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Epidemiology and public health significance of bovine tuberculosis in cattle in the highlands of Cameroon

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Copyright statement

Epidemiology and public health significance of bovine tuberculosis in cattle in the highlands of Cameroon

by

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Abstract

Bovine tuberculosis (TB) is a contagious neglected zoonosis of cattle that is prevalent but under-investigated in Cameroon, hence this study was designed to assess the epidemiology of bovine TB in cattle, risks for *M. bovis* infection in cattle and humans; and public health implications of zoonotic bovine TB in the highlands of Cameroon. A retrospective study of meat inspection records (1994 – 2010) was done to estimate the prevalence of TB lesions in slaughtered cattle in the North West region. The prevalence of bovine TB and anti-bovine TB antibodies in live cattle based on tuberculin skin tests (2 surveys) and immune-chromatographic assays respectively were carried out in the Western and Adamawa highlands of Cameroon. The performance of the tuberculin tests for bovine TB diagnosis in cattle using various tuberculin skin test cut-off points against the detection of anti-bovine TB antibodies (hypothesised risks of exposure) was compared. Suspected TB lesions from slaughtered cattle and infected human sputa were cultured on Lowentein – Jesen and Middlebrook 7H9 media to isolate mycobacteria agents for molecular genotyping using genomic deletion analysis and spoligotyping. Risk factors for exposure and transmission of zoonotic bovine TB infection of cattle and cattle professionals, and its public health significance were determined using structured questionnaires.

Seventeen years of meat inspection record revealed that suspect TB lesions were identified in 599 of 129,165 slaughtered cattle at the Bamenda abattoir. The lungs and associated lymph nodes (over 60%) were the most affected tissues. Other results showed that the prevalence of anti-bovine TB antibodies in cattle in the study regions was 37.17%. Chi square statistics revealed that irrespective of the tuberculin test cut-off value ($P<0.05$; $\chi^2>48$), strong associations existed between the detection of anti-bovine TB antibodies and disease status. A 95% confidence interval analysis of the comparative cervical tuberculin tests revealed that the prevalence rates were 4.67% – 7.15%, 12.02% – 15.67% and 20.56% – 24.98% at the ≥ 4mm, ≥ 3mm and ≥ 2mm cut-off points, respectively. Overall, the best test performance was realised at ≥ 3-mm, though the ≥ 2-mm cut-off point predicted more positive reactors. Age, sex, breed and husbandry practices served as significant ($P<0.05$) risks to the prevalence and exposure of bovine TB in cattle. The feedbacks from cattle professionals suggested that there was high possibility of cattle to cattle and cattle to human transmission of bovine TB such as intimate and repeated animal / animal and animal / human interactions, consuming unpasteurised milk and eating raw meat. Genomic deletion analysis of cultured isolates showed evidence of *M. tuberculosis* from cattle and M*. bovis* from human while spoligotyping identified five cattle *M. bovis* strains; and four spoligotype patterns that had not been previously described anywhere.

The study has important epidemiological and public health implications requiring prompt and decisive actions from the Cameroonian authority towards controlling zoonotic bovine TB in both humans and animals. A multidisciplinary approach is needed for further collaborative research and effective control strategies such as enhancing the awareness of people to this deadly disease through continuous education, proper food handling and personal hygiene, healthy husbandry practices and maintenance of the environment.

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List of Abbreviations

ADP	Adamawa Plateau
AIDS	Acquired immunodeficiency syndrome
AT	Avian tuberculin
AU/IBAR	African Union/Inter African Bureau on Animal Resources
BecA	Bioscience Eastern and Central Africa
BT	Bovine tuberculin
CI	Confidence Interval
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme Linked Immunosorbent assay
FAO	Food and Agricultural Organisation
HIV	Human immunodeficiency virus
ILCA	International Livestock Centre in Africa
LJ	Lowentein-Jensen media
MAC	*Mycobacterium avium-intercellulare* complex
MAPIA	Multiple antigen print immunoassay
MINEPIA	Ministère de l'Elevage, des Pêches et des Industries Animales (Ministry of Livestock, Fisheries and Animal Industries)
MoPH	Ministry of Public Health (Ministère de la Santé Publique)
MTC	*Mycobacterium tuberculosis* complex
OIE	World Organisation for Animal Health (Office International des Epizootiques)
OR	Odds Ratio
PCR	Polymerase chain reaction
PPD	Purified Protein Derivative
RD	Region of Difference (Genomic)

RR	Relative Risk
SDA	Strand Displacment Amplification
SDS	Sodium Dodecyl Sulphate
SE	Standard error
Se	Sensitivity
Sp	Specificity
SICCT	Single Intradermal Comparative Cervical Tuberculin skin test
SICCT-AT	Single Intradermal Comparative Cervical Tuberculin skin test for detecting positive reactors to avian tuberculin PPD
SICCT-BT	Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis
SIT	Single Intradermal Tuberculin skin test
SIT-AT	Single Intradermal Tuberculin skin test for detecting positive reactors to avian tuberculin PPD
SIT-BT	Single Intradermal Tuberculin skin test for the diagnosis of bovine tuberculosis
TB	Tuberculosis
TAE buffer	Tris – Acetate – EDTA buffer (Tris – Acetate – Ethylenediamine tetraacetic acid buffer)
	Tris (hydroxymethyl) aminomethane – EDTA buffer (Tris (hydroxymethyl) aminomethane – Ethylenediamine tetraacetic acid buffer
TST	Tuberculin skin test
UV	Ultra violet
VNTR	Variable number tandem repeats
WHC	Western Highlands of Cameroon
WHO	World Health Organisation
ZN	Ziehl-Neelsen
γ-IFN	gamma interferon

Dedication

To the loving memory of **Ahanwi Taibatu AWAH-NDUKUM** (19th July 2002 – 30th December 2002) who was indeed blessed to bring the spirit of laughter into our home. May her gentle soul rest in perfect peace; AMEN.

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First, I thank the Almighty God for preserving me and for the abundant grace He continues to shower on me. I have always been interested in higher education; and combining research, teaching and out-reach activities is a goal I value greatly.

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Author's Declaration

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

This study was financed with the aid of a Commonwealth Academic Scholarship from the Commonwealth Scholarship Commission in the United Kingdom.

A programme of advanced study was undertaken which included Postgraduate modules in Research Skills & Methods and Laboratory Based Teaching Methods & Practice as well as associated studies and skill development courses organised by the Graduate School and other societies of the university. The field survey and some laboratory analyses were conducted in the highland regions of Cameroon and Mycobacteriology Laboratory of the Bioscience eastern and central African (BecA) Hub in the University of Buea, Cameroon.

Part of the molecular analysis (Spoligotypying) was done at the Veterinary Laboratory Agency, Weybridge, UK.

Relevant scientific seminars and conferences were attended at which some results were presented. External institutions were also visited for consultation purposes and papers have been submitted for publication in journals.

Associated research activities

Published article

Awah-Ndukum, J. Kudi, A. C. Bradley, G. Ane-Anyangwe, I. N. Fon-Tebug, S. and Tchoumboue, J. (2010). Prevalence of bovine tuberculosis in abattoirs of the Littoral and Western highland regions of Cameroon: a cause for public health concern. *Veterinary Medicine International* **2010** (495015), 8 pages; doi:10.4061/2010/495015.

Poster presentations at conferences

1. Awah-Ndukum J., Kudi A. C., Bradley G., Bah G. S. and Ane-Anyangwe I. (2011): Bovine tuberculosis in cattle: true prevalence and risk factor appraisal in cattle and cattle professionals in the highlands of Cameroon. In: *IMT / DVTD Joint Colloquium on Zoonoses and Neglected Infectious Diseases in Africa* (Eds Anita et al.); pp 34. Johannesburg, South Africa.
2. Awah-Ndukum J., Kudi A. C., Bah G. S., Bradley G., Ane-Anyangwe I., and Titanji V.P.K. (2011): Bovine tuberculosis in Cameroon: Current Status in cattle and challenges for the future. In: *Impact, Limitations and Needs in Developing Countries*. The 1st International Congress on Pathogens at the Human – Animal Interface (ICOPHAI) (Eds Kazwala et al.); pp 70. Addis Ababa, Ethiopia.

Oral presentations at conferences

3. Awah-Ndukum, J. Kudi, A. C. Bradley, G. and Ane-Anyangwe, I. N. (2011). Preliminary report on the zoonotic significance of tuberculosis in cattle in Cameroon. In: *Animal Health and Sustainable Livestock Production.* Proceedings of the XVth International Congress on Animal Hygiene organised by the International Society of Animal Hygiene (Eds Kofer and Schobesberger), pp. 193 – 196. Vienna, Austria.
4. Awah-Ndukum, J. Kudi, A. C. Bradley, G. and Bah, G. S. (2011): Assessment of cut-off points during tuberculin skin test for the diagnosis of bovine tubercuolosis in Cameroonian cattle. In: *Advances in animal biosciences: Food security-challenges and opportunities for Animal science* (Eds Baxter et al), p. 150. Nottingham, UK: Proceedings of the British Society of Animal Science and the Association of Veterinary Teaching and Research Work.
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7. Awah-Ndukum, J. Fon-Tebug, S. Tchoumboue, J. Kudi, C. and Bradley, G. (2009). Zoonotic importance of bovine tuberculosis in Cameroon: Current status and factors limiting control. In: *The role of the veterinarian in sustainable development and environmental protection* (Eds Zoli et al), p. 25 – 38, Yaoundé, Cameroon. Proceedings of 3rd African Veterinary Congress.

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Chapter 1

Literature review

1.1 Introduction

Tuberculosis (TB) is a disease of animals and man caused by pathogenic members of the genus *Mycobacterium* and characterized by progressive development of granulomatous lesions or tubercles in the lung tissue, lymph nodes, or other organs (Blood and Radostits 1989; Thoen et al. 2009). It is the most important zoonosis associated with enormous economic losses in the animal industries and severe hazards to human health worldwide, particularly in the developing world (WHO 2002; Kiboss and Kibitok 2003; Ayele et al. 2004; WHO 2009). TB is the leading cause of human death due to a single infectious agent (O'Reilly and Daborn 1995; Cosivi et al. 1998; Larson 2000a; Tan et al. 2003; Thoen et al. 2009) with over 9.27 million new cases and nearly 2 million deaths recorded every year (WHO 2009; 2010). When the right combination of medication is made available and taken by the patient, TB can be cured more than 95% of the time; and in certain targeted populations, the manifestations of the dieseae can be attenuated by vaccination and even prevented by chemotherapy (Murray 2004). However, it still accounts for about 25% of all avoidable deaths among adults in the world (Murray et al. 1990; Porter and McAdam 1994; Ayele et al. 2004).

The devastating effects of the disease have varied widely with time and in different regions; but its occurrence has largely been influenced by man-made factors, such as urban crowding and poverty (Fätkenheuer et al. 1999). Most

pathogenic mycobacterium species affecting man and animals are members of the *Mycobacterium tuberculosis* complex (MTC) (Biet et al. 2005; Une and Mori 2007). Early understanding of cross species infection or zoonotic nature of these agents began at the time that humans domesticated and lived closely with animals (Biet et al. 2005). The causative agents have since spread to all groups in the human population and constitute major threats to human health globally (Tan et al. 2003; Ayele et al. 2004; Thoen et al. 2009). TB co-infection with the Human Immunodeficiency virus (HIV) and rapidly spreading Acquired immune deficiency syndrome (AIDS) or the HIV/AIDS epidemic has significantly worsened the situation (Fätkenheuer et al. 1999; Larson 2000b; Corbett et al. 2002; Corbett et al. 2003; WHO 2005; Corbett et al. 2006). Also, the widespread development of drug-resistant strains has complicated the treatment of TB in humans and significantly increased the cost associated with the use of multiple drug therapy (Thoen and Ebel 2006; Thoen et al. 2009). Although TB is a major cause of human deaths, the real extent of human TB due to zoonotic agents is not known (O'Reilly and Daborn 1995; Ashford et al. 2001; Thoen et al. 2009). Over 70% (6 million) of humans co-infected with TB and HIV/AIDS live in sub-Saharan Africa (O'Reilly and Daborn 1995; Cosivi et al. 1998; Corbett et al. 2006) where bovine TB represents a potential health hazard to both animals and humans (Ayele et al. 2004).

In the industrialised countries, significant progress has been made towards eliminating the disease from human and animal populations. The awareness of the public health hazard and economic impact to animal production of TB in these countries has resulted in the design and implementation of successful long term programmes for control and / or eradication, based on the “test and slaughter” method for animals and efficient health systems for human cases

(Citron 1988; Abernethy et al. 2006; Berrada 2006; Enarson 2006; Goodchild and Clifton-Hadley 2006; Pavlik 2006a; Pavlik 2006b; EFSA 2007). Although TB control programmes have averted millions of deaths worldwide, their effects on transmission and incidence rates are not yet widely detectable (Dye et al. 2009). For example, potential risks still remain for global trading in animals and animal products on animal and public health (WHO 1994b; Ayele et al. 2004). Also, *M. bovis* is virulent for cattle but can infect humans and cause disease with pathology similar to *M. tuberculosis*, which is virulent for man (Biet et al. 2005).

In Africa, bovine TB represents potential hazards to both animals and humans as over 85% of cattle and 82% of the human populations live in areas where the disease is prevalent and only partially controlled or not controlled at all (Ayele et al. 2004). Many of these countries are underdeveloped and lack sufficient financial and technical resources to support control programmes (Thoen et al. 2009). Thus, in most of Africa, animal TB remains a neglected public health hazard and an under-investigated animal health and production problem (Kaneene and Pfeiffer 2006; Zinsstag et al. 2006). There is also the tendency to under- estimate the prevalence and problem in regions where the disease has been reported. Effective monitoring and reduction of the resurgent TB in the African continent would require comprehensive knowledge of the disease status in various human and animal populations. A proper understanding of the magnitude and distribution, risk factors, transmission routes and reservoirs of the disease cannot be overemphasised.

The aim of this review is to summarize available epidemiologic information on bovine TB and human TB. Risk factors for the exposure and transmission of zoonotic TB due to *M. bovis*, populations at risk and existing control measures

in Africa with particular attention to Cameroon in the Central African sub-region will be examined.

1.2 Aetiology of tuberculosis in human and cattle

TB in humans and animals is caused by the tubercle bacilli of the *Mycobacterium tuberculosis* complex (MTC) and other environmental non-tuberculous or atypical mycobacteria (Raviglione et al. 1995; Falkinham 3rd 1996; Harris and Barletta 2001; Biet et al. 2005; Une and Mori 2007). Many atypical mycobacteria are saprophytes, commensals, and symbionts, in a wide variety of environmental reservoirs such as natural and municipal water, soil, aerosols, protozoa, animals and humans (Primm et al. 2004) and members of the *Mycobacterium avium-intracellulare* complex (MAC). They have been reported to cause significant morbidity and mortality to humans and impact economic loss to livestock (animals and birds) industries (Falkinham 3rd 1996; Harris and Barletta 2001; Primm et al. 2004; Biet et al. 2005; Une and Mori 2007).

Most pathogenic mycobacteria have slow growth rates and do not grow outside of a host except when cultured on selective laboratory media; are resistant to acid decolourising stains and appear microscopically as slender rods (tubercle bacilli) that are 0.3 – 0.6 µm in diameter and 1.5- 3.0 µm in length (Thoen et al. 2009). They cannot tolerate harsh environments such as prolonged exposure to heat, direct sunlight and dry conditions but can survive for long periods under cold, dark, and moist conditions (Goodchild and Clifton-Hadley 2001; Philips et al. 2003).

Most MTC and MAC bacteria are of veterinary and medical importance (Harris and Barletta 2001; Ayele et al. 2004; Primm et al. 2004; Biet et al. 2005; Thoen

et al. 2006; Une and Mori 2007; Thoen et al. 2009) but the MTC bacteria are highly pathogenic and are distinguished based on taxonomic characteristics, distinct phenotypic differences and nucleotide similarities (Falkinham 3rd 1996; van Soolingen et al. 1997; Aranaz et al. 1998; Aranaz et al. 1999; Ayele et al. 2004; Biet et al. 2005; Brudey et al. 2006). All MTC bacteria evolved from a common ancestor (Figure 1) and are characterised by 99.9% similarity at the nucleotide level and identical 16S rRNA sequences (Brosch et al. 2002; Smith et al. 2006a; Thoen et al. 2009). The typical MTC agents: *Mycobacterium tuberculosis, M. bovis, M. africanum, M. microti* and *M. canetti* cause TB in an exceptionally wide range of domestic and wild mammalian hosts including humans (O'Reilly and Daborn 1995; van Soolingen et al. 1997; Kremer et al. 1998; van Soolingen et al. 1998; Harris and Barletta 2001; Aranaz et al. 2003; Biet et al. 2005; Brudey et al. 2006; Une and Mori 2007; Thoen et al. 2009). Of these species, *M. tuberculosis* and particularly *M. bovis* are highly pathogenic and very closely resemble each other. The MAC is comprised of *M. avium ssp. avium, M. avium ssp. paratuberculosis, M. avium ssp. silvaticum, M. avium ssp. hominissuis,* and *M. intracellulare* (Falkinham 3rd 1996; Biet et al. 2005).

Human TB is caused mainly by *M. tuberculosis* but *M. bovis* the etiological agent of bovine TB can also be responsible for the human disease, making *M. bovis* an important zoonotic species (Blood and Radostits 1989; Cosivi et al. 1998; Biet et al. 2005; OIE 2009) while *M. africanum* is problematic to humans in tropical Africa (Cosivi et al. 1998). Human and other non-host specific infections caused by *M. avium, M. microti,* and *M. canettii* have also been documented (Falkinham 3rd 1996; van Soolingen et al. 1997; Kremer et al. 1998; van Soolingen et al. 1998; Biet et al. 2005).

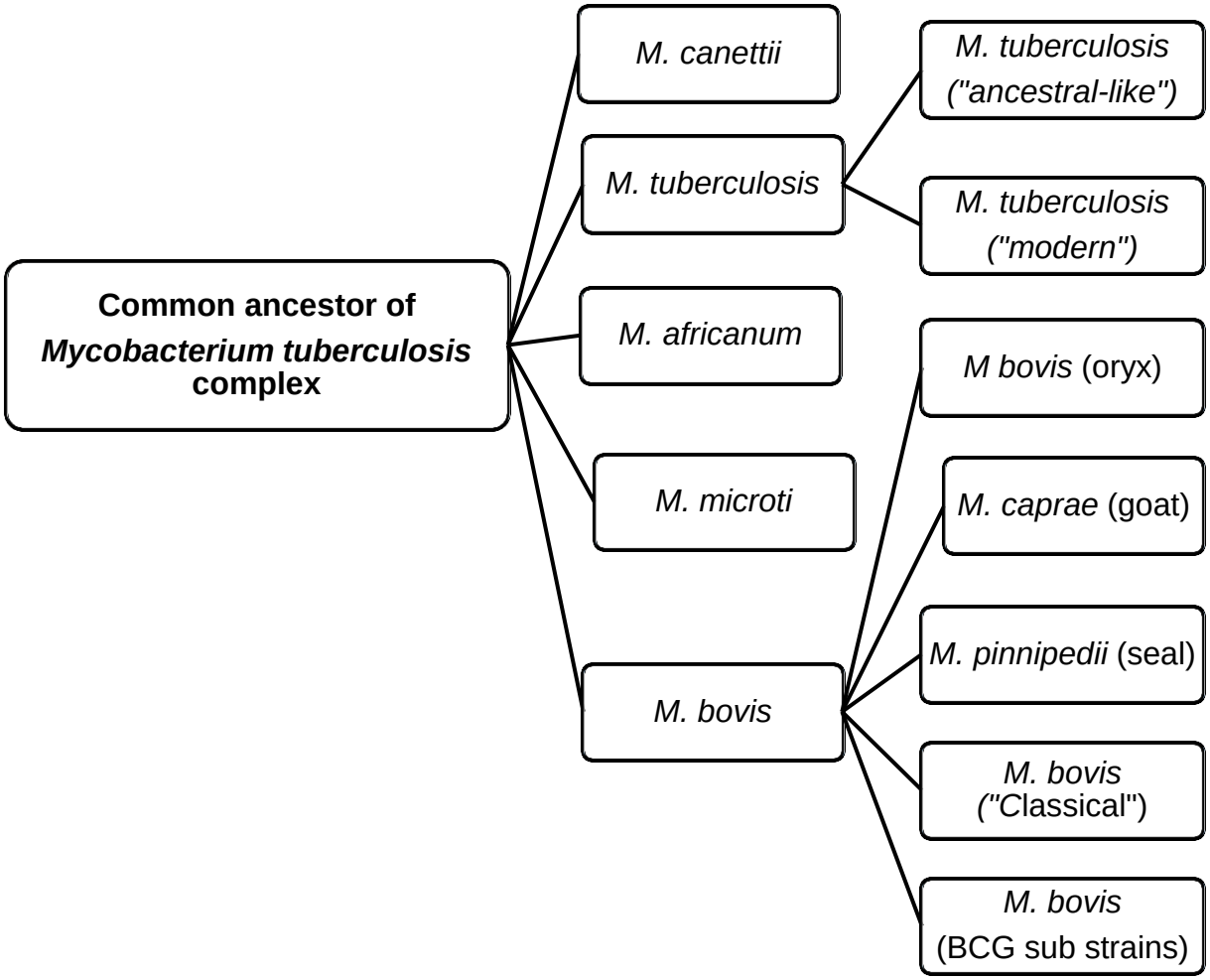


Figure 1: Summary of proposed evolutionary scheme of *Mycobacterium tuberculosis* complex from a common ancestor.

*: The scheme is based on Brosch et al. (2002) and Thoen et al. (2009). During evolution, the ancestral progenitor underwent various deletions (eg loss of RD^{can}, RD9, RD4, …) giving origin to micro-organisms of the *Mycobacterium tuberculosis* complex.

