SURVEY OF TUBERCULOSIS AND TUBERCLE BACILLI IN FULANI HERDS, "NONO" AND SOME HERDSMEN IN ZARIA AREA, NIGERIA.

BY

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"And Verily in cattle (too), will ye find an instructive sign. From what is within their bodies between excreations and blood, We produce for your drink, - milk pure and agreeable to those who drink it."

(Al-Nahl, xvi:66)

"Among Scientists are collectors, classifiers and collective tidiers-up: many are detectives by temperament and many are explorers: some are artists and others artisans. There are poet-scientists and philosopher-scientists and even a few mystics".

- Sir Peter Medawar.

D E C L A R A T I O N

I hereby declare the originality of this work, carried out by me in the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria under the supervision of Dr. A.A. Adesiyun, Professor P.B. Addo and Dr. S.N.A. Saidu.

The work of other investigators is duly acknowledged and referred to in this write up. No part of this thesis has previously been submitted else where for a degree or diploma.

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C E R T I F I C A T I O N

This thesis entitled "Survey of tuberculosis and tubercle bacilli in Fulani herds, "nono" and some herdsmen in Zaria area by Shehu, Ladan Mohammed, meets the regulations governing the award of the degree Master of Science of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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D E D I C A T I O N

This work is dedicated to the memory of my late father JUSTICE SHEHU MOHAMMED BELLO who died the very week I registered for this program. May the Almighty ALLAH (S.W.T.) grant him eternal rest. (Amen Thumma Amen).

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ABSTRACT 9

Mycobacteria are distributed all over the world with the exception of a few countries. Among die-eases produced by mycobacteria is tuberculosis, which is one of the most important zoonoses so far known to man. It is against this background that the present study was designed to explore further, the prevalence of these, organisms in Fulani herds and the environment in general, through tuberculin test survey of Fulani cattle, and to culturally examine fresh milk and sputum from such herds and market-based fermented milk, "nono".

Of 732 heads of cattle in twenty selected herds in and around Zaria, 5-00 were screened for mycobacteriosis using the tuberculin test. Fifty nine (11.8%) heads of cattle were detected to be reactors, while 43(8.6%) and 398 (79.6%) were suspects and non reactore respectively. Only one herd had no reactors nor suspects. Amongst reactors, the incidence increased with age, with 6.9% reactors in the 0-1 year age group, while those over five years old had 28(20.9%) reactors. The difference was statistically significant ($P < 0.05$, X^2).

Management system had an effect on the incidence of tuberculin test reactors with purely nomadic animals showing a greater number of reactors (72.88%) than intensively (0.0%) and semi-inteneively (27.12%.) managed farms. Sex had no effect on rate of reactor rates as 13(11.4%) of 114 males and 46(11.9%) of 386 females were positive. The difference was not statistically significant ($P> 0.05$; X^2). Out of the 90 fresh milk samples examined, only 1 (1.1%)was found to be presumptively positive for Mycobacterium species. Four (13.8%) of the 29 sputum samples examined from cattle milkers and herders were positive for the same organism. Eleven (4.2%) of 262 ready-to-drink market "nono" samples yielded mycobacteria. Of the 9 sputum samples from volunteer "nono" hawkers, 2(22.2%) yielded mycobacteria. The pH of "nono" and fresh milk had no effect on the frequency of isolation of mycobacteria from such sources.

A total of 18 mycobacterial isolates were obtained from all samples tested, 11 of which were obtained from ready-to-drink fermented milk, "nono", 1 from fresh cow milk obtained directly from the udder, while 4 and 2 were from sputum samples of herders and volunteer "nono" hawkers respectively. The only typical Mycobacterium isolated, M. bovis. was cultured from fresh milk. The remaining 17 isolates obtained from "nono" and sputum were atypical. Of the atypicals

isolated, 7 were Scotochromogenic, namely M. scrofulaceum (2 isolates), M. gortionae (aquae) (4) and M. flavescena (1). Six isolates were non-chromogenic, M. gastri (4) and M. triyiale (2); while the remaining 4 were rapid growers (Group IV) tentatively identified as M. smegmatis.

It is concluded that mycobacteria are wide spread in Fulani herds, herders, fresh milk and fermented milk (nono) . sold in markets in Zaria and perhaps else where, and this may constitute a health hazard to families in close association with the animals and consumers of locally fermented milk (nono). It is imperative that good sanitary practices should be employed during preparation and marketing of "nono".

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CHAPTER 1

INTRODUCTION

Mycobacteria constitute the group of micro-organisms known to be responsible for the production of debilitating diseases of man, domestic animals, birds and other warm blooded animals often characterised by chronicity and high fatality rate (Myers, 1968; Stead, 1971; Ellis, 1974; Youmans, 1979).

The word Mycobacteria originated from Greek words Myces-fungus, and bacterion - small rod. Mycobacteria are therefore fungus-rodlike organiams (Runyon et al., 1974). The specific epithet mycobacteria was assigned to this group of organism by Lehmann and Neumann in the year 1896, a few years following the discovery of the organisms by a German Scientist - Robert Koch (Collins and Grange, 1983).

Although mycobacteria are to a limited extent gram-positive, they are not readily stained by the popular Grams method, but once stained they resist decolorization by acids and alcohols, and are therefore quite often referred to as acid and alcohol fast bacilli.

They are aerobic non-motile, slightly curved or straight rods 0.2 - 0.6 by 1 - 10 um in length (Runyon et al, 1974), but of recent Anonymous, (1988) reported the occurrence of mycobacteria in ultra-fine spherical

forms, that are 20 times smaller than the conventional rods.

The pathogenic members of the group, quite often referred to as the 'typical' that produce the more important human and animal infections, have enjoyed the leadership of the group in terms of importance for nearly three-quarter of a century but of recent, the other members of the group generally referred to as the atypical, anonymous, opportunistics, among others, that were initially considered non pathogenic, are now gaining wider recognition in terms of disease production in man and animals. The typical members of the group constitute Mycobacterium bovis (bovine tubercle bacillus), Mycobacterium tuberculosis (Human tubercle bacillus) and Mycobacterium avium.

The prevalence of mycobacteria in cattle dates back to the times of Robert Koch (Collins and Grange 1983). Since then, they have been recorded increasingly with a variety of disease conditions in cattle, and from the common stand point of veterinarians and physicians, the most important clinical manifestation of tuberculosis in bovines is mastitis (McKay, 1941).

The progress which has been made towards the total eradication of tuberculosis has changed the pattern of its present distribution, and countries which at the turn of the century that had the highest prevalence, now have the lowest, while countries in South America,

Asia and Africa, which probably acquire the disease by way of Europe, are today the problem areas (Alhaji 1976b).

The economic losses to the cattle industry due to mycobacterial diseases is so great that it is difficult to estimate. Losses result from milk and meat condemnation (Stamp and Wilson, 1946; Eid, 1976), death of offspring, decrease in life span of breeding animals, insufficient feed utilisation, decreased milk production, infertility, poor condition, interference with herd improvement programs, and low market value (Alhaji 1976b).

Myers (1968) reported the condemnation of 50,000 carcasses of cattle with tuberculosis in Minnesota (U.S.A.) alone. World Health Organisation/Food and Agricultural Organisation report 1951 projected the possibility of reduction of 10-25% efficiency in an infected cattle (Alhaji, 1976b).

Losses to the human population in terms of money used in purchasing drugs to treat infected persons as well as for funding tuberculosis research programs is great (Miles and Maat, 1984). A lot of money was and is still being utilized for the establishment of diagnostic laboratories, and compensation of slaughtered tuberculous animals.

In addition to the economic loss, mycobacteria have been found to be of direct health hazard to the

human population. There are several reports in which it has been associated with diseases of human concern (Karlson and Carr, 1970; Wigle et al, 1972; Damsker et al., 1974; Tsukamura et al., 1983; Chan et al., 1984; Palmer and Watanakunakorn, 1984; Pitchenic et al., 1984; Warren et al., 1984; Gribeth et al., 1985; Horsburgh et al., 1985; Rolston et al., 1985; Goldman, 1987).

Furthermore, because of their ubiquitous nature (Horsburgh et al., 1985) and the wide variety of susceptible host, mycobacteria have been isolated from a number of sources including faeces, mastitic udders (Gajinder et al., 1957), tap waters and soils (Ellis, 1974). They have also been reported from fresh and fermented milk samples of bovine origin (Chapman and Spheight, 1968; Hosty and McDurmont 1975; Alhaji and Shnurrenberger, 1977; Appuswamy et al., 1980); as well as sputum samples of human origin (Kotian et al., 1981; Kuze and Uchihira, 1984).

In a study done in this environment by Alhaji (1976a) attention was mostly given to animals in government farms with little importance attached to Fulani herds from where raw milk used in preparing "nono" and other products emanate.

Based on the frequency with which these organisms are isolated from a number of food products meant for human consumption, along with the ease with which they are disseminated between man and animals, there is

therefore the need to carry out further epidemiologic studies, especially in Nigeria where literature on these organiams is scanty.

This project was therefore designed with the following specific objectives:-

i. to determine the prevalence of Mycobacterium species in selected fulani herds through tuberculin testing of their cattle;

ii. to screen milkers in various herds for mycobacteria through cultural examination of sputum samples; and

iii. to determine the frequency of isolation of Mycobacterium species from raw milk and fermented milk products in local markets in the vicinity of herds tested.

CHAPTER 2

LITERATURE REVIEW

2:1 Historical background

Since time immemorial tuberculosis has been a scourge of man and other domestic animals whose exact nature remained questionable for sometimes (Collins and Grange, 1983).

Tuberculosis as a disease of man was described in Italian writings two thousand years before Christ was born (Kleeberg, 1975). Though tuberculosis of spine was found in Egyptian mummies, as well as in the early stone age people and Peruvian mummies, Hippocrates was the first to observe and describe clinical 'phthis', as tuberculosis was then referred to in domestic animals (Myers and Steel, 1969). The epidemic nature of tuberculosis was also recognised by the Greek and Roman empires. Sylvius, a Roman Scientist was the first to describe the nodular growth of lesions characteristics of tuberculosis and termed these lesions 'tubercle' (Ayanwale, 1984).

The danger of eating meat from tuberculous cattle was found in Mosaic laws and the German regulations prohibiting the sale of tuberculous meat (Collins and Grange, 1983). The trade and movements of cattle from different parts of the world contributed in no small way to the spread of the disease to virgin areas

particularly in Europe.

Tuberculosis ravaged the entire world; and several English writers described the disease in different forms. Clement in 1893 for example, referred to tuberculosis as an enemy which probaly destroyed more lives annually than all of the so-called scourges or wars put together (Ayanwale, 1984).

During the 18th century, the Germans, believed the disease in cattle was related to human syphilis and therefore termed it "frazosenkrankheit" (The French disease). The unflattering epithet was derived from the fact that for centuries syphilis had been known as French disease (Collins and Grange, 1983). An earlier observation on the nature and epidemiology of the disease was noted by Klenke who in 1846, made an observation on the higher frequency of tuberculous lymphadenitis among infants given cow milk than among those breast fed, and he thus incriminated cows milk as the most likely source of the disease.

Earlier on, an association between the 'Scrofula' unique characteristics of tuberculous lesion and consumption of cow milk had been noted by Carmichael in 1810. Some years later, a French Military Surgeon - Villemin, in 1868, inoculated rabbits with tuberculous materials from diseased cows and found that they developed tuberculosis. Incidently he also observed the virulent nature of infected material in young

rabbits (Collins and Grange, 1983).

The turning point in the history of mycobacteria in general and tuberculosis in particular, occurred on March 1882, when a German Scientist, who was later to be honoured with the title of the 'father of modern clinical bacteriology' - Robert Koch, publicly announced that he had observed and cultured the bacillus responsible for tuberculosis (Groschel, 1982). The name Robert Koch will no doubt remain in the history of bacteriology without parallel.

Robert Koch inoculated guinea pigs with tuberculous material and was able to prove the infectious nature of the tubercle bacilli from human and animal cases (Groschel, 1982). Among one of his numerous contributions that survived the test of time and which applies not only to mycobacteria alone, but other microorganisms as well; was what is today popularly known as the "Koch's postulates."

Following this momentous discovery, Koch did not give his bacillus any specific name but merely termed them 'tuberkelbacellain' - bacillus of tuberculosis. However in the same year of the discovery of tubercle bacilli (1882), Paul Erhlich discovered the acid-fast nature of mycobacteria and the specific name "bacterium tuberculosis" was proposed by Zopf, a year later. The term tuberculosis as earlier mentioned was coined from a Latin word meaning small lumps or nodules that appropriately describe the basic pathologic lesion

characteristic of the disease in affected individuals (Collins and Grange, 1983).

In the year, 1896 Lehmann and Neumann assigned the species to the genus Mycobacterium. At first, Koch and others considered that there was only one type of tubercle bacillus affecting man and animals. At that time the idea became so firmly entrenched that, Smith in 1896, wrote that "the absolute identity of tubercle bacilli has been so generally assumed that any one who would attempt to question its identity must be prepared to meet considerable sceptism". Nevertheless it was demonstrated that there were small but definite differences in strains from human and bovine source. In particular, bovine strain were found to be shorter and grew less vigorously in vitro than their human counterpart (Collins and Grange, 1983). Smith (1896), also found that bovine bacilli were more virulent for a range of animals especially the rabbits. This conclusion has been reached previously by Villemin (1868), during his experiment with tuberculous tissues. Koch in 1901, changed his opinion on the absolute identity of the tubercle bacilli, but maintained that the human subject is immuned against infection with bovine bacillus, or is so slightly susceptible, that it is not necessary to counteract risk of infection. A year later he further declared that "I feel justified in maintaining that human tuberculosis differs from

bovine and cannot be transmitted to cattle."

This erroneous conclusion however, encouraged Koch and his associate to prepare a vaccine for veterinary use from human tubercle bacilli supposeldly attenuated by ageing (Collins and Grange, 1983). This vaccine termed "tauruman" and its kind produced by Von Behring and his colleagues achieved some popularity, until Griffith (1913) demonstrated that vaccinated cattle excreted viable and virulent human bacilli in their milk.

Koch's view stimulated the interest of two other scientists - Calmette and Guerrin, in their choice of a bovine strain for the preparation of a vaccine BCG for use in man. In 1890, in an attempt to find an effective means of treating tuberculosis, - Koch came up with a product that proved to be of diagnostic significance.

