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First Insight into Molecular Epidemiology of Tuberculosis Infection in Slaughtered Sheep Intended to Human Consumption in Cameroon: The Case of New-Bell's Slaughterhouses

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Abstract

Objective: Tuberculosis (TB) remains understudied in Cameroonian sheep which are very consumed by human beings and which nevertheless live in close contact with cattle in which TB remains endemic. We carried for the first time a six months descriptive cross sectional study on slaughtered sheep in the major slaughter-house of Douala, from November 2013 to April 2014.

Methods: About 2922 slaughtered sheep were successively inspected for tuberculosis through visual examination and palpation of carcasses, lungs, livers, hearts, intestine, inner carcasses and lymph nodes. Ziehl-Neelsen staining, culture on Lowenstein Jensen solid media supplemented with Pyruvate or Para Nitro Benzene (PNB) and molecular techniques (Genotype Mycobacterium CM/AS assay and Spoligotyping) were used to identify atypical mycobacteria and *Mycobacterium tuberculosis* Complex (MTBC) species.

Results: From the 2922 sheep slaughtered, 810 presented tuberculosis like lesions corresponding to an apparent prevalence of 27.72% (810/2922). Ziehl-Neelsen examination confirmed Acid fast bacilli in 77.78 % (630/810) of cases, corresponding to apparent prevalence of 21.56%. Liver was significantly the most affected by tuberculosis like lesion with 40.74% of case ($\chi2=67.02$, $p<0.0001$). The use of para-nitro-benzoïc acid showed that 10.74% of all detected cases are caused by atypical mycobacteria, for which molecular typing identified, *Mycobacterium fortuitum*, *Mycobacterium interjectum* and Mycobacterium sp. Moreover, spoligotyping reveals that 4.44% of cases were due to *M. tuberculosis* represented by it ubiquitous T lineage SIT53. No *M. bovis* or *M. caprae* were identified.

Conclusion: This result shows implication of *M. tuberculosis* and the high prevalence of atypical mycobacteria in sheep intended for human consumption in Cameroon.

Keywords: Atypical mycobateria; *Mycobacterium tuberculosis*; *Mycobacterium fortuitum*; *Mycobacterium interjectum*; Sheep; Tuberculosis; Molecular epidemiology

Introduction

Tuberculosis is still a neglected disease in animals in the Sub-Saharan countries, where it is less studied [1]. *M. bovis* is the principal agent of animal tuberculosis and its transmission to humans constitutes a public health problem as this species is naturally resistant to pyrazinamid, one of the main anti-tuberculosis drugs [2]. In Africa, animal tuberculosis remains endemic and it causes significant losses in the animal industry, with implications for food safety and trade [3].

In Cameroon the breeding sector provides income of about 30% to rural population [4,5]. Sheep represents the second important livestock in Cameroon after cattle though it's a great source of animal protein for the general population. Despite his usefulness, many reports of the Ministry of Livestock, Fisheries and Animal Industries and scarce research based only in cattle livestock, showed that animal tuberculosis is endemic and it is one of the main causes of seizures in abattoir [1,6-9]. Only few regions (two) in Cameroon have data on animal tuberculosis and these were essentially on bovine tuberculosis (tuberculosis in cattle). Moreover, all the ten regions are concerned by bovine tuberculosis.

It is now known that bovine tuberculosis like lesions are not only caused by a member *Mycobacterium tuberculosis* complex (MTBC) but also by Non Tuberculosis Mycobacteria (NTM) or atypical mycobacteria which in human need different treatment from that of patients infected by *M. tuberculosis* complex strains [10]. Accurate identification of *Mycobacterium tuberculosis* and NTP is thus, essential for TB control. We used in this study a new identification algorithm proposed by the International Union against Tuberculosis

and Lung Disease (IUATLD), consisting by the use of p-nitrobenzoic acid (PNB) susceptibility testing permitting the differentiation between *M. tuberculosis* and NTM [11].

The aim of this study is to evaluate for the first time the prevalence of animal tuberculosis and the contribution of NTM and MTBC in sheep livestock in Cameroon. This will permit to gain more information which may significantly impact TB control in Cameroon.