1.3 Host range of tubercle bacilli

The ability of MTC bacteria to infect a wide variety of mammalian species has been attributed to their different routes of transmission from infected to susceptible hosts (Kaneene and Pfeiffer 2006). Table 1 summarises the host range of the principal tuberculous agents of humans and cattle. The rate of transmission is determined by the infectiousness of the host expelling the pathogens, the concentration of the organisms and length of exposure; and immune status (or susceptibility) of the host at risk (Menzies and Neill 2000; Tiruviluamala and Reichman 2002). Similarly, the susceptibility levels vary with different host species, pathogenic mycobacteria, route of exposure, and infective dose and virulence of the tubercle bacilli strain (Biet et al. 2005; Une and Mori 2007; OIE 2009; Thoen et al. 2009). The relative susceptibilities of various animal species and spread of zoonotic tubercle bacilli are shown in Table 2. Susceptible non-specific hosts that become infected can serve as reservoir or “spillover” hosts with sporadic infections or persistent disease within affected animal populations if these maintenance hosts are present in the environment (Blunden and Smith 1996; Gunn-Moore et al. 1996; Gupta and Katoch 2005; Cassidy 2006; Corner 2006; Ellis et al. 2006; Monies et al. 2006; Pollock et al. 2006; Une and Mori 2007; Shrikrishna et al. 2009).

TB is not commonly reported in wild animals, except when they have been exposed to infected domestic animals or humans. Infected wild animals may be spillover cases where the disease is present but cannot be maintained in the wildlife population. However, several wildlife species have been recognized as reservoirs of *M. bovis,* though cattle are the most probable natural reservoir hosts (Kaneene and Pfeiffer 2006; Thoen et al. 2009).

Table 1 : Principal mycobacteria of humans and animals

Species	*M .tuberculosis*	*M. bovis*	*M. avium*
Man	P	P	0
Domestic animals			
Canines – Dogs	P	P	0
Felines – Cats	0	P	0
Bovine – cattle	P	P	0
Ovine – Sheep	0	0	P
Caprine – Goats	0	P	P
Porcine – Pigs	P	P	0
Equine – Horses	0	0	
Avian in general – Poultry	0	0	P
Wildlife (*M. bovis* reservoirs)			
– Ungulates (eg: Buffalos, Bisons, …	0	P	
– Cervids (eg: Deers, Lechwe, Elk, Wapiti,..	P	P	
– Badgers, Brushtail possums, wild boars, other rodents, …	P	P	
Wildlife (*M. bovis* potential reservoirs)			
Others: Cloven hoofed animals (eg the Kudus, Ilama, Some Deers, Giraffe, Ferrets, Oryx, Wild Goats, Impala, Yak, Eland, Wildebeest, …	P	P	
Wildlife (Carnivores & scavengers – Wild canines & felines)			
Foxes, Tiger, Coyotes, Wolf, Lions, Cheetah, Leopard, Lynx, Raccoon, Black Bear, Opossums, Bush Pigs, Warthogs, …	P	P	
Wildlife (Non-human primates and non-primates)			
– Monkeys/Apes, Gibbon. Mayotte, Baboons, Gorillas, ….;	P	P	0
– Rhinoceros, Hares, ….; Elephants,	P	P	0
– Birds : Psittacids, …	P	0	0

Pathogenic power: P = high; 0 = exceptional or rare

The table is adapted from Thoen et al. (2009), Une & Mori (2007) and Biet et al. (2005).

Table 2 : The relative susceptibilities of various animal species and spread of zoonotic tubercle bacilli

Group	Species 1	No. of bacilli in lesions[a]	Species 2	Susceptibility of infection with three types of tubercle bacilli[a]			Spread
				Bovine	Human	Avian	
1	Primitive humans #1	1		5	5	1	5
	Monkeys	2	Great apes	3	2	3	5
			Asian monkeys	5	5	2	
			African monkeys	4	4	2	
			South American monkeys	2	2	2	
	Guinea pigs	1		5	5	2	1
	Rabbits	2		1	5	4	1
	Mice	3		1	5	4	1
2	Modern humans #2	1		2	2	1	5
	Elephants	3		3	3	1	
	Cattle	1		1	4	1	5
	Goats	1		1	4	2	1
	Pigs	1		2	4	2	1
3	Chickens	4		1	1	3	4
4	Horses , etc	3		1	2	1	1
5A	Dogs	2		2	2	0	0
5B	Cats	3		1	4	2	1
	Ferrets	5		1	5	2	0
5C	Hamsters	4		5	5	1	0

The maximum value for each feature in this table is 5. The value for spread represents the degree of case with which tuberculosis spreads naturally between members of any one species.

#1: aboriginal people; #2: contemporary human.

[a]: The rating scale is as follows : 1: not likely; 2: rare; 3: occasional; 4: common; 5: classical

Source : (Une and Mori 2007)

1.4 Routes of transmission of the tubercle bacilli

There are several routes of entry and transmission of the tubercle bacilli (Figure 2); but the respiratory and gastrointestinal tracts are the primary routes and less frequently incisions in the skin (Neill et al. 1994; Goodchild and Clifton-Hadley 2001; Philips et al. 2003; Ayele et al. 2004; Biet et al. 2005; Kaneene and Pfeiffer 2006; Wilsmore and Taylor 2008). The location, nature and extent of tuberculous lesions vary with the route of exposure and also affects how the organism are shed from the infected host (Palmer et al. 2001; Kaneene and Pfeiffer 2006). Externally draining abscesses can serve as a source of infection in a wide variety of susceptible host populations and the tubercle bacilli particularly *M. bovis,* can persist in environmental samples for long periods (Goodchild and Clifton-Hadley 2001; Philips et al. 2003; Wilsmore and Taylor 2008).

Respiratory transmission through direct inhalation of contaminated aerosols (Figure 2), is the most important route of infection in groups of susceptible hosts that remain in repeated close contact or in a confinement with infected individuals (Goodchild and Clifton-Hadley 2001; Hussain et al. 2003; Cassidy 2006; Kaneene and Pfeiffer 2006; Palmer and Waters 2006). Transmission by inhalation requires a lower number or infective dose of organisms compared to the other routes (Francis 1971; Goodchild and Clifton-Hadley 2001; Cassidy 2006). Usually infected hosts generate aerosols and mucous containing the agents when they cough or sneeze. Also, this mode of transmission is effective in intensively managed animals kept in a limited space, free-ranging animals that maintain social or familial groups (Kaneene and Pfeiffer 2006) and in common spots where animals gathering occur such as communal grazing, drinking points, vaccination centres and night enclosures (Ayele et al. 2004).

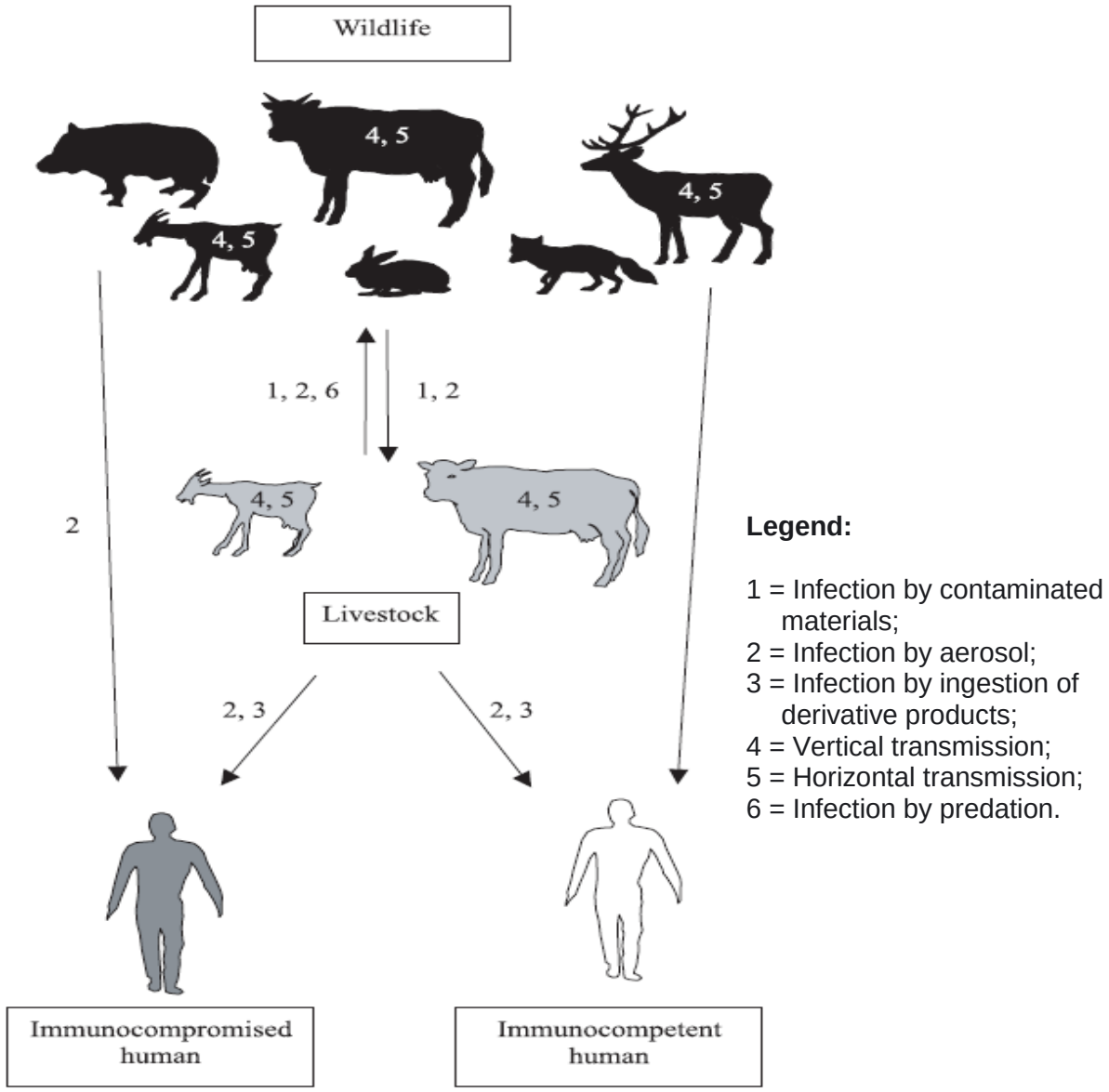


Figure 2 : Possible transmission pathways of *M. bovis* between the environment, wildlife, livestock and humans

The figure is adapted from Biet et al. (2005)

Inhalation of the infectious bovine TB agents is the most probable and principal route among yearlings and adult cattle. The minimum infective dose required to establish infection by the respiratory tract could be up to 1000 times less than through the oral route (Cassidy 2006). In fact, Palmer and Waters (2006) estimated that doses as low as 1–5 bacilli and 10–20 million bacilli can lead to infection through aerosol and the oral routes, respectively.

The oral route is accomplished when feed or water contaminated with mucous, nasal secretion, saliva, discharging lesions, faeces and urine that contain the infective organism; and unpasteurised milk or raw meat from an infected animals are consumed by the healthy host (Moda et al. 1996; Ayele et al. 2004; Biet et al. 2005; Kaneene and Pfeiffer 2006; Palmer and Waters 2006). Also, the ingestion of *M. bovis* directly from cows to nursing calf and indirectly from contaminated pasture or farm tools to other animals (O'Reilly and Daborn 1995; Cosivi et al. 1998; Ayele et al. 2004; Good 2006; Goodchild and Clifton-Hadley 2006; Delahay et al. 2007) could be common in some regions.

Other modes of transmission though less common, include the transcutaneous mode of transmission of *M. bovis* from animals to humans who handle infected carcasses, with the spread of infection through cuts and abrasions; for example butcher's wart in humans (Grange and Yates 1994). For animals, the transcutaneous mode could primarily result through bites of infected animals such as domestic cats, ferrets and badgers (Kaneene and Pfeiffer 2006). Congenital infections, genital transmission and vertical transmission have also been noted to occur when the reproductive organs are affected (Neill et al. 1994; Cosivi et al. 1998; Philips et al. 2003; Figueiredo et al. 2008), but these modes of transmission are rarely reported in regions with strict eradication programmes (Ayele et al. 2004).

The dissemination of TB due to *M. bovis* from animals to human and *M. tuberculosis* from human to animals; and within human or animal populations involves the cyclic patterns of animal to animal, animal to human, human to animal and human to human transmissions (Cosivi et al. 1998; Ayele et al. 2004; LoBue 2006; Evans et al. 2007; Thoen and LoBue 2007; Thoen et al. 2009). The oral route (consumption of contaminated unpasteurised milk or raw meat) is the most important route for transmitting zoonotic TB from animals to humans. Transmission of TB from humans to cattle is rare and human to human spread of *M. bovis* is considered less efficient than for *M. tuberculosis* (van Soolingen 2001). Nonetheless, transmission is more among HIV/AIDS infected humans since immunosuppression increases their susceptibility to the infection (Ayele et al. 2004). In general, the incidence of *M. bovis* infection in humans (usually pulmonary TB) is higher in rural areas with infected herds and also among individuals whose occupation exposes them to infected herds such as veterinarians, abattoir and farm workers (Daborn et al. 1996; Moda et al. 1996; Ayele et al. 2004). Urban dwellers usually develop the extra-pulmonary form of TB since the gastrointestinal route is the frequent mode of transmission to them (Daborn et al. 1996; Ayele et al. 2004). Therefore, differences in the clinical presentation of human TB due to *M. bovis* are also related to the route of infection of the agent (Palmer and Waters 2006).

1.5 Manifestations of tuberculosis in cattle and human

The pathogenesis of bovine TB in cattle is not as well understood as for TB in humans. Advances and findings in the field of human TB using various small animal models of *M. tuberculosis* infection are extrapolated to better understand

bovine TB (Gupta and Katoch 2005; Cassidy 2006; de la Rua-Domenech 2006b; Palmer and Waters 2006; Pollock et al. 2006). The routes of transmission of TB agents determine the distribution pattern of lesions in the infected hosts (Biet et al. 2005; Palmer and Waters 2006). Tubercle bacilli have evolved adapted abilities to avoid immune clearance and induce chronic lesions ensuring their transmission. Lesions are therefore elicited as mechanisms to limit spread of the bacillus, thereby preventing early death of the host (Cassidy 2006; Palmer and Waters 2006). The pathogenesis of TB, host immune response to tubercle bacilli, dissemination and combination of tuberculous lesions in the initial focus of infection and regional (i.e. draining) lymph nodes have been documented (Griffin and Buchan 1994; Neill et al. 1994; Dannenberg 2001; Neill et al. 2001; Smith 2003; Gupta and Katoch 2005; Cassidy 2006; de la Rua-Domenech 2006b; Palmer and Waters 2006; Pollock et al. 2006; Thoen and Barletta 2006).

TB is characterized by progressive development of granulomatous lesions or tubercles in affected tissues / organs (Blood and Radostits 1989; McAdams et al. 1995; Cassidy 2006; Liebana et al. 2008). Tuberculous lesions have been reported to be distributed mostly in the respiratory tract and associated lymph nodes of naturally infected cattle and humans (Francis 1971; Collins and Grange 1983; Blood and Radostits 1989; McAdams et al. 1995; Cassidy et al. 1998; Cassidy 2006; Palmer and Waters 2006; Liebana et al. 2008), particularly in portions of the lungs close to the pleural surface (Cassidy 2006). Predominant findings of lesions in the retropharyngeal, submandibular and parotid lymph nodes also exist in a considerable proportion of animals, suggesting potential foci of excretion on the upper respiratory tract surface (Corner 1994; Neill et al. 1994). Palmer and Waters, (2006) also cited

evidences of lesions on the laryngeal and other upper respiratory tract surfaces in human tuberculous patients who were considered highly infectious.

An initial infection through the respiratory route may progress in some individuals to manifest as follows:

- rupture of subpleural infectious foci into the pleural space, resulting in tuberculous pleuritis
- extensive caseous pneumonia
- enlargement of the tuberculous focus into a bronchus leading to extensive endobronchial spread throughout one or both lungs
- rupture of a tuberculous focus into a pulmonary vessel with haematogenous spread leading to the acute disseminated disease (McAdams et al. 1995).

This initial infection, also termed primary TB may resolve spontaneously in most individuals (Thoen et al. 2009), the healed lesions appearing on chest radiograph as calcified parenchymal nodules (Leung et al. 1992; Thoen et al. 2009). Due to factors yet poorly understood, TB can reactivate months or years after containment, though many patients may remain entirely asymptomatic or have non-specific symptoms of chronic respiratory infection (e.g. fever, weight loss, productive cough and haemoptysis) (Thoen et al. 2009). Haematogenous spread of the tubercle bacilli to other body structures may follow the pulmonary reactivation to produce the extrapulmonary form (McAdams et al. 1995).

However, there is a wide range of clinical manifestations to both pulmonary and extrapulmonary human TB due to *M. tuberculosis* and *M. bovis* infections.

Inhaled contaminated droplets initially lodge in the respiratory tract would result in local inflammatory reaction followed by spread to regional lymph nodes in the thorax and haematogenous dissemination to the head and abdomen (Liebana et al. 2008; Thoen et al. 2009). The mediastinal, retropharyngeal and tracheobronchial lymph nodes are primary landmark for infection through this pathway (Corner 1994; Neill et al. 1994; Palmer and Waters 2006; Liebana et al. 2008). A low-grade fever and symptoms of respiratory illness may also be present. Common symptoms of human TB patients include cough, haemoptysis, dyspnoea, chest pain, night sweating, anaemia, tachycardia, lung-auscultation finding, fever, low body-mass index and low mid-upper arm circumference (WHO 2004a; Wejse et al. 2008; OIE 2009). Similar clinical manifestations occur in animal TB (Blood and Radostits 1989; OIE 2009). For example in cattle signs usually become visible at the *advanced stage* of the disease (Corner 1994; Shitaye et al. 2006) and mainly in adult or old animals (Oloya et al. 2006). Some infected livestock are apparently in healthy condition showing no evidence of infection but lesions may be found during slaughter / meat inspection (Murray et al. 1991; Shitaye et al. 2006).

Bovine TB in cattle is a chronic and *wasting* (weight loss) disease and other *non-specific* clinical signs include anorexia, drop in production (eg: drop in milk yield), chronic intermittent cough (may be productive), dyspnoea and enlarged regional lymph nodes in advanced cases which may rupture (Blood and Radostits 1989; OIE 2009).

1.6 Diagnosis of tuberculosis

a) Clinical symptoms

Human TB and bovine TB are difficult to diagnose based only on clinical manifestations. Due to similarities in clinical manifestations, contagious bovine pleuropneumonia, *Pasteurella* or *Corynebacterium pyogenes* pneumonia, traumatic pericarditis and chronic aberrant liver fluke infestation (Blood and Radostits 1989; Gracey and Collins 1992; Grist 2008) are often differentially diagnosed for bovine TB in cattle.

b) Detection of cellular immunity

Various techniques to determine cellular immunity for the diagnosis of TB have been described. Tuberculin skin tests (TST) using purified protein derivatives (PPDs) of *M. tuberculosis, M. bovis* and *M. avium* are widely used to critically detect TB infection in humans and animals. However, blood tests that also measure cellular immunity such as the gamma-interferon and lymphocyte transformation assays have been used as ancillary tests to the TSTs for improved detection of the preclinical stages of TB in live subjects (Hoge et al. 1994; Gonza´lez-Llamazares et al. 1999; Ameni et al. 2000; de Lourdes Garcia-Garcia et al. 2000; Brock et al. 2001; Ameni and Tibbo 2002; Cousins and Florisson 2005; de la Rua-Domenech et al. 2006a; Coad et al. 2008; Kim et al. 2009). The lymphocyte proliferation test is uncommonly used in cattle, but may be useful in wildlife and zoo animals (CFSPH 2009). Actually both tuberculin skin and gamma interferon (IFN-γ) tests have been used to maximise the efficacy of detecting latent TB and early stages of the disease in immunocompromised hosts (Ameni et al. 2000; Kim et al. 2009; Ameni et al. 2010a).

The gamma-interferon (IFN-γ) test, an in vitro immunoassay based on the specific release of IFN-γ as the indicator of a response to the *M. bovis* antigen, was described by Wood *et al.* to enhance sensitivity, specificity, and to reduce handling events during tuberculin skin screening (Alicia et al. 2006). IFN-γ can be detected using an enzyme immunoassay and the sensitivity of the IFN-γ test was higher compared to the TST and the specificity of the IFN-γ assay was 90.6 to 98.6% (Alicia et al. 2006; de la Rua-Domenech et al. 2006a). However, the performance and specificity of both tuberculin skin and IFN-γ tests can be affected by co-infecting agents, the most frequent being the presence of other mycobacterial infections such as paratuberculosis leading to dual infections of bovine tuberculosis and paratuberculosis (Alicia et al. 2006). False positive reactors with the TST and/or the IFN-γ tests, most probably caused by cross-reactivity with *M. avium* subsp. *paratuberculosis*, have been reported (Biet et al. 2005; Alicia et al. 2006; de la Rua-Domenech et al. 2006a). Though the TST are widely used for international field diagnosis of bovine TB in live animals (de la Rua-Domenech et al. 2006a; de la Rua-Domenech et al. 2006b), they may not be appropriate to eradicate bovine tuberculosis in herds with dual mycobacterial infections.

c) Detection of humoral immunity

Tests of humoral immunity employing the Enzyme-linked immunosorbent assays (ELISAs), immunochrommatographic (lateral flow) assay and other serologic based tests may complement tests of cellular immunity in anergic hosts. However, anti-TB antibodies titres are inconsistent and rise only in the late stages of infection while a limited cocktail of selected *Mycobacterium* antigens (e.g.: ESAT-6, MTSA-10, MPTS1, MPT63, MPB59, MPB64, MPB70, MPB83) are employed to detect circulating antibodies (Lyashchenko et al. 1998;

Pollock et al. 2001; Banerjee et al. 2003; Cousins and Florisson 2005; Lyashchenko et al. 2007; Lyashchenko et al. 2008).

