Mycobacterial Diseases

Addo et al., Mycobact Dis 2016, 6:5
DOI: 10.4172/2161-1068.1000229

Research Article OMICS International

Prevalence of Tuberculosis Infection in a Cohort of Cattle that Enters the Food Chain in Accra, Ghana using Bovigam

Kennedy Kwasi Addo*, Vida Yirenkyiwaa Adjei, Gloria Ivy Mensah and Dolly Jackson-Sillah

Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

***Corresponding author:** Kennedy Kwasi Addo, Department of Bacteriology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana; **Tel:** +23324333486; **Fax:** +233 302502182; **E-mail:** kaddo@noguchi.ug.edu.gh

Rec date: September 27, 2016; **Acc date:** December 06, 2016; **Pub date:** December 12, 2016

Abstract

Tuberculosis (TB) continues to be an important public health problem worldwide. Currently there are 286 TB cases per every 100,000 people in Ghana. This figure is three times higher than the TB burden estimated by the World Health Organization. The numerical contribution of bovine TB to the general TB burden is unknown. Herdsmen, livestock workers, and veterinarians and the general public are at high risk of contracting bovine TB. Zoonotic TB caused by *Mycobacterium bovis* is present in animals in most developing countries including Ghana. Unfortunately, activities to check and control cattle with TB infection from entering the food chain are often inadequate or unavailable. This study therefore aims to determine the prevalence of TB infection in a cohort of cattle that enters the food chain in Accra, Ghana. A cross sectional study involving five major abattoirs was conducted in the Greater Accra region between the periods of September 2012-June 2013. After routine inspection of live cattle by veterinary officials, 10 mL of blood was drawn from 94 cattle before slaughter and tested for TB infection using BOVIGAM. Six (6.4%) of the 94 cattle screened, were positive for TB infection. All except one abattoir had at least one cattle testing positive. Although the study recorded a lower prevalence of 6.4%, all animals tested were deemed fit for slaughter by veterinary officials and was to enter the food chain. The low sensitivity of routine abattoir inspection for infected organs and negative results of post-mortem examination reinforces the need for more sensitive screening tool such as BOVIGAM.

Keywords: Bovine Tuberculosis; BOVIGAM; Gamma interferon (γ-IFN); Abattoir; Ghana

Introduction

Tuberculosis (TB) is caused by a group of bacteria collectively known as the *Mycobacterium tuberculosis* complex [1]. The commonest strain of *Mycobacterium* found among TB patients in Ghana is the *M. tuberculosis* followed by *Mycobacterium africanum* and *Mycobacterium bovis* [2]. *M. bovis* is virulent for cattle but can infect other animals and humans causing disease and pathology similar to *M. tuberculosis*, which is naturally pathogenic for man [3,4]. It has been established that 3% of pulmonary TB in Accra, Ghana is caused by *M. bovis* [2] which raises concern about possible aerosol transmission between cattle and human population or within the human population. Despite rigorous control efforts, the current global estimates indicate that 1/3 of the world's population has TB infection and 5-10% of these individuals if HIV negative will develop active TB during their lifetime, contributing to a global annual incidence of approximately 9.2 million cases [5]. A study in the Ho district of the Volta Region of Ghana revealed a prevalence rate of 3.1% bovine TB infection in cattle and 5.9% within a cluster [6] whilst and others have also indicated transmission from humans to animals and vice versa [7]. Abattoirs provide ideal settings for screening of cattle because they are the last point for inspection before slaughter and it is critical that TB is detected at this time to prevent TB-infected cattle in our food chain.