2.2. The Organism and disease produced

Apart from being uniquely acid alcohol fast, mycobacteria produce nodular growth in affected organs (Youmans, 1979).

The typical members of the group in addition to producing classical pulmonary tuberculosis in lungs have the ability of producing local infection in almost all organs of the body and as such clinical manifestation of the disease produced generally reflects the function of the affected organ.

Tuberculous infections of organs other than the lungs are often referred to as extra-pulmonary (Farer et al., 1979).

For so long, such extra-pulmonary lesions have appeared in the literature under a variety of names such as 'phthis', consumption, pott's disease (spine), lupus vulgaris (skin), scrofula (cervical lymph nodes). Lesions due to mycobacteria have been reported from some of the important organs of man and animals like the brain and spinal cord (Santamaria - Jaureigui et al., 1984), and mammary glands of cow (MacKay, 1941) where they produce mastitis. They have also been reported from human milk (MacKay, 1941). Stuart and Harvey (1951) also reported successful isolation of mycobacteria from bovine mastitic udder.

On the other hand the atypicals as they are so called, are being reported with greater frequency all over the world. Robbinowitsch in 1927, drew attention to the acid fast bacilli resembling mycobacteria (Kotian et al., 1981). He felt that these saprophytic organisms could cause disease. Pinner (1935) gave the name atypical mycobacteria and proved the association of certain chromogenic acid fast bacilli with human diseases and therefore emphasised the need for proper bacteriologic assessment (Kotian et al., 1981).

The pulmonary lesions produced by some of the atypicals closely resemble those produced by M. tuberculosis roentogenologically and pathologically

(Johnson et al, 1961) and to a limited extent clinically, though there are distinct differences in virulence treatment and prognosis.

Mycobacteria are believed to be responsible for a number of infections in man and animals, some of which could be fatal. Karlson et al. (1970) documented six cases of tuberculous infection at Mayo Clinic (United States of America) some of which ended fatally. Similarly, Wigle et al. (1972) reported mycobacterial infections in man. Other human conditions reported from various parts of the world include brain lesions (Levy, 1973), skin ulcers (Baker, 1974), tuberculosis of spine (Anon, 1974a), multiple bone lesions (Ellis, 1974), pulmonary infections (Weizman et al., 1980), disseminated infections of various organs (Savage and Dellinger, 1983), lung cancer (Tsukamura et al., 1983), osteo-arthritis and tendosynovitis of fore fingers (Demoulin et al., 1984) pulmonary infections (Engbaek et al., 1984; Warren et al., 1984), pericarditis (Palmer et al., 1984; Santamaria-Jauregui et al., 1984), and a variety of multiple infections of other organs from different parts of the world (Narain et al., 1968; Prowse and Cavannagh, 1976; Farer et al., 1979; Kotian et al., 1981; Horsburgh et al., 1985).

2.3. Classification

To the founding fathers of bacteriology, the classification and nomenclature of pathogens was

straight forward. Tuberculosis was caused by tubercle bacillus and leprosy was caused by leprosy bacillus and when these pathogens were allocated to the genus Mycobacterium, this posed no problem, because they merely became M. tuberculosis and M. leprae respectively (Collins et al. 1984). This golden age of simplicity was however short-lived as it soon became apparent that tubercle bacilli were not all identical, and that some tuberculous like infections were caused by other mycobacterial spcies that normally exist as saprophytes in the inanimate environment (Collins et al., 1984).

Because of the confusion that arosed in the nomenclature and classification of mycobacteria (Collins and Yates, 1984), the term tubercle bacilli was reserved for M. tuberculosis comprising other organisms like M. bovis and M. africanum as variants or sub species, while mycobacteria other than tubercle bacilli are simply classfied as "others", "atypicals", "anonymous", "opportunists" (Collins et al., 1984). Accordingly, other names still used from time to time are "MOTT" (Mycobacterium other than tubercle bacilli) "non-tuberculous mycobacteria". To this names, Collins et al. (1984) have recently added a poetically inspired epithet "nyrocine mycobacteria".

For epidemiological purposes, Collins et al., (1984) and Collins and Yates (1984), have therefore

classified the tubercle bacilli into the following:

1. Mycobacterium tuberculosis. The classical human variety.

2. Mycobacterium tuberculosis. The Asian variety. Originated from Southern India.

3. Mycobacterium tuberculosis var. bovis, the classical bovine variety, synonymous with M. bovis, infections with which are not necessarily of bovine variety.

4. Mycobacterium tuberculosis African 1 var: Corresponds to M. africanum of the type isolated mainly in patients from West Africa.

5. Mycobacterium tuberculosis African II var. corresponds to M. africanum of the type isolated mainly in patients from East Africa.

Others (atypicals)

Isolation of this group of mycobacteria has become so frequent during the 1950s as to result in confusion both in the laboratories and in hospitals, but Timpe and Runyon in 1959; elaborated a syetem of classification whose acceptance and success has stemed from its simplicity and acceptability to clinical problems (Alhaji, 1985). Grange (1984) also shed some light on the classification of the M. avium group. Ealier on, Xalabarder (1961), reviewed the problems of

classification of the atypicals. Chapman (1977), reported the Timpe Runyon classification as outlined in TABLE 2:1 below.

TABLE 2:1 Timpe Runyon classification of mycobacteria on the basis of pigmentation and growth rate.

Group	Pigment	Growth rate	Colonical features
I.	Photochromogenic	12-14 days	Rough
	M. kansasii		Eugonic
	M. marinum		
	M. simiae		dysgonic
II.	Scotochromogenic	10-14 days	Smooth
	M. scrofulaceum		Eugonic
	M. szulgi		
III.	Non photochromogenic	14-21 days	Dysgonic
	M. avian complex		
	M. intracellulare		
	M. xenopi		
IV	Rapid growers	5 - 7 days	Eugonic
	M. phlei		
	M. fortuitum		
	M. ulcerans		
	M. smegmatis		
	M. chelonei		

The list of mycobacteria approved by the International Committee on Systemic Bacteriology that is accepted world wide is made up of over 41 species or strains majority of which are saprophytic (Collins et al., 1984).

2.4: General characteristics of mycobacterial organisms

2.4.1 Mycobacterium tuberculosis

The organism is rod-shaped straight or slightly curved, occurring singly often showing beading (Runyon et al., 1974). It is a slow grower on solid media for example Loweinstein Jensen with colonies that are usually raised, matte with irregular margin (Runyon et al., 1974; Youmans, 1979; Kent et al., 1985). In liquid media M. tuberculosis growth is flocculent with pellicle and sediment in 2-3 weeks. Among unique features of this organism are cord formation, catalase negative reaction at 68°C, nitrate reduction, growth inhibition by isoniazid, para-amino salicylic acid, and rifampicin at 5mcg/ml (Youmans, 1979). M. tuberculosis produce disease in man; domestic animals and primates. Experimentally it has been found to be pathogenic for guinea pigs and hamsters, but relatively non-pathogenic for rabbits (Runyon et al., 1974).

2.4.2 Mycobacterium bovis

The organism occurs as a moderately long rod with poor growth on primary isolation on glycerol-containing

media (Runyon et al., 1974). It is a non-pigmented slow grower that takes three weeks or longer to appear on solid media for example Loweinstein Jensen (Youmans, 1979). Mycobacterium bovis is slightly anaerobic with small rounded buff coloured colonies on solid media often with irregular edges. In liquid media M. bovis forms granular sediment seldom with a pellicle.

Some unique features of M. bovis include inhibition by tricarboxylic acid hydrazide (T.C.H) at 2 mcg/ml, cord formation, catalase negative reaction at 68°C, negative nitrate reduction test. The organism is pathogenic to man, domestic and wild animals, guineapigs and rabbits (Runyon et al., 1974).

The bacillus Calmette - Guerin (BCG) comforms to the properties described for M. bovis except that it is much attenuated in pathogenicity and grows well on glycerinated media (Runyon et al., 1974).

2.4.3 M. avium

It is a slow growing long sometimes filamentous rod whose colonies are usually non-pigmented and appear in 2-3 weeks on primary isolation and about a week on sub culture (Runyon et al., 1974; Youmans, 1979). The organism grow better at 40°C. It grows in suspension in liquid media such as phosphate buffered saline. Unique features of M. avium include the production of smooth glistering non-pigmented colonies, negative tween hydrolysis, negative niacin reaction, poor cord

formation, positive nitrate reaction (Youmans, 1979). The organisms is pathogenic mainly to domestic fowl, but could also affect man and other domestic animals (Runyon et al., 1974).

2.4.4 M. kansasii

The organism is a morderately long slow growing rod that exhibit cross-barring on incubation. The organism is buff coloured but becomes pigmented on exposure to light, and M. kansasii lose cord formation occasionally and some of its unique features include photochromogenic reaction in the presence of light (Runyon et al., 1974). It is catalase positive at 35°C and 68°C and hydrolyses tween 80 in 5 days. It produces pulmonary infection in man.

2.4.5 M. marinum

It is also a moderately long slow growing rod that produces cross-bars at 24-30°C on solid media. Its colonies are usually non-pigmented or buff colored, but on brief exposure to light turns brilliant yellow (Runyon et al., 1974; Youmans, 1979). In liquid media M. marinum grows with turbidity with viscous sediment that suspends on shaking. The organism does not reduce nitrate and rarely form niacin but hydrolyses tween 80 in less than five days. It affects mainly fish, but also produces "swimming pool granulomas" in man which usually heals spontaneously (Runyon et al., 1974).

2.4.6 M. scrofulaceum

The organism is short or filamentous with yellow or orange colonies in seven or more days on solid media. Colonies of the organism are usually round, glistering and pigmented in darkness and in light (Runyon et al., 1984). In liquid media it grows with turbidity. It forms no cords, is niacin negative and does not reduce nitrate, nor hydrolyse tween-80. It affects man particularly children (cervical lymphadenitis), but of limited pathogenicity to experimental animals.

2.4.7 M. gordonae

It is a slow growing Mycobacterium that produces pigment in the dark which intensifies on exposure to light. It grows with turbidity in liquid medium hydrolyses tween 80 in five days; does not reduce nitrate and does not produce niacin. It has been mainly reported from tap water.

2.4.8: M. xenopi

The organism is a slow grower that produces pigment both in the dark and on exposure to light (Youmans 1979). It has occasionally been associated with chronic pulmonary disease of man and infrequently genitourinary tract infection of man.

2.4.9. M. terrae

M-terrae is a non-photochromogenic slow grower that reduces nitrates; hydrolyses tween 80, produces

catalase at 68°C but no niacin. It has been reported mainly from soil and sputum of man (Runyon et al., 1974).

2.4.10 M. triviale

M. triviale is a non-chromogenic Mycobacterium that produces rough heaped non-pigmented colonies on solid media. It is niacin negative, but reduces nitrate, catalase positive at 68°C, and hydrolyses tween 80 in five days. It also grows fairly well in medium containing five percent sodium chloride (Runyon et al., 1974).

2.4.11 M. gastri

It is a non photochromogenic organism that produces smooth to rough colonies on solid medium. It hydrolyses tween-80 in five days but does not produce catalase at 68°C. It does not produce niacin nor reduce nitrate. It has been isolated mainly from human gastric lavage and soils (Runyon et al., 1974).

2.4.12 M. chelonei

The organism is a rapid grower that grows in less than one week with smooth colourless round colonies. It grows on MacConkey agar in five days (Youmans, 1979), it does not reduce nitrate, and does not produce niacin. It is found mainly in soil but affects man (Runyon et al., 1974).

2.4.13 M. ulcerans

M. ulcerans is a slow grower that takes about 3 weeks to grow with small round colorless colonies. It grows better at 30°C but rarely at 37°C. It does not produce niacin, does not reduce nitrate, but is catalase positive at 68°C (Runyon et al., 1974). It causes skin ulcers in man.

2.4.14 M. flavescens

The organisms produce intensely orange coloured colonies which adhere strongly to the medium in less than one week (Runyon et al., 1974) and could be pathogenic to man.

2.4.15 M. leprae

The organism is an obligate intracellular parasite of man that does not grow in vitro (Youmans, 1979). Baccilli are however reported to range in size from 0.2 to 0.5 by 1.5-8um in length with parallel sides and rounded ends (Runyon et al., 1974; Youmans, 1979).

2.5 Epidemiology

2.5.1. Distribution and Prevalence

Animals

Although the epidemiology of tuberculosis and mycobacteria in general has remained poorly understood for quite sometime (MMWR, 1982, Collins and Grange, 1983), the significant contributions of Hampton in 1930s should not go unnoticed (MMWR, 1982).

Mycobacteria have a world wide distribution and are found in all continents of the world except Antartica (Horsburgh et al., 1985). Mycobacteria, being ubiquitous in nature, have been isolated from a number of environmental source such as water and soils and contaminated ponds (Galli-Vellerio and Bolnaud, 1927; Mollahan and Romer, 1961; Appuswamy et al., 1980).

The progress which has been made towards the total eradication of bovine tuberculosis has changed the pattern of the present distribution (Alhaji, 1976a). Countries which at the turn of the century, had the highest prevalnce of the disease now have the lowest while countries in South America, Asia and Africa which probably acquire the disease by way of Europe are today the problem areas. Tuberculosis was known to be widely disseminated in countries where animals are housed together for long periods and in large herds (Alhaji, 1976b). It was particularly prevalent amongst dairy herds in the vicinity of large European cities, but by 1930s countries such as Finland (Huhtala, 1953), Denmark and Norway (Francis, 1947) have been reported free of disease. A five per cent reactor rate was reported in United States of America following the start of eradication program (Johnson et al., 1961). In Australia the situation was about the same (Francis, 1947). In India at the beginning of the century the

prevalence was between 1.6 to 16 per cent in cattle and 3 to 25 per cent in buffalos, but by 1973, the corresponding figures have gone down to 5-10 per cent and 4.6 per cent respectively (Nagaraja et al., 1973).

Apparently, tuberculosis had been rare in parts of Africa and Asia where animals constantly live in the open. However, the introduction of the European breeds of cattle and the subsequent development of intensive agriculture rapidly changed the distribution of the disease in these areas. For instance, Ellwood (1975) reported a high prevalence among the cattle population in Malawi, while Eid (1976), also recorded a prevalence as high as 25 per cent in some areas in Northern Nigeria but in a comparative tuberculin survey only 4 per cent prevalence rate was recorded by Alhaji (1976a).

