Safety in Mines Research Advisory Committee

Final Project Report

To establish the presence of *M. tuberculosis* in the underground workplaces on the gold mines and by so doing develop strategies to reduce the prevalence of tuberculosis

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Executive Summary

Tuberculosis was and still is the infectious disease responsible for most deaths on mines since the turn of the century. The annual incidence rate has recently increased dramatically and now exceeds 2 000 per 100 000 despite the availability of effective treatment.

Tuberculosis programmes which exceed the requirements of the national guidelines are present on most mines but the epidemic, now confounded by the HIV/AIDS virus continues. Development of disease, previously thought to be reactivation of childhood infection has now been shown to occur as a result of recent infection in ± 50% of cases.

This study sought to establish by means of environmental sampling the areas of transmission with particular emphasis on the underground environment. However, extensive sampling and varied laboratory methods including the most recent molecular techniques, failed to demonstrate the presence of *M. tuberculosis*.

This study found significant numbers of non-tuberculous mycobacteria particularly in the underground water and mud samples. In recent studies, non-tuberculous mycobacteria (NTM) have been found to be responsible for ± 12% of the cases of mycobacterial pulmonary disease on the gold mines. Effective chlorination of water will reduce this hazard.

Tuberculosis infection results from person to person transmission and disease develops when immunity is impaired. Control measures should be concentrated on the early diagnosis and treatment of infectious cases and environmental measures limited to areas such as hospitals and clinics where infectious patients are likely to congregate.

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GLOSSARY

ACQUIRED IMMUNO-DEFICIENCY SYNDROME (AIDS) A multiplicity of secondary diseases caused by primary infection with the HIV.

ALVEOLUS One of the terminal saclike dilations of the alveolar ducts in the lungs.

AMPHOTERICIN An antibiotic agent used for the treatment of fungal infections.

BRONCHIOLUS One of the finer divisions of the bronchial tubes less than 1mm in diameter.

CLUSTERS A group of individuals resembling each other in some way e.g. patients infected by the same strain of MTB.

CONTROL To verify an experiment by means of another with the crucial condition omitted.

DEOXYRIBOSE NUCLEIC ACID (DNA) The type of nucleic acid containing deoxyribose as the sugar component and found principally in the nuclei of the cells.

EPIDEMIOLOGY The science dealing with the distribution and determinants of disease.

HUMAN IMMUNODEFICIENCY VIRUS (HIV) The retrovirus responsible for AIDS.

IMMUNITY An inherited, congenital, or naturally or artificially acquired status in which various factors result in resistance to disease.

INCIDENCE The amount or extent of an occurrence e.g. the number of new cases of a disease.

INFECTIOUS CASE A person capable of transmitting disease.

INHIBITORS Metals and other unknown airborne particulate matter which result in negative readings.

MEDIUM (BACTEC, Lowenstein-Jensen, Middlebrook) Substances used for the cultivation of mycobacteria.

NON-TUBERCULOUS MYCOBACTERIA (NTM) Organisms belonging to the genus Mycobacterium and classified into 4 groups by Runyon.

NUCLEIC ACIDS Substances of large molecular weight found in chromosomes.

POLYMERASE CHAIN REACTION (PCR) A technique for amplifying nucleic acids.

REACTIVATION A dormant endogenous infection which causes disease.

SAPROPHYTE Organisms that grow on dead organic matter.

SENSITIVITY The ability of a test to identify correctly those who have the disease.

SPECIFICITY The ability of a test to identify correctly those who do not have the disease under study.

TUBERCULOSIS (TB) A specific disease caused by the presence of *Mycobacterium tuberculosis* organisms.

ZIEHL-NEELSEN STAIN A method for staining acid fast bacilli.

1 INTRODUCTION

South African gold miners have the highest rates of tuberculosis (Mycobacterium tuberculosis/MTB) in the industry and possibly in the world (Dr MAC La Grange, personal communication). Gold mining is a physically demanding occupation often performed several kilometers underground for up to 8 hours per day. The air is dusty and contaminated and there is a possibility that tuberculosis may be transmitted underground. Most miners live in hostels sharing rooms with others, where transmission may also occur. Transmission may also occur in hospitals and medical station clinics.

