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Survival of *Mycobacterium bovis* Following Heat Treatment of Infected Tissues obtained from Slaughtered Cattle in Sokoto Metropolitan Abattoir, Nigeria

Abdurrahman Hassan Jibril*, Hameed Garba Sharubutu, Bello Rabiu Alkali, Bashir Muhammad Bello and Abdullahi Abdullahi Raji

Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto, Nigeria

***Corresponding author:** Abdurrahman Hassan Jibril, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto PMB 2346, Nigeria, Tel: +234 60 23 21 34; E-mail: jibrilah50@yahoo.com

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Abstract

This study was carried out to determine the viability and transmissibility of heat treated *Mycobacterium bovis* from gross tuberculous lesions obtained from Sokoto metropolitan abattoir. A total of 25 samples were collected for a period of 6 weeks, 13 (76.5%) of lungs and 5 (62.5%) of lymph nodes were positive on Ziehl-Neelsen stain. Positive samples were subjected to a temperature and time combination of about 1000C for 20 minutes. Acid fast bacilli was demonstrated in 25.0% (4/9) lungs and 40.0% (2/3) of Lymph nodes. This study used guinea pig (*Cavus porcellus*) as an infection model and were categorized into control and experiment groups. Experimental group (18) were inoculated with heat treated *Mycobacterium bovis*, while Control group (6) were inoculated with non-heat treated positive residue, and were examined post mortem for the presence of tuberculosis lesion 35 days post inoculation day (pid). Samples from lesions taken for direct microscopy to check for Acid Fast bacilli. At necropsy pulmonary granuloma and congestion with diffuse hepatic and splenic necrosis was observed. In conclusion, *Mycobacterium bovis* shows heat resistance and could retain its pathogenicity following cooking. There should be strict adherence to meat inspection procedure in abattoirs and governments should provide diagnostic laboratories in our existing abattoirs to augment meat inspection procedures.

Keywords: *Mycobaterium bovis*; *Carvia porcellus*; Ziehl neelsen; Viabilty

Introduction

Mycobacterium bovis causes tuberculosis (TB) mainly in cattle but has a broad host range and causes disease similar to that caused by *M. tuberculosis* in humans [1]. It belongs to the *M. tuberculosis* complex (MTBC) that comprises the closely related human pathogens *M. tuberculosis* and *M. africanum* [2]. *Mycobacteriums* are predominantly rod shaped about 0.5 µm wide and variable in length. Spores, flagella and capsules are absent [3]. *Mycobacterium bovis* can be demonstrated microscopically on direct smears from clinical samples and on prepared tissue materials. The acid fastness of *M. bovis* is normally demonstrated with the classic Ziehl-Neelsen stain, but a fluorescent acid-fast stain may also be used [4]. Bovine tuberculosis is an infectious disease caused by *M. bovis* that affects cattle, other domesticated animals and certain free or captive wildlife species. It is usually characterized by formation of nodular granulomas known as tubercles [4]. It is characterized by the formation of granulomas in tissues and organs, more significantly in the lungs, lymph nodes, intestines, liver and kidneys [5]. Currently, BTB in humans is becoming increasingly important in developing countries like Nigeria as humans and animals are sharing the same micro-environment and dwelling premises especially in rural areas [6]. Rural inhabitants and some urban dwellers in Nigeria still consume unpasteurized and soured milk potentially infected with *M. bovis* [6]. The human cases of tuberculosis associated with *M. bovis* infection, both pulmonary and extra-pulmonary have been described in Nigeria [6-8]. From the limited survey researches which have been reported over the last 30 years in the country, prevalence of bovine tuberculosis due to *M. bovis* ranges from 2.5% in 1976 to 14% in 2007. The disease has been on the increase as demonstrated by the tuberculin test reports of Alhaji [9], Ayanwale [10], Shehu [11] and Abubakar [6]. While bovine tuberculosis is currently a relatively minor disease problem in the developed world, the disease remains problematic in several countries, particularly Great Britain, Ireland and New Zealand [12,13]. Even in countries where the disease has been officially 'eradicated', such as the United States of America (USA), problems remain, due to relatively frequent, but sporadic outbreak [13]. Furthermore, the disease is an important zoonosis, with a particular threat presented in those tropical countries where the disease remains endemic, and may represent a particular risk to people infected with human immunodeficiency virus (HIV) [14]. *Mycobacterium bovis* has been identified in humans in most countries where isolates of mycobacteria from human patients have been fully characterized. The incidence of pulmonary tuberculosis caused by *M. bovis* is higher in farm and slaughterhouse workers than in urban inhabitants [4]. This study determined the viability of *Mycobacterium bovis* following heat treatment and the transmissibility of heat treated *Mycobacterium bovis* in guinea pigs (*Cavia porcellus*).