Therefore the benefits of using multiple diagnostic tests to detect mycobacteria infected animals and humans cannot be overemphasized. It would be interesting to combine anti-bovine TB antibodies detection assays with tuberculin skin and or IFN-γ techniques for the accurate detection of *M. bovis* infected in animals.

d) Post mortem examinations

TB diagnosis could also be based on post mortem findings of tuberculous lesions such as abscess with yellowish pus and tubercles which may be caseous or sometimes 'gritty' calcification in carcasses and during slaughter / meat inspection in abattoirs (Grossklaus 1987; Murray et al. 1991; Gracey and Collins 1992; FAO 1994; Grist 2008). Detection of bovine TB in most African countries are based on the post mortem findings of tuberculous lesions (Asseged et al. 2004; Shitaye et al. 2006). However, not all infected animals present lesions at carcass inspection while gross visible lesions suggest that the disease is at an advanced or late stage (Corner 1994; Shitaye et al. 2006).

e) Demonstration of the tubercle bacilli

Presumptive findings based on histopathological techniques and microscopic demonstration of acid-fast bacilli have also been described (Chakravorty et al. 2005; Johnson et al. 2008). Direct smears from clinical samples (e.g. sputum) or suspected tissues typically lymph nodes are stained with the Ziehl - Neelsen stain, a fluorescent stain or immunoperoxidase techniques to demonstrate the acid-fast tubercle bacilli under the microscope (Strong and Kubica 1985; WHO

1998a). The diagnosis is confirmed following growth and isolation of the *Mycobacterium* on various selective media (e.g.: Lowentein-Jesen media, Middlebrook broth). Incubation of mycobacterial culture takes up to eight weeks for *M. tuberculosis* growth and twelve weeks for *M. bovis* (Strong and Kubica 1985; WHO 1998b; a; OIE 2009). Further characterisation of the organism can be achieved by performing various biochemical tests (Nitrate reduction, Niacin and Catalase tests), culture characteristics (morphology of colony) and direct polymerase chain reaction (PCR) based genomic deletion typing for the presence or absence of various regions of difference (Frothingham 1995; Brosch et al. 2002; Parsons et al. 2002; Smith et al. 2006a; Warren et al. 2006; Müller et al. 2009a; Ameni et al. 2010b). Detailed molecular typing or genetic fingerprinting techniques (e.g. Spoligotyping, Variable Number Tandem Repeat) may be used to further differentiate, characterise and geographically map different strains of *Mycobacterium* species (Frothingham and Meeker-O'Connell 1998; van Soolingen et al. 1998; Frothingham et al. 1999; Sritharan and Sritharan 2000; Watterson and Drobniewski 2000; Drobniewski et al. 2003; Chakravorty et al. 2005).

f) Other diagnostic techniques

An unconventional trial in Tanzania by Poling et al. (2010) found that giant African pouched rats can be trained to detect TB infected sputum samples and suggested that the rats may be useful for human TB diagnosis in developing countries. Animal (e.g. guinea pig) inoculation as a diagnostic technique for TB is rarely done (Chambers et al. 2001), but may be necessary if histopathology suggests TB and mycobacterium cultures are negative. Also, radiographic imaging has been employed for TB diagnosis in dogs and cats (CFSPH 2009)

and is regularly used in humans (Leung et al. 1992; de Vries and van Hest 2006; Thoen et al. 2009).

1.7 Control / eradication and treatment of bovine and human tuberculosis

Control and eradication programs for bovine TB, human TB and zoonotic TB of humans due to *M. bovis* are based on early accurate detection and removal of infected animals, chemotherapy of infected humans and vaccination of target populations to attenuate or prevent the manifestation of the disease (Citron 1988; Abernethy et al. 2006; Good 2006; Goodchild and Clifton-Hadley 2006; Pavlik 2006a). The test-and-slaughter policy is the basis for international bovine TB control and eradication programs using the TST to detect affected herds (and re-test) periodically and removing reacting cattle (Gilbert et al. 2005; Abernethy et al. 2006; Good 2006) that may shed the infective organism.

In many industrialised countries there is “effective” compulsory reporting of *M. bovis* infection of all animals, quarantine of infected herds, tracing and re-testing of animals in contact with bovine tuberculin skin positive reactors, movement restrictions of cattle herds *not* yet tested for TB as well as controlled animal movement out of known TB infected herds and endemic areas (Citron 1988; Gilbert et al. 2005; Abernethy et al. 2006; Good 2006; Goodchild and Clifton-Hadley 2006; Pavlik 2006a; OIE 2008; 2009). However, the test-and-segregation program, a modified form of the test-and-slaughter policy, may be more useful for developing countries, where the test-and-slaughter policy cannot be practicable for the whole cattle population (WHO 1994b). Thus, interim measures to segregate infected herds and phased slaughter of reactors

are done. In most countries with strict TB eradication programmes, the test-and-segregation strategy made up the early stages followed by the test-and-slaughter methods in the final stage (CFSPH 2009) and infected slaughter / meat cases during inspection are traced back to the originating farms (Defra 2008). Informed farm management decisions such as proper sanitation and disinfection are also important to reduce the spread of *Mycobacterium* within and between herds as well as the risks of exposure and transmission of bovine TB infection to humans (Wilsmore and Taylor 2008).

The occurrence of *M. bovis* in wildlife reservoir hosts complicates eradication efforts. Culling to reduce population density can decrease animal TB transmission but the situation must be assessed carefully to avoid unanticipated effects such as the economic benefit and increase scattering members of the infected species (Donnelly et al. 2007; Smith et al. 2007; CFSPH 2009). The development of TB vaccines for wildlife reservoirs (Hughes et al. 1996; Ayele et al. 2004) and use in situations where the test-and-slaughter policy is totally impracticable (WHO 1994b) is also being considered as an alternative. Also, human TB due to *M. bovis* is rare in countries where raw and poorly cooked meat are not consumed; and pasteurization of milk and milk products are components of bovine TB eradication programs (WHO 1994b; Ayele et al. 2004). *M. tuberculosis* infection and zoonotic TB of humans can be treated successfully with antimicrobial drugs but there is widespread drug resistance (Chalmers et al. 1996) and untreated infections are usually fatal.

1.8 Epidemiological burden of human tuberculosis

1.8.1 Global status of human tuberculosis

During the 18th century TB was the major cause of death of all infectious diseases throughout Europe and other regions of the world (Crimi et al. 2005). Improved socio-economic, surveillance, hygienic and therapeutic conditions have since led to progressive decrease in the incidence rates in rich countries (Raviglione et al. 1993; Raviglione et al. 1995; Ayele et al. 2004). However, in the mid – 1980s, an apparent increase in TB notifications was observed in the industrialized countries (Crimi et al. 2005) and a rapid widespread re-emergence in developing countries resulted into a serious emergency being declared in the sub-Sahara African region (Murray et al. 1990; WHO 1994a; Tan et al. 2003; WHO 2004b). TB incidence in Africa was rapidly increasing, contrary to overall global observations of decline due to the emergence of HIV/AIDS epidemics (O'Reilly and Daborn 1995; Cosivi et al. 1998; Fätkenheuer et al. 1999; Corbett et al. 2003; WHO 2005; Corbett et al. 2006). TB was found to be the major opportunistic infection in HIV infected persons (Raviglione et al. 1993; Fätkenheuer et al. 1999; WHO 2005).

Figures 3 and 4 present the effect of HIV infection on TB incidence and prevalence in Africa which is highly affected by HIV/AIDS against people with HIV in the world. A similar trend reported earlier in Europe and other industrialised countries was not only associated to the spread of HIV/AIDS but also to the deterioration of public health in general and of TB control in particular (Citron 1988; Dasgupta et al. 2000; WHO 2002; Crimi et al. 2005; Dale et al. 2005; Farah et al. 2005; Schneider et al. 2005; Thoen et al. 2006; Müller et al. 2008b). The 25 to 44 year age group, drug addicts in urban areas,

people engaged in unprotected sex and immigrants from countries with high incidence of TB were considered to be most at risk (Raviglione et al. 1993; Codecasa et al. 1999; Zellweger 1999; Schneider et al. 2005). World Health Organisation's (WHO) assessment of the TB epidemic status (Figures 5 and 6) revealed an estimated 9.27 million new TB cases in 2007 with over 1.37 million (14.8%) being HIV-positive (WHO 2009).

The WHO statistics were higher than the estimated 9.24 million incident TB cases and 0.7 million TB / HIV-positive cases for 2006 (WHO 2008) as well as for previous years (Dye et al. 1999; WHO 2002; Corbett et al. 2003; Dye et al. 2005; Maher 2005; WHO 2005; 2006b; Dye et al. 2009). These numbers showed that most human TB cases were in Asia (55%) and Africa (31%) with smaller proportions in the Eastern Mediterranean Region (6%), the European Region (5%) and the Region of the Americas (3%). The 2007 TB burden according to country, as for previous years, showed that many countries with the highest estimated human TB incidence rates were in Africa, a phenomenon linked to high rates of TB / HIV co-infection (Dolin et al. 1994; Dye et al. 1999; Corbett et al. 2003; WHO 2006a; 2007; 2008; 2009). Furthermore, the highest HIV-positive / TB cases peak noted in 2005 of 1.39 million (WHO 2009), as for previous years was in Africa (79%) followed by the South-East Asia Region (11%) (Dye et al. 1999; Corbett et al. 2003; Dye et al. 2005; Maher 2005; WHO 2009). Of the estimated 1.38 million deaths associated with TB in 2007, ≈ 456,000 (33%) were among HIV-positive people which was equivalent to ≈ 26% deaths in HIV-positive and HIV-negative people worldwide and ≈ 23% of the estimated 2 million HIV-related deaths (WHO 2008; 2009).

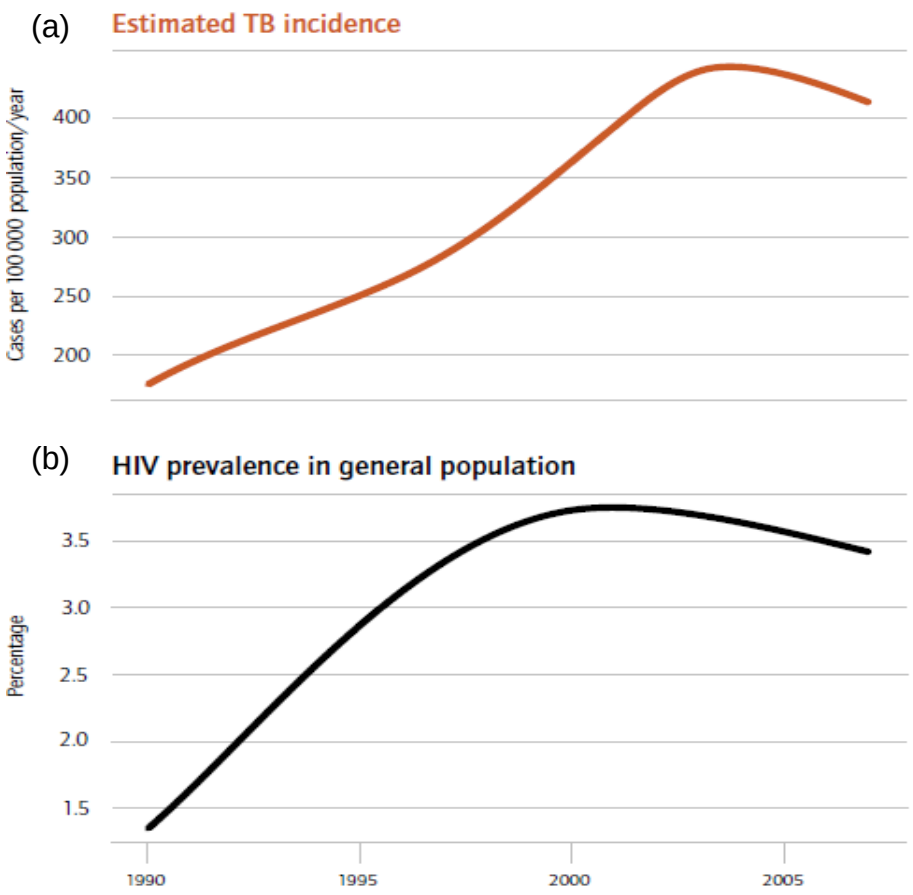


Figure 3: (a) Estimated incidence of human tuberculosis and (b) prevalence of HIV for the African sub-regions, most affected by HIV (Africa high-HIV), 1990–2007.

Source: (WHO 2009)

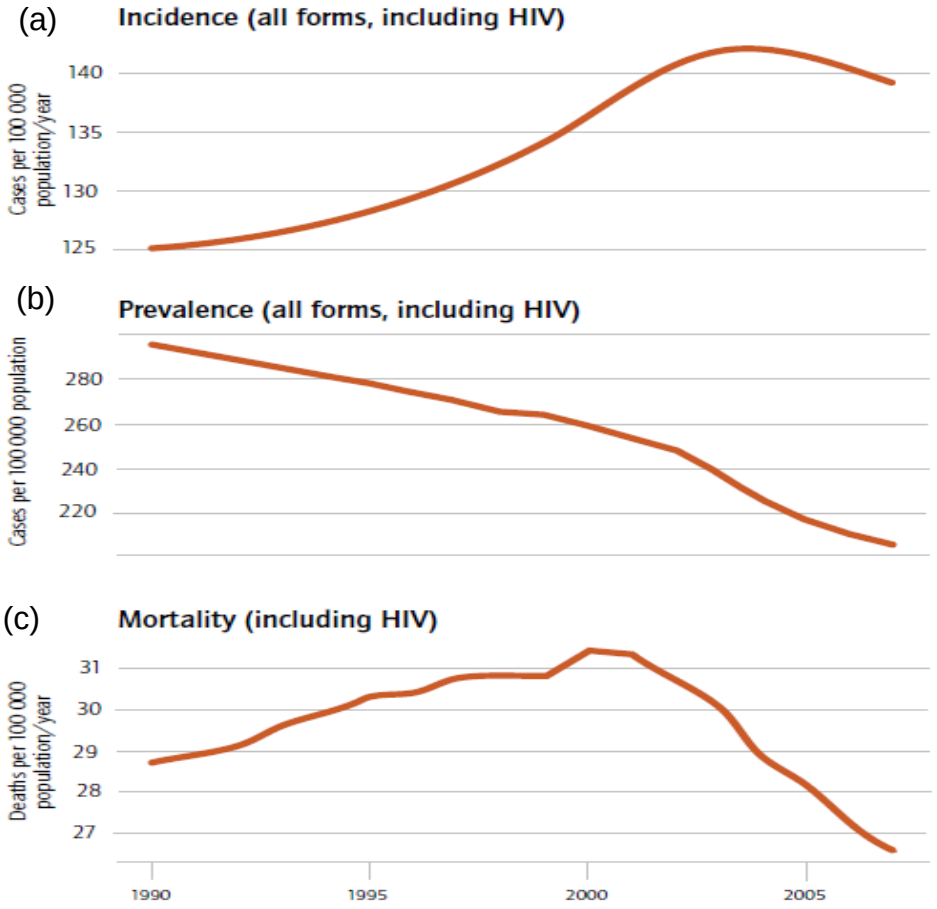


Figure 4 : Global rates of human tuberculosis (a) incidence, (b) prevalence and (c) mortality, including in people with HIV, 1990–2007.

Source: (WHO 2009)

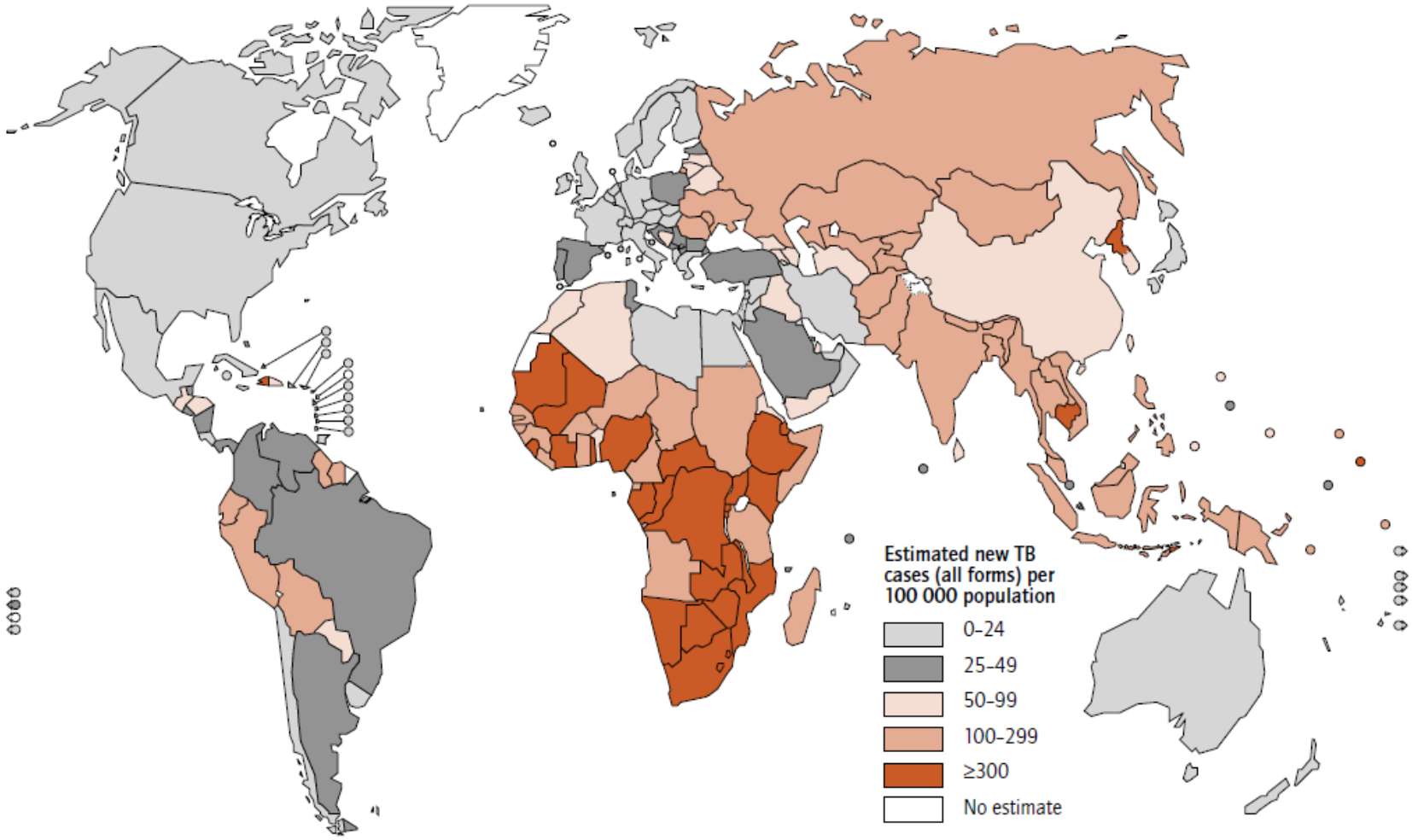


Figure 5: Estimated human tuberculosis incidence rates, by country, 2007.

Source: (WHO 2009)

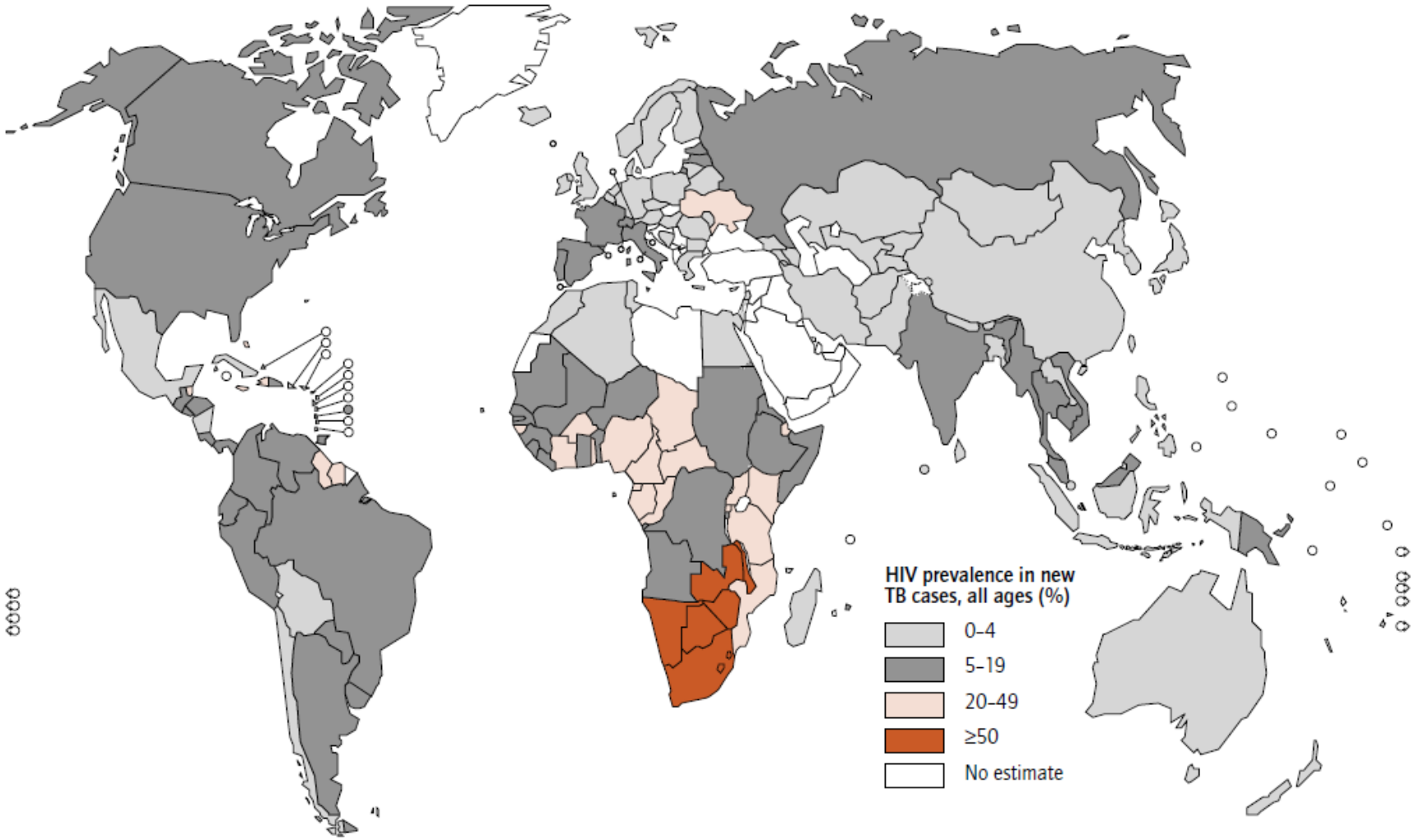


Figure 6: Estimated HIV prevalence in new human tuberculosis cases, by country, 2007

Source: (WHO 2009)

The estimated global incidence and prevalence of TB cases and burden of TB / HIV co-infection rates are presented in Tables 3 and 4, respectively. The increasing trend of TB cases and TB / HIV co-infection in developing countries reported in recent WHO reports (2000 to 2007) are similar to earlier prediction estimates of TB incidences and mortality during 1990 – 2000 by Dolin et al. (1994), which they considered conservative because of the under-reporting of TB cases.