Methods

Slaughterhouse sampling

The sheep's slaughterhouse of central market in New-Bell district of Douala subdivision 2($4°01'26.3''$N; $9°42'53.4''$E) was chosen to screen slaughtered sheep. We made the choice due to its geographical situation (it is located in one of the populous markets of the town of Douala), the diverse origin of the animals coming from almost all the breeding regions in Cameroon, which are slaughtered there and by the existence of a system of notification of all the TB cases.

Sampling

Sampling for tuberculosis lesions occurred during routine inspection by veterinaries surgeon from the Ministry of Livestock, Fisheries and Animal Industries (MINEPIA) and us. This inspection was done according to the government's legislation regulating veterinary health inspection and notification of contagious animal diseases and the recommendation of the World Organization for Animal Health (OIE) [12,13]. After obtaining consent from the relevant authorities, sampling was done from November 2013 to April 2014. About 2922 sheep were successively inspected for tuberculosis during this study. Lesion identification was done by postmortem examination of carcasses as earlier described [14]. This consisted of visual examination and palpation of lungs, liver, bowel, spleen, heart, and inside of the carcass of each slaughtered sheep for the research of caseous nodules, incision of thoracic, mesenteric lymph nodes and other nodes of the carcass for the research of tuberculous granulomas with caseous center.

Lesion collection

A sample was taken according to the World Organization for Animal Health recommendation [13], from any carcass presenting suspect lesions of tuberculosis or a fragment of lymph node presenting a caseous granuloma with or without purulence. Samples were transported dry in sterile plastic containers under controlled conditions and conveyed immediately to the laboratory of the Tuberculosis Research (LTR) of the Biotechnology Center (BTC) of the University of Yaounde I, where they were preserved at $-20°$C. Information relating to each animal (type of lesion, sex, animal age and origin) was recorded postmortem.

Processing of samples

The processing of lesions samples was based on grinding and decontamination procedure using sodium lauryl sulfate as described in our previous study [7,15]. Briefly, the samples were first carved on sterile Petri dish using a sterile scalpel, then ground in a mortar by using a pestle before addition of sterile distilled water. Using a sterile

50 ml centrifuge tube with a screw cap, equal amounts of specimen and sodium lauryl sulfate were added. The centrifuge tube was capped and its content was vortexed until the specimen was liquefied. The mixture was allowed to stand at room temperature for 45 minutes with permanent gentle shaking by Khan Shaker. Prepared phosphate buffer was added to the mixture in the centrifuge tube and mixed, then centrifuged for 20 minutes at 3000 g. The supernatant was carefully decanted and 2 ml of sterile distilled water was added to re-suspend the sediment. The suspension was inoculated onto Lowenstein-Jensen (LJ) slopes with pyruvate and/or glycerol, para-nitro-benzoic acid and incubated at $37°$C for 8 to 12 weeks. All smears were stained by the conventional Ziehl-Neelsen method for the identification of acid-fast bacilli (AFB) and observed under a light microscope [12].

Molecular analysis

The GenoType® Mycobacterium CM/AS was used to identify mycobacteria from any positive culture media, according to the manufacturer's instructions. The results were interpreted using a template sheet and interpretation charts supplied by the manufacturer. Standard spoligotyping was done as described by Kamerbeek and colleagues [16], using a commercially available kit (Isogen Bioscience BV, Maarssen, The Netherlands) for identification and typing of MTBC strains of positives culture on LJ and LJ+P media.

Data analysis

The spoligotypes patterns were introduced to the SITVIT_WEB database and assigned to a SIT number. Spoligotyping-based phylogeographic clades were assigned according to signatures provided by Brudey and collaborators [17]. The Pearson Chi-square or Fisher's exact test was used to estimate the association between the sex, the infected organs, and the result of Ziehl-Neelsen, using statistical software R version 2.15.3 (www.r-project.org).

Results

Postmortem inspection findings

Examination of the 2922 sheep carcasses, presented 810 sheep with the typical macroscopic lesions of tuberculosis, mainly characterized by caseous tubercles with a yellowish appearance (Figure 1a to 1f). The organs or tissues from which tuberculosis lesion were taken were liver (55.55%), bowel (27.40%), lung (8.88%), lymph nodes (6.66%), muscle and spleen (0.74%). Among these, liver appeared to be the most frequently infected organ (χ^2=67.02, p<0.0001).