Over the years, *in vivo* intradermal comparative tuberculin skin test has been used as standard test for diagnosis of TB in cattle worldwide, in spite of lacking the sensitivity and specificity [8]. Monitoring bovine TB in cattle by bacteriological assay is not feasible, costly, time consuming and most laboratories are ill equipped [9]. In recent years, gamma interferon (γ-IFN) assay has been used for detection of bovine TB in that it detects the cytokine γ-interferon which is predominantly released by T-cells after in vitro stimulation with bovine Purified Protein Derivative (BvPPD) and avian Purified Protein Derivative (AvPPD) [10,11]. BOVIGAM® is an example of gamma interferon (γ-IFN) assay used for the diagnosis of bovine TB infection in cattle. Animals infected with *M. bovis* can be identified by measuring the cytokine interferon gamma (IFN-γ) against tuberculin, an antigen used to aid in the diagnosis of TB infection. Tuberculin Purified Protein Derivatives (PPD) antigens are presented to lymphocytes in whole blood cultures and the production of IFN-γ from the stimulated T cells is detected using a monoclonal antibody-based sandwich enzyme immunoassay (EIA). Lymphocytes from uninfected cattle do not produce IFN-γ to tuberculin PPD antigens and hence IFN-γ detection correlates with infection.

This study aimed to determine the prevalence of TB infection in a cohort of cattle that enter the food chain in Accra, Ghana using Bovigam.

Material and Method

Study design

This was a cross sectional study involving five major abattoirs namely: Tema abattoir (GIHOC), Madina abattoir, University of Ghana farms (Legon Abattoir), Accra abattoir and Amasaman abattoir in the Greater Accra region of Ghana. Abattoir practices, including the extent of examination of live animals (ante mortem) and carcasses

(post mortem) and what is done when there is a visible lesion was observed and recorded by a trained veterinarian.

Sample size

Ninety-four cattle slaughtered for consumption were involved. Because slaughtering of cattle was not regular in the selected abattoirs only 94 animals were available for sampling during the period of sample collection.

Sample collection

Sample collection was done shortly after the animals had been inspected and declared fit for slaughter. 10 mL of blood was drawn from the jugular vein for Bovigam test between September 2012 and June 2013. Handling of blood from cattle and the laboratory analysis was conducted at the Noguchi Memorial Institute for Medical Research.

Bovigam test

Stage one-whole blood culture

A minimum volume of 5 mL of blood from each animal was collected into a heparinised blood collection tube and mixed evenly. Three 1.5 mL aliquots of heparinised blood from each animal were aseptically dispensed into wells of 24-well tissue culture trays. The wells were labeled; Phosphate Buffered Saline (PBS), AvPPD and BvPPD. 100 µL of PBS, (NIL Antigen control), avian PPD and bovine PPD were aseptically added to the appropriate wells containing previously dispensed blood. The culture tray was swirled ten times both clockwise and counter clockwise on a flat smooth surface to mix for 1 minute. The culture tray containing blood and antigens was incubated for 16-24 h at 37°C in a humidified atmosphere. 500 µL of plasma was harvested into sterile Eppendorf tubes and stored at -20°C prior to ELISA.

Stage two - Bovine IFN-γ EIA

All test plasmas and reagents except the Conjugate 100X concentrate were brought to room temperature (22+/-3°C) before use. Freeze dried component was reconstituted based on the manufacturer's instruction.

50 µL of Green Diluent was added to the required wells. 50 µL of test and control samples were also added to the appropriate wells containing the Green Diluent. The control samples were added last. The plates were then placed on a microplate shaker to mix the content thoroughly for 1 min, after which they were incubated at room temperature on the microplate shaker at a setting of 600 rpm for 1 h. The content was poured out and washed six times with freshly prepared wash buffer. After the six washes, plates were placed face down on clean filter paper and allowed to drain and flick several times over absorbent paper to remove excess wash buffer as possible. 100 µL of freshly prepared conjugate reagent was added to the wells and incubated on a microplate shaker set at 600 rpm for 1 h (conjugate reagent was prepared based on manufacturer's instruction). The content was again poured out and washed six times with wash buffer. 100 µL of freshly prepared enzyme substrate solution was added to the wells and mixed thoroughly in a microplate shaker. The plates were covered and incubated on the microplate shaker set at 600 rpm in a dark area for 30 min. 50 µL of the enzyme stopping solution was added to each well and agitated gently, while care was taken not to transfer chromogen from well to well. Absorbance of each well was measured within 5 min of terminating the reaction using a microplate reader fitted with 450 nm filter. The absorbance value was used to calculate the results. Blood plasma collected from cattle having an OD value greater than 0.100 above that of avian PPD and nil (PBS) antigen, indicates the presence of *M. bovis* infection.

