negative cultures are kept for between four and eight weeks before being discarded.

With the increasing use of antibiotics prior to obtaining cultures more cases of brucellosis, even in the acute stage, have yielded negative cultures. Of 1,644 cases of abattoir-associated brucellosis reported to the Center for Disease Control in U.S.A. in the period 1960 - 1971, only 283 (17 per cent) were confirmed by culture; 1,361 (85 per cent) were diagnosed serologically (Center for Disease Control, 1975).

Cultural techniques are now superior to guinea pig inoculation for the detection of Brucellae, except when such specimens are derived from a highly contaminated source (Spink, et al, 1952).

Serological Tests in Brucellosis

The differentiation and identification of the two types of antibody (19S macroglobulin and 7S microglobulin) formed in the patients sera during infection with brucellae is the basis of the serological diagnosis of brucellosis, especially chronic brucellosis (Kerr et al, 1966; Coghlan and Weir, 196 ; Reddin, et al, 1965; Wilkinson, 1966).

Because the rate of isolation of Brucellae, in particular Br. abortus from blood or tissues is low, laboratory diagnosis of brucellosis is based mainly on the results of serological tests, the results of which depend on the clinical form and stage of the infection (Kerr et al, 1968).

Currently available serological tests for brucellosis include:

(A) the standard tube agglutination test;

(B) the card agglutination test;

(C) the slide agglutination test;

(D) the acidified plate agglutination test (Rose bengal plate test);

(E) the 2-mercaptoethanol agglutination test;

(F) the anti-human globulin (Coombs) and centrifugation agglutination tests;

(G) the complement-fixation test;

(H) the indirect haemagglutination test (IHT);

(I) the direct and indirect immunofluorescence test.

The Agglutination Tests

The standard agglutination test detects the presence of agglutinating antibodies, which may be a mixture of macroglobulin (IgM) and microglobulin (IgG), depending on the stage of the disease. It is usually positive after two weeks of active disease (Christie, 1974).

IgG antibody is the most important antibody in brucellosis. It may or may not be demonstrated by the agglutination test: if it agglutinates, this ability is not destroyed by 2-mercaptoethanol; it readily fixes complement; its presence when not agglutinating is revealed by the anti-human globulin test (Coombs test) Heddin et al, 1965; Kerr et al, 1958).

The sero-agglutination tests are the most widely used diagnostic procedures and they have been rendered much more reliable since W.H.O. established an International Standard or anti-brucella abortus serum in 1953: the Expert Committee on Standardisation recommended the

adoption of a Unit System for standard sera which enables the agglutinating strength of all sera to be stated in the same terms (WHO, 1953). This will ensure a valid comparison with the results obtained in different countries with different methods. It also enables a worker in any country to know at once the sensitivity of the test he employs.

The reliability of the agglutination test depends on the antigen that is used. In human medicine there is a wide variability in the antigens and test procedures being used, with accompanying variability in test results. Most antigens differ in their cell concentrations, and since an inverse linear relationship exists between the concentration of the bacterial cells and the titres obtained in the test, the titre of a particular serum will vary with the antigen employed unless the density of the cells is comparable in each case (Kerr et al, 1968; Bothwell, 1952).

There is also a need for adopting a standardized method for performing the various available agglutination tests. For the standard tube agglutination test, the procedures used by Spink et al, (1952) in the United States and Kerr et al, (1968) in U.K. have proved satisfactory in several laboratories. With each batch of test sera, the International Standard for anti-brucella abortus serum is included as the positive control and standardization of the titre obtained in International Units (I.U.) (App. I).

The agglutination test in the presence of 2-mercaptoethanol (for distinction of IgM and IgG agglutination) is carried out as for the standard agglutination test except for the diluent which is normal saline containing 0.05 M 2-mercaptoethanol in place of phenol saline. The agglutination test, when carried out with a suitable antigen and a satisfactory

technique nearly always gives significantly positive and reliable results with high IgG antibody level, in the presence of active infection. The test may however be positive in moderately high titres in people who have never suffered from overt or active disease but who have been repeatedly exposed to infection, either by drinking raw milk or by occupational exposure. The agglutinating antibodies of such exposed type of persons fall over a period of time without further contact but the residual antibodies may still remain IgG.

There are also those with low or moderately high agglutination titres found during the investigation of cases of pyrexia of unknown origin or during serological surveys such as those of blood donors. The antibodies in some of these cases have proved to be mercaptoethanol-sensitive and have failed to fix complement. They are IgM antibodies that are residual from past infection, either overt or subclinical, through contact or through the drinking of raw infected milk and are no longer indicative of active infection. Low titres might similarly be obtained in districts where brucellosis is endemic.

In some patients in whom the diagnosis of chronic brucellosis is proved by culture of the organism, the agglutination test may even be negative at all dilutions (Kelly, et al, 1960). Therefore, the finding of negative agglutination tests do not exclude infection. Repeated tests should be carried out in such patients with symptoms and signs strongly suggestive of chronic brucellosis, and the detection of microglobulin (IgG antibodies) may be very helpful. Though a diagnosis of brucellosis can be made with certainty only if IgG antibody is present, it must also be remembered that the presence of IgG antibody may not

necessarily signify active infection, but may be due to constant exposure to brucellae, as with veterinary surgeons. The WHO Expert Committ on Brucellosis cautioned that extreme care should be taken in interpreting levels of agglutinins of less than 100 IU/ml (WHO, 1964a). A titre of 100IU is very suspect; a titre of 100 IU in combination with clinical symptoms and signs of brucellosis is proof of disease (Spink, 1956).

Slide Agglutination Test

A slide agglutination test performed with whole blood and blue stained antigen was introduced by Brumpt (1940) and Castaneda (1950, 1961) for quick screening. When agglutination occurs, clumped particle forming a blue ring, surround a red centre of liberated red cells. Because of the low sensitivity of this test low false positive titres are eliminated. Castaneda developed a surface fixation test which detects an antigen-antibody complex on filter paper. From an examination of 21800 cattle samples Kretschmer (1967) concluded that the reliability of the surface test was not significantly below that of the tube agglutination test, while most of the non-agglutinating antibodies were detected as well. The surface-fixation test is therefore considered to be a time- and cost-saving procedure. It can be performed and interpreted easily, but standardization of the antigen preparation is needed (WHO, 1964a).

Acidified Plate Test (Rose and Roepke, 1957; Lambert and Amerault, 1962)

The technique is described by Alton and Jones (1967), Alton et al, (1975). 0.03 ml of an acidified antigen is added to respectively 0.08, 0.04, 0.02, and 0.01 ml of serum (equivalent to respectively 200, 100, 5[illegible], and 25 IU/ml). The testing is done at room temperature. The antigen-

serum mixture is spread, rotated and read after eight minutes. Low titres are easily missed. Non-specific agglutinins are inhibited by the low pH. The inhibition of specific agglutinins depends on the height of the titre and the pH of the antigen.

Recently the acidified plate test has been used frequently under the name of Rose bengal plate test (RBPT). The antigen is stained and concentrated to increase its sensitivity. The test can be performed either as a card test or plate test.

One drop of antigen is mixed with an equal amount of undiluted serum on a glass plate at room temperature. The results are read after five minutes on a white background as either positive - showing agglutination - or negative - showing no agglutination.

Another technique, using serially decreasing quantities of serum, enables the examiner to read the test in eight minutes.

The reliability of the RBPT in veterinary medicine has been established by Morgan et al, (1960), Morgan (1969), and Davies (1971). The application of the RBPT to human medicine needs further investigation according to the WHO/FAO Committee on Brucellosis (WHO, 1971).

Cox (1968) advocated the use of the rapid agglutination test when working amongst the nomadic tribes in the Northern parts of Uganda and Kenya. However, preliminary studies in Nigeria had shown that the Rose-Bengal plate test was ineffective on human sera (Falade: personal communication).

The Brucellosis Card Agglutination Test

This is a macroscopic, agglutination procedure utilizing disposable materials, a stained buffered whole cell antigen suspension of Brucella

abortus, and contained in compact kits of minimal size. The card test is a relatively sensitive qualitative means to measure the presence or absence of IgG agglutinins. The card test has been developed to have comparable results with the mercaptoethanol tube agglutination and complement-fixation tests in the screening of cattle sera. It has been found most useful during the Brucellosis Eradication Programme in the United States (Nicoletti, 1967). The test has also been improved upon and adopted in the diagnosis of human brucellosis (Nicoletti and Fadai - Ghotbi, 1971; Buchanan, et al, 1974) by employing the method recommended by United States Department of Agriculture (Nicoletti, 1967; O'Reilly and Cunningham, 1971). Buchanan and co-workers (1974) found no false-positive reactions with the card agglutination test and this suggests that the card test is probably superior to previously described rapid slide agglutination tests which gave many false-positive reactions (Casteneda, 1961; Cox, 1968; Hall and Manion, 1953; Schubert, 1953). The greater specificity of the improved card test may be due to the use of an acidified standardized antigen which minimizes IgM agglutination antibody effects (O'Reilly and Cunningham, 1971). The card agglutination test offers the advantage of immediate bedside results since it can be performed in four minutes. A positive card test in a symptomatic patient would provide laboratory support for treating the patient immediately and might result in treatment two to five days earlier than if the physician waited for completion of the standard tube agglutination tests. However, a negative result would not rule out a diagnosis of brucellosis, especially if the serum was obtained during the first week of the patients' illness, when IgM agglutinins were mainly produced.

The Antihuman Globulin Test (Modified Coombs' Test)

Inhibition prozones or slight to absent agglutination in the lowest series of dilutions of the serum has been reported as a cause of false-negative agglutination tests (Feinberg and Wright, 1951; Glanchur, et al, 1961; Schuherdt, 1951; Zinneman et al, 1959; Zinneman et al, 1964). When serum containing IgG antibody is mixed with a suspension of brucella cells, agglutination often does not occur, though the antibodies adhere to and become, as it were, part of the antigenic mosaic of the cell wall. If rabbit serum containing anti-human globulin (AHG) is now added, the AHG reacts with the human globulin on the brucella cell and agglutination takes place (Coombs, Mourant and Race, 1945; Wilson and Morrifield, 1951). Two types of antiglobulin technique of Coombs have been described (WHO, 1971). In the first, a previously titrated antiglobulin reagent (serum of a rabbit immunized against human globulin) is added to the saline-washed Brucella antigen after the standard agglutination test has been performed. This is designed to detect incomplete or blocking antibody, as well as the presence of antibody at serum dilutions beyond those at which it can be detected by the standard agglutination test. The demonstration of incomplete antibodies or the extension of the titre is interpreted as a positive result, and expressed in I.U.

In the second type of antiglobulin test, the test serum is heated to 70°C, mixed with Brucella antigen, and after a short period of incubation the cells are washed. An antiglobulin reagent is then added.

An automated method for the determination of incomplete antibodies in the Coombs anti-human globulin test has also been described (Petrow et a

1973). Hall and Manion (1953) found centrifugation during 15 minutes at 4000 rpm as efficient as, and simpler and cheaper than, the Coombs' test in the estimation of non-agglutinating antibodies.

The Coombs test is a suitable procedure for making an early diagnosis as incomplete antibodies appear earlier than the agglutinating ones. It is also a useful test for establishing a diagnosis of chronic brucellosis (together with 2-ME test and CFT).

Complement Fixation Test

Of the immunoglobulins produced during Brucella infection, IgG fixes complement but IgM does not. In human infection, the IgM antibodies appear earlier and disappear later than the IgG (Brodhagu and Fey, 1955). The same holds true for animal infections. If active infection persists, the complement-fixation test (CFT) remains positive because of the presence of microglobulin antibody.

In veterinary medicine the CFT is an important and much used test in cases where a preliminary screening survey by the agglutination test is not conclusive as to vaccination or infection of an animal.

In human medicine the CFT is an essential test to diagnose activity of the disease, particularly in cases of chronic brucellosis. In the presence of a low or negative agglutination titre, a positive CFT indicates a still active disease. In the acute stage of the disease, the standard agglutination titre is usually so high that the CFT does not contribute to the diagnosis.

The CFT is subject to great variability and results depend on minor differences in technique and the type of antigens available. Standardising of the CFT is even more important than standardization of the

agglutination test. The standard international serum of anti-Brucella abortus might be useful for the CFT and its use has been recommended by the joint FAO/WHO committee on Brucellosis (WHO, 1971). Titres of test sera should be given relative to the titre of the standard serum.

Immunofluorescence Test

The indirect antibody test is a specific and sensitive method of detecting antibodies in sera of man and animals, and may be positive when the agglutination test is negative (WHO, 1971; Hiegeleisen, Bradshaw and Moody, 1962).

The direct fluorescent antibody test and the method using fluorescent labelled antiglobulin (two-stage method), can be used for detecting brucellae in certain pathological materials, particularly when they are heavily contaminated as in aborted materials and isolation of the agent may be impossible (WHO, 1971). Corbel (1973) proposed counter-staining with Evans blue to suppress tissue autofluorescence.

The fluorescent antibody technique is very useful in epidemiological surveys when many sera have to be screened (Edwards et al, 1970). The equipment required for this test is relatively expensive, preparation of the reagents is a complex task, and interpretation of the results requires well-trained personnel and careful attention to necessary controls. If the laboratory is adequately equipped and staffed, and the technique properly used, the immunofluorescence test can contribute greatly to basic studies of pathogenesis and immunogenesis of brucellosis. It can also be recommended for routine diagnosis along with other conventional bacteriological and serological examinations (Hiegeleisen, et al, 1962; WHO, 1971).

The Passive Haemagglutination Reaction

Indirect (passive) haemagglutination (IHA) tests using a lipopolysaccharide antigen obtained from brucella cells have been studied extensively in the USSR by Versilova et al, (1974) in the diagnosis of human and animal brucellosis. The data obtained in the USSR suggest that the IHA test may be more specific and sensitive than the standard agglutination test. The specificity of IHA test was not verified by isolation of brucella in culture of specimens obtained from positive persons and animals. However, the method deserves further evaluation and may be worthy of inclusion in the battery of serological tests used to confirm suspicious or negative serological tests (WHO, 1971).

The specificity and sensitivity of this test depend on the antigen used for sensitization of sheep erythrocytes: Versilova et al (1974) have developed a lyophilized erythrocyte antigen and other workers have also prepared soluble antigens derived from brucella cells for use in IHA test and CFT (Chen and Elberg, 1969).

The Brucellin Intradermal Skin Test

This is used to detect the sensitivity to Brucella antigens. A positive test indicates that the body has at some time reacted to the presence of brucella antigen and become sensitive to it. It has no other significance and its value is limited. There is no correlation between this reaction and the level of agglutinins. The test can be negative not only when specific agglutinins are present but even when the organism has been grown from the blood.

The WHO/FAO Expert Committee (WHO, 1971) suggest (1) that a persistent negative test excludes brucellosis, (2) that a positive test in

an area of low incidence may have some diagnostic significance, (3) that it is useful in epidemiological surveys, but (4) that if it is the only positive finding, great caution should be exercised in making a diagnosis of chronic brucellosis in a patient with vague symptoms, since in some countries up to 25 per cent of the normal population are positive reactors (Spink et al, 1952). Also it is important to bear in mind the fact that vaccinated individuals may have positive skin-tests.

One of the disadvantages of intradermal injection of brucellin is that it may provoke the formation of agglutinins, thus rendering the interpretation of positive agglutination tests difficult (Bradstreet et al, 1970; Report, 1972). Many investigators have not found the test of value, (Spink et al, 1952; Christie, 1974), and some even suggest that its use in the diagnosis of chronic brucella infection in the absence of other supporting laboratory findings should be discouraged (Spink, 1950).

D: SUMMARY OF LABORATORY DIAGNOSTIC CRITERIA FOR HUMAN BRUCELLOSIS

The different techniques and methods used for the diagnosis of human brucellosis vary depending on the objectives of the diagnosis:

(a) diagnosis of individual cases;

(b) screening and mass surveys to evaluate the prevalence and distribution of human brucellosis in a particular area.

The fourth report of the joint FAO/WHO Expert Committee on Brucellosis recommended the following guide-lines (WHO, 1964a):

(a) Diagnosis of Individual Cases:-

(1) Culture

(2) Serological tests, including serum agglutination test, complement fixation test, Coombs test.

With the increased tendency to employ antibiotics for febrile conditions of unknown aetiology, positive diagnosis by haemoculture is less frequently possible, and increasing reliance in being placed on the results of agglutination tests and on the clinical picture (Spink et al, 1952; WHO, 1964a). For practical purposes, therapy with the antibiotics does not interfere with the diagnostic value of agglutination test (Spink et al, 1952). For chronic brucellosis the standard agglutination test alone helps very little in the diagnosis. The 2-mercaptoethanol, Coombs's test and CFT are therefore the usual method of diagnosis (Christie, 1974) and these may be supplemented by blood culture (Kerr et al, 1966). The 2-ME test is perhaps most useful for evaluating whether chronic brucellosis is present in patients with positive standard agglutination tests who developed illness more than six months previously. If the 2-ME test and CFT are negative, the case is not one of chronic brucellosis (Buchanan et al, 1974; Kerr et al, 1966; Coghlan and Weir, 1967; Reddin et al, 1965; Wilkinson, 1966).

(b) Screening and Mass Surveys: The practicable and useful tests include:

(1) the serum agglutination test;

(2) the intradermal test;

(3) the whole-blood agglutination test; and

(4) the surface-fixation test.

The accumulated experience of investigators over many years has demonstrated that the agglutination test, if properly performed, is an invaluable diagnostic aid. It is to be recalled that in the

classical and highly informative studies on human brucellosis carried out by the Mediterranean Fever Commission the agglutination reaction was clearly demonstrated to be quite dependable as a diagnostic procedure (Spink, et al, 1952). Most screening and mass surveys for the incidence of brucellosis in man (and animals) has been carried out by means of the standard agglutination test which is a simple and economic procedure (Buchanan, et al, 1974).

E: TREATMENT OF HUMAN BRUCELLOSIS
(Regardless of the Species of Brucella Causing Disease)

General Points to Note in Therapy

1. In the treatment of brucellosis, it is necessary to consider the acute and subacute forms separately from the chronic form of the disease.

2. Antibiotic treatment is recommended for all stages of the disease. It is commonly thought that since Brucella is an intracellular pathogen relatively inaccessible to antibiotics, brucellosis is incurable and patients only need reassuring. This is not so, and prolonged antibiotic treatment would seem advisable. Treatment should be started early, and the temptation not to treat patients diagnosed late who are improving must be resisted: they may deteriorate suddenly while being observed (Williams, 1973).

Choice of Antibiotics: Tetracycline seems to be the most suitable antibiotic (Spink, 1960; WHO, 1971). Chloramphenicol has some value in treatment but penicillin has no place in therapy. While Streptomycin and Sulphonamides prove singularly ineffective when given alone, success has been reported when they are used in combination and also when streptomycin and tetracycline have been combined (Ramsay and Emond, 1967).

Sharma (1965) reported successful treatment of four cases of brucellosis using nalidixic acid (one gram 4 times daily for 10 days).

Co-trimoxazole (trimethoprim, 80 mg, and sulphamethoxazole, 400 mg per tablet) has also proved effective in a number of patients. Sensitivity tests using commercially prepared discs on bovine isolations of Br. abortus showed that 47 per cent were resistant to co-trimoxazole, 1.1 per cent to tetracycline, and 4.7 per cent to streptomycin (Williams, 1973).

Most adult patients respond to oral tetracycline 0.5 g, six-hourly for six weeks, together with intramuscular streptomycin, 1.0 g daily for the first three weeks of treatment (WHO, 1971a). In severe illness tetracycline should be prescribed for another six weeks at least (Williams, 1973), and it may also be necessary to administer the drug parenterally, particularly in cases with demonstrable localized lesions (WHO, 1971).

Relapse or reinfection, which is indistinguishable, is an indication for a further course of treatment with a combination of tetracycline and streptomycin. Occasionally in chronic brucellosis the response even to prolonged treatment is poor, suggesting that some of its manifestations may be due to immune mechanisms which do not depend on a persisting endogenous focus of infection.

Precautions During Antibiotic Treatment: The following warning in the Third Report of the Joint FAO/WHO Expert Committee on Brucellosis (WHO, 1958) should be kept in mind: "The indiscriminate and uncritical administration of antibiotics for brucellosis offers certain hazards. In some critically ill patients, the initial dose of antibiotic may be followed by a severe reaction, including shock. Even in patients with unques-

tionable acute brucellosis, antibiotics should be used with caution, and the advantages and disadvantages considered. Undesirable side-effects, including nausea, vomiting and diarrhoea, may seriously interfere with the comfort of the patient. Disturbed intestinal function may persist in some cases for weeks and months after therapy has been discontinued. Stomatitis and pruritus ani are disturbing side-effects. Occasionally, severe pseudo-membranous enterocolitis associated with antibiotic-resistant staphylococci may occur."

3. Corticosteroid Therapy

Corticosteroids and immunosuppressants are not recommended in ordinary cases of brucellosis. Their use with antibiotics, however, may be justified in certain conditions. They may help, along with antibiotics, in patients with severe septicaemic brucellosis to prevent toxic reactions following the first few doses of antibiotics. Bleeding due to thrombocytopenia is another rare but urgent indication. Corticosteroids may also be used in certain visceral forms of brucellosis (WHO, 1971). Steroids should be given for only a few days, at moderately low dosage, equivalent to 300 mg cortisone daily for 1 to 5 days are recommended by Christie (1974).

During chronic stagesof the disease, treatment with corticosteroids would seem inappropriate, for these drugs sometimes are lethal but chronic brucellosis is not (William, 1973).

Treatment of Acute and Subacute Brucellosis

A. General Treatment: A patient with acute brucellosis requires supportive therapy, including bed rest, good nursing and adequate diet. If the patient is mentally disturbed, sedation may be required. If there

is much pain, aspirin 650 mg eight-hourly may help, its disadvantage being that it may increase the sweating. Tepid sponging may be necessary if the temperature is very high.

In many patients, the temperature and symptoms disappear on this general treatment only, without specific treatment, and this should be recognized in evaluating any new treatment.

B. Specific Treatment: Antibiotic treatment is recommended once the diagnosis is made. They shorten the acute stage and destroy extracellular brucellae. Brucellae are intracellular organisms, they may be protected from the action of drugs and emerge later to cause complications or relapse. However, early and adequate antibiotic treatment will probably reduce the incidence of relapses and complications.

Evaluation of Patients Response to Specific Treatment:

In view of the frequency of spontaneous recovery, the following points are recommended by the WHO Expert Committee on Brucellosis in judging the effectiveness of any therapy (WHO, 1971):

a) clinical improvement within one week;

b) the failure to recover brucella from the blood, or from other tissues or sites, when previous results were positive;

c) a reduction in the frequency of complications;

d) a reduction in the frequency of relapses;

e) a drop in antibody titre.

Treatment of Chronic Brucellosis

The patient with chronic brucellosis without obvious complications presents a different therapeutic problem. The first thing is to be sure that the diagnosis is correct; next is to decide on treatment.

The patient should be given one full three week's course of tetracycline. If the patient has occasional bouts of fever, treatment is best given then, for the fever may sometimes be due to the presence of extracellular brucellae and also circulating organisms to which drugs are accessible: but fever is sometimes due to the release of endotoxin or other products from dead organisms and, when this is so, antibiotics cannot be expected to help (Christie, 1974). The response of patients with chronic brucellosis to antibiotics is less than in the acute phase of the disease because some of its manifestations may be due to immune mechanisms which do not depend on a persisting endogenous focus of infection.

If the patient is hypersensitive to brucellar antigens, desensitisation may be worth trying and is recommended (WHO, 1964a). Desensitisation must be distinguished from treatment by intramuscular or intravenous doses of brucellar antigen (Griggs, 1948; Castaneda, 1941) which was used in the past in some cases of acute brucellosis with painful localized infections (WHO, 1971) but not now used (Christie, 1974).

A few cases of brucellar spondylitis and arthritis may require surgical intervention, in addition to antibiotic treatment.

Physiotherapy may also be a useful adjunct to treatment.