The apparent prevalence of tuberculosis-compatible lesions on the basis of macroscopic observation was 27.72% (810/2922). Female sheep presented more significant (χ^2=52.06; p<0.0001) tuberculosis like lesion than male with 38.9% (768/1974) and 4.45% (42/948) respectively.

Ziehl-Neelsen stain findings

Acid Fast Bacilli (AFB), using Ziehl-Neelsen stained method, were detected in 630 (77.77%) of the 810 cases (Table 1). This corresponded to the apparent tuberculosis prevalence of 21.56%. There was no statistical association between the tissue lesions origin and Ziehl-Neelsen staining result in this study (p=0.6358).
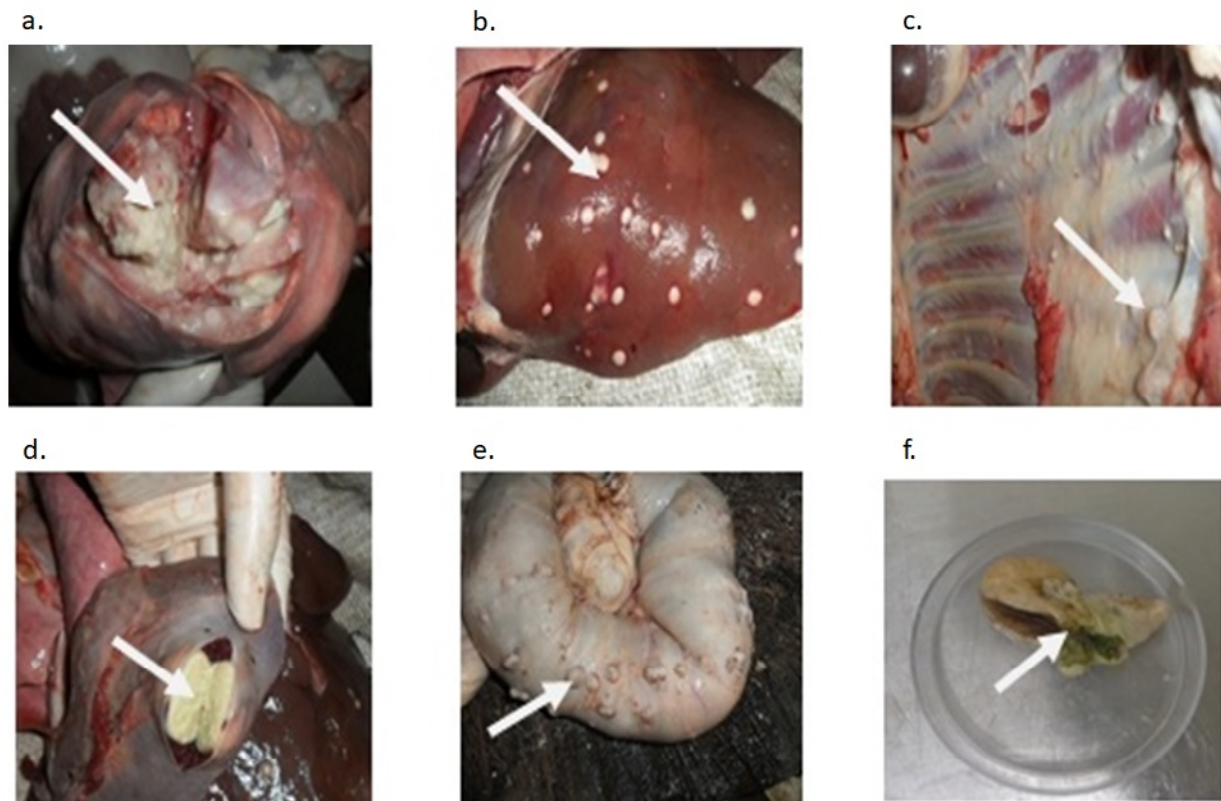
Figure 1: Example of some tuberculosis lesions identified on sheep slaughtered in the abattoir of New-Bell (Cameroon). **a.** Caseous lesion on sheep lung; **b.** Hepatic lesions caseo-limestones generalized; **c.** Caseous nodular lesions on the sheep body in the milliary tuberculosis in abattoir of New-Bell (Cameroon); **d.** Spleen caseous lesion; **e.** Caseous Nodules in intestinal fabric; **f.** Greenish discharge mesenteric lymph nodes.

