

Bovine tuberculosis: Occupational hazard in Abattoir workers

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Abstract: The study was carried out to access *Mycobacterium bovis* as occupational hazard among abattoir staff in Enugu. Blood samples were collected from 50 subjects at Artisan and Ogbete market abattoirs with 25 samples from each location. DNA was extracted from blood samples using Relia prep DNA spin column method and screened for Tuberculosis using Nested Polymerase Chain Reaction (PCR) with specific Tuberculosis primer; Insertion sequence 6110 (IS6110) while Restriction Fragment Length Polymorphism (IS6110- RFLP) method was used to differentiate between *Mycobacterium bovis* and *Mycobacterium tuberculosis* using Nar I digestion enzyme. Statistical tools used to analyze the data were: Chi-square, fishers exact test and non parametric t test. Out of 50 samples analysed, 7 (14%) were positive in PCR method. After using Nar I digestion enzyme on the positive samples; 3 (6%) of the blood samples were positive for *Mycobacterium tuberculosis* while the remaining 4 (8%) were found to be *Mycobacterium bovis*. There was no statistically significant difference between the positive samples of the causative agents ($P>0.05$). Age distribution of tuberculosis cases in humans showed that individuals between the age-group of 16 to 45 years were significantly affected ($P<0.05$). The result of the study shows that detection of *M. bovis* in abattoir staff confirms that they are prone to occupational hazard.

Keywords: Tuberculosis, *Mycobacterium bovis*, *Mycobacterium tuberculosis*, DNA, Abattoir.

I. Introduction

Tuberculosis continues to be an important disease both in humans and animals; it causes morbidity, mortality and economic loss worldwide. Human and animal tuberculosis are widespread in Africa and are caused by organisms with very close genetic and antigenic similarities: *Mycobacterium tuberculosis* and *Mycobacterium bovis*, respectively both cause identical and clinically indistinguishable disease in humans [1]. The occurrence of *Mycobacterium bovis* disease in human, domesticated and wild animals confirms the relevance of this zoonosis [2]. Currently, bovine tuberculosis (BTB) in humans is becoming increasingly important in developing countries, as humans and animals are sharing the same micro-environment and dwelling premises, especially in rural areas [2].

The link between animal and human tuberculosis has long always been known to be strong, as shown by the works of Villemin in 1865, Davies 2006, and Koch in 1882[3] which demonstrated the cross adaptability of the tubercle bacilli from one species to another to cause disease; pointing out the danger that tuberculosis could be transmitted from animals to humans [3]. This was corroborated in 1902 by Ravenel, who demonstrated *Mycobacterium bovis* in a child with tuberculous meningitis. The current increasing incidence of tuberculosis in humans, particularly in immunocompromised persons, has given rise to a renewed interest in the zoonotic importance of *M. bovis*, especially in developing countries [4].

Mycobacterium bovis is a zoonotic organism and should be treated as a risk/hazard group III organism with appropriate precautions to prevent human infection occurring. In many cases, the course of the infection is chronic and signs may be lacking, even in advanced cases when many organs may be involved. When present, clinical signs vary; lung involvement may be manifested by a cough, which can be induced by changes in temperature or manual pressure on the trachea. Dyspnoea and other signs of low-grade pneumonia are also evidence of lung involvement. Human to human airborne transmission of *M. bovis* does occur and it may be important where human immunodeficiency virus (HIV) infection in humans is prevalent.

Tuberculosis is not only a public health concern but also an occupational health concern, that is, it can be directly related to work. Most people aged between 16 and 65 years work as part of a team rather than in isolation. Given that tuberculosis is usually transmitted through the air, potential tuberculosis contacts and infections may occur in the workplace [5].

Determining which TB cases are caused by *M. bovis* is an essential first step to elucidating the epidemiology of *M. bovis* tuberculosis, which in turn would support the development and implementation of appropriate prevention strategies. Tuberculosis caused by *M. bovis* and tuberculosis caused by *M. tuberculosis* cannot be distinguished chemically, radiographically, or pathologically in individual patients, thus the identification of these causative agents requires laboratory testing [2]. Several strategies for typing *M. bovis* isolates on the basis of DNA polymorphisms have arisen in recent years. Techniques commonly used internationally include restriction fragment length polymorphism (RFLP) analysis, spoligotyping, Pulse-field gel electrophoresis and PCR-based techniques. RFLP analysis has been demonstrated to be a robust and highly

discriminatory typing procedure due to the availability of multiple DNA probes for the detection of polymorphic loci within the *M. bovis* genome and has been the method of choice [6].