1.8.2 Global trends in the incidence of human tuberculosis

Human TB is not uniformly distributed in the global populations. While it is very low in some industrialised countries (< 5% of the population), about 80% of all cases occur in about 22 poor countries (WHO 2009). According to WHO's 2009 report on global TB control and supported by other findings (Dye et al. 1999; Corbett et al. 2003; Dye et al. 2005; Maher 2005; Dye et al. 2009; WHO 2009) the global incidence of human TB per capita peaked around 2004 and is in decline (Figure 3). The peak and subsequent decline followed a similar pattern to the trend in HIV prevalence in the general population. This explained the increase in number of incident TB cases in absolute terms (Tables 3 and 4), while the incidence rates per capita dropped with population growth. In the African, Eastern Mediterranean, European and South-East Asia regions, the decline in TB incidence per capita was more than compensated by increases in population size. From the start of reliable data recording by WHO member countries in 1995, the trends in TB incidence rates have been observed (Table 3) to vary with regions (WHO 2009).

Table 3: Estimated incidence and prevalence rates of human tuberculosis in WHO regions (per 100 000 population), 1990 – 2007

WHO Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007
Incidence[a]																		
African	168	183	194	206	218	230	242	257	275	296	319	343	364	379	383	379	371	363
The Americas	57	55	54	52	50	48	46	45	43	42	40	39	38	37	35	34	33	32
Eastern Mediterranean	110	109	110	109	109	109	109	108	108	107	107	106	106	106	105	105	105	105
European	37	36	37	38	40	42	45	47	47	50	51	51	50	49	49	49	49	49
South-East Asia	202	201	199	198	196	195	194	192	192	190	188	187	187	185	184	183	182	181
Western Pacific	129	127	126	124	123	121	120	119	119	116	115	114	113	112	111	110	109	108
Global	125	125	126	126	127	128	129	131	131	134	136	138	141	142	142	141	140	139
Prevalence[a]*																		
African	324							384			436	461	480	497	501	500	487	475
The Americas	82							72			51	50	48	46	43	41	38	38
Eastern Mediterranean	227							258			203	200	187	181	172	159	150	139
European	52							73			68	67	63	62	60	55	52	51
South-East Asia	554							524			417	390	370	237	309	296	286	280
Western Pacific	320							230			260	255	250	235	218	207	201	197
Global	296							277			259	254	248	237	225	217	210	206

a : Incidence and prevalence estimates include TB in people with HIV.
Adapted mainly from WHO (2009). Except stated otherwise the source is : (WHO 2009);
* Prevalence estimates for 212 countries in-1997: (Dye et al. 1999)

Table 4 : Estimated epidemiological burden of tuberculosis / HIV co-infection in WHO regions, 1990 – 2007

WHO Region	1997*	2000	2001	2002	2003	2004	2005	2006	2007
Incidence rates of TB / HIV- positive -Number (per 100,000 population)									
African	515	123	133	142	148	149	145	141	136
The Americas	25	4	4	4	4	4	4	4	4
Eastern Mediterranean	16	3	3	3	3	3	3	4	4
European	10	2	2	3	4	4	4	5	5
South-East Asia	64	9	9	9	9	9	9	9	8
Western Pacific	9	2	2	2	3	3	3	3	3
Global	640	17	19	20	21	21	21	21	21
Prevalence rates of TB / HIV- Positive -Number (per 100,000 population)[#]									
African	7302				28	42	34	39	68
The Americas	510				5.2	1	≤ 1	1.2	2
Eastern Mediterranean	107				2.1	≤ 1	≤ 1	≤ 1	2
European	84				3.9	≤ 1	≤ 1	≤ 1	2
South-East Asia	2364				2.9	2	2	1	4
Western Pacific	307				1.2	≤ 1	≤ 1	≤ 1	1
Global	10675				11	6	5	5	10
Prevalence of HIV in incident TB cases of all ages[b#] (%)									
African	34				33	33	28	22	38
The Americas	16				5.8	10	7.9	6.4	11
Eastern Mediterranean	23				2.3	2.4	2.1	1.1	3.5
European	14				4.3	4.7	4.6	3.0	9.8
South-East Asia	24				3.6	3.9	3.9	1.3	4.6
Western Pacific	18				1.3	1.4	1.0	1.2	2.7
Global	23				12	13	11	7.7	15

b : Prevalence of HIV in incident TB cases of all ages;
Adapted mainly from WHO (2009). Except stated otherwise the source is (WHO 2009);
* Incidence and prevalence estimates of TB / HIV positive in 1000 for 212 countries in 1997: (Dye et al. 1999);
# for 2003: (Maher 2005); # for 2004, 2005 and 2006: (WHO 2006a; 2007; 2008) respectively.

Comparing the average rate of change of TB incidence and prevalence between the periods 1995 and 2007 confirmed that the trend was fastest in African countries and in the Eastern European sub-region with high HIV/AIDS prevalence but slower in the other regions (Corbett et al. 2003; Corbett et al. 2006; Corbett et al. 2007; Dye et al. 2009; WHO 2009). Overall as shown in Table 3, while the TB case rates dropped slowly or approximately stabilised in almost all the WHO epidemiological sub-regions, it increased in African countries including those with low HIV/AIDS prevalence rates (WHO 2009).

1.8.3 Current status of human tuberculosis in Cameroon

TB remains a common disease in Cameroon, with a current annual incidence of almost 200 cases per 100,000 populations (WHO 2009). Its control is hampered by unfavourable socio-economic conditions, the interaction with the HIV epidemic and widespread anti-TB drug resistance (Kuaban et al. 2000a). Table 5 presents the inextricable link that exist between the re-emerging TB and the emerging HIV/AIDS epidemics in Cameroon (WHO 2004b; 2006a; 2007; 2008; 2009), with the HIV seroprevalence in TB patients serving as a ‘sentinel’ for HIV seroprevalence in the general population (Noeske et al. 2004).

In fact the prevalence of human TB in Cameroon is known to be high and increasing, especially among the economically active 21 to 40 years age group and in immunosuppressed hosts as well as hastening death of HIV/AIDS patients (Noeske et al. 2004; Ane-Anyangwe et al. 2006; WHO 2008). In 2009, 40% of TB patients in the country were also HIV-positive (WHO 2011). However, a 12 year study in Cameroon (Table 6) found HIV prevalence in both the general adult population and adults with TB to increase steadily. A strong

positive linear relationship noted in the TB / HIV co-infection has been reported (Noeske et al. 2004; WHO 2004b) with 29% to 32% of TB cases in the general adult population at any point in time being HIV seropositive (Noeske et al. 2004). Thus, adult TB patients appeared to constitute a proxy for the increase in HIV seroprevalence rates in the general Cameroonian adult population. Recent studies in the general population have reported even higher prevalence rates of HIV/AIDS among incident TB cases of all ages ranging between 31% and 43% (WHO 2006a; 2007; 2008; 2009). Due to the high magnitudes and ever increasing trend of both infections in Cameroon, Noeske et al. (2004) have speculated that even after the HIV incidence in the general population would have reached a peak and starts declining, TB / HIV co-infection epidemics will probably still continue to grow since TB disease can occur at different moments in the course of HIV infection.

1.9 Epidemiology of bovine tuberculosis

1.9.1 Burden of bovine tuberculosis in the World

Bovine TB negatively affects animal welfare and productivity worldwide with significant economic losses in some countries (Pollock and Neill 2002). Infected cattle are important sources of infection for healthy cattle, but wildlife reservoirs of infection have also been reported in many regions (Wedlock et al. 2002; Philips et al. 2003; Kaneene and Pfeiffer 2006; Thoen et al. 2009), thereby complicating the epidemiological picture. Bovine TB is an FAO – OIE "List B" disease because of its important socio-economic and public health impact (Benkirane 1998; OIE 2008).

Table 5 : Estimated epidemiological burden of TB in Cameroon, 2000 – 2007

Parameter	2000	2001	2002	2003	2004	2005	2006	2007
Incidence[a] rates of TB (per 100,000 population)	168	181	194	202	204	202	197	192
Prevalence[a] rates of TB (per 100,000 population)	228	241	240	227	228	2103	201	195
Incidence rates of TB / HIV- positive -Number (per 100,000 population)	77	83	88	91	91	89	86	83
Prevalence rates of TB / HIV- Positive -Number (per 100,000 population)				20	20	16	15	41
Prevalence of HIV in incident TB cases of all ages[b] (%)				-	31	26	15	43

a : Incidence and prevalence estimates include TB in people with HIV.

b : Prevalence of HIV in incident TB cases of all ages;

Adapted from WHO (2005; 2006a; 2007; 2008; 2009).

Table 6 : HIV seroprevalence rates in the general adult population and in adult smear-positive pulmonary tuberculosis patients in Cameroon, 1989 – 2000

Year	HIV seroprevalence rate in genaral population (%)	HIV prevalence rate among adult pulmonary TB patients			References
		Rate (95%CI)	Number of patients included	Place of study	
1989	0.9	2.9 (± 2.8)	137	Hôpital Jamot	Ndumbe et al. (1991); Trébucq et al. (1990)
1990	1.3	4.1 (± 1.6)	± 600	Hôpital Jamot	Garcia-Calleja et al. (1992a; 1992b; 1993b)
1991	2.2	9.9 (± 4.6)	162	Hôpital Jamot	Garcia-Calleja et al. (1992a; 1993a; 1993b); WHO (1993); Kuaban et al. (1992b)
1992	2.9	11.4 (± 4.8)	166	Hôpital Jamot	Ndumbe et al. (1993); UN-HIV/AIDS/WHO. (2002); Kuaban et al. (1992a)
1993	3.4				UN-HIV/AIDS/WHO. (2002)
1994	4.0	16.6 (± 4.8)	235	Hôpital Jamot	Mbopi Keou et al. (1998); Kuaban et al. (1997);
1995	4.8	16.8 (± 2.8)	671	Hôpital Jamot	MoPH. (1996); Kuaban & Bercion (1996);
1996	5.25				MoPH. (1997)
1997	5.5	21.6 (± 7.7)	111	Hôpital Jamot	MoPH. (1998a; b) ; Kuaban et al. (2000a);
1998	6.5	22.2 (± 3.3)	600	West province	MoPH. (1999); Kuaban et al. (2000b);
1999	7.73				UN-HIV/AIDS/WHO. (2002); MoPH. (2001);
2000	10.8	29.0 (± 4.5)	396	West province	MoPH. (2001); Noeske et al. (2001).

Hôpital Jamot: the main TB hospital in the capital, Yaounde;
HIV: Human Immunodeficency virus;
WHO: World Health Organization; UN-HIV/AIDS/WHO: United Nations Programme on HIV/AIDS/World Health Organization
MoPH: Cameroon Ministry of Public Health.

This table is based on Noeske et al. (2004) with further additions.

Bovine TB is also a serious zoonosis transmitted to humans through the consumption of contaminated raw or poorly cooked animal products (e.g.: fresh milk and meat), inhalation of aerosols from infected animals and contamination of breaks in the skin (Blood and Radostits 1989; Ayele et al. 2004; Kaneene and Pfeiffer 2006; Wilsmore and Taylor 2008; CFSPH 2009).

Strict control / eradication programmes have eliminated or nearly eliminated bovine TB from domesticated animals in many industrialised countries (Citron 1988; Gilbert et al. 2005; Abernethy et al. 2006; Good 2006; Goodchild and Clifton-Hadley 2006; Pavlik 2006a; CFSPH 2009). However, endemic areas and infected herds still continue to be reported in some industrialised countries (Good 2006; Goodchild and Clifton-Hadley 2006) because of the presence of bovine TB in wildlife species (e.g.: wild white-tailed deer in the United States of America, badgers in the U.K. and Ireland, and brush-tailed opossums in New Zealand) which share the same environment as cattle (O'Reilly and Daborn 1995; Bruning-Fann et al. 2001; Aranaz et al. 2004; Good 2006; Mathews et al. 2006; Delahay et al. 2007). Increasing rate or persistence of bovine TB has also been linked to husbandry trends of increasing herd sizes and changes in cattle genotypes (Goodchild and Clifton-Hadley 2006).

For example, the trend in cattle population and bovine TB incidence based on TST statistics since 1960 in Ireland showed considerable reduction (from 2.99% to 0.33% annually) rather than eradication of bovine TB with herd infection rates stabilizing at between 5.6% and 8.2% (Good 2006). Although a small proportion (about 7.4%) of herds in Great Britain were affected by bovine TB in 2007 (EFSA 2007), the number of confirmed herd breakdowns has been increasing since 1990 at an average rate of 18% per annum (Defra 2005; EFSA 2007).

The percentages were much higher in “hot spots”, areas with long established bovine TB prevalence in cattle and with a high risk of local transmission, and accounted for between 5% and 20% of the current overall incidence of bovine TB in the country (Defra 2005; Goodchild and Clifton-Hadley 2006). The existence of “hot spots” represents isolated clusters with expanding fronts of infection but preventing new hot spot areas from developing would be through tracing infected cases, pre-movement testing of cattle herds, survey of wildlife reservoirs (Goodchild and Clifton-Hadley 2006) and removal of all test positive animals.

1.9.2 Burden of bovine tuberculosis in Africa

Bovine TB is widespread in Africa, parts of Asia and some Middle Eastern countries (Cosivi et al. 1998; Ayele et al. 2004). The disease represents a potential health hazard to both animal and human populations in Africa; and as in most developing countries remain largely under-estimated and under-investigated. Reported data are largely insufficient to determine the true regional epidemiological information of the disease (Ayele et al. 2004; Zinsstag et al. 2006). However, there are sufficient evidences to indicate that bovine TB is present in almost all African countries (WHO 1994b; Daborn et al. 1996; Benkirane 1998; Cosivi et al. 1998; Ayele et al. 2004; Zinsstag et al. 2006) and often at high prevalence rates in domestic and wild animals.

The relatively little attention paid to *M. bovis* and failure to control and eradicate bovine TB in Africa are due to many factors such as economic constraints, lack of adequate legislation or failure to implement that which does exist (AU/IBAR 2006; Biffa et al. 2009), limited diagnostic capacity and high costs of a

sustainable testing programme (Daborn et al. 1996; Ayele et al. 2004; Zinsstag et al. 2006; Anaelom et al. 2010). These factors are mainly politico-economical in nature but could be further extended to include problems of social unrest due to political instability and ethnic conflicts resulting in mass displacement of human and animal populations (AU/IBAR 2006; Ameni et al. 2010b). Other limiting factors include lack of veterinary expertise, inadequate diagnostic facilities, poor communication networks; insufficient collaboration between neighbouring countries, lack of quarantine facilities and policies, free movement and smuggling of live animals across state boundaries (Daborn et al. 1996; Ayele et al. 2004; AU/IBAR 2006; Ameni et al. 2010b). Furthermore, various national animal disease control preferences are often absorbed by actions against the higher incidence of other acute and fatal animal diseases such as Contagious Bovine Pleuropneumonia, Foot and Mouth disease, various arthropod – borne diseases and other parasitic diseases (Ayele et al. 2004).

The detection of bovine TB in Africa is carried out usually on the basis post mortem examination during abattoir meat inspection, TST and rarely on bacteriological techniques (Ameni et al. 2000; Asseged et al. 2004; Shitaye et al. 2006; Shitaye et al. 2007), though the consistency of using a particular test varies among countries. For example, the TST is common in Ethiopia for detecting bovine TB (Shitaye et al. 2007) while in Cameroon and Nigeria, identification of TB lesions at meat inspection in abattoirs is used to detect the disease (Doufissa 1993; Awah-Ndukum et al. 2005; Cadmus and Adesokan 2009). An overview on the reported occurrences of *M. bovis* in animals in some African countries from 1992 to 2001 are summarised in Table 7.

Table 7 : Bovine tuberculosis in cattle in 43 African countries, 1992 – 2001*

Country	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
Algeria	+	+	+	+	+	+	+	+	+	+
Angola	+	+	+	+	+	+	+	+	+	NR
Botswana	+	NR	000	1993	NR	NR	NR	NR	NR	NR
Burkina Faso	+	+	NR	+	+	+	+	+	+	+
Cameroon	**+**	**+**	**NR**	**NR**	**?**	**+**	**+**	**+**	**+**	**+**
Cape Verde	NR	+	NR	NR	?	?	NR	1987	NR	NR
Central African Republic	?	?	+	+	+	NR	NR	NR	NR	NR
Chad	+	+	+	+	-	+	+	NR	NR	NR
Comoros	?	?	NR	NR	NR	NR	000	NR	NR	NR
Ivory Coast	+	+	+	+	+	+	+	+	+	+
Democratic Republic of Congo	NR	NR	NR	NR	NR	+	NR	NR	NR	NR
Egypt	+	+	+	+	+	+	+	+	+	+
Eritrea	+	+	+	NR	+	+	+	+	+	+
Ethiopia	+	+	+	+	+	-	-	NR	NR	+
Gabon	NR	NR	NR	+	NR	NR	NR	NR	NR	NR
Gambia	NR	NR	NR	NR	-	NR	NR	NR	NR	NR
Ghana	+	+	+	+	+?	+	+	NR	+	+
Guinea	-	NR	-	-	-	NR	NR	NR	NR	NR
Kenya	000	NR	NR	NR	+	+	+	+	+	+
Lesotho	-	NR	NR	NR	NR	NR	NR	NR	+	NR
Libya	+	?	+	NR	NR	+	+	+	+	+?
Madagascar	+	+	+	+	+	+	+	+	NR	NR
Malawi	+	+	+	NR	NR	+	+	+	+	NR
Mali	+	NR	NR	NR	+	+	+	+	+	NR
Mauritius	+	+	NR	NR	+	NR	NR	NR	NR	NR
Morocco	+	+	+	-	+	+	+	+	+	NR
Mozambique	+	+	+	NR	1985	+	+	+	NR	NR
Namibia	1984	1984	+	+	+	1995	1995	1995	1995	1995
Niger	+	+	+	+	NR	+	+	NR	NR	+
Nigeria	+	+	+	NR	+	NR	NR	NR	NR	+
Reunion (FR)	+	NR	NR	NR	+	+	+	+	+	+
Sao Tome & Principe	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
Senegal	+	NR	NR	NR	-	-	-	NR	NR	NR
Seychelles	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
South Africa	+	+	+	+	+	+	+	+	+	+
Sudan	+	NR	NR	NR	1992	1992	1992	1992	1992	1992
Swaziland	NR	+	NR	+	+?	+	+	NR	+	+
Tanzania	+	NR	NR	+	NR	+	+	+	+?	+
Togo	+	NR	NR	+	NR	+	NR	NR	+	+
Tunisia	+	+	+	+	+	+	+	+	+	+
Uganda	+	+	+	+	+	+	+	+	+	+
Zambia	+	NR	-	+	-	+	+	NR	+	NR
Zimbabwe	NR	NR	1990	1990	1990	1990	1990	1990	1990	1996

*: data were extracted from the following sources: 1) FAO/OIE/WHO Animal Health Yearbooks, Rome, Italy; 2) OIE, 1992 – 1997, and OIE World Animal Health in 1997, Parts 1 and 2, Paris; 3) OIE, 1998 – 2001. NR: disease not reported; 000: never reported; 1987: year of last occurrence; ? : suspected but not confirmed; +: disease reported; - : no information available; +? : Serological evidence or isolation of causative agent, no clinical disease.

Source : (Ayele et al. 2004; Zinsstag et al. 2006)

However, recent studies have further recorded various bovine TB prevalence rates in Ghana, Mali, Nigeria, Cameroon and Chad of West and Central Africa (Du-Sai and Abdullahi 1994; Ankugah 2002; Awah-Ndukum et al. 2005; Cadmus et al. 2006; Diguimbaye-Djaibé et al. 2006; Zinsstag et al. 2006; Müller et al. 2008a; Okaiyeto et al. 2008; Ngandolo et al. 2009) and in Eritrea, Ethiopia, Uganda, Tanzania, Zambia and South Africa of Eastern and Southern Africa (Cook et al. 1996; Weinhäupl et al. 2000; Omer et al. 2001; Ameni et al. 2003; Ameni and Wudie 2003; Teklu et al. 2004; Bernard et al. 2005; Kazwala et al. 2006; Oloya et al. 2006; Swai et al. 2006; Shitaye et al. 2007; Inangolet et al. 2008; Regassa et al. 2008; Durnez et al. 2009; Fetene and Kebede 2009; Munyeme et al. 2009). These reports confirmed that bovine TB is prevalent in the 4 geo-political regions of sub-Saharan Africa, but definitive regional prevalence status cannot be estimated due to inadequate data from several key countries (e.g.: Cameroon in Central Africa).

1.9.3 Current status of bovine tuberculosis in Cameroon

Information on bovine TB in Cameroon is sparse. Available data extracted from FAO-OIE-WHO Animal Health Yearbooks (1992–1997) and OIE World Animal Health in 1997 and OIE, 1998–2001 presents the occurrence of bovine TB in Cameroon as low and sporadic or not reported; and no information with respect to other animals are available in these reports. However, preliminary studies for the detection of TB lesions in slaughtered cattle in parts of the country recorded rates ranging 0.67% – 4.28% and also observed that TB lesions were 3 – 5 times more prevalent than other pathologies at slaughter / meat inspections (Awah-Ndukum et al. 2005; Fon-Tebug 2009).