Since the early decades of this century it has been contended that transmission of tuberculosis may occur underground and Mavrogordato found tuberculosis in 8 of 46 rats kept underground for varying periods[1]. Watkins Pitchford, the first Director of the South African Institute for Medical Research (SAIMR) and of the Miners' Phthisis Bureau, examined the sputa of over 300 miners after completion of their shift. Just over 15% showed the presence of acid fast bacilli on microscopy only. As a control, he obtained sputum specimens from surface workers and only 2.5% had acid fast bacilli in their sputa[2].

However, Harvey Pirie carried out a survey of environmental samples in 1916 (100 mud, 14 air and 33 sputa) and found only one sputum positive for MTB. Fifty eight samples contained acid fast bacilli, almost certainly non-tuberculous mycobacteria (NTM). He concluded that the underground environment cannot be regarded as highly infectious and that person to person "droplet infection" remains the most important means for the spread of tuberculosis[3].

At the end of the 1960s and in the early 1970s, Prof. Webster and Dr Palmhert, Head of the Bacteriological Department of the Pneumoconiosis Research Unit, obtained samples of underground mud and soil from two gold mines and using culture methods found viable MTB organisms (Prof. Webster, personal communication). The present study was undertaken to validate these findings.

Chlorination of mine water is undertaken to destroy organisms causing acute enteritis and not primarily to control mycobacteria. Routine testing for faecal coliforms is performed at weekly intervals but chlorine estimations are not regularly done at hospital laboratories. Once NTM were identified in mine water, special arrangements were made with a mine hospital laboratory, to test for chlorine levels in the water specimens used for culturing for both coliforms and NTM.

2 OBJECTIVES

a) To determine whether the underground mining environment contained areas where tubercle bacilli could be found
b) If MTB were found, to develop methods to eliminate these organisms.

Following approval by the Safety in Mines Research Advisory Committee (SIMRAC) the study was extended to include surface areas.

3 MATERIALS & METHODS

3.1 Methods

The study was undertaken on six gold mines, five on the West Rand and one in Free State, with a total workforce of 70 000 people. The sampling methods determined at the beginning of the study were to obtain specimens from areas where people congregated, both at work and during transport to and from the shaft. Any likely specimen of sputum was to be collected. Water samples were to be taken from reservoirs, dams, stopes and travelling ways, as well as water discharged from the mines and found in regional dams. Mines close to the SAIMR where management approved the study were chosen, as samples had to be processed on the day of collection.

Environmental sampling was done in two phases. Firstly water, mud, sputa and cigarette butts were tested followed by air samples. Samples were placed in sterile containers, sealed and transported in cool boxes to the SAIMR and the University of the Witwatersrand laboratories for processing.

3.2 Laboratory techniques for the detection of mycobacteria

3.2.1 Sputa, water, mud and cigarette butts were decontaminated with 3% sodium hydroxide and then neutralized with 50mls of buffer. Following centrifuging at 3 000 rpm for 15 minutes the supernatent was discarded and the sediment planted on 12B BACTEC and Lowenstein-Jensen media (+ 0.5ml Panata) and incubated for eight weeks. Growth indices were checked at weekly intervals.

3.2.2 The air filters received on Middlebrook 7H10 media, containing amphotericin B were incubated in carbon dioxide (CO_2) at 37°C for 4 weeks after which colonies were stained with Ziehl-Neelsen and examined microscopically.

3.2.3 Air filters not already on media were divided, one half plated on Middlebrook 7H10 media and the other half dissolved in sterile water and planted on Lowenstein-Jensen media and 12B Bactec media and incubated in 5% CO_2 at 37°C.

3.2.4 The analysis at the Witwatersrand medical school used a novel qualitative approach namely the polymerase chain reaction (PCR) for detecting <u>airborne</u> *M. tuberculosis*. This does not require purification and culturing of samples. The objective of this technique is to increase the sensitivity and specificity of the test and reduce the time necessary to detect the pathogen. This also eliminates problems due to contamination by fungi and bacteria. The Roche AMPLICOR testkit was used and the NIOSH/0900 recommended method followed[4] . Note that the PCR technique is not available for airborne NTMs other than *Mycobacterium avium* complex.

3.3 Air Sampling Equipment

3.3.1 MRE type 113A gravimetric dust sampler

This machine supplied by the NCOH, Industrial Hygiene division, samples 2.5 l/min of air. The first stage is the size selector, a 4 channel horizontal plate elutriator which limits particle size to <7µ, the respirable fraction. Since it was assumed that there would be significant numbers of bacilli in the air in the TB wards, and this machine sampled smaller volumes, it was only used in the hospital.