Tuberculosis is an important disease in humans and animals worldwide. It is estimated that 1.5 to 2 million people die from tuberculosis each year. A total 95% of cases occur in people in developing countries including Nigeria. TB remains a major public health problem worldwide. The disease is widespread and affecting livestock and human health in Africa [7]. It is amongst the fastest killer diseases in Nigeria today [8] and in developing countries as a whole [9].

Occupational hazard are the major source of mortality and morbidity and mortality among workers, many animal workers are exposed to many hazardous situation in their daily practice. Different types of occupational hazard encounter by animal workers were documented by Awosile [10].

This work is therefore an attempt at contributing to this knowledge, with the following:

- To estimate the risk of exposure to *M. bovis* in abattoir staff using molecular techniques.
- To monitor the frequency of *M. tuberculosis* in same population.

II. Sample Collection

Blood samples were collected from 50 subjects; 25 from each abattoir at Artisan and Ogbete market abattoirs, both in Enugu, Ngeria. The age and sex of the subjects were noted. 5mls of venous blood was collected from each subject into EDTA bottle by venepuncture after the cubital forsal of the arm has been swabbed with 70% alcohol. Samples were labelled accordingly with the laboratory number given to each sample, then transported to the laboratory in a cooler containing ice pack and stored at -20°C.

2.1 Extraction of Genomic DNA (gDNA) using Relia Prep DNA Spin Column

- Blood samples were allowed to thaw and then mixed thoroughly for 10 minutes at room temperature.
- 20µl of protein K (PK) solution was dispensed into 1.5 ml microcentrifuge tubes.
- 20µl of the blood sample was added to each of the tubes containing protein K solution and mixed briefly.
- 20µl of Cell Lyses Buffer (CLD) was added to each tube; the tubes were capped, mixed by vortex for 20 seconds and incubated at 56°C for 10 minutes.
- ReliaPrep binding column were placed in empty collection tubes and labelled according to the number of samples. Incubated tubes were removed from the heating block, then 250µl of Binding Buffer was added to each tube; the tubes were capped and vortex for 10 seconds.
- The contents of the tubes were added to the ReliaPrep Binding Column, capped and centrifuged for 1minute at 14000 rpm.
- Collection tubes that contain flow through were removed and discarded as hazardous waste.
- Binding columns were placed into fresh collection tubes and labelled accordingly; 500µl of column wash solution (CWD) was added to each column and centrifuged for 3 minutes at 14000 rpm. The flow through were discarded. This step was repeated twice for a total of 3 washes.
- The columns were placed in clean 1.5 ml microcentrifuge tube; 100µl of elution buffer (Nuclease free water) was added to each column and centrifuged for 1minute at 14000 rpm. ReliaPrep Binding Columns were discarded.
- DNA elute were labelled properly and stored at 4°C.

1. Polymerase Chain Reaction (PCR)

The primer sequence for the Mycobacterium PCR:

IS6110-Outer-F	CGGGACCACCCGCGCAAAGCCCGCAGGAC
IS6110-Outer-R	CATCGTGGAAGCGACCCGCCAGCCCAGGAT
IS6110-Inner-F	CCTGCGAGCGTAGGCGTCGG
IS6110-Inner-R	CTCGTCCAGCGCCGCTTCGG

HPLC grade. Manufactured by Integrated DNA Technology, Belgium.

3.1 Method: Nested PCR

The basic technique of PCR includes repeated cycles of amplifying selected nucleic acid sequences. Each cycle consists of three steps: (a) a DNA denaturation step, in which the double strands of the target DNA are separated; (b) a primer annealing step, performed at a lower temperature, in which primers anneal to their complementary target sequences; and (c) an extension reaction step, in which DNA polymerase extends the sequences between the primers. At the end of each cycle (each consisting of the above three steps), the quantities of PCR products were theoretically doubled. The whole procedure was carried out in a programable thermal cycler and 35 thermal cycles result in an exponential increase in the total number of DNA copies synthesized. PCR was set in 2 rounds.

