

Detection of *Mycobacterium tuberculosis* Complex in Lung Specimen of Slaughtered Cattle and Goats By a DNA Based multiplex- Polymerase Chain Reaction and Ziehl-Neelsen Methods In Jos, Nigeria.

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28 ABSTRACT

29 **Aims :** Determine percentage of acid fast bacilli (AFB) present in samples by Ziehl-Neelsen
 30 (ZN) stain , detect members of *Mycobacterium tuberculosis* complex using a multiplex PCR
 31 (m-PCR),compare number of positive samples detected by ZN stain and m-PCR, determine
 32 prevalence rates of *Mycobacterium tuberculosis* complex between the two animal species
 33 studied and the rates of detecting agents of tuberculosis using Ziehl-Neelsen (ZN) technique and a
 34 Multiplex-Polymerase Chain Reaction (M-PCR) in lung specimens of slaughtered cattle and goats in
 35 the study area.

36 **Place and Duration of Study :** Samples were analyzed at the Central Diagnostic Laboratory and
 37 Molecular Biology Departments of the National Veterinary Research Institute Vom. This work was
 38 carried out between July-October 2010.

39 **Results : Study Design :** Experimental

40 **Methodology :** Our PCR amplified the 245 base pair (bp) fragment which is specific for this group
 41 of *Mycobacteria* while ZN exploited the acid-fast nature of the organisms. We examined one
 42 hundred lung samples, fifty each from cattle and goats.

43 Out of the fifty (50) lung samples from cattle, 15(30%) were positive by ZN while 9 (18%) were
 44 positive by PCR. Among the 35 lung samples from cattle that were negative by ZN smear
 45 microscopy, one (1) was positive by M-PCR. All 50 lung samples from goats were negative by the
 46 two diagnostic tools used in this study. Results obtained in this study showed 0% prevalence of
 47 MBTC in goats and 18% prevalence in cattle. The study showed a high prevalence of tuberculosis
 48 among cattle sampled in the area of study, as such, preventive and curative measures have to be
 49 stepped up in controlling the zoonosis.

50 **Keywords:** Cattle; Goats; m-PCR; *Mycobacterium tuberculosis* complex; Ziehl-Neelsen.

51

52 INTRODUCTION

53 Members of the *Mycobacterium tuberculosis* complex are slow-growing, aerobic intracellular and
 54 acid-fast bacilli that belong to the family, mycobacteriaceae. These bacteria are the causative agent
 55 of tuberculosis in humans and animals, [1]. *Mycobacterium bovis* is closely related to the species
 56 *Mycobacterium tuberculosis* (aetiologic agent of tuberculosis in human) and *Mycobacterium avium*
 57 (aetiologic agent of tuberculosis in birds). *Mycobacterium bovis* can also cross the species barrier
 58 and cause tuberculosis (Tb) in humans [2]. Due to their close relationship, the *Mycobacterium*
 59 species causing tuberculosis form the *M. tuberculosis* complex (MTBC) which include; *M.*
 60 *tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. bovis* Bacillus Calmette-Guerin (BCG), *M.*
 61 *Caprae*, *M. Pinnipedii*, and *M. cannettii* [3]. These species are the causative agents of tuberculosis in
 62 human and animals and their near relatedness, can be verified by the degree of similarity at the DNA
 63 level (DNA relatedness of 85 to 100%) [4].

64 Despite the genetic close relationship that exists among members of the *M. tuberculosis*
 65 complex, they differ in their epidemiology and in their importance for tuberculosis in humans. *M.*
 66 *tuberculosis* is the major cause of tuberculosis and, according to the latest figures of the World
 67 Health Organization (WHO), infects more than one-third of the world's population [5].
 68 *Mycobacterium tuberculosis* usually infects humans, but it can also infect animals which have
 69 contact with infected humans. *M. africanum* is predominantly isolated from different parts of Africa.
 70 In certain regions, *M. africanum* represents up to 60% of the isolates obtained from patients with
 71 pulmonary tuberculosis . *M. microti* causes tuberculosis mainly in small rodents like voles [4].
 72 However, up till now its importance for TB in human has remained unclear [4]. *M. cannettii* can
 73 cause Tb in humans, but so far only a few *M. cannettii* strains have been isolated and its
 74 epidemiological contribution to human Tb is uncertain [6].

The host range of *the Mycobacterium tuberculosis* complex is broad and it can cause Tb in domestic animals like cattle and goats, wild animals and humans [7]. Tb in domestic animals remains an important disease in many countries of the world, causing significant economic losses. Tb, limits live stock production and affects their quality. It is transmitted to humans through consumption of contaminated milk, improperly cooked meat from infected animals and contaminated dairy products. Infected milk and milk products have been found to be the sources of primary tuberculosis in children and infants, mainly extra pulmonary forms: (miliary, meningeal or lymphatic nodes-localized) [8].