TABLE VI.1

CLINICAL FEATURES OF HUMAN BRUCELLOSIS REPORTED BY DIFFERENT AUTHORS

Author (Year) Country Organism	Spink (1956) U.S.A. Br.abortus	Huddleson (1947) U.S.A. Br.ab.+suis	Dalrymple Champneys (1960) England Br.abortus
Number of Patients	121	230	1,500
SYMPTOMS (in %)			
Weakness	93	100	76
Sweating	77	84	78
Pain:			
General	67	30	55
Headache	65	64	64
Backache	40	...	...
Joint pain	37	25	55
Neck pain	33	...	...
Neuralgia	5	...	...
Abdominal	19	7	19
Anorexia	72	75	60
Constipation	16	60	30
Diarrhoea	10	...	5
Cough	25	30	3
Nervous disturbance	43	50	6
Insomnia	30	35	3
SIGNS (in %)			
Fever	98	100	100
Undulant fever	0	15	75
Splenomegaly	40	33	22
Lymph glands enlargement	39	...	4
Hepatomegaly	20	...	6
Weight loss	...	90	2
Skin rash	5	11	7
Orchitis	2	5	1
Spondylitis	4	...	...
Arthritis	...	2	1
Cardiac involvement	6	1	...

CHAPTER VII

BRUCELLOSIS AS A WORLD PROBLEM AND REVIEW OF METHODS OF CONTROL IN VARIOUS COUNTRIES.

A: SOCIO-ECONOMIC PROBLEMS

Brucellosis is of particular importance among the zoonoses, not only because of its direct impact on man's health but also because of the economic losses and reductions in food supplies for which it is responsible. Therefore, the important aspects of brucellosis as a world problem are essentially two: (a) the public health significance, and (b) the economic loss to animal industry (WHO, 1964a, 1971), caused by the abortus species.

Its public health significance includes not only the direct or indirect transmission of the disease from infected animals to man, with the consequent illness, physical incapacity, and loss of manpower, but also the serious dimunition of much needed animal proteins which are essential to human health and well-being. This is particularly true in countries with a developing economy and animal husbandry.

In many countries of the world, brucellosis contributes largely to a low standard of living, and the World Health Assembly (WHO, 1969b) recognising this requested both FAO and WHO to assist member countries in assessing, in a more analytic and systematic manner, the economic

losses caused by brucellosis and the cost of services set up to deal with it. WHO has therefore drawn up guidelines for such studies which are required whenever funds are requested from national and international sources (WHO, 1971; Guidelines, 1972). It is suggested that an economic analysis should include the following principal costs of the disease:

1. Direct and indirect costs of the disease in the individual, e.g.:
   (a) medical fees, and costs of hospitalization, nursing, and drugs;
   (b) loss of income and loss of productivity, as a result of absence from work.
2. Costs to the animal industry from:
   (a) abortion, causing loss of potential adult livestock both for replacement in herds and for human consumption in the form of meat, milk, and milk products;
   (b) weakling animals resulting from premature birth, causing loss of revenue-producing products;
   (c) lowered prices of animals intended for export from a brucellosis-affected area, and lowered prices of milk and milk products as a result of local ordinances prohibiting the use of products from diseased herds;
   (d) effects of infertility;
   (e) loss of national and international markets;
   (f) decreased output of meat and other animal products from herds that are infected with brucellosis;
   (g) condemnation of meat.

3. Administrative costs on a national level, including the costs of research, control, and eradication programmes, Such costs may include:

(a) government compensation for the replacement of diseased animals that are eliminated;

(b) workmen's compensation for disability;

(c) costs of testing-programmes;

(d) costs of vaccines and their administration;

(e) costs of enacting and enforcing legislation (expenses of veterinary and medical services, legislators, etc.);

(f) costs of informing the public and professional groups about the programmes;

(g) costs to other necessary projects, as a result of the need to give priority for manpower, facilities, supplies, etc., to brucellosis control.

B: PREVENTION AND CONTROL OF BRUCELLOSIS IN MAN

In the prevention or control of an infectious communicable disease, three main methods are generally used, viz:

a) limitation and control of the reservoir of infections;

b) control of the method of transmission, and

c) attempts to increase the resistance of the population.

The problem of brucellosis is primarily a veterinary one: if animal reservoirs of the disease could be eliminated, human infection would automatically cease. Therefore the theoretical approach to the prevention and control of brucellosis is simple. Infected animal reservoirs can be detected and removed from herds; milk can be rendered safe

by pasteurization before being consumed; meat and meat products can be subjected to adequate heat-treatment before being eaten; and cattle, goats, sheep, pigs and man can be immunized against the disease.

In practice the matter is not so simple. The elimination of extra-human reservoirs presents formidable problems, but much can be expected from attempts to control their size or from their abolition in prescribed areas. Various methods have been adopted to bring this about. With a self contained small or medium sized herd it may be possible to eradicate infection entirely by protecting against infection from manure or other animals, by provision of calving boxes which ensure segregation during parturition and re-testing of non-reactors at regular short intervals (for example, every two months or so) so as to eliminate all animals as soon as they become positive. With larger herds this method may not prove effective particularly if there is imperfect control over infection from other sources. A serious problem is presented by the infected non-reactor which may abort or excrete Br. abortus in the milk and so contaminate other animals in the herd. Control of brucellosis in areas of nomadic animal husbandry is particularly difficult to achieve.

High costs are a serious hindrance to programmes based on the test-and-slaughter policy. The initial expense may be beyond the resources of veterinary services in many developing countries, even when it is clearly justifiable on a cost-benefit basis (Abdussalam and Fein, 1975).

Milk and Milk Products: Legislation should be enacted to require pasteurization of milk and milk products (WHO, 1971). Brucellae are killed by a lower time-temperature combination than that required for Mycobacterium tuberculosis or Mycobacterium bovis. Hence, where milk cannot

be pasteurized it is sufficient merely to raise the temperature of the milk to boiling point and then to cool it immediately. The cream of infected milk is usually more heavily contaminated than the rest of the milk, because the fat globules rising to the surface carry the organisms with them. A higher time-temperature combination should be used for pasteurization of cream derived from unpasteurized infected milk than for the rest of the milk.

Meat and Meat Products: The transmission of brucellosis by meat and meat products can be prevented by subjecting them to adequate heat treatment. It is important to note that the organism can survive pickling and smoking, as well as chilling and freezing (WHO, 1971).

In the interest of public health, it is justified to adopt strict measures concerning the delivery of brucella-infected animals to abattoirs, the conditions under which they are slaughtered, and the methods of inspecting and judging the meat. Personnel should be well protected against the risk of infection by wearing gloves and goggles, and they should be kept under medical surveillance. Health education of livestock farmers and abattoir personnel is important.

Immunization: Adequate heat treatment of milk, meat and their products has no effect on human brucellosis due to contact with infected animals. Vaccination of animals has proved successful in preventing spread of brucellosis in herds and it is practised extensively in veterinary medicine. However, eradication of the disease by removal of infected animals from herds is the only completely satisfactory solution (Christie, 1974).

Vaccination of man as a method of controlling brucellosis has been practised widely in the USSR for more than two decades, and the vaccine

prepared from Br. abortus strain 19-BA is given to population groups occupationally exposed to infection with Br. melitensis (Versilova, personal communication). Vaccination of man is considered by many authors as a poor approach to the problem, though it may be the best available method when, due to poor standards of husbandry, adequate control of infection in animals is not possible (Versilova, 1961; Versilova, 1965; Regamey, et al, 1970; Roux and Serre, 1971). In Russia between 4 and 5 million people have been vaccinated annually with a fall in morbidity from 100 to 100,000 inhabitants in 1952 to none in 1965 (Regamey et al, 1970). The USSR authorities consider that vaccination is a valuable adjunct to sanitary measures (Versilova - personal communication).

Human vaccination has disadvantages in that the vaccine may produce severe untoward reactions or illness in some individuals, and that it may render the vaccinee hypersensitive to future contact with brucella antigen (Christie, 1963; Spink et al, 1962; Pappagianis et al, 1966). Also it produces persistent antibodies, which are sometimes difficult to distinguish from post-infection antibodies. Therefore the Expert Committee on Brucellosis emphasizes that human vaccination is a temporary protective measure against brucellosis where there is great danger of occupational infection, e.g. for owners, and handlers of sheep and goats, for workers in abattoirs and meat industry, and for veterinarians and laboratory workers. The Committee considers vaccination in man as a supplement to strict application of sanitary and hygienic measures. The decision on whether to carry out vaccination in man must be taken in agreement with the national or local health authorities WHO, 1971). Roux and Serre (1971) recommend full serological investigation and skin

testing of exposed workers before giving vaccine. Primary and booster vaccinations should be given only to those occupational groups that are highly exposed to Br. melitensis infection, and only if the intradermal test is negative (WHO, 1971). The advantages of vaccination in man have to be weighed against the risk enumerated above.

C: CONTROL AND ERADICATION OF BRUCELLOSIS IN ANIMALS

1. Diagnosis of Infection in Herds: Isolation of the organism from milk, placenta, vaginal discharge and foetal stomach content is the most certain method of diagnosing infection in herds, but it is time-consuming and almost impracticable. Serum agglutination tests in cattle are reliable indicators of infection, provided the animal has not been recently vaccinated with Br. abortus strain 19. In goats, sheep and swine agglutination tests are not so reliable as in cattle, but are still the most practicable method used (Christie, 1974).

In recent work, some of the old-established serological tests for the diagnosis of bovine brucellosis have been evaluated. For example the standard serum agglutination test was found to give low titres in cattle from which Renoux et al (1968) were able to isolate Br. abortus. Nicoletti (1969) found that this test detected only 52 per cent of the culture positive cattle. Alton et al (1975) confirmed that standard serum agglutination test frequently gave low titres in infected animals in a comparative evaluation of this test with the Rose Bengal (RBT) and CFT. Also the test gave low titres in a large number of animals from which no brucella organism could be isolated. The RBT correctly classified almost all the culture - positive cattle but was more often positive in

culture-negative cattle than the standard serum agglutination test. The CFT, however, identified all culture-positive cattle and was considered superior to the standard serum agglutination test except in cattle vaccinated with the 45/20 vaccine (vide infra).

The milk ring test is a sensitive indicator of the excretion of brucella agglutinins in cow's milk. The mechanism of the test depends on an antigen - antibody reaction. The sample of milk is poured into a test-tube and the cream rises to the surface, carrying with it any agglutinins in the milk. Haematoxylin-stained brucella antigen (Wellcome product) is added, and this reacts with the agglutinins causing clumping and the formation of a blue ring. The test can be applied to a bulk herd sample and, if positive, it can then be applied to samples of milk from single cows or from groups of cows till the individual excreters are detected. When a ring test is positive, the presence of brucellae in the milk may be confirmed by isolation on culture or by guinea-pig inoculation. However, in practice a positive ring test is sufficient evidence of infection. The ring test is less reliable for goat milk because false positive result may be obtained in the presence of mastitis (Ojo: personal communication).

2. Vaccination of Animals: As long ago as 1906, Bang showed that the inoculation of non-pregnant heifers with a living Br. abortus vaccine conferred some degree of immunity as shown by a lower abortion rate. It was also found that the more virulent the strain the greater the degree of subsequent immunity but in practice this proved dangerous both for pregnant animals in the herd and for human beings who might contract infection from milk.

Immunization of Cattle: Various vaccines have been used to immunize cattle, but the living attenuated vaccine, Br. abortus strain 19 (S19), remains pre-eminent because of its stability, safety, reliability and ease of production (WHO, 1964a). The S19 was introduced into the United States by Buck in 1930 and it has been used with good effect. The vaccine may be used to vaccinate calves between four and eight months of age, for non-pregnant heifers and for cows not more than four months pregnant. A single injection of one dose (60 to 80 X 10^9 viable cells) is sufficient to maintain immunity for at least seven years (WHO, 1971) or five pregnancies (McDiarmid, 1957). Unlimited multiplication in the uterus and udder is prevented so that abortion and infection of the milk are much less frequent than in unimmunized animals. This in turn leads to a diminution in the total amount of infective material excreted and thus lessens the danger of infection both of other animals in the herd and of human beings consuming raw milk.

The protection offered by S19 vaccine does not appear to wane with age, but the titre of agglutinins falls rapidly. This is of great importance, for vaccination of calves does not upset agglutination tests in the adult animals. If vaccine is given to adult cows it protects them against acquiring infection but it has no effect on an infection already present. The agglutination test becomes positive and there is no simple way of differentiating between animals positive because of previous infection and those positive because of vaccination. Hence, vaccination of adult cows is not recommended if the objective is to eradicate the disease within five years. Proper governmental control is extremely important to ensure the production and usage of vaccines with desirable

qualities (WHO, 1970).

Other Vaccines Used in Cattle Immunization

Inactivated Vaccines: (a) Brucella abortus strain 45/20 adjuvant vaccine is a suspension of killed organisms incorporated in a water-in-oil adjuvant. The immunogenicity of strain 45/20 adjuvant vaccines is influenced by the kind of adjuvant employed, hence the degree of protection with all lots of vaccines produced in different laboratories has not been consistent. Also, some strain 45/20 adjuvant vaccines are not totally non-agglutinogenic, a property which is considered to be an advantage over strain 19 vaccine. Yearly re-vaccination with strain 45/20 may be necessary since little is known about the duration of immunity in cattle (WHO, 1971).

(b) H38 adjuvant vaccine is prepared from formol-killed Br. melitensis strain 53 H38, incorporated in a water-in-oil adjuvant. A single dose of 3 ml (4.5×10^{11} killed organisms) is administered subcutaneously, comprehensive studies have shown that H38 adjuvant vaccine produces a serviceable immunity in cattle against virulent Br. abortus. The vaccine is safe for use irrespective of age, sex, or reproductive status of the cattle. Animals develop significant sero-agglutinins and complement fixing antibodies which usually recede below diagnostic level within six months and in milk and seminal fluid within one month after vaccination (WHO, 1971).

(c) Pilet-Bonneau Vaccine (Bonneau et al, 1970) is prepared by the total saturation of the superficial antigenic sites of killed strain 19 organisms with anti-Brucella serum. Preliminary studies show that satisfactory levels of short-term immunity were demonstrated in a number of

cattle (WHO, 1971).

Rev. 1 Vaccine: Controlled studies have demonstrated that this living attenuated vaccine is almost as effective as strain 19 vaccine in protecting cattle against Br. abortus and Br. melitensis infection. However, the Expert Committee on Brucellosis recommends that the use of Rev. 1 vaccine should be limited to countries where Br. melitensis infection is known to exist (WHO, 1971).

D: REVIEW OF THE PROGRAMME FOR THE CONTROL AND ERADICATION OF BRUCELLOSIS IN DIFFERENT COUNTRIES

Conditions in different countries throughout the world, and also in different parts of the country, vary so much that a single universal programme for the control and eradication of brucellosis is not possible. Complete eradication of brucellosis from animals should be the final objective of all countries (WHO, 1964a) and this should always be the aim with cattle (Christie, 1974). In a country with reasonable standards of dairy farming management, elimination of brucellosis requires only determination, co-operation and money (Craig and Wright, 1967).

The following are examples of different systems of Brucella control and their results in various countries under varying local conditions (Van der Hoeden, 1964): In some countries, especially the Scandinavian countries, the brucellosis has been brought under control. In 1938, a campaign against bovine brucellosis was launched in Finland, where the initial infection rate was three to four per cent of herds. The radical control programme then initiated yielded very satisfactory results. In 1956, infectious abortion was completely wiped out among the Finnish livestock and consequently brucellosis was also eradicated from the population of the country. In Norway, energetic nation-wide measures were

undertaken in 1934, with financial aid from the Government. Without milk-testing and vaccination, the disease has been practically eliminated within a period of 10 years. Similarly, eradication of brucellosis has been achieved in Sweden (Bjorkman and Bengtson, 1962).

The wide-spread infection among the dense cattle population of Denmark required unabated activity. With the full support of the Cooperative Dairies and a large subvention from the state, a collective campaign-programme was set up. The original infection-rate of 25 per cent of the herds in 1944 had declined to less than five per cent towards 1957.

In the Swiss Canton of Zurich some 15 per cent of the cattle herds were infected in 1951, in 1957 the figure was only one per cent. In Yugoslavia the percentage of cows reacting to Brucella was 4.1 in 1951, and 1 in 1956. In the U.S.S.R. the corresponding percentage was estimated at almost 8 in 1932, 3 in 1940 and 1.5 in 1953; human morbidity has been constantly declining because of reduction of infection in animals and the use of strain 19-BA vaccine in occupationally exposed groups (Versilova, 1965).

In the U.S.A., the first officially organized efforts to combat bovine infectious abortion were made in 1934, when a Cooperative State-Federal-Brucellosis-Eradication-Programme was instituted (Busch and Parker, 1972). Great improvement was achieved when, in 1941, vaccination with strain-19 was added to the programme. Since 1947, a uniform nation-wide campaign has been carried out, which is flexible enough to cope with the different herd and regional conditions. In the two decades following 1934, approximately 132 million head of cattle in 11 million

herds have been blood tested. The number of infected herds fell in this period from 38 to 14.2 per cent, and that of the infected animals from 10 to 2.6 per cent. Between 1941 and 1955 more than 21 million calves were vaccinated and during the following three years, 20.9 million. In 1958 the infection rate of all bovines throughout the USA had fallen to 1.6 per cent, while 15 states and Puerto Rico were certified as having been completely free from brucellosis. Swine brucellosis is however still prevalent especially in the Midwest and some difficulties have been met in efforts to eliminate it. These consist of lack of suitable diagnostic tests, expense of testing and difficulty of tracing animals tested in slaughter-houses to breeding farms.

The incidence of brucellosis in man reached a peak in the United States in 1947 with more than 6,000 reported cases (4.4 cases per 100,000 population). The cooperative eradication programme and associated control measures, such as pasteurization of milk, have resulted in a steady reduction of human cases. At present less than 200 cases of brucellosis are reported every year (0.1 per 100,000 population): these are mainly in meat packing workers and other occupationally exposed to infected animals and products, and are due to Br. suis of swine origin (Busch and Parker, 1972).

In the United Kingdom, bovine brucellosis has been brought under control since the introduction of compulsory test-and-slaughter programme in November 1972, but complete eradication of the disease from the entire cattle population is yet to be achieved (McDiarmid, 1973).

Although, in many developing countries it will be difficult, on socio-economic grounds, to inaugurate active eradication programmes, the

Expert Committee on Brucellosis (WHO, 1971) strongly recommends the immediate adoption of control measures that can be put into practice in these areas, and that target dates be put on control programmes which should be followed by a long-range eradication programme. With sheep, goats and pigs, herd management is much more difficult than in cattle herds and one may have to aim at control, rather than eradication.

Efficiency and economy in controlling and eradicating brucellosis can be accomplished by utilising surveillance procedures to detect infected herds. The milk ring test is especially valuable for locating infected dairy herds with a minimum of effort and cost. The Expert Committee on Brucellosis advocates the widest possible use of the milk ring test, and believes that it can be applied in most countries. (WHO, 1971). In areas where the milk ring test is not practicable, e.g., in beef herds, serological testing of blood samples collected from animals moving in trade through markets, stockyards and abattoirs is of great value in locating foci of infection: this is referred to as 'market testing'. The Expert Committee therefore favours the development of animal identification system, so that each reactor animal in the market testing can be traced back to its herd of origin and each potentially infected herd can thereby be tested.

Vaccination programmes can be used effectively in areas with very high prevalence of brucellosis to reduce the infection to a point where procedures based on surveillance and testing with the elimination of reactors are possible without serious economic loss to owners of infected herds. Vaccination is regarded as an important step in the direction of complete eradication. Approved vaccines are also useful in

maintaining the brucellosis protected status of herds and areas that are subject to exposure from adjacent sources of infection.

As eradication campaigns advance and the incidence of brucellosis is reduced in a given area, it becomes important to limit the extension of infection that may occur through normal movements of animals, both within and between countries. This can best be achieved through legislation. For example, cattle introduced into brucella-free herds, areas, or countries must be from brucella free herds, areas or countries.

All control programmes must include the maintenance of a high level of environmental sanitation and personal hygiene, with adequate steps being taken to reduce the intensity of exposure. This is of particular important in herds with goats, sheep and pigs where eradication may be difficult.

The establishment of a register of brucella-free herds, under constant supervision and subject to periodic re-testing, is necessary in order that such herds may serve as a source of replacements for herds in which the eradication of brucellosis is being attempted.

SECTION THREE

WORK CARRIED OUT BY THE AUTHOR

CHAPTER VIII

P I L O T S T U D Y :

SERO-EPIDEMIOLOGICAL EVALUATION ON BRUCELLA ANTIBODIES IN IBADAN

A: INTRODUCTION

Clinical impressions usually are the first indication of either occurrence of, or absolute increase in, diseases: but these observations have to be tested in population statistics.

The need for the present epidemiological study was indicated by Dr. E. A. Lewis, consultant physician at the University College Hospital, who successfully treated with antibiotics two clinically diagnosed cases of acute brucellosis, both with serum antibodies for Br. abortus above 400 i.u. The husband of one of the patients was later investigated and found to have symptoms suggestive of acute brucellosis with high anti-brucella antibodies (400 i.u.) (Lewis: personal communication). About the same period, an epidemiological report on Brucellosis in the British Medical Journal (Anon. 1973) mentioned a case of acute brucellosis in a man with a cervical vertebral abscess who had recently arrived in United Kingdom from Northern Nigeria. The report implied that the infection did not originate from the United Kingdom, and that Nigerians probably

had the disease. The level of suspicion, and therefore the rate of diagnosis, of human brucella infection among the majority of practising physicians in the country was at that time low. This could possibly be attributed to the fact that only few health laboratories in Nigeria undertake routine diagnosis for brucellosis.

Therefore a retrospective review of serological results of all patients seem at the University College Hospital, Ibadan with a clinical diagnosis of pyrexia of undetermined origin (P.U.O.) and whose blood samples were sent to the department of Medical Microbiology for Brucella saline agglutination test (S.A.T.) between January 1970 and December 1973 was undertaken in order to determine the prevalence of brucella infection among P.U.O. patients.

A serological epidemiological study was also carried out in Ibadan between August and December 1973 to find out the prevalence of human brucella infection, to determine the serological criteria for laboratory diagnosis of human brucellosis, to identify the group of people at special risk of infection, and to determine the source of infection and possible mode of transmission. In addition, the various serological tests employed in the diagnosis of brucellosis were compared in order to select the primary test for further epidemiological studies.

B: MATERIALS AND METHODS

(i) Retrospective Clinical Study: The laboratory records of all patients with a clinical diagnosis of P.U.O. and whose blood samples were sent for S.A.T. between January, 1970 and December, 1973 were reviewed. Only the saline Brucella agglutination test was performed on these sera

as they were processed routinely in the serology section of the laboratory.

(ii) Prospective Epidemiological Study: Sources of Sera: About 5 ml of blood samples were obtained from each of 1480 blood donors, composed of various groups of people working in Ibadan, aged between 18 and 55 years. In addition, 120 sera from school children in different parts of Ibadan and aged between 6 and 17 years were available for serological examination for Brucella antibodies. The sera were divided into two categories (Table VIII.1).

Group A: Those representing a cross-section of the general population, which include school children, pregnant women attending the ante-natal clinic (A.N.C.) of the University College Hospital (U.C.H.), Ibadan, male patients attending the Venereal Diseases (V.D.) clinic of the U.C.H., and male blood donors at U.C.H. Blood Bank: those engaged in animal husbandry or abattoir work are excluded among the V.D. patients and blood donors.

Group B: Those occupationally exposed to the risk of Brucella organism infection, which include slaughtermen, meat inspectors at abattoirs in Ibadan, veterinarians, dairy farm and meat shop workers, herdsmen at cattle control post and workers at the University of Ibadan Research Farm.

The sera were separated from the clotted blood samples within 24 hours of collection and kept at $-20^{o}C$ until serological tests were performed on them.

Serological Tests: The collected human sera were screen-tested using the following serological methods:

1. The Standard Saline Tube Agglutination Test: All the 1600 sera were tested by this test, using the method described by Kerr et al (1968) against three different available antigens: Weybridge Br. abortus concentrate diluted 1 in 50, Wellcome Br. abortus and Br. melitensis concentrated suspension diluted 1 in 15 (Kerr et al, 1968), and Bacto-Brucella abortus tube antigen (Difco Laboratories, U.S.A.).

