Full Length Research Paper

The Use of Lateral Flow Technique (Rapid Kit Test) in the Determination of Prevalence of Bovine Tuberculosis (bTB) in Cattle from Two Abattoirs in Abuja, Nigeria

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Abstract
Bovine tuberculosis (bTB) is a zoonotic disease that is hazardous to both man and animals. A huge economic loss which could be direct or indirect is associated with the disease. The endemicity of the disease in Nigeria poses significant threat to food safety, food security, meat quality, international trade and the general health of the public. The fact that Mycobacterium bovis is resistant to some first line antibiotics in the treatment of Tuberculosis (TB) and TB is generally diagnosed using Ziehl-Neelsen staining method in humans disposes its victims of having drug resistant TB in the phase of treatment. The lateral flow technique which involves the use of a solid phase chromatographic immunoassay for the qualitative detection of Mycobacterium bovis antigen in serum or plasma was used in this study to determine the prevalence of bovine tuberculosis. Blood serum was collected from a total of 185 cattle based on the calculated sample size after which the screened animals were subjected to postmortem inspection, 5 tuberculous tissue samples were obtained and screened using ZN staining method. The prevalence rate of 17.3% (32/185) was obtained and all 5 tissue samples were positive for Acid Fast stain respectively. The only risk factor associated with bTB infection in these cattle was the sex (female) (P = 0.01). Based on the results obtained it was eminent that a lot of cattle are infected with bTB which cannot be identified by means of postmortem inspection only which further reiterates the need for active surveillance in order to curtail the spread of the disease being actively spread in the cattle and human population. Furthermore, if the disease is to be eradicated in Nigeria, the issue of active disease surveillance can no longer be compromised due to its effective role in the early detection of positive cases.

Keywords: Bovine Tuberculosis, Prevalence, Lateral flow technique, Abattoirs, Nigeria

Introduction

Bovine Tuberculosis (bTB) is a chronic infectious and contagious zoonotic disease of domestic, wild animals and humans (Radostits *et al.*, 2002). Human and bovine tuberculosis (bTB) are two forms of tuberculosis that cause significant disease in mammals (Beals *et al.*, 2007). It is characterized by the formation of granulomas (tubercles) in tissues and organs which is more significant in the lungs, lymph nodes, intestine, liver and kidneys (Shitaye *et al.*, 2007). *Mycobacterium bovis* primarily affects the bovine specie, other species which could be infected by this organism include; camels, pigs, sheep, goats, horses, dogs, cats, badgers, lions, elephants, deers, primates and man (Ayele *et al.*, 2004).The disease is zoonotic and therefore of public health significance (O'Reilly and Daborn, 1995). Human tuberculosis is mainly caused by *Mycobacterium tuberculosis* but in regions where bovine tuberculosis is prevalent in animals, human TB cases due to *Mycobacterium bovis* may occur (Theon *et al.*, 2009). Zoonotic bovine tuberculosis is present in most developing countries where surveillance and control activities are often inadequate or unavailable (Cosivi *et al.*, 1995). Bovine tuberculosis in Nigeria is endemic and the situation is further complicated by the fact that active surveillance of the diseaseis rare while the passive surveillance of the disease in our abattoirs is not fully adhered to couple with lack of movement control of cattle across our local and international borders.

In Africa, the occurrence of tuberculosis due to *Mycobacterium bovis* in human is difficult to determine accurately because of technical problems in isolating the microorganism (Collins and Grange, 1993). Currently bovine tuberculosis in human is becoming increasingly important in developing countries like Nigeria as human and animals are sharing the same micro-environment and dwelling premises especially in rural areas (Abubakar, 2007). Historically, tuberculosis caused by *Mycobacterium bovis* in human was associated with consumption of unpasteurized milk and this is still the most important route of exposure in developing countries (Wilkins *et al.*, 2008). Rural inhabitants and some urban dwellers in Nigeria still consume unpasteurized and soured milk potentially infected with *Mycobacterium bovis* (Abubakar, 2007). The human cases of tuberculosis associated with *Mycobacterium bovis* infection, both pulmonary and extra pulmonary has been documented in Nigeria (Abubakar, 2007, Idigbe, *et al.*, 1986; Cadmus *et al.*, 2004). From the limited survey which has been reported over the last 30 years in the country, prevalence of bovine tuberculosis due to *Mycobacterium bovis* ranges from 2.5% in 1970 to 14% in 2007. The disease has been on the increase as demonstrated by tuberculin test reports (Abubakar, 2007; Ayanwale, 1984; Shehu, 1988).