3.2 Agarose Electrophoresis

2% of agarose gel was prepared by dissolving 2g of agarose in 100ml of 1x TAE buffer; 10µl of Ethidium Bromide was added. It was sterilized in microwave oven for 3minutes at medium temperature. The gel was poured and allowed to solidify. The gel was set in electrophoretic tank that contained 1x TAE buffer. 10µl of 2nd round PCR products were mixed with 2µl of 6x loading buffer and loaded into the gel with 100bp DNA ladder on the first lane and controls were set on the next two lanes, followed by PCR product of the samples. Electrophoresis was run for 30minutes, then UV transilluminator was used to visualize the products and pictures were taken.

3.3 Restriction Fragment Length Polymorphism Analysis

Only positive samples for IS6110 were run for restriction enzyme digestion. 15µl each of the PCR products were transferred into 1.5ml tube, 5µl of restriction enzyme was added to each tube and mixed by repeated pipetting. The tubes were sealed with PCR caps, spun briefly and incubated at 37^{oc} for 12hours. The products were run on 3.0% agarose gel and pictures were taken.

All analysis was carried out at Safety Molecular Pathology Laboratory, Faculty of Health Sciences and Technology, University of Nigeria, Enugu Campus.

III. Result

4.1 Blood samples from abattoir staff in enugu

Lanes labeled 3, 4, 5, 6, 7, 8, 9, 10 in figure 1, below are 7(14%) of infected samples which shows band at 123bp on agarose gel electrophoresis. After using Nar 1 digestion enzyme on the positive samples; 3(6%) of blood samples were positive for Mycobacterium tuberculosis (Fig. 2) while the remaining 4(8%) were found to be Mycobacterium bovis. In figure 2; lanes labeled 8, 9, and 10 are 3 fragmented blood samples from abattoir staff after using digestion enzyme and this confirms the presence of Mycobacterium tuberculosis in the samples. Lanes labelled 8 and 9 were from Ogbete abattoir staff while the last is one of the blood samples from Artisan abattoir staff. Lanes labelled 4, 5, 6 and 7 remained single (un-fragmented) at 123bp and this shows the presence of Mycobacterium bovis in those samples; all these four samples were from Artisan abattoir staff. Two abattoirs were screened with equal number of samples collected from each abattoir in Enugu. After the analysis; 2(8%) of 25 blood samples collected from Ogbete main market abattoir staff were positive for tuberculosis of which 1(4%) was found to be M.bovis and the other 1(4%) was M. tuberculosis while 23(92%) were negative. Statistically, there was no significant difference (P>0.05).

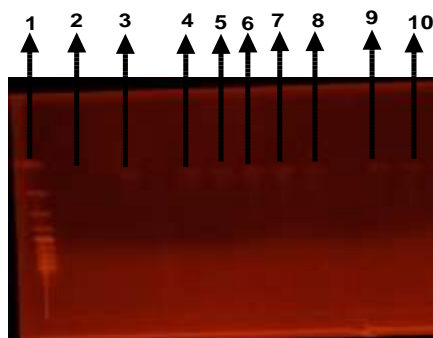


Fig 1 : PCR product on agarose gel showing Lane 1-100bp, Lane 2- negative control, Lane 3- positive control, Lane 4,,5,6,7,8,9,10 - Tuberculosis positive samples.

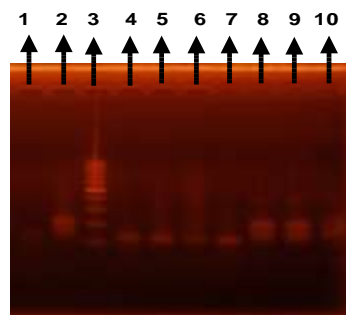


Fig 2: PCR product on agarose gel after using Nar 1 digestion enzyme. Lane 1- M. bovis positive control, Lane 2- M. tuberculosis positive control, Lane 3- 100bp ladder, Lane 4,5,6,7- M. bovis positive samples, Lane 8,9,10- M. tuberculosis positive samples.

Table 1: PCR result on blood samples from different abattoir in Enugu.

Abattoir	No of Blood Sample	Total +ve	Total -ve	M. bovis	M.tb
Ogbete	25	2(8%)	23(92%)	1(4%)	1(4%)
Artisan	25	5(20%)	20(80%)	3(12%)	2(8%)
TOTAL	50	7(14%)	43(86%)	4(8%)	3(6%)

Table 2: Age distribution of M.tuberculosis and M. bovis in abattoir staff in Enugu.