Human

Until the 1920s, no extensive work has been undertaken to highlight the prevalence of the disease in man (Myers, 1968). Tuberculosis was reported to be responsible for the death of about 97,336 persons in the United States of America alone in 1920 (Myers, 1968). Greenland, where tuberculosis used to account for one third of all death in the fifties is now reported free of the disease (Iverson, 1971).

Levy, (1973) documented thirty six cases of tuberculoma of the brain in Malawi, Rhodesia and

Zambia.

The substantial reduction of tuberculosis in England and Wales during the early sixties gave rise to potentially dangerous misconceptions about the level of the disease which resulted into some negeligence (Katz et al., 1985). It was regarded as rare with only about 11,280 notifications in 1970 in England. About 1465 death in the same year were attributed to tuberculosis in England which not only exceeded those due to other notifiable infectious disease but also exceeded the total of all notifiable infectious disease put together. An unjustifiable belief that the disease was rare led to delay in diagnosis with subsequent delay in initiation of treatment, and hence prolongation of period of infectivity (Springett, 1972).

The distribution of tuberculosis usually follows a particular pattern. It has been found to be associated with poor environmental conditions (Springett, 1972; Katz et al., 1985), and in addition it has also been reported to be more prevalent among older men living in inferior accomodations with low dietary intake and it has also been found to be strongly associated with alcoholism (Katz et al., 1985). It has been further claimed that most death due to tuberculous agent occur in poor individuals as a result of failure on part of the patient to seek proper medical attention in the event of infection (Springett, 1972). But of recent Fenstenstein (1984) reported a number of cases of

active tuberculosis amongst hospital doctors in London. The problems of tuberculosis could sometimes be due to failure on the part of physician to provide the patients with the most suitable drugs or a complete drug regimen that will last the period of treatment (Springett, 1972).

Anon. (1979) in England is also of the view that social deprivation, especially amongst the lower class of the society, could lead to a high attack rate due to inability or decreased ability to mount resistance against the invading bacilli, also overcrowding at working places and homes could lead to a rapid spread of the disease. Poor standards of hygiene and failutre to take advantage of preventive measures at disposal are also contributory.

From the literature, most reported human infections were from America, Europe, and a few other developed nations and no doubt the scanty nature of cases from Africa and other developing nations is a true reflection of the little work done on the epidemialogy of mycobacteria in these areas. Infections no doubt exist among Africans and Asians since of recent, several outbreaks in the western world were linked with African and Asian immigrants into those countries (Chatterjee, 1974; Prowse and Cavanagh, 1974).

Despite a substantial fall in the incidence of

human and bovine tuberculosis in the developed countries, as a result of large scale developmental programs and provision of adequate health facilities and medical care, new cases of infections are recorded daily.

With the failure of a good number of laboratories to recognise mycobacteria and also the frequency with which they are often misdiagnosed, an attempt made to assess the overall incidence of Mycobacterium africanum and other atypical mycobacteria in the United Kingdom, proved abortive (Collins and Yates, 1984). However, shortly there after, the latter reported from the United Kingdom 87 (1.2%) M. africanum infection in human population.

With the recent advance in better diagnostic technics, the trend of mycobacterial infection among the human population has changed, and cases due to atypical mycobacteria are now assuming greater proportion, especially in the developed countries where there is proper documentation. A strong correlation was reported between the isolation of M. scrofulaceum and M. avian intracellulare from flood plains of four rivers in the United States of America and a positive tuberculin reaction among naval recruits that dwell around the rivers (Brooks et al., 1984). Also of recent several cases due to M. malmoense and M. africanum were reported from Europe and United States of America (Warren et al., 1984; Santamaria - Jauregui,

1984). From Asia, several human infections with atypical mycobacteria were reported (Kotian et al., 1981; Kuze and Uchihira, 1984). In Denmark seven per cent of all lung infection were reported to be due to atypical mycobacteria (Engbaek et al., 1984). Baker (1974) related a case of M. ulcerans to swampy area and cited a good number of outbreaks of a similar nature in Uganda, New Guinea, and Ibadan (Nigeria) which were all traced to swampy areas. Thirteen cases of infection with M. avium - intracellulare were reported from Europe (Horsburgh et al., 1985). In America and Japan, M. kansasii was found to be responsible for a greater percentage of infections due to atypical mycobacteria (Tsukamura et al., 1983). A very dangerous trend that has of recent been observed in the pattern of diseases produced by the atypicals is the high frequency with which they occur along side important human diseases such as croh's disease (Gitnich, 1984; Thayer et al., 1984), lung cancer (Boncheck, 1971) and Acquired Immuno Deficiency Syndrome - AIDS (Chan et al., 1984; Pitchenick et al., 1984; Goldman, 1987).

2.5.2. Predisposing factors to infection with mycobacteria

Animals

Among the animal population, overcrowding (Alhaji 1976b) is known to facilitate the spread of mycobacteria amongst housed animals. Other factors

include poor nutrition, poor medical attention and over-crowding at watering points.

Man

Human beings have been reported to acquire infection more readily with mycobacterial organisms when exposed to a number of predisposing factors. Such factors include poor living conditions (Anon, 1979) alcoholism, inadequate knowledge of the organisms and the disease they produce (Aitken et al., 1984) as well as old age. Majority of cases documented in the literature were people over fourty years old (Damsker et al., 1974, Prowse and Cavannagh, 1976; Palmer and Watanakunakorn, 1984; Horsburgh et al., 1985). However children under fifteen years of age have also been reported susceptible (Prowse and Cavannagah, 1976).

2.5.3: Susceptibility and Pathogenicity

There is some degree of host susceptibility to the various species of Mycobacterium, although cross-infections are known to occur, with animal species being more susceptible to bovine tubercle bacillus, while man on the other hand appears more susceptible to the human specie (Collins and Grange, 1983). Dog has been found to be more susceptible to the human species (Alhaji, 1976b).

Of the domestic animals cattle and pigs are more readily susceptible to either natural or artificial infection with bovine bacillus (Krishnaswamy et al.,

1974). Although infection in cattle with human species does not often result in progressive diseases, Stuart and Marshall (1952) indicated that such bacilli could remain viable for sometimes in the organs and tissues affected, with frequent excretion from milk without apparent signs of the disease. Stenius (1938) cited a fatal case in a calf traced to a cow man who was looking after an infected herd. Earlier on Griffith in 1913, reported of symptomless cows excreting virulent human bacilli in their milk following experimental inoculation or natural infection (Collins and Grange, 1983).

Horse

It is remarkable how seldom tuberculosis is diagnosed in the horse even in the countries where tuberculosis of other animals is common (Luke, 1958). There seems to have been very few surveys carried out in horse populations. There is also limited information regarding susceptibility under experimental conditions and indeed there is also a general agreement regarding the low incidence of the disease in horses passing through the battoir (Luke, 1958; Leeslie and Davis, 1958).

Calmette in 1927 (Luke, 1958) stated that barely one horse in 15,000 showed evidence of tuberculosis. No single case was recorded among those slaughtered over a 10 year period at London zoo. From Germany,

Bywaters (1949) during a period of six years found only 3 cases among 80,000 horses examined. Of the 11,544 examined by Grant (1949) in Scottish abattoirs, no case was recorded, but from Ireland, Dromey (1949) recorded 4 cases among 980 examined. Of all the isolations made, about 80 percent were the avian type and progressive in nature.

Swine

A number of reports indicated that they are susceptible to both natural and artificial infection with avian bacillus (Luke, 1958); but from all indications they have been found susceptible to the human bovine and avian strains. Mycobacterium bovis has been found to cause widespread infection in pigs with encapsulated granulomas in affected organs and indeed such pigs continue to shed the organisms until towards the terminal stages of the disease (Fichanler and Osborne, 1966). The latter reported an outbreak of tuberculosis in a swine herd traced to consumption of infected cow offal. Several authors have cited isolations of human strain of bacilli from pigs. Buttler and March (1927) reported - investigating an outbreak of tuberculosis in swine fed swill from a tuberculous sanatorium. Infections resulting in serious outbreaks have also been reported in swine fed offal from tuberculous cattle.

Ovine (Sheep)

Though tuberculosis is a rare disease in sheep, there are reported cases of sheep acquiring infection with bovine strain. The first recorded case of tuberculosis in Great Britain in sheep was by M'Fadyean in 1900 (Luke, 1958).

Caprine (goats)

Though rarely susceptible, there are reports of goat succumbing to mycobacterial infection (Luke, 1958).

Canine (Dogs)

Dogs acquire infection more readily with human bacilli but infections with M. bovis is also a possibility. Ayanwale and others (1983) reported a case in a dog traced to bovine strain thought to have been acquired from eating abattoir scraps.

Feline (Cats)

Cats acquire infection more readily with the bovine strain as a result of their milk drinking habit (Snider, 1971) but infections with human strains is also a possibility.

Primates

Primates especially in zoological gardens have been reported susceptible to M. bovis (Ruch, 1959).

Wild Animals

These are also susceptible to infection with mycobacteria (Alhaji, 1976b; Collins and Grange, 1983) and as such, could serve as reservoir for human and animal populations.

Birds

Although birds are more readily susceptible to infections with the avian specie, parrots have been found unique in their susceptibility to mycobacteria in that they are generally less susceptible to the avian specie, and infections mostly occur on exposure to human cases (Ackerman et al., 1974). Ackerman and his associates (1974) documented an unusual case in a parrot in the United States of America with lesions restricted to the head region of the bird.

Human

As early as 1907, it was known that bovine bacillus can be responsible for serious form of tuberculosis in children (Alhaji 1976b). Fatal cases of tuberculosis in Australia in children majority of whom were below the age of five were traced to M. bovis acquired through the consumption of raw milk (Francis, 1947). Earlier on Mackay (1941) cited a number of reports in which bovine bacilli were isolated from lactating women in the United States of America. Infections in man due to M. bovis were also documented by Wigle et al. (1972) and Damsker et al. (1974). From

Nigeria Alhaji and Shnurrenberger (1977) reported isolating M. bovis from the sputum of a farmer. Mycobacterium bovis is reported to behave in the same manner with human bavilli once it enters the body, and such infected individuals could remain a constant source of infection to animals and other fellow human beings (Collins and Grange, 1983).

Similarly Karlson and Carr (1970) reported six human cases of tuberculosis in an American hospital between 1954 and 1968, which were thought to be bovine type. Mycobacteria are also reported from human milk samples (MacKay, 1941), There was the report of a herdsman who had to undergo surgery (lobectomy) following infection with M. bovis he acquired from a herd he lost to tuberculosis a fews months back (Baldwin, 1968). Infections in man with atypical mycobacteria have also been reported from America and Asia (Barker, 1974; Kotin et al, 1981; Tsukamura et al., 1983).

2.5.4 Transmission

Animals

At the end of the last century, there was a unanimous view that tuberculosis in cattle was spread by way of the respiratory tract. However, during the early decade of this century, opinion differed as to mode of transmission of pulmonary tuberculosis in

cattle. While some workers felt that pulmonary tuberculosis was due to inhalation of the bacillus, (Collins and Grange, 1983), others felt that the lesions in the lungs were always secondary to an initial lesions in the alimentary canal. The debate was finally settled by Lange in 1931, who convincingly demonstrated that the earlier view had been the correct one (Collins and Grange, 1983).

Further compelling experimental proof was provided by several other workers later and so it became firmly established that the principal route of transmission of bacilli among herds of cattle is the aerogenous one (Collins and Grange, 1983), although oral means could also be possible (Stenius, 1938).

At present it is a well known fact that transmission occurs more readily amongst housed or crowded animals, hence the higher incidence amongst dairy cattle than amongst beef cattle kept under range conditions (Alhaji, 1976b). Air inhaled by housed cattle become contaminated by droplet of expectorate or dust arising from dried excretion and when transmission does occur by ingestion the source of infection is usually infected milk, food or water contaminated with infected discharges and feaces. Alhaji (1976b) reported further that infection is also possible through coitus or possibly the broken skin. Among a set of calves examined, Stamp and Wilson (1946), found the congenital means of transmission predominating.

Infection has earlier on been documented via the teat canal at medication (MacKay, 1941). Also in support of the concept of congenital form of transmission were Collins and Grange (1983).

Transmission in domestic animals other than cattle follows a similar trend to that of cattle, but more often than not, transmission follows the pattern of association with the source (Luke, 1958). Inspite of the fact that transmission mostly occur through direct inhalation of infected droplets, oral route at watering points, could also be a possibility.

In pigs invariably the oral route serves as the major means of acquiring infection, particularly via infected offals not properly cooked as cited earlier on.

Human

In man, tuberculosis has been associated with poor environmental conditions, age and nutrition, cigarette smoking and alcoholism (Springett, 1972) but other means of transmission are oral or aerogenous.

Around the 1940s, there was a widely held opinion that bovine tubercle bacilli (M. bovis) in man was always acquired by alimentary route, whereas human tuberculosis was almost always acquired by inhalations (Collins and Grange, 1983). The idea that cattle could directly infect man by the pulmonary route was barely considered, until after a number of studies were

conducted.

Today it is a well established fact that bovine tubercle bacilli can reach their human target through a number of means, such as direct inhalation of contaminated air, oral through consumption of contaminated or infected meat, milk and their products. A number of cases in children linked to consumption of contaminated milk werecited by Collins and Grange (1983). Transmission has also been reported possible via direct contact with the infective agent as well as through vaccination sites (Damsker et al., 1974).

2.6 Control and eradication

Eradication efforts over the past fifty years have only succeeded in wiping out tuberculosis in a few develoed countries (Alhaji 1976b). Many authors have thrown some light on factors that could militate against the total eradication of mycobacterial diseases. Their ubiquitous nature (Horsburgh et al., 1985), the ease with which they are interchanged between man and animals, their progressive nature in some animals (Collins and Grange, 1983), and the wide range of reservoir hosts (Alhaji 1976b) are just but a few examples of such factors. Other factors include the relative ability of the organism to withstand the action of phagocytic cells of the infected host (Draper, 1981), their resistance to changes in pH

(Brooks et al., 1984) and ability to withstand pasteurization temperatures (Chapman and Spheight, 1968).