For the standard tube agglutination test (SAT), the procedures used by Spink et al, (1952) and Kerr et al, (1968) have proved satisfactory in several laboratories; the method of Kerr and co-workers has been adopted in the present study. This involved serial dilutions of serum made in 0.5 ml volumes of phenol-saline, and to each tube was added an equal volume of diluted antigen. A series of serum dilutions beginning at 1 in 10 were made, and in order to avoid missing a "positive" because of a prozone (due to blocking antibody of IgG type), dilutions were made up to at least 1 in 400. The tubes were kept at 37°C in a waterbath for 48 hours and read with a magnifying mirror. The end point (titre) was taken in each series as that tube with the highest serum dilution showing a clear pattern of agglutination deposit and clear supernatant.

2. The Card Agglutination Test: All the 1600 sera were tested by this test, in humidity chambers in room temperature (22°C).

0.03 ml of serum was placed onto the 'tear drop' area of the card and two drops of antigen were added. A stirrer was used to mix the serum and antigen and the card rocked forward and backward continuously for four minutes.

The result was qualitatively measured and expressed as either positive when there were visible agglutination clumps or negative when there were no clumps.

3. The Anti-human Globulin (Coombs) and Centrifugation Agglutination for Detection of Non-agglutinating Antibodies in the Prozone Phase of Standard Tube Agglutination Test: Sera which showed negative reactions by the saline tube agglutination were tested by this method, using the method described by Kerr et al (1968) with Weybridge Br. abortus antigen diluted 1 in 20 and Wellcome Br. abortus and Br. melitensis concentrated suspension diluted 1 in 6.

The same range of serum dilutions was prepared in 0.5 ml volumes as for the standard agglutination test; an equal volume of the more concentrated antigen (than in the SAT) was added to each. The tubes were incubated at 37°C for 24 hours and any tubes showing agglutination were recorded and not proceeded with further. (The titres of sera showing agglutination were less than that of the SAT because of the more concentrated antigen used in the test: Kerr et al, 1968). Tubes not showing agglutination were centrifuged at 2000 r.p.m. for 15 minutes, and the supernatant was discarded. The centrifuged deposit was thoroughly washed three times by resuspension in normal saline in order to remove all traces of human protein. After the final washing the cells were resuspended in 0.45 ml normal saline and to each tube 0.05 ml of suitably diluted anti-human globulin (Burroughs Wellcome and Co.) was added and mixed by shaking (as recommended by the manufacturers). The tubes were kept at 37°C for a further 24 hours and examined for agglutination, which if present, was due to the anti-human globulin (AHG) reaction with the non-agglutinating antibody attached to the bacterial cells.

4. The 2-Mercaptoethanol Agglutination Test: Sera which showed positive reactions in the standard saline tube agglutination test or Coombs test were again retested and also simultaneously by the mercaptoethanol test (agglutination test in the presence of 2-mercaptoethanol).

The procedure for carrying out the mercaptoethanol test (2-ME test) was the same as in the standard tube agglutination test except for the substitution of 0.05 M 2-Mercaptoethanol in place of phenol-saline as the diluent. Reading and recording the results were also similar to those described for the standard tube agglutination test.

The difference between the titres in the SAT and the 2-ME tests indicates the nature of the immunoglobulin present; therefore both tests were carried out at the same time and under identical laboratory conditions.

5. The Complement-fixation Test (CFT): As in the case of 2.ME test, only sera which showed positive reaction in the SAT or Coombs test were tested by the CFT, employing the method described by Kerr et al, 1968, using only Wellcome Br. abortus antigen.

The CFT were carried out in WHO plastic plates using a four-volume technique with veronal buffer (Oxoid) as diluent. The serum dilutions commenced at 1 in 4. The Wellcome Brucella abortus complement-fixing antigen, diluted to an optimal concentration (1 in 55 dilution) was used and two per cent sensitized sheep red blood cells (by the Wellcome haemolytic serum) constituted the haemolytic system. The overnight fixation technique at $4^{o}C$ was used, with predetermined haemolytic units of Burroughs Wellcome preserved guinea-pig serum. (The haemolytic units of complement determined by titration of complement in the presence of

antigen and normal (pooled negative) serum used in the CFT was 1.[illegible] units). Antigen and serum controls were included in each batch of tests.

The titre of the serum was expressed as the reciprocal of the highest serum dilution that produced approximately 50 per cent lysis of the red cells. The antigen and the serum controls should show complete lysis for the results of each batch of tests to be valid; otherwise the tests would be repeated. It is important to note that anti-complementar activity in fresh uncontaminated human sera is frequently seen in Africans in Nairobi and Kampala (Cox; personal communication), and in Ibadan (Osoba: personal communication).

Control Sera: The Second International Standard Anti-Brucella abortus serum was titrated along with every batch of the above five tests in order to enable the titre of the test-sera to be expressed in International Units (I.U.) (App. I). In addition, the commercial standard serum produced by Burrough Wellcome & Co. was included with each batch of test.

C: RESULTS

The results of agglutination using the Weybridge antigen (diluted 1 in 50) were comparable to those of Wellcome antigen (diluted 1 in 15). But the Bacto-Brucella abortus antigen gave lower titres as compared with the other two mentioned agglutinable suspensions: this was probably due to the fact that a more concentrated suspension was employed in the Bacto-Brucella abortus antigen (Kerr et al, 1968).

Generally, apart from the non-reactive sera and in another 25 sera where the titres were the same, the [illegible] abortus antibodies were

higher for all the positive sera than the anti-Brucella melitensis antibodies, usually by at least two or three tubes. The Card test, the 2-mercaptoethanol agglutination test, the Coombs test and the Complement-fixation test were all negative.

Table VIII.2 summarises the anti-Brucella abortus titres produced by sera from all the 1600 persons by the S.A.T. when the Wellcome Br. abortus antigen was employed: the overall sero-positivity rate was 50.2 per cent. It will be observed that while 51.2 per cent of the people in Group A had no detectable agglutinins in their sera, 43.4 per cent of those in Group B showed negative sero-reactivity. On the other hand, 19.4 per cent of those in Group B had Brucella abortus agglutinins titre of 100 I.U. and above, while only 10.4 per cent of those in Group A had agglutinins at these titre levels. The prevalence of infection is highest in the population of working age.

Among the occupationally exposed workers in Group B (Table VIII.2), the slaughtermen and labourers working in the Ibadan City Council (I.C.C.) controlled abbatoirs had the highest overall seropositivity rate (of 85 per cent). Seven of these people had high antibody titres (400 I.U. and above). The staff at the Government cattle control post at Bodija with 58 per cent seroreactivity were in two groups consisting of eight clerks and eleven field workers. It is significant that only those employed in field work had antibodies against Br. abortus. There were also different categories of workers among the 19 Meat Shop Staff: eight slaughtermen, six meat sellers, three cashiers and two administrative clerks. All the eight slaughtermen and two of the three meat sellers had Brucella abortus agglutinin. Among the 22 veterinary staff examined,

there were three veterinary surgeons, who also supervise the Dairy farm at Iwo Road and all of them gave positive agglutination test. The remaining veterinary staff were mainly involved in looking after dogs and similar small animals, and they only occasionally engage in field work with veterinary surgeons. Forty-five per cent of the workers at the University of Ibadan Research Farm had agglutinins in their sera.

Table VIII.3(a) compares the agglutination titres of 316 blood donors and 270 men occupationally exposed to risk of Brucella infection, and table VIII.3(b) shows the test of significance between the same two groups. More people in the exposed group had agglutinins in their sera at 100 I.U. and above than did the general population ($P<0.001$). It is however noteworthy that 24 (7.62 per cent) of the blood donors tested had titres of 100 I.U. and above.

Inquiry during the survey revealed that most of the occupationally exposed people tested had been engaged in livestock farming for periods ranging between 2½ and 37 years (mean 7 years). Table VIII.4 shows the age and sex distribution, length of time occupationally in contact with cows and history of consumption of fresh (unpasteurized) cow's milk in eleven people with agglutinin titres at 400 I.U. and above. All these eleven people with high antibody looked well and were symptom-free at the time their blood samples were collected. The 2-ME test was negative for these eleven, showing that they did not have active brucella infection.

The age and sex distribution of brucella sero-reactivity among the different healthy population groups investigated is shown in table VIII.5. Among the 120 school children whose sera were tested, only eleven (9.2%) had agglutinins and all those with reactive sera were above

10 years of age. There was no significant difference between the prevalence of infection among boys and girls below the age of 18 years ($p > 0.05$). Similarly, the infection rates in the older group of the population (18 years and above) only reflect their occupation: no difference was found in the prevalence of infection among males and females.

Table VIII.6 summarises the antibody titres among patients with a clinical diagnosis of P.U.O. investigated at University College Hospital, Ibadan for possible Brucella infection between January, 1970, and December, 1973. The number of patients investigated increased from 5 in 1970 to 31 in 1973. Similarly, the number of patients with titres of 100 I.U. and above increased from zero in 1970 to eight in 1973. Over 60 per cent of all the sera received in the laboratory for Brucella agglutination test (S.A.T.) examination came from the neurology unit. Also six of the eight sera with high titre of 100 I.U. and above were obtained from patients with neurological disorders.

D: DISCUSSION AND INTERPRETATION OF SEROLOGICAL RESULTS

The definitive laboratory test for confirming a (clinical) diagnosis of brucellosis is a positive culture. However, in epidemiological studies, cultural methods are often impracticable and unsuitable. Because of the use of antibiotics either through self-medication or during concurrent treatment of other microbial infections, particularly common in east tropical countries, the results of cultural methods are likely to be very disappointing. Therefore more cases of brucellosis are now diagnosed serologically than by any other means.

In the present study, serological tests formed the basis for the objective laboratory diagnosis of human brucellosis. Serological

surveys are accepted methods of epidemiological investigations of many bacterial infections, including brucellosis in man and animals (WHO, 1959; Paul, 1966). A study of the antibody distribution by age, sex, and occupation throws useful light on the rate of spread of infection as well as the age at which infections are acquired and the impact on different population groups - ie., age specific rates under conditions of poverty and affluence, urban or rural living, or according to occupational groups in a given population (WHO, 1959).

Serological epidemiology seems to be of particular value among populations that have special habits, and for which morbidity data and vital statistics are scanty or completely lacking; that is, in populations characterized by primitive health facilities and a dearth of existing medical information (Paul and White, 1973). Information collected in such special sero-epidemiological surveys can be of immense value to those formulating programmes of control: the development and maintenance of sound public health programmes depend on knowledge of the prevalence and distribution of disease (WHO, 1959).

One of the objectives of the present pilot study was to compare the different available serological tests for brucellosis and to determine the serological criteria most appropriate for Nigeria. The choice of the five serological tests being evaluated in this study was based on the experience and findings of several previous workers in other countries on this subject as already discussed in the section dealing with the Laboratory Diagnosis of Brucellosis. Basically the five tests can be considered under two broad headings: Agglutination Tests and Complement-fixation Test. The agglutination reaction was clearly demonstrated to

be quite dependable as a diagnostic procedure as evidenced in the classical and highly informative studies on human brucellosis carried out by the Mediterranean Fever Commission (Spink, et al, 1952). Agglutinable antigen suspensions which were obtainable in Nigeria from different commercial companies were tested in order to determine their reliability and reproducibility. Also, the simplicity, inexpensiveness, sensitivity and selectivity attached to all procedures are locally evaluated. Finally the acceptability of the methods to be community and health authorities is also considered.

The serological diagnosis of human brucellosis is complicated by many factors. These include:

(a) the production of cross-reactive brucella agglutinins in persons previously infected or recently vaccinated with Vibrio cholerae or Francisella tularensis (Eisele, et al, 1947;

(b) low agglutinin levels in early or chronic infections, prozone and blocking antibody phenomena;

(c) difficulties in technique and interpretation of different serological tests, for example, the determination of limits of normal values in a particular test;

(d) the differences in the reproducibility and sensitivity of the tests in the hands of different investigators;

(e) in certain areas the lack of good quality laboratory reagents, laboratory equipment or trained personnel results in additional problems.

While tularaemia, a natural disease of rodents and lagomorphs that may also infect man (causing ulceration of the skin, lymphadenitis, fever and sometimes septicaemia - WHO, 1974), has not been known to occur in Nigeria, the country experienced an epidemic of cholera in 1971 (Lewis et al, 1972). During that epidemic, cholera vaccine was given to many people in Ibadan, Though, the vaccine is now only given to international travellers from Nigeria and there was no epidemic of cholera in Ibadan at the time this pilot study was carried out, it was felt by the author that a thorough investigation on the incidence of cross-reactivity between brucella and cholera in Nigeria should be undertaken: the full report of this study which is summarised in the next chapter of this thesis did not reveal any significant cross-reacting brucella agglutinins due to cholera in the community investigated.

Comparing the five different serological tests used, the card test was the simplest: this test was rapid and relatively specific for detection of IgG brucella agglutinins, but it had the disadvantage that the Kits for the test had to be obtained through special import licence after obtaining permission from the Director of Veterinary Services of Nigeria. The Saline Brucella agglutination test (S.A.T.) was also easy to perform: the method was sensitive and it detected elevated brucella agglutinins in many people, but it was not specific for determination of activity of infection. The S.A.T. was obviously easier to perform than the 2-ME agglutination, the Coombs agglutination test and the CFT. In addition, the use of International Standard serum and standardised method described by Kerr et al (1968) have increased reproducibility and accuracy of the S.A.T.

The Card test, 2-ME and C.F.T. were useful for the detection of IgG antibodies to brucella organisms and thus for the detection of disease activity. However, the 2-ME was found to be less complex and easily standardized than the C.F.T.

In conclusion, the S.A.T. was found to be the most useful single serological method to screen for brucella infection, and it has therefore been chosen as the primary method for further epidemiological survey in the present project. The other serological tests and cultural method would, however, be reserved for special studies, particularly for the diagnosis of active brucellosis.

The results of the epidemiological study clearly distinguish between people at special risk of infection by nature of their occupation and those that are thought not occupationally exposed to brucella infection. Environmental and occupational factors seem to play a great role in the age and sex prevalence and distribution. The differences in the rate of seropositivity almost certainly reflects the incidence of subclinical brucellosis among the different groups of people included in the survey. The slaughtermen at the abbatoir and the meat shops, and field workers at the cattle control post are specially exposed to risk of infection and they showed a relatively higher level anti-Brucella antibody. That they were infected by constant contact with infected animals or their products has been demonstrated in this study, since if contact with infection is broken off, the humoral antibodies disappear slowly and gradually within one year (Henderson and Hill, 1972). The shortest contact period among the eleven people with high antibody level was two and a half years. The route of infection is usually through

cuts and abrasions on the skin or through the eyes from splashed blood from infected animals, since many slaughtermen and livestock farmers in this country are not protected at work. Aerosol infection through the muccous membrane of the respiratory tract and conjunctivae is also a strong possibility. The three veterinary surgeons were probably infected by contact through the skin since they all carry out surgical and intra-uterine manipulations in recently aborted cows. Vaccination of animals with Br. abortus 519 live vaccine is not presently practised in Ibadan. Most of the dairy farmers and some of the abbatoir workers admitted to drinking raw milk, which was the probable source of their infection.

It is noteworthy that as high as 48.8 per cent of the people considered to belong to the 'healthy' population group had Brucella abortus agglutinins in their sera: children under 10 years were however free from infection in Ibadan. None of them drank untreated milk or engaged in brucella work. Their source of subclinical infection might be from infected roast meat (barbecue) served at parties, a practice now very common among Nigerian elites. However, the possibility of infection through bites by infected mosquito or flies could not be completely excluded in tropical Africa, and this aspect is worth looking into in future.

From the results of this study, brucella antibody titre at 100 I.U. and above could be regarded as the significant level indicative of brucella infection, past, latent or current, among people in Ibadan who are not constantly exposed to infection and therefore an indication for further definitive diagnostic tests. Below this level of antibody titre, there seems to be no significant difference between those constantly

exposed and the rest of the population constituting unexposed control group. The low-grade antibody titres found among the majority of the human population investigated in Ibadan probably relates to the endemicity of bovine brucellosis in southern part of Nigeria (Esuruoso, 1974b). However, it is important to note that when the clinical diagnosis of brucellosis is reasonably certain, antibody titres lower than 400 I.U. might provide supportive evidence of disease in such individuals. On the other hand, high antibody titre may be found without overt symptoms among persons occupationally exposed to repeated infection. For example, none of the patients with antibody titres 400 I.U. and above showed any symptoms or signs of being unwell or suggestive of brucellosis.

Many infected people probably do not develop clinical illness since none of the 1600 people had serological evidence of active brucellosis: the high agglutinins (of mainly IgM type) only pointed either to very recent contact with the causative organism when only IgM was stimulated or latent/spontaneously recovered illness when the level of IgG had fallen beyond detection by agglutination and complement fixation tests (Reddin et al, 1965; Kerr et al, 1966; Wilkinson, 1966; Coghlan and Weir, 1967; Christie, 1974; Elberg, 1973). Since Br. abortus is the most predominant strain encountered in animal and human population in Nigeria, this observation of low level of pathogenicity in the face of high degree of infectivity is completely in agreement with that of Christie (1974), and might therefore partly explain the low index of clinical awareness of this important zoonotic infection in this country. However, whenever facilities for laboratory diagnosis are available, the index of suspicion among practising physicians could be increased when more patients

with P.U.O. and neurological disorders would be investigated for possible brucella infection. This was the observation at the University College Hospital, Ibadan during 1972 and 1973 when increased diagnostic facilities for human brucellosis became generally available to physicians. With increasing index of suspicion in hospital practice, more cases could be detected (Lewis: personal communication) and this could help in finding the correct incidence of human brucellosis in Nigeria.

E: SUMMARY

The findings and observations obtained from the present pilot study have revealed that:

(a) high prevalence of human brucella infection exists in Ibadan, Nigeria, and the sources of infection include direct and indirect transmission from infected animals, particularly cows; significantly higher prevalence of infection occur in occupationally exposed group of the population;

(b) the Saline Brucella agglutination test (S.A.T.) is very sensitive and easy to perform, and it also yields the most standardized results of the serological tests: it is also a simple and inexpensive test and this is very relevant in research work in a developing country like Nigeria where laboratory resources are limited; a titre of 100 I.U. and above in the S.A.T. is regarded as the significant level indication of brucella infection in the community investigated.

(c) the epidemiological pattern of brucella infection in occupationally exposed population requires further and detailed investigations in different parts of Oyo State, and later throughout Nigeria.

TABLE VIII.1

PILOT EPIDEMIOLOGICAL STUDY: Sources of Sera

SOURCES	NUMBER		TOTAL
	Male	Female	
GROUP A:			
1. Pregnant women attending Ante-Natal Clinic, U.C.H.	-	713	713
2. Male Patients attending V.D. Clinic, U.C.H.	163	-	163
3. School Children in Ibadan	63	57	120
4. Blood Donors	316	-	316
GROUP B:			
5. Abbatoir Workers	83	2	85
6. Veterinary Workers	20	2	22
7. Dairy Farm, Ivo Road, Ibadan.	44	-	44
8. Meat Shops in Ibadan	13	6	19
9. Cattle Control Post, Bodija, Ibadan.	18	1	19
10. Research Farm, University of Ibadan.	92	7	99
TOTAL (%)	812 (50.75%)	788 (49.25%)	1,600 (100%)

TABLE VIII.2

SERO-EPIDEMIOLOGICAL EVALUATION OF BRUCELLA ABORTUS ANTIBODY IN IBADAN

SOURCE	TITRE (I.U.)						Total
	Less than 25	25	50	100	200	400 and above	
GROUP A:							
1. Pregnant women attending A.N.C., U.C.H., Ibadan.	297	157	162	75	20	2	713
2. Male patients attending V.D.Clinic, U.C.H., Ibadan.	78	32	38	14	1	0	163
3. School Children	109	9	[illegible]	1	0	0	120
4. Blood Donors	187	61	43	15	7	2	316
TOTAL GROUP A (%)	672 (51.2%)	259 (19.7%)	244 (18.6%)	105 (8.0%)	28 (2.1%)	4 (0.3%)	1,312 (100%)
GROUP B:							
5. Abbatoir Workers in Ibadan	13	16	23	14	12	7	85
6. Veterinary Workers	12	4	4	1	0	1	22
7. Dairy Farm, Iwo Road, Ibadan.	24	9	9	1	1	0	44
8. Meat Shops in Ibadan	9	5	1	2	1	1	19
9. Cattle Control Post, Sodija, Ibadan.	8	2	5	2	1	1	19
10. Research Farm, University of Ibadan.	59	13	15	9	2	1	99
TOTAL GROUP B (%)	125 (43.4%)	49 (17.0%)	57 (19.8%)	29 (10.1%)	17 (5.9%)	11 (3.8%)	288 (100%)
GRAND TOTAL (GROUPS A AND B) (%)	797 (49.8%)	308 (19.3%)	301 (18.8%)	134 (8.4%)	45 (2.8%)	15 (0.9%)	1,600 (100%)

TABLE VIII.3(a)

COMPARISON BETWEEN TITRES OF BLOOD DONORS AND MEN OCCUPATIONALLY EXPOSED TO RISK OF INFECTION

CATEGORY	T I T R E S (I.U.)						
	LESS THAN 25 (%)	25 (%)	50 (%)	100 (%)	200 (%)	400 AND ABOVE (%)	TOTAL
BLOOD DONORS	188 (59.47)	61 (19.3)	43 (13.61)	15 (4.15	7 (2.22)	2 (0.63	316 (100)
MALE AT RISK OF INFECTION (Abbatoir Workers, Veterinary Workers, etc.).	118 (43.7)	45 (16.67)	53 (19.63)	28 (10.37)	15 (5.56)	11 (4.07)	270 (100)

TABLE VIII.3(b)

COMPARISON BETWEEN TITRES OF BLOOD DONORS AND MEN OCCUPATIONALLY EXPOSED TO BRUCELLA INFECTION: TEST OF SIGNIFICANCE

CATEGORY	Titres less than 100 I.U.	Titres at 100 I.U. and above	TOTAL
BLOOD DONORS	292	24	316
SPECIAL RISK GROUP	216	54	270

χ^2 = 19.42

p = Less than 0.001

TABLE VIII.4

INQUIRY ON ELEVEN PEOPLE WITH HIGH ANTIBODY LEVEL

Case No.	SEX	Age (years)	Length of time in contact with cows (years)	Consumption of fresh cows milk	Saline agglutination Titre(I.U.)	2-ME Titre or Card Test or C. F. T.
1.	M	35	12	Yes	More than 400	Negative
2.	M	53	37	Yes	More than 400	Negative
3.	M	40	4	No	400	Negative
4.	M	50	2½	No	More than 400	Negative
5.	M	40	15	No	400	Negative
6.	M	27	7	No	More than 400	Negative
7.	M	45	7	Yes	More than 400	Negative
8.	M	37	8	Yes	400	Negative
9.	M	38	6	Yes	400	Negative
10.	M	28	6	Yes	400	Negative
11.	F	23	4	No	400	Negative

TABLE VIII.5

AGE AND SEX PREVALENCE OF BRUCELLA SERO-REACTIVITY AMONG HEALTHY HUMAN POPULATION IN IBADAN

SEX	Age groups (in years) 6-9 (%)	10-13 (%)	14-17 (%)	18 and over (%)	Total Prevalence of Infection (%)
Male	0/15 (0%)	1/18 (5.6%)	5/30 (16.7%)	213/479 (44.5%)	219/542 (40.2%)
Female	0/14 (0%)	0/15 (0%)	5/28 (17.9%)	416/713 (58.3%)	421/770 (48.8%)
Total Prevalence of Infection (%)	0/29 (0%)	1/33 (3.0%)	10/58 (17.2%)	629/1192 (52.8%)	640/1312 (48.8%)

TABLE VIII.6

PATIENTS WITH A CLINICAL DIAGNOSIS OF P.U.O. INVESTIGATED FOR POSSIBLE BRUCELLA ABORTUS INFECTION AT U.C.H. IBADAN BETWEEN JANUARY, 1970 AND DECEMBER, 1973

YEAR	Total Number of Micro-biological Samples Received	Number of Patients with P.U.O. Investigated	PATIENTS' TITRE (I.U.)					
			Less than 25	25	50	100	200	400 and above
1970	48,274	5	4	-	1	-	-	-
1971	34,859	6	6	-	-	-	-	-
1972	39,472	21	10	1	5	3	2	-
1973	38,451	31	12	5	6	5	1	2
TOTAL	161,056	63	32	6	12	8	3	2

CHAPTER IX

EFFECT OF CHOLERA VACCINATION ON BRUCELLOSIS IMMUNOLOGY IN NIGERIA

1: INTRODUCTION

The production of cross-reactive brucella agglutinins in persons previously infected or recently vaccinated with Vibrio cholerae has been documented by earlier workers (Steele, et al, 1947, Feeley, 1969). Two-and-a-half years before the commencement of the present epidemiological investigation of human (and animal) brucellosis, Nigeria experienced its first-ever recorded cholera epidemic which was caused by Vibrio cholerae biotype el tor (Ogawa serotype). The epidemic in Ibadan, capital of Western State of Nigeria started on 3rd January, 1971, reached its peak about the third week in February, and was virtually at an end by early May, 1971 (Lewis, et al, 1972). Many thousands of people had clinical attacks of cholera during that epidemic. In addition, many Nigerians received cholera vaccination. Although only sporadic cases of cholera were being reported in Ibadan at the time of this investigation, and cholera vaccine was only being given to Nigerians travelling abroad, it was still thought necessary by the author to find out what influence the previous cholera epidemic in Nigeria might have had on the immunological

responses of human brucellosis, since occasional cholera patients who have not received treatment have been found to have high brucella agglutination titres persisting for one or more years (Buchanan, et al, 1974).