Results

Blood samples were collected from cattle declared as "good for slaughter" upon clinical examination by veterinary officers. Blood samples were collected from 94 cattle from 5 abattoirs, namely Legon (6), Accra (26), Madina (24), Amasaman (20) and GIHOC (18).

Of the 94 cattle screened for TB infection using the Bovigam test, 6 (6.4%) were positive for TB infection. All except Legon abattoir had at least one cattle testing positive (Table 1). We could not find an association between Bovigam result and abattoir of cattle ($\chi 2$ test: p=0. 841).

Abattoir	No. of cattle tested	Bovigam (IFN-γ assay)	
		Positive	Negative
Legon	6	0	6
Accra	26	2	24
Madina	24	1	23
Amasaman	20	1	19
GIHOC	18	2	16
Total	94	6	88

Table1: Bovigam results by facility.

Discussion

More than a decade ago, the TB prevalence in cattle around the Accra plains was estimated at 11-19 % using the intradermal tuberculin (ITT) skin test [12]. This present study using the Bovigam test, however, recorded a much, lower prevalence of 6.4%. This figure does not suggest a reduction in TB cases in cattle, but is rather a reflection of the test used which is more specific than the ITT used in the previous study. This assertion is supported by several studies that have reported that the ITT is less sensitive and specific than the Bovigam [10,13-15].

In our study, all the animals tested with the Bovigam, had been deemed fit for slaughter, after routine examination by veterinary officials. The examination involved evaluation of the general body condition scored as 1 "good", 2 "bad" and 3 "very bad" as well as inspection of inguinal lymph node and prescapular lymph node judged as normal or enlarged. In spite of the animals being declared "free of TB infection" by clinical inspection, 6 out of the 94 were positive for TB infection by the Bovigam test. The TB infection could have been missed during the clinical inspection because the infection was in the early stages or latent.

At the early stages of TB infection or latent, the pathological changes caused by the bacilli are not yet profound enough to be detectable during routine abattoir inspection. From this period till the

development of detectable signs of bovine TB, cellular immune responses will however be detectable earlier than the pathological changes caused by the disease (e.g., visible lesions), and before the bacterial loads exceed the numbers necessary for the detection of *M. bovis* from tissue samples by culture[16]. This is one major advantage of Bovigam over other tests that rely on detection of bacillus or signs of active infection. Bovigam essentially detects infection rather than disease so can be used as a screening tool in large herds to prevent the spread of infection by detection and subsequent quarantine of infected cattle early before visible signs of TB are seen.

Compared to other studies in Turkey [17] and Cameroon[18] which recorded a TB prevalence of 49.2% and 60%, respectively, in cattle bound for slaughter, the prevalence of 6.4% recorded in our study is on the lower side.

The low prevalence notwithstanding, it is still a cause for concern as these cattle had been adjudged to be fit for slaughter and was to enter the food chain. Our study also highlights the low sensitivity of routine abattoir inspections for infected organs and reinforces the need for more sensitive screening tools such as the Bovigam. A recent study in Ethiopia has revealed that the probability of missing an animal with a TB lesion during routine abattoir inspection is 95.24% [19].

Additionally, in many of such cases even post mortem investigation does not yield any positive results, because due to the low bacteria load or latent stage of the disease, organs would appear normal. Consumption of undercooked meat from such cattle could potentially lead to infection.

Also, considering the fact that many animals are slaughtered outside the abattoir system where there are no clinical examinations, the prevalence of TB in cattle entering the food change will be much higher.

Acknowledgements

This work was funded by the University of Ghana Research Fund Number: URF/5/ILG-006/2011-2012 and supported partially by the consortium Afrique One "Ecosystem and Population Health: "Expanding Frontiers in Health" funded by the Wellcome Trust (WT087535MA) and Afrique One – ASPIRE (107753/A/15/Z). The authors thank the staff of the Department of Bacteriology, Noguchi Memorial Institute for Medical Research for their support during the analysis of the samples. The authors also acknowledge the Heads of the Accra, GIHOC, Madina, Legon and Amasaman abattoirs for their support during the collection of the samples.