Treatment of TB with antibiotics is not universally successful, even in humans, receiving multiple drug therapy for several months is not easily adhered to; *M.bovis* is naturally resistant to one of the first-line drugs used for the treatment of TB in humans. In order to eliminate the risk of antibiotic-resistant strains of *M. bovis* infecting the human population, where multiple-drug resistant strains of *M. tuberculosis* are already a significant public health problem, it is critical to ensure that such strains of *M. bovis* are not artificially selected in animal populations (Skuce *et al.*, 2011).

The current study is aimed at determining the prevalence of bovine tuberculosis in Abuja based on the study that was conducted in Karu and Kubwa abattoirs using the lateral flow technique.

Materials and Methods
Study area
Abuja is the capital of Nigeria located in the central region of the country just north of the confluence of rivers Niger and Benue. It is bordered by the state of Niger to the West and North, Kaduna to the Northeast, Nassarawa to the east and south, and Kogi to the southwest lying between the latitude 80 50'N and 8.8330'N, and longitude 70 10'E and 7.1670'E. It covers total area of 7,315 km^2, of which the actual city occupies 275.3 km^2 and a total population of 1,405,201 according to 2006 census. It is situated within the Savannah region with moderate climatic conditions. The territory is currently made up of six local councils, comprising the city of Abuja and five Local Government Areas, namely: Abaji, Gwagwalada, Kuje, Bwari, Kwali. The study was conducted in two major abattoirs in randomly selected area councils (Bwari and Abuja municipal area councils) managed by the Federal Capital Territory Administration.

Cattle are brought and slaughtered from livestock markets, Fulani herds and private farms mostly from Northern part of the country and neighboring African countries of Cameroun, Chad, Mali and Niger. Abattoir operations are not standard and unhygienic, giving room for potential contamination of the premises.

Fig. 1. Map of Abuja, Indicating the regions where the two abattoirs are located (red colored)

Calculation of sample size
The method of Sample size used for this study was calculated according to the method described by Thrushfield (1997) as follows:
N = 1.962 Pexp (1-Pexp) / d^2 Where n= the sample size, Pexp (the expected prevalence) = 12.5%, d= the desired absolute precision= 5%.N = Minimum sample size + an additional 10% of randomly selected animals in cases some samples may not be used; P = prevalence rate 12.5% (103); d = desired absolute precision 5% (0.05); Appropriate value for standard normal deviation at 95% C.I (1.962); Therefore 1.96^2x 12.5% x (1 – 12.5)/0.05^2= 168. Simple random sampling was used in the selection of animals that were used in the study.

Sample collection

Animals were examined just before slaughter to record data such as the body condition score (good, fair or poor), age (determined as adult or young), sex and breed. One hundred and seventy six (185) blood samples and five tissue samples of lesions suggestive of bTB from the 185 cattle was obtained. About 5mls of blood was collected in tubes from the jugular vein from slaughtered animals. Postmortem meat inspection was conducted to identify bTB lesions from the 185 cattle whose blood samples were collected for the study (visceral organs and lymph node were inspected through careful visual palpation and incision procedures for nodules and granulomatous lesions as described by Corner, 1994). Tissue samples were aseptically collected in sterile screw–capped plastic containers on ice packs in a Coleman box and transported to the laboratory for analysis. The blood samples collected in tubes were also taken to the laboratory for analysis. All samples were properly labeled before transporting to the laboratory where they were analyzed.