B: MATERIALS AND METHODS

Many thousands of Nigerian muslims travel to Saudi Arabia during the yearly pilgrimage to Mecca and Medina. One of the medical requirements for Nigerians at Jeddah Airport in Saudi Arabia is a currently valid cholera vaccination. During the 1975 pilgrimage, a longitudinal survey was planned, in collaboration with medical officer chosen to accompany the pilgrims of Oyo State origin to Saudi Arabia, in which serial blood samples were taken from some intending pilgrims in Ibadan before and after they received cholera vaccination. Only pilgrims who were not occupationally exposed to risk of Brucella abortus infection were included in the study. All vaccinees had the recommended two doses of killed cholera vaccine which consisted of Inaba and Ogawa strains (Batch Numbers 653 p4 Lister Institute and 3L024 BDH Ltd., London).

The sera from these blood samples were tested in a batch for the presence of Br. abortus agglutinins using the standard tube agglutination method described by Kerr et al, (1968).

C: RESULTS

Blood samples were collected from 74 persons before they had cholera vaccination, and in 46 of these (62.2%) was it possible to obtain two more blood samples after cholera vaccination: the first post-immunization sample was collected within 2 to 3 weeks, while the second

post-immunization sample was collected within 8 to 12 weeks of cholera vaccination.

The detailed results of the agglutination tests performed on these sera are shown in Table IX.1. The overall titres of Brucella agglutinins were significantly higher in the immediate post-immunization sera than in the control (pre-immunization) sera ($p = 0.00003$). Though the titres of sera collected after two months of receiving cholera vaccination were still significantly higher than the titres obtained in the initial (pre-immunization) sera ($p = 0.0132$), the level of significance was much lower than in the immediate post-immunization period. In addition, there was a significant fall in the titres of sera obtained after two to three months, compared with sera collected two to three weeks after immunization ($p = 0.00003$), as calculated from the "t" distribution of the paired samples, using the Hewlett - Packard HP-65 programmable calculator (Table IX.1).

D: DISCUSSION AND CONCLUSION

The findings of this study support the earlier view of Eisele et al (1947) that cholera infection or immunization could lead to the production of cross-reacting agglutinins against Brucella organisms. However, this influence on the immunological response of human brucellosis did not seem to last for a long period of time, and in this respect it contrasts the findings of Buchanan, et al (1974) who found that occasional high brucella agglutinin titres might persist for one or more years in some cholera patients who have not received treatment. In the present study, the rate of fall of Brucella agglutinin titres over a

period of two to three months was found to be highly significant and it was presumed that the effect of cholera vaccination on Brucella serology would be completely nullified after six months.

The obvious deduction from this present study is that cross-reacting brucella agglutinins, due to cholera immunization or the disease itself consequent on the cholera epidemic which occurred in Nigeria about two and half years before the present brucellosis epidemiological study in Western Nigeria was begun, could not have had any significant influence whatsoever on the brucella sero-reactivity of the population studied. Similar conclusion was reached by Albert et al (1974) in epidemiological study carried out on the influence of cholera immunization and the disease on the immunological responses of human brucellosis during the last epidemic of cholera in Africa. Therefore, in Africa, cholera does not seem to oppose the sero-immunological diagnosis of human brucellosis.

TABLE IX.1

EFFECT OF CHOLERA VACCINATION
ON BRUCELLA ABORTUS AGGLUTINATION (TITRE IN I.U.)

No.	PRE-VACCINATION SERA	POST-VACCINATION	
	I*(0 DAY)	II*(2-3 WEEKS)	III*(8-12 WEEKS)
1.	100	400	200
2.	100	200	100
3.	100	100	50
4.	50	200	200
5.	50	200	100
6.	50	200	100
7.	50	100	100
8.	50	100	50
9.	50	100	50
10.	50	50	50
11.	50	50	50
12.	50	50	25
13.	50	50	25
14.	25	100	50
15.	25	100	25
16.	25	50	50
17.	25	50	25
18.	25	50	25
19.	25	25	50
20.	25	25	50
21.	25	25	≤ 25
22.	25	25	≤ 25
23.	≤25	100	≤ 25
24.	≤25	100	≤ 25
25.	≤25	50	50
26.	≤25	50	50
27.	≤25	50	≤ 25
28.	≤25	50	≤ 25
29.	≤25	50	≤ 25
30.	≤25	25	25
31.	≤25	25	25
32.	≤25	25	25
33.	≤25	≤25	25
34.	≤25	≤25	25
35-37	≤25	25	≤ 25
38-46	≤25	≤25	≤ 25

*"t" distribution of paired samples:
(a) Comparison between I and II gave p = 0.00003
(b) Comparison between I and III gave p = 0.0132
(c) Comparison between II and III gave p = 0.00013

CHAPTER X

EPIDEMIOLOGICAL INVESTIGATIONS OF BRUCELLA INFECTION IN GOVERNMENT-OWNED SETTLED HERDS IN OYO STATE

Both the Federal and the state governments have shown considerable interest in the improvement and development of livestock industry in Nigeria, and large sums of money are being invested on the project each fiscal year. Because of this, there has been increasing attention paid to the importance of reproductive disease in dairy and beef cattle. Infertility and abortions have been observed among cattle under various management regimes: an earlier systematic investigation of bovine brucellosis in different types of herds in Western State, which included government-owned farms and some nomadic and settled Fulani herds, revealed a high prevalence of the disease (Esuruoso, 1974a, 1974b). Therefore, three government-owned cattle herds were subjected to detailed investigation in order to determine the important factors involved in the transmission of Brucella organisms to man.

A: CATTLE HERDS INVESTIGATED

1. The Upper Ogun Cattle Ranch: This farm is owned and managed by the Oyo State Development Corporation. The cattle ranch is situated on the Upper Ogun Farm Estate near Iseyin, about 142 kilometres northwest of

Ibadan. The estate consists of 10500 hectares of land, of which 1360 hectares are planted pasture, with good grass (Giantstar and elephant grass) mixed with legume centrocema, and 2,730 hectares of cleared bush. There are also 5,600 hectares of bush grazing, and the rest of the estate is covered by a cashew plantation 440 (hectares), buildings (which include staff residential apartments and offices) and irrigation streams.

At the commencement of this investigation (July/August, 1975), there were over 4,000 cattle consisting mainly of N'Dama and also Keteku and N'Dama/Keteku crosses. Most of the N'Dama were originally imported from Guinea, Sierra Leone, and the Congo some 20 years ago. There were a total of 97 permanent workers: consisting of veterinary assistants, tractor drivers, herdsmen, clerical staff, etc. About two-thirds of the workers live on the farm with members of their families. There is no resident Veterinary officer: the farm is under the care of a Veterinary officer based at Fashola Farm 60 kilometres away. There is no school or any form of medical services: these social facilities are available and obtainable privately in Iseyin, a town about 32 kilometres from Upper Ogun. Transport services are very infrequent and it takes over one hour with the local lorry transport to travel to Iseyin on the rough and untarred road.

From the data collected from the administrative office on the farm, between six and eight workers are off sick daily, and the chief complaints are usually fever, backache and malaise. The majority of those that are sick first take to self-medication using the easily available local herbs, then later analgesics (Aspirin, Cafenol, Phensic and APC) and antibiotics (Tetracycline capsules) which are bought in Iseyin. When there is no

improvement, they finally attend the Fatimo Catholic Hospital at Iseyin, and the common diagnoses are malaria and Pyrexia of undetermined origin (P.U.O.).

The cattle in this estate are generally considered healthy and the annual crop of calves is reported to be satisfactory (Esuruoso, 1974a). However, abortions, stillbirths and retained placentae occur occasionally but are seldom reported by the herdsmen. There are also reports of some cows which have been barren for up to four consecutive years. Vaccination against brucellosis has never been carried out on the animals on this farm. Previous investigation carried out in 1972 on 116 cattle in the herd revealed a Brucella abortus sero-positivity rate of 34.5 per cent (Esuruoso, 1974a). The workers on this farm have never been investigated for evidence of brucellosis.

2. Fashola Multiplication Farm: The Farm is now under the management of the Oyo State Ministry of Agriculture and Natural Resources, with a Senior Agricultural Superintendent in charge. The farm is situated in Fashola village, which is 24 kilometres north-west of Oyo and approximately 80 kilometres from Ibadan. The farm covers over 6000 hectares of land and consists of cattle multiplication, piggery and poultry sections. The N'Dama herd at Fashola is one of the earliest to be established in the Oyo State more than 25 years ago. The farm was managed during the early stage with the assistance of the United States Agency for International Development (USAID) until the late 1960's when the Western State government took over the direct control of the farm management.

Vaccination of cattle against brucellosis and other communicable diseases was regularly carried out on this farm until 1964, when the

last bovine brucellosis immunization was undertaken. Previous investigation has revealed a high prevalence rate (60%) of bovine brucella infection in this farm (Esuruoso, 1974a).

During the present investigation (November/December, 1974), there were about 750 cattle and over 300 pigs on the farm. There were 120 permanent workers, engaged in various jobs, including a resident veterinary officer (who also supervises Upper Ogun Cattle Ranch). Less than half of the workers live in the residential quarters on the farm. There is one primary school in the village, but medical and health services are obtainable in Oyo. The daily average number of workers on sick-off is between four and five: the commonest complaints are fever, backache, piles, night sweats and malaise.

3. Dairy Farm, Iwo Road, Ibadan: The farm is situated at 10 kilometre on Ibadan-Iwo road and it is now managed by the Oyo State Ministry of Agriculture and Natural Resources. It covers an area of over 2000 hectares. There were 210 cattle on the farm at the time (June, 1975) the present investigation was carried out. There were 44 workers, engaged in various occupation including milking of cows, milk pasteurization (Flash technique), cattle herding and grazing, slaughtering, veterinary work and clerical duties. None of the workers live on the farm. There is also a veterinary doctor attached to the farm.

The first two farms at Upper Ogun Estate and Fashola are the largest collection of range cattle in Oyo State, and they serve both as Multiplication and distribution centres. Cattle are sold to butchers and individuals for slaughter at abattoirs and private homes respectively. Also cattle from these two farms are distributed widely to individual

last bovine brucellosis immunization was undertaken. Previous investigation has revealed a high prevalence rate (60%) of bovine brucella infection in this farm (Esuruoso, 1974a).

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farmers and cooperative societies wishing to start their herds, or for interbreeding with the local cattle.

The cattle at the Dairy farm, Iwo Road, Ibadan were obtained from Upper Ogun Cattle Ranch and Fashola Multiplication Farm. The Dairy farm is mainly for fresh milk production which is sold to the public, but occasionally cows from this farm are slaughtered for human consumption.

B: MATERIALS AND METHODS

Blood samples were collected from all available workers in the three government-owned settled herds. Also blood samples were obtained (with the help of a veterinary doctor) from as many cows as were available during the periods of investigation in each of the three farms. The sera were separated from the whole blood within 24 hours of collection and kept at -20°C until standard saline and 2-ME agglutination tests were performed on them (Kerr et al, 1968).

C: RESULTS

The results of the detailed human and bovine brucellosis survey carried out between December, 1974 and August, 1975 in the three government-owned farms are shown in Tables X.1, X.2 and X.3.

At the cattle Ranch in Upper Ogun Estate, 23 (42.6%) of the 54 people tested were positive for Brucella antibodies and five (9.3%) persons showed evidence of active human brucellosis. Fifteen (23%) of the 65 cattle tested had high level of Brucella antibodies (100 I.U. and above) in their sera and 17 (26.2%) showed evidence of active bovine brucellosis.

At the Fashola cattle multiplication centre, 103 (99%) of the 104 people tested were positive for Brucella antibodies and 77 (74%) had antibodies titres at 100 I.U. and above. Only one person showed evidence of active brucellosis. Thirty-four (55.8%) of the 61 cattle tested had high level of Brucella antibodies (100 I.U. and above) in their sere and 38 (63%) showed serological evidence of active brucellosis (ie, positive 2-ME test).

In the survey carried out at the Dairy Farm, Iwo Road in Ibadan, only two (4.6%) of the 44 workers had high titre of brucella agglutinins in their sera. The rate of bovine sero-positivity above 100 I.U. was 26.4 per cent. The 2-ME test was negative on all the sera tested from both the human and bovine populations in this farm.

D: DISCUSSION

The results of this study show that the overall human Brucella abortus sero-positivity rate was closely related to the overall bovine infection rate in each of the three farms investigated. Also, the proportion of workers with high and significant Br. abortus antibodies (100 I.U. and above) appeared to be directly related to the level of active infection in the cattle population. Hence at Fashola where 62.3% of the cattle had serological evidence of active infection, 74% of the workers recorded antibody titres 100 I.U. and above. Similarly at Upper Ogun Cattle Ranch where 26% of the cattle population tested had serological evidence of active brucellosis as shown by positive 2-ME test, only 9.3% of the workers recorded significant high antibody titre. In addition, at Iwo Road Farm, where none of the cattle tested showed evidence

of active disease, only 4.6% of the workers had titres at 100 I.U. and above, but none of these workers had antibodies over 200 I.U. It is significant to note that the Dairy Farm is located on the outskirts of Ibadan, where medical facilities are easily obtainable by ill workers.

The prevalence of active infection detectable in the human population in two of the three farms investigated seemed to be related to the number of cattle per human population and also the frequency of contact of the workers to infected animals. At the Upper Ogun Estate where the ratio of cattle to human population was the highest and majority of the workers were living on the farm, probably sharing the same stream with the cattle, 9.4 per cent of the workers tested had serological evidence of active brucellosis, whereas at the Fashola Farm with fewer cattle and less human contact with animals, the active infectivity rate was only one per cent. At the Dairy Farm, Iwo Road, where none of the workers lived on farm, and therefore human contact with infected animals was kept to the minimum, there was no case of active human brucella infection detected serologically.

The high rate of infection with low level of active disease in all the three farms investigated was probably related to the biological behaviour of Br. abortus, which is known to have a high degree of infectivity but low level of pathogenicity in man (Christie, 1974). Consequently the infection remains latent or retrogresses in the majority of people, even among those constantly exposed to infection, as in the group here studied. The majority of the occupationally exposed people with high significant titres probably had subclinical infection. The full clinical syndrome of undulant fever is produced in only a small proportio

of infected persons. The factors determining the development of clinical disease are still undetermined in individual cases.

Protection against many bacterial and viral diseases by naturally acquired active immunity, resulting from subclinical infection, is now well recognised. Subclinical infection giving rise to antibody formation in different groups of people, occupationally or accidentally exposed to Br. abortus, has been reported by several workers in Europe and North America (Dooley, 1932; Cayton, 1967; Foley et al, 1970; Foley and O'Flynn, 1971; Henderson and Hill, 1972). Repeated subclinical infection in the dairy farming community is thought to confer protection against brucellosis, since undulant fever rarely attacks these group of workers with previous demonstrable antibody against Br. abortus (Henderson and Hill, 1972). Similar finding was obtained at the Iwo Road Dairy farm during the present investigation.

The presence of subclinical infection, with varying levels of humoral antibody to Br. abortus among people constantly in contact with infected cows should therefore always be borne in mind by physicians if misdiagnosis of some fatal disease simulating brucellosis is to be avoided. Henderson and Hill (1972) suggested that antibody titre against Brucella abortus in one of the occupational groups or someone with past history of exposure should not be accepted as the only explanation of the patient's condition. This needs to be re-emphasised particularly in tropical areas, where many clinical conditions can simulate acute brucellosis, and at the same time contact with infected animals seems to be widespread.

All the people with serological evidence of active brucella infection detected during this investigation at Upper Ogun Estate and Fashola Multiplication Farm were further subjected to more detailed clinical and laboratory examination, and they were offered treatment: the results of these findings are summarised in chapter XIII of this thesis. The economic and social problems of brucellosis in these farms are also considered in chapter XIV.

E: SUMMARY

The rate and level of human Brucella abortus infection in the three government-owned settled herds investigated in Oyo State depend on many factors, including:

(1) the geographical location of the farm: whether the herd is located in the town with easily available medical facilities for ill workers or the herd is located in a remote farm settlement without any form of medical and other social services;

(2) the number of cattle per human population;

(3) the rate of active infection in the cattle herd;

(4) the level of human exposure and contact with infected animals: the contact rate is much higher in farm settlements where most of the workers are also resident on the farm than in farms where majority of the workers live away from the farm;

(5) imported bovine infection from neighbouring countries: cattle imported from the Republics of Niger, Chad and Guinea were found to show serological evidence of Brucella abortus infection (Esuruoso, 1974).

Finally, the finding of high antibody titre against Br. abortus in people occupationally exposed to infected animals should not always be taken as evidence of disease, since the majority of these people probably have subclinical infection and therefore protection (immunity) against brucellosis. However, all persons with serological evidence of active infection, as revealed by 2-ME test, Card test, or CFT should be further investigated and treated for the disease.

TABLE X.1

HUMAN AND BOVINE BRUCELLOSIS SURVEY:
UPPER OGUN CATTLE RANCH (U.N.D.C.). (JULY/AUGUST 1975)

Titre (I.U.)	H U M A N				C A TT L E			
	Saline Agglutination Test		2-ME Test		Saline Agglutination Test		2-ME Test	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
400 and above	3	(5.5)	2	(3.7)	6	(9.2)	3	(4.6)
200	1	(1.9)	1	(1.9)	4	(6.2)	3	(4.6)
100	1	(1.9)	0	(0.0)	5	(7.7)	3	(4.6)
50	10	(18.5)	1	(1.9)	16	(24.6)	3	(4.6)
25	8	(14.8)	1	(1.9)	14	(21.5)	5	(7.7)
Less than 25	31	(57.4)	49	(90.6)	20	(30.8)	48	(73.9)
Total	54	(100)	54	(100)	65	(100)	65	(100)

TABLE X.2

FASHOLA FARM: HUMAN AND BOVINE BRUCELLOSIS SURVEY
(NOVEMBER/DECEMBER, 1974)

Titre	H U M A N				C A T T L E			
	Saline Agglutination Test		2-ME Test		Saline Agglutination Test		2-ME Test	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
400 and above	10	(9.6)	1	(1)	22	(36.1)	15	(24.6)
200	16	(15.4)	0		5	(8.2)	6	(9.8)
100	51	(49)	0		7	(11.5)	3	(4.9)
50	25	(24)	0		10	(16.4)	3	(4.9)
25	1	(1)	0		8	(13.1)	11	(18.0)
Less than 25	1	(1)	103	(99)	9	(14.7)	33	(37.7)
Total	104	(100)	104	(100)	61	(100)	61	(100)

TABLE X.3

IWO ROAD DAIRY FARM: HUMAN AND BOVINE BRUCELLOSIS SURVEY (JUNE 1975).

Titre	H U M A N		C A T T L E	
	Saline Agglutination Test		Saline Agglutination Test	
	No.	(%)	No.	(%)
200	1	(2.3)	4	(11.7)
100	1	(2.3)	5	(14.7)
50	9	(20.4)	9	(26.5)
25	9	(20.4)	6	(17.7)
Less than 25	24	(54.6)	10	(29.4)
T o t a l	44	(100)	34	(100)

CHAPTER XI

THE INCIDENCE AND SEASONAL PREVALENCE OF HUMAN BRUCELLOSIS AMONG OCCUPATIONALLY EXPOSED PEOPLE IN OYO STATE

A: INTRODUCTION

A high prevalence of subclinical infection with accompanying naturally acquired immunity has been found among different occupational groups involved in the livestock industry in Oyo State of Nigeria. Only a small percentage of the people investigated had serological evidence of active brucellosis. Most of the people investigated had been engaged in cattle farming for varying periods, ranging from six months to over 20 years. It was very difficult to determine the incidence of infection among these farm workers, and therefore the prevalence of brucella infection could only be estimated in all the farms investigated.

In order to be able to determine the incidence of human brucella infection among occupationally exposed individuals in Oyo State, a three-year longitudinal study was carried out at the Institute of Agricultural Research and Training, University of Ife, Moor Plantation, Ibadan, in collaboration with a Veterinary doctor at the Institute.

B: THE INSTITUTE OF AGRICULTURAL RESEARCH AND TRAINING (I.A.R. & T.)

This Institute has a school of Animal Health which runs an in-service training programme, comprising two separate courses leading to the award of either a certificate or a diploma degree in Animal Healt Candidates admitted for the Certificate (Animal Health Assistant) course are fresh from secondary school. Candidates applying for the two-year diploma (Animal Health Superintendent) course must have passed the Animal Health Assistant Certificate Course with at least two years of meritorious livestock field experience.

The School of Animal Health therefore has four sets of Veterinary Students every year, comprising those in the first and second year Certificate (Animal Health Assistant) Course, and those in the first and second year Diploma (Animal Health Superintendent) Course. Each academic year starts in October and ends in June of the following year. Those in the first year Certificate Course are fresh from the secondary school without previous formal Veterinary work and they are expected to have the same Brucella abortus sero-reactivity as the general healthy population, but this also depends on the individual's previous exposure to infected animals in his area of origin. The second year Certificate Students must have had moderate exposure to animals in course of their training, particularly in the large animal veterinary clinic and some of the government-owned settled herds previously described.

All the students in the diploma course should have had a reasonable exposure to animals for at least four years, during their certificate course training and the compulsory minimum two years livestock field

work as Animal Health Assistants.

The other group of people occupationally exposed to risk of infection at the Institute are the workers, including the instructors (Veterinary doctors and other officers), the herdsmen, the slaughtermen and labourers. These workers have been in the employment of the institute for periods ranging from two to twelve years (with an average of four and half years).

During the three-year period of study, the number of cows on the school teaching farm ranged between 97 and 108 (average 105).

C: MATERIALS AND METHODS

Five mls. of blood samples were collected from each of the students in both classes of the Certificate and Diploma courses at the School of Animal Health, Institute of Agricultural Research and Training, Moor plantation, Ibadan. The first blood samples were obtained in December 1973, and subsequently repeated yearly in December 1974, December 1975 and January 1977. Thus, those students in the first year Certificate course in 1973/74 Academic Session (whose blood samples were taken in December 1973) would be in the second year Certificate course when their blood samples were collected in December 1974. Similarly some of the students in the 2nd year Certificate course who passed out in June 1974 would be in first year Diploma course when their blood samples were taken in January 1977, having spent two years between July 1974 and September 1976 as field Veterinary Assistants in different livestock farms in Oyo State. These latter students were the only group that could be followed up during their Certificate course as well as the

Diploma course.

Blood samples were also collected from all the workers and available cows on the school farm during each of four visits made in December 1973, July 1974, December 1974 and July 1975 in order to determine the seasonal prevalence of brucellosis in human and animal populations. The month of December represents the dry season, while July represents the rainy (wet) season in Ibadan.

The sera from the clotted blood samples were tested for the presence of Brucella abortus antibodies using the standard saline agglutination, and 2-ME agglutination on positive SAT sera only.

D: RESULTS

In this three-year longitudinal survey, the serological results for each year of study are shown in Table XI.1. The results for students in the corresponding year of study did not differ from one another, and therefore they were added and considered together for the purpose of statistical analysis (Table XI.2). Higher Br. abortus antibody titres were found among the students in the diploma course who had worked for at least two years on commercial farms than among the certificate course students, most of whom probably had never been in contact with infected animals. This difference among the students in the two separate courses was found to be significant at titres of 100 I.U. and above ($p < 0.001$). However, there was no significant difference ($p > 0.05$) between the titres found among the first and second year of study of corresponding courses showing that the students were probably not exposed to any significant Br. abortus infection during the course of their training at

the I.A.R. & T.