Respiratory droplet is the transmission vehicle of pulmonary tuberculosis in exposed adults especially among slaughter – house and meat processing industry workers [9]. Animal and human Tb, emerging or re-emerging and caused by species of the *M. tuberculosis* complex, are wide-spread and affect the animal industries and human health in Africa [10]. Most human tuberculosis due to *M. bovis*, occur in young individuals due to ingestion of contaminated milk or through breaks in the skin. As a result of this, cervical lymphadenopathy, intestinal lesions, chronic skin tuberculosis (Lupus vulgaris), and other non-pulmonary forms are very common [11].

Bovine Tb is a multi-host infection with slow progression of disease in the individual. Tuberculosis is a major opportunistic infection in human Immunodeficiency Virus (HIV)-infected persons. The epidemic HIV infection in Nigeria, in which Tb in animals and the environmental conditions, enhancing zoonotic transmission, could make animal Tb infection, a serious public health threat to persons particularly at risk [10-11].

96 In Nigeria, bovine tuberculosis is an endemic bacterial disease of cattle, judging from the
97 reported prevalence from various abattoirs in the country [12]. Nigeria ranks fifth among the 22 high
98 TB burden countries in the world. One of the available reports in Nigeria of diagnosis of bovine TB
99 by bacteria culture from abattoir specimens showed that *M. bovis* was isolated in 62.5% of cases, *M.*
100 *tuberculosis* in 14.0% and *M. avium* and unclassified *Mycobacteria* in 9.5% [12].

101 A report from Sabon Gari Local Government Area of Kaduna State, showed that out of 32
102 nomadic Bunaji cows, with a history of persistent coughing, emaciation, superficial
103 lymphadenopathy using rapid bovine tuberculosis Antibody test, 4 were positive for antibodies to *M.*
104 *bovis* and were culled to avoid further spread of the diseases [13]. Another study carried out in four
105 abattoirs in North – Eastern Nigeria, two from Borno State in Maiduguri and Damboa, and two from
106 Yobe State in Damaturu and Gashua, showed prominent tuberculous lesions in lungs and associated
107 lymph nodes in slaughtered cattle [12].

108 Documentation of bovine tuberculosis in goats in Nigeria was reported by Ojo [14], on the
109 basis of gross lesions without culture confirmation. Live stock owners in Nigeria normally graze
110 cattle and goats together, and this practice poses a high risk for transmission of bovine tuberculosis
111 among these animals [14]. This practice is especially a threat to goats in Nigeria because several
112 reports have described bovine TB in cattle in Nigeria [15]. However, reports of diagnosis in goats in
113 Nigeria are lacking.

114 Molecular epidemiologic techniques such as deletion typing and spoligotyping have been used
115 to characterize members of the *M. tuberculosis* complex and to provide information on transmission
116 of mycobacterial diseases between animals and human [16]. However, because of limited resources
117 and lack of expertise, these techniques are not commonly used in most developing countries such as
118 Nigeria, where tuberculosis is endemic [17].

119 In Nigeria, as in most resource-limited countries, the Ziehl-Neelsen's (ZN) smear microscopy
120 method is used in clinical laboratories for the confirmation of clinical Tb, and for detection of Tb

agents (acid fast bacilli) in biological samples. It is not very specific and sensitive because it requires 10^3 to 10^4 organisms per ml of specimen such as sputum or tissue homogenate and detects only acid fast bacilli [18]. This makes the ZN method less dependable than the other methods such as the DNA based PCR method.

Among these methods, the culture method is still the gold standard as isolates can be used to study the morphology, physiology and biochemical characteristics of the organism. It is also more specific and sensitive than ZN microscopy. Nevertheless, despite being the gold standard, most routine laboratories do not culture for *M. tuberculosis* complex. This may be as a result of the fastidious nature of the organism, the cumbersome nature of its manipulation in the laboratory as well as the slow growth rate (3 – 8weeks) when cultured. However, the PCR methods have proven to be more rapid, highly specific and more dependable for diagnosis than other methods. Moreover, in recent studies the spoligotyping method has shown to be useful for the identification of species of the *M. tuberculosis* complex.

Reports on the use of PCR for the detection of MTBC in biological samples date over a decade [19]. Most of the studies, however, were performed in non-endemic western countries with only a few [20] from some endemic nations of sub-Saharan Africa [21]. The IS6110 sequence is specific for the MTBC [22]. The sequence, which undergoes several inverted repeats in the bacterial chromosome, has been cloned and used for detection and for molecular epidemiological studies of the MTBC group [20].

Justification and Scope of Study

Little information is available on the use of ZN and m-PCR for specific and efficient detection of members of *M. tuberculosis* complex in biological specimens in the study area .This present study compared the rates of detection of Tb agents(*Mycobacterium tuberculosis* complex) (MTBC) by ZN microscopy and IS6110 DNA based m- PCR assay, in lung specimens from slaughtered cattle

145 and goats at the Jos central abattoir, Plateau state, Nigeria. It also attempted to establish the
 146 prevalence of Tb caused by MTBC group in cattle above goats in the study area.