Some surveys based on single intradermal tuberculin test (SIT) in Northwest Cameroon detected bovine TB in 26% of 166 dairy cows controlled by a "parastatal" (Muchaal 2002) and 3% – 13% depending on the type of husbandry for 2,492 cattle (Merlin and Tsangueu 1985). Similarly, Tanya et al (1985) noted 1.4% of zebus and 2.8% of dairy (Holsteins & their crosses) cows were infected with bovine TB in an experimental livestock station (n=1,395) in the Adamawa region. Nfi and Ndi (1997) recorded an overall 14.8% (42% of zebus & 9.02% of cross breeds) bovine TB detection based on the single intradermal comparative cervical tuberculin test (SICCT) in another experimental station (n=142) in the West region. While, bovine TB prevalence of 10.6% using SIT and 2.7% using SICCT were recorded (n=890) in the Northern regions (Martrenchar et al. 1993). However, preliminary immuno-chromatographic assay (rapid anti-TB-antibodies detection) revealed that 60% of 90 zebu cattle tested were positive reactors (Fon-Tebug 2009). Although the above studies were limited by diagnostic techniques, area coverage and sample sizes, the findings actually confirmed that bovine TB is endemic in cattle in Cameroon and recommended that systematic studies of the prevalence rates, validation of diagnostic tests in the Cameroon conditions, evaluation of existing control measures and zoonotic implication of bovine TB in Cameroon should be undertaken.

1.10 Status of human tuberculosis due to *M. bovis*

1.10.1 Burden and risk of Zoonotic tuberculosis due to *M. bovis*

The *Mycobacterium tuberculosis* complex has been described to originate from a common ancestral progenitor that underwent deletions of various genomic regions of difference (RD) (e.g. loss of RD^{can}, RD9, RD4, RD1, …) (Brosch et al.

2002; Smith et al. 2006a; Müller et al. 2009a; Thoen et al. 2009). These bacteria particularly *M. bovis* have since spread to all groups in the human and animal populations; and constitute major threats to human health as well as animal health and production (Tan et al. 2003; Ayele et al. 2004; Une and Mori 2007; Thoen et al. 2009). Although TB is a leading cause of human deaths due to a single infectious agent in the world today (O'Reilly and Daborn 1995; Larson 2000a; Tan et al. 2003; Thoen et al. 2009), the extent of human TB due to *M. bovis* is not known but *M. bovis* seems to account for only a small percentage of the cases of TB reported in humans. TB is of great economic importance in the animal industries (wild and domestic) worldwide, especially in countries where little information is available on the incidence of *M. bovis* infection in humans (Cosivi et al. 1998; Enarson 2006; Pavlik 2006a; Pavlik 2006b; Thoen et al. 2006; Zinsstag et al. 2006; Defra 2008). However, *M. bovis* has been estimated to account for less than 0.5% – 7.2% of human TB in most industrialised countries and 10% – 15% in most developing countries (Cosivi et al. 1998; Ashford et al. 2001; de la Rua-Domenech 2006b; a). Also, approximately 85% of the cattle and 82% of the human populations of Africa live in areas where animal TB is either partly controlled or not controlled at all (Ayele et al. 2004; Shitaye et al. 2006). An overview of reports of human TB due to *M. bovis* in some regions of the world from 1966 – 2005 are shown in Table 8.

Table 8 : Human tuberculosis due to *Mycobacterium bovis* in industrialized countries distributed by decade, regions and countries of origin

Data reported for some industrialized countries*						Some epidemiological case reports and reviews distributed by decade and region or countries of origin#				
Country	**Reference**	**Years**	**No**	**% of *M. bovis* of total TB**	**Pulmonary (% of total *M. bovis*)**	**Regions / Countries**	**1966 – 1975**	**1976 – 1985**	**1986 – 1995**	**1995 – 2005**
Australia	Cousins and Williams (1995)	1970 – 94	240	0.43 – 3.1	71.6[a]	Western Europe (1)	37	10	31	36
England	Grange and Yates (1994)	1977 – 90	232	1.2	40	Eastern Europe (2)	13	9	4	0
Germany	Krebs and Kappler (1982)	1975 – 80	236	4.5	73.7	United States / Canada	7	7	16	10
Ireland										
Rural	Cormican and Flynn (1992)	1986 – 90	17	6.4	70.6	Latin America (3)	3	1	5	3
Urban	Collins et al. (1987)	1982 – 85	9	0.9	88.8					
New Zealand	Brett and Humble (1991)	1983 – 90	22	7.2	31.8	Australia / New Zealand	0	1	6	2
Spain	Sauret et al. (1992)	1986 – 90	10	0.9	50	Africa (4)	5	1	2	11
Sweden	WHO (1994)	1983 – 92	96	2	-	India, Israel, Taiwan, Turkey	0	0	3	3
Switzerland	Anonymous (1994)	1994	18	2.6	-	WHO/OIE/FAO/IUATLD	2	0	3	3
U.S	Karlson and Carr (1970)	1954 – 68	6	0.3	33.3	Total	67	29	70	68
	Dankner et al. (1993)	1980 – 91	73	3	52[b]; 12[c]					

a : Overall percentage includes 80.6 % males and 51.2% females; b : Adults. c : Children

(1) France, Germany, Netherlands, Italy, United Kingdom, Denmark, Sweden, Switzerland, Spain, Ireland, Norwegian and Finland.

(2) Czech Republic, Russia, Slovakia, Romania, Poland, Hungary and Croatia.

(3) Argentina, Brazil and Mexico.

(4) Algeria, Congo, Burundi, Tanzania, Ghana, Nigeria, Uganda, South Africa and Madagascar. Source: A computer literature search (INTERNET, MEDLINE/Pubmed.com, April 6, 2005).

The table is based on * Cosivi et al. (1998) and # Thoen et al. (2006).

Reliable information is not generally available on the incidence of human TB due to *M. bovis* in developing countries since poor attention is given to the problem due to limited diagnostic facilities. Indeed, zoonotic TB is neglected in most African countries and techniques for differentiating between organisms are not widely accessible (Cosivi et al. 1998; Zinsstag et al. 2006; Thoen and LoBue 2007; Marcotty et al. 2009). In countries where bovine TB is endemic and not controlled or partially controlled, human TB due to *M. bovis* may occur resulting from ingesting contaminated milk and milk products, other fresh animal products (ex: contaminated raw beef) and by inhaling cough spray from infected cattle (Collins and Grange 1987; Moda et al. 1996; Cosivi et al. 1998; Etter et al. 2006; Thoen et al. 2006; Shitaye et al. 2007). Also, human infection by *M. bovis* is clinically indistinguishable from that caused by *M. tuberculosis* (Cosivi et al. 1998; de la Rua-Domenech 2006b; Thoen et al. 2009) and can lead to pulmonary and extrapulmonary TB.

Cervical lymphadenopathy, intestinal lesions, chronic skin TB (lupus vulgaris) and other extrapulmonary forms are common in human *M. bovis* infection (Cosivi et al. 1998; Kazwala et al. 2001a). Furthermore, quantification of human losses due to TB cannot be determined easily since lives are involved but losses in animals can be estimated on the basis of condemnation of carcasses during meat inspection, payment of compensations to the owners, shorter lifetime or death of animals, payment for veterinary services, sterility, reduced beef and milk production and restrictions in trade of live cattle and fresh cattle products (e.g.: milk, beef).

Progress in the global control of human TB has been challenged by several factors in developing countries and also within industrialised countries where the disease occurs (WHO 2008; Thoen et al. 2009; WHO 2009). These factors

include population growth, close association of TB with poverty, co-infection of TB and HIV/AIDS epidemic, deficiencies in control measures and migratory movements (Corbett et al. 2003; Corbett et al. 2006; Thoen et al. 2006; WHO 2008; Thoen et al. 2009; WHO 2009). However, other situations also become significant when the human TB is due to *M. bovis* and bovine TB is endemic in the region. Zoonotic TB can occur as a result of occupational or accidental hazard among cattle farmers, handlers of fresh cattle products, veterinarians and migrants from countries where bovine TB is endemic (O'Reilly and Daborn 1995; Ameni et al. 2003; Ayele et al. 2004; Etter et al. 2006; Shitaye et al. 2007; Regassa et al. 2008). *M. bovis* infected cattle professionals may also be source of infection to cattle (Cosivi et al. 1998; Ayele et al. 2004) as well as other persons they come in contact with (Ocepek et al. 2005; Evans et al. 2007; Thoen et al. 2009; Etchechoury et al. 2010).

Few studies have reported the isolation of *M. bovis* from humans suffering from pulmonary TB in parts of Africa such as Cameroon, Egypt, Nigeria, Democratic Republic of Congo and Tanzania (Cosivi et al. 1998; Kazwala et al. 2001a; Niobe-Eyangoh et al. 2003; Zinsstag et al. 2006). Also, an epidemiologic association between tuberculin skin positive cattle and human TB has been observed in Zambia (Cook et al. 1996; Regassa et al. 2008). These findings suggest zoonotic bovine TB transmission to humans is supported by transient influences on the sensitivity to TST of cattle exposed to human TB cases and coincidental environmental mycobacteria (Cosivi et al. 1998). The threats to public health of human TB due to *M. bovis* in Africa therefore require urgent collaborations of veterinary and medical professionals, biomedical and ecological (environmental) experts, public health services, social workers and policy makers.

1.10.2 Current status of human tuberculosis due to *M. bovis* in Cameroon

There is a dearth of information on the epidemiology of different MTC strains in Cameroon. However, molecular characterization of mycobacterium confirmed *M. bovis* in one human case (Niobe-Eyangoh et al. 2003). Zoonotic TB due to *M. bovis* may therefore be a real human health problem with severe public health implications which is under estimated and not investigated in the country.

Bovine TB is endemic but poorly controlled in cattle in Cameroon (Doufissa 1993; Awah-Ndukum et al. 2005) and cattle business is important in the livelihood of livestock rearing communities. Opportunities exist in these communities for the transmission of *M. bovis* to humans due to very close human-livestock contacts and through the consumption habits of unpasteurised milk and raw meat. Surveillance of bovine TB in the country is mainly through the detection of tuberculous lesions during slaughter / meat inspection (Doufissa 1993; Awah-Ndukum et al. 2005). Bovine TB could be widespread in cattle destined for human consumption. Cattle rearing communities are most exposed and at risk of developing zoonotic bovine TB and could serve as source of the disease with serious implications to the general public health. The relative importance of *M. bovis* in the human TB problem in Cameroon cannot be overemphasised. The prevalence of HIV infection is high and bovine TB exists in livestock but is poorly controlled and many opportunities exist for the zoonotic spread.

Chapter 2

Rationale and research frame work

2.1 Rationale of the study

There is increasing contact between humans and animals worldwide due to increasing population growth and density, especially in poor developing countries where livestock offers important socio-economic, cultural and religious pathways out of poverty (Perry et al. 2002; OIE 2005). Animal keeping therefore plays a vital role in their livelihood and provides them with many products: food protein, work power, animal wealth ("living bank"), cash income and precious natural fertilizer (Perry et al. 2002). Also, animal and human diseases contribute greatly to poverty in developing countries due to the ineffectiveness, costliness and inappropriateness of control programmes (Perry et al. 2002). Many of these diseases are zoonotic, including TB and are on the increase (WHO 2002; Kiboss and Kibitok 2003; Tan et al. 2003; Anaelom et al. 2010). Poverty therefore is not only a predisposing factor for these conditions, but also a consequence of them.

TB is strictly controlled in most industrialised countries but population growth, widespread poverty, migratory movements, deficiencies in control measures and the HIV/AIDS epidemic continue to boost TB numbers and its devastating effects in most of Africa including Cameroon (Cosivi et al. 1998; Perry et al. 2002; Corner 2006; Zinsstag et al. 2006; WHO 2008). Bovine TB is prevalent in

cattle in many African countries (Cosivi et al. 1998; Ayele et al. 2004; Zinsstag et al. 2006; McCrindle and Michel 2007). However, the exact prevalence of bovine TB, different mycobacterial strains, reservoir hosts, public health implications and extent of human TB due to *M. bovis* are unknown on the continent.

The test-and-slaughter policy has proved to be effective in many bovine TB eradication programmes worldwide and is likely to remain the basis of national and international elimination of bovine TB. However, its application is not yet practicable in many developing countries because of logistical, political and financial constraints. Evaluation of alternative strategies that are technically and economically appropriate as well as preventing transmission of the infection should be the primary objective. Further factors that limit bovine TB control in many African countries include poor implementation of existing legislation against the disease, poor monitoring and notification, lack of collaboration between services in charge of local ecosystems, inadequate veterinary and medical professionals, deficiencies in public education about zoonotic bovine TB and sub-standard husbandry practices (Daborn et al. 1996; Ayele et al. 2004; AU/IBAR 2006; Anaelom et al. 2010).

In many communities of sub-Saharan Africa, man and animals share the same microenvironments and water sources, especially during drought and dry seasons. The hygiene of these microenvironments is usually very poorly managed. Also, the food preferences and consumption habits of many people include fresh animal products such as raw milk and raw meat. Bovine TB is very widespread in over 60% of Africa (Cosivi et al. 1998; Ayele et al. 2004) and approximately 85% of cattle and 82% of human populations in the continent live in areas where bovine TB is endemic and either partly controlled or not

controlled at all (Ayele et al. 2004). The potential for spread of *M. bovis* to humans is real if a bovine TB infected animal is introduced into the community. The prevalence of human TB is high and complicated in Africa by many factors including population growth, HIV/AIDS epidemic and poverty (Perry et al. 2002; WHO 2009). TB in cattle is therefore a human health issue on the continent and knowledge about the implication of bovine TB to public health needs to be developed and disseminated for effective control. Accurate diagnosis of bovine TB in cattle, at different stages of the disease and under various environmental conditions is necessary to provide very useful epidemiological information and facilitate the development of effective strategies against bovine TB in cattle and zoonotic TB due to *M. bovis* in humans.

The existence of animal TB in Cameroon has since been established based on macroscopic lesions at meat inspection but also on historical and clinical findings and the infrequent use of TST of cattle (Merlin and Tsangueu 1985; Doufissa 1993; Muchaal 2002; Awah-Ndukum et al. 2005; Fon-Tebug 2009). However, the magnitude and distribution of bovine TB in cattle are not known, and opportunities exist in many livestock rearing communities for the zoonotic transmission of *M. bovis* such as close human-livestock interactions in limited microenvironments shared by cattle and humans. There is dearth of information of the threat of human *M. bovis* infections in the country but the veterinary and medical services are increasingly becoming concerned. The importance of TB in indigenous cattle populations and a high interrelationship between bovine TB and human TB are being speculated. In fact *M. bovis* has been reported in one human TB subject in West Cameroon (Niobe-Eyangoh et al. 2003) indicating that zoonotic bovine TB poses a real public health problem. Thus, to determine the involvement of bovine TB in the morbidity and mortality of TB in Cameroon,

a broad investigative approach on the epidemiology of the disease in cattle and humans, identification of the TB causing agents and their sources, routes of transmission and associated risk factors need to be conducted. The possibility of a cattle – human – cattle cyclical zoonotic TB transmission developing, the existence of an epidemiological link between TB in humans and TB in cattle, and the presence of reservoir hosts of the bovine tubercle bacilli in Cameroon must be seriously considered. The vulnerability of HIV-infected and non- HIV infected individuals to TB also needs to be addressed. Though the contact between humans and cattle, and thus risk of public exposure to bovine TB would be expected to be high, the level of public awareness and empowerment of veterinary and human surveillance programmes relating to TB control are not clear.

Meat inspection provides very significant insight into the prevalence of many animal diseases (FAO 1994; Hinton and Green 1997; Grist 2008) including TB. However, diagnosis of bovine TB based on post mortem examination at meat inspection may be confused with lesions caused by *Nocardia*, *Corynebacterium* and other granuloma causing organisms (Blood and Radostits 1989; Gracey and Collins 1992; FAO 1994; Grist 2008). The introduction of confirmatory techniques such as bacteriological isolation and molecular genotyping of the mycobacterial strains circulating in cattle and human populations are necessary for proper understanding of the epidemiology of TB in Cameroon. Lack of quarantine measures, free movements and smuggling of live animals from neighbouring Nigeria, Chad and Central African Republic where bovine TB is widespread (Du-Sai and Abdullahi 1994; Diguimbaye et al. 2004; Cadmus et al. 2006; Diguimbaye-Djaibé et al. 2006; Abubakar 2007; Okaiyeto et al. 2008;

Müller et al. 2009a; Ngandolo et al. 2009) can promote the transmission of *M. bovis* into Cameroon, between the neighbouring countries and within regions.

The highland regions of Cameroon, made up of the Western highlands and Adamawa plateaux, are major cattle producing areas and contribute over 60% (ILCA 1992) of the estimated 6 million cattle population in Cameroon. The regions are among the top populated in the country with average population densities of over 100 inhabitants and 20 cattle per Km^2 (Pamo 2007); and cases of suspected TB lesions in slaughtered cattle have also been reported (Doufissa 1993; Awah-Ndukum et al. 2005; Fon-Tebug 2009). The Bamenda city abattoir in this study is the largest in the Western highland regions and provides all the daily beef requirements of over one million inhabitants of the Bamenda city and neighbouring areas. The study regions (bordered by bovine TB endemic countries to the West by Nigeria and East by Chad and Central African Republic) are made up of ethnic groups with passionate traditions for livestock rearing.

2.2 Aim and objectives of the study

2.2.1 Aim of the study

Assessment of the epidemiology of bovine TB in cattle, risk factors for exposure and transmission of *M. bovis* and public health implications of zoonotic TB due to *M. bovis* in the highlands of Cameroon that would provide key information required for the control of TB in man and livestock in Cameroon.

2.2.2 Objectives of the study

A. Epidemiological investigations

- To determine the trend of detection of suspected TB lesions in slaughtered cattle from 1994 – 2010 in the Bamenda city abattoir of the Western highlands of Cameroon.
- To determine the prevalence of bovine TB in cattle based on TST in the highlands of Cameroon; using the single intradermal comparative cervical tuberculin (SICCT) and single intradermal tuberculin (SIT) tests.
- To determine the prevalence of circulating anti-bovine TB antibodies in cattle using the lateral flow method (immunochromatographic assay) in the study regions.
- To compare different cut-off points of TST against the anti-bovine TB antibodies assay for the maximum detection of bovine TB in Cameroonian cattle.
- To evaluate risk factors associated with the exposure and transmission of zoonotic TB due to *M. bovis* in the highlands of Cameroon.
- To compare the reactions of various breeds of cattle in the highlands of Cameroon to natural bovine TB infection on the basis of responder frequencies to TST and anti-bovine TB antibodies assay.

B. Mycobacterial culture and molecular genotyping

- To culture and isolate mycobacterium agents from suspected TB lesions in cattle and infected human sputa in the highlands of Cameroon.
- To determine and characterise the isolates by molecular genotyping techniques; using genomic deletion analysis and spoligotyping.

Chapter 3

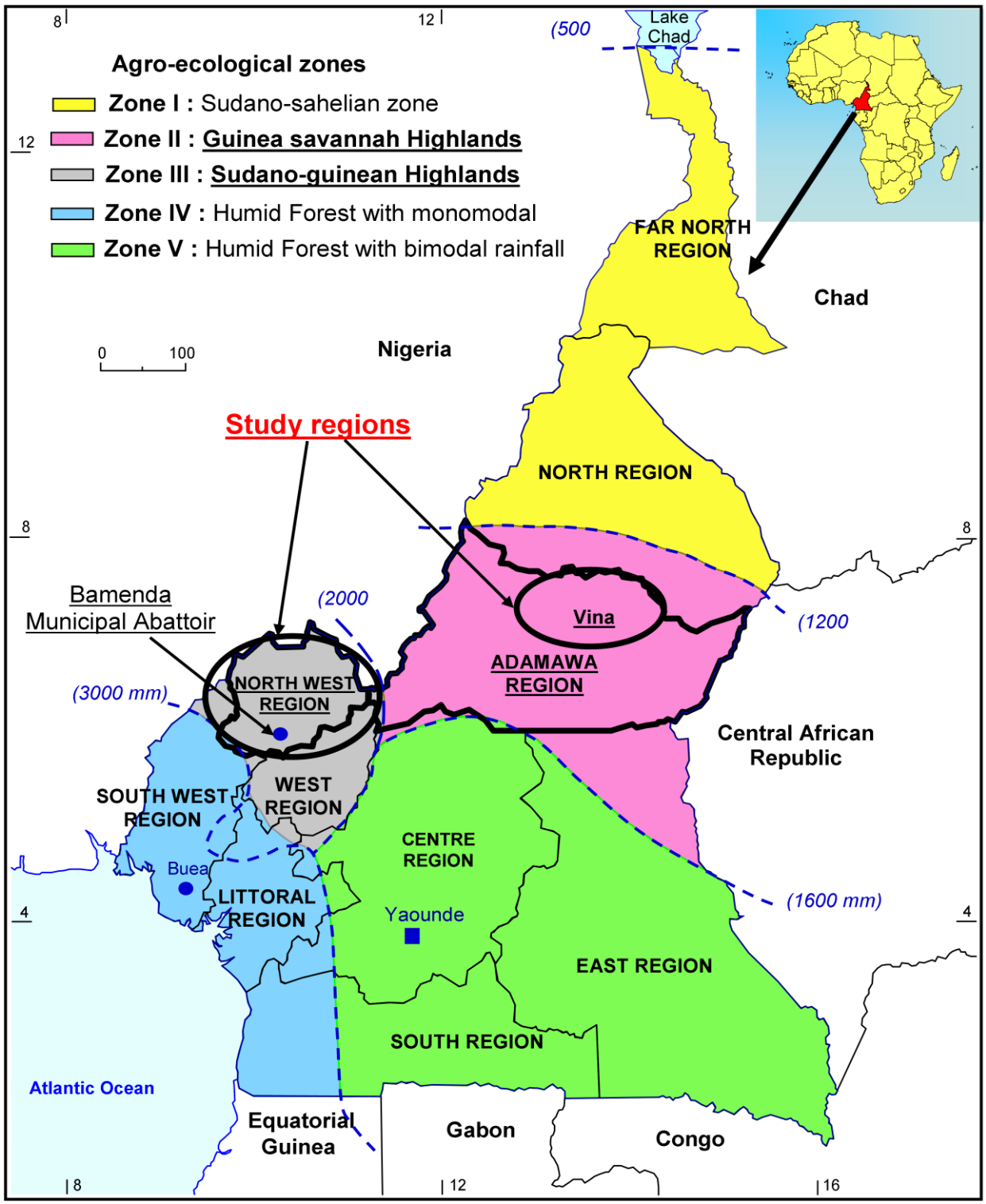
Materials and Methods

3.1 Geography and choice of study sites

Study of the prevalence of bovine TB in cattle based on TST was carred out from May to September 2009 and covered the wide geographical highlands of Cameroon include the Western highland regions (sudano – guinean zone) bordered to the northeast by the Adamawa plateaux (guinea – savannah zone). These zones are two out of the five agro-ecological zones of Cameroon (Figures 7 and 8) and the areas were the Northwest region (5°–7°N and 10°–11°E and 35, 926 km^2 in area) in the sudano-guinean zone and the Vina area (6 – 7°30'N and 12°30' – 14°E and 17,196 km^2) of the Adamawa region (4°30'– 8° & 11°–15°E and 63,691 km^2) in the sudan type zone with transitions of zone guinean savannah.