A significant rise in Br. abortus titres was demonstrated in eight of thirteen students in whom follow up was possible between the blood samples taken during their second year certificate course in December 1973 and further blood samples obtained in January 1977 during their first year Diploma course, after they had spent two years in various livestock farms in Oyo State (Table XI.2).

Table XI.3 shows that there was no difference in the seasonal prevalence of brucellosis among the 41 workers and 81 cows tested in I.A.R. & T. school farm in Ibadan during both the dry and rainy seasons.

None of the human and cattle sera tested were positive when the 2-ME method was performed on positive S.A.T. sera. Therefore, no serological evidence of active brucellosis was detected in this teaching farm, even among the diploma students, some of whom showed evidence of recently acquired infection.

E: DISCUSSION

The occupational hazard to which livestock farmers are exposed, in terms of brucella infection, was evidently demonstrated by the results of this longitudinal study. Because of the modern livestock management facilities available at the school training farm coupled with adequate medical attention in Ibadan, the students go through their courses without much exposure to contaminated environment and infected animals. However, when these Animal Health Assistants are posted to government-owned or private herds in the rural areas, where traditional methods of livestock management are widely practised, and without basic medical

facilities and adequate water supply, they sooner or later acquire brucella infection from infected animals as well as from contaminated environment.

Within two years of constant exposure to sources of Br. abortus, many of the students who were seronegative during the certificate course were found on returning for the diploma course to have become seropositive, some of them with titres as high as 400 I.U. Although none of them had symptoms or showed serological evidence of active infection, it might be very difficult to exclude the disease in some of these people, since cultural techniques were not routinely performed on all the people investigated. However, the school authorities decided to offer 'epidemiological treatment' to all students who had a titre of Br. abortus antibody of 100 I.U. and above. Forty persons received the treatment, which consisted of Tetracycline 500 mg qds for two weeks, and they were later followed-up with further serological tests: the results of this treatment would be considered in the chapter which deals with Treatment of Brucellosis.

The prevalence of brucella infection in the I.A.R. & T. farm was studied over two seasons and no seasonal variation, between the dry and rainy (wet) seasons, was found. Though the numbers of people and animals tested were small, this finding might indicate that in settled herds in southern Nigeria, the dry season probably does not impose a natural limit on the rate of brucella infection of cattle and human. In previous national bovine brucellosis surveys, Esuruoso (1974a) found the settled herds in Western State to be more heavily infected than those in nomadic herds of Northern States. [illegible] he gave for the difference

in infection rate among the cattle was that the nomadic herding in the intense savanna heat of the North probably imposes a natural limit to the rate of bovine brucella infection. Climatic variation is probably not as important as the level of active infection in the cattle population: this observation has also been previously noted in earlier section of this thesis (Chapter X). In addition, a greater chance of infection due to closer contact in settled herds than in nomadic herds may explain the higher infection rate.

F: SUMMARY

The present three-year longitudinal study has clearly demonstrated the advantages of modern and scientific livestock management over the traditional and extensive methods of animal husbandry in the prevention and control of bovine and human Brucella abortus infection. The occupational hazard, in relation to brucellosis, to which livestock farmers are exposed in Oyo State is closely related to the level of infection in the animal population, the level of environmental contamination and hygiene on the farm. This study has also revealed that many farmers could acquire subclinical brucellosis within two years of occupational constant exposure to source of infection.

There was no seasonal variation found in the prevalence of brucella infection among the human and cattle population studied at the I.A.R. & T. farm in Ibadan: climatic variation is therefore considered to play no important role in the prevalence of brucellosis in Southern Nigeria.

TABLE I.1

SERO-EPIDEMIOLOGICAL OBSERVATIONS AMONG STUDENTS AT I A R & T, IBADAN OVER A 3-YEAR PERIOD

SALINE AGGLUTINATION Titre I.U.	December '73 1973/74 Academic Year				December '74 1974/75 Academic Year				December '75 1975/76 Academic Year				December '76 1976/77 Academic Year			
	Cert. Course		Diploma Course		Cert. Course		Diploma Course		Cert. Course		Diploma Course		Cert. Course		Diploma Course	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
400	0	13	1	0	0	0	1	2	0	0	0	1	0	0	2	1
200	0	0	0	0	0	0	2	1	0	0	1	1	1	1	1	1
100	0	0	1	0	0	0	2	1	1	1	2	3	0	1	3	2
50	2	2	1	1	2	2	2	1	3	1	3	2	1	2	3	4
25	2	2	0	1	0	2	2	1	4	1	2	1	4	3	4	1
Less than 25	15	25	14	7	18	14	9	11	16	14	6	8	12	13	12	7
TOTAL TESTED	19	29	17	12	20	18	18	17	24	17	14	16	18	20	25	16
STUDENTS' CODE (Letters refer to key below)	A	B	C	D	E	A	F	C	G	E	H	F	G	I	B	H

KEY TO STUDENTS' CODE:-

A = 1st year Certificate course 1973/74 session, and 2nd year Certificate course 1974/75 session.
B = 2nd year Certificate course 1973/74 session, and 1st year Diploma course 1976/77 session.
C = 1st year Diploma course 1973/74 session, and 2nd year Diploma course, 1974/75 session.
D = 2nd year Diploma course 1973/74 session.
E = 1st year certificate course 1974/75 session, and 2nd year Certificate course 1975/76 session.
F = 1st year Diploma course 1974/75 session, and 2nd year Diploma course 1975/76 session.
G = 1st year Certificate course 1975/76 session, and 2nd year Certificate course 1976/77 session.
H = 1st year Diploma course 1975/76 session, and 2nd year Diploma course 1976/77 session.
I = 1st year Certificate course 1976/77 session.

TABLE XI.2

I A R & T LONGITUDINAL SURVEY: TOTAL BLOOD SAMPLES TESTED

SALINE AGGLUTINATION Titre (I.U.)	STUDENTS (Dec. 1973 - Jan. 1977)				S.A.T. in 13 Follow-up Students	
	CERTIFICATE COURSE		DIPLOMA COURSE		1st sample during 2nd Yr. Cert. course (Dec. 1973)	2nd sample during 1st Yr. Dip. course (Jan. 1977)
	1st Yr. No.	2nd Yr. No.	1st Yr. No.	2nd Yr. No.	No.	No.
400 and above	0	0	4	4	0	2
200	1	1	4	3	0	0
100	1	2	8	9	0	2
50	8	7	9	8	1	2
25	10	8	8	4	0	2
Less than 25	61	66	41	33	12	5
TOTAL	81	84	74	61	13	13

TABLE VI.3

I A R A T SURVEY

SEROLOGICAL PREVALENCE OF BRUCELLOSIS IN I A R A T, IBADAN

SALINE AGGLUTINATION Titre (I.U.)	Dry season (Dec. 1973/Dec. 1974)		Rainy season (July 1974/July 1975)		Total Prevalence	
	Human No.	Cattle No.	Human No.	Cattle No.	Human No.	Cattle No.
400 and above	0	1	0	1	0	2
200	0	4	1	5	1	10
100	1	6	1	7	2	13
50	5	8	3	12	8	21
25	7	8	5	8	12	11
Less than 25	8	13	10	9	18	24
Total Tested	21	39	20	42	41	81

CHAPTER XII

EPIDEMIC INVESTIGATION OF BRUCELLOSIS IN IGBO-ORA

A: INTRODUCTION AND LIVESTOCK ACTIVITIES IN IBARAPA DIVISION

Igbo-ora, a rapidly developing town in Ibarapa division of Oyo State, lies about 100 kilometres south-west of Ibadan. The town has a Rural Health Centre (R.H.C.) which is jointly run by the government of Oyo State and the University of Ibadan Medical School, and is popularly known as the Ibarapa Community Health Project. The population of Ibarapa Division is approximately 250,000; Igbo-ora alone has a population of over 25,000.

The vegetation in Ibarapa division is savanna, with many shrubs and good plant grass, suitable for livestock farming. There are over 20 pri- semi-nomadic and settled-cattle herds in Ibarapa division, each herd consisting of between 150 and 300 cows. Most of these herds belong to the Fulanis who herd down their cows from the northern states of the country. There is a veterinary office in Igbo-ora which serves the whol of Ibarapa division. The staff of this office consists of a qualified veterinary doctor, one Animal Health superintendent, two Animal Health Assistants and four veterinary attendants/labourers. The veterinary

services available to livestock farmers include: (a) advice on sanitation on the farm; (b) teaching the traditional herdsmen how to apply modern scientific methods to livestock management; (c) diagnostic and therapeutic services for animal diseases; (d) investigation and control of epidemics in the animal population. (The supervision of abattoirs is under the control of the public health superintendent at the Rural Health Centre). The veterinary services are considerably limited by inadequate facilities and resources: for example there is no equipped laboratory to carry out diagnostic work, hence help is sought from the Vet. Clinic in Ibadan; there are frequently inadequate supplies of essential drugs and vaccines; and there is no suitable transportation system to encourage the veterinary workers to visit the individual herds regularly.

There was no known available data in the Rural Health Centre at Igbo-ora suggestive of previous brucella infection in Ibarapa division. However, an epidemiological survey on the prevalence of human brucella sero-reactivity carried out on 51 Igbo ora butchers in June 1974 revealed that fifteen (29.5%) were sero-positive, but only eight (15.6%) had high titres of 100 I.U. and above. None of the butchers had serological evidence of active brucellosis. Two years after this investigation, Igbo-ora experienced an acute shortage of cows for slaughter at the abattoirs, and meat, which was usually available at reasonable cost to the people, became an expensive commodity. Investigations jointly carried out by the staff of the public health and veterinary offices in the town found that the Fulani herdsmen were refusing to sell out as many of their cows as before to the butchers. Further, it was later revealed that the herdsmen in Ibarapa divisio[illegible] staff and many losses from recurrent

bovine abortions and infertility in the recent past (Abass and Adeoye, personal communications).

The Fulani nomads, who own over 90 per cent of the total cattle population in Ibarapa division and manage their herds in the traditional method, willingly sell cows to butchers for slaughter at abattoirs or to private individuals only in one of two circumstances: (i) whenever they are in need of money for an urgent expenditure, for example, marriage or during the muslim pilgrimage to Saudi Arabia; and (ii) when there is a high and favourable calf crop : many cows would then be sold at cheap rates to butchers and this would reflect in cheap cow meat available to the consuming public. Therefore when the calf crops became depleted and unfavourable, the Fulani herdsmen held to their remaining cattle stock, because Fulanis count their wealth primarily in terms of the total number of cows they have. He would sell occasionally to maintain himself and his family. During the present investigation, some herdsmen also complained of being unwell and unable to look after their cattle properly.

The socio-economic significance of the situation at Igbo-ora required an urgent investigation and practical solution, and in collaboration with the veterinary doctors at Igbo-ora and Mokola, Ibadan, three randomly selected herds, all around Igbo-ora, were screened for Br. abortus infection, as the possible cause of the large-scale abortion and infertility among the cattle population in Ibarapa division. The results of this investigation, which revealed an epidemic of active bovine brucellosis, with human involvement, in Igbo-ora, are reported and discussed in this chapter.

B: MATERIALS AND METHODS

Blood samples were collected from 40 randomly selected (out of approximately 500) cows in the three herds chosen for investigation (stratified sampling method was employed).

Pooled milk specimens, from each of the three herds tested, were collected into sterile containers.

Additional blood samples were taken from twelve Fulani herdsmen who owned the cows: some of them had symptoms suggestive of brucellosis.

The sera obtained from the bovine and human blood specimens were tested for the presence of IgM and IgG types of Brucella abortus agglutinins by the tube agglutination method, using phenol-saline and 2-mercaptoethanol as diluents. In addition, the card test was performed on all the blood samples. The results of the tube agglutination tests were expressed in international units (I.U.), while the results of the card test were recorded as either negative or positive.

The three samples of pooled milk were tested for the presence of Br. abortus agglutinins by the milk ring test (MRT).

C: RESULTS

All the 40 (100%) cows examined had serological evidence of active brucellosis: both the card test and the 2-ME test were positive in all of them (Table XII.1(A)). Of the twelve herdsmen examined, six (50 per cent had significant titres of 100 I.U. or greater and all these six people had serological evidence of active brucellosis as revealed by positive 2-ME and Card tests (Tables XII.1, A and B). One person with a low SAT

titre of 25 I.U. (and negative 2-ME test) had a positive card test. Nine (75%) of the twelve herdsmen tested gave symptoms suggestive of acute brucellosis, and the card test was positive in seven (77.8%) of them. Those people with serological evidence of active infection were invited to the R.H.C., Igbo-ora for proper examination and further investigation, but they all refused to come. They were therefore offered free treatment, consisting of tetracycline 500 mg 6 hourly for three weeks and multivite tablets, one thrice daily, as a supplement. When the Card test was repeated two weeks after the completion of treatment, three (42.8%) out of the seven with previous positive card test still had serological evidence of active brucellosis, and therefore a repeat course of treatment was given to them.

There was close correlation between tube agglutination and card tests. All the cattle with positive SAT and 2-ME tests were also card test positive. Similarly, all the herdsmen with significant SAT had positive 2-ME and card tests. Only one sample with less than 100 I.U. SAT was card test positive: this sample was however 2-ME negative.

The three pooled milk samples were positive by the Brucella abortus milk ring test.

D: DISCUSSION

The circumstances which led to the present investigation and the eventual discovery of a large-scale brucellosis epidemic in the cattle population around Igbo-ora were very informative and instructive about the hazards caused by this zoonosis in Nigeria. Under normal conditions, the Fulani nomads usually resist attempts to investigate their herds

especially when it involves the collection of blood samples. Therefore previous epidemiological studies on the prevalence of brucellosis in Fulani-owned herds in the country had to be carried out on slaughter surveillance at abattoirs (Esuruoso, 1974b). However, because of prevailing economic hardship they were experiencing due to recurrent abortions and infertility among their cattle population, and also ill-health among themselves, these Fulani nomads cooperated with the present investigators. They obviously wanted something done quickly to arrest the worsening situation in their herds. Similarly, the Ibarapa Community wanted an urgent solution to the soaring cost and unavailability of cow meat in the markets. The investigation was therefore timely and served as a big relief to all concerned.

The laboratory methods chosen for this investigation were simple, inexpensive and accurate. There was close correlation between the results of the tube agglutination (SAT and 2-ME) and card tests. All the cattle and more than 50 per cent of the people investigated had active brucellosis, and their sera contained antibodies of the IgG type. The 2-ME test and the buffered acid antigen of the card test are designed to detect these: the results of this investigation has further strengthened earlier findings of others (Nicoletti and Fadai-Ghotbi, 1971; Morgan, et al, 1969) that the brucellosis card test is an efficient aid to diagnosis of acute human infections.

E: CONTROL MEASURES

The very high prevalence of active brucellosis (100%) which was discovered in the three herds investigated called for a concerted effort on the best practicable solution to deal with the ravaging bovine epidemi

particularly when concomitant active human disease was also present. In addition, the socio-economic effect of the epidemic was beginning to be felt by the community, which was experiencing acute shortage of 'staple' cow meat in the markets, and this could worsen the existing protein malnutrition in this area. The human population with evidence of active infection were treated with standard antibiotic therapy (Williams, 1973). Slaughter technique, as a method of dealing with bovine brucellosis in developed countries could not be suggested in this situation because of the poor state of our livestock industry and lack of compensation to farmers by the government. Also, the Fulanis would vigorously oppose this type of control measure. Since probably all the cattle were affected in these herds, sanitary control of livestock grazing was suggested and put into practice, and new animals were prohibited from entering into the infected herds: routine vaccination against bovine brucellosis is not practised in Oyo State. The Veterinary division of the Oyo State Ministry of Agriculture and Natural Resources, Ibadan, was informed about this devastating disease in the areas investigated. The Ministry promised to look into it and in addition planned to embark on Br. abortus -S19 immunisation programme in the immediate future. Also, places to be selected for the establishment of new livestock farms in the areas would have to be based on proper sanitary advice.

The overall active infection rate among the occupationally exposed Fulani on these farms depended mainly on socio-economic factors which effected the degree of contact between these people and their cows with active disease. Since brucellosis is not generally transmitted from person to person the prevention of human infection depends mainly upon the

control and elimination of this disease in animals (WHO, 1971). But since the complete eradication of infection in animals was not feasible in the herds investigated here, some practicable preventive measures were suggested to prevent further infection among the human population occupationally exposed: (a) hygiene and improvement in the working conditions on the farm to limit contact to a minimum; (b) early diagnosis of active disease in occupationally exposed people; (c) avoidance of drinking raw milk, until regular screening services could be provided; (d) Bovine Vaccination programme, in collaboration with other states of the country because of the continuous free inter-state movements of cattle in Nigeria.

F: BRUCELLOSIS SURVEY IN OYO NORTH

Following the successful investigation of the causes of epidemic abortion and infertility among the cattle population around Igbo-ora, many farmers became aware of the importance of brucellosis in the livestock economy. There were therefore several requests from many cooperative societies and individuals for screening tests on their herds. Such epidemiological surveys, employing cluster sampling technique, were conducted on four different sized herds in various geographical areas of the Oyo north division of the state between September, 1976 and February, 1977. The surveys were carried out in collaboration with the staff of the veterinary centre, Mokola, Ibadan. Since we were only interested in detecting herds with active bovine brucellosis, the card test was used throughout the investigation. The farm workers were not examined during these investigations. The results are summarized in Table XII.2. The active infection rate ranged between 4 and 21 per cent. There was a

correlation between the infection rate and the level of abortion in the four herds investigated: Lanihuns Livestock had not been performing very well in terms of calf crops during the previous two seasons (Awoseyi: personal communication). Similar serological surveys have now been planned for many more herds in the state by the Ministry of Agriculture and Natural Resources (Veterinary Section) before the government embarks on control programme throughout the state: the Federal government has already set up a committee to look into the socio-economic importance of brucellosis in Nigeria (Falade: personal communication).

G: RECOMMENDATIONS

The first essential step in brucellosis control programme in Oyo State is therefore the provision of adequate facilities for early diagnosis of the infection. These laboratories would provide, among other things, the essential diagnostic services on the farm, regular screening of fresh milk and farm workers. The present epidemic might probably have been detected earlier if there was a laboratory service at the veterinary office, Igbo-ora. The high rate of human and animal infections in different parts of Oyo State require that full cooperation must be established between the medical and veterinary professions for a realistic approach to the control of this important zoonosis. This cooperative approach had made the present investigation a possibility. For example, abattoir surveillance is best performed by the veterinary public health department, under the direction of a veterinarian with good laboratory support. In recent years, the role of the veterinarian in public health has become prominent, not only due to his knowledge of the zoonoses, but

also because of his extensive experience in "herd medicine" (WHO, 1976). The present status of such diseases as brucellosis and tuberculosis can be determined by well organized abattoir surveys. The animal health assistant is frequently, because of his close association with the farmer, the first person to suspect an outbreak of any zoonosis and is able to feed back information to the human and animal health authorities, so that adequate steps may be taken to trace human exposure and to prevent spread among animal population. In this respect, a two-way notification system for reporting zoonoses would probably be a practical step in the right direction, as suggested by McDiarmid (1973).

TABLE XII.1

BRUCELLOSIS OUTBREAK IN IGBO-ORA (MARCH 1976)

A. TUBE AGGLUTINATION TEST

Titre i.u.	Human Sera				Cattle Sera			
	Saline Test		2-ME Test		Saline Test		2-ME Test	
	No.	%	No.	%	No.	%	No.	%
1,600 and above	0	0	0	0	20	50	20	50
800	2	16.7	0	0	6	15	6	15
400	2	16.7	1	8.3	2	5	2	5
200	2	16.7	2	16.7	4	10	2	5
100	0	0	1	8.3	4	10	2	5
50	0	0	2	16.7	2	5	6	15
25	4	33.3	0	0	2	5	2	5
less than 25	2	16.7	6	50.0	0	0	0	0
Total	12	100	12	100	40	100	40	100

B. CARD TEST

	Human Blood		Cattle Blood
	Before Treatment	After Treatment	
Total number Tested	12	7	40
Number Positive	7	3	40
Per cent Positive	58.3%	42.8% (Or 25% of all the 12 tested).	100%

TABLE XII.2

BRUCELLOSIS SURVEY IN OYO NORTH (Card Test)

Date	Herd Tested	Total Tested	No. Positive	% Positive
22/9/76	Shaki Stock Farm	52	2	3.8%
22/9/76	Lanihuna Farm	33	7	21.2%
11/1/77	Igbeti Nomadics	201	28	13.9%
6/2/77	Oke-ho Nomadics	189	21	11.1%

CHAPTER XIII

CLINICAL ASPECTS OF HUMAN BRUCELLOSIS IN OYO STATE

A: INTRODUCTION

In studying occupational diseases which affect mainly people on farm settlements in remote rural areas, it is often difficult to conduct a detailed follow-up clinical investigation. The problems encountered are even greater in the developing countries where the rural areas are neglected and left without good communications. Many people have to travel long distances on foot or bicycle in order to obtain some form of medical service. Therefore it is almost impossible to request farm workers who are found to be suffering from an illness during epidemiological surveys to report for further investigations and treatment in a far-away hospital. Those of them who have private herds are often reluctant to stay away from their farms. In government-owned farms, some farm workers are employed on daily paid basis and they will therefore lose their pay if they are absent from the farm. Hence, further clinical studies on farm workers are probably best carried out in the respective farms. This will however impose certain limitations on full clinical examinations and other laboratory investigations.

B: MATERIALS AND METHODS

(i) Selection of Patients: Within these unavoidable limitations resulting from the level of socio-economic development of the area studied, the following groups of people were selected for clinical investigations:

(a) thirteen persons found during epidemiological investigations with serological evidence of active brucella infection;

(b) twenty-five persons found with anti-Brucella agglutinins of 200 I.U. and above at Fashola Cattle Multiplication Farm;

(c) the forty persons with a titre of 100 I.U. and above who were offered treatment by the authorities of I.A.R. & T., Moor Plantation, Ibadan;

(d) sixty-one patients admitted to the University College Hospital, Ibadan with a provisional clinical diagnosis of Pyrexia of undetermined origin (P.U.O.) or Septicaemia between January and June, 1976;

(e) twenty-five patients attending the Neurology Clinic, U.C.H., Ibadan between April and June, 1976;

(f) twenty-three patients with clinical diagnosis of hepatosplenomegaly and those with immunoproliferative diseases (e.g., Leukaemia, Hodgkin's disease, etc.) seen at the U.C.H., Ibadan between January and June, 1976;

(g) pregnant women with P.U.O. associated abortion seen in U.C.H., Ibadan between June and September, 1975.

(ii) Methods of Study: The selected farm workers at Upper Ogun and Fashola Farm were seen on the farm and they were interviewed individually in the Yoruba or Hausa language (with the help of an interpreter) for

common symptoms suggestive of brucellosis. The information collected was recorded on a standard form (App. II). (Suggestive questioning was avoided as much as possible during the interview). Only physical examination could be done on a table provided by the farm supervisors. Apart from other supportive therapy, the specific antibiotic treatment offered to these workers consisted of either tetracycline (500 mg 6 hourly for two weeks for those without serological evidence of active disease and for three weeks for those with positive 2-ME or Card tests) or Vibramycin (100 mg twice daily for two weeks). Repeat serological examination was performed on those treated two to four weeks later. Two criteria were employed in judging the effectiveness of antimicrobial therapy: (a) symptomatic improvement, and (b) either negative 2-ME or Card test in cases of previous IgG-type agglutinins or a four-fold (significant) reduction in titre in cases of previous IgM-type agglutinins.

The 40 asymptomatic persons treated with tetracycline capsules (500 mg 6 hourly for two weeks) at I.A.R. & T., on the basis of high anti-Brucella agglutinins had a repeat serological test three to four weeks after completion of antibiotic therapy. None of this group of people had symptoms suggestive of Brucella infection, and therefore no medical examination was performed on them.