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171 MATERIALS AND METHODS

172 Study Area

173 Lung samples of slaughtered cattle and goats were collected from the central abattoir in Jos,
 174 Plateau State, Nigeria. The research work was carried out at the Central Diagnostic
 175 Laboratory and Molecular Biology Departments of the National Veterinary Research
 176 Institute, Vom,

177 Sample Size

178 The sample size was determined using the formula:

$$N = \frac{Z^2 \times P \times q}{d^2} \quad (Benneth \text{ et al. }, 1991)$$

182 Where: Z = 95% confidence limit

183 N = Minimum sample size

184 P = Local Prevalence

185 q = 1 – P

186 d = Tolerable error (5%)

187 The local prevalence of bovine tuberculosis in Jos, Plateau State was 15.4%.

188 Therefore,

$$N = \frac{0.95^2 \times 0.154 \times 0.846}{0.05^2} = 47$$

191 One hundred (100) lung samples were collected (50 each from slaughtered cattle and goats).

192 **Sample Collection and Preparation**

193 Lung samples were collected from slaughtered cattle and goats into sterile universal containers and
 194 taken to the Laboratory for detection of any member of the *Mycobacterium tuberculosis* complex by
 195 ZN stain microscopy and multiplex PCR. All samples were collected between September and
 196 December 2010.

197 Lung samples were homogenized using sterile sea-sand, mortar and pestle. 1ml of phosphate buffer
 198 saline was added to about 2g of lung sample when homogenizing. The homogenate of each lung
 199 sample was strained into 1.5ml micro-centrifuge tubes, corked and stored at – 80°C until ready for
 200 use. Smears were made from lung homogenates and stained by Ziehl-Neelsen method and observed
 201 under the light microscope (x100) for detection of acid-fast bacilli. The stored homogenates were
 202 further used for detection of *M. tuberculosis* complex by the m-PCR assay.

203 **DNA Extraction**

204 Total DNA was extracted based on the protocol provided in the kit used (Zymo Research Genomic
 205 DNATM – Tissue Mini – Prep, (Catalog: Numbers, D3005 and D3051), which was purchased from
 206 Inqaba Biotech Company in Pretoria South Africa. The protocol was as below:

- 207 1. To a tissue sample homogenate of 95µl, 95µl of 2x digestion buffer and 10 of
 208 proteinase – K were added and mixed properly by vortexing in a 1.5ml centrifuge tube.
- 209 2. It was incubated at 55°C for 3 hours on a heating block.
- 210 3. 700µl Genomic lysis buffer was added to the tube and mixed thoroughly by vortexing
 211 and centrifuged at 10,000 Xg for one minute to remove insoluble debris.
- 212 4. The supernatant was transferred to a Zymo-Spin tm IIC column in a collection tube and
 213 centrifuged at 10,000 Xg for one minute.
- 214 5. 200µl of DNA pre-wash buffer was added to the spin column in a new collection tube
 215 and centrifuged at 10,00Xg for a minute.

216 6. 400µl of g – DNA wash buffer was added to the spin column and centrifuged at
217 10,000Xg for one minute.

218 7. The spin column was transferred to a clean micro-centrifuge tube. 30µl DNA elution
219 buffer was added to the spin column and incubated for 5 minutes at room temperature
220 then, centrifuged at 14,000 Xg for 30 seconds for the elution of the DNA. This was
221 repeated for all the lung samples.

222 DNA Amplification

223 PCR was performed in a 50µl reaction volume comprised of 26.5µl nuclease-free water
224 (Fermentas®), 5µl dNTPs (10mM), 1µl each of JB21, + JB 22 primers for 500bp (JB21, 5¹–
225 CGTCCGCTGATGCAAGTGC-3¹ and JB22, 5¹-CGTCCGCTGACCTCAAGAAAG-3¹)
226 INS1+INS2 primers for 245bp (INS1, 5¹–CCTGCGAGCGTAGGCGTCGG-3¹ and INS2, 5¹–
227 CTCGTCCAGCGCCGCTTCG-3¹), 0.5µl taq DNA polymerase and 5µl extracted DNA. The total
228 reaction mixture was submitted to an initial denaturation at 94°C for 5 minutes, then to 30 cycles of
229 denaturaion at 94°C for 1 minute, annealing at 68°C for 1 minute, and elongation at 72°C for 7
230 minutes and cooling at 4°C for as long as there was time.