AGE	Total	Number Pos (M. tuberculosis)	Number Pos (M. bovis)
16-25	10	1	1
26-35	20	1	2
36-45	13	1	1
46-55	4	0	0
≥ 56	3	0	0
TOTAL	50	3	4

In table 2; out of 2 positive samples detected between age 16 to 25, 1 was found to be M. tuberculosis and other 1 was M. bovis. From age 26 to 35, 1 out of 3 samples screened was M. tuberculosis while the remaining 2 were M. bovis; 1 M.tuberculosis and 1M. bovis were found in 2 positive samples detected between age 36 and 45. Out of the total number 7 positive samples detected in 50 blood samples screened; 3 were found to be M. tuberculosis while 4 were M. bovis. Age 16 to 45 were significantly affected ($P < 0.05$)

In Ogbete abattoir, out of 24 blood samples screened from male, only 1 was positive for tuberculosis and it was found between age 36 to 45 while the only female screened was also positive for tuberculosis. 2,6,10,3,3 are numbers of males screened between ages 16 to 25, 26 to 35, 36 to 45, 46 to 55 and 55 and above respectively. Total number of 2 positive samples were detected in Ogbete abattoir out of 25 blood samples screened (Table 1).

In Artisan abattoir, no female was screened. From 8 males screened between age 16 to 25; 2 were positive for tuberculosis likewise 2 positive were found among 13 males screened between age 26 to 35, while 1 positive was detected among 3 males screened between age 36 to 45. Only 1 was screened between ages 46 to 55 and was negative while none was screened between ages 56 and above. From Artisan abattoir, total numbers of 5 positive were detected out of 25 blood samples screened. There was no statistically significant difference between the two abattoirs ($P > 0.05$).

IV. Discussion

Two main abattoirs were screened in Enugu namely; Ogbete and Artisan abattoir with total number of 25 blood samples collected from apparently healthy workers of each abattoir. 7(14%) of 50 blood samples collected were positive for tuberculosis, out of which 4(8%) were found to be M.bovis and this is in agreement with the review study conducted by Abubarka, who stated that prevalence of human tuberculosis due to M.bovis in Nigeria ranges from 2.1% to 14% [11]. But in contrary to the report presented by Ofukwu, who stated that in Nigeria, zoonotic tuberculosis due to M.bovis is said to account for 5% of all cases of tuberculosis in human [12]. Cadmus also stated that confirmed studies today in Nigeria have shown between 3.1% to 11.1% cases of M.bovis in human patients suffering from pulmonary or extra-pulmonary tuberculosis [13]. During the process of this study, it was found that most of the animals slaughtered in Enugu abattoir came from Northern parts of this country. One of the cattle marketers/butchers in new artisan explained that they usually get their animals from cattle dealers in the North and most of these cattle are being imported from neighbouring countries like Chad, Cameroun, and Niger. Aliyu, estimated the prevalence rate of bovine tuberculosis among slaughtered cattle in Northeastern Nigeria in 2004 as follows; Borno 1.63%, Taraba 9.83%, Yobe 5.5% while 53.30% was reported in Gombe [14].

In this study, out of the 4(8%) that was found to be M.bovis from blood samples collected from abattoir staff, 1(2%) was detected from Ogbete abattoir while 3(6%) are from Northerners who settled in Artisan market for their business. There was no statistically significant difference between the two abattoirs ($P > 0.05$). Sex-associated prevalence rate have been reported by some researchers but during the time of sampling for this study, only one female was available while 49 blood samples were collected from male workers of the two abattoirs studied. Total prevalence rate of 6(12%) was detected in male workers of abattoir. The findings of Milan [15], Chemala [16], Itah [17], Bikom [18] and Nwachukwu [19] confirmed this trend. Occupation and lifestyle put men at higher risk than female persons [20].

Age distribution of TB cases in humans showed that individuals between the age-group of 16 to 45 years were significantly affected ($P < 0.05$) (Table 5). This is similar to the finding of Nwanta [20] in Enugu State, Nigeria and Chamala [16] in china. This is probably due to the fact that individuals in this age-group are able-bodied men and women with higher exposure to the risk factors. It may also be connected to the high incidence of HIV/AIDS among young adults in Nigeria [21], as infection with HIV/AIDS facilitates the process towards active TB when exposed. Okodua [22] and Acholonu [23] corroborated this fact, when they reported significantly higher HIV/AIDS and TB co-infection in individuals belonging to the age-group, 21 to 50 years,

in Benin-City and Imo state, respectively. This finding is of great concern because of its socio economic impact on the state and the nation at large, as this group of persons constitutes the manpower of the economy.