3.3.2 Airborne biological samplers

These two machines were manufactured by a local company, DMEM Corporation, and loaned to us. The units were designed to have manually selected high and low flow rates: the high rate at 60 l/min and the low rate at 10 l/min, for use in either clean or dirty conditions. A meter records the sampling time and a gas meter the volume of air. All particles are sampled including "fallout dust" particles larger than 20 µ which only remain airborne for a few minutes. These machines were not used underground as they are too bulky and not intrinsically safe.

3.3.3 SAS – Super 90

This machine, manufactured in Italy, was purchased for the study and calibrated at PBI International. The flow rate is 90 l/min and all airborne particles are sampled. This was the only machine used to sample underground air as it is easily portable and safe.

3.4 Data analysis

Data was entered and analysed using EPI INFO 6.04 (World Health Organization, Geneva).

4 RESULTS

4.1 Sampling results of water, mud, cigarette butts and sputa (culture)
Two hundred and eighty five specimens were analysed (Table 1)

TABLE 1. NUMBER OF SPECIMENS ANALYSED

SPECIMEN	NUMBER	%
Water	193	67.7
Mud	82	28.7
Cigarette butts	6	2.1
Sputa	3	1.1
Unknown	1	0.4
TOTALS	285	100.0

NTM were cultured from 47 (16.5%) of the samples (see Tables 4 and 5 for sample details). However, no MTB were found. (Table 2).

TABLE 2. CULTURE RESULTS OF WATER, MUD, CIGARETTE BUTTS AND SPUTA

SPECIMEN	NUMBER	%
NTM	47	16.5
Bacteria	163	57.2
No growth	75	26.3
TOTALS	285	100.0

Characterisation of the NTM was done where possible and whilst no *M. kansasii* (the commonest pathogen in mine patients) were identified, *M.gordonae* and *M. scrofulaceum* were detected (Table 3).

TABLE 3. CHARACTERISATION OF NTM CULTURED

ORGANISM	NUMBER	%
M. gordonae	10	21.3
M. scrofulaceum	7	14.9
NTM's (not characterized)	30	63.8

No statistical difference was found between the number of NTMs found in underground and surface water (Table 4) or potable and service water.

TABLE 4. CULTURE RESULTS OF WATER SPECIMENS

SPECIMEN	NTM	NO GROWTH	BACTERIA
Underground	23	26	68
Surface	9	25	42
x^2=3.75 p=0.15 (no significant difference)			

Underground mud contains significantly more NTMs than surface soil (Table 5).

TABLE 5. CULTURE RESULTS OF MUD SPECIMENS

SPECIMEN	NTM	NO GROWTH	BACTERIA
Underground	12	4	39
Surface	3	15	9
$x^2=23.7$ $p=>0.001$(HIGHLY SIGNIFICANT)			

4.2 ANALYSIS OF WATER FOR CHLORINE CONTENT

Of the 105 specimens tested for chlorine, NTM grew in 18% (Table 7).

TABLE 6. CHEMICAL AND CULTURE ANALYSIS OF WATER SPECIMENS

TOTAL CHLORINE Mg/l	PRESUMPTIVE COLI	FAECAL COLI	NO GROWTH	NTMs
<0.10	35	21	18	11
0.10-<0.20	10	4	6	4
0.20-<0.30	5	2	1	1
0.30-<0.40	-	-	2	-
0.40-<0.50	1	-	1	2
>0.50	1	-	1	1
TOTALS	52	27	29	19

Note that a single specimen may contain more than one bacterial species.

4.3 CULTURE RESULTS OF AIR SAMPLES

4.3.1 Hospital Air Samples

As most of the published reports documenting TB transmission occurred in hospitals [5,6,7], samples were taken from a mine hospital serving TB patients from five mines.

Twenty air samples were analysed with volumes ranging from 600 16 800 litres and all three types of air sampling machines used (Table 7). Six specimens were taken from a TB isolation ward (occupied by four terminally ill patients), eleven from the TB wards and three from the TB outpatients department.

Despite the finding of MTB in cultures from sputa from all patients in the Isolation ward, and one being sputum positive on staining, no MTB were

Isolated from the air samples.