231 Amplicons and 100 DNA base pair (Fermentes®) molecular markers were loaded in 2% agarose gel
232 stained with ethidium bromide 10glml (Promega®) with 6x loading dye (Fermentas®) and
233 electrohoresed at 100Volts for 45minutes. Two hundred and forty five based pair (bp) target DNA
234 fragments, specific for MTBC were viewed under ultraviolet illumination. An in house
235 *Mycobacterium tuberculosis* complex positive lung sample was used as a positive control while all
236 the constituents of the PCR master mix without the DNA extracts, was used as the negative control.
237 The m-PCR products were snapped and recorded

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240 RESULTS

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242 From the 100 lung samples processed, 50 were from slaughtered cattle and goats. Out of the 50 lung
243 samples from cattle, 15 (30%) were positive for Acid fast bacilli (AFB) by Ziehl-Neelsen stain
244 microscopy, while 35 (70%) were negative. Also, out of the 50 lung samples from slaughtered goats,
245 none (0%) was positive for AFB by ZN stain microscopy.

246 Comparatively, 9(18%) lung samples from cattle were positive for *Mycobacterium tuberculosis*
247 complex group by multiplex – polymerase chain reaction, amplifying the 245 base pair fragment.
248 However, there was no amplification of the 500bp fragment, which is sometimes specific for *M.*
249 *bovis* (Figures 1, 2, and 3). All the lung samples from slaughtered goats were negative for both AFB
250 by ZN stain microscopy and MTBC using an m-PCR assay.

251 In this study, all the lung samples from slaughtered goats were both negative for AFB by ZN-smear
252 microscopy and by the M-PCR respectively. Among the 35 lung samples from cattle negative by ZN
253 smear microscopy, only one (1) was positive by M-PCR, amplifying the 245bp fragment.

254 Statistically, the chi-square analysis at 95% confidence limit, showed a higher prevalence of bovine
255 tuberculosis in cattle and that prevalence in goats was zero (0%) by both ZN stain microscopy and
256 m-PCR assay, there was (Tables 1 and 2). With a chi-square value of $X^2=17.647$ it means that, there
257 is a significant relationship between the animal type and the high prevalence of the disease (Table 1).

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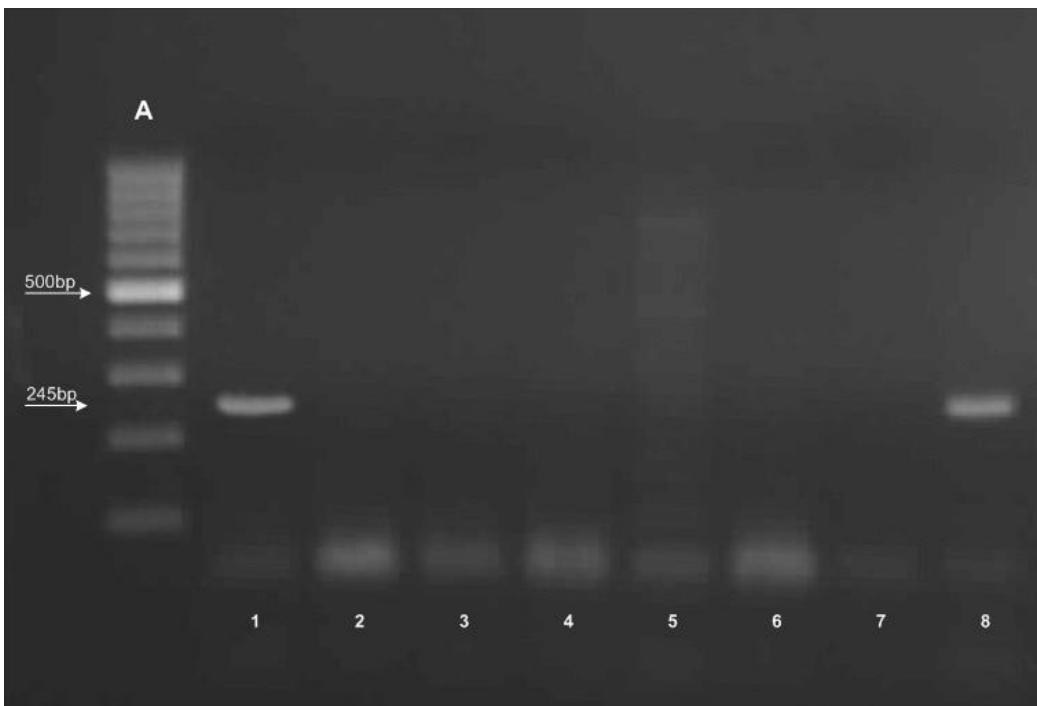
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TABLE 1: Detection of Acid fast Bacilli in Lung Samples from Slaughtered Cattle And Goats Using Ziehl-Neelsen Stain Microscopy

Type of Animal	Number of lung samples tested	Number (%) positive for Acid fast bacilli
Cattle	50	15 (30)
Goat	50	0 (0)
Total	100	15 (30)