References

1. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, et al. (2002). A new evolutionary scenario for the Mycobacterium tuberculosis complex. Proc Natl Acad Sci USA 99: 3684-3687.
2. Addo KK, Owusu-Darko K, Yeboah-Manu D, Caulley P, Minamikawa M, et al. (2007) Mycobacterial Species Causing Pulmonary Tuberculosis at the Korle Bu Teaching Hospital, Accra. Ghana Med J 41: 52-57.
3. Biet F, Boschiroli ML, Thorel MF, Guilloteau LA (2005) Zoonotic aspects of Mycobacterium bovis and Mycobacterium avium-intracellulare complex (MAC). Vet Res 36: 411-436.
4. Kaneene JB, Pfeiffer D (2006) Epidemiology of Mycobacterium bovis: Mycobacterium bovis infection in animals and humans. Blackwell Publishing Ltd. Iowa, USA 34-48.
5. WHO (2009) WHO report Global tuberculosis control: Epidemiology, strategy, financing. World Health Organization. Geneva, Switzerland.
6. Ankugah D (2000) http://ugspace.ug.edu.gh/bitstream/handle/123456789/7094/
7. Fetene T, Kebede N, Alem G (2011) Tuberculosis infection in animal and human populations in three districts of Western Gojam, Ethiopia. Zoonoses Public Health 58: 47-53.
8. Lilenbaum W, Ribemro ER, Souza GN, Moremra EC, Fonseca LS, et al. (1999) Evaluation of an ELISA-PPD for the diagnosis of bovine tuberculosis in field trials in Brazil. Res Vet Sci 66: 191-195.
9. Corner LA (1994) Post-mortem diagnosis of Mycobacterium bovis infection in cattle. Vet microbial 40: 53-63.
10. Ryan TJ, Buddle BM, de Lisle GW (2000) An evaluation of the gamma interferon test for detecting bovine tuberculosis in cattle 8 to 28 days after tuberculin skin testing. Res Vet Sci 69: 57-61.
11. Gormley E, Doyle MB, Fitzsimons T, McGill K, Collins JD (2006) Diagnosis of Mycobacterium bovis infection in cattle by use of the gamma-interferon (Bovigam®) assay. Vet Microbiol 112: 171-179.
12. Bonsu OA, Laing E, Akanmori BD (2001) Prevalence of tuberculosis in cattle in the Dangme-West district of Ghana, public health implications. Acta Trop 76: 9-14.
13. Gonzales-Llamazares OR, Gutierrez CB, Alvarez Nistal D, de la Puente R, et al. (1999) Field evaluation of the single intradermal cervical tuberculin test and the interferon-assay for detection and eradication of bovine tuberculosis in Spain. Vet Microbiol 70: 55-66
14. Cagiola M, Feliziani F, Severi G, Pasquali P, Rutuli D (2004) Analysis of possible factors affecting the specity of the gamma interferon test in tuberculosis free cattle herds. Clin Diagn Lab Immunol 11: 952-956.
15. Wood PR, Corner LA, Rothel JS, Baldock C, Jones SL, et al. (1991) Field comparison of the interferon gamma assay and the intradermal tuberculin test for the diagnosis of bovine tuberculosis. Aust Vet J 68: 286-290.
16. Vordermeier M, Whelan A, Ewer K, Goodchild T, Clifton-Hadley R, et al. (2006) The BOVIGAM assay as ancillary test to the tuberculin skin test. Gov Vet J 16: 72-80.
17. Ozturk D, Pehlivanoglu F, Kale M, Ali Ta, Guldali Y, et al. (2010) In Vitro Diagnosis of Bovine Tuberculosis by γ-Interferon Assay. Kefkas Vet J 16: 875-878.
18. Ndukum JA, Kudi AC, Bradley G, Ane-Anyangwe IN, Fon-Tebug S, et al. (2010) Prevalence of bovine tuberculosis in abattoirs of the Littoral and Western highland regions of Cameroon: A cause for public health concern. Vet Med Inter 2010: 61-69.
19. Ewnetu L, Melaku A, Birhanu A (2012) Bovine Tuberculosis Prevalence in Slaughtered Cattle at Akaki Municipal Abattoir Based on Meat Inspection Methods. Glob Vet 5: 541-545.