Organs	Number of sheep presenting tuberculosis macroscopic lesions	Ziehl-Neelsen Staining			
		Positive	Percentage of positivity	Negative	Percentage of negativity
Liver	450	330	73	120	27
Lymph node	54	48	89	6	11
Bowel	222	174	78,38	48	21,62
Muscle	6	6	100	0	0
Lung	72	66	91,7	6	8,3
Spleen	6	6	100	0	0
Total	810	630		180	

Table 1: Macroscopic and microscopic identification of tuberculosis according to sheep organ.

Culture findings

From the 810 detected cases only 123 were positive in culture.

Non tuberculosis mycobacteria identification

Among the culture positive cases, 87 were positive in PNB indicating that 10.74% of cases were due to NTM. The used of the GenoType® Mycobacterium CM assay allows us to identify

Mycobacterium interjectum, *Mycobacterium fortuitum* and Mycobacterium sp. (Figure 2).

Mycobacterium tuberculosis complex strain identification

Only 36 (4.44%) cases were positive in LJ and LJ supplemented with 0.4% of Pyruvate. Spoligotyping showed that all these cases were due to *M. tuberculosis*. No *M. bovis* nor *M. caprea* were identified. The use of SITVITWEB permitted to identify all these *M. tuberculosis* strains as belonging to the Ubiquitous T lineage called SIT53 (Table 2).

Spoligotype profile	SIT	Number of Strains	Lineage
▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▪▫▫▫▫▪▪▪▪▪▪	53	36	T1

Table 2: Spoligotype profile of *M. tuberculosis* identified in Slaughtered sheep in Cameroon.
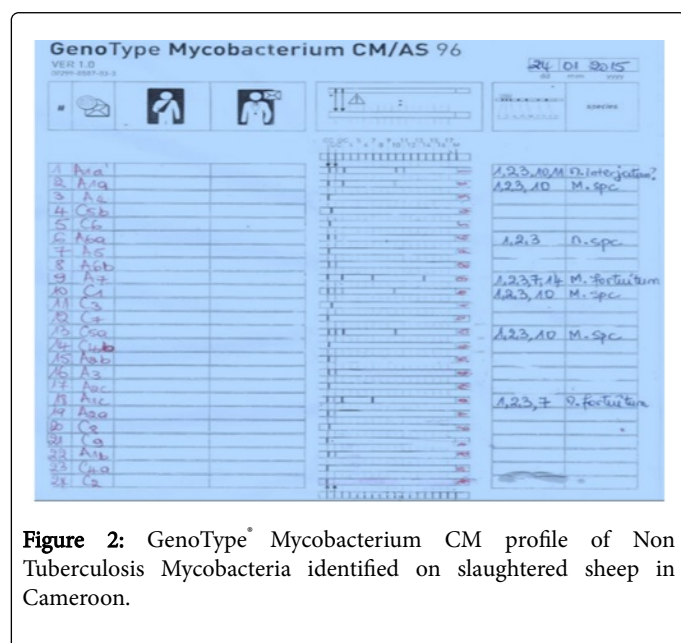
Figure 2: GenoType® Mycobacterium CM profile of Non Tuberculosis Mycobacteria identified on slaughtered sheep in Cameroon.

Discussion

The detection of the lesions during a meat inspection of slaughtered animals is the only way for identifying animal tuberculosis in Cameroon. Many studies have speculated that sheep are resistant to tuberculosis [18-20], but our study shows a very high prevalence of TB-like lesions and very high acid fast bacilli prevalence in slaughtered sheep. This prevalence is very high compared to what is observed in other countries even by the use of comparative intradermal tuberculin skin test or ELISA test. Among these countries was Algeria (4. 4%) [21], Ethiopia, 1.4% [22], Bangladesh, 9.15% [23]. However in Spain a study obtained a more high prevalence (59.4%) [18].