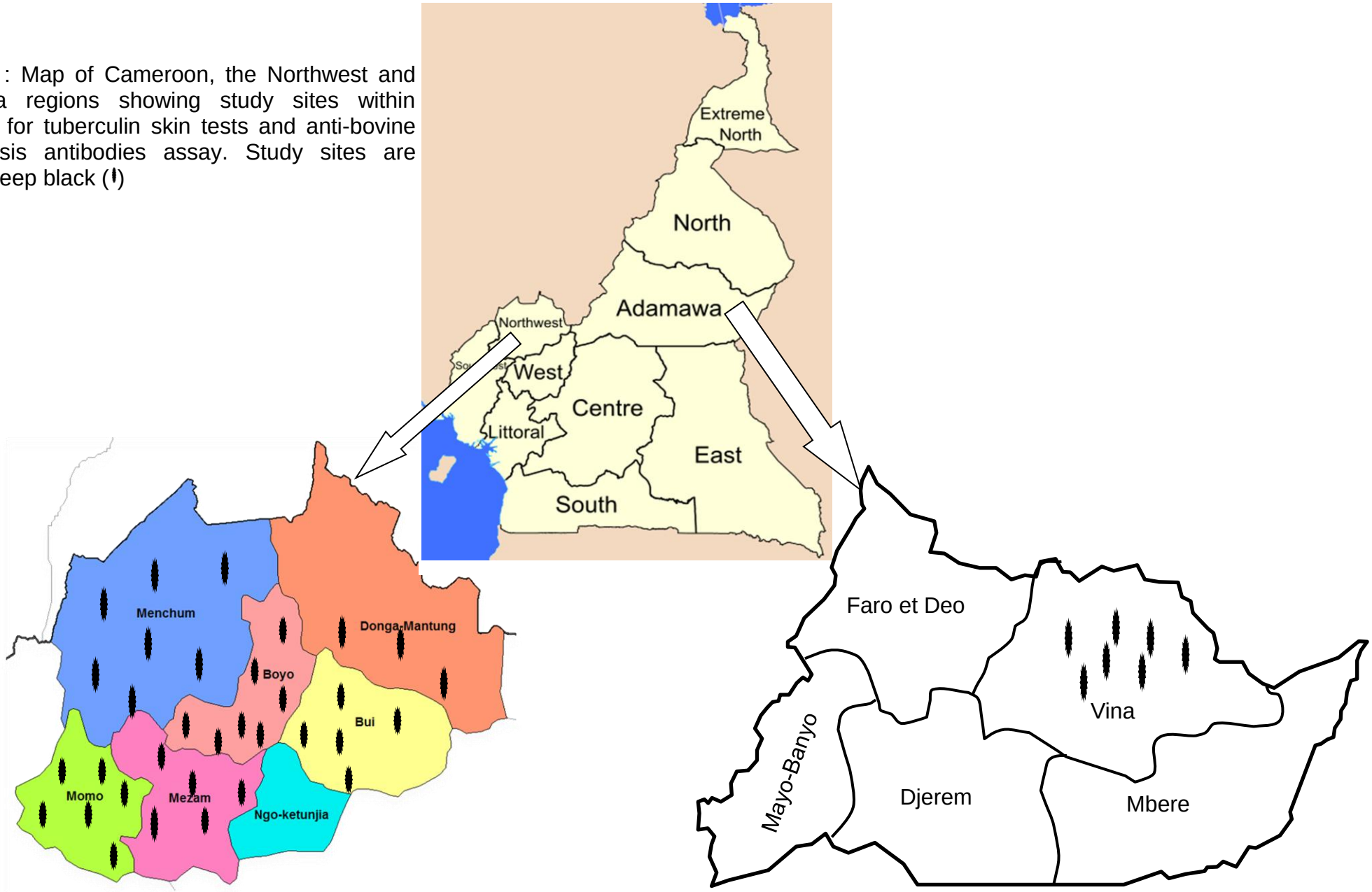
Both highland areas are characterised by high altitude (1000 – 2650m), cool temperature (20 ± 5°C), heavy rainfall (1500 – 2000mm), high humidity (70 ± 5%) and savannah vegetation with forest galleries (Gwanfogbe et al. 1983; Neba 1999). The Western highlands rises in steps from the west and experience an equatorial climate, two major seasons: a long wet season (mid-March to Mid-October) and a short dry season (mid-October – Mid-March). The higher elevations give the regions cooler climate than the rest of the country. Rainfall levels are reduced in the Adamawa plateau regions which is larger and northward and as the Sudan climate becomes predominant (Neba 1999).

Figure 7 : Map of Cameroon showing agro-ecological zones and study regions[#].



#: The borders of the study regions are in bold: The Northwest region and Vina area in the Adamawa region

Figure 8 : Map of Cameroon, the Northwest and Adamawa regions showing study sites within Divisions for tuberculin skin tests and anti-bovine tuberculosis antibodies assay. Study sites are shaded deep black (▮)



These regions account for over 60 % of the country's esimated 6 million cattle population (ILCA 1992; Pamo 2007) and has an overall average density of over 100 people and 20 cattle per Km^2 (Pamo 2007). The choice of the study areas was following reports of suspected tuberculous lesions in slaughtered cattle in abattoirs in the regions and the presence of communities with strong cultures of livestock rearing. The ethnic groups in the study sites were mostly agro-pastoralists with varied customs and also kept other livestock such as sheep, goats, horses, donkeys and fowls. The Bamenda municipal abattoir was used for a retropective study of the prevalence of bovine TB based on detection of suspected TB lesions from meat inspection records of slaughtered cattle (1994 – 2010) and for the collection of tuberculous lesions in slaughtered cattle for mycobacterial culture (March 2009 – April 2010).

3.2 Animals and management practices in the study regions

The animals used in this study were reared under the extensive or traditional pastoral, the semi-extensive and intensive or "zero grazing" management systems. The animals on extensive and traditional pastoral systems were kept in a free range, grazing on natural pastures and drinking points as well as went on transhumance during periods of scarcity. These animals were made up of the indigenious zebus (eg: White Fulani, Gudali, Red Bororo, Namchi, Exotic and various cross breeds – Figure 9), scarcely supplemented with concentrate feed diets and received little or no professional veterinary attention.

Animals in the semi-extensive system were grazed on communal and natural pastures but were given concentrate feed supplements, received frequent

veterinary attention and did not go on transhumance. Animals reared under the intensive and “zero grazing” systems were dual purpose animals destined primarily for milk production and secondarily for beef; and received frequent veterinary attention and concentrate supplement. The animals on intensive system were often grazed in limited paddocks and fodder was often brought to them. Animals on “zero grazing” were always provided fodder and did not graze at all. Exotic, upgraded or exotic crossbreeds and arbitrarily selected local zebus made up the animals (Figure 9) raised under the semi-extensive, intensive and “zero grazing” management systems. The animals used in this study were owned by individuals, group of farmers, cooperatives or government institutions.

Traditional pastoral husbandry and transhumance, with scarce supplementary feeding and limited veterinary care provided to the animals were commonly practised; and less frequently the intensive, “zero grazing” and semi-intensive systems. The transhumance system was employed during periods of drought (dry season) and involved long distant relocations of herders and their herds (leaving their families behind), sharing of available natural or communal grazing and overcrowding at night enclosures and watering points that were always heavily contaminated with faeces and urine.

White Fulani

Gudali

Red Mbororo

Local zebu (Mixed Zebu crossbreeds – ? : Red Mbororo x White Fulani x Gudali)

Holstein

Namchi

Figure 9 : Some breeds of cattle found in the highland regions of Cameroon and used in this study.

3.3 Epidemiological investigations

3.3.1 Abattoir bovine tuberculosis prevalence study

Routine post mortem examination of slaughtered carcasses at meat inspections for the detection of gross pathologies including TB lesions was carried out by a team of resident veterinary staff based on procedures adopted by MINEPIA[1] as stipulated by the law regulating Veterinary health inspection and notification of contagious animal diseases in Cameroon (MINEPIA 2002). Briefly, the procedure employed visual examination, palpation and incision of the lungs, liver and kidneys; lymph nodes of the thoracic and head regions; the mesenteric lymph nodes, other lymph nodes and tissues/organs of the body. For condemnation, the Veterinary inspector would seize the whole carcass if generalised TB was detected, otherwise only the parts drained by affected lymph nodes and affected tissues / organs were condemned (FAO 1994; MINEPIA 2002).

In this study, retrospective analysis of meat inspection records (January 1994 to December 2010) to determine the trend of detection of suspected TB lesions in cattle slaughtered in the Bamenda municipal abattoir was done. There was no pre-slaughter TB testing schemes in the abattoir but ante-mortem inspection for general examination of good health was done. Animals that showed signs of ill-health were usually not slaughtered but kept for further appraisal before slaughter can be recommended in the subsequent days. The slaughter / meat inspection records of approximately 129,165 slaughtered cattle were scrutinised while data on tuberculous and other pathological cases were extracted as found in each case.

[1] Ministère de l'Elevage, des Pêches et des Industries Animales (Ministry of Livestock, Fisheries and Animal Industries)

3.3.2 Prevalence of bovine tuberculosis by tuberculin skin tests and anti-bovine tuberculosis antibodies assay in cattle

3.3.2.1 Estimation of sample sizes

Two separate TST exercises of cattle were carried out between March – September 2009 and May – September 2010 in two wide agro-ecological highland zones (Western highlands and Adamawa plateaux) of Cameroon (Figure 8). Seven study sites being Administrative Divisions, were surveyed to minimise the effect of variations within the regions. Both survey periods coincided with the transition of the dry to rainy season and a return to familial grazing of animals that went on transhumance from the chosen study areas. However, most transhumance locations and migration sites were within the highland regions, as well as across the country's borders to Eastern Nigeria for some herds in the Northwest region.

Selection of cattle herds was carried out based on random-number generation from records of annual livestock vaccination campaigns (CBPP, pasturellosis, black quarter) of cattle keeping communities, cattle owners and locations of herds at the MINEPIA Regional Delegations. All animals within selected herds were tested except recently calved cows (within 2 months post-partum) and calves less than 6 months old because of immuno-suppression in lactating cows and the high maternal antibodies in calves that de-sensitizes them to tuberculin (Costello et al. 1997; Shirima et al. 2003). Dairy herds were significantly fewer than beef herds and were always included in the list for testing in localities where they were present. Based on a bovine TB prevalence range of 26% recorded by Muchaal (2002) in an earlier single intradermal TST of cattle in peri-urban centres of Bamenda in the Western highlands of

Cameroon, the total number of cattle required to be tested to detect at least one infected animal with 95% confidence and a desired absolute precision of ≥ 5% was calculated using previously described formula (Putt et al. 1988; Pfeiffer 2002; Thrusfield 2007).

Thus $$\boldsymbol{n} = \frac{\mathbf{1.96^2}\boldsymbol{Pexp}(\mathbf{1}-\boldsymbol{Pexp})}{\boldsymbol{d^2}}$$ …… Equation 1

Where n = required sample size

Pexp = expected prevalence;

d = desired absolute precision.

An expected prevalence of ≈ 30% gave a required minimum sample size of 323 in each division. A total simple random sample size of 2,261 animals was estimated for the bovine TB prevalence study. However, group stratifications (sex, age, breed, animal husbandry practices) and minimal variability of estimated values were needed; and maximum allocations were based on the size of cattle rearing communities and number of herds within the Divisions while also taking into consideration costs, season and road accessibility. The selection procedure was repeated in some localities until the required number of cattle to be tested was met because a farmer's willingness to participate was never guaranteed. It was also not possible to list all herd owners in a locality and or the sizes of herds before the test. Most pastoral farmers were reluctant to reveal the number of herds or estimate number of animals in their herds because of their cultural beliefs.

Information relating to the location, type of production (beef or dairy) and management system, breed, sex and age of the animal were noted. Age

estimation were noted as provided by the owners and or determined as described by Turton (1999). The body condition scores of the animals based on three main conditions of fat, medium and lean reported by Nicholson and Butterworth (1986) and breeds as described by MINEPIA (MINEPIA 2002) and Blench (1999) were noted.

3.3.2.2 Prevalence of bovine tuberculosis based on tuberculin skin tests

During the period of March to September 2009, the first TST were carried out. Skin responses to the Single Intradermal Comparative Cervical Tuberculin skin (SICCT) and Single Intradermal Tuberculin skin (SIT) tests were performed using both Avian (AT) and Bovine (BT) tuberculin purified protein derivates (Lelystad Biologicals, Lelystad, Netherlands). A total of 2,853 cattle (61 herds in 33 village communities) in six Administrative Divisions (Donga and Matung, Menchum, Bui, Mezam and Boyo) of the Sudano-Guinean Western highland region and 23 herds (7 village communities) in the Vina Division of the Guinea-savannah Adamawa plateau region (Figure 8) were tested for bovine TB infection (SICCT-BT and SIT-BT).

Intradermal injections of 0.1ml each of AT (2500 $IUml^{-1}$) and BT (3000 $IUml^{-1}$) were administered in two sites, ≈ 12 cm apart from each in the middle third of the right neck region. A correct intradermal injection was confirmed by palpating a small grain-like swelling at each injection site. The skin thickness was measured prior to and 72 (±6) hours after injecting the tuberculin using a 0.01 mm graduated digital calliper. The same person measured the skin before and when the test was being read while the test interpretation was based on the observation, palpation and recorded increase in skin fold thickness. Placed ear

tags and skin tattoos were used to identify test animals and herds' men also had memorised features for each animal under their care (eg vocals, body markings, mannerisms). However, the right horn or horn bud were also marked with red cosmetic nail varnish to guarantee that all tested animals were presented when the results were being read. The OIE-recommended cut-off standards were used to interpret SICCT and SIT test responses (OIE 2009). The existence of bovine TB had been established in the country (Doufissa 1993; Martrenchar et al. 1993; Awah-Ndukum et al. 2005). However, the sensitivity and specificity findings obtained by Ameni *et al.* (2000) in the similar tropical conditions of Ethiopia were used to correct the observed prevalence of SICCT-BT reactors (section: 3.8.2 below) because of lack of data for the exact Cameroon environment. The data obtained by Pollock et al (2003) was used to adjust the observed prevalence of SIT-BT reactors.

Briefly for SICCT-BT test, a reaction was considered to be positive if the increase in skin thickness at the BT site of injection was ≥ 4 mm greater than the reaction shown at the site of the AT injection. The reaction was inconclusive and negative if the increase in skin thickness at the BT site of injection was from 1 to 4 mm greater and < 1 mm less than increase in the skin reaction at the AT site of injection. Thus, SICCT-BT skin response was given by $(BT_{72} – BT_0) – (AT_{72} – AT_0)$

Where: BT_0 and AT_0 = the measures of skin fold thickness prior to injecting BT and AT; while BT_{72} and AT_{72} = the measures 72 hours after injecting BT and AT.

For SIT-BT, a positive reactor was considered if the skin-fold thickness at the BT site of injection or $(BT_{72} – BT_0)$ was ≥ 4 mm increase; negative if the increase was < 2 mm and inconclusive if the increase was ≥ 2 mm and < 4 mm.

3.3.2.3 Anti-bovine tuberculosis antibodies assay

The level of exposure of cattle to bovine TB using the Rapid anti-bovine TB antibodies (anti-bovine TB Ab) assay (Anigen Bovine Tb Ab®, BioNote Inc., Korea) was assessed using data from 807 (20 herds) of 1,381 cattle (40 herds) screened for bovine TB using the TST between May and September 2010. The cattle / herds were similarly sampled as previously described (sections: 3.1, 3.2 and 3.3.2.1 above) in Mezam and Bui Divisions of the western highlands which showed high bovine TB prevalence rates in the previous survey and also in the Vina division in the Adamawa plateaux. Some herds not screened in the first survey were selected during the second screening.

However, prior to injecting BT and AT for the second tuberculin tests, about 5ml of blood was collected by jugular venopuncture to extract serum for the detection of anti-bovine TB Ab using the lateral-flow-based rapid test (Anigen Bovine TB Ab®, BioNote Inc., Korea), as described by the manufacturer. Briefly, in the ready-to-use disposable test kit, 10 μl of test serum was poured into the sample well (S) and after 1 minute, 3 drops of developing buffer placed in the buffer well. The lateral flow test was performed under ambient temperature and the result was interpreted after 20 minutes (but not later than 30 minutes). The presence of two purple coloured bands within the result window, the test area (T) and control (C) line, indicated antibodies positive result whereas no band in the test area in addition to a visible control purple line was negative (Figure 10). An invalid test was one where no coloured band was visible within the result window. The appearance of a control colour band, for positive or negative assays, indicated that the test was working properly.

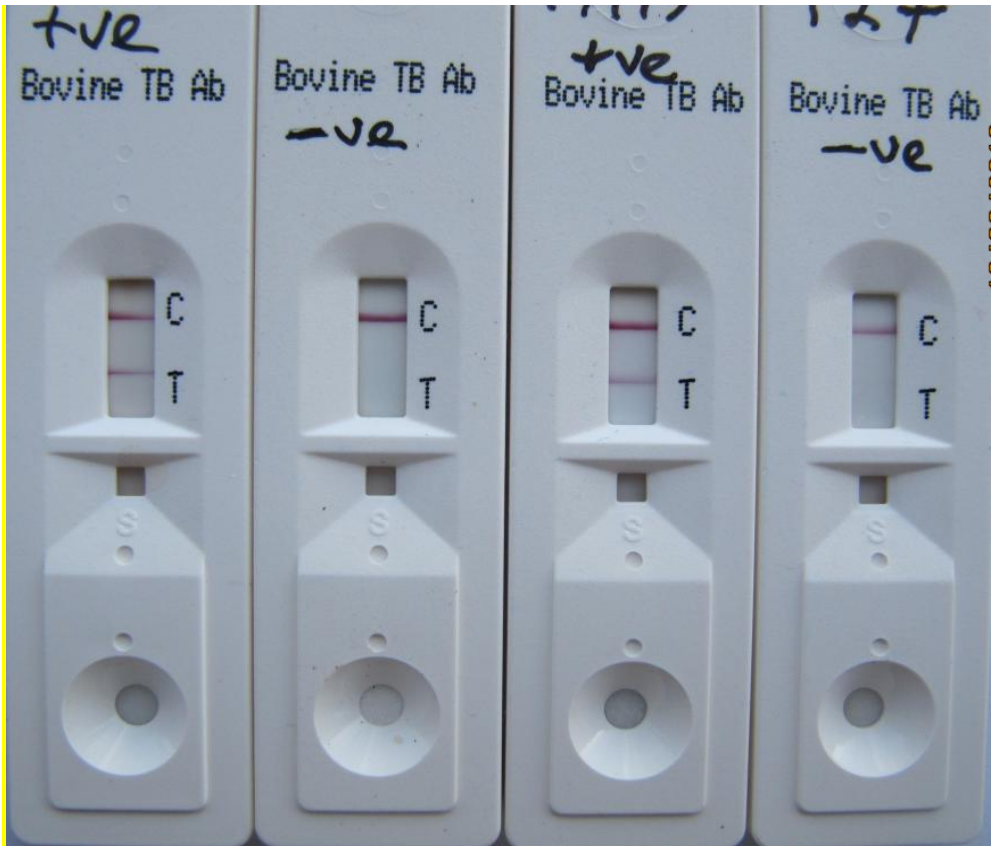


Figure 10 : Four lateral-flow kits showing anti-bovine tuberculosis antibodies test results of four cattle

The presence of two purple coloured bands within the result window, the test area (T) and control (C) line, indicated antibodies positive (from left: 1st and 3rd) result whereas no band in the test area in addition to a visible control purple line was negative (from left: 2nd and 4th).

3.3.3 Comparison of different tuberculin skin test cut-off points and classification of reactors for the diagnosis of bovine tuberculosis

To assess the SICCT-BT cut-off points in a local Cameroonian context, analysis was performed using data from 807 (20 herds) of 1,381 cattle in 40 herds screened during the second TST period of May – September 2010. These animals were subjected to the detection of circulating anti-bovine TB antibodies to define their disease exposure status and risk of disease (see 3.3.2.3 above) and the TST to define the disease status. The geography and choice of study sites, selection of herds and animal types, husbandry practices and sampling procedures for TST have been described earlier (section: 3.1 and 3.2 above).