In human beings factors such as cultural and religious inclinations (Macauley, 1958), manlnutrition and alcoholism (Anon., 1979, Snider, 1985) failure to seek proper medical attention and, or refusing to take medication to completion (Springett, 1972) are known to interfere with control and eradiciation programs. Side effects such as optic neuritis that accompany some of the antituberculous drugs particurlay the newer ones (Santamaria-Jauregui et al., 1984) also interfere with control and eradication programs.

Problem of compensation from slaughtered animals (positive reactors), particularly in the developing countries, and total absence of strict government policies on the sale of products such as unpasteurised milk that could be of health hazard as well as shortage of drugs and medical personnel interfere with eradication programs. Lack of restriction on the sale of antituberculous drugs also constitute a stumbling block to eradication efforts (Siddiqi, 1976). The recent increase in the incidence of mycobacterial infections concurrently with dangerous diseases such as Acquired Immuno Deffidency Syndrom (AIDS) (Goldman, 1987) has become a further complicating factor.

2.6.1 Animals

Standard Control Methods

The standard method of controlling bovine tuberculosis proved effective in many countries, but these methods are expensive and require considerable manpower and the cooperation of livestock owners (Alhaji 1976b). Both test and slaughter of infected animals which was first started in Ontaria (Canada) long ago (Wigle et al., 1972) and Bange method of segregation and gradual replacement with fresh stock (Stenius, 1938; Wilson and Howes, 1979) could be used along with tuberculin tests. However, this involves heavy expenditure from payment of compensations for slaughtered tuberculous animals, segregation of reactors and for the replacement of animals.

However Blood and Henderson (1968) advocated the segregation of reactors method as the only method of achieving control where the prevalance of tuberculosis is low in an infected herd.

In Iran, the incidence of bovine tuberculosis dropped from 30 per cent in the sixties to 16.6 per cent by 1972 following the introduction of slaughter policy (Keyhani et al., 1973).

In Germany, Ostertags method of 1913 whereby only animals with clinical or bacteriologic evidence of disease were removed did not survive the test of time. This was because in infected animals, the disease may

be latent or lesions may be minimal in the lungs but open and slowly progressive thereby escaping clinical or bacteriologic diagnosis (Collins and Grange, 1983).

BCG vaccination

Following its success in man, it was advocated as a first step in animal eradication programs (Aspinall, 1965). Calves are vaccinated annually and immunity is achieved six weeks after vaccination. However its use in calves has fallen out of favour throughout the world because of problems of annual revaccination and interferrence with eradication programs (Mares, 1972; Ellwood, 1975).

Chemotherapy and Chemoprophylaxis

A good number of conventional anti-tuberculous drugs such as isoniazid® have been found useful when concurrently used with slaughter policy for eradication purposes (Alhaji, 1976b).

Eradication program is usually interfered with by a number of problems such as the one due to "non specific reactors", which cause problem of intepretation of results in situations where the incidence of the disease is extremely low (Alhaji 1976b) and most of the non-specific reactions have been attributed to atypical mycobacteria, Nocardia, fungi, and other saprophytic mycobacteria. Francis (1968) advocated strongly the use of tuberculin test in

eradication programs and at present Australia is the best example of a country where tuberculin test is being employed nation-wide on large scale for eradication of tuberculosis.

In the swine industry, the incidence of mycobacteriosis could be brought down through proper treatment of offal meant for feeding purposes (Fichandler et al., 1966).

2.6.2 Man

Control of tuberculosis in man dates back to the early part of the 20th century when in 1917, Dr. Varrier - Jones advocated the use of sanatorium for isolation purposes. According to Bryder (1984), Sir Varrier-Jones studied individual cases temperamentally, psychologically, physically and socially. In essence, he attached more importance to patient's personality in his approach to diagnoses and treatment. A good number of drugs have also been reported to be effective in the treatment of human tuberculosis, and of recent, newer drugs are reported to have shown promising result (Engbaek et al., 1984, Gangadharan et al., 1984; Santamaria-Jauregui et al., 1984). Useful suggestions made that could be of help in eradication programs includes enlightening the public on the danger of tuberculosis, proper medical attention and the use of vaccines (Chapman and Spheight, 1968; Michison, 1972; Kathipari et al., 1982; Wolter et al., 1982).

While a few authors were strong advocates of pasteurisation of milk, MacKay (1941), strongly showed some reservation. In his opinion, pasteurization does nothing other than produce an artificially "safe" milk containing the inert products and organisms of virulent disease.

According to Sprigett (1972) to achieve effective control of tuberculosis, there is the need for prompt initiation of diagnosis and effective chemotherapy through an increase in the level of awareness in the minds of physicians and the provision of proper diagnostic facilities. This view was shared by Stead (1971) and Rosen and Kimmey (1972).

Different countries adopt different control methods for example in some European countries compulsory chest radiography and tuberculin testing of all immigrants particularly those from Africa and Asia was suggested. This was then to be followed by application of appropriate treatment to those found positive and BCG vaccinations to those found free of the disease.

In a recent preview of tuberculosis in hospital doctors in London, Fenstenstein (1984) cited a good number of reports from United States of America (U.S.A.) and Canada in which some doctors were found responsible for disseminating infections to their patients and vice-versa. These observations led to the establishment of a number of organisations such as "The

Joint Tuberculosis Committe of the British Thoracic Society" and "The Department of Health and Social Service - DHSS" to produce some regulations binding on hospital employees. Sequele to that, a number of staff working in the Paediatric Departments were made to produce evidence of a satisfactory chest radiograph, certifying them to be free of tuberculosis six month to the time in question. The need for pre-employment screening and thorough follow-up is examplified best by a recent survey in Leeds in which among five doctors with a strong reaction to BCG heave test, three were discovered with active type of tuberculosis six month after the tests (Belfield et al., 1984).

In most of the developing countries, there is therefore the need to educate the general public on tuberculosis, and also the need for proper meat inspection at abattoirs and slaughter slabs, and also the need to discourage the sale of raw milk directly to the general public for consumption (Alhaji, 1976a). Such an increase in awareness has led to a demand for safe milk in the absence of government legislation, by the public in other parts of the world (Anon, 1940).

2.7: Procedure for isolation of mycobacteria

The presence of acid fast bacilli in clinical specimens may be confirmed by microscopy supported by cultural examinations. Identification by smear examinations may not be enough for definitive diagnoses

(Kent and Kubica, 1985). The efficiency with which mycobacteria are isolated from specimen depends very much on the manner in which the specimen is obtained and handled. Specimens need therefore be collected with utmost care and promptly transported to the laboratory.

Over the years, attempts to isolate mycobacteria from clinical specimens with subsequent development of routine isolation technics, have met with a lot of complications (Camaron and Castles, 1946; Corper and Nelsen, 1949; Bishop and Neuman, 1970; Bates, 1974; Barksdale and Kim, 1977). Of recent Kent and Kubica (1985), have reviewed a simple procedure routinely used for the isolation and identification of mycobacteria from clinical specimens.

2.7.1 Sample collection

To get the most ideal sputum specimen, the mouth need to be properly rinsed with water to minimise residual food particles and drug residues that might interfere with microbial growth. Early morning productive cough is ideal.

In an individual where sputum proves difficult to obtain, other technics such as the inhalation of five percent warm aerosolized hypertonic saline solution could be employed to induce coughing and production of specimen (Kent and Kubica, 1985). Other specimen of value include laryngeal swab, gastric lavage, bronchial

washings and transtracheal aspirations. In extra-pulmonary conditions, specimens could be in the form of surgically excised tumors, body fluids: urine and pus.

Aseptically collected fluid specimens such as spinal fluids, pleural pericardial, synovial, blood, pus and bone marrow are directly inoculated into isolating media such as Dubos broth, Middlebrooks 7H-9 broth at the ratio of 1 part of specimen to five to ten parts liquid medium and incubated at 37°C with daily shaking by hand. Growth is transferred to solid media at a later stage (Kent and Kubica, 1985).

2.7.2 Isolation

Aseptically collected specimens are generally inoculated onto the isolating media as opposed to routine clinical specimens for example sputum, that are often contaminated with rapidly growing organisms (Strong and Kubica, 1981; Kent and Kubica 1985). Contaminated specimens are generally subjected to a harsh digestion - decontamination procedure before final inoculation onto solid media such as Loweinstein Jensen (LJ), Stonebrink, and Middlebrook. Amongst a number of reagents useful in digestion-decontamination exercise are N-acetyl-L-cystaine (NALC) - sodium hydroxide, zephiran trisodium phosphate (ZTSP), sodium hydroxide, oxalic acid and sulphuric acid.

2.7.3 Identification

Not all mycobacteria isolated from clinical materials are tubercle bacilli. Some are saprophytic contaminants (Collins and Grange, 1983). Though the search for a simple test to identify reliably a strain and preferably to distinguish human from bovine variants has not been a fruitful one, a number of tests are known to be of value (Johnson et al., 1961; Harrigton and Karrlson, 1966; Youmans, 1979; Levy-Frevault et al., 1982).

As no single method has been found entirely reliable, combination of tests are used. Commonly used among such tests are growth rate, pigment production, arylsulfatase, carbon source, catalase, iron uptake, niacin production, nitrate reduction, growth on MacConkey, and sodium chloride tolerance (Kent and Kubica, 1985). Others are tellurite reduction, tween-80 hydrolysis, urease test and tween opacity test.

2.8. Tuberculin testing

Tuberculin testing has been a subject of great importance to the veterinary profession ever since. Jenner was the first scientist to pay attention to the question of hypersensitivity in his inoculation against small pox, but his work was largely forgotten until the time of Robert Koch who observed the reaction of injected tuberculin on infected animals and recorded the tuberculin type of hypersensitivity or delayed type

of hypersensitivity as it is popularly known (Lamonnt, 1947).

Koch in 1990, was the first to observe that a few hours following the injection of tubercle bacilli, a small inoculation wound was created, followed by an inflamatory phase and eventual healing with a scar formation at the injection site. Koch at first thought his injection of tuberculin would have markedly curative properties and his premature publication raised hope all over the world. However, the tuberculin eventually got relegated to the position of a diagnostic agent (Lamont, 1947; Groschel, 1982; Collins and Grange, 1983).

Tuberculin testing properly developed by Von pirquet around the nineteen hundred (MMWR, 1982) was believed to have played a very significant role in tuberlulosis eradication programs in countries such as Finland and Australia (Stenius, 1938; Francis et al., 1978a; Francis et al., 1978b).

According to Stenius (1938) Finland adopted the use of intradermal tuberculin test way back in 1910 following the declaration of tuberculosis as a notifiable disease in 1899 by the Animal Disease Law of the country. Similarly in its cooperative program for Eradication of Bovine Tuberculosis, the United States Bureau of Animal Industry was reported to have employed the use of intradermal tuberculin test since 1920 for its eradication programs.

2.8.1 Tuberculin

The tuberculin is believed to be the antigen that when injected into the body ellicits the delayed type of hyper-sensitivity that is characteristic of mycobacterial infections (Lamont, 1947). For quite sometime the nature of tuberculin reaction was a subject of controversy and according to Alhaji, (1976b), several authors have expressed their opinion on this subject matter. For many years the tuberculin reaction was looked upon as a unique immunological event, but it is now clear that several other types of immune reactions have the same basic mechanism, thus it is possible to demonstrate "tuberculin type" hyper-sensitivity to other bacteria ·fungi and viruses (Alhaji, 1976b; Macauley, 1958). Autosensitization diseases (Lamont, 1947) as well as rejection of vascularized tissue homeografts are also immune responses with the same basic mechanism.

The reactions is described as being "cellular sensitivity" or "delayed hypersensitivity". It is "cellular" because the reaction is believed to be mediated by viable sensitized cells of the thymus-drived or "T-cell" group of lymphocytes, and it is "delayed" because there is a lag period between contact of the sensitized host with an eliciting dose of antigen and the onset of reaction (Alhaji, 1976b). This phenomenon of cellular immunity as classically

examplified by the tuberculin, has long been considered the main host response to tuberculous infections but Alarcon-Segorvita and Fishbein (1971) and Nash and Douglass (1980) have reported the participation of humoral immunity in tuberculosis. They found a rise in the levels of Immunoglobulins IgG , IgM, and to a lesser extent IgA in tuberculous individuals and therefore strongly advocated the usefulness of their findings in serological procedures. Similarly, a number of authors have explored the use of serological technics in the diagnosis of tuberculous infections (Yugi and Nozaki, 1972; Muscoplat et al., 1975; Daniel et al., 1985).

Nevertheless, the high prevalence of false-positives among test positive animals with no visible lesions and lack of clear serological differentiation between healthy cattle and infected, constitute a limiting factor to such tests (Yugi and Nozaki, 1972). The antigenic components of tuberculo protein have been reviewed by several authors (Guesoni, 1961; Janot et al., 1974).

2.8.2: Types of tuberculins

The 3 types of tuberculins in use are old tuberculin (O.T.), Heat concentrated synthetic medium (HCSM) tuberculin and the Purified Protein Derivative (PPD) tuberculins (Alhaji 1976b). Although several countries are reported to have used the old tuberculin

in those days, the PPD tuberculin which is a pure form of trichloro acetic acid precipitated tuberculo protein developed in 1931 by Seibert (MMWR, 1982) is becoming increasingly used in Europe, South Africa and some developing nations at the expense of other types of tuberculins due to among other factors its purity, specificity and relative ease of standardization (Van Waveren, 1965; Alhaji, 1976b).

As a diagnostic agent, the tuberculin has been subjected to test by a number of workers with results that seemed quite interesting (Stenius, 1938; Lamont, 1947; Johnson et al., 1961; Vet. staff Ministry of Agriculture and Fisheries 1947). Various modes of preparations of tuberculins have been documented (Morgan 1940; Veterinary staff of Ministry of Agric. and Fisheries 1947). In Nigeria Ayanwale (1984) pioneered successfully the production of P.P.D. tuberculin that could be of future use in eradication programs.

A recent breakthrough in the diagnoses of bovine tuberculosis is the discovery from Japan of a product TAS-1D3 that is abput fourty times more potent than P.P.D. tuberculin (Yano et al., 1984a; Yano et al., 1984b).

2.8.3 Test Reaction Procedure and Interpretations

To appreciate fully the tuberculin test or reaction one requires to study how it works.