The use of PNB and GenoType® Mycobacterium CM/AS permitted to identify NTM in 10.74% of sheep presenting gross TB-like lesion. This result can justify the high prevalence of AFB observed but remain difficult to understand since cattle and local sheep live in closely proximity but many TB studies in cattle in Cameroon did not found any atypical mycobacteria [7,8]. An explanation could be the fact that many sheep sold and slaughtered in Douala is coming from Chad passing through Far North. In Chad, some study has showed that

38.3% cattle TB-like lesion, are due to Non tuberculosis mycobacteria (atypical mycobacteria) [24], and knowing that in the countries of Central Africa, cattle and sheep live in close contact, we think therefore that Chadian sheep and cattle might share the common source of TB contamination and when this sheep arrive in Douala it is directly sold for human consumption and is very rarely mixed with Cameroonian cattle. This assertion is supported by the fact that *M. fortitum* which have been identified mainly in sheep slaughtered in Cameroonian had been described as dominant in Chadian slaughtered cattle sheep. This assertion needs however to be verified with a large transversal study in Chadian and local Cameroonian since one study done in many regions of Cameroon, have identified *M. furtitum* in Cattle slaughtered [25].

This result can be of great interest in public health and can be disturbing since *M. fortitum* may cause infection in patients with or without HIV infection [26]. Moreover Non Tuberculous Mycobacteria (NTM) can be associated to increasing virulence outbreaks and emergence of antibiotic resistance [27], it may also lead to ill-treatment of the patients to whom a non-adverse treatment would be administered.

The female sheep was significantly more associated to tuberculosis like lesions than male in our study. This result is in line with that obtained in Algeria [21]. This could be explained by the fact that the females are maintained in the herd longer in order to maintain the reproductive performance of the herd and therefore increasing their chance to be exposed to the TB infection [28]. Our result is nevertheless contrary to that obtained by Tschopp et al., in Ethiopia, who showed that the males like the females are more at the risk to develop tuberculosis [29].

An interesting result of this study was the identification of *M. tuberculosis* in Sheep TB like lesion, only represented by it ubiquitous T family clad SIT 53. Identification of *M. tuberculosis* in animal has been also described [8,29-31]. The *M. tuberculosis* lineage SIT 53 has been more descript in human TB in Cameroon but never in cattle, goat, pig or sheep [8,32-35]. Knowing that human remain the true host for *M. tuberculosis*, the finding of *M. tuberculosis* in sheep may traduce an indirect zoonosis case as it has been suggested for cattle [8]. This result shows the urge to make a large epidemiological study to evaluate the degree of implication of *M. tuberculosis* in animal tuberculosis in Cameroon. This may significantly impact TB control in Cameroon.

The absence of *M. bovis* and *M. caprea* among slaughtered sheep in Cameroon can be intriguing but it have longer known that tuberculosis caused by *M. bovis* is rare in sheep, mainly because of the nature of sheep husbandry and the fact that sheep are rarely exposed to infectious material [36]. Moreover others studies also did not identified *M. bovis* and *M. caprea* in sheeps [37]. Nevertheless, the fact that 630 specimens were Ziehl-Neelsen stain positive but that only 123 were positive in culture may also explain the absence of *M. bovis* or *M. caprea* because of the difficulty of recovery of these MTBC species by culture. In fact, difficulties to recover *M. bovis* due to its cultural habit have been longer highlight [38].

Conclusion

This study is the first study on molecular epidemiology of tuberculosis in sheep in Cameroon. It shows a high prevalence of TB in sheep due principally by *M. tuberculosis* and the implication of atypical mycobacteria represented mostly by *M. fortitum* and *M. interjectum* in the sheep TB like lesions. This highlight the urge to

make a large study in Cameroonian livestock in order to identify the risk factors associated to *M. tuberculosis* transmission in animal in Cameroon.

Conflict of Interest Statement

I would like to undertake the responsibility for this submitted manuscript, I did not receive reimbursements, fees, funding, or salary from an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future. I did not hold any stocks or shares in an organization that may in any way gain or lose financially from the publication of this manuscript, either now or in the future. Moreover, I did not hold or was currently applying for any patents relating to the content of the manuscript even received reimbursements, fees, funding, or salary from an organization that holds or had applied for patents relating to the content of the manuscript. In the best of my knowledge I did not have any other financial and non-financial competing interests.