Materials and Methods

Sample collection

Twenty five suspected visible lesions of lungs and lymph nodes were collected from Sokoto metropolitan abattoir during routine meat inspection and transported in polythene bags to the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto. Acid fast staining techniques was used

to determine the presence of *Mycobacterium bacilli*. Samples were collected for a period of three months.

Sample processing and staining

Visible tubercle lesions were cut, defattened, digested and crushed. A small sample of the residue was transferred to a clean glass slide and was fixed at 80°C for 15 minutes. Methods described by Gerhardt et al. [15,16] were used in Acid-fast staining of smears with carbolfuschin, Acid alcohol (95% ethanol and Concentrated Hydrochloric acid), Methylene blue and Distilled water respectively. Smears were view using a light microscope.

Heat treatment

All tissues from positive samples were subjected to 100°C for a period of 30 minutes. Heat treated samples were afterward subjected to Acid-fast staining and microscopy to check for *Mycobacterium bacilli*.

Inoculation

Twenty four guinea pigs were collected from a TB free breeding laboratory and was divided into group A (Eighteen) and B (Six). Group A (Experiment group) were inoculated with positive heat treated sample orally and intranasally, while group B (Control group) were inoculated with residue of positive non heat treated sample. The animals were observed for 35 days tuberculosis incubation period before humanely slaughtered and post mortem examination was performed. This is in accordance with the regulated procedural methods of the Federation of European Laboratory Animal Science Association (FALSEA, 2005).

Post mortem examination

The animals were euthanized using pentobarbitone overdose before post-mortem. Pulmonary and extra pulmonary lesions were observed and recorded. Tissues from lungs, liver and spleen with lesions compatible to Tuberculosis were taken for microbiological examination and with the aid of Acid fast staining methods the tissues were examined for the presence of mycobacterium bacilli.

Data analysis

Data was exported to GraphPad InStat® Version 3.05 for windows and was analyzed using Fisher's Exact test for contingency table.

Results

During this study period a total of 25 gross lesions of tuberculosis from lung and lymph nodes of cattle were collected from Sokoto metropolitan abattoir. 13 (76.5%) of lungs and 5 (62.5%) of lymph nodes were positive to acid fast before heat treatment (Table 1) while 4 (25.0%) of lungs and 2 (40.0%) of lymph nodes showed acid fast bacilli after heat treatment (Table 2).

Gross Pathology

At necropsy, gross visible pulmonary primary lesions affecting both lobes of the lung was observed. The lungs appeared congested and consolidated with the presence of granuloma on palpation. Extra pulmonary lesions were also observed with edema and multifocal areas of hepatic and splenic necrosis.

Key: A=Rod like bacilli (Figure 1)

B=Areas of hepatic necrosis (Figure 2)

C=Areas of pulmonary congestion (Figure 3)

Tissue	No of Sample	Negative Samples	Positive Samples	% positive
Lung	17	4	13	76.5%
Lymph nodes	8	3	5	62.5%
Total	25	7	18	72.0%
p value=0.0870, Relative risk=2.039 (0.8023- 5.183) at 95% Confidence interval				

Table 1: Zieehl-Nielson staining test of Samples collected from abattoir before heat treatment.

Tissue	No of Sample	Negative Samples	Positive Samples	Prevalence
Lung	13	9	4	25.0%
Lymph nodes	5	3	2	40.0%
Total	18	12	6	33.3%
p value=1.00, Relative risk=0.7692 (0.1997-2.963) at 95% confidence interval				

Table 2: Ziehl Neelson staining test of heat treated samples.

Figure 1: Photomicroscopy of Acid fast bacilli after heat treatment x 100.