TABLE 7. CULTURE RESULTS OF AIR SAMPLES TAKEN IN HOSPITAL

Ward	Number of samples	Volume/litres per sample	Results
15	4	8 000	Negative
15	2	600	Negative
13	1	16 800	Negative
13	4	1 000	Negative
Isolation ward	3	600	Negative
Isolation ward	3	1 000	Negative
TB OPD	3	3 000	Negative

4.3.2 Underground Air Samples

Samples of underground air were taken using the SAS model and identical media plus Amphotericin (Table 8). Twenty samples were taken at different areas, including the cages, crush areas, stopes and bank and transport areas. Again no MTB or NTM organisms were isolated and all the plates were grossly contaminated with bacteria and fungi.

TABLE 8. CULTURE RESULTS OF AIR SAMPLES TAKEN FROM UNDERGROUND AREAS.

Number	Area	People	Time/min	Volume/litre	Results
1	Cage	44	20	1800	Negative
2	Walkway	65	20	1800	Negative
3	Bank H	357	240	16800	Negative
4	Raise centre 17	15	20	1800	Negative
5	Return raise 17/18	11	20	1800	Negative
6	Waiting place 18	29	20	1800	Negative
7	Refuge bay 18	15	25	2250	Negative
8	Tunnel 18	10	40	3600	Negative
9	Crush	50	20	1800	Negative
10	Shaft	100	20	1800	Negative
11	Cage	44	20	1800	Negative
12	18 Level	350	20	1800	Negative
13	Subshaft cage	50	20	1800	Negative
14	24 level station	90	20	1800	Negative
15	24 level haulage	30	20	1800	Negative
16	24 level stope	20	20	1800	Negative
17	25 level stope	20	20	1800	Negative
18	Refuge bay	10	20	1800	Negative
19	24 level waiting area	40	20	1800	Negative
20	18 level bank	120	20	1800	Negative

4.3.3 Surface Air Samples

Thirty five air samples were taken from various surface sites including a gold plant (Table 9), multiple areas in the hostels (including areas housing known TB patients on treatment) and transport vehicles (Table 10). No mycobacteria were isolated and all the plates were contaminated with other bacteria and fungi.

TABLE 9. CULTURE RESULTS OF AIR SAMPLES TAKEN IN A GOLD PLANT

Number	Area	People	Time/min	Volume/litres	Results
1	Area 1	4	15	1350	Negative
2	Area 2	4	15	1350	Negative
3	Area 3	150	420	29 400	Negative
4	Change house	4	15	1350	Negative
5	Change house	3	15	1350	Negative
6	Mill	24	20	1800	Negative
7	Relining area	25	20	1800	Negative
8	Change house	15	15	1350	Negative
9	Change house	20	15	1350	Negative
10	Training centre	4	10	900	Negative

TABLE 10. CULTURE RESULTS OF AIR SAMPLES TAKEN IN HOSTELS AND TRANSPORT VEHICLES.

Number	Area	People	Time/min	Volume/litres	Results
1	Hostel change	14	14	1260	Negative
2	Kitchen	95	45	3150	Negative
3	Hostel room	14	480	33600	Negative
4	Medical station	280	405	1012	Negative
5	Medical station	350	540	1350	Negative
6	Hostel room (TB)*	13	25	2250	Negative
7	Hostel room (TB)*	14	25	2250	Negative
8	Hostel room (TB)*	10	60	5400	Negative
9	TV hall	18	30	2700	Negative
10	Train	50	30	2700	Negative
11	Train	60	30	2700	Negative
12	TV hall	24	30	2700	Negative
13	Training centre	30	25	2250	Negative
14	Hostel bar	500	20	1800	Negative
15	Kitchen No 2	400	25	2250	Negative
16	Train station	90	20	1800	Negative
17	Bus	60	6	540	Negative
18	Kitchen No 4	88	20	1800	Negative
19	Medical station No 2	17	12	1080	Negative

20	Bar	55	10	900	Negative
21	Hostel subway	69	10	900	Negative
22	Medical station No 4	30	20	1800	Negative
23	Kitchen No 5	78	12	1080	Negative
24	Ambulance	8	20	1800	Negative
25	Ambulance	16	20	1800	Negative

*** Known TB patients resided in these rooms.**

4.4 AIR SAMPLING USING THE PCR TECHNIQUE FOR DETECTION OF MTB

The PCR method was validated by directly innoculating MTB organisms from cultures and sputa from infectious patients on to the filter plates. All these controls tested positive for MTB.