References

1. Awah JN, Kudi AC, Bradley G, Ane-Anyangwe I, Titanji VPK, et al. (2012) Prevalence of bovine tuberculosis in cattle in the highlands of Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skin tests of live cattle. Vet Med 57: 59-76.

2. Kleeberg HH (1984) Human tuberculosis of bovine origin and public health. Rev sci tech 3: 33-54.

3. FAO (2012) Bovine tuberculosis at the animal-human interface ecosystem. EMPRES Bulletin of animal diseases transboundary 40: 2-9.

4. Hamadou S (2001) A new framework for the exercise of animal health activities in Cameroon. Afr Agri 294: 30-31.

5. Ly C (2007) Animal health and poverty in Africa. Agriculture, livestock and poverty in West Africa, ed. HRD.

6. 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.

7. Koro FK, Bouba, Foko E, Ngatchou AF, Eyangoh S, et al. (2013) First insight into the current prevalence of bovine tuberculosis in cattle slaughtered in Cameroon: The case of the main abattoirs of Yaoundé and Douala. Br Microbiol Res J 3: 272-279.

8. Koro FK, Ngatchou AF, Portal JL, Gutierrez C, Etoa FX, et al. (2015) The genetic population structure of Mycobacterium bovis strains isolated from cattle slaughtered at the Yaoundé and Douala abattoirs in Cameroon. Rev Sci Tech 34: 1001-1010.

9. Awah JN (2005) Prevalence of bovine tuberculosis at the SODEPA Douala abattoir, Cameroon (1995-2003). Cameroon J Exp Bio 1: 116-120.

10. Wagner D, Young LS (2004) Nontuberculous Mycobacterial infections: A clinical review. Infection 32: 257-270.

11. Affolabi D, Sanoussi ND, Odoun M, Anandi M, Koukpemedji L, et al. (2013) Rapid low-cost identification of Mycobacterium tuberculosis complex using p-nitro-benzoic acid (PNB) as inhibitor and the resazurin microplate assay (REMA): A preliminary study. Af J Microbiol Res 7: 3135-3138.

12. MINEPIA (2000) The sectoral strategy of livestock, fisheries and animal industries, in Cabinet Management: Yaounde, Cameroon.

13. OIE (2009) Bovine tuberculosis in OIE Terrestrial manual, Geneva.

14. Murray G, Biddle R, Miller P (1991) Efficiency of inspection procedures for the detection of tuberculous lesions in cattle. Aust Vet J 68: 217-218.

15. Tacquet A, Tison F (1961) New technique of isolation of mycobacteria by sodium lauryl sulphate. Ann Inst Pasteur 100: 676-680.

16. Kamerbeek J, Schouls L, Kolk A, Agterveld MV, Soolingen DV, et al. (1997) Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. J Clin Microbiol 35: 907-914.

17. Brudey K, Driscoll JR, Rigouts L, Prodinger WM, Gori A, et al. (2006) Mycobacterium tuberculosis complex genetic diversity: Mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. BMC Microbiol 6: 23.

18. Muñoz MM, Romero B, Cerro AD, Gortazar C, Garcia-Marin JF, et al. (2015) Sheep as a potential source of bovine TB: Epidemiology, pathology and evaluation of diagnostic techniques. Transbound Emerg Dis 63: 647-657.

19. Radostits OM, Gay CC, Blood DC, Hinchelift KW (2000) Disease caused by bacteria-Mycobacterium in A text book of Disease of cattle, sheep, pig, goat and horses (9th Edn), Veterinary Medicine, Harcourt Publisher Ltd., London, pp: 909-918.

20. Thoen CO, Steele JH, Gilsdorf MJ (2006) Mycobacterium bovis infection in animals and humans (2nd Edn) Blackwell Publishing, UK.

21. Sahraoui N, Brahim ME, Tazerart F, Hadjadja FZ, Habbas N, et al. (2013) Tuberculosis in small ruminants in Algeria. Bull Anim Health Prod Afr 60: 1-2.