Microscopy (Ziehl-Neelsen staining method)

The processing of lesions was based on the OIE recommendation for digestion and decontamination procedures. The tissues were first homogenized by using a pestle and mortar as described by OIE (2009). This was then followed by decontamination in a 15ml centrifuge tube containing equal amount of homogenized specimen and NALC (N- acetyl- l –cysteine), NaOH (containing 4%NaOH, 2.9% sodium citrate). The tube containing the mixture was allowed to stand for 15 minutes at room temperature until the specimen was digested followed by neutralization using 6ml phosphate buffer. The mixture was then centrifuged at 3000×g for 15min. The supernatant was carefully decanted; 2ml of phosphate buffer was added to resuspend the sediment. Finally, smears of the homogenates of each specimen were made on slides and were stained by the Ziehl-Neelsen (Z-N) method as described by Elmer, (1992). Presence of acid-fast bacilli was suggestively positive for bTB.

Lateral flow technique

This technique involves the use of a solid phase chromatographic immunoassay for the qualitative detection of *Mycobacterium bovis* antigen in serum or plasma. Recently, the MPB 70 protein was revealed to be a highly species specific protein secreted by *Mycobacterium bovis*, resulting in the development of the Antigen (Ag) Rapid Bovine TB Antigen (Ag) test kit. The principle of this technique involves the use of immunochromatographic assay using Direct Sandwich Method (Rec. MPB70 Ag capture)-(Antibody in blood)-(Rec.MPB70 Ag detector) Capture and detector material: Recombinant MPB70 Antigen in order to detect antibodies (IgM, IgG) against *Mycobacterium bovis* with a sensitivity of 90% versus bTB confirmed by bacterial isolation 85.1% versus Purified Protein Derivative (PPD) test and a specificity of 98.6% versus Purified Protein Derivative (PPD) test. In the technique used, there is no cross reaction against *Mycobacterium avium, Mycobacterium tuberculosis*. Blood samples collected were centrifuged and the serum was used for the above mentioned technique.

Results

The number of serum samples analyzed as shown in Table 1; the average daily slaughter figure in the two abattoirs was 87 cattle, out of which 73 were in Karu and 14 were in Kubwa abattoirs. A total of 185 samples were analyzed, out of which 155 were from Karu and 30 from Kubwa as indicated in Fig. 2 below. The prevalence of bovine tuberculosis in cattle screened at the two abattoirs; Karu and Kubwa are as shown in Table 2 and Fig. 3 below. Out of a total of 185 serum samples screened at both abattoirs, 32 (17.3%) were positive for the lateral flow technique. From the 185 cattle from which blood for serum was taken, 5 (2.7%) had visible lesions suspected to be bovine tuberculosis as shown in Table 2. All the 5 suspected tissue samples were positive for the Ziehl-Neelsen test as shown in Table 2. All tissue samples analyzed were from female and all were positive for Ziehl-Neelsen test.

Out 185 serum samples 48 were from female and 137 were from male as shown in Table 3. And Fig. 4. Fourteen (14) of the 48 were positive for lateral flow technique giving a prevalence of 29.2% in females while 18 of the 137 were positive for lateral flow technique giving a prevalence of 13.1% in males. Table 3 and Fig.4 below shows the prevalence of bovine tuberculosis in males (13.1%) and in females (29.2%). All the tissue samples obtained were possible for Ziehl-Neelsen stain and the tissue samples were all obtained from the female cattle (Table 3). Table 4 and Fig. 5 below shows the body condition score of the cattle that were tested for bTB. Out of those with fair body condition score, 6 were sero-positive, 28 were sero-negative and they constitute 17.6% of those with fair body condition score. Twenty three (23) of those with good body condition score were sero-positive constituting 16.2% of those with good body condition score. Lastly, 3 out of 6 of those with poor body condition score were sero-positive constituting 50% of those with poor body condition score. Analysis as indicated in Table 5 shows that there is a significant P value (P = 0.01) as relates to sex. P value is said to be significant when it is ≤0.05.