TABLE 11. VALIDATION RESULTS OF PCR METHODS (CONTROLS)

Number	Specimen	TB internal	PCR RESULT
1	TB culture	Positive	Positive
2	TB culture	Positive	Positive
3	Patient 1, sputa +	Positive	Positive
4	Patient 2, sputa ++	Positive	Positive
5	Patient 3, sputa +++	Positive	Positive
6	Patient 4, sputa +	Positive	Positive

Fifteen samples of underground air were tested and all were negative (Table 12), as were 32 samples of surface air (Table 13).

TABLE 12. RESULTS OF UNDERGROUND AIR SAMPLES USING PCR

Sample	Location	People	Time/min	Volume/litres/ sample	TB PCR
1	7# crush	100	15	1 350	Negative
2	7# lamproom	250	15	1 350	Negative
3	7# surface bank	1220	420	29 400	-
4	7# level 2 station	160	20	1 800	-
5	7# waiting area	57	15	1 350	Negative
6	2/41 stope entrance	12	15	1 350	-
7	7# surface check	11	15	1 350	Negative
8	Cage	40	30	2 700	-
9	Level 20	350	240	16 800	Negative
10	Level 20	2	60	5 400	Negative
11	Level 20	4	240	2 660	-
12	Waiting area	?	60	5 400	-
13	Bank area	180	60	5 400	-

14	Level 20	?	60	5 400	-
15	Level 20	2	60	5 400	-

TABLE 13. RESULTS OF SURFACE AIR SAMPLES USING PCR

Sample	Location	People	Time/min	Volume/litres/ sample	TB PCR	Volume/ litres
1	Hostel kitchen	170	20	1 800	-	1800
2	Staff mess	5	15	1 350	-	1350
3	Union mess	11	15	1 350	-	1350
4	Hostel pay hall	490	20	1 800	Negative	1800
5	Hostel pay hall	520	20	1 800	Negative	1800
6	Hostel pay hall	600	20	1 800	-	1800
7	Hostel No2	2100	420	29 400	-	29400
8	Hostel No 1	1900	420	29 400	-	29400
9	Training centre	55	480	33 600	Negative	33600
10	Hostel room	6	20	1 800	-	1800
11	Hostel room	26	20	1 800	Negative	1800
12	Hostel room	7	20	1 800	Negative	1800
13	Induction hall	40	20	1 800	-	1800
14	Induction hall	15	20	1 800	Negative	1800
15	Toilet	19	20	1 800	Negative	1800
16	Admin area	16	20	1 800	Negative	1800
17	Admin area 2	21	20	1 800	Negative	1800
18	Toilet	7	20	1 800	Negative	1800
19	Upcast shaft	?	480	33 600	-	33600
20	Bar	170	15	1 350	Negative	1350
21	Bar	480	15	1 350	Negative	1350
22	Bar	370	15	1 350	-	1350
23	First Aid class	22	15	1 350	-	1350
24	First Aid class	10	15	1 350	-	1350
25	TB drug dispensary	10	15	1 350	Negative	1350
26	Payhall	675	15	1 350	Negative	1350
27	Kitchen	7	15	1 350	Negative	1350
28	Toilet	250	60	5 400	Negative	5400
29	Hostel visitors room	6	60	5 400	Negative	5400
30	Hostel visitors room	6	60	5 400	Negative	5400
31	Hostel visitors room	?	60	5 400	Negative	5400
32	Hostel visitors room	9	60	5 400	-	5400

5 DISCUSSION

The key to tuberculosis control is early detection and effective treatment of infectious patients[8,9].Another aspect in the control of infectious diseases is to detect and reduce the number of infectious particles in the environment. This study concentrated on the environment and whilst no MTB organisms were found in 413 specimens (water, cigarette butts, mud, sputa and air), numerous NTM were found.

Mycobacterium tuberculosis

M. tuberculosis is carried on airborne droplets produced when patients with pulmonary tuberculosis cough, sneeze or speak. Larger particles containing numerous bacilli do not remain airborne and if inhaled do not reach alveoli. Small droplets (1 -5μ) remain airborne for long periods and when inhaled pass down the bronchial tree and implant in a respiratory bronchiole or alveolus[9,10] Specific immunity usually limits multiplication and spread. Techniques that reduce the number of droplets, such as good ventilation (6 air changes per hour) and ultraviolet irradiation of air in the upper areas of the room, will reduce transmission as will effective treatment which reduces the number of bacilli in the sputum.