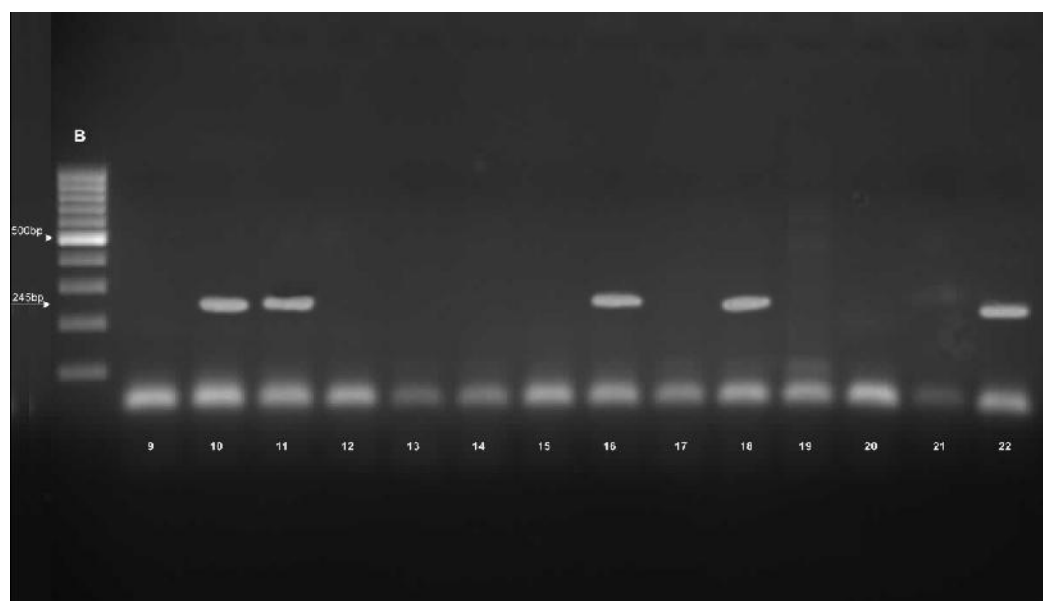
$\chi^2 = 17.647$ DF = 1 P = .05

Figure 1: Agarose Gel Electrophoresis of IS6110 m-PCR for Detecting *M. tuberculosis* Complex from Lung Samples of Slaughtered Cattle



Lane A: 100bp DNA ladder (Fermentas).Lane 1: m- PCR amplification of 245bp fragment diagnostic of MTBC. Lane 7: Negative control. Lane 8: Positive control

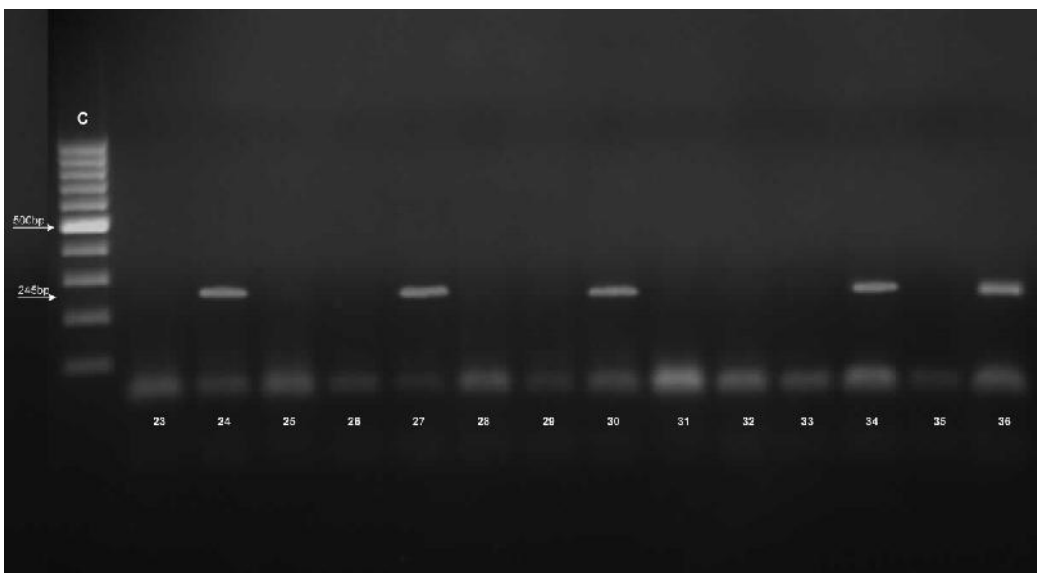
272 **Figure 2:** Agarose Gel Electrophoresis of IS6110 m-PCR for Detecting *M. tuberculosis* Complex
 273 from Lung Samples of Cattle



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 275 Lane B: 100bp DNA ladder (Fermentas®). Lane: 10, 11, 16 and 18: m-PCR amplification of the 245bp fragment diagnostic for MTBC. Lane 21:
 276 Negative control. Lane: 22: Positive control.