Butchers handle a large number of animals (both infected and non-infected) on daily basis. Slaughtered animals were usually dressed and processed without any protective clothing and during the course of processing carcasses, food and drinks are usually handle and consumed with blood stained hands, a potential source of infection to the butchers and their families. One of the first studies indicating *Mycobacterium bovis* zoonotic transmission between cattle and humans in Africa was conducted in Tanzania where the same *Mycobacterium bovis* spoligotype was isolated from man and cattle [24]. In Nigeria, Simeon reported that approximately 13% of tuberculosis in human is caused by *M.bovis* [25].

V. Conclusion

In conclusion, the result of this study shows 14% prevalent rate of tuberculosis infection in abattoir workers out of which 8% prevalent rate of *Mycobacterium bovis* were recorded. Detection of *M. bovis* in abattoir staff confirms that they are prone to occupational hazard.

VI. Recommendation

For the effective control of Bovine tuberculosis in Nigeria, it is worthwhile to apply the following measures as fundamental practice:

- Proper inspection of animal by veterinarian before slaughter in abattoir is very necessary.
- Government should create a strong policy that will guide the movement of animal from one place to the other, especially from other country.
- Vaccination should be taken as paramount importance.
- Abattoir staff, herdsman and others that are working or have closely relationship with animals should be orientated on the personal hygiene in order to prevent occupational hazard.
- In general, information about zoonotic disease and their potential impact on human health should be disseminated appropriately.

References

- [1] Denis M., Keen DL, Parlane NA., Storset AK., Buddle BM. Bovine natural killer cells restrict the replication of *Mycobacterium bovis* in bovine macrophages and enhance IL-12 release by infected macrophages. *Tuberculosis.*, 2007, Vol. 87: Pp53–62.
- [2] Ogundejì E. B, Onyemelukwe N. F, Nwuko A.C, Onuoha M. N, Ogundejì A. O, Osaretin J.E, Eze K. A, Olofu J. E, Jik A. W, Kemza S.Y and Okafor N.C. Molecular detection of *Mycobacterium bovis* in cattle milk in Enugu State, Nigeria. *Journal of Natural Sciences Research*, Vol.5(12), 2015. Pp 42-47.
- [3] Davies PDO. Tuberculosis in humans and animals: are we a threat to each other? *Journal of Royal Society of Medicine* , 2006., Vol. 99(10): Pp539-540.
- [4] Radostits OM, Gay CC, Blood DC, Hincheliff KW. Disease caused by bacteria – *Mycobacterium*. In: *Veterinary Medicine: A Text Book of Disease of Cattle, Sheep, Pig, Goat and Horses*. 9th ed. Harcourt Publisher Ltd., London, 2000 Pp 909-918.
- [5] Rodríguez Bayarri M.J, Madrid San Martín F. Pulmonary Tuberculosis as an Occupational Disease. *Arch Bronconeumol*. 2004., Vol. 40 (10), Pp463-472. DOI: 10.1016/S1579-2129(06)60358-3
- [6] Rory O. B, Orla F, Eamon C, Don. O. G and mark R. Identification of Novel DNA probe for strain Typing *Mycobacterium bovis* by Restriction Fragment length polymorphism Analysis. *Journal of clinical microbiology*. 2000., Vol.38: Pp.1723-1730.
- [7] Corbett, E.L., Marston, B., Churchyard, G.J and De Cock, K.M. Tuberculosis in sub-Saharan Africa: Opportunities, challenges, and change in the era of antiretroviral treatment. *Lancet*, 2006. Vol. 367(9514): Pp926-937.
- [8] Anosike, P. Why we are waging war against tuberculosis-Damian Azubuiké Foundation In: *The Sun Newspaper*, 2011, March, 29: 23.
- [9] Seyed, M.A and Nejad, S. The causes of death among patients with Tuberculosis in Khuzestan, Iran. *Pak. Journal. Medical Sciences.*, 2008. Vol. 24(2): Pp217-220
- [10] Awosile B, Oseni O, Omoshiha E. Hazaed Exposures of workers of Animal related Occupation in Abeokuta Southwestern, Nigeria. *Journal of Veterinary Advances.*, 2013. Vol. 3(1): Pp9-19
- [11] Abubakar U.B, Ameh J.I , Abdulkadir I.A, Salisu I, Okaiyeto S.O and Kudi A.C. Bovine Tuberculosis in Nigeria: A Review. *Veterinary Research*, 2011, Vol. 4(1): Pp24-27.
- [12] Ofukwu,R.A. Studies on the Epidemiology of bovine and Human Tuberculosis in Benue State, Nigeria. A Ph.D dissertation, faculty of veterinary medicine, University of Nigeria ,Nsukka, 2006, Pp. 15
- [13] Caduns S.I falodun O.I, Fagade O.T. Methods of sputum decontamination with emphasis on local tuberculosis laboratories. *Africa journal of medical sciences*, 2011, Vol.40(1): Pp5-14.
- [14] Aliyu M.M, Adamu J.Y, and Bilyaminu Y.A. Current prevalence of Tuberculous Lesions among slaughtered cattle in Northeastern state of Nigeria, 2009.
- [15] Milan F, sanchez M, Toledo P, Ramirez C, Santilan MA. Descriptive Study of Bovine and Human TB in Queretor, Mexico. *Jan-mar Review. Latinoam Microbiology*, 2000, Vol. 42(1): Pp13-19.
- [16] Chamala DD, Nie S, Duan O. Retrospective Descriptive Study of adult tuberculosis in Wuhan, China. *International Journal of Tuberculosis of the Lung Discussion*, 2004, Vol.B(6): Pp730-736.
- [17] Itah AY, Udofia SM. Epidemiology and endemicity of pulmonary Tuberculosis in southeastern Nigeria. *Southeast Asian Journal of Tropical Medicine. Publishers*, 2005, Vol.18: Pp253-258.
- [18] Bikom PM, Oboegbulem S.I. Prevalence of suspected tuberculous lesions in cattle slaughtered in Cross River State Abattoirs, *Nigerian Journal of Animal Production*, 2007, Vol. 34(2): Pp301-305.
- [19] Nwachukwu NC, Orji A, Kanu I, Okereke HC. Epidemiology of pulmonary Tuberculosis in some parts of Abia state, Nigeria. *Asian Journal of Epidemiology*. 2009, Vol. 2(1): Pp3-19.