Recently, a study of 488 gold miners using both molecular (DNA fingerprinting) and conventional epidemiology revealed that 50% of patients with pulmonary tuberculosis acquired their disease in the mining community. These results seem to dispel the long held belief that tuberculosis on the mines occurs as a result of reactivation of childhood infection. However, that study failed to identify “hot spots” of transmission which might be amenable to environmental controls. There was clustering of 31 cases who slept in the same rooms, indicating that their disease was acquired on the mines, whilst 88 cases sharing rooms with an infected case, had different strains of MTB indicating reactivation of previous disease (Dr J Murray personal communication) [11].

Schaffer and Loudon successfully aerosolized and sampled mycobacteria in the laboratory environment.[12] However, no one has successfully sampled air generated by expulsion from the human respiratory tract[13] for MTB-containing bio-aerosols or droplet nuclei. Historical attempts to culture MTB from other environments such as water and mud failed, and more recent efforts by Drs Laing[14] and Corbett (1998, personal communication)[15] have also not been successful. In this study, we were also unable to culture any MTB organisms from the environmental samples. This failure to detect MTBs in the environment may be due to inherent difficulties in culturing naturally occurring aerosolized tubercle bacilli, or the bacteria may be viable and infectious but not culturable. Alternatively, the concentration of airborne bacilli may be too low and thus simply cannot be detected using standard bio-aerosol sampling methods. Researchers in other countries have also been unsuccessful in detecting MTB organisms by culturing air samples (Paul Jensen, National Institute for Occupational Hygiene, personal communication).

To overcome the problems of sample purification and culturing we tried a novel qualitative approach for the detection of airborne MTB organisms. This technique is a DNA diagnostic method involving PCR which should detect 20 or more MTB organisms in a sample[16]. Since its first application in the diagnosis of tuberculosis in 1989 by Brisson-Noel et al, the PCR has become the most widely used technique for amplifying nucleic acids from mycobacteria. The objectives of this technology are to reduce the time necessary to detect the pathogens in clinical samples; to increase sensitivity and specificity and to simplify the test by automation and incorporation of non-isotopic detection formonts[17]. The AMPLICOR test kit manufactured by Roche, was used in this study.

As this method is relatively new and local experience limited, several controls were submitted before environmental sampling commenced. These were directly contaminated with organisms from tuberculosis cultures and sputa from known infectious patients. All these controls were positive but all subsequent environmental samples contained no MTB organisms.

As the samples taken represent only a minute portion of the mine environment, one cannot infer from this or any previous study that mine dust is entirely free of MTB organisms. Since most samples were taken from areas where people congregate and tubercle bacilli might reasonably be expected, we conclude that MTB's, unlike MTNs, do not thrive or even survive for any length of time in mining environments.

Enarso states that tuberculosis is an infectious disease caused by a bacillus which is inefficient as regards transmission and potential to cause disease and predicts that this inefficiency will result in a gradual decline in the incidence of disease without any intervention[18]. This gives us hope that the disease could be eliminated from society with modern programs. Despite comprehensive programmes, the annual incidence of TB on gold mines is 2 000 per 100 000 and increasing, almost certainly due to silica dust exposure and HIV infection. Therefore, TB control on mines should concentrate on reducing the levels of silica dust in the workplace, limiting the spread of HIV, and diagnosing and treating miners with TB as soon as possible.

Non-tuberculous mycobacterium

The non-tuberculous mycobacteria include all mycobacterium species that are not members of the *M. tuberculosis* complex hence the previous terminology MOTT – mycobacterium other than tuberculosis. The organisms are free-living environmental saprophytes which have been isolated from a variety of habitats such as soil, water and aerosols and it is now generally accepted that most human pathogens are found in natural waters including tap water [19,20].

Before the advent of AIDS and in the absence of person-to-person spread of NTMs, pulmonary infection was thought to be due to an aerosol route of infection[21]. However in AIDS patients infection can occur via the lungs, gastro-intestinal tract or both. Recent studies have shown an increase in NTM disease on mines most commonly due to *M.kansasii* [22]. Previous studies by Cowie[23] and Nel[24]and Corbett[15] also documented that *M kansasii* was the most common pathogen particularly in cases who had previous tuberculosis (Table 14).