Table 1: The number of samples analyzed in Karu and Kubwa abattoirs

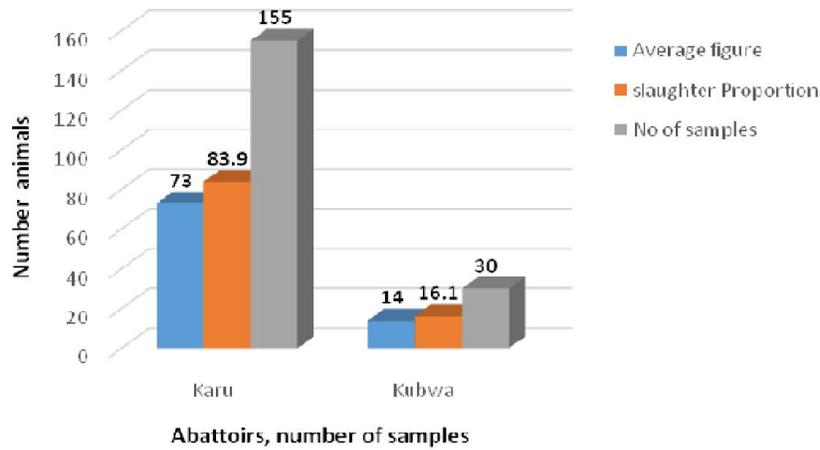| Study site	Average figure	Slaughter Proportion	No of samples
Karu	73	83.9	155
Kubwa	14	16.1	30
Total	87	100	185

Fig. 2. Showing the number of samples analyzed in Kubwa and Karu abattoirs

Table 2: Prevalence of bovine tuberculosis in cattle slaughtered at Karu and Kubwa abattoirs

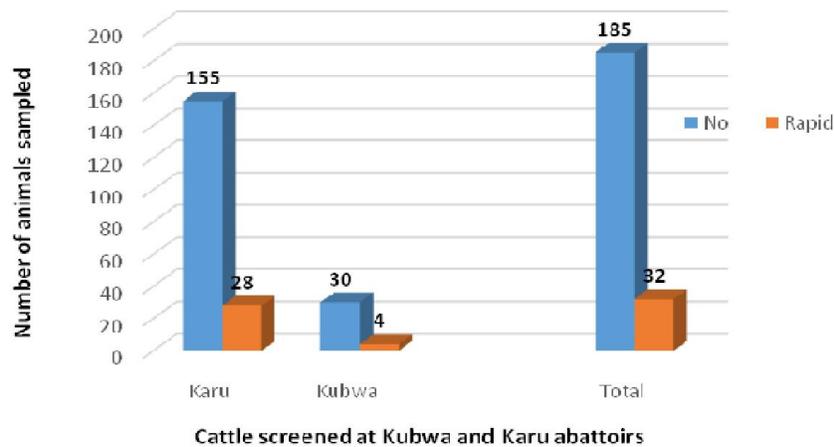| Site	No of samples	Rapid test positive	Percentage %	Ziehl-Neelsen positive	Percentage %
Karu	155	28	18.1	4	80
Kubwa	30	4	13.3	1	20
Total	185	32	17.3	5	100

Fig 3: Distribution of seropositives among cattle slaughtered at Karu and Kubwa abattoirs, F.C.T, North Central Nigeria

Table 3: Prevalence of bovine tuberculosis in male and female cattle slaughtered at the two abattoirs