Briefly, prior to injecting AT and BT (Lelystad Biologicals, Netherlands) anti-bovine TB Ab detection assay using the lateral flow method (section: 3.3.2.3 above) was carried out as described by the manufacturer (Anigen Bovine Tb Ab®, BioNote Inc., Korea). Also, the TST were performed as described earlier (section: 3.3.2.2 above). The interpretations of various observations of increase in skin fold thickness of the tuberculin tests were compared to the OIE-recommended ≥ 4-mm cut-off point (OIE 2009). However, the following SICCT-BT cut-off points: ≥2 mm; ≥3 mm and ≥4 mm were assessed for positive reactor status with the corresponding skin fold thicknesses: ≥1 to <2 mm, ≥1 to <3 mm and ≥1 to <4 mm classified as inconclusive reactors, respectively. SICCT-BT was noted as negative if skin response was <1 mm. SIT-BT interpretations were done using skin fold thickness of ≥ 4 mm, ≥ 2 mm to < 4 mm and < 2 mm for positive, inconclusive and negative reactors, respectively (OIE 2009). These cut-off points were assessed against the demonstrated circulating anti-bovine

TB antibodies status and also classified (Figure 10) as adapted from Martrenchar et al. (1993) to determine the cut-off zone and risk group of reactors for further consideration.

Detailed post-mortem examination of SICCT-BT positive reactors after testing for the presence of TB lesions and subsequent laboratory confirmation were not done due to the impracticalities of slaughtering TST positive reactors in the study. However, the detection of circulating anti-bovine TB Ab to define the disease exposure status in each of the tested cattle was used for assessing the TST which was assumed to define the disease status at the different cut-off points. An animal was classified as exposed to bovine TB when anti-bovine TB Ab was detected and negative or non-exposed when anti-bovine TB Ab was not found in its blood or serum. The sensitivity and specificity of SICCT-BT at ≥2 mm; ≥3 mm and ≥4 mm cut-off points were calculated against the anti-bovine TB antibodies assay results. The findings were compared to those obtained elsewhere (Ameni et al. 2000; Pollock et al. 2003; Ameni et al. 2008a) and also used to determine test performance and accuracy (see 3.8.2 below) in the Cameroon environment.

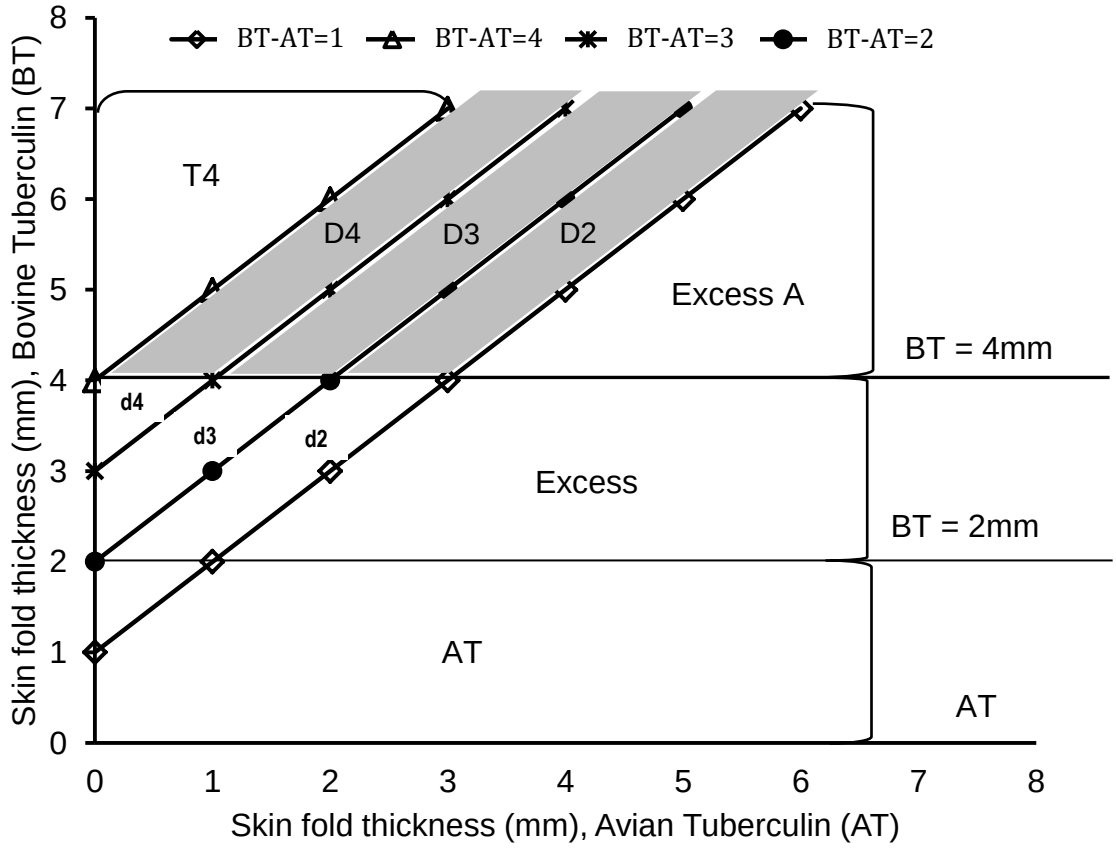


Figure 11 : Classification of cattle according to their possible tuberculin skin test responses at different cut-off points

The figure was adapted from Martrenchar et al. (1993).
Where:

BT = (BT_{72} – BT_0) = skin fold thickness at the injection site of bovine tuberculin at 72 hours

AT = (AT_{72} – AT_0) = skin fold thickness at the injection site of avian tuberculin at 72 hours

(D+d) = SICCT-BT doubtful responses. The skin responses (D2+d2), (D3+D2+d3+d2) and (D4+D3+D2+d4+d3+d2) are for ≥1 mm to <2 mm, ≥1 mm to <3 mm and ≥1 mm to <4 mm cut-off ranges, respectively.

Excess d4 (Xd4) = d4+d3+d2 = SICCT-BT doubtful responses (≥ 4-mm cut-off point) and classified as SIT-BT doubtful responses (when 1 mm ≤ (BT – AT) < 4 mm and 2 mm ≤ BT < 4mm)

Excess d3 (Xd3) = d3+d2 = SICCT-BT doubtful responses (≥ 3-mm cut-off point) and classified as SIT-BT doubtful responses (when 1 mm ≤ (BT – AT) < 3 mm and 2 mm ≤ BT < 4mm)

Excess d2 (Xd2) = d2 = SICCT-BT doubtful responses (≥ 2-mm cut-off point) and classified as SIT-BT doubtful responses (when 1 mm ≤ (BT – AT) < 2 mm and 2 mm ≤ BT < 4mm)

Excess D4 (XD4) = (D4+D3+D2) = SICCT-BT doubtful responses at ≥ 4-mm cut-off and classed as SIT-BT positive animals (when 1 mm ≤ (BT – AT) < 4 mm and BT ≥ 4mm).

Excess D3 (XD3) = (D3+D2) = SICCT-BT doubtful responses at ≥ 3-mm cut-off point and classed as SIT-BT positive animals (when 1 mm ≤ (BT – AT) < 3 mm and BT ≥ 4mm).

Excess D2 (XD2) = (D2) = SICCT-BT doubtful responses at the ≥ 2-mm cut-off and classed as SIT-BT positive animals (when 1 mm ≤ (BT – AT) < 2 mm and BT ≥ 4mm).

T4 = SICCT-BT positive animals at ≥ 4 mm cut-off point (when (BT – AT) ≥ 4 mm).

T3 = (T4+XD4+Xd4) = SICCT-BT positive animals at ≥3 mm cut-off point (when (BT – AT) ≥ 3 mm)

T2 = (T3+XD3+Xd3) = SICCT-BT positive animals at ≥ 2 mm cut-off point (when (BT – AT) ≥ 2 mm).

Excess A (XA) = Animals classed as SIT-BT positive animals and infected with atypical mycobacteria according to SICCT-AT (when BT ≥ 4 mm and (BT – AT) < 1 mm).

Excess AD (XAD) = Animals classed as SIT-BT doubtful responses and infected with atypical mycobacteria according to SICCT-AT (when 2 mm ≤ BT< 4 mm and (BT – AT) < 1 mm).

AT = Animals infected with atypical mycobacteria according to SICCT-AT and classed as SIT-BT negative animals (when BT< 2 mm and (AT – BT) > 0 mm).

3.4 Questionnaire survey

Risk factors for the exposure and transmission of bovine TB infection of cattle and cattle handlers were examined by structured questionnaire surveys conducted to collect information on a range of variables relating to animal management and practices, the habits and level of awareness about TB of willing cattle professionals in the study regions. The targeted cattle handlers included ethnic groups with a tradition of cattle keeping including the Fulani, Bororo, Foulbe, butchers, cattle owners and herdsmen / herdsboys, “Buyam sellems” (meat traders), and other cattle professionals. The professionals were visited in their communities, herds, abattoirs, meat shops, cattle markets and other targeted sites. Animal health and production technicians (veterinarians, para-veterinarians and some extension Agricultural workers) were surveyed at

their offices or job sites to appreciate their level of awareness and implementation of the existing legislation on bovine TB control. Also, regular visits to the Bamenda Regional hospital and other private clinics (belonging to individuals and churches) that carried out conventional human TB control programmes and equipped or associated with mycobacteriological laboratories were carried out. Willing TB patients diagnosed through ZN microscopy of acid-fast bacilli in their sputa responded to questions about their lifestyle and interactions with animals as well as donated sputa (78 cases) for mycobacterial culture and molecular studies.

The highland regions of Cameroon are made up of many multi-cultural communities (whether involved in traditional livestock production or not) with distinct vernaculars. Considerable time and patience were needed to obtain maximum cooperation of the traditional cattle professionals; and where necessary a trusted and knowledgeable intermediary was engaged. The questionnaire surveys covered the period of March to September 2009 and May to September 2010 (same period as the second TST). The questionnaires were divided into the following main sections: animal husbandry and practices, basic demographic information of respondents, their lifestyle, habits and awareness of zoonotic TB (Table 9). Predesigned questionnaires were pre-tested on 81 willing animal handlers. All respondents in the study signed a consent and confidentiality form. Explanatory analysis of 645 of approximately 1000 filled questionnaires was performed (namely 489 of 600 cattle handlers; 77 of 250 animal health and production technicians and 84 of 150 human TB patients).

Table 9 : The main areas of animal management and practices, habits and awareness of zoonotic tuberculosis of respondents asked in the questionnaires

Risk factor group	General description
General	Sex, age, marital status, level of education, occupation, religion, tradition and duration of cattle keeping, residence, ethnic group, participation in similar study in future.
Animal management	Herd type, herd size, husbandry system, animal type and breed, restocking status, ownership, care-taking, feeding practices, body condition of animals
Animal ownership and care-taking?	Reason, sources of cattle, number and type of cattle, other livestock, reasons and rate of exploitation of herds, size of household (involved in animal business)
Housing of animals (especially at nights),	Free roaming, night enclosures, fenced areas, sheltering paddocks, well-constructed housings
Contact with animal (human-animal interactions)	Type and degree of interactions with cattle, contact with other livestock, drink raw milk, eat raw milk, keeping other animals, abattoirs, cattle markets, vaccination campaign, communal dips,
Contacts of owned cattle with other cattle (animal – animal interactions)	Use of same bulls for breeding (group bull), animal breaking fence or bound (coming in or going out), contact with other livestock, transhumance, communal grazing, cattle market, going to or coming from cattle market, vaccination centres, drinking spots, communal dips,
Awareness and recognition of Human TB?	Previous contact/knowledge, hospital consultations, "Njangie[2]" and group meetings, mode of transmission (milk, meat, aerosols), humans affected by bovine TB?
Awareness and recognition of TB in animals?	Previous contact/knowledge, "Njangie" and group meetings, Veterinary service, Post mortem findings in carcass, know bovine TB is zoonotic, mode of transmission (milk, meat, aerosol), cattle be affected by human TB?
Other diseases in owned animals?	Recognition (clinical symptoms), persistence of disease, incidence of disease in owned and adjacent herds, morbidity rate, mortality rate
Vaccination programmes?	Vaccines?, routine vaccination?
Request of Veterinary services?	Reasons for veterinary attention, sick animals, disease monitoring, disease routinely screened, deworming, average number of veterinary visits per year,
Bovine TB detection and control?	Diagnostic methods? Screening frequency, testing service/agency, meat inspection and condemnation, awareness and implementation of bovine TB control law, action after test (if positive result), acceptance of routine testing, payment for bovine TB test,

[2] Group of persons with common objectives and meeting regularly to improve their targeted social, economic, cultural and or religious goals

3.5 Mycobacterial culture and molecular genotyping

3.5.1 Collection of suspected tuberculous lesions in slaughtered cattle

During the period March 2009 to April 2010 intensification of meat inspection and collection of tuberculous lesions for mycobacterial culture were carried out in the Bamenda city abattoir. Diagnosis was based on identifying characteristics macroscopic lesions (abscess, tubercles) on palpation and incision during meat inspection as guided by MINEPIA (2002) with supplementary knowledge of TB pathogenesis and distribution of lesions in infected cattle (Gracey and Collins 1992; FAO 1994; Grist 2008). The origin, breed, sex and age of the animals were noted. Age estimation was determined as described by Turton, (1999) while the breed was obtained as previously described (Blench 1999; MINEPIA 2002). A total of 219 suspected lesions were collected in sterile plastic bags and stored in sample boxes at –20°C for up to 3 weeks before processing and incubation on Lowentein-Jesen (LJ) and Middlebrook 7H9 media was done.

3.5.2 Collection of human sputa for mycobacterium culture

Regular visits were made to public and private hospitals in the highland regions of Cameroon that carried out conventional TB control programmes and were equipped or associated with a mycobacteriology laboratory. Sputa from 92 willing participants with microscopic demonstrations of acid-fast bacilli following Ziehl-Neelsen (ZN) staining were collected for mycobacterial culture and characterisation. Infected sputa were collected in sterile plastic sample bottles

for mycobacteriological culture or stored at 2 – 8°C for a maximum of 24 hours before incubation on Lowentein-Jesen (LJ) media.

3.5.3 Mycobacterium culture of specimens from cattle and humans

The suspected tuberculous lesions and infected sputa were incubated using pyruvate and glycerol enriched Lowentein-Jesen (LJ) slants (Appendix 1) and modified Middlebrook 7H9 broth base (Becton Dickinson Diagnostics, UK) following standard procedures. All processing of the bovine and human specimens was done in biosafety level 2 cabinet. Ziehl-Neelsen (ZN) staining and microscopic demonstrations of acid-fast bacilli (Appendix 2) were used to confirmed successful inoculation and growth (Strong and Kubica 1985; WHO 1998b; a).

Briefly, suspected tuberculous cattle specimens were cut into tiny pieces and then homogenized separately in 0.85% saline in sterile blenders to obtain fine pieces. Frozen samples were allowed to thaw to room temperature before processing. The cattle tissue homogenates and suspected human sputa were decontaminated with equal volumes of sterile 4% NaOH; mixed well by shaking for a few seconds and allowed to stand for 10 minutes at room temperature before neutralization with 1 mol/L HCl using phenol red as the indicator. Neutralization was achieved when a yellowish colour change of the suspension was attained; which was centrifuged at 3,000 rpm for 15 min. The supernatant was discarded leaving about 2ml or re-suspended in 1 – 2ml of distilled water (if required) and spread generously (~ 0.3 ml) on the LJ slants in quadruplets as follows: 2 LJ medium enriched with glycerol and 2 LJ medium enriched with

Pyruvate. A mycobacterial growth indicator tube (MGIT) medium or modified Middlebrook 7H9 broth base with proven performance containing polymixin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin (PANTA) (Becton Dickinson Diagnostics, New York, USA) was also used to improve mycobacteria isolation. Incubation at 37°C for up to 12 weeks (depending on media) with weekly observation for growth of colonies was done. On observation of visible growth, a few colonies were gently mixed into one drop of sterile saline and smeared on a clean, grease-free microscopic slide, heat – fixed using the Bunsen burner flame without burning and stained by the ZN method (Appendix 2) to confirm the presence of acid-fast bacilli.

Briefly, the smeared slide was flooded with ZN carbol fuchsin, steamed without boiling gently with the Bunsen burner flame from the underside for 5 min. It was then rinsed gently with plenty of water until all free stain was washed away. The slide was flooded with 3% acid-alcohol decolourising solution for 2 – 3 minutes until the red colour disappeared, then rinsed again with water and the excess water drained. The slide was then flooded with Methylene blue counter stained for I minute, rinsed thoroughly with water, excess water drained from the slide and the smear allowed to air dry without blotting. The smear was systematically examined under a microscope (100 x oil immersion objective) for the presence of acid-fast bacilli. The presence of at least three bacilli in 100 immersion fields was recorded as positive. Smears in which no acid-fast bacilli were seen in 100 fields were considered negative.

3.5.4 Harvest of colonies and biochemical characterisation of isolates

Cultured *Mycobacterium* on LJ slants were harvested by gently scraping colonies into sterilised screw-cap vials containing 1 ml – 1.5 ml physiologic saline while the modified Middlebrook 7H9 broth (MGIT medium) were centrifuged at 3,000 rpm for 20 min and the supernatant discarded leaving the bacterial pellet in about 2ml of suspension. On observation of colonies and prior to complete harvesting into vials, ZN staining-microscopy (Appendix 2) and biochemical characterisations (Niacin production, Nitrate reduction and Catalase enzyme activity) of the isolates were carried out following standard procedures (Appendices 3 – 5) (Strong and Kubica 1985; WHO 1998b; a). The isolates were killed using heat as previously described (Bemer-Melchior and Drugeon 1999; Warren et al. 2006) with few modifications such immersing the vials so that the suspensions are completely below water level in a water-bath at 100°C for 20 minutes. To ensure death of colonies, all suspensions were inoculated upon cooling to room temperature on LJ slants or MGIT medium as described earlier (section: 3.5.3) with weekly observation for up to 4 weeks. The killed samples were stored in a freezer (– 20°C) and transported to the University of Plymouth, UK and Veterinary Laboratory Agency, Weybridge for molecular genotyping. However, the samples were kept at – 80°C once in the UK until molecular analyses.

It is worth noting that viable TB cultures were not imported or used in the UK studies and mycobacterium culture of speciemens in Cameroon was done according to accepted safety regulations. The cultured DNA samples were inactivated and shown to be sterile, before importing into the UK.

3.5.5 Molecular characterisation of mycobacterial isolates

3.5.5.1 Genomic deletion typing of mycobacterial isolates

The genomic typing of the isolates was developed to differentiate MTC members known to cause TB in humans and animals based on deletion typing of various genomic regions of difference (RD) (Brosch et al. 2002; Warren et al. 2006; Müller et al. 2009a). MTC members are 99.9% similar at the nucleotide level and comparative genomic analysis have showed that all of them evolved from a common ancestor through sequential DNA deletions with precise genomic locations (Brosch et al. 2002; Pinsky and Banaei 2008). However, identification of hypervariable regions between the *M. bovis* and *M. tuberculosis* genomes and several loci exist that show significant diversity across the bacilli strains have been reported (Gordon et al. 2001). The evolutionary scheme of MTC is based on the presence or absence of conserved deleted regions and on sequence polymorphisms in five selected genes (Brosch et al. 2002).

The complete genome sequence of the best-characterized strain of *Mycobacterium tuberculosis*, H37Rv, has been determined and analysed in order to improve our understanding of the biology of this slow-growing pathogen and management interventions (Cole et al. 1998; Gordon et al. 1999; Gordon et al. 2001; Brosch et al. 2002). The genome comprises 4,411,529 base pairs, contains around 4,000 genes, and has a very high guanine + cytosine content that is reflected in the biased amino-acid content of the proteins (Cole et al. 1998). However, whole-genome comparisons of Mycobacterium tuberculosis and Mycobacterium bovis BCG-Pasteur together with the complete genome sequence of M. tuberculosis H37Rv revealed the presence of 10 deletions (RD1–RD10) in M. bovis BCG genomic DNA relative to M. tuberculosis. Seven

of these regions, RD4–RD10, were also found to be deleted from M. bovis, with the three M. bovis BCG-specific deletions being identical to the RD1–RD3 loci (Gordon et al. 1999; Gordon et al. 2001).

Heat killed acid-fast isolated bacilli in this study were subjected to a multiplex Polymerase Chain Reaction (PCR) deletion typing method (Warren et al. 2006). The presence or absence of RD9 and RD4 were checked for all the mycobacteria isolates followed by RDAf1 deletion typing of RD4 deleted isolates. Sequences of the Flanking and Internal sets of primers and their positions in *M. tuberculosis* H37Rv or *M. bovis* 2122 strains are shown in Table 10. A Flanking primer indicated the absence of the RD while the associated Internal primer indicated the presence of the RD being analysed.

The RD multiplex-PCR reactions were performed in 96-well plates and contained per reaction 8 µl DNA template, 1 µl each of 10 pmol / µl Flanking and Internal primer (Eurofins MWG Operon, Ebersberg, Germany) and 10 µl HotStarTaq DNA polymerase (Qiagen, Hilden, Germany) to give a total PCR reaction[3] volume of 20 µl. Amplification was initiated by incubation at 95°C for 15 minutes, followed by 45 cycles at 94°C for 1 minute, 61°C for 1 minute and 72°C for 2.5 minutes. After the last cycle, the samples were incubated at 72°C for 10 minutes and later maintained at 4°C until electrophoresis was performed. The products were electrophoresed using 1.5% agarose gel in 1x Tris-Acetate-EDTA (TAE) running buffer (pH 8.03). SYBR Safe dye at a ratio of 1:10000, 100 bp DNA ladder and orange 6 x loading dye were used in the gel electrophoresis and visualised using the Quantity1® Bio Imaging System (Bio-Rad Laboratories Ltd, USA).

[3] After several trials PCR products were obtained at the described formulation. DNA free water was not included because the isolates were much diluted during harvesting using 1 – 1.5 ml physiological saline.