Test Reaction

According to Lamont (1947) when the tubercle bacilli invades the body, certain products result from the growth and break down of the bacillus. The body then responds by producing "antibodies" to neutralize these products. In the circulation, these "antibodies" sooner or later become fixed to tissue cells-lymphocytes. The body is now in a hypersensitive state and the tuberculin type of reaction appears on the introduction of "antigens' - tuberculin. On injection of tuberculin into such a sensitized body, a reaction occurs between the "antibodies" and the tuberculin as a result of which toxic products are formed and it is the effect of these toxic substances in the body which leads to the development of the so-called tuberculin type of reaction. The injection of the tuberculin into a sensitized individual sets inflammatory reaction in motion characterized by capillary dilatation and an outpouring of fluid and cells. Leucocytes, mainly polymorpho nuclear (PMN) type collect at the site in the very early states but in the later stages mononuclear leucocytes predominate. In the early stages the reaction may not be visible macroscopically, but it gradually increase reaching a maximum 72 hours post injection. On reaching its peak; the area becomes firm and tense and as resolution commences it takes on the diffuse character. In case of very severe

reactions cell death occurs with infiltration of the site by giant cells. Current immunological concept is of the view that the whole process outlined above involves three components (Seth et al., 1981) namely:

i) Sensitization of thymic-drived lymphocytes against a macrophage processed antigen

ii) Lymphokines production by sensitized T-lymphocytes

iii) Inflamatory reaction probably induced by the pharmacologically active lymphokines released at the site of antigen deposition.

Test Procedure

In the original tests, old tuberculin (KOT) made from M. tuberculosis was injected subcuta-neously and the animal's temperature checked two hours later. A rise in temperature indicates positive reaction (Alhaji, 1976b). Among sites used for this reaction are gingival, opthlmic, intrapalpebral and nasal inoculations. Of these, intradermal has been universally adopted for testing cattle in different parts of the world.

Intradermal test

The double intradermal test that has been widely used in 1930s (Green, 1933) involves a second injection of tuberculin at the site of the first injection fourty eight hours later and read twenty four hours after the second injection. However a trial by the Ministry of

Agriculture and Fishereies (1947), has found as unnecessary the second injection. The single intradermal test then became the official method of test in Britain and other countries (Alhaji, 1976b). The comparative test was used to distinguish cattle infected with M. tuberculosis and those sensitized by M. paratuberculosis and M. avium by a number of workers including Stenius (1938). In Irelands the Stomont test was widely used around the 1940s, and though time consuming, it was reported to detect with ease tuberculous pregnant cows that normally fail to react to other skin tests.

The Intravenous test

The intravenous tuberculin test based on a rise in body temperature four to six hours post injection had the advantage of detecting anergic animals and new reactors in herds with large population of animals (Larsen and Kopecky, 1965).

Other tests of significance were the subcutaneous short thermal test, conjunctival and palpebral of primates (Alhaji, 1976b).

Of all the technics outlined above, the intradermal continues to be the most satisfactory and should not be replaced as stated in the conclusions drawn by the representatives of Food and Agricultural Organisation and World Health Organisation at their second symposium on eradication of bovine tuberculosis

in Rome in 1960 (Alhaji, 1976b).

Administration of tuberculin

The concentration of tuberculin used for test varies from country to country due to differences in standardization but most P.P.D. tuberculins are generally known to be used at concentrations of 0.5 mg/ml for avian type, 1mg/ml for bovine type and 2mg/ml for human type all using a dose of 0.1ml (Huitema, 1973; Kleeberg, 1975; Leslie et al., 1975).

Needle size also varies with the official conducting the test, but 25-28 gauge 1/8" - 1/2" needle (depending on site of inoculation) have wide applicability (Larsen et al., 1957).

Intepretation of test reaction

Intepretations of tuberculin reactions usually present some problems. A number of instances are reported where diseased animals failed to respond to test (false negative), conversely there are several reports of tuberculin negative animals with no visible lesions at slaughter, that reacted positively - false positive (Anonymous, 1940; Lamont 1947; Johnson et al., 1961).

The tuberulin test being biological in nature is subject to limitations. Misintepretations could be due to faulty technic or equipment (Anon.,1940; Johnson et al., 1961), nature of the organisms, dose and route of

exposure among others (Muscoplat et al., 1975). Anergy-absence of skin reaction in individuals known to have developed cell mediated immune response could also interfere with intepretations (Seth et al., 1981). Bonchek (1971) had reported that, malignant conditions of the immune system could produce anargy in patients under test.

2.8.4 Sensitivity and Specificity of tuberlin test

Assessments or comparisons of tests must be based on sound criteria, and of those available, sensitivity and specificitty are the most important (Francis et al., 1978a).

Sensitivity is a measure of the population of diseased animals detected by a screening test, in relation to total number of diseased animals in the population being screened (true positives); while specificity is a measure of the population of non-diseased animals, whose response is negative to a screening test in relation to the number of non-diseased animals in the population being screened - true negatives (Francis et al., 1978b). Inherent in these concepts is that sensitivity and specificity tend to be inversely related.

Despite the belief that tuberculins can only be compared in the field more accurately, if all animals shall be slaughtered and autopsied, screening tests mentioned above can be applied in eradication programs

(Francis et al., 1978a; Francis et al., 1978b). According to the latter authors, in situations where the prevalence of disease is high, the most sensitive test should be used for eradication purposes. However, where the prevalence is low and the disease is confirned to an area or a herd, specificity becomes more significant. For diagnotic purposes, specificity test to a large extent depends on the quantities and qualities of tuberculin used, and many countries including Australia have adopted the use of P.P.D. in screening programs for eradication purposes (Francis et al., 1978a).

Although the skin of the caudal fold has been reported as being less reactive than the skin of the neck (Larsen et al., 1950), the tuberculin test using the caudal fold region has given very good overall results. Tuberculin test using this region has given high specific results (Lepper et al., 1977; Francis et al., 1978a). According to Francis et al., (1978a) and Francis et al. (1978b); reactions in true positives becomes more visible at 72 hours rather than 96 hours post injection, also reaction in tuberculous cattle to the 0.4 mg dose (largest ever produced) were larger than those of 0.2mg dose. In the false positives, on the other hand, there was no significant difference observed in reaction size to the 0.2 or 0.4 mg dose.

Francis et al. (1978a) were of the view that the caudal fold test using 0.4 mg dose of bovine PPD read

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72 hours post injection provides the highest sensitivity and specificity in any given test.

Recently, a screening test program was employed in assessing risk factors in population of food handlers in the United States of America (Judson et al., 1983). Similarly, based on results of field test of attested herds, Herbert et al., (1955), sounded a strong warning on tuberculin test result interpretations, which they felt should be considered as tentative pending experimental proofs.

2.9 Economic and Public Health Significance of Mycobacteriosis

Diseases due to mycobacteria are no doubt among diseases of major concern to the human population especially on the basis of direct and indirect devastating effects on livestock population and direct health hazard to the human population (Alhaji, 1988 - Personal Communication).

2.9.1. Economic Significance

Economically, a lot of money has been and is still being spent to purchase drugs for treating human cases as well as for compensating slaughtered tuberculous animals (Alhaji, 1976b).

Losses occur through meat and milk condemnations (Stamp et al., 1946; Rosen and Kimmey, 1972; Eid, 1976), loss of offspring, decreased milk production,

infertility, poor conditions resulting in low market value and interference with herd improvement programs (Alhaji, 1976b). According to Francis (1968), annual loss to Australian Government from bovine tuberculosis alone could reach two hundred and fifty million American dollars ($250 million).

In America alone, over 50,000 cattle were sent to rendering tanks around the year 1918 (Rosen and Kimmey, 1972).

Also for quite sometimes, mycobacteria have been associated with mastitis in cattle (McKay, 1941; Stauart et al., 1951) and in addition to the adverse effect on milk yield, the possibilities of acquiring infections from such sources particularly in young children, dates back to the early part of the nineteenth century (Collins and Grange, 1983).

The relative high frequency with which mycobacteria are isolated from market milk samples, thereby rendering such products unfit for human consumption is of economic significance.

Results of tuberculin test surveys all over the world (Nagaraja et al., 1973; Ellwood, 1975; Eid, 1976) just to mention but a few, have clearly indicated that a large number of the cattle population in some parts of the world habour mycobacteria and indeed millions of such animals have ended up being slaughtered with a good number of them unfit for human consumption thereby resulting in heavy losses to affected countries.

In countries where the danger of tuberculosis is foremost in the minds of the governments of such countries, money is being spent in establishing hospitals, sanatoria and clinics (Ellis, 1974). For example, Miles and Maat (1984), recently reported s successful supervised out-patient tuberculosis treatment program in an open refugee camp in the Thai-Cambodian border that involved a lot of money. In a preventive screening program among food handlers in Colorado (United States of America), Judson et al., (1983) reported that an average of fourty five American Dolla was needed per person.

2.9.2. Public Health significance

Tuberculosis is one of the most important zoonoses. Its effect on man could be direct or indirect. Indrectly, infection, could occur through the consumption of contaminated foods, such as milk and other milk products, while direct means could be through exposure as in direct contact with a tuberculous patient. Direct infection could also occur through inhalation of contaminated aerosol (Collins and Grange, 1983).

The ubiquitous nature of mycobacteria resulting in their isolation from a number of sources (Anon, 1978) is also of public concern. The frequent isolation from market-based milk samples (Alhaji and Snurrenberger, 1977; Appuswamy et al., 1980), and the relative ability

of mycobacteria to withstand pasteurization temperatures (Chapman and Spheight, 1968) is also of serious concern.

The literature on disease conditions associated with human mycobacteriosis is voluminous (Narain et al; Damsker et al; 1974; Prowse and Cavannagh, 1976; Farer et al., 1979; Kotian et al., 1981). Of recent, the atypicals have been found to be associated with important disease conditions in man such as Chron's disease (Gitnich, 1984; Chiodini et al., 1984); Lung cancer (Rolston et al., 1985); and Acquired Immune Deficiency Syndrome - AIDS (Chan et al., 1984; Pitchenick et al., 1984; Goldman 1987).

At the beginning of the twentieth century, tuberculosis resulted in the death of nearly 0.1 million people in the United States of America alone (Myers, 1968; Rosen and Kimmey, 1972) and around the 1970s, it resulted in the death of over 1,000 people in England (Springett, 1972). Wigle et al., (1972) reported 31 active cases of tuberculosis some of which were associated with consumption of unpasteurised milk.

According to a recent report by the world experts on tuberculosis, an estimate of ten million cases may occur throughout the world each year, four to five million of them highly infectious, and two to three million resulting in death (MMWR, 1982).

2:10 Situation In Nigeria

Mycobacteria are prevalent in Nigeria but an objective assessment of their epidemiology is by no means a small task. This is due to the fact that despite the knowledge of their existence both in the human and animal populations as well as the environment, the literature on mycobacteria appears to be sketchy. The relative scarcity of literature may be partly due to the limited number of field studies so far undertaken on mycobacteria. Furthermore, not all hospitals keep adequate records on mycobacterial diseases. Routinely in most of the hospitals, identification of the organiams which are mostly done by direct smear examinations stops at the generic level. This is not surprising since mycobacteria are not only fastidious but they require anything between a few days to several weeks to grow on culture media. Infact media used for routine isolations are very expensive and not readily available. In that respect, epidemiologic assessment can only be made on the basis of the limited field surveys recently carried out and the records of cases from abattoirs and hospitals.

The first report of the existence of the presence of mycobacteria in West Africa as the causative agent of bovine tuberculosis was in 1913 by Ziemann in Camerouns, according to the Veterinary Department Report, Nigeria, (Alhaji, 1976b). Subsequent reports of cases among slaughtered cattle were made in the

Medical Annual Report of 1916-1918. The first report of the existence of bovine tuberculosis in Nigeria based on tuberculin test results, subsequently followed by post mortem and laboratory examinations was made by Manley (1929).

Abattoir reports on mycobacteria are found in all parts of the country. From Calabar abattoir between 1937 and 1939, 364 cases of tuberculous lesions were reported in 2300 bovine carcasses, and between 1939 and 1947 the same abattoir reported that 960 of 10,042 cattle slaughtered had tuberculous lesions. The 1973 annual report of the then South Eastern State of Nigeria recorded tuberculous lesions from several other cases.

At the Maiduguri abattoir between 1963 and 1964, of the 2640 carcasses examined, 56 had tuberculous lesions (Northern Nigerian Annual Report of the Ministry of Agriculture and Forest Resources , 1967). From the records of Annual Reports of Northern Nigeria between 1971 and 1973, despite the fact that some of the specimens sent for examination went bad due to inadequate preservation, a few isolations were made, and in addition, M. tuberculosis was reportedly isolated from a mongoose when specimens from zoo animals were examined.

Eid (1976) and Alhaji (1976a) reported a high prevalence of tuberculous lesions from abattoir samples

in Northern Nigeria.

Tuberculin testing of cattle in Nigeria was sporadic until the early 1950's when Veterinary officers in the then Sokoto province were active in conducting tuberculin tests in herds around Birnin Kebbi and Argungu.

In 1956, a World Health Organisation tuberculosis survey team at Katsina tested a "known exposed" herd in which three of the 13 cattle tested were found to be infected after slaughter (Alhaji, 1976b).

The Shika Quarterly report of March 1957 contained a report of tuberculin test conducted on a herd of 158 cows with negative results. Reports of bovine tuberculin test increased during the 1960s and a good number of tuberculin test surveys were undertaken thereafter (Eid, 1976; Alhaji, 1976b).

From the public health point of view based on hospital records of Annual Report 1971-72 of Health Ahmadu Bello University, Zaria; records of carcass condemnations from abattoirs, as well as the relative high frequency with which mycobacteria are recorded from milk samples (Alhaji, 1976b; Alhaji and Schnurrenberger, 1977), it can be concluded that mycobacteria are prevalent in Nigeria and indeed a good percentage of the Nigerian populace stands the risk of infection with mycobacteria.

CHAPTER 3

MATERIALS AND METHODS

3.1.1. Tuberculin testing of animals

Five hundred heads of cattle from twenty representative Fulani herds (nomadic, semi-nomadic and intensive) in and around Zaria were selected and tuberculin tested. In herds with overall population of less than thirty animals, efforts were made to test all animals, otherwise only lactating cows and their calves along with a randomly slected few were screened from herds with large population of animals. All animals were tuberculin tested using the single caudal fold (S.C.F.) technic. Test procedure and intepretations of the Michigan Department of Agriculture (1962) were used.