In collaboration with many physicians at the University College Hospital, Ibadan, blood samples were collected from different groups of adult patients with provisional diagnoses of P.U.O., Septicaemia, Hepatosplenomegaly, Leukaemia, Hodgkin's disease, and neurological disorders: the sera from these patients were screened for anti-Brucella agglutinins. In addition blood cultures, for pathogens and in

particular for Brucella abortus isolation, were done on some patients with clinical diagnoses of P.U.O. or Septicaemia, and a few neurological patients. Each culture consisted of 5 ml of aseptically taken venous blood inoculated into each of three bottles, one containing 70 ml of glucose broth, the second containing 80 ml of thioglycollate broth for the detection of anaerobic organisms, and the third bottle containing 50 ml of trypticase soy broth with added 10 per cent carbon dioxide for the detection of Brucella organisms. Cultures were incubated at 37°C for seven days, with subcultures made either when growth was apparent in the original culture bottles or routinely at the end of the week's incubation time. Subcultures from the first two bottles were made to Blood Agar and MacConkey media: the Blood Agar sub-cultures were incubated both aerobically and anaerobically, using Gaspak anaerobic jars and an atmosphere of hydrogen with 10 per cent CO_2 provided by the Gaspak generators. Sub-culture from the trypticase soy broth bottle was made onto tryptose agar and the plates were examined after three days of aerobic incubation for Brucella colonies. All isolates were identified by standard bacteriological methods (Cowan 1974).

Between June and September 1975, a prospective study was carried out, in collaboration with one obstetrician at the University College Hospital, Ibadan, to find out the association between brucella infection and human abortion (particularly in patients with P.U.O.). All selected cases had blood films for malarial parasites, P.C.V., W.B.C. and differential counts, Brucella serological tests by Card test (and tube agglutination in some cases), Widal agglutination test for typhoid fever, and culture of blood, urine, vaginal discharge, foetal products and

particular for Brucella abortus isolation, were done on some patients with clinical diagnoses of P.U.O. or Septicaemia, and a few neurological patients. Each culture consisted of 5 ml of aseptically taken venous blood inoculated into each of three bottles, one containing 70 ml of glucose broth, the second containing 80 ml of thioglycollate broth for the detection of anaerobic organisms, and the third bottle containing 50 ml of trypticase soy broth with added 10 per cent carbon dioxide for the detection of Brucella organisms. Cultures were incubated at 37°C for seven days, with subcultures made either when growth was apparent in the original culture bottles or routinely at the end of the week's incubation time. Subcultures from the first two bottles were made to Blood Agar and MacConkey media: the Blood Agar sub-cultures were incubated both aerobically and anaerobically, using Gaspak anaerobic jars and an atmosphere of hydrogen with 10 per cent CO_2 provided by the Gaspak generators. Sub-culture from the trypticase soy broth bottle was made onto tryptose agar and the plates were examined after three days of aerobic incubation for Brucella colonies. All isolates were identified by standard bacteriological methods (Cowan 1974).

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placenta for Brucella organisms. Also, some pregnant women having fever, but without abortion, were investigated along similar line, as controls.

C: RESULTS

(i) Clinical Features:

There was a total of 13 farm workers who were detected on the basis of positive 2-ME and Card tests as suffering from active brucellosis: five persons in Upper Ogun Estate, one person in Fashola Farm, and seven nomadic herdsmen at Igbo-ora. In addition, detailed clinical data were obtained from 25 other farm workers, who had high anti-Brucella agglutinins of 200 I.U. and above, at Fashola Farm. The common symptoms in these two groups of serological positive workers are listed in Table XIII.1. The duration of symptoms varied from one week to six months. Fever and pain were the two leading symptoms.

The 13 persons with serological evidence of active brucellosis were males aged between 25 years and 55 years. They had been involved in animal husbandry for periods ranging between six and eighteen years (average: eleven years). They all agreed to taking raw milk for many years. Ten of them were married with children, the remaining three were below 30 years of age and unmarried. Nine of the 13 persons were herdsmen (including the seven at Igbo-ora), three were veterinary birth attendants and the remaining one person was the Veterinary Superintendent in charge of Upper Ogun Farm. Only four of the eleven people with backache came to U.C.H., Ibadan for X-ray examination, and one was found to have severe cervical spondylotic changes at C5/6 level. All the seven herdsmen in Igbo-ora refused to attend the hospital for further investigation.

placenta for Brucella organisms. Also, some pregnant women having fever, but without abortion, were investigated along similar line, as controls.

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(i) Clinical Features:

There was a total of 13 farm workers who were detected on the basis of positive 2-ME and Card tests as suffering from active brucellosis: five persons in Upper Ogun Estate, one person in Fashola Farm, and seven nomadic herdsmen at Igbo-ora. In addition, detailed clinical data were obtained from 25 other farm workers, who had high anti-Brucella agglutinins of 200 I.U. and above, at Fashola Farm. The common symptoms in these two groups of serological positive workers are listed in Table XIII.1. The duration of symptoms varied from one week to six months. Fever and pain were the two leading symptoms.

The 13 persons with serological evidence of active brucellosis were males aged between 25 years and 55 years. They had been involved in animal husbandry for periods ranging between six and eighteen years (average: eleven years). They all agreed to taking raw milk for many years. Ten of them were married with children, the remaining three were below 30 years of age and unmarried. Nine of the 13 persons were herdsmen (including the seven at Igbo-ora), three were veterinary birth attendants and the remaining one person was the Veterinary Superintendent in charge of Upper Ogun Farm. Only four of the eleven people with backache came to U.C.H., Ibadan for X-ray examination, and one was found to have severe cervical spondylotic changes at C5/6 level. All the seven herdsmen in Igbo-ora refused to attend the hospital for further investigation.

Blood culture for Brucella abortus organism which was attempted on all the 13 people was negative. None of the patients were ill enough to require admission into hospital.

The other twenty-five workers at Fashola Farm who were investigated only on evidence of high anti-Brucella IgM-type agglutinins consisted of twenty-two males and three females, aged between 28 years and 50 years. Seventeen were permanent staff, and the remaining eight were employed seasonally on daily-paid basis. All the 25 workers (including the seasonal workers) had been involved in animal husbandry on the farm for periods ranging between seven and fifteen years (average twelve years). The three women among this group had children and none of them gave a history of previous abortion (miscarriage). Ten of the permanent staff on this farm were invited to U.C.H., Ibadan for further investigations consisting of P.C.V., W.B.C. and differentials, blood film for malarial parasites, blood culture for Brucella organisms, and X-ray examination for those with backache. In all the ten people investigated, apart from the high SAT titre, and in one patient with S. haematobium in his urine, no further abnormality was detected in the laboratory investigations. However, three of the ten people had significant inguinal lymphadenopathy and two had splenomegaly (4.c.m. and 6 c.m. enlargement). No radiological abnormalities were found. All those that attended the hospital were seen as outpatients, and none looked ill enough to warrant admission.

Patients with P.U.O. and Septicaemia (January to June, 1976)

During the six month period, January to June, 1976, 1820 blood cultures from 1571 patients were received in the laboratory, including samples from 61 patients with an initial diagnosis of Pyrexia of undetermine

Blood culture for Brucella abortus organism which was attempted on all the 13 people was negative. None of the patients were ill enough to require admission into hospital.

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During the six month period, January to June, 1976, 1820 blood cultures from 1571 patients were received in the laboratory, including samples from 61 patients with an initial diagnosis of Pyrexia of undetermined

origin. They were made up of 32 male and 29 female adult patients (ie, 15 years and above). In 21 (34.4%) of the 61 patients, the causes of the P.U.O. were due to bacterial septicaemia. Salmonellae were isolated from the blood cultures from ten of these patients, with Klebsiella spp. and Staphylococcus aureus being isolated from four other patients each. Proteus spp., Esch. coli, and Ps. aeruginosa accounted for the remaining three patients. Brucella organism was not isolated from any of the 61 patients.

The result of the serological tests on the 61 patients with P.U.O./ Septicaemia is shown in Table XIII.2. Though 14 (23%) had high anti-Brucella agglutinin of 100 I.U. and above, all but one of the sera tested were negative with either the 2-ME or Card test or CFT, showing that Brucella infection was probably the cause of P.U.O. in only one patient. All the 21 patients with confirmed bacterial septicaemia had agglutinin levels below 100 I.U.

Neurological Patients (April - June, 1976)

The details of the clinical features and results on the 25 patients attending the Neurological Clinic at the University College Hospital, Ibadan, who were screened for Brucella infection, are summarised in Table XIII.3. All the patients were seen and assessed by one consultant Neurologist. Blood cultures performed on 16 of the 25 patients were negative: these included all the five patients with positive 2-ME test.

Fourteen (56%) of the patients screened had high level of anti-Brucella abortus agglutinins of 100 I.U. and above, and five (20%) of them had serological evidence of active Brucella infection as revealed by the 2-ME agglutination test. Four of these with IgG-type agglutinins

were engaged in occupations which brought them in contact with animals: constant occupational exposure to infected animals might possibly be responsible for their seroreactivity.

Brucella Sero-reactivity Among Patients with Immunoproliferative Disorders

Blood samples for Brucella agglutination test were obtained from only 23 patients with clinical diagnosis of either Leukaemia (10) or Hodgkin's Disease(4) or Hepatosplenomegaly, including cases of Idiopathic Tropical Splenomegaly Syndrome(9). The clinical diagnoses were made by a consultant clinical Haematologist, who also collected about 5 ml of blood sample from each patient before treatment.

Only three (or 13.0%) of the 23 patients had agglutinin titres of 100 I.U. and above and they were all 2-ME test negative (Table XIII.4).

Brucellosis and P.U.O.-associated Human Abortion

Throughout the three month period (June to September 1975), when this study was undertaken, only two patients with P.U.O. and abortion were seen and investigated. In these two cases no cause was detected for the abortions which were regarded as spontaneous. The S.A.T. was less than 25 I.U., and the 2-ME and Card tests were negative in both patients.

(ii) Treatment Results

All the thirteen patients with serological evidence of active brucellosis were treated free of charge on the farm with tetracycline capsules (500 mg 6 hourly for three weeks) together with multivite tablets as supplements and other symptomatic supportive therapy. The detailed serological results, before and after treatment, are shown in Table XIII.5.

Four of the five patients treated at Upper Ogun Farm Estate responded satisfactorily to antibiotic chemotherapy and the 2-ME test was negative after treatment, though high titre IgM-type agglutinins could still be detected in the sera of two of them. The only patient who did not respond to the three-week course of tetracycline capsules at the Upper Ogun Estate had severe cervical spondylosis, and he was referred to the orthopaedic surgical unit for further management.

The only patient treated for active disease at Fashola Cattle Multiplication Farm responded satisfactorily to antibiotic chemotherapy. Four of the seven Fulani herdsmen with active brucellosis showed satisfactory response to the first course of antibiotic treatment. However, these Fulanis did not cooperate fully during the clinical studies because they were more concerned about the fate of their cattle, and they could not understand why they were being treated instead of their cattle. Apparently they were ignorant of the possible transmission of animal disease to human beings.

The 25 workers at Fashola Farm with high IgM-type antibody were divided randomly into two groups: one group consisting of 13 workers (including two women) were treated with vibramycin capsules (100 mg twice daily for two weeks) and the other group consisting of 12 workers (including one woman) were treated with tetracycline capsules (500 mg 6 hourly for two weeks). All the patients were also given supportive symptomatic treatment as indicated, including multivite supplements. The twelve patients on tetracycline completed their two-week course of treatment. On the other hand, only nine of the 13 patients having vibramycin completed the two-week treatment: the remaining four patients discontinued

with treatment because of untoward side effects of high dosage of ibromycin, mainly nausea, loss of appetite and vomiting. However, all twenty-one patients who completed the treatment showed symptomatic (clinical) improvement, but without any significant fall in Brucella abortus antibody titres. These four patients who failed to complete their course of treatment did not show any clinical improvement and the antibody titres in three of them did not change: in one of them, the antibody titre rose from 200 I.U. to 800 I.U. within a period of five weeks.

The 40 students at the I.A.R. & T. at Moor plantation in Ibadan who were treated with tetracycline capsules did not show any significant fall in their Brucella abortus antibody levels when re-examined after the completion of chemotherapy.

D: DISCUSSION

Generally follow-up of patients in tropical countries is notoriously disappointing and frustrating: for instance, all the seven herdsmen in Igbo-ora refused to attend the hospital for further investigation, and only four of the remaining six farm workers at Upper Ogun and Fashola could attend the hospital for follow-up. There were various reasons which contributed to the poor attendance at hospitals, including transport difficulties, long distance to hospitals, long waiting in hospitals before patients could see a doctor and collect medicine, loss of pay (for daily-paid workers), and wide-spread practice of self-medication with traditional and 'imported' medicines. Therefore thorough clinical and laboratory investigations were impossible under these circumstances, and the

author had to cope with these unavoidable difficulties which were the direct consequenceof the country's level of socio-economic development.

Clinical information obtained from the thirteen patients and other 25 farmers with high Brucella agglutinins of 200 I.U. and above showed that the duration of symptoms varied between one week and six months, but none of the affected people were ill enough to be considered for admission into the hospital. According to the criteria for admissions into the few beds in government hospitals in Oyo State of Nigeria, a patient must be acutely or seriously ill to be considered by a physician for admission, hence many patients with chronic illnesses are usually seen on outpatient basis. This might probably explain why people suffering from brucellosis were never seen among the inpatients, as the findings in the present clinical studies indicate. Another probable reason is the low level of pathogenicity of the prevalent abortus species. Hence, it could probably be concluded that patients who are likely to have brucella infection are the workers in farm settlements and they do not attend hospitals, hence hospital data on the prevalence of this important zoonosis in Nigeria are likely to be inaccurate. Some form of medical services should be provided for the rural community, especially the occupational groups in the livestock and allied industries to cater for their immediate primary health care and prophylaxis. This activity ordinarily comes under industrial or occupational medicine (Hamilton, 1943), which at present is only available in a few industries in big towns in Nigeria. This field of industrial medicine had attracted the interests of many epidemiologists in the past (Gafafer, 1943; Hamilton, 1943; Reid, 1954), and it deals with

the nature, degree and rates of common illnesses that people in different occupations acquire.

In Southern Nigeria, where over 60 per cent of the working population are involved in agricultural occupation, health and social services should be given high priority in rural areas where farm settlements are located. This would ensure adequate coverage of the majority of the population and also yield good returns to the economy, thus improving the cost-benefit and cost-effectiveness of government's investments on social and health services. At present medical services are concentrated in capital cities and few other big towns.

The pattern of symptoms presented by the 38 people interviewed were very similar to those observed by earlier workers (Huddleson, 1947; Spink, 1956; and Dalrymple-Champneys, 1960). Fever and pain were the most prominent symptoms, and genital symptoms were uncommon. But unlike the findings of previous authors, many patients did not complain of sweating. This might be explained on the basis of the rarity of Brucella melitensis infection in Southern Nigeria: drenching perspiration which is common in Malta fever and in suis infection, is usually absent in abortus infection, and when it occurs, it is relatively mild (Christie, 1974). There were also few people with lymph gland enlargement and hepatosplenomegaly in the present series. In Kenya, hepato-splenomegaly was a common clinical feature of brucellosis amongst the Wakambas and Pokot in Machakos District (Manson-Bahr, 1956; Cox, 1966).

Only one patient was found with radiologically confirmed cervical spondylosis: this low rate was not surprising because majority of those with serological evidence of active disease could not be fully

investigated in the hospital. The patient was one of those who did not respond to the first course of antibiotic therapy and he was eventually referred for orthopaedic management.

Fourteen of the 40 patients with P.U.O., and in whom the blood cultures were negative for bacteria isolation had high Brucella agglutinins of 100 I.U. and above. One of these 14 patients was found to have serological evidence of active brucellosis which was possibly responsible for the P.U.O. in this patient. This gave a prevalence rate of only 1.6 per cent among the total 61 in-patients with an initial diagnosis of P.U.O./ Septicaemia during a six-month period in U.C.H., Ibadan. In India, the incidence of brucellosis amongst P.U.O. cases has been reported as between 0.9 and 6.4 per cent (Joshi, and Markash, 1971; Mathur, 1969). The present study which seems to be in agreement with those of many other previous authors, suggests that brucellosis should be considered as one of the differential diagnoses of febrile illnesses in Oyo State of Nigeria. This infection is often overlooked as P.U.O. and enteric fever (Mathur, 1969). However, in tropical countries, where many diseases can simulate acute brucellosis and contact with infected animals is widespread, the presence of subclinical brucella infection with varying levels of humoral antibody to Br. abortus should also be borne in mind by physicians if misdiagnosis of some fatal diseases is to be avoided. During the initial phase of the present study, a patient with past history of exposure to infected cows and consumption of fresh milk presented with symptoms and signs suggesting acute brucellosis and he also had a positive saline S.A.T. of 100 I.U. The 2-ME test and Blood culture for Brucella organisms were negative, but X-ray examination revealed severe spondylotic changes

at L3/4 level. He was admitted to the medical ward of the University College Hospital, Ibadan and started on a combination of tetracycline and streptomycin, as recommended by Williams (1973). Because his symptoms and S.A.T. persisted after adequate chemotherapy he was further investigated and he was later found to be suffering from Hodgkin's disease. This case lends support to the suggestion of Henderson and Hill (1972) that antibody titre against Br. abortus in one of the occupational groups or someone with past history of exposure should not be accepted as the only explanation of the patient's condition.

The results of the study on 25 neurological patients seen at the University College Hospital, Ibadan, during the three month period, April to June, 1976, clearly revealed a very high prevalence of brucella infection among this group of patients. Fourteen (56%) of the 25 patients had significant high agglutinins of 100 I.U. and above, and five (20%) of them were found with serological evidence of active brucellosis. It was, however, not possible to isolate Brucella organisms from any of these patients, partly because only one blood culture was done on each of the 16 patients at the out-patient clinic and partly because of the recognised difficulty in cultivating Brucella organisms from clinical samples (Wilson and Miles, 1964; Williams, 1973), and the widespread use of antibiotics in Ibadan (Alausa et al, 1975).

This finding of a high prevalence of brucella infection among patients with neurological disorders agrees with the impression obtained in the pilot (retrospective) study which was undertaken during the early stage of this investigation. The possibility of brucella infection causing some of the neurological disorders in Nigerians had been thought of

by a few practising Neurologists at the University College Hospital in the late 1960's, but there was then inadequate laboratory support to confirm this clinical impression (Bademosi: personal communication). It is therefore hoped that many patients would benefit from the findirgs of the present investigation, as more and more patients with neurological disorders due to brucellosis would henceforth be detected and treated earlier than before.

Involvement of the Central nervous system by Brucella organisms with protean neurological and psychiatric manifestations has been recognised by many earlier authors (Hughes, 1897; Evans, 1934; Roger and Poursines, 1938; Spink, 1956; Sehadevan et al, 1968; Mathur, 1969). Marked fatigue, weakness, backache and cability are all indications of neurological and psychiatric complications of brucellosis. Though psychiatric patients were not investigated in the present study, it is recommended that this group of patients should be screened for brucellosis in future.

The low pattern of sero-reactivity found among hospital patients who presented primarily because of either hepatosplenomegaly (including Idiopathic Tropical splenomegaly syndrome (I.T.S.S.) or Leukaemia or Hodgkin's disease suggests that brucellosis probably does not contribute significantly to immunoproliferative disorders in Southern Nigeria. This finding, which differs from the earlier report from East Africa might possibly be explained on the known causes of hepatosplenomegaly in the two regions: while in Nigeria malaria is a common cause of hepato-splenomegaly I.T.S.S. (David-West: personal communication), in Kenya and other parts of East Africa, malaria and [illegible] common causes of hepatosplenomegaly (Owen, 1970). Further studies are however recommended in order

to unravel the association between brucellosis and immunoproliferative disorders in Nigerians. It will be recalled that one patient who was diagnosed as a case of brucellosis, on the basis of clinical features and positive saline S.A.T. but failed to respond to adequate therapy, was later found to have Hodgkin's disease.

Involvement of the reproductive system, in either male or female, has not been demonstrated in this investigation. It must be accepted, however, that the small number of patients, of both sexes, actually investigated would not permit any conclusion on this interesting and controversial aspects of brucellosis. Abortion being one of the most devastating effect of brucella in animals, its potential occurrence in women has generated much concern. Unfortunately, studies on the abortive effect of brucella infection in humans are scarce. Spink (1956) reviewed the genital complication of brucellosis, including its abortive effect, and he concluded that there was no definitive evidence that brucellae produce abortions in human subjects any more frequently than do other species of bacteria. However, in a more recent study carried out in Isfahan, Iran, which is a highly endemic area for Brucella melitensis infection, six (11.8%) of 51 women with second-trimester abortion had brucellosis (Sarram et al, 1974). Placental and foetal materials showed positive culture for brucella in five cases, and the card test was positive in all the six patients. The authors therefore concluded that their findings might indicate a cause-effect relationship between brucella infection and second-trimester abortion. The positive cultures found in placenta, various foetal tissues, and foetal heart blood were further evidence for the intrauterine infection of the foetus with brucella. It is important

to note that all brucella organisms cultured in the Iranian study were Br. melitensis. Similarly, the few sporadic positive foetal cultures o brucella reported in the literature were also mostly Br. melitensis (Smith, 1968). However, Poole, et al, (1972) had described a case of second-trimester abortion in a mother who, on serological evidence, had contracted acute brucellosis, and Br. abortus biotype 2 was later isolated from the amniotic fluid.

The discrepancy betwee the abort ve effect of brucella infection in domestic animals and in human beings has been attributed to the presence of erythritol in the animal (Smi , 1968). Erythritol is a growth stimulant for brucellae. It is found concentrated in the foetal fluid, the foetal placenta and chor on of susceptible animal species but it is not found in the human placenta.

Antimicrobial Therapy: The tetracyclines have been widely accepted as the most suitable antibiotics for the treatment of brucellosis (Spink, 1960; WHO, 1971; Williams, 1973; Shafe , 1973). Two groups of patients were selected for treatment, consisting of those with evidence of active brucellosis and those who were considered to be suffering from subclinic infection, in order to determine the criteria for treatment of brucello among the population investigated. The majority of patients with activ infection who took the antibiotics for complete three weeks showed satisfactory response to treatment in terms of clinical improvement and significant drop in antibody titres. On the other hand, among those with sub-clinical disease, there was no significant drop in antibody titres after treatment, though most of the people who completed either the Vibramycin or the Tetracycline for two weeks had symptomatic improvement

It was difficult to rely entirely on symptomatic improvement as many Nigerians derive some form of satisfaction after taking antibiotic capsules or injections (Alausa et al, 1975). Unfortunately, there was no control group with high agglutinin titre but without antibiotic treatment for comparison as this practice would seem to be against the declaration of Helsinki on the recommendations guiding doctors in clinical research (World Medical Association, 1964), particularly at the early phase of the present investigation when there were no proper guidelines for treatment of brucellosis in Nigeria. However, when the results of the two groups were compared, it was obvious that all those with serological evidence of active brucellosis benefited from chemotherapy, and that antibiotic treatment of those with only high IgM-type agglutinins was probably unnecessary. But this later group of farm workers who probably had developed immunity should be screened periodically in order to detect evidence of active infection, possibly resulting from lower resistance, as early as possible.

E: SUMMARY AND RECOMMENDATIONS

An attempt has been made in this chapter to demonstrate how essential it is for both clinicians and epidemiologists to work closely in order to obtain the complete clinical picture and natural history of a disease, as previously emphasised by Morris (1975). Hence clinicians and epidemiologists should be in continuous professional contact (Fletcher, 1963) to maintain this desirable continuity. Sir James Spence (1950) observed over two decades ago that "if clinical research is to be used to get the full picture of disease, it must equip itself to carry observations beyond hospitals." The findings of the present study has lent

support to this observation. The relationship between animal husbandry and brucellosis among the population of Oyo State of Nigeria is very strong. The majority of those with clinical and subclinical diseases would be missed if doctors see only patients that attend the hospital, since the greater proportion of those affected are in the farm settlements in remote rural areas.

Among patients seen in hospitals, special attention should probably be paid to those with neurological disorders who are seen in the outpatient clinics with vague symptoms, usually referring to the musculoskeletal systems: more cases of active brucellosis, requiring antibiotic treatment can then be detected.