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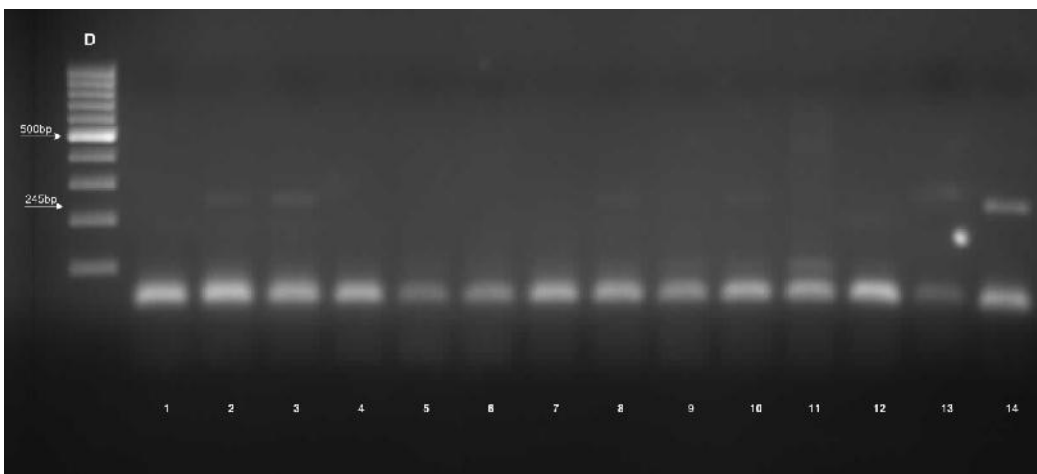
Figure 3: Agarose Gel Electrophoresis Of IS6110 M-PCR for Detecting *M. tuberculosis* Complex
From Lung Samples Of Cattle



Lane C: 100bp DNA ladder (Fermentas).Lanes 24, 27, 30 and 34: m-PCR amplification of the 245bp fragment diagnostic for MTBC.

Lane 35: Negative control, Lane 36: Positive control.

Figure 4: Agarose Gel Electrophoresis of IS6110 m-PCR for Detecting *M. tuberculosis* Complex from Lung Samples of Slaughtered Goats



Lanes 1-12, showed no amplification of both 245bp and 500bp fragments. Lane 13: Negative control. Lane 14: Positive control.

Table 2: Detection of the MTBC group in lung samples from Cattle and Goats using m-PCR

Type of Animal	No Tested	M-PCR	
		No (%) Positive	No (%) Negative
Cattle	50	9 (18)	41 (82.0)
Goat	50	0 (0)	50 (100)
Total	100	9 (18)	91 (91)

$\chi^2 = 9.890$ DF = 1 P = .05

296 DISCUSSION

297 The incidence of bovine tuberculosis has been observed to be increasing in many parts of the world,
 298 especially in third world countries where, the standard of living is poor, such as in Asia and Africa
 299 [1]. In this study, one hundred lung samples were tested for *Mycobacterium tuberculosis* complex
 300 group, 50 lung samples each were collected from cattle and goats respectively. Two methods were
 301 used for the detection of MTBC groups in the lung samples: Ziehl-Neelsen stain microscopy and m-
 302 PCR methods.

303 The results obtained from this study, showed that tuberculosis was more prevalent in cattle than in
 304 goats. This agrees with [12], who reported a 62.5 % prevalence in cattle against 0.36% in goats in
 305 four northern states. The wide disparity of the prevalence of TB between cattle and goats have been
 306 traced to an innate resistance of goats to TB [15]

307 Out of the 50 lung samples from slaughtered cattle, 15 (30%) were positive for Acid fast bacilli
 308 (AFB) by Ziehl-Neelsen stain microscopy, while all of the 50 lung samples from slaughtered goats
 309 were negative for AFB by both ZN staining and m-PCR methods. This does not agree with the
 310 findings of Ojo, [14], where 4 strains of *M. bovis* and 1 strain of *M. tuberculosis* were isolated from
 311 goats in Ibadan, Nigeria.

312 In this study, the results obtained by the m- PCR technique, showed that amplification at the 245bp
 313 target region by INSI and INS2 primers was obtained and that, there was no amplification at the
 314 500bp fragment by the JB21/JB22 primers. This agrees with [23], who reported a 13.3% failure of
 315 *M. bovis* isolates to produce the 500bp fragment. This failure was traced to mutations which occurred
 316 along the insertion sequence of the organism, leading to alterations of the diagnostic base pair
 317 fragments. This resulted to failure of the JB21\JB22 primers to amplify the 500bp fragment
 318 diagnostic for *M. bovis*. It is possible that members of the MTBC responsible for TB in the animals
 319 tested, lacked the 500bp fragment.

320 Amplification of the 245bp fragment is diagnostic for the MTBC-group while amplification of 500bp
 321 fragment is diagnostic for *M. bovis* [24]. PCR using primers JB21 and JB22 has been considered to
 322 be highly reliable in identifying *M. bovis*, presenting 100% concordance with the conventional
 323 microbiological method. However, the absolute specificity of JB 21 and JB 22 (500bp) primers to
 324 *M. bovis* has been disputed by some studies which reported that 13.3% (4/30) of *M. bovis* isolates
 325 failed to produce the 500bp fragment [25]. Using specific primers for IS6110 sequence, the 500bp –
 326 negative isolates were identified as belonging to the MTBC groups, leading to causing the authors to
 327 suggest that those isolates may lack the genomic target for JB21 and JB22 primers for 500bp
 328 fragment.