Animals were restrained and then identified by serial numbering at the rump region prior to injection of the bovine tuberculin as shown on PLATE I. A site 1/3 of the distance from the base of the tail on the right side was chosen and cleaned with dry cotton wool or tissue paper. One-tenth millilitre purified protein derivative (PPD) bovis (U.S. Veterinary licence No. 107) at the concentration of 1mg/ml was then carefully deposited intradermally using the 2ml McLintock pre-set syringe No. 622848 - (PLATE II). Efforts were made to avoid flow back from the injection site while at the

same time maintaining consistency of injection site. Seventy-two hours post-injection sites were examined by visualization (PLATE III), palpation (PLATE IV) and measurement using Verniers callipers and response recorded. Animals with response (swelling) 4mm and above were considered as reactors and those with less than 4mm as suspects, while those with no swelling at all, were classified as non-reactors.

In addition to the test, data which included breed sex and age of each animal tested were obtained. The history from the herdsman and the cornual ring method were used to determine the conservative age of each animal tested. Table 3:1 is the list of herds screened and specimens obtained.

Animals were further grouped into 3 based on management system as follows, nomadic (those always on the move and graze in the field with little or no supplementation in diet), semi-intensive (those partly kept indoors, but atimes graze in the field with partial supplementation in diet); and intensive (where animals are permanently kept indoors with full supplementation in diet) as shown in Chapter 4 Table 6.

3.1.2. Collection of fresh milk from animals and sputum from milkers and herders

The aspect of collection of fresh milk from cows under test was in most cases carried out prior to the tuberculin test in an effort not to get animals

excited, thereby making the test not only difficult, but atimes impossible. In such situations where animals got excited; collection of milk samples had to be deferred to the 3rd day when tuberculin test reactions were read.

A total of 90 fresh milk samples were collected from all the herds tested. From each animal, approximately 10ml of milk was obtained from each quarter and pooled into clean sterile univeral bottles (PLATE V). Bottles were properly identified, tightly capped and conveyed to the laboratory ice-cooled and stored at -20^{o}C until processed.

A total of 29 sputum specimens of milkers, herders, and in some instances other members of the family were collected in wide-mouthed disposable plastic containers, properly identified and conveyed to the laboratory ice-cooled and remained frozen till processed. The number of specimens and types obtained are shown in Table 3:1.

Table 3:1: Location of Herds Screened and types of specimens obtained.

Herd Number	Location of herd	Population of Animals in herd	Type and number of animals tuberculin teated per herd				Type and Number of Specimens Obtained	
			Calves	Cows	Bulls	Total	Milk	Sputum[a]
1.	Birnin Gwari Road	69	10	36	3	49	11	1
2.	Anguwar Mallam I	20	5	13	2	20	2	1
3.	Anguwar Mallam II	29	11	16	2	29	2	1
4.	Birnin Gwari Road V	14	2	9	2	13	2	2
5.	Birnin Gwari Road VI	67	20	39	3	62	9	1
6.	Jaji I	110	10	19	6	35	3	2
7.	Kafin Damisa	34	5	25	0	30	0	1
8.	Across A.B.U. Dam I	37	11	20	5	36	5	2
9.	Across A.B.U. Dam II	7	1	4	1	6	0	1
10	Jaji II	45	4	10	2	16	3	1
11	Yakasai	13	1	10	2	13	0	2
12	Dogarawa	24	6	10	2	18	7	1
13	Soba	42	10	20	5	35	13	2
14	Jaji III	48	8	18	4	30	7	0
15	Jaji IV	28	2	9	2	13	3	0
16	Zaria city	16	2	10	1	13	2	1
17	Fatima village	32	10	18	4	32	12	5
18	Marke	14	3	9	2	14	1	1
19	Biye I	72	10	12	3	25	5	2
20	Biye II	11	2	8	1	11	3	2
Total	-	732	133	315	52	500	90	29

[a]Collected from milkers or other members of the household.

Table 3.2: Markets screened for Mycobacterium species and types of sputum.

Market Location	No. of "nono" Samples	No. of sputum Samples
Giwa (GW)	32	1
Jaji (JJ)	13	3
Pan Ladan (PL)	14	0
Sabo (SB)	52	0
Samaru (SM)	38	2
Shika (SK)	27	0
Tudun Wada (TW)	39	0
Wusasa (WS)	16	1
Zaria City (ZR)	31	2
Total 9	262	9

3.1.3. Collection of ready-to-drink "nono" samples and sputum from milk hawkers.

"Nono" samples were obtained from local markets at the vicinity of tested herds known to be supplied by such herds. A total of 262 samples were obtained, properly identified and conveyed to the laboratory ice-cooled and remained frozen till processed. Sputum samples from "nono" sellers were also collected. Table 3:2 shows the list of markets screened and number of samples obtained from such markets.

3.2. Laboratory processing of Specimens.

3.2.1. Determination of pH of specimen

Prior to processing, the pH of all specimen except sputum samples were determined using a pH meter (KENT EIL 7045/46).

3.2.2. Mycobacteriologic examination of specimens

Processing and culturing of milk and 'nono' samples:

Milk obtained from individual animals were processed separately except in a few instances where herd-pooled milk were obtained in addition to the individual samples. Processing of fresh milk and 'nono' specimens was carried out as described in the C.R.C. Manual of Clinical Laboratory Procedures (Anon, 1970), and in Laboratory Methods in Veterinary Mycobacteriology U.S.D.A. Ames (Anon, 1974b). Briefly, 40ml of milk was centrifuged in a 50ml screw capped

tube at 1641 relative centrifugal force (R.C.F.) for 15 minutes. The supernatant was discarded and the sediment resuspended with 20ml of four per cent sodium hydroxide (NaOH) containing phenol red indicator. Suspension was then allowed to stand for 30 minutes before being neutralized by drop-wise addition of 6N hydrochloric acid until the colour changed from purple to pink. The specimen was then centrifuged again at 1641 R.C.F. for 20 minutes.

The supernatant was discarded until about 2ml of fluid was left in the tube. The sediment was suspended in the last 2 ml after adding a few pellets of NaOH. Using steril Pasteur pipette, Loweinstein Jensen (LJ) medium slants in screw capped tubes (125 x 20mm) were inoculated. The L.J. media were prepared according to the instructions of the manufacturers. The sediment from each sample was inoculated onto four slants of L.J. agar (two with glycerol and the other two without glycerol), one from each of these set (i.e. one with glycerol and the other without glycerol) was wrapped in aluminium foil. All inoculated tubes were incubated at 37^{o}C for 8 weeks. In addition a smear of the sediment of each specimen was stained by the Ziehl-Neelsen technic and examined for acid and alhocol fast bacilli (A.A.F.B.).

Sputum samples were similarly processed as outlined in the Laboratory Methods in Veterinary Mycobacteriology (Anon, 1974b). About 2-5ml of

specimen were transfered to sterile 50-ml screw-capped centrifuge tubes, and 10-15ml of NaOH containing phenol red indicator added. Tubes were tightly closed and shaken to homogenize the specimen. Tubes were then allowed to stand on the bench for 30 minutes before being neutralized slowly with hydrochloric acid (HCL) until the colour changed from purple to pink. The rest of the procedure was as described for milk samples.

All inoculated tubes were incubated at 37°C and examined daily for the first fourteen days and weekly thereafter for 8-10 weeks for evidence of growth.

Subsequently colonies were subcultured. Growth time and culturing characteristics were noted. Smears of the colonies weremade and then stained by Z-N technic. The slides were examined under the microscope using oil immersion objective for presence of acid fast bacilli. The rest of the colonies were kept in the refrigerator before they were finally transferred to enriched Dubos broth with sterile serum in preparation for biochemical differentiation.

3.2.3. Biochemical testing of isolates

Cultures that showed evidence of growth were subjected to a number of tests with a view to characterising organisms. Methods routinely used in the differentiation of Nocardia from mycobacteria includes - Nitrophenol test of Vestal (1975); growth on yeast-glucose-agar medium at 28°C, hydrolysis of

casein, and decomposition of tyrosine of Gordon and Mihm (1962). Others include Z-N staining characteristics, growth rate, pigmentation, tween 80 hydrolysis, catalase production (Alhaji, 1977); growth under anaerobic and microaerophilic condition and niacin production. The last five test were employed and all cultures that were found to grow rapidly (in less than one week), with filamentous acid-fast variable rods on Z-N staining, deep orange pigmentation on L-J medium, negative tween hydrolysis and catalase production and no growth at all under microaerophilic condition were presumed to be Nocardia.

The remaining isolates were then subjected to the following biochemical tests as outlined by Kent and Kubica (1985).

(a) Catalase.

(i) The Semi quantitative test

Loweinstein Jensen medium was prepared and dispensed in 5ml amounts into 20 x 150mm screw cap test tubes and inspissated at 85°C for 50 minutes with tubes in an upright position. Tubes were then incubated at 37°C for 24 hours to check for sterility. L-J tubes were then inoculated with a loop-full of suspected M. bovis growth from an actively growing slant and re-incubated with caps loosened for two weeks at 37°C. On the day of the test a 10% solution of Tween 80 was prepared by mixing 10ml of Tween 80 with 90ml of

distilled water. The mixture was autocalved for 10 minutes at 121°C, properly swirled immediately after autoclaving and on cooling to resolubilize. Just before use, an equal part of 10 per cent tween 80 and 30 per cent hydrogen peroxide (superoxol^{R}) were mixed. To each tube under test 1.0ml of freshly prepared Tween-peroxide mixture was added, cap replaced loosely and allowed to stand at room termperature for 5 minutes before measuring the height of the column of bubles above the medium surface. Readings were recorded in millimeters. An uninoculated tube was used as a negative control and M. bovis sensinogen as a positive control.

(ii) Heat-stable catalase test (at pH 7 and 68°C).

A 0.067M phosphate buffer, pH 7 was prepared as outlined by Kent and Kubica (1985) and 10 per cent tween 80 and 30 per cent hydrogen peroxide were prepared as described in the "semi-quantitative method".

Several spadeful of growth from culture slants of actively growing test organisms were suspended in 0.5ml of 0.067M phosphate buffer, pH 7, contained in 16 x 125-mm screw capped tubes. Tubes were then incubated in a 68°C water bath for 20 minutes. Following the incubation the suspensions were later cooled to room temperature before the addition of 0.5ml of Tween-peroxide mixture. Tubes were recapped loosely and

examined for up to 20 minutes for the formation of bubbles before discarding. An un-innoculated tube was used as a negative control. This test is used to differentiate M. bovis from other species.

(b) Growth rate

Enriched Dubos broth in sterile 20 x 150mm screw capped tubes were prepared as outlined by Kent and Kubica (1985). Several colonies of the organisms on L.J. were aseptically picked and inoculated into the broth base. Inoculated tubes were then incubated for 1 week at 37°C with daily shaking to detect any evidence of growth. Several ten-fold dilutions of organism were prepared from bacterial suspension in broth. On preparation, 0.1ml of each of the dilutions prepared were inoculated on L.J. egg base media and tubes incubated at 37°C and examined daily for one week, and weekly there after for 8-10 weeks for evidence of growth.

(c) Pigment production

Growth in enriched Dubos broth was also used for this test.

Cultures in enriched Dubos broth were sufficiently diluted and inoculated on to ten sets of L.J. slants per isolate (five with glycerol and the other five without glycerol), four tubes from each set (i.e. four with glycerol, and four without glycerol) were properly

wrapped in aluminium foil. Two shielded (wrapped) tubes from each set (i.e. two with glycerol and two without glycerol) were incubated at 25°C, while the other two were incubated along with one non-shielded tube from each set at 37°C. On noticing growth on the non-shielded tubes, a shielded tube each from the two different media and incubation temperatures were exposed and examined for pigmentation, leaving the other shielded tubes from the two incubation temperatures as controls. Shielded tubes that showed no evidence of growth on exposure, were then exposed to 60-W tungsten bulb placed 25cm away with caps loosen, and then re-incubated for 2-3 days before finally read for pigmentation.

(d) Growth on MacConkey Agar without crystal violet.

MacConkey agar plates were prepared according to the directions of the manufacturer and 15-20 millilitres of medium poured per disposable petri dish.

Cultures used for the test were grown for two weeks in freshly prepared enriched broth base. Plates were then streaked for isolation with cultures and M. bovis sensinogen as control, and incubated in open petri dish connister at room temperature (25°C). Plates were examined at 5 and 11 days for evidence of growth of M. bovis.

(e) Niacin production test.

Four per cent aniline used for the test was prepared by adding 4mls of colourless aniline to 96ml of 95 percent ethanol and stored in brown bottles in the refrigerator. Ten per cent cyanogen bromide was prepared by dissolving 5g of cynogen bromide in 50ml of distilled water (in the safety hood) and stored in the refrigerator in a tightly capped brown bottle. To egg-base medium culture slants, 1.0ml of sterile distilled water was added. Tubes were kept horizontally so that the fluid covered the entire surface of the medium. Fifteen minutes extraction time was allowed, before 0.5ml of fluid from each culture was transfered to clean 16 x 125mm steril screw cap tube. Half a millilitre of the 4 per cent aniline was added to each tube followed by 0.5ml of 10 percent cyanogen bromide. M. tuberculosis was used as a positive control and an un-inoculated tube as a negative control. Immediate development of any yellow color in the tubes was considered positive.

(f) Nitrate reduction test.

To prepare 0.01M sodium nitrate in 0.022M phosphate buffer, pH 7.0, the following were dissolved in 100ml distilled water - 0.085g $NaNO_3$, 0.117g KH_2PO_4, and 0.485g Na_2HPO_4. $12h_2O$, and sterilized by autoclaving.

Reagent #1, was prepared by the addition of 50ml of conc. HCL to 50ml of distilled water.

Reagent #2, was prepared by dissolving 0.2g of sulfanilamide in 100.0ml of distilled water.

Reagent #3, was prepared by dissolving 0.1g of N-naphthylethyline diamine dihydrochloride in 100ml of distilled water.

To clean sterile 16 x 125mm screw cap tubes; 0.2mls of sterile distilled water was added, into which 2 loopful of growth from a 4-week-old culture on L.J. was added and emulsified. This was then followed by the addition of 2.0mls of sodium nitrate substrate. The tubes were shaken by hand and incubated upright for 2 hours in a 37°C water bath. On removal from the water bath, the following were added immediately, one drop of reagent 1; two drops of reagent 2; and two drops of reagent 3. Tubes were immediately examined for development of a pink-to-red color. To all negative tubes, a small amount of zinc dust was added for further confirmation. A tube of M. tuberculosis and another uninoculated tube were used as positive and negative controls respectively.