- [20] Nwanta, J.A ., Umeonigwe,C.N., Abonyi, G.E and Onunkwo,J.I. Retrospective study of bovine and Human tuberculosis in abattoirs and hospitals in Enugu State, southeast. *Journal of Public Health and Epidemiology*. 2011, Vol. 3 (7), Pp. 329 –336.
- [21] World Health Organisation. Bringing Vets and Medics together to deal with the bovine tuberculosis in the United Republic of Tanzania. In: *The Control of Neglected Zoonotic*, 2006. Pp. 5.
- [22] Okodua MA, Nwobu GO. Taffeng YM, Ongey JY. Agwu E. Incidence Of Hiv-Related Pulmonary Tuberculosis In Edo State, Nigeria Shiraz Electronic Medical Journal, 2004, Vol.5(1), www.semi.suns.ac.ir/vol5/jan2004.htm. accessed on 14th December 2009.
- [23] Acholonu ADW, Njoku A, Dunbar A. Prevalence of Tuberculosis and HIV infection in Imo State Nigeria. www.willyestherfoundation.org. Accessed on 3rd march, 2010
- [24] Damina M.S., Owoludun O.A., Chukwukere S., Ameh J.A., Aliyu M.M. The use of Deletion Analysis in the Detection of *Mycobacterium bovis*, *Mycobacterium tuberculosis* and *Mycobacterium africanum* among Slaughtered Cattle in Plateau State, North Central Nigeria. *Nigerian Veterinary Journal*, 2011, Vol. 32(1); Pp9 – 15.
- [25] Simeon Cadmus, Di Palmer, Melissa Okker, James Dale, Karen Gover, Nover Smith, Keith Jahans, R. GylN Hevinson, and Stephen V. Gordon. Molecular Analysis of Human Bovine Tubercle Bacilli from a local setting in Nigeria. *Journal of clinical Microbiology*. 2006, Vol. 44: Pp29-34.