22. Kassa GM, Abebe F, Worku Y, Legesse M, Medhin G, et al. (2012) Tuberculosis in goats and sheep in Afar Pastoral Region of Ethiopia and isolation of Mycobacterium tuberculosis from goat. Vet Med Int.

23. Rahman MM, Khan MFR, Nazmul-Hussain KHMN, Rahman MB (2013) Prevalence of bovine and avian tuberculosis in sheep and goat population of Bangladesh. Sci J Microbiol 2: 1-8.

24. Diguimbaye CD, Vincent V, Schelling E, Hilty M, Ngandolo R, et al. (2006) Species identification of non-tuberculous mycobacteria from humans and cattle of Chad. Schweiz Arch Tierheilkd 148: 251-257.

25. Egbe NF, Muwonge A, Ndip L, Kelly RF, Sander M, et al. (2015) Abattoir based estimates of mycobacterial infections in Cameroon. Sci Rep 6: 24320.

26. Agheli A, Tehranirad M, Cofsky R (2006) An unusual presentation of Mycobacterium fortuitum: Massive isolated empyema in a patient with HIV. Med Gen Med 8: 90.

27. Khosravi AD, Mehrabzadeh RS, Farahani A, Jamali H (2015) Molecular identification of clinical isolates of Mycobacterium fortuitum by random amplified polymorphic DNA (RAPD) polymerase chain reaction and ERIC PCR. J Clin Diagn Res 9: 1-5.

28. Boukary AR, Thys E, Mamadou S, Rigouts L, Matthys F, et al. (2011) Mycobacterium bovis tuberculosis in Sub-Saharan Africa. Ann Med Vet 155: 23-37.

29. Rea T, Kidist B, Abraham A, Esther S, Meseret H, et al. (2011) Bovine tuberculosis at a cattle-small ruminant-human interface in Meskan, Gurage region, Central Ethiopia. BMC Infect Dis 11: 318.

30. Fischer RJ, Johnson TL, Raffel SJ, Schwan TG, Johnson TL (2009) Mycobacterium bovis and M. tuberculosis in goats, Nigeria. Emerg Infect Dis 15: 2066-2067.

31. Deresa B, Conraths FJ, Ameni G (2013) Abattoir-based study on the epidemiology of caprine tuberculosis in Ethiopia using conventional and molecular tools. Acta Vet Scand 55: 8-15.

32. Niobe-Eyangoh S, Kuaban C, Sorlin P, Thonnon J, Sola C et al. (2003) Genetic biodiversity of Mycobacterium tuberculosis complex strains from patients with pulmonary tuberculosis in Cameroon. J Clin Microbiol 41: 2547-2553.

33. Ngo Niobe ES, Kuaban C, Sorlin P, Thonnon J, Vincent V, et al. (2004) Molecular characteristics of strains of the Cameroon family, the major group of Mycobacterium tuberculosis in a country with a high prevalence of Tuberculosis. J Clin Microbiol 42: 5029-5035.

34. Koro FK, Kamdem YS, Fotso FP, Noeske J, Gutierrez C, et al. (2013) Population dynamics of tuberculosis bacilli in Cameroon assessed by spoligotyping. J Clin Microbiol 51: 299-302.

35. Francioli KK, Boock AU, Leslie KA, Jurgen N, Cristina G, et al. (2016) Genetic structure and drug susceptibility patterns of Mycobacterium tuberculosis complex strains responsible of human pulmonary tuberculosis in the major rearing region in Cameroon. BioMed Res Int 2016: 12.

36. Francis J (1958) Tuberculosis in animals and man: A study in comparative pathology. Cassell, London, pp: 357.

37. Kassa GM, Abebe F, Worku Y, Legesse M, Medhin G, et al. (2012) Tuberculosis in goats and sheep in Afar Pastoral Region of Ethiopia and isolation of Mycobacterium tuberculosis from goat. Vet Med Int 2012: 869146.

38. Keating L, Wheeler P, Mansoor H, Inwald J, Dale J, et al. (2005) The pyruvate requirement of some members of the Mycobacterium tuberculosis complex is due to an inactive pyruvate kinase: Implications for in vivo growth. Mol Microbiol 56: 163-174.