(g) Sodium chloride tolerance (5%).

Two bottles of egg-base medium were prepared according to standard formulation outlined in the Laboratory Manual of Kent and Kubica (1985). To one

batch of 300ml of complete egg medium, 15g of NaCl was added and properly mixed before dispensing into L.J. tubes. The second batch of egg medium was used as a growth control and contained no NaCl. A 7-8ml amounts of each medium was dispensed into 18 x 150mm screw cap tubes and inspissated in slanted position at 85°C for 50 minutes. Sterility was checked later by incubation of tubes overnight at 37°C. Both the NaCl containing slants and the non-NaCl containing slants were later inoculated with 0.1ml of saline suspensions from fresh slant cultures. Tubes were incubated at room temperature and examined for evidence of growth. Test slants with more than 50 colonies were considered as positive and the final reading was made after 4 weeks incubation.

(h) Tween 80 hydrolysis.

A phosphate buffer, pH 7 was prepared by mixing 61.1ml of 9.47g of anhydrous $Na_2 HPO_4$ in one litre of distilled water, and 38.9ml of 9.07g of KH_2PO_4 in one litre of distilled water.

To the 100ml, pH 7 phosphate buffer, 0.5ml of Tween 80, and 2ml of a 0.1 per cent aqueous solution of Neutral Red were added. The substrate was then dispensed in 2ml amounts into 16 x 125mm screw cap tubes and sterilized at 121°C for 10 minutes. Sterile substrate in screw cap tubes were later inoculated with spadeful of test organiams from actively growing young

cultures and incubated at 37°C. Tubes were examined after 1, 5, and 10 days for a change from amber to pink or red colour. An uninoculated tube M. bovis sensinogen tubes were used as negative controls.



PLATE I: Restraint and identification of cattle during the study.



PLATE II: Injection of PPD tuberculin into the caudal fold of cattle.



PLATE II: Injection of PPD tuberculin into the caudal fold of cattle.

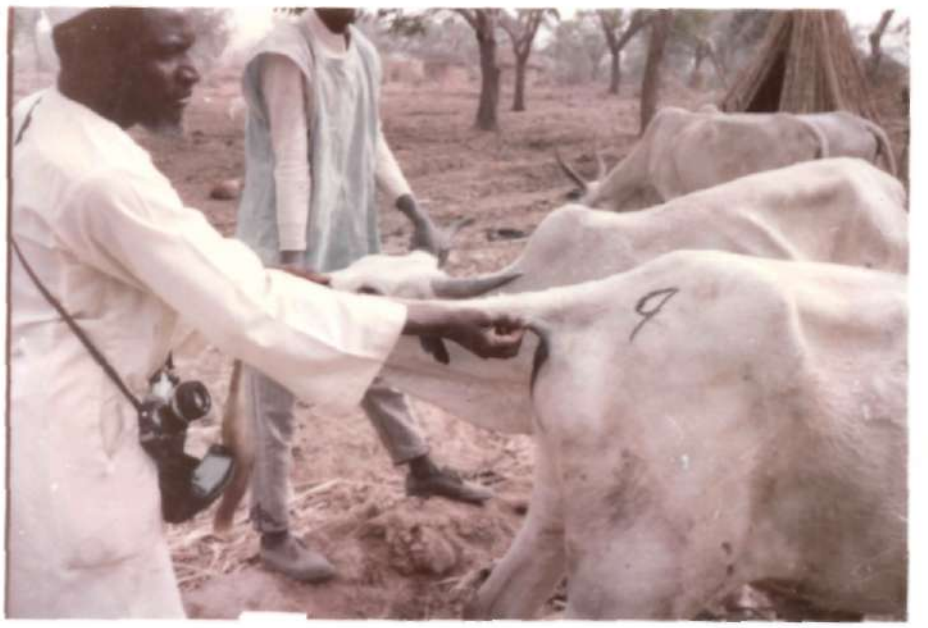


PLATE III: Assessment of a positive tuberculin test reaction by visualization



PLATE III: Assessment of a positive tuberculin test reaction by visualization



PLATE IV: Assessment of a tuberculin test reaction by palpation.



PLATE IV: Assessment of a tuberculin test reaction by palpation.



PLATE V: Collection of fresh milk directly from the udder of cattle in Fulani herds.

CHAPTER 4

RESULTS

The tuberculin test results.

Table 4:1 shows the results of tuberculin test carried out in the twenty selected Fulani herds. Of a total of 500 animals tuberculin tested, 59(11.8%) were reactors, and 398(79.6%) were non-reactors, while 43(8.6%) were inconclusive reactors. The highest number of reactors was recorded from herd number 12, where amongst the 18 animals tested, 7(38.89%) were reactors to the test. Four herds (2, 3, 4 and 7) recorded no positive reactors at all; and also relatively low number of inconclusive reactors came from these herds as well. However, of all the herds, tested, only one (Herd 7) was found to be one hundred per cent free of demonstrable exposure to mycobacterial infection from which non of the animals tested reacted. This herd was identified as a fattening ranch made up of predominantly "Gudali" breed of cattle.

Age distribution of tuberculin reactors

The age distribution of animals tested and their tuberculin test results are shown on Table 4.2. More than half of the animals tuberculin tested were between 0-1 and over 5 years age group. A general increase in prevalence rate with age was observed, as those animals older than 5 years had highest reaction rate of

28(20.9%). Nearly half (43.46%) of overall tuberculin reactors were over 5 years of age. Similarly, the highest number of suspect (inconclusive) reactors was also recorded from this age group. The reactor rate amongst cattle older than five years was significantly higher than in other age groups ($P < 0.05$; X^2).

Sex distribution of animals tested

The sex distribution of animals tested and their tuberculin test reactions are shown in Table 4:3. Thirteen (11.4%) of 114 males and 46(11.9%) of 386 females tested, were reactors and the difference was not statistically significant ($P>0.05$; X^2).

Distribution of infection by management.

Table 4:4, shows the prevalence of mycobacterial infections in cattle by management system. In cattle kept under the nomadic system of management, 43 (13.7%) of 315 tested, were reactors, while 16(10.3%) of 155 heads of cattle managed semi-intensively were reactors. None of the 30 heads of cattle kept under the intensive management systems were reactors, nor were there suspect (inconclusive) reactors in this age group. Animals kept under the nomadic system had a significantly higher ($P<0.05$; X^2) reactor rate than those intensively managed.

Presumptive biochemical test results.

The biochemical test results and presumptive

identities of mycobacterial isolates from milk, sputum from herders and "nono" hawkers and "nono" samples are shown in Table 4:5.

The overall presumptive results were as follows, one isolate of M. bovis was identified, 7 were scotochromogenics, namely, M. scrofulaceum (2), M. gordonae (aquae) (4), and M. flavescens (1); 6 were non chromogenics, namely, M. gastri (4) and M. triviale (2); and 4 isolates were rapid growers (Group IV) tentatively identified as M. smegmatis. Identification was based on a total of 8 biochemical tests in addition to cultural characteristics. Of 18 isolates, only 1 (5.5%) was a typical Mycobacterium, M. bovis, while the remaining 17 (94.5%) were all atypicals.

Distribution of Mycobacterium species by source.

Table 4:6 shows the species of Mycobacterium isolated from the various sources. The only M. bovis isolate was cultured from fresh milk in a herd. Of the seven isolates of scotochromogens cultured, 5 were from "nono", and 2 from sputum samples of herders. Of the 6 isolates of non-chromogens, 2 were cultured from "nono", and the remaining 4 from sputum samples of herders and "nono" hawkers. The 4 group IV members, M. smegmatis, were cultured from "nono".

Mycobacterium species from fresh milk and sputum

The frequency of isolation of Mycobacterium

species from fresh milk and sputum samples is shown in table 4:7. Of the 90 milk samples cultured, only 1(1.1%) was positive for Mycobacterium species, while 4(13.8%) of 29 sputum samples contained the organisms. Both sputum samples collected from milkers in herd No.19 were positive for Mycobacterium species. The pH values of the milk samples collected, ranged from 6.20 to 7.74, while the mean value was 6.28. There was no observed relationship between the pH values of raw fresh milk and frequency of isolation of Mycobacterium species.

Mycobacterium species from "nono" and sputum

The pH values ranged from 4.00 to 5.21, while the mean value was 4.68. Statistically the pH value had no effect on the frequency of isolation of Mycobacterium species. Only 2(22.2%) of 9 sputum samples collected from "nono" sellers were positive for Mycobacterium species, one sample each from Jaji (33.3%) and Zaria City (50.0%) markets.

A total of 11(4.2%) of 262 "nono" samples collected from nine markets around Zaria, were culturally positive for Mycobacterium species (Table 4:8). Six (66.7%) of the nine markets where samples were collected had Mycobacterium species positive samples with the highest occurrence detected in Shika market where 4(14.8%) of 27 "nono" samples were contaminated with Mycobacterium species.

TABLE 4:1 Prevalence of Mycobacterium spp. infection in various herds on the basis of tuberculin test result.

Herd No.	No. of animals tested	Management system	Classification of reaction		
			No. (%) of Reactors*	No. (%) of Inconclusive Reactors**	No.(%) of Non Reactors***
1.	49	Semi-Intensive	3(6.1)	4(8.1)	42(85.71)
2.	20	Nomadic	0(0.0)	2(10.0)	18(90.0)
3.	29	Nomadic	0(0.0)	1(3.4)	28(96.5)
4.	13	Semi-intensive	0(0.0)	1(7.7)	12(92.3)
5.	62	Nomadic	5(8.1)	4(6.5)	53(85.5)
6.	35	Nomadic	6(17.1)	2(5.7)	27(77.1)
7.	30	Intensive	0(0.0)	0(0.0)	30(100.0)
8.	36	Nomadic	2(5.6)	5(13.9)	29(80.6)
9.	6	Nomadic	2(33.3)	1(16.7)	3(50.6)
10.	16	Nomadic	4(25.0)	2(12.5)	10(62.6)
11	13	Semi-Intensive	3(23.1)	3(23.1)	7(53.8)
12	18	Nomadic	7(38.9)	2(11.1)	9(50.0)
13	35	Semi-intensive	5(14.3)	3(8.6)	27(77.1)
14	30	Nomadic	7(23.3)	2(6.7)	21(70.0)
15	13	Nomadic	4(30.8)	0(0.0)	9(69.2)
16	13	Semi-Intensive	2(15.4)	3(23.1)	8(61.5)
17	32	Semi-Intensive	3(9.4)	0(0.0)	29(90.6)
18	14	Nomadic	1(7.1)	2(14.3)	11(78.6)
19	25	Nomadic	3(12.0)	3(12.0)	19(76.0)
20	11	Nomadic	2(18.2)	3(27.3)	6(54.5)
Total	500	-	59(11.8)	43(8.6)	398(79.6)

*These are animals with ≥4mm tuberculin test reaction
**These are animals with less than 4mm tuberculin reaction
***These are animals with no reaction at all.

Table 4:2: Age distribution of animals tested and their tuberculin test reactions

Age (Years)	No. of animals Tuberculin Tested	No. (%) of Reactors	No. (%) of Inconclusive Reactors	No. (%) of Non-Reactors
0 - 1	131	9(6.9)	9(6.9)	113(86.2)
>1 - 2	35	2(5.7)	3(8.6)	30(87.7)
>2 - 3	32	2(6.2)	3(9.4)	27(84.4)
>3 - 4	84	7(8.3)	4(4.8)	73(86.9)
>4 - 5	84	11(13.1)	7(8.3)	66(78.6)
>5	134	28(20.9)	17(12.7)	89(66.4)
Total	500	59(11.8)	43(8.6)	398(79.6)

TABLE 4:4: Effect of Management on tuberculin test reactions of 500 heads of cattle tested

Management System	No. of Herds Tested	No. of Animals Tuberculin Tested	No.(%) of Positive Reactors	No.(%) of Incon-clusive Reactors	No. (%) of Non-Reactors
Purely Nomadic	13	315	43(13.7)	29(9.2)	243(77.2)
Semi Intensive	6	155	16(10.3)	14(9.0)	125(80.7)
Intensive	1	30	0(0.0)	0(0.0)	30(100.0)
Total		500	59(11.8)	43(8.6)	398(79.6)

TABLE 4:5. Biochemical test results of isolates Mycobacterium spp.

Isolate No.	Source	Biochemical Characteristics: Niacin Production	Nitrate Production	Sodium Chloride test(5%)	Catalase test(mm*): Semi quant.	Heat stable (68°C)	Growth in McConkey in days: 5	Growth in McConkey in days: 11	Tween hydrolysis in days: 5	Tween hydrolysis in days: 10	Pigmentation: Photochr.	Pigmentation: Scotoch	Pigmentation: Nonphot.	Presumptive Identity
PL 11(1)	Pan Ladan (PL)	-	±	-	3	-	-	-	±	±	-	-	+	M. gastri
SK 10	Shika (SK)	-	+	+	45	7	-	-	+	+	-	-	+	Grp IVb M. [illegible]
KD 15	Kerd (15)	-	-	-	5	-	-	-	-	-	-	-	+	M. kansasii
SK 11	Shika (SK)	-	+	+	32	4	-	-	+	+	-	-	+	Grp IVb M. [illegible]
KD 17 SPT	Kerd (17)	-	+	+	10	2	-	-	+	+	-	-	+	M. triviale
SPT 1	Kerd (20)	-	+	+	50	55	±	±	+	+	-	-	+	M. triviale
SPT 1	Kerd (19)	-	-	-	27	10	-	-	-	-	-	+	-	M. scrofulaceum
SK 22	Shika (SK)	-	-	-	2	-	-	-	-	-	-	-	+	M. gastri
SPT 2	Kerd (19)	-	-	-	15	10	-	-	+	+	-	+	-	M. gordonae (aquae)
JJ SPT 2	Jaji (JJ)	-	-	-	4	-	-	-	±	±	-	-	+	M. gastri
MS 8	Mussaa (MS)	-	-	-	5	±	-	-	+	+	-	+	-	M. flavescens
ZR SPT	Zaria (ZR)	-	-	-	1	-	-	-	±	±	-	-	+	M. gastri
ZR 25	Zaria (ZR)	-	-	-	46	15	-	-	-	±	-	+	-	M. gordonae (aquae)
SB 15	Sabo (SB)	-	-	-	47	-	-	-	+	±	-	+	-	M. gordonae
SB 35	Sabo (SB)	-	-	-	50	15	-	-	-	-	-	+	-	M. scrofulaceum
SK 12	Shika (SK)	-	+	+	56	15	-	-	+	±	-	-	+	Grp IVb M. [illegible]
GW 24	Giwa (GW)	-	-	-	35	5	-	-	-	±	-	+	-	M. gordonae (aquae)
SB 36	Sabo (SB)	-	-	-	35	10	-	-	-	-	-	-	+	Grp IVb M. [illegible]
M. bovis zensin-ogen +ve control		-	-	-	25	1	+	-	±	-	-	-	+	Control (+ve)

a = Millimetres b = Isolates that need further identification + = Positive reaction (response) - = Negative reaction (response) ± = Inconclusive

Table 4:6. Source of presumptive mycobacterial isolates

Species	No of isolates	Source
M. bovis	1	Fresh milk (1)
Group II Scotochromogens		
M. scrofulaceum	2	Sputum of herder (1), "Nono" (1)
M. gordonae (aquae)	4	Sputum of herder (1), "Nono" (3)
M. flavescens	1	"Nono" (1).
Group III Non-chromogens		
M. gastri	4	"Nono" (2), sputum of "nono" hawkers (2).
M. trivialw	2	Sputum of herders (2).
Group IV Rapid growers		
M. chelonatia	4	"Nono" (4)

Table 4:7 Freqency of isolation of *Mycobacterium* species from fresh milk and sputum and pH values of samples by source.