Discussion

This study revealed that *Mycobacterium bovis* was recovered from the lungs than the lymph nodes 35 days from post inoculation day (pid).This differences seen maybe due to the fact that lungs is the initial tissue where disease is initiated before being disseminated to draining lymph nodes. However there the differences observed is statistically insignificant (p value >0.05). Report by Palmer et al. [17] indicated that *Mycobacterium bovis* was recovered from tonsils and medial retropharyngeal lymph nodes at 15 days post inoculation, however, gross lesions were seen on 28-42 days post inoculation. In

contrast, cattle inoculated intranasally develop lesions as early as 7 days after inoculation [18]. This difference may be due to host species differences or differences in route of inoculation, inoculum strain, or inoculum dosage. This study also showed that *Mycobacterium bovis* could resist high temperature and retains its infectivity/pathogenicity. Study conducted in Brazil by Clarence et al., [19] identified Mycobacterium from pasteurized milk. Grant et al. [20] has demonstrated thermal inactivation of *Mycobacterium bovis* at 63.5°C for 30 minutes following pasteurization of milk. Most of the lesions observed are found in the lungs, Liver and spleen. The pulmonary and extra pulmonary lesions observed is in agreement with the study of Pritchard et al. [21] that observed necrotic lesions in the lungs, liver and lymph nodes of badger following experimental inoculation [22-25].

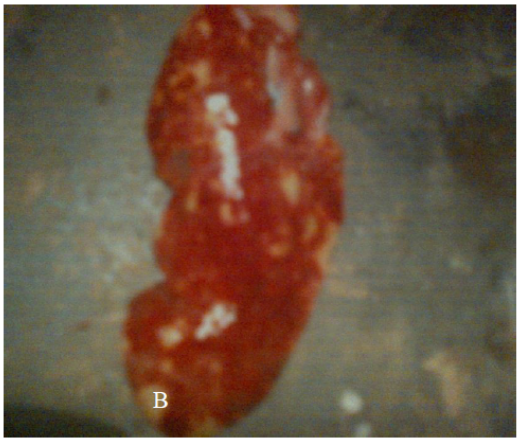

Figure 2: Photograph of a guinea pig liver showing areas of necrosis x 100.

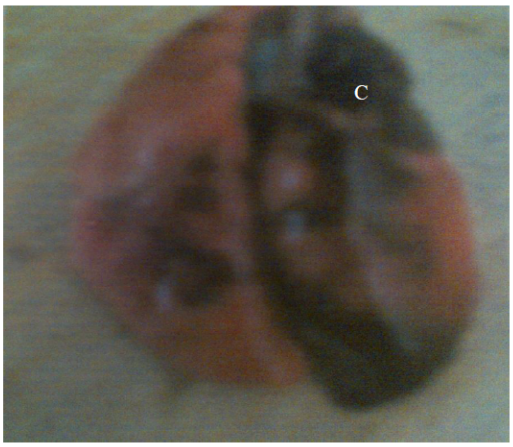

Figure 3: Enlarged and congested lung lobes x 100.

Conclusion

From this study tuberculosis lesion were observed more in the lungs than the lymph nodes and when this lesion are processed under cooking temperature, some of the *Mycobacterium bovis* retained their pathogenicity and infectivity with possible chances of causing disease in guinea pigs (*Cavus porcellus*). Lesions observed are concentrated in the lungs following experimental infection, although extra pulmonary lesions were also observed.

Recommendation

There should be strict adherence to meat inspection procedure in abattoirs and governments should provide diagnostic laboratories in our existing abattoirs to augment inspection procedures.