TABLE XIII.1

HUMAN BRUCELLOSIS - COMMON SYMPTOMS IN OYO STATE

Symptoms	Group 1 (13 patients)		Group 2 (25 patients)	
	2-ME and Card Tests Positive		High level IgM-type Agglutinins	
	No.	%	No.	%
Fever	13	100	24	96
Backache	11	84.6	12	48
Body pain	11	84.6	11	44
Joint pain	10	76.9	9	36
Weakness	4	30.8	8	32
Testicular pain	5	38.5	4/22	18.2
Night sweats	9	69.2	3	12
Constipation	0	0	2	8

TABLE XIII.2

BRUCELLA SERO-REACTIVITY AMONG IN-PATIENTS WITH SEPTICAEMIA/P.U.O. AT THE UNIVERSITY COLLEGE HOSPITAL, IBADAN

TUBE AGGLUTINATION METHOD

TITRE (I.U.)	SALINE AGGLUT. TEST		2-ME TEST	
	No.	%	No.	%
Over 400	2	3.3	0	0
400	1	1.6	1*	1.6
200	4	6.6	0	0
100	7	11.5	0	0
50	8	13.1	0	0
25	8	13.1	0	0
Less than 25	31	50.8	60	98.4
TOTAL	61	100	61	100

*Serum was also positive with the Card Test and Complement-Fixation Test (C.F.T. Titre = 16).

TABLE XIII.3

BRUCELLOSIS AMONG NEUROLOGICAL PATIENTS SEEN AT U.C.H., IBADAN

No.	Lab. Code	Age (yrs)	Sex	Clinical Diagnosis	Laboratory Findings*		Animal Occupation (Yes/No)
					Saline Agglut. (Titre i.u.)	2-ME Test (Titre i.u.)	
1	O.O.	28	M	Guillain-Barre Syndrome	> 400	40	Yes
2	A.A.	15	F	Focal Epilepsy (Post-anoxic encephalopathy)	400	20	Yes
3	R.B.	17	M	Dementia (Post-convulsive)	200	20	No
4	O.S.	26	M	Pain both legs (Post allergic myelitis)	200	20	Yes
5	P.O.	32	F	Backache	200	Negative	No
6	S.A.	15	M	Dementia (Post-seizure encephalopathy)	100	Negative	No
7	F.W.	29	M	Anxiety Neurosis	100	Negative	No
8	O.M.	11	M	Epilepsy	100	Negative	No
9	W.P.	18	M	Backache	100	20	Yes
10	S.J.	25	M	Right Internal Carotid Artery Syndrome	100	Negative	No
11	S.K.	40	M	Focal Epilepsy	100	Negative	No
12	M.O.	46	F	Backache	100	Negative	Yes
13	L.R.	50	M	Neurogenic Bladder	100	Negative	No
14	O.R.	36	M	Tension Headache	100	Negative	No
15	A.T.	30	M	Cervical Cord lesion Radiculopathy	50	Negative	No
16	O.M.	45	M	Headache;(R)ptosis	50	Negative	No
17	G.R.	12	F	Jacksonian Epilepsy	50	Negative	No
18	O.S.	37	M	Headache	50	Negative	No
19	A.F.	40	M	Slipped disc	50	Negative	No
20	A.E.	39	F	Temporal Lobe Phenomenon	50	Negative	No
21	O.E.	34	M	Tension Headache	50	Negative	Yes
22	S.Y.	20	M	Optic Atrophy	50	Negative	No
23	E.A.	18	F	Anxiety Neurosis	25	Negative	No
24	A.S.	43	M	Post-traumatic Syndrome	25	Negative	No
25	F.B.	49	F	Backache.Anxiety	< 25	Negative	No

*Blood cultures performed on 16 patients were negative.

TABLE XIII.4

BRUCELLA SERO-REACTIVITY*
AMONG PATIENTS WITH IMMUNOPROLIFERATIVE DISORDERS

Clinical Diagnosis	Anti-Brucella abortus Agglutinins (Titre in i.u.) Saline Tube Agglutination						TOTAL
	< 25	25	50	100	200	400	
Leukaemia	5	2	2	1	0	0	10
Hodgkin's Disease	1	1	2	0	0	0	4
Hepatosplenomegaly (+ Idiopathic Tropical Splenomegaly Syndrome)	2	3	2	1	1	0	9
TOTAL	8	6	6	2	1	0	23
Percentage	34.8	26.1	26.1	8.7	4.3	0	100

*All sera tested were 2-ME negative.

TABLE XIII.5

THIRTEEN PATIENTS WITH ACTIVE BRUCELLOSIS TREATED WITH TETRACYCLINE (500 mg 6-hourly for Three Weeks)

Farm Location	Saline Agglutination Test (Titre i.u.)		2-ME Agglutination Test (Titre i.u.)		Card Agglutination Test	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
Upper Ogun Farm						
1	800	100	400	Less than 25	NT	NT
2	400	50	400	Less than 25	NT	NT
3	400	200	200	50*	NT	NT
4	200	50	50	Less than 25	NT	NT
5	100	Less than 25	25	Less than 25	NT	NT
Fashola Farm						
6	400	50	400	Less than 25	NT	NT
Igbo-ora Fulani Herds						
7	800	200	400	50	Positive	Positive
8	800	Less than 25	200	Less than 25	Positive	Negative
9	400	50	200	Less than 25	Positive	Negative
10	400	50	100	50	Positive	Positive
11	200	50	50	25	Positive	Positive
12	200	25	50	Less than 25	Positive	Negative
13	25	Less than 25	Less than 25	Less than 25	Positive	Negative

NT = Test not performed.

*Patient with cervical spondylotic changes at C5/6 level.

CHAPTER XIV

SOCIO-ECONOMIC EFFECTS AND CONTROL OF BRUCELLOSIS IN OYO STATE

The socio-economic effects of brucellosis in any community are generally considered under (a) its public health significance, and (b) its economic repercussions.

A: THE PUBLIC HEALTH SIGNIFICANCE OF BRUCELLOSIS IN OYO STATE

A disease is considered to be of public health importance if (a) it is common, (b) it causes a high morbidity or mortality, and (c) its treatment takes up much time with loss of working hours, or costs a lot of money. The present investigation has clearly shown that bovine brucellosis is endemic in Oyo State and that direct or indirect transmission of the disease from infected animals to man is common. The human infection rate is also high as revealed by a high sero-positivity rate in various areas. Some of those infected would eventually develop ill-health with physical incapacity: the disabilities associated with brucellosis include backache, fatigue, tiredness and persistent fever. Sometime, some of the daily paid workers, who continue to be sick or absent from work, are laid-off, and new workers are immediately employed in their positions: unemployment is still a major social problem in Nigeria.

Brucellosis has a low mortality rate. Hospitalisation is also

uncommon but many people who have brucellosis, often improperly diagnosed, probably attend the outpatient clinics of many hospitals, in Oyo State. Treatment is time-consuming and antibiotics have to be given over a long period before 'cure' is achieved. This has an undesirable economic effect on many Nigerians since the majority of those under treatment have to pay for these drugs which can be very expensive.

The public health effects of brucellosis on the various communities studied are sometimes dependent on availability of medical services. Where medical attention is not immediately available, as in Upper Ogun Estate, and the workers have to cover long distances to get to the nearest government hospitals, the rate of absenteeism would be higher than in other livestock farms which are situated either in the urban areas, as the Dairy Farm in Ibadan, or near a town with medical services such as Fashola Multiplication Farm, via Oyo (Table XIV.1). In the remote rural areas without any form of health services, self-medication with local herbs and orthodox drugs is widely practised, and this usually lead to chronic ill-health with incapacity to work efficiently on the farm. Loss of manpower and pay is a characteristic feature of the rural community in Nigeria because of the daily-paid system of employment. On the other hand, workers in permanent employment in government-owned farms in urban centres can take permission to attend a near-by hospital, without loss of income; in addition, they are entitled to free medical treatment in government hospitals. There is no compensation at present in Nigeria for disabled farm workers, especially for those privately employed.

Another important feature of the public health effects of brucellosis is ignorance about the mode of transmission of the disease. This is evident by the refusal of the Fulani nomads at Igbo-ora to attend the Rural Health Centre in the town for treatment: these herdsmen with active brucellosis were not convinced that they could contract a disease from their cows. Also, the initial treatment which was offered on the farm yielded poor clinical results, possibly because these herdsmen failed to complete their antibiotic treatment.

Finally, the acute shortage of animal protein which was experienced at Igbo-ora during the epidemic of bovine brucellosis illustrates another important public health effect of this disease on any community. In Oyo State and other parts of Nigeria, cow meat is the main source of animal protein for the majority of the population: animal proteins are essential to human health and well-being. Therefore, acute shortage of cow meat in any part of the country worsens the already existing protein under-nutrition, especially among the rural population (Oyenuga, 1976). The Federal government's decision to import chilled meat to ease the problem of protein under-nutrition emphasised the seriousness of the national plight in meat supplies. In addition, the Nigerian Livestock and Meat Authority (Emergency Meat Supply Project) was established: the Authority was charged with the following responsibilities: (1) manpower training at all levels of animal production; (b) provision of capital in the form of generous loans to livestock farmers; (c) establishment of animal breeding centres with health services for animals and farmers throughout the country; (d) introduction of modern marketing and distribution systems in the country; and (e) provision of modern abattoirs and meat inspection services by veterinary staff (Awosoyi: personal communication).

B: THE ECONOMIC REPERCUSSIONS OF BRUCELLOSIS IN OYO STATE

The various governments in Nigeria now offer financial incentives to farmers of all categories in order to increase food production. Meat shortages can be overcome by either increasing the number of livestock or increasing the quantity of meat obtainable per animal. Unfortunately, in the presence of a high incidence of brucellosis in both the human and animal population (Table XIV.2), it may be difficult to achieve a high level of animal production. An economically viable livestock industry is unrealistic under the present situation in Oyo State. Direct losses in meat production from abortion, infertility and weight loss are some of the economic consequences of endemic bovine brucellosis. Infertility brings about prolonged periods between lactations and in an infected herd, the average intercalving period can be prolonged by several months, as it was observed in the Fulani herds at Igbo-ora. This is an important economic aspect in herds where the calves represent the main source of income. In addition, there is loss of revenue from poor quality hides and skin: in future, importing countries might refuse infected animal products, with resulting loss in foreign exchange by the country. Active bovine brucellosis is therefore a devastating disease in Oyo State and its effects are directly opposed to the aspirations of livestock industries.

The following figures based upon officially recorded incidences may give an indication of the total annual economic loss in different countries: in the U.S.A. originally 100 million dollars (in 1947), which has been reduced to an estimated 40 million dollars in 1957; in France in 1953 37 milliard old Fr. frcs.; in Argentina 172 million pesas; in

Germany (in 1946) 200 to 250 million R. Mk.; in the Netherlands (in 1956) 45 million H.fl.; in Switzerland (1945) 20 million Sw. frcs. In the U.S.S.R. the number of infected animals has been estimated in the millions and the economic losses from abortion are accordingly extraordinarily high (Van der Hoeden, 1964). Though no comprehensive data are available on the overall economic loss to the livestock industry due to brucellosis in Oyo State, and indeed for the whole country, the figures would probably run into millions of Naira (₦) annually.

C: REALISTIC CONTROL MEASURES AGAINST HUMAN BRUCELLOSIS IN OYO STATE

A disease may be said to have been controlled when its prevalence is reduced to a level where it is no longer a major public health problem. Control measures have one or several of the following objectives: prevention of spread, reduction of transmission, or lowering morbidity and mortality in the affected population. Control is quite different from eradication, which means ending transmission, and eliminating reservoirs of infection in the definitive hosts. The ideal ultimate aim of any control measure is, of course, eradication. Brucellosis is of economic as well as medical concern, the expenses of which could be reduced through increased funds for control and eventual eradication.

In order to eradicate a zoonotic disease, such as brucellosis, certain criteria have to be met:

1. The overall prevalence and incidence of the disease in both animal and human populations must be determined by well-planned epidemiological surveys.

2. Financial losses shown by the above surveys must convince the government that eradication would be a profitable exercise for the country/state as a whole, taking into consideration the place of livestock industry in the country's socio-economic growth. Also prospective benefit must be shown for the livestock owners.

3. The eradication plan must be practical and realistic, with ultimate success assured. For instance, understanding of the social habits and also human activities may be important for a successful control and eradication programme. These are social habits such as eating of roast or partially cooked meat and raw milk which may lead to the transmission of the disease.

4. Available diagnostic tests should be reliable and easy to apply.

5. A public awareness must exist of the dangers of this disease to human population as well as to animals: the farming community as a whole must appreciate the true value of eradication in the broadest sense, through proper and well-planned public health education. Unless it is understood in the livestock industry that it is not economic to live with brucellosis, there is a danger that complacency may impair progress from control to eradication.

6. Full cooperation must be established between the veterinary and medical professions for accurate reporting of the disease - a two-way notification system would be a practical step in this direction. Reporting of zoonoses is an important aspect in the development of a control/eradication programme. It is through proper and prompt reporting of a disease to the appropriate authorities that prompt control action can be taken. Administrative practice as to what diseases are to be reported

and how they should be reported varies a great deal from one country to another. For example, in Great Britain, "The Zoonoses Order 1975" which came into operation on 14th July, 1975 empowers a veterinary inspector to investigate and control animal diseases due to the organisms of the genera Salmonella and Brucella. The order also provides that any of these diseases becomes reportable in any animal species when organisms have been isolated by laboratory tests (Statutory Instruments, 1975). This type of specific order does not operate in Nigeria at present.

Problems Facing Brucellosis Control Programmes in Oyo State

Though the enormous economic losses and the serious hazard to human health demand enforcement of energetic measures, proper control programmes leading to eventual eradication of brucellosis in Oyo State, and indeed other parts of Nigeria, is not feasible at present because of the following reasons:

1. It will be difficult to inaugurate active eradication programmes based on test-and-slaughter policy because of lack of resources, in terms of money, manpower and materials. The initial expense would be beyond the present resources of the veterinary services. Also, the manpower and materials to carry out such extensive eradication programmes are at present not available. This was borne out clearly during the epidemic of bovine brucellosis in Igbo-ora, when the state government was notified but could not immediately help the poor farmers financially or with technical aid. The existing shortage of animal protein in the country is perhaps the most important reason why the government would probably not consider embarking on eradication policy.

2. Livestock management in the state is largely unscientific. More

than half of the herds are controlled by the Fulani nomads, who are illiterates and ignorant of the mode of transmission of brucella organisms from cattle to human beings. These nomads move from one place to another in search of good pasture for their cattle, and they usually object to examination and serological testing of their cows. The government livestock farms are usually located in remote rural areas, and therefore their management is left mainly under the control of livestock attendants who are semi-illiterate. Though trained veterinary personnel are supposed to be in-charge, they do not live near the farm because of poor social amenities, such as water, electricity, health services and schools for their children. These veterinarians visit the farms periodically, hence they cannot claim to be effectively in-charge of the routine and daily management of the livestock. The local people, who are always around, therefore manage these farms in the traditional method, which is semi-nomadic and under insanitary conditions. Generally, control of brucellosis in areas of nomadic animal husbandry is difficult to achieve (Abdusalam and Fein, 1975).

3. There is free movement of cattle and other animals between the various states in the country, and also between Nigeria and her northern neighbours. In fact, over 90 per cent of cows belonging to the nomadic herdsmen were originally derived from the northern parts of the country. They are usually herded down from the north across many states to the south. It is hoped that after establishment of many more cattle ranches in various parts of the country, free movement of animals would be controlled.

4. At present, there is no control measure against infected animals in the abattoirs. The numerous abattoirs that exist operate without

any health control measures whatsoever. In fact, it is not yet resolved whether inspection at slaughterhouses and meat-packing factories should be under the veterinary or medical services. Environmental and personal hygiene in many slaughter slabs is very poor indeed. Also meat sellers can be seen on the streets hawking the commodity in the most deplorable and unhygienic state. In fact, meat selling is regarded as a dirty occupation in Oyo State, and it is left entirely in the hands of the illiterates.

5. Majority of livestock farmers consume raw milk, and the social habit of taking 'half-done' roast meat is increasing among the elites in many parts of Nigeria. Apparently, many people, including the educated, are ignorant about the zoonotic diseases in general. Hence, there is very little public health awareness of the dangers of these group of diseases in Nigeria.

6. Regular exchange of information and collaboration, such as demonstrated throughout this study, between the Veterinary and Health Services are lacking in the country.

Realistic and Practicable Control Measures

Human brucellosis is mainly an occupational disease in Oyo State and probably in other parts of Nigeria. Significantly higher infection and disease rates are found among herdsmen, slaughtermen, veterinarians and other occupationally exposed people than the general population. The main factors responsible for this higher prevalence of infection among livestock workers are: (a) exposure to infected cows during their normal occupation; (b) the adverse insanitary conditions under which these workers carry out their duties; and (c) the low social and economic

status attached to the livestock occupation. Therefore, properly organised control measures can be tackled along these three major identifiable contributing factors:

1. Control Against Exposure to Infected Cows: The establishment of State-Federal Cooperative Brucellosis Control Programme through legislation, as was the case during the initial control programme in the United States in 1934 (Busch and Parker, 1972) would be a practical step in the right direction for a long-term solution to the problem of bovine brucellosis in Nigeria. The Federal government should be responsible for the interstate and international control regulations against importation of infected animals into the country and also from one state to another within the country. The Federal government should also ensure that infected animals or their products are not exported to other countries. The various state governments should be responsible for the control programme within their state. The establishment of different programmes for each state, or groups of states within identical identifiable epidemiological factors, would be a more realistic approach than proposing a general (blanket) control policy for the whole country.

Since a slaughter policy is impracticable on socio-economic grounds, vaccination programmes for beef cattle in settled herds should be introduced in Oyo State because of the present high prevalence of brucellosis. This might help to reduce the level of infection to a point where economic loss to owners of infected herds are reduced to the minimum. Also with the reduction in the level of active infection in the animal population, human infection would be greatly reduced or eradicated. Vaccination of nomadic herds is likely to present some difficulties, therefore every

effort should be made to mount a surveillance and testing programme for those cattle entering the Oyo State borders from other states. The card test will be useful for this surveillance programme: reactors should be separated and herded separately from non-infected animals.

Vaccination of specially exposed people, as suggested by Versilova (1965) in the Soviet Union, is not considered suitable in the Nigerian situation, even in the presence of prevailing poor standards of beef and dairy-farming management and great danger of occupational infection. Apart from the well-known disadvantages associated with human vaccination (Christie, 1974; Spink et al, 1962; Pappagianis et al, 1966), the vaccine is usually indicated for population groups occupationally exposed to infection with Br. melitensis (WHO, 1971; Versilova: personal communication). However, human brucella infection in Nigeria is largely caused by Br. abortus, which is recognised to be of low pathogenicity for man. In addition, the vaccine has to be imported into Nigeria, where at present there are no facilities for quality control of microbiological products. Therefore, on medical and economic grounds, human vaccination probably has no place in the immediate control measures of brucellosis in Nigeria: the risk involved probably outweighs the advantages to be derived.

2. Control Against Infected Environment and Animal Products: Environmental spread of brucella infection requires that there should be careful control of working conditions and periodic regular screening of workers in contact with animals. Hence scientific farming should be encouraged by the government and proper slaughtering and meat-packing houses should be built in many parts of the state. Therefore, one

important aspect of any brucellosis control is the provision of adequate facilities for the early diagnosis of the infection. This would probably involve the establishment of industrial or occupational medical services which would be supported by many peripheral public health laboratories at the local levels. These are at present non-existent in the whole country. These laboratories would provide, among other things, the essential diagnostic services and medical surveillance of farm workers, animals and fresh milk. Communicable disease control is greatly facilitated by early detection of infection and determination of the epidemiology of an outbreak (WHO, 1972). But before these facilities are provided by the government, the Veterinary Services Division should teach the farmers how they can maintain proper sanitary and hygienic condition on their farms. In addition, strict measures should be adopted concerning the delivery of brucella-infected animals to abattoirs, the conditions under which they are slaughtered, and the methods of inspecting and judging the meat. Personnel should be well protected against the risk of infection by wearing well-covered uniforms, gloves and goggles to prevent direct infection through abraded skin, and the conjunctivae. Raw milk and meat should be subjected to adequate heat treatment to prevent transmission of brucellosis by these products. It is important to note that brucella organisms can survive pickling and smoking (WHO, 1971), a common practice among many Nigerian elites.

Health education of livestock farmers and abattoir workers is important. Full cooperation of the cattle owners and handlers is most desirable during control programme. Proper and well-planned public health education is perhaps the most important epidemiological control measure

when a long term objective is envisaged. The principal task of public health education is to implant motivation in the minds of the livestock farmers and the populace so that people, after they are made to understand the mode of transmission of zoonosis, can willingly comply with health regulation requirements. Public health measures directed at the control of infectious diseases, and its ultimate eradication can be put into effect with any hope of success only when a solid socio-economic structure of the community exists with proper understanding of the goals of public health. Modern audio-visual technique serves as a valuable tool for teaching prevention of disease in man and animals.

3. Improvement of the Socio-Economic Status of Livestock Farmers:

About 70 per cent of the population of this country live in the rural areas, where the livestock farms are mainly located. For so long now, life in rural areas has been dull and unattractive due to inability of successive governments to provide the basic amenities. In addition, living and working conditions of all categories of workers in the rural areas are deplorable and unprogressive, with few prospects for economic progress. Unless these man-made obstacles are removed, the desired goal of the huge investment on agriculture in the country would not be realised. Therefore it is in the best interest of the country as a whole that every effort should be made to improve the socio-economic level of the rural majority, particularly in the livestock farm settlements. These amenities will serve as incentives to farmers to stay on their farms.

It is therefore gratifying that the Federal government has recently established a Basic Health Scheme for the whole country whose primary function will be the provision of the primary health needs of the rural

population. Also the establishment of local government administration in the country provides a great opportunity for the development of the rural areas. It is hoped that the Basic Health Scheme Agency would work closely with the local councils to achieve greater benefit. It is however important to establish peripheral (local) laboratories to ensure adequate diagnostic facilities and surveillance for communicable diseases, which will add to the success of the primary health care delivery system to the community.

The local government authorities should pay particular attention to the construction of rural roads, provision of good water supply, building of schools and rural electrification. A good and reasonable network of roads in the rural areas will ensure easy communication between the rural farm establishments and the urban areas. Free movement of food products to the markets in the urban areas will also be ensured. The farm workers will also have access to the more developed social facilities, including hospital health services, when they are available in the nearest urban centre. Adequate pipe borne water supply is important to ensure proper personal hygiene and adequate sanitation on the farms. If wells are used, they should be of the protected type: surface water is easily contaminated. In one farm visited during the present study, all washings by the farmers were being carried out in an irrigation lake where the animals also drink water (Fig. XIV.1).

Rural electrification has a lot to its credit which probably outweighs its heavy cost, such that any investment in it is not wasted. Apart from raising the standard of living in rural areas socially, it has several economic advantages like encouraging small-scale industries,

hospitals, veterinary services, schools and other social amenities. These establishments also attract and provide employment opportunities for young school leavers in the locality, and help to halt the rural migration to urban areas. With increasing social stability in the rural areas, more highly skilled personnel, including veterinary officers, doctors and teachers are likely to live in the locality, thus ensuring greater attention by the veterinary workers to scientific livestock management. This approach will also encourage adult education which is an essential requirement for a successful health education of the illiterate farmers.

The control of the effect of the present adverse working conditions and the social class factor on the prevalence of human brucellosis in Oyo State will eventually have to be tackled through industrial hygiene legislation, such as the William-Steiger Occupational Safety and Health Act in the United States (Public Law, 1970), which is not yet applicable in Nigeria. It is however hoped that with greater awareness of the risk involved in animal husbandry amongst the group of workers directly involved, they would organise themselves into a trade union which could negotiate for improved working conditions with the responsible management. This would probably only apply to those in government-owned herds; the situation in privately-owned herds will be more difficult to tackle unless there is a government intervention by a legislation.

D. CONCLUSION

Though medical research plays an important part in the processes leading to eventual disease control, alone it cannot eliminate ill-health and disease, without simultaneous economic and social progress to ensure a minimum decent standard of living for the masses, especially those living at present in the remote rural areas and farm settlements. With better education, higher income and improved social amenities, the masses will strive for better housing and sanitation, and their contact with many infectious diseases will then fall by itself, with medical intervention only accelerating the process. But, while waiting for a rise in the economic, social, and cultural level which may be a long time coming, the prevailing problems should be tackled with the tools presently available through proper organization and deployment of our scarce resources.