TABLE 14. THE PROPORTION OF ALL MYCOBACTERIAL CULTURES DUE TO NTM AND THE SPECTRUM OF NTM ORGANISMS ON THE SOUTH AFRICAN GOLD MINES

Study	NTM %	%•	*M. kansasii*	*M. avium* complex	*M. scrofulaceum*	OTHER
1977 Nel n=5	5	-	4	-	1	-
1983 Cowie n=234	29	12.4	23 (79%)	2 (8.7%)	1 (4.3%)	2
1995 Sonnen-burg n=341	24	7	19 (79%)	2 (8.3%)	2 (8.3%)	1
1993-96 Corbett n=594	206	-	161 (78%)	15 (7%)	30 (15%)	-
1977-88 Cowie n=691	144	20.8	90 (62.5%)	4 (2.8%)	46 (30.6%)	4
1995 Sonnenberg n=135	27	20	15 (55.6%)	7 (25.9%)	1 (3.7%)	4

• **Proportion of all positive mycobacterial cultures which isolated an NTM**

NTM disease is thus common both in SA miners and miners in other parts of the world[25] who have lung damage from silica dust exposure and previous TB. NTMs were isolated from 47 (16.5%) of the environmental samples and of these seven were *M. scrofulaceum*, the organism responsible for pulmonary disease in several studies [15,22,23]. In this study, many NTM were not fully characterised due to difficulties with culture methods and one assumes that many of these were pathogens.

The only water specimen taken from a humidifier in an acclimatization centre contained an NTM organism which we were not able to fully characterize. It is however, well known that *M. kansasii* is frequently found in tap water where it can survive for up to 12 months [12,13]. Isolation of this organism is difficult as it appears to

thrive only intermittently[26]. Many miners are thus exposed to a potential pathogen whilst undergoing heat tolerance testing and acclimatization.

Routine testing of underground water is undertaken on many mines but acclimatization centres are not included. Moreover, as previously noted, tests for NTM are not performed and chlorine estimations rarely done. In this study, total chlorine (chlorine combined with nitrogenous products) was measured. Free chlorine, the active form of chlorine, has to be determined within one hour of collection of the specimen, and this was not possible in this study. We found that most bacteria and NTM are destroyed by the concentrations of the chlorine found in mine water but several NTM were isolated when the chlorine level was above 0.4 mg/L. The upper limit of chlorine levels should not exceed 1mg/L because of potential toxicity. However, no mine sample contained this amount and chlorination could be increased provided that regular testing was undertaken.

Whilst cardio-pulmonary disease due to MTB is compensable in SA, disease due to NTM may, or may not, be compensated. Many cases submitted to the Medical Bureau for Occupational Diseases (MBOD) are compensated are based on clinical, radiological and sputum tests for acid fast bacilli, and culture results which have identified whether the infection is due to tuberculosis or a non-tuberculous mycobacterium are not available. This is ironical, as when patients have been thoroughly investigated and found to have NTM disease on culture results, they may not receive compensation. Mycobacterial disease due to MTB and NTM diagnosed at autopsy, is compensated as there is no way of differentiating diseases caused by different mycobacteria in post mortem tissue.

6 CONCLUSION

The failure to detect MTB in the environment indicates that TB programmes should be concentrated on the early detection and effective treatment of patients with active disease. On the other hand NTMs were detected in the environment, and more careful attention to water chlorination should reduce, although not eliminate their presence.

7 RECOMMENDATIONS

7.1 Chlorination of service water to levels above 0.4 mg/L but below 1mg/L will reduce but not entirely eliminate NTM in mines. In addition to routine checks for *E. coli*, mine water should be tested for NTM and chlorine levels.

7.2 Humidifiers should be emptied, cleaned and sterilised weekly and the water in these systems tested for NTM. Reservoirs supplying acclimatisation centers should also be regularly cleaned and tested.

7.3 The MBOD should clarify the status of NTM disease with regard to compensation.

7.4 Tuberculosis is a disease resulting from person-to-person transmission and control programmes should concentrate on the early detection and effective treatment of infectious patients, both to cure the patient and prevent transmission of the disease.

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