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References

1. Ayele WY, Neill SD, Zinsstag J, Weiss MG, Pavlik I (2004) Bovine tuberculosis: an old disease but a new threat to Africa. Int J Tuberc Lung Dis 8: 924-937.
2. Wayne LG, Kubica GP (1986) The mycobacteria. In: Sneath PHA and Holt JG, (editors). Bergeys manual of systematic bacteriology. Williams & Wilkins Co., Baltimore: 2: 1435-1457.
3. Clemens DL, Horwitz MA (1995) Characterization of the Mycobacterium tuberculosis phagosome and evidence that phagosomal maturation is inhibited. J Exp Med 181: 257-270.
4. Office International Des Epizootics (OIE) (2009) Bovine Tuberculosis (Version adopted by the World Assembly of Delegates of the OIE in May 2009) (Chapter 2.4.7).
5. Shitaye JE, Tsegaye W, Pavlik I (2007) Bovine tuberculosis infection in animal and human population in Ethiopia: A review. Vet Med 52: 317-332.
6. Abubakar IA (2007) Molecular epidemiology of Human and Bovine tuberculosis in the Federal Capital Territory and Kaduna State Nigeria (Ph. D Thesis), Plymouth University, UK.
7. Idigbe EO, Anyiwo CE, Onwujekwe DI (1986) Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. J Trop Med Hyg 89: 143-148.
8. Cadmus SIB, Atsanda NN, Oni SO, Akang EEU (2004) Bovine tuberculosis in one cattle herd in Ibadan Nigeria. Veterinarni Medica: 49: 406-412.
9. Alhaji I (1976) Bovine tuberculosis: A general review with special reference to Nigeria. Veterinary Bulletin 46: 829-841.
10. Ayanwale FO (1984) Studies on the epidemiology of bovine tuberculosis in some states of southern Nigeria (Ph. D thesis) University of Ibadan, Ibadan, Nigeria, pp. 184.
11. Shehu LM (1998) Survey of tuberculosis and tubercle bacilli in Fulani herdsmen, Nono and some herdsmen in Zaria, Nigeria (MSc thesis). Ahmadu Bello University, Zaria.
12. Clifton-Hadley RS, Wilesmith JW (1995) An epidemiological outlook on bovine tuberculosis in the developed world. In Proc. 2nd International Conference on Mycobacterium bovis (Griffin F, de Lisle G) (eds), 28 August-2 September, Dunedin, New Zealand, University of Otago Press, Otago, 178-182.
13. Essey MA, Koller MA (1994) Status of bovine tuberculosis in North America. Vet Microbiol 40: 15-22.
14. Daborn CJ, Grange JM, Kazwala RR (1996) The bovine tuberculosis cycle-an African perspective. Soc Appl Bacteriol Symp Ser 25: 27S-32S.

15. Gerhardt P, Murray RGE, Costilow RN, Nester EW, Wood WA, et al. (1981) Manual of methods for general microbiology. ASM Press, Washington.
16. Gerhardt P, Murray RGE, Wood WA, Krieg NR (1994) Methods for general and molecular bacteriology, ASM Press, Washington.
17. Palmer MV, Waters WR, Whipple DL (2003) Lesion development in white-tailed deer (*Odocoileus virginianus*) experimentally infected with Mycobacterium bovis. Vet Pathol 39: 334-340.
18. Cassidy JP, Bryson DG, Pollock JM, Evans RT, Forster F, et al. (1998) Early lesion formation in cattle experimentally infected with Mycobacterium bovis. J Comp Pathol 119: 27-44.
19. Leite CQ, Anno IS, Leite SR, Roxo E, Morlock GP, et al. (2003) Isolation and identification of Mycobacteria from livestock specimen and milk obtained in Brazil. Mem Inst Oswaldo Cruz 98: 319-323.
20. Grant IR, Ball HJ, Rowe MT (1996) Thermal inactivation of several Mycobacterium spp. in milk by pasteurization. Lett Appl Microbiol 22: 253-256.
21. Pritchard DG, Stuart FA, Brewer JI, Mahmoud KH (1987) Experimental infection of Badgers (Meles meles) with Mycobacterium bovis. Epidemiol Infect. 98: 145-154.
22. Niemann S, Richter E, Rüsch-Gerdes S (2000) Differentiation among members of the Mycobacterium tuberculosis complex by molecular and biochemical features: evidence for two pyrazinamide-susceptible subtypes of M. bovis. J Clin Microbiol 38: 152-157.
23. Collins CH, Grange JM (1983) The bovine tubercle bacillus. J Appl Bacteriol 55: 13-29.
24. Amanfu W (2006) The situation of tuberculosis in four northern states of Nigeria (Ph.D thesis) Ahmadu Bello University, Zaria, Nigeria. pp. 236.
25. O'Reilly LM, Daborn CJ (1995) The epidemiology of Mycobacterium bovis infections in animals and man: A review. Tuber Lung Dis 7 Suppl 1: 1-46.