329 With the above limitation that may occur using PCR, it is possible that 18% lung samples which
 330 produced amplification of the 245bp fragment only, may lack the genomic target for the JB21 and
 331 JB23 primers for 500bp fragment, as suggested by [25].

332 In this study, out of the 30% positive for tuberculosis by ZN staining, it was observed that only 18%
 333 were positive by multiplex – PCR. Several reasons may be responsible for differences in results.
 334 Some of the isolates may lack the genomic targets for both JB21 and JB22 and INS1/INS2 primers
 335 respectively. Alternatively, they may be members of the MTBC requiring the use of Specific primers
 336 for confirmation, or environmental mycobacterium species that do not belong to MTBC.

337 Members of the MTBC undergo mutations constantly and as such, primer specificity cannot be relied
 338 upon over a long period of time. These mutations can be identified by PCR amplification of target
 339 loci and subsequent analysis by PCR-SSCP (Single strand conformation polymorphism), probe
 340 hybridization; sequencing etc one of such assay is commercially available. While this assay has been
 341 found promising and reproducible [26], it is not applicable to a number of strains and correlation of
 342 60% has been observed in some of these studies due to unknown mutations.

343 Cultivation of MTBC, though not fully proven, is considered the gold standard for the diagnosis of
344 tuberculosis in both humans and animals. However, this gold standard lacks sensitivity and is
345 negative in specimens from majority of paucibacillary cases. This poses a great dilemma for
346 comparing gene amplification methods which are vastly more sensitive but have danger of false
347 positivity or negativity due to contamination. While PCR is specific for other gene amplification
348 assays, other data should be carefully interpreted and should be correlated with clinical, histological,
349 cytological, biochemical, imaging and therapeutic parameters [26].

350 Due to various limitations of false negativity and danger of false positivity, no guidelines for
351 application of gene amplification methods are readily available. American Thoracic Society [27]
352 recommended using PCR as additional evidence in specimens smear positive for AFB with smear
353 negative cases, cautious approach of using gene amplification as one of the evidences would be
354 preferable [28], which should be repeated in cases of doubt. The use of ZN-stain microscopy as a
355 diagnostic tool for tuberculosis is still very much in use in third world countries like Nigeria because
356 it is cheap and affordable.

357 In this study, the detection MTBC by m-PCR method was 18%. This prevalence of bovine
358 tuberculosis is higher than the 15.4% reported earlier by [29]. In their study, they examined with
359 human subjects (sputum samples) while in this study, slaughtered cattle and goats (lungs samples)
360 were used. Therefore, care should be taken in comparing the results from the two studies because of
361 differences in subjects, methods used and infecting agent identified. It is highly probable that the
362 majority of the MTBC species detected from cattle in this study could be *M .bovis*, but additional
363 work will have to be carried out to establish this.

364 It can however be concluded that, the prevalence of bovine tuberculosis in cattle in this environment
365 is high and one reason for this high prevalence rate, may probably be attributed to the lack of
366 national and state control strategies. However, other factors may be involved, taking into
367 consideration the limitations of this study.

Moreover, as tuberculin testing of human, cattle and other susceptible animals is not routinely performed, the detection of bovine tuberculosis is restricted mainly to the observation of meat inspectors at the abattoirs. This inspection on the other hand is often not consistently carried out by the inspectorate agency. This no doubt poses a great threat to the unsuspecting consumers. This has economic and public health implications in Jos due to the zoonotic nature of the organism.

The possibility of zoonotic transmission of bovine tuberculosis from animals to humans has been reported by several authors [14] but its degree is not clear because of inadequate follow-up and documentation. However, cultural practices exist that could facilitate transmission between cattle and humans. For example, prior to sale, cattle are fattened in close proximity to farmers' homes. After being sold at markets, cattle are often slaughtered in nearby abattoirs, where the butchers wear minimum protective clothing and process offal from diseased carcasses with their bare hands. The close association between farmers and animals is exemplified by the Fulani herdsmen, who live their entire lives with their animals, offering ample opportunity for zoonotic transmission of infection.

In the course of this study, it was observed that, animals brought to the Jos main abattoir were not examined or inspected before slaughter and also, meat inspection was not carried out before sale. In order for meat inspection to be an effective means of monitoring the level of bovine tuberculosis in the study area, all predilection tissues and organs should be thoroughly examined during inspection and condemned if contaminated by MTBC species.