Identity of Herd	Fresh milk				Sputum*	
	Total No. of Samples Collected	No. (%) positive for *Mycobacterium* spp.	pH Values		Total No. of samples Collected	No. (%) positive for *Mycobacterium* spp.
			Means	Range		
1	11	0(0.0)	6.80	6.70 - 7.74	1	0(0.0)
2	2	0(0.0)	6.89	6.97 - 6.90	1	0(0.0)
3	2	0(0.0)	6.67	6.62 - 6.73	1	0(0.0)
4	2	0(0.0)	6.47	6.23 - 6.71	2	0(0.0)
5	9	0(0.0)	6.62	6.54 - 6.71	1	0(0.0)
6	3	0(0.0)	6.61	6.52 - 6.78	2	0(0.0)
7	0	0(0.0)	0.00	0.00 - 0.00	1	0(0.0)
8	5	0(0.0)	6.71	6.52 - 6.87	2	0(0.0)
9	0	0(0.0)	0.00	0.00 - 0.00	1	0(0.0)
10	3	0(0.0)	6.50	6.48 - 6.52	1	0(0.0)
11	0	0(0.0)	0.00	0.00 - 0.00	2	0(0.0)
12	7	0(0.0)	6.62	6.40 - 6.82	1	0(0.0)
13	13	0(0.0)	6.56	6.21 - 6.81	2	0(0.0)
14	7	0(0.0)	6.69	6.54 - 6.80	0	0(0.0)
15	3	1(33.3)	6.67	6.62 - 6.72	0	0(0.0)
16	2	0(0.0)	6.30	6.20 - 6.40	1	0(0.0)
17	12	0(0.0)	6.55	6.30 - 6.83	5	1(20.0)
18	1	0(0.0)	6.92	6.92	1	0(0.0)
19	5	0(0.0)	6.88	6.71 - 7.20	2	2(100.0)
20	3	0(0.0)	6.53	6.35 - 6.72	2	1(50.0)
Total	90	1(1.1)	6.28	6.20 - 7.74	29	4(13.8)

*Sputum of milkers, herders and other members of the family.

Table 4:8. Frequency of isolation of Mycobacterium spp. in "Nono" samples and their pH values

Market	"Nono"*				Sputum**	
	No. of Samples Collected	No. (%) positive for Mycobacterium spp.	pH values		No. of Samples Collected	No. (%) positive for Mycobacterium spp.
			Mean	Range		
Giwa (GW)	32	1(3.1)	4.63	4.00 - 4.95	1	0(0.0)
Jaji (JJ)	13	0(0.0)	4.82	4.62 - 5.10	3	1(33.3)
Pan Ladan (PL)	14	1(7.1)	4.77	4.50 - 5.00	0	0(0.0)
Sabo (SB)	52	3(5.8)	4.18	4.10 - 5.00	0	0(0.0)
Samaru (SM)	38	0(0.0)	4.65	4.31 - 4.95	2	0(0.0)
Shika (SK)	27	4(14.8)	4.63	4.11 - 4.50	0	0(0.0)
Tudun Wada (TW)	39	0(0.0)	4.84	4.10 - 5.21	0	0(0.0)
Wusasa (WS)	16	1(6.3)	4.60	4.19 - 4.92	1	0(0.0)
Zaria City (ZR)	31	1(3.1)	4.62	4.12 - 5.10	2	1(50.0)
Total	262	11(4.2)	4.68	4.00 - 5.21	9	2(22.2)

* = Fermented (unpasteurised) milk samples

** = Sputum of 'nono' sellers.

CHAPTER 5

DISCUSSION

The tuberculin test results in the present study which established that 11.8% of cattle tested in Fulani herds were reactors, is suggestive of the fact that 59 of 500 heads of cattle screened had been exposed to mycobacteria. It is however realised that other microorganisms like Nocardia spp could also elicit delayed hypersensitivity reactions, but swellings may not have 4 millimetre or more diameter used to determine reactions in the present study (Stenius, 1938; Michigan Department of Agriculture, 1962). The 11.8% tuberculin reaction rate recorded here is much higher than the 0.2% earlier reported using the same method for a government farm of 529 animals also in Zaria area (Alhaji 1976c). It is also higher than the 1.5% reaction rate reported by Eid, (1976) for cattle in semi-settled and settled herds in North western part of Nigeria. The reactor rate recorded in this study is also slightly higher than the 7.8% cumulative reactor rate recorded by Ayanwale (1984) from a university farm in the Southern part of Nigeria.

The reactor rate recorded in the present study however, agrees with those reported in other countries such as Iran (Keyhani et al., 1973) and Malawi (Ellwood, 1975), but at variance with a rate of 50%

reported for cattle population in some parts of India (Nagaraja et al., 1973).

Management of cattle herds has been demonstrated to influence the rate of spread of infection, or incidence of mycobacteriosis moreso that over 90% of infection by typical mycobacteria, particularly, M. bovis is by airborne transmission (Alhaji, 1976; Collins and Grange, 1983). Crowding and housing under poor sanitary conditions are also known to facilitate spread of tuberculosis (Alhaji, 1976c). In the present study, of 500 animals tuberculin tested, 315 (63.0%) were from purely nomadic herds, 155 (31.0%) from semi-intensively managed farms, and 30 (6.0%) from intensively managed herds. The highest tuberculin reactor rate of 13.7% was however recorded for the purely nomadic herds, while all the heads of cattle intensively managed were negative (0.0%). This rather surprise finding may be explained in part, by the fact that cattle under the nomadic managed system are always on the move and are exposed to animals of unknown health status from other herds while grazing or at watering points, particularly during the dry season. This practice will definitely increase exposure potential to Mycobacterium species from infected animals. Intensively managed farms may however keep off mycobacterial infections out of the herds if introduction of newly purchased animals is controlled and the health of such animals checked. It is

pertinent to mention that the thirty heads of cattle from the intensively managed herds in the present study were from one farm.

It was of interest to observe that there was a general increase in tuberculin reactor rate with age in the present study with the highest rate of 20.9% in cattle over five years old. This has epidemiologic and public health implications because the Fulanis normally dispose off old cattle in their herds either to other livestock owners or for slaughter. This practice could therefore facilitate the spread of tuberculosis to other herds. while at slaughter in local abattoirs (slaughter slabs) or private slaughter where proper meat inspection is not done routinely, the risk of beef-borne tuberculosis cannot be ignored. Tuberculosis has been documented to be transmitted orally (Luke, 1958).

From the view point of milk and milk-borne tuberculosis, the finding of a relatively high tuberculin reactor rate in Fulani herds, is of significance despite known tuberculin test limitations (Macauley, 1958). This is because the Fulani herds are the major sources of raw milk and milk products to most of the populace, particularly in the rural and semi-urban communities. The situation is further compounded by the fact that most of the milk is consumed raw, and the milk-product particulary "nono" and "man shanu"

(local butter) are not exposed to any heat treatment. Although it is known that mycobacteria even resist pasteurization temperature (Chapman and Spheight, 1968). Infected cattle may therefore be shedding the organisms in their milk or contaminating the environment. The isolation of Mycobacterium bovis from a fresh milk sample is therefore of public health significance because consumers of raw milk or milk products from such cows shedding the organisms stand the risk of contracting tuberculosis. Mycobacteria have been isolated from a number of milk sources elsewhere (Chapman et al., 1965; Appuswamy et al., 1980). There are several reports of milk-borne tuberculosis in the literature (Karlson and Carr, 1970; Wigle et al., 1972; Collins and Grange, 1983; Horsburgh et al., 1985). The risk of spread of M. bovis in the herd by human herders exposed through consumption of raw milk containing the organisms is also high. Human beings contracting infection from their cattle as a result of close association with them and vice-versa have been documented (Stenius, 1938; Kotian et al., 1981).

The finding that four human herders were positive culturally for Mycobacterium species infection is also an indication that transmission of infection could go either way, cattle to man to cattle. Man has been reported susceptible to various strains of mycobacteria (Karlson and Carr, 1970; Wigle et al., 1972; Dameker et

al., 1974). It is also known that the human strain of M. tuberculosis may infect animals although not showing clinical manifestations, but such infected animals have been shown to shed the organisms in their milk (MacKay, 1941; Stuart and Harvey, 1951).

The isolation rate (1.1%) for mycobacteria from raw milk in the present study is lower than a reported 6.0% isolation rate from mastitic milk in India and America (Gajinder and Sengupta, 1951; Chapman et al., 1965).

The finding that "nono" sold in markets in Zaria area, and sputum samples of Fulani women that hawk the products around markets had isolation rates of 4.2% (11 of 262) and 22.2% (2 of 9) respectively for mycobacteria, is a source of concern. This is because "nono" is a very popular drink in Northern Nigeria amongst infants and adults, and this may constitute a source of mycobacteriosis in human consumers. Although poor sanitary conditions that accompany preparation of "nono" may be a source of mycobacterial contamination of the product, infected hawkers shedding the organisms in their sputum may also contaminate it. Mycobacteria have been isolated from a number of environmental sources such as soils and ponds (Galli - Vellerio and Bolnaud, 1927 ;Brooks et al., 1984) and are ubiquitous in nature (Horsburgh et al., 1985). The common practice by Fulani hawkers of fraudulently adding

stream or well water to increase the volume of market-bound "nono" makes it a potential source of contamination of the "nono".

It was hardly a suprise to obtain mycobacterial isolates from "nono" - a highly acidic milk product since species of Mycobacterium have been reported to survive low acidic pH (Lowrie, 1983; Brooks et al., 1984; Growle et al., 1984; Gandgadharam and Pratt, 1984).

Although only one typical Mycobacterium, M. bovis, was isolated in the present study, with the remaining 17 mycobacterial isolates being atypical, contrary to the findings of Alhaji and Schnurrenberger (1977), this finding does not reduce the significance or public health risk. This is because a number of atypical mycobacteria have been associated with human mycobacteriosis from different parts of the world (M. ulcerans - Barker, 1974; Kotian et al., 1981; M. avium intracellulare and M. scrofulaceum - Brooks et al., 1984; M. gordonae Chan et al., 1984; M. intracellulare - Demoulin et al., 1984; M. avian intracellulare - Engbaek et al., 1984; Kuze and Uchihira, 1984; Pitchenick et al., 1984; Proust and Wiles, 1984; M. fortuitum - Santamaria - Jauregui et al., 1984; M. molmoense Warren et al., 1984; M. fortuitum / M. chelonei - Rolston et al., 1985). Michell et al., (1984) have also reported the isolation of M.

scrofulaceum from skin ulcers in Koalas in Australia. This species of Mycobacterium was also isolated from "nono" and sputum of herders in this study. However, it may be necessary to determine the pathogenicity of the atypical mycobacteria isolates in the present study to fully assess their significance.

Based on the findings in the present study, it is concluded that mycobacteria are widespread in the environment particularly in cattle in Fulani herds based on tuberculin testing. Culturally, it was established that raw milk could be a source of tuberculosis to consumers with the isolation of Mycobacterium bovis.

Human herders shedding mycobacteria may be source of infection for their cows, and may contaminate fresh milk drawn from cows. The isolation of mycobacteria from Fulani hawkers and from their "nono" makes consumption of unpasteurised "nono" a potential source of mycobacteriosis in human consumers.

There is therefore a need for the government to constantly monitor the prevalence of tuberculosis in cattle particularly in Fulani herds which serve as the major source of milk and milk products for the populace. This could be achieved through the long overdue nation-wide tuberculin test survey of cattle. Detected tuberculin positive animals should be slaughtered and tuberculous positive meat condemmed. Owners of such condemned animals should be

appropriately compensated by the government.

It is also imperative to educate the Fulanis on sanitary practices during "nono" preparation and marketting, and no doubt the present Federal Government policy on Nomadic Education is a step in the right direction.

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Grouping (range = 0.50)

Cell	Frequency
11.20-11.70	9
11.71-12.21	16
12.22-12.72	5

The y ordinate for the drawing of the normal probability curve is computed using the relationships:

At $\bar{x}$: $y = 0.3989 . \frac{n.c}{\sigma}$

At $x \pm \sigma$: $y = 0.242 . \frac{n.c}{\sigma}$

At $x \pm 2\sigma$: $y = 0.54 . \frac{n.c}{\sigma}$

where $\bar{x}$ = mean strength

n = number of samples

σ = standard deviation

C = cell size or range in strength

thus

At $\bar{x}$: y = 16.50

At $\bar{x} \pm \sigma$: y = 10.01

At $\bar{x} \pm 2\sigma$: y = 2.23

Note:

Note that statistical analysis for other mixes (B & C) were carried out but only the results are shown in chapter four.