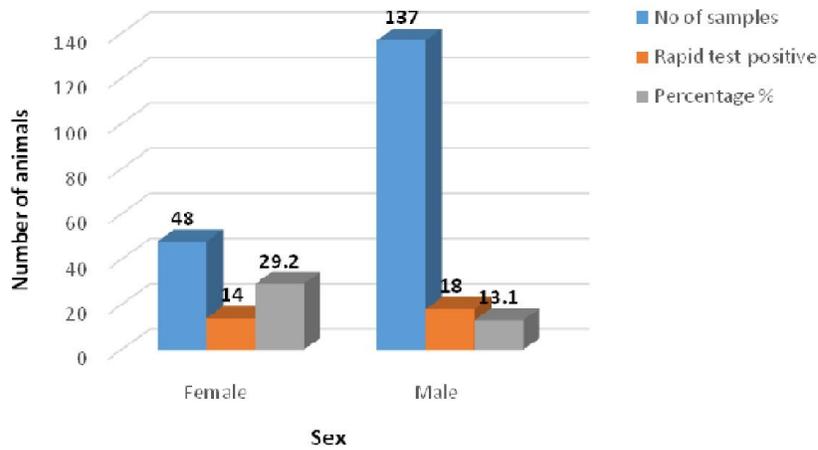| Sex	No of samples	Rapid test positive	Percentage %	Ziehl-Neelsen positive	Percentage %
Female	48	14	29.2	5	10.4
Male	137	18	13.1	0	0

Fig 4: Sex distribution of cattle screened at Karu and Kubwa abattoirs

Table 4: Body condition data of sero-positive cattle slaughtered at the two abattoirs

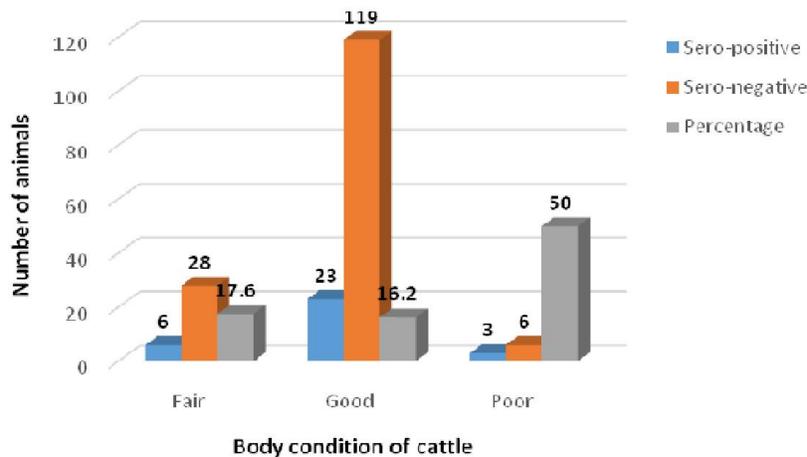| Body Condition of Cattle	Sero-positive	Confidence Interval 95%	Sero-negative	Confidence Interval 95%	Percentage %
Fair	6	[7.2-36.4]	28	[12.5-25.4]	17.6
Good	23	[53.3-86.3]	119	[70.4-84.1]	16.2
Poor	3	[2.0-25.0]	6	[1.5-8.3]	50.0

Fig 5: Showing body condition data of sero-positive cattle slaughtered at the two abattoirs

Table 5: Bivariate analysis of sero-positive cattle slaughtered at the two abattoirs in F.C.T, North Central, Nigeria, March, 2013

Variable	Odds Ratio	Confidence Interval (CI) 95%	P-value
Body condition	1.3	[0.6 -3.2]	0.3
Sex	2.7	[1.2 – 6.0]	**0.01**