Major improvements in animal and human health, within the concept of meat consumer protection and eradication of epizootic tuberculosis during the 1960s in developed countries were achieved, when drastic destruction of organs with relevant or suspicious lesions at meat inspection was the main strategy employed [30]. Rigorous meat inspection tracing of TB lesions back to the animal farms, pasteurization of raw milk before consumption, continuous tuberculin testing of human and domestic animals susceptible to TB and prompt treatment of infected humans are complementary to eradication or consistent reduction of zoonotic bovine TB. [30]

Health education on the prevalence of bovine tuberculosis, the mode of transmission, prevention and control is needed in the study area. This is because, most of the butchers and other abattoir workers often wear little or no protective outfit when slaughtering the animals and handling of their viscera organs. This attitude may be due to lack of proper education on the part of the workers or lack of knowledge of the high prevalence of this important zoonotic disease by the public health workers and their agencies in the Jos central abattoir.

However, bovine TB and zoonotic TB due to *M. tuberculosis* complex group are poorly investigated and controlled in most African countries including Nigeria [31]. The cattle and goats slaughtered at the abattoir in the study originated from different major cattle rearing areas of the country. Nevertheless, the sources or farms from where the animals were reared before being brought for slaughter at the abattoir were not known.

The incidence of bovine tuberculosis in cattle herds in Nigeria will continue to increase unless there is a concrete effort by the Nigerian government to formulate a comprehensive policy on the control of bovine tuberculosis as is done in the developed countries for example in Europe. If this is not done, the zoonotic spread to humans as documented by [15] will increase and this may have a very devastating effect on the people when one considers the epidemic of HIV/AIDS in the country.

Limitations of Study

1. The health status of the animals used in this study was not known.
2. Information on vaccination of the animals was not provided.
3. During sampling, cross contamination of samples was not prevented, since knives used for cutting lung samples were not sterilized before use.
4. Samples were collected randomly without looking out for suggestive T.B lesions.
5. Information on the source or farm from where each animal was reared before being brought for slaughter was not provided.

417 **Conclusion**

418 The observations from this study have shown that cattle may be more susceptible to tuberculosis than
419 goats. This was shown by the various tests carried out, for the detection of members of the
420 *Mycobacterium tuberculosis* complex in both slaughtered cattle and goat.

421 By the statistical analysis using Pearson-chi square test, the prevalence of the disease in cattle was
422 significant and had relationship with the animal type. Nevertheless, limitations cannot be ruled out,
423 which makes this work subject to further research. It has been shown that the best technique for
424 detecting the various species of the MTBC is spoligotyping [32]. In this study, spoligotyping was
425 not carried out.

426 Bovine tuberculosis is underestimated, but endemic in slaughtered cattle in Nigeria [15]. The test
427 and segregation, with removal of infected livestock, intensification of slaughter and meat inspection,
428 laboratory analysis of lesions for isolation, and confirmation of isolates by molecular typing for
429 strain differentiation and identification are practical intervention for bovine TB in the study area.
430 They will clarify the epidemiology of animal and human TB including zoonotic bovine TB for
431 effective control to be developed.

432 Finally, TB is prevalent in cattle destined for human consumption in Nigeria with serious public
433 health implications. The general public is at risk, and infected individuals can serve as source of
434 infection. Many opportunities exist for the emergence of zoonotic TB and necessitate further study
435 into the modes of transmission and link between human and bovine TB through molecular
436 techniques. Targeted control of infected animal populations, concerted veterinary and medical
437 efforts, active involvement of the populations at risk, and good health systems are essential for
438 effective control.

439 From the foregoing, the following recommendations can be made:

1. More of such research should be carried out in the study area to see if results obtained will be in concordance with the results obtained from this research to establish the prevalence of bovine tuberculosis among cattle above goats.
2. An all encompassing research can be carried out in the study area, involving more techniques for the identification of the members of *Mycobacterium tuberculosis* complex in domestic animals and animal farmers and handlers in order to establish the prevalence of tuberculosis in both humans and animals in the area.
3. Financial support of research in the study area should be encouraged, to enhance purchase of the necessary biologics required for speciation of the members of the *Mycobacterium tuberculosis* complex, in order to identify the particular species responsible for tuberculosis in the area.
4. Adequate animal inspection before sale and slaughter, should be encouraged and annual tuberculin testing of animals and animal handlers should be carried out to reduce the prevalence of tuberculosis in that area.

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Competing Interest

Authors have declared that no competing interests exist.

463 **Authors' Contributions :**

464 Chukwu Isioma Doris : Did the sampling, ran the DNA extraction and wrote most of the manuscript

465 Chukwu Otuh Okoh Chukwu : Wrote the Introduction

466 Kandakai- Olukemi : Read and corrected the entire manuscript

467 Owolodun Olajide : Prepared the PCR master mix for the m-PCR assay

468 Chika Nwosuh : Assisted in purchasing the extraction kits and primers used for this work

469 Godwin Agada : Did the Ziehl-Neelsen staining for this work

470 Benshak Audu : Assisted in DNA extraction and the PCR assay

471 Chollom Solomon : Assisted in writing the results and preparing the manuscript for publication

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