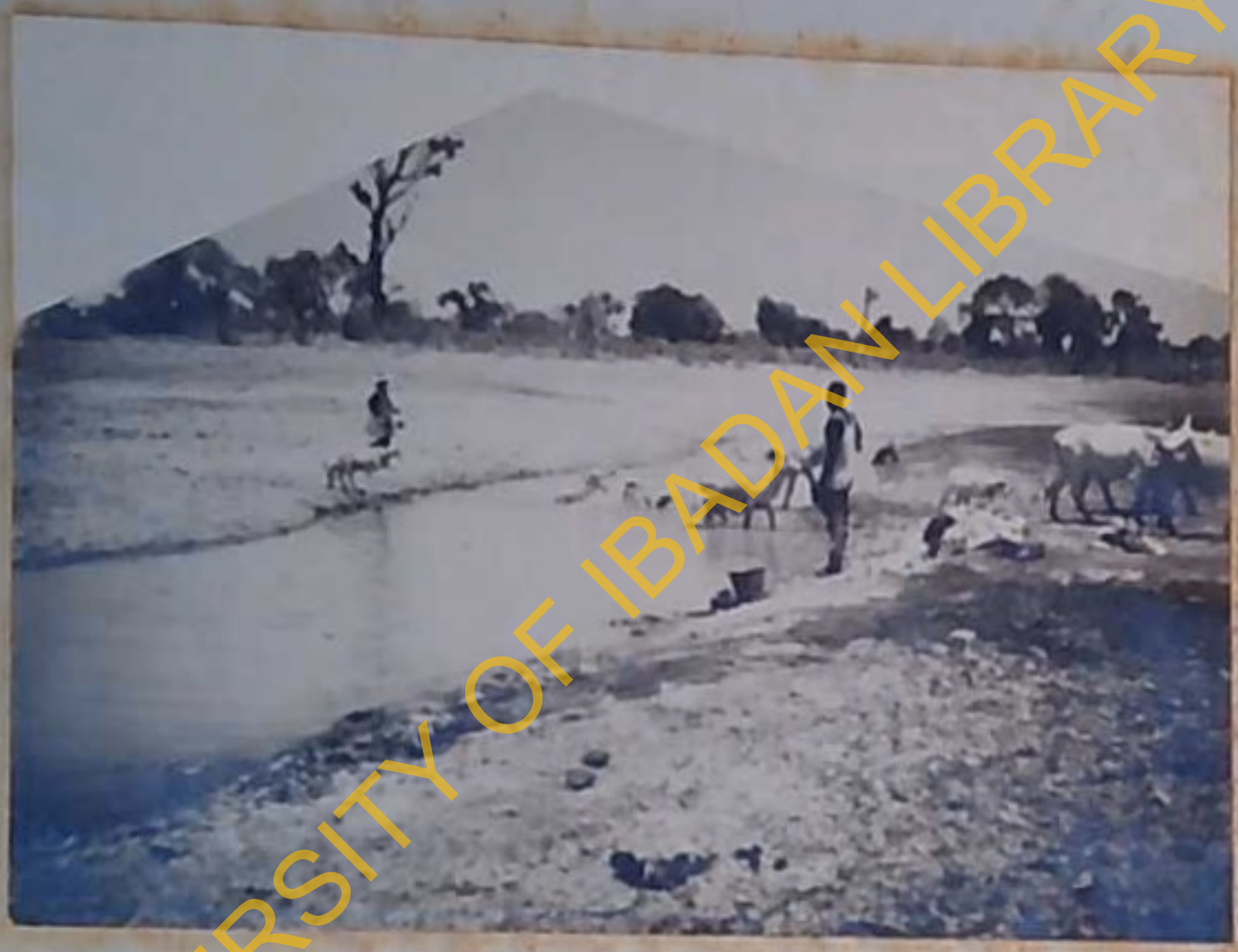

Fig.XIV.1: Irrigation Lake in a Farm Settlement: showing cattle, sheep and farmers utilising water from the same source.

TABLE XIV.1

PUBLIC HEALTH SIGNIFICANCE OF HUMAN BRUCELLOSIS IN OYO STATE

Place of Study/ Type of Survey	Category of People Investigated	Total Number Studied	Active Brucellosis Requiring Treatment		Absenteeism Rate	
			No.	% of Total	Number/ Population	%
1. Igbo-Ora 'Epidemic' Study	Private Farm and Nomadic Herdsmen	12	7	58.3	9/12	75
2. Upper Ogun Estate Survey	Government Farm Workers (Farm Settlement)	54	5	9.3	6-8/98 (7/97)	7.2
3. Fashola Cattle Multiplication Farm Survey	Government Farm Workers (Partial Farm Settlement)	104	1	1.0	4-5/120	3.75
4. Neurology Clinic U.C.H., Ibadan Screening Survey	Hospital Out-patients	25	5	20.0	.	.
5. P.U.O. Cases, U.C.H., Ibadan. Screening Survey	Hospitalised Patients (In-patients)	61	1	1.6	.	.

TABLE XIV.2

ECONOMIC EFFECT OF BOVINE BRUCELLOSIS ON LIVESTOCK INDUSTRY IN OYO STATE

Place of Study	Category of Farm	Total Number of Animal Investigated	Animal with Active Brucellosis		Abortion Rate (Source of data)
			No.	% of Total	
1. Igbo-Ora Nomadic Herd	Private	40	40	100	Very high (Cattle owners observation)
2. Fashola Settled Herd	Government-owned	61	38	63.3	42% (Esuruoso 1974b)
3. Upper Ogun Settled Herd	Government-owned	65	17	26.2	Low (Esuruoso 1974a)

CHAPTER XV

GENERAL SUMMARY AND CONCLUSIONS

The term "brucellosis" is applied to disease, occurring in many species of animals and man, attributable to infection with organisms of the genus Brucella, the first member of which was discovered by Sir David Bruce in 1886. Brucellosis is a zoonosis, primarily a disease of domestic animals which under special circumstances, may be readily transmitted to man.

The relationship of the disease in animals to human infection was first recognised in 1906 when Themistocles Zammit, a Maltese physician and also a member of the British Mediterranean Fever Commission, showed that man acquired Malta fever from drinking infected goat's milk: this observation was outstanding and one of the most significant landmarks in the epidemiology of brucellosis. The first practical method in the prevention and control of human infection was demonstrated in 1908 when the ban on the consumption of unboiled goat's milk among the troops stationed in Malta led to a remarkable drop in the cases and deaths of undulant fever among the military population.

Brucellosis in man and animals may be caused by any of the main three species of Brucellae. Brucellosis is at present one of the most widespread and economically the most ravaging of more than 100 zoonoses that are recognised. Its distribution is practically world-wide and prevails wherever domestic animals and man cohabit. Infection caused

by the abortus species has occurred in nearly every country of the world: the present study confirms previous finding that it is responsible almost exclusively for the disease in both animal and man in Oyo State of Nigeria. Generally , human infection is closely related to methods of animal husbandry, food habits, standards of hygiene, and economic activities. Therefore, the epidemiological picture varies from area to area in different parts of the world, and has to be studied carefully in planning control and eradication programmes. However, figures on the incidence and prevalence of brucellosis may be deceptive, for in most of the developed countries where investigations are actively carried out, the incidence usually appears high whereas in the majority of developing and tropical African countries, without facilities for diagnosis, few cases are usually reported though infection may be rife.

In Nigeria, where livestock management is still largely unscientific and bovine brucellosis is known to be endemic, there are only scanty data on the epidemiology and clinical presentation of human brucellosis. Therefore, the present study was undertaken with the objectives of collecting adequate and reliable data on the prevalence, incidence,, distribution and other important determinants of human brucellosis in Oyo State of Nigeria in order to be able to formulate a realistic interim and long term control programme. The study also aimed at expanding medical and applied scientific knowledge in the field of brucellosis and occupational medicine in Nigeria. This study covered a period of three and half years, between September 1973 and February 1977.

A brief socio-economic description of Nigeria and the areas covered by the study revealed that over 70 per cent of the total 55 milli

Nigerians live in rural areas and farm settlements, which are under-developed and neglected in terms of basic social amenities. Agriculture, including livestock, is the mainstay of the Oyo State economy, and approximately 50 per cent of the State's labour force are engaged in agricultural work. Cow meat is the most readily available animal protein to the people of Oyo State. Most of the livestock are derived from the northern states of the country and some cows are also imported from the neighbouring African countries. The medical services provided by the state government are grossly inadequate and unevenly distributed, with the rural areas virtually neglected. The present epidemiological study was undertaken largely in several farm settlements scattered all over Oyo State, but the central unit for the study was based at the Department of Medical Microbiology, University College Hospital, Ibadan, where also the clinical aspects of the study were carried out.

An initial pilot sero-epidemiological evaluation on human brucella antibodies carried out on 1600 people of various occupation and age groups living in Ibadan during the first four months of the study revealed an overall sero-positivity rate of 50.2 per cent. Significantly higher prevalence of infection was found among the occupationally exposed population, including herdsmen, abattoir workers and veterinarians, than the general population, including blood donors, pregnant women and school children. The sources of human infection included direct and indirect transmission from infected animals, particularly cows, to the farmers and abattoir workers. A titre of 100 I.U. and above in the Standard Saline Brucella Tube Agglutination (S.A.T.) was considered by statistical comparison between the occupationally exposed and non-exposed groups as the significant level indication of brucella infection. A prospective

longitudinal study carried out to determine the effect of cholera vaccination or the disease itself which occurred as an epidemic in Ibadan in early 1971 did not reveal any significant cross-reacting brucella agglutinins due to Vibrio cholerae which persisted for more than three months. Therefore, cholera does not seem to oppose the sero-immunolcgical diagnosis of human brucellosis in the community investigated.

Epidemiological investigations carried out in the government-owned settled herds in Oyo State showed that the important factors determining the rate and level of human Brucella abortus infection include: (1) The geographical location of the farm: where the herd is located in the town with easily available medical facilities for all workers, the level of human infection was found to be lower than in the rural farm settlements without medical and other social facilities. (2) The number of cattle per head of population and frequency of contact with infected animals: at the Upper Ogun Farm Estate where the ratio of cattle to human population was the highest and majority of the workers lived on the farm, 9.4 per cent of those tested had serological evidence of active brucellosis. On the other hand, at the Fashola Farm with fewer cattle and less human contact with infected animals, the active infectivity rate was only one per cent. (3) The rate of human infection was directly proportional to the rate of active bovine brucellosis. (4) Imported bovine infection from neighbouring countries.

A high prevalence of subclinical infection as shown by high antibody titre against Br. abortus without evidence of disease, in people occupationally exposed to infected animals was also found in all the three farms investigated.

A three-year longitudinal study was carried out among the students

at the Institute of Agricultural Research and Training (I A R & T), Moor Plantation, Ibadan in order to be able to determine the incidence of human brucella infection in the occupationally exposed individuals in Oyo State. The findings revealed that many farmers acquired subclinical brucellosis within two years of occupational constant exposure to infected animals. The study also demonstrated the advantages of modern and scientific livestock management (as practised at I A R & T) over the traditional and extensive methods of animal husbandry (as practised in most government-owned and privately-owned settled and nomadic herds) in the prevention and control of bovine and human Brucella abortus infection. Within two years of constant exposure to sources of infection, many of the students who were sero-negative during the certificate course were found on returning for the diploma course two years later to have become seropositive, some with S.A.T. titres as high as 400 I.U.

The prevalence of brucella infection in the I.A.A. & T farm was studied over two seasons, and no seasonal variation, between the dry and rainy (wet) seasons, was found: climatic variation was therefore considered unimportant in the prevalence rate of brucellosis in southern Nigeria.

An outbreak of active bovine brucellosis, with human involvement, occurred at Igbo-ora during the period of this study. The results of the investigation carried out revealed 100 per cent active infection rate in the cattle population and over 50 per cent active brucellosis among the herdsmen. Nine (75 per cent) of the twelve herdsmen tested gave symptoms suggestive of acute brucellosis. The serological methods used for the investigation were the saline tube agglutination test (S.A.T.), the 2-mercaptoethanol tube agglutination test (2-ME test), and the card

test, as well as the Brucella abortus milk ring test (M.R.T.). There was close correlation between the results of the tube agglutination tests (S.A.T. and 2-ME) and the card tests, showing that the latter test was a satisfactory aid to diagnosis of acute human infections. All the three pooled milk samples from the three herds investigated were positive by the M.R.T.

The socio-economic effects of this large-scale brucellosis epidemic in Igbo-ora were very informative and instructive about the hazards caused by Br. abortus infection in Nigeria. The Fulani nomads, who under normal conditions would have resisted attempts to investigate their herds, cooperated during the investigation because of the economic hardship they experienced due to poor condition of their stock. Similarly, the Ibarapa community experienced an acute shortage of cows for slaughter at the abattoirs, and cow meat, which was usually available at reasonable cost to the low income people, became a scarce and expensive commodity.

The practical problems of controlling active bovine brucellosis in a developing economy were highlighted, as the ideal 'slaughter technique' of dealing with this type of situation in developed countries was not feasible because of the poor state of livestock industry in Oyo State and the opposition of the Fulanis to this type of control measure. In addition, the State government had no operating control programmes to deal with this situation. When the veterinary division of the Oyo State Ministry of Agriculture was informed of this devastating epidemic and the socio-economic implications, they were unable to offer immediate aid to the farmers. To tackle this problem, and also to prevent further spread, sanitary control of livestock grazing was introduced and the entry of new animals into the infected hers prohibited. Routine

vaccination against bovine brucellosis was then not in practice in Oyo State, but the government promised to embark on this in the immediate future, as part of a National Brucellosis Control Programme being envisaged by the Federal government of Nigeria. The human population with active brucellosis were treated with standard antibiotic therapy and they were advised to stop consuming raw milk obtained from infected cows. Following the successful investigation of the causes of epidemic abortion and infertility among the cattle population and chronic ill-health among the herdsmen in Igbo-ora, many farmers in the State became aware of the socio-economic importance of brucellosis in the livestock industry. Several requests were received from many cooperative societies and individuals for serological screening tests on their herds and the occupationally exposed people; cases of bovine brucellosis were detected in other herds.

The difficulties usually encountered in studying the full clinical aspects of an occupational disease, such as brucellosis, which affects mainly people on farm settlements in rural areas in developing countries were highlighted throughout the entire period of the present study. Because of the lack of basic social amenities in the rural areas and poor communication links between the areas and the urban centres where the essential social amenities are concentrated, it is often impossible to request farm workers who are found to be suffering from an illness during epidemiological surveys to report for further investigations and treatment in a far-away hospital. In addition, owners of private herds are often reluctant to stay away from their farms. Therefore, people in the rural areas in Nigeria indulge in self-medication with traditional

and orthodox medicines. These unavoidable limitations resulting from the level of socio-economic development of the area studied made thorough clinical and laboratory investigations impossible. Clinical follow-up of patients was very poor.

Clinical findings among patients attending the University College Hospital, Ibadan showed that only a few cases of brucellosis are likely to be diagnosed, as the greater proportions of those with clinical and subclinical diseases live on farm settlements in remote rural areas. The majority of those with clinical brucellosis had neurological disorders and presented with vague symptoms referring to the musculoskeletal systems. It was therefore recommended that both clinicians and epidemiologists should work closely in order to obtain the complete clinical picture and natural history of brucellosis in Oyo State of Nigeria.

In a developing country like Nigeria where there is a need for an increase in the production of animal protein to cater for the increasing population, endemic bovine brucellosis is a devastating disease which prevents a high level of animal production. Although a high priority is being given to the establishment of more cattle ranches in Oyo State and other parts of Nigeria in the current (1975-1980) agricultural development plan, a high incidence of bovine and human brucellosis will render an economically viable livestock industry impossible. Though no comprehensive data are available, the economic loss due to brucellosis, if estimated, would probably run into millions of naira (₦) annually.

Complete eradication of bovine brucellosis, and hence human infection is not feasible in Oyo State at present because of the poor state of animal husbandry and the huge cost involved in slaughter policy,

However, realistic control measures, based on the improvement of the existing agricultural, social and health systems in the State are possible. Immediate interim control measures include hygiene on the farms and abattiors, avoidance of raw milk (especially in the rural areas) and vaccination programmes for beef cattle in settled herds. Vaccination of nomadic herds will be difficult: therefore, every effort should be made to mount a surveillance and testing programme for those cattle entering the Oyo State borders from other parts of the country and neighbouring countries. The card test has been shown to be useful in the present study for this type of programme. Sero-positive cattle should be herded separately from non-infected animals. In addition, every effort should be made to improve the socio-economic level of the rural population in order to encourage trained farmers to stay on their farms. Therefore, the local government should pay particular attention to the construction of rural roads, provision of good quality pipe borne water, building of schools and rural electrification.

The long-term control measures should include (1) the establishment of clinical and laboratory services for the early diagnosis of infection in both the animal and human population, and also the treatment of farmers with active infection; (2) health education of livestock farmers and abattoir workers on the mode of transmission of brucellosis and the necessity for appropriate hygienic measures, (3) full co-ordination in the medical and veterinary professions to ensure a two-way notification system for this important disease; (4) widespread pasteurisation of fresh milk; (5) the establishment of State-Federal co-operative Brucellosis Control Programme, which will be responsible for the individual State's control

policy, and also the inter-state and International control regulations; and (6) industrial legislation which aim at improving the working conditions of the livestock farmers.

SUGGESTED FUTURE STUDIES

The results of the sero-epidemiological studies of different population groups in Oyo State have indicated that there is a need to carry out a large scale epidemiological survey of Nigeria to determine the overall socio-economic significance of human brucellosis in the Nigerian community, in order to formulate a realistic National Control Programme.

In addition, the results of the present study have indicated that future studies are required to evaluate the role played by brucellosis among patients (in Oyo State) with pyrexia of undetermined origin (P.U.O.), pregnant women with second-trimester abortions and patients with neuro-psychiatric disorders.

Little success has so far been achieved in the attempts to immunize human beings against brucellosis. Many authors have reservations about advisability of recommending human vaccination because of the known inherent risks associated with the currently available vaccines, which have however proved successful in animals. Therefore, more basic or fundamental research is still needed in the field of brucella immunology in order to find a safe and effective human immunizing agent for both primary and booster vaccinations, especially for those occupationally-exposed workers in areas with endemic bovine brucellosis, as it is at present in Oyo State of Nigeria. Suitable human vaccination will be of great advantage in reducing the level of human disease ir

many developing countries, where livestock management is still largely unscientific and large numbers of the population are being exposed to risks of brucellosis, without immediate or direct access to effective medical care.

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APPENDIX I

THE INTERNATIONAL STANDARD FOR ANTI-BRUCELLA ABORTUS SERUM (ISABS) (STANDARDIZATION AND INTERPRETATION OF THE "SERUM AGGLUTINATION TEST")

The Joint FAO/WHO Expert Committee on Brucellosis recommended that published papers including data on serological or milk tests in brucellosis should always indicate the sensitivity of the test used, and therefore the meaning of titres, by stating the titre at which 50% agglutination is obtained when the ISABS is tested with the antigens and methods concerned (WHO, 1953)[1].

The WHO Expert Committee on Biological Standardization later recommended the adoption of a Unit system (WHO, 1954)[2].

DESCRIPTION OF THE ISABS: The second ISABS (which was used during the present study) was established in 1958[3] and replaced the first International Standard. It was prepared from the serum of cow which had been infected experimentally with *Brucella abortus* biotype 1, strain 544 (FAO/WHO reference strain) six years previously and which had remained infected throughout this time. The serum was diluted to a suitable titre with *Brucella*-negative bovine serum. The diluted serum was distributed in 1 ml volume into neutral-glass ampoules and freeze-dried. The ampoules were filled with nitrogen at about atmospheric pressure and sealed. The average weight of dried material in each ampoule has been determined as 95.52mg. with a standard deviation of 3.04 per cent. The antibodies in the standard have been shown to consist entirely of immunoglobulin G (FAO/WHO Brucellosis Reference Centre, Weybridge, England).

THE INTERNATIONAL UNIT: The International Unit (I.U.) is defined as the activity contained in 0.09552mg. of the International Standard. Each ampoule therefore contains 1000 I.U. (WHO, 1969)[4].

SOURCE OF ISABS: The ISABS used in the present study was obtained, free of charge, from the International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey, England.

RECONSTITUTION OF ISABS: The material in each ampoule was reconstituted in 0.5 per cent phenol in physiological saline. Care was taken to ensure that the entire contents of the ampoule were completely resuspended.

CONTROL OF AGGLUTINATION TESTS: The ISABS was titrated along with every batch of agglutination tests. This allowed the potency of the sera being tested to be expressed in I.U. by comparing their titres with that of the ISABS.

Suppose, for example, the titre at which 50 per cent agglutination was obtained when the ISABS was tested by the antigen and method used was 1/500, a titre of 1/50 of a test serum with the same antigen and method would contain:

$$\frac{1000 \times 50}{500} = 100 \text{ I.U. per ml.}$$

ADVANTAGES OF ADOPTING AN INTERNATIONAL UNITAGE SYSTEM: The statement of agglutinin content in I.U. can have but one meaning because the I.U. has been defined by international agreement as indicating a given amount of Brucella antibody. It also ensures a valid comparison with the results obtained in different countries with different antigens and methods.

It enables a worker in any country to know at once the sensitivity of the test concerned and the real meaning of any diagnostic or other titres recommended (WHO, 1964)[5].

Appendix I: Literature Cited

1. World Health Organization (1953) Techn. Rep. Ser., No. 67.

2. World Health Organization (1954) Techn.Rep. Ser., No. 86.

3. World Health Organization (1958) Techn. Rep. Ser., No. 384.

4. World Health Organization (1969) Techn. Rep. Ser., No. 413.

5. World Health Organization (1964) Techn. Rep. Ser., No. 289.

APPENDIX II

PROFORMA: SURVEY OF HUMAN BRUCELLA INFECTION IN OYO STATE OF NIGERIA

DATE ..... LOCATION/STATION .....

NAME ..... IDENTIFICATION MARK .....

..... AGE ..... SEX .....

MARITAL STATUS ..... NO. OF CHILDREN AGES (YRS.) .....

ANIMAL OCCUPATION (NO. OF YEARS) ... .

.....

CONSUMPTION OF RAW MILK/ROAST MEAT (BARBECUE) .....

(A) CLINICAL FEATURES

SYMPTOMS	YES/NO	DURATION
- Fever		
- Chills or rigors		
- Loss of appetite		
- Constipation		
- Diarrhoea		
- Debility (loss of wt., etc.)		
- Weakness		
- Muscular pain		
- Articular pain		
- Eye involvement (type)		
- Nocturnal sweats/Excessive sweat ..		

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- Eye involvement (type)		
- Nocturnal sweats/Excessive sweat ..		

SYMPTOMS

	YES/NO	DURATION
- Insomnia		
- Nervous irritability		
- Neuralgia		
- Abdominal pain or swelling		
- Cough (? bronchitis)		
- Dysuria		
- Testicular swelling/pain		
- Skin rash		

Others (including family history)

SIGNS

- Nutritional status (and weight)
- Lymph glands enlargement
- Arthritic effusions
- Osteomyelitis/localised bone abscess
- Abdominal distension/tenderness

  (Inflammation of visceral organs: Enlarged Spleen/Liver)
- Peripheral and C.N.S. abnormalities
- Tenderness in the spinal column
- Cardiovascular system (Pulse and heart sounds)
- Other clinical findings

(B) LABORATORY TESTS

Brucella abortus Saline Agglutination Test (I.U.) ..

2-ME Test (I.U.) .. ..

Card Test .. ..

Widal Test (Titre) .. ..

Blood culture (i) Routine culture .. .. ..

(ii) Brucella culture .. .. ..

Blood film for Malaria Parisite .. .. ..

PCV .. ..

W.B.C. and Differentials .. ..

Blood film .. ..

Genotype .. ..

Urine examination .. ..

Radiological Investigations .. ..

Other Tests .. ..

(C) TREATMENT GIVEN

Tetracycline (500mg. 6 hrly.) for ..... days

Vibramycin (100mg. twice daily) for ..... days

Urfamycin (500mg. 6 hrly.) for ..... days

Other drugs .....

Brucella abortus agglutination Titre ..... (I.U.) after treatment

2-ME/Card Test ..... (I.U.) after treatment

Other Remarks.