Discussion

Serum analysis with lateral flow technique revealed a prevalence of 17.3% which is higher than the prevalence value of 12.5% using the same lateral flow technique reported by Danbirni *et al.*, (2012) but lower than prevalence value of 60% reported by Awah*et al*., (2012). The difference may be due to number of animals and location. The prevalence of 2.7% was revealed based on the visual identification of lesions in tissues of cattle slaughtered which is similar to prevalence of 2.8% reported by Igbokwe *et al.*, (2001) but higher than values of 0.6% reported by Cadmus *et al*., 2008. The difference may be due to the number of animals used for the study and for detection of bovine tuberculous lesions in particular depend on the work load, time and the diligence of the inspector

conducting the examination Corner *et al.*, (1990). Despite this, detection of tuberculous lesions in abattoirs can be affected due to early infection or parasites, non-specific reactions and due to infection other than *M. bovis* as reported by Corner, (1994) and other irregularities of abattoir meat inspections as was documented by Edwards *et al.* (1997). The tissue stained with Ziehl-Neelsen revealed a prevalence of 100% as all 5 tissue samples were positive indicating higher prevalence rate than what was obtained by Awah*et al.*, (2012) who in their study reported a prevalence value of 31%. The difference could be attributed to the proportion of the tissues with lesions detected. The prevalence from this study suggests that meat being sold for public consumption is either diseased or exposed to several levels of contamination which could endanger the public health. The risk of disease transmission from abattoir to the public at large is very high.

The sero-prevalence was more in females 29.2% than in males 13.1% (OR=2.7, 95% CI=1.2-6.0), prevalence due to visual identification of lesions on tissues was 2.5% in females and 0% in males as well as in the Ziehl-Neelsen test 100% in females and 0% in males. This is consistent with the herd prevalence of 50% reported by Firdessa *et al.*, (2012) where 94% of the positive cases were females. This demonstrates conditions as a major influence on the prevalence on the prevalence of bTB as reported by Ameni*et al.*, (2006). Intensification, stress and overcrowding, are possible explanations for such relationship. The same results of higher prevalence in females than males was revealed in the study of Maxwell *et al.*, (2012).The higher prevalence in cows suggest that they shade the infection for a long time spreading the disease to other animals through feces, close contact and to humans through consumption of milk and close contact with animal handlers.

The sero-prevalence according to the body score of the cattlewere 17.6%, 16.2% and 50% for Fair, Good and Poor body condition respectively. This is similar to a study reported by Firdessa*et al.*, 2012 where the body condition scoring of the nearly 3,000 animals suggested no significant differences between tuberculin reactors and non-reactors and a poor correlation was also seen among the 36 slaughtered animals (Firdessa *et al.*, 2012). Over time and with repeated meat inspections butchers acquire ample knowledge about the nature of pathologies that can lead to condemnation of carcasses just from observing the activities of the veterinary staff. Unruly butchers could obstruct inspection of their animal carcasses or hide lesions from unassisted inspectors. Similar findings have been reported by Cadmus in Nigeria that pathological cases including zoonoses in slaughtered animals were missed due to uncooperative attitudes of butchers in ensuring thorough meat inspection (Cadmus *et al.*, 2008)

Conclusion
The study establishes the presence of the Infection in the animals slaughtered with higher prevalence in female as in the two Abuja abattoirs. The study also confirmed that routine abattoir surveillance of bovine tuberculosis through identification of lesions in meat under reports the prevalence of the disease indicating the need to implement active surveillance of bovine tuberculosis in the country.

Recommendation
Based on the findings of this study, the following recommendations are hereby suggested; The Federal Capital Territory (FCT) Department of Agriculture and Rural Development which manages these abattoirs should; Carry out further screening of cattle for bovine tuberculosis to establish the true prevalence of the disease in the FCT; Encourage livestock farmers to screen their animals regularly to control the disease at farm level. Organize public enlightenment campaigns aimed at highlighting the zoonotic importance of bovine tuberculosis among abattoir workers who are at high risk of acquiring the disease because close contact. Have regular surveys organized by the Federal and State Ministries of Agriculture on Bovine Tuberculosis among abattoir workers and others at risk because of their occupation as a lot of data could be generated for the National Animal Disease Surveillance and Response (NADIS) of Federal Department of Veterinary and Pest Control Services for its control. Non-Governmental Organizations or Animal Insurance Organizations should formulate a policy of compensating owners of positive animals or condemned organs to augment efforts of government.

Acknowledgements

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