Table 10 : Polymerase Chain Reaction primer sequences for genomic deletion typing of mycobacterial isolates from cattle and humans and their position in *M. tuberculosis* H37Rv or *M. bovis* 2122 strains

Region of difference	Primer set	Sequence	Position in *M. tuberculosis* H37Rv (bp)	Position in *M. bovis* 2122 (bp)
RD9	Internal			
	RD9-INT_FW	5' CGATGGTCAACACCACTACG 3'	2330997-2331016	-
	RD9-INT_RV	5' CTGGACCTCGATGACCACTC 3'	2331361-2331342	-
	Flanking			
	RD9-FL_FW	5' GTGTAGGTCAGCCCCATCC 3'	-	2313855-2313873
	RD9-FL_RV	5' GCCCAACAGCTCGACATC 3'	-	2314327-2314310
RD4	Internal			
	RD4-INT_FW	5' CAAGGGGTATGAGGTTCACG 3'	1703142-1703161	-
	RD4-INT_RV	5' CGGTGATTCGTGATTGAACA 3'	1703631-1703612	-
	Flanking			
	RD4-FL_FW	5' CTCGTCGAAGGCCACTAAAG 3'	-	1692533-1692549
	RD4-FL_RV	5' AAGGCGAACAGATTCAGCAT 3'	-	1692996-16922977
African 1	Internal			
	Af1_FW	5' ACTGGACCGGCAACGACCTGG 3'	664100	-
	Af1_INT	5' CGGATCGCGGTGATCGTCGA 3'	664449	-
	Flanking			
	Af1_FW	5' ACTGGACCGGCAACGACCTGG 3'	664100	-
	Af1_RV	5' CGGGTGACCGTGAACTGCGAC 3'	669951	-

RD = Regions of difference; INT = Internal; FL = Flanking; FW = Forward; RV = Reverse;
Primer sets for RD9, RD4 and African 1 were obtained from Eurofins MWG Operon, Ebersberg, Germany.

The expected results were as follows:

I. **RD9 deletion typing**

a. **Internal primers:** The presence of the RD9 region (such as in *M. tuberculosis*) generated a PCR product of 364bp while strains with the RD9 region deleted generated no PCR product with the Internal primers (RD9Int_FW and RD9-Int_RV).

b. **Flanking primers:** The presence of RD9 generated a PCR product size of 2501 bp (not visualised) while its absence (such as in *M. bovis* BCG) generated a PCR product of 472bp with Flanking primers (RD9-FL_FW and RD9-FL_RV).

II. **RD4 deletion typing**

a. **Internal primers:** The presence of RD4 region (such as in *M. tuberculosis*) generated a PCR product of 489bp while strains that have the RD4 region deleted (absent) generated no PCR product with Internal primers (RD4-Int_FW and RD4-Int_RV).

b. **Flanking primers:** However, strains that have the RD4 region deleted (such as *M. bovis* BCG) generated a PCR product of 447bp with Flanking primers (RD4-FL_FW and RD4-FL_RV).

III. **African 1 deletion typing**

a. **Internal primers:** Strains that have the Af1 region intact (AF2122/97) generated a PCR product of 349bp while no product was generated when the Af1 region was deleted with Internal primers (Af1_FW and Af1_INT).

b. Flanking primers: Strains with the Af1 region deleted (CHAD491) generated a PCR product of 531bp while no product was generated when the Af1 region was intact with the Flanking primers (Af1_FW and Af1_RV).

3.5.5.2 Spoligotyping of *Mycobacterium bovis* isolates

Fifty five isolates from TB lesions of Red Bororo and White Fulani cattle identified as *M. bovis* strains by deletion of both RD9 and RD4 were selected for spoligotyping (spacer oligonucleotide typing) as described by Kamerbeek et al. (1997) with minor modifications at the Veterinary Laboratory Agency – Weybridge, United Kingdom[4].

The method was based on the detection of DNA polymorphism within the direct repeat (DR) locus and relies on the prior amplification of the DNA sequence of the highly polymorphic DR locus in the chromosome of *M. tuberculosis* complex. The individual DR regions were all potential targets for *in vitro* PCR amplification (the oligonucleotide primers were obtained from Eurofins MWG Operon, Ebersberg, Germany), and the variation in spacers was exploited to obtain different hybridization patterns of the amplified DNA. The amplified products were then hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequences within the DR that had been sequenced from a number of *M. tuberculosis* complex strains.

Briefly, the direct repeat (DR) region was amplified by PCR with oligonucleotide primers derived from the DR sequence. About 25 µl of the following reaction

[4] The candidate could not be granted access into VLA labs and the spoligotyping method was performed by Dr Noel Smith and Dr Jim Dale (External collaborators).

mixture was used for the PCR: 12.5 µl of HotStarTaq Master Mix[5] (QIAGEN), 2 µl of each primer (20 pmol each), 5 µl of the suspension of heat-killed cells, and 3.5 µl of distilled water. The mixture was heated for 15 minutes at 96°C and then subjected to 30 cycles of 1 minute at 96°C, 1 minute at 55°C and 30 seconds at 72°C. The amplified product was hybridized to a set of 43 immobilized oligonucleotides, each corresponding to one of the unique spacer DNA sequence within the DR locus.

Prior to hybridization all buffers were pre-warmed to 60°C. About 20 µl of the PCR products including positive and negative controls[6] were added to 150 µl of 2 X SSPE[7] / 0.1% Sodium dodecyl sulphate (SDS). The mixture was incubated at 99°C for 10 minutes and cooled immediately on ice to denature the PCR product. The membrane was washed for 5 minutes in 250 ml of 2 X SSPE / 0.1% SDS at 60°C and placed in the miniblotter supported by a cushion; such that the slots were perpendicular to the line of pattern of oligonucleotides. Residual fluid was removed by aspiration and the slots were filled with 150 µl of the diluted PCR products and then hybridized for 60 minutes at 60°C on a horizontal surface in a hybridization oven without shaking to avoid contamination of adjacent slots.

After hybridization, the samples were removed from the miniblotter by aspiration and the membrane taken out (using forceps) of the miniblotter for washing and detection of the hybridized DNA. The membrane was washed twice in 250 ml of 2 X SSPE / 0.5% SDS for 10 min at 60°C each washing, placed in a rolling bottle and allowed to cool. The membrane was incubated in 1:4,000 diluted

[5] this solution provides a final concentration of 1.5 mM $MgCl_2$ and 200 µM each deoxynucleoside triphosphate

[6] *M. tuberculosis* ($H37R_V$) and *M. bovis* (2122/97) were used as positive controls and DNA free H_2O as the negative control

[7] 1 X SSPE is 0.18 M NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA [pH 7.7]

streptavidin-peroxidase conjugate [8] (Boehringer Ingelheim Ltd, UK) for 45 minutes at 42°C in the rolling bottle, washed twice in 250ml of 2 X SSPE / 0.5% SDS for 10 minutes at 42°C each washing and then rinsed twice with 250 ml of 2 X SSPE for 5 min at room temperature.

The hybridized DNA was detected using enhanced chemiluminescence (ECL) method (Amersham) as specified by the manufacturer. Patterns were identified by using 1 to indicate the presence of a spacer and 0 for the loss of a spacer. The spoligotypes were named according to the international website database for *M. bovis* (Mbovis.org).

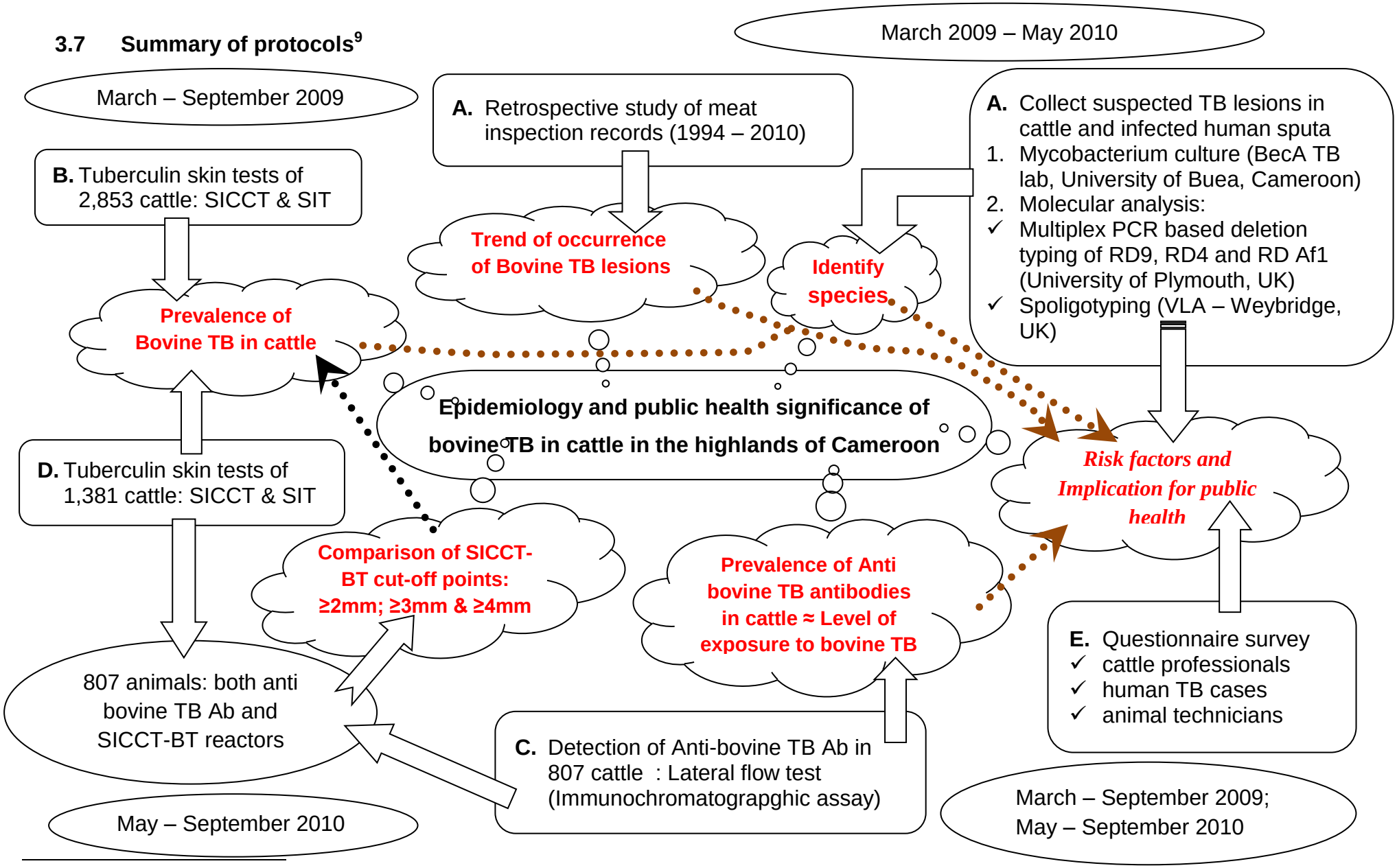
3.6 Ethical, Health and Safety issues

Risk assessments were done to avoid hazards to all persons and animals involved in the project. Project approval and ethical clearances were obtained from the Faculty of Science and Technology, University of Plymouth ethics committee and the required authorities in Cameroon (National Ethics Committee, Regional delegations of MINEPIA and MINSANTE in the Northwest and Adamawa) for the study. An ATAS (Academic Technology Approval) certification was also a requirement to carry out the research in the United Kingdom. Apart from the minor jugular vein puncture for blood collection, intradermal injections of avian and bovine tuberculins and procedural restraining manipulations for safety purposes, the live animals were not subjected to suffering. Slaughtering and dressing of cattle carcasses were done as described by the Cameroon veterinary services (MINEPIA 2002). All laboratory analyses using suspected TB samples (alive or dead) were carried out in

[8] 2.5ml Strptavidin + 10 ml secondary buffer

laboratories equipped with at least a category II Biosafety cabinet. Also, test kits and reagents were obtained from recognised manufacturers. The purpose of the study was explained to targeted participants usually with the assistance of resident veterinarians, local community leaders and trusted intermediaries. A herd was tested for bovine TB after an informed consent was given by the owner. Human participants were included in the study after giving their informed consent. The consents of cattle professionals including butchers were also obtained before their slaughtered animals were used in the work.

3.7 Summary of protocols[9]



[9] A, B, C, D and E : represent the main sequence the research activities. There was overlapping of some activities such as E was actually an overlap of A / B / C / D.

3.8 Data management and statistical analysis

3.8.1 Prevalence of bovine tuberculosis

Retrospective data of suspicious TB lesions and other pathologies detected during meat inspections in the Bamenda abattoir, results from individual animal TST and anti-bovine TB antibodies assay were entered in Microsoft Excel® 2007 spread sheet together with other epidemiological data and information about management systems. The data were validated by determining the number of valid observations and missing values and exported to SigmaPlot® (Systat Software, Inc) for further analysis.

3.8.1.1 Trend of bovine tuberculosis based on the detection of tuberculous lesions in slaughtered cattle

Frequency distribution of the annual and monthly prevalence rates of the detection of TB lesions and the other pathologies (non-TB lesions) were generated as described earlier (Putt et al. 1988; Petrie and Watson 1999; Thrusfield 2007). A prevalence rate was determined as the proportion of carcasses with TB lesions of the total carcasses examined and the 95% confidence intervals or standard error calculated as previously described (Putt et al. 1988; Petrie and Watson 1999; Greiner and Gardner 2000). The Mann-Whitney test was used to compare the differences in observation of TB lesions between the dry and wet season; and yearly durations (Petrie and Watson 1999; Greiner and Gardner 2000). The regression model was used to evaluate the association between the recorded TB and non-TB pathologies, and the effect of season (month of year) and yearly duration on the detection of bovine TB lesions.

3.8.1.2 Prevalence of bovine tuberculosis based on tuberculin skin tests

De la Rua-Domenech (2006a) had cited that the specificity and sensitivity of the SICCT test could vary from 88.8% to 100% and 52.0% to 100%, respectively. Specificity and sensitivity for TST in cattle have not been obtained in Cameroon but the diagnostic performance of the tests was assumed to be constant across the regions used. The OIE-recommended cut-off point of ≥4-mm (OIE 2009) and the other cut-off points (≥2-mm, ≥3-mm, ≥4-mm) were applied for positive results from one group of animals to another.

Due to the impracticality of slaughtering TST reacting cattle in this study, the results of earlier trial by Ameni et al. (2000) and Pollock et al. (2003) were used to correct the observed prevalence of reactors to the SICCT-BT and SIT-BT, respectively. Ameni et al. (2000) found specificity and sensitivity to the SICCT test and their confidence intervals in cattle to be 100% (95% CI: 59.8 – 100%) and 90.9% (95% CI: 69.4 – 98.4%), respectively. While Pollock et al. (2003) obtained 90% for specificity and 86.4% for sensitivity to SIT-BT test of cattle at optimal cut-off of 4 mm. Thus, the observed prevalence (P_o) rates were corrected using the Rogan-and-Gladen formula to obtain the true prevalence (P_t) rates as stated earlier (Putt et al. 1988; Greiner and Gardner 2000; Thrusfield 2007). Thus :

$$Pt = \frac{Po+Sp-1}{Se+Sp-1} \qquad \text{…….. Equations 2}$$

Where: Pt = True prevalence;
Po = Observed prevalence
Sp = Specificity;
Se = Sensitivity

The 95% confidence intervals or standard error were also calculated as previously described (Putt et al. 1988; Petrie and Watson 1999; Greiner and Gardner 2000; Thrusfield 2007).

Individual cattle prevalence was defined as the number of cattle with skin test-positive response at the ≥4-mm cut-off point per 100 cattle tested. The McNemar's test approximating the Chi-square distribution and normal distribution techniques were applied to compare and determine the level of significant differences between the studied variables. The difference between the effects of different risk factors on prevalence was analyzed using the regression analysis. The odds ratio (OR) was calculated to assess the strength of association of different factors with the prevalence of bovine TB. For age, four age grouping were undertaken for the whole population sampled. However, the data was also distributed into 2 age groups (less than or equal to 4 years (Young) and more than 4 years (adult/old)) as cattle in the study locations usually reach maturity at between 3 and 4 years.

3.8.2 Comparison of different tuberculin skin test cut-off points and lateral flow assay for the detection of bovine tuberculosis

The classification of TST reactors according to different cut-off points has been described earlier in figure 10 (Section: 3.3.3).

The anti-bovine TB antibodies assay results from individual cattle (807 animals) and TST at the ≥2-mm, ≥3-mm and ≥4-mm cut-off points were entered into Microsoft Excel® and also exported to SigmaPlot® (Systat Software, Inc) for further analysis. Frequency distribution of the detection rates of anti-bovine TB

antibodies which defined the level of exposure of tested cattle (hypothesised risk factor of disease) and skin responses to the TST which determined the disease status of bovine TB in the study were generated. The overall accuracy of the anti-bovine TB antibodies assay used in this study had been noted to be ≥ 85.5% compared with TST (Anigen Bovine Tb Ab®, BioNote Inc., Korea). Also, the performance of the anti-bovine TB antibodies assay was assumed to be unvarying over the study sites and cattle exposed to bovine TB would produce anti-bovine TB antibodies and be positive reactors.

The frequency of exposure, risk of disease and odds ratios (disease and exposure odds ratios) and their 95% confidence intervals were determined (Table 11) at each TST cut-off point to assess the degree of association between the TST and anti-bovine TB antibodies assay (Thrusfield 2007). Furthermore, the sensitivity and specificity values and their 95% confidence intervals were calculated (Putt et al. 1988; Thrusfield 2007) and used to correct observed prevalence rates to the true prevalence rates using the Rogan-and-Gladen formula (Equation 2 : see 3.7.1.2 above).

Table 11 : The 2 x 2 contingency table of possible diagnostic results

		True status* (Diseased status)		Totals
		Positive	Negative	
Exposed status# (Hypothesised risk factor)	Positive	a	b	a+b
	Negative	c	d	c+d
	Totals	a+c	b+d	a+b+c+d

*: provided by tuberculin skin test

#: provided by anti-bovine TB antibodies assay

The measures of associations were determined as previously described (Thrusfield 2007) as follows:

- The Chi square (χ^2) test of association (with one degree of freedom) was stated as

$$\chi^2 = \frac{n(|ad-bc|-\frac{n}{2})^2}{(a+b)(c+d)(a+c)(b+d)} \quad \text{…….…….. Equations 3}$$

- The Risk of disease (R_{ed}) and no disease (R_{end}) in exposed animals are

$$R_{ed} = \frac{a}{(a+b)} \quad \text{and} \quad R_{end} = \frac{b}{(a+b)} \quad \text{…….. Equations 4 and 5}$$

- The Risk of disease (R_{ued}) and no disease (R_{uend}) in unexposed animals are

$$R_{ued} = \frac{c}{(c+d)} \text{ and } \quad R_{uend} = \frac{d}{(c+d)} \quad \text{…….. Equations 6 and 7}$$

- The frequency of exposure (P_{fed}) and non-exposure (P_{fued}) of diseased animals are given as

$$P_{fed} = \frac{a}{(a+c)} \quad \text{and} \quad P_{fued} = \frac{c}{(a+c)} \quad \text{…….. Equations 8 and 9}$$

- The frequency of exposure (P_{fend}) and non-exposure (P_{fuend}) of non-diseased animals are given as

$$P_{fend} = \frac{b}{(b+d)} \quad \text{and} \quad P_{fuend} = \frac{d}{(b+d)} \quad \text{…….. Equations 10 and 11}$$

- Odds of disease in exposed (OR_{ed}) and unexposed (OR_{ued}) animals are

$$OR_{ed} = \frac{a}{b} \quad \text{and} \quad OR_{ued} = \frac{c}{d} \quad \text{…….. Equations 12 and 13}$$

- Odds of exposure of diseased (OR_{fed}) and exposure of non-diseased (OR_{fuend}) animals are

$OR_{fed} = \frac{a}{c}$ and $OR_{fuend} = \frac{b}{d}$ …….. Equations 14 and 15

- Both the disease odds ratio (OR_d) which is the ratio of OR_{ed} to OR_{ued} and based on prevalence and the exposure odds ratio (OR_e) which is the ratio of OR_{fed} to OR_{fuend}

$OR_d = OR_e = \frac{ad}{bc}$ …….. Equation 16

And the approximate 95% confidence interval for the odds ratio (OR) was estimated as

$OR^{1\pm1.96/\chi}$ …….. Equation 17

where: $\chi = \sqrt{Chi\ square} = \sqrt{\chi^2}$

Also, the Sensitivity (Se) and Specificity (Sp) of SICCT-BT and SIT-BT against the anti-bovine TB antibody assay were calculated as follows:

$S_e = \frac{a}{a+c}$ …….. Equation 18

and $S_p = \frac{d}{b+d}$ …….. Equation 19

The validity of each SICCT-BT cut-off point (≥2 mm; ≥3 mm and ≥4mm) was determined by computing the confidence intervals (CI) for sensitivity and specificity. The accuracy of the tuberculin test at cut-off points of ≥2 mm; ≥3 mm and ≥4 mm to detect the presence of the disease was determined as described by Thrusfield (2007).

Positive test predictive value

$$\frac{(Pt \times Se)}{(Pt \times Se)+\{(1-Pt)\times(1-Sp)\}}$$ ……. Equation 20

Negative test predictive value

$$\frac{(1-Pt)\times Sp}{\{(1-Pt)\times Sp\}+\{Pt\times(1-Se)\}}$$ ……. Equation 21

Where: Pt = True prevalence

Sp = Specificity

Se = Sensitivity

The likelihood ratios (LR+ and LR-) were also used to evaluate the diagnostic performance of each cut-off point (Thrusfield 2007) as follows:

$LR+ = Se/(1 - Sp)$ …….. Equation 22

$LR- = (1 - Se)/Sp$ …….. Equation 23

The method of Wilson (1927) cited by Thrusfield (2007) was used to calculate the 95% confidence intervals. The lower range was given by $(A - B)/C$ and upper range by $(A + B)/C$.

Thus : $A = 2r + 1.96^2$ …….. Equation 24

$B = 1.96\sqrt{1.96^2 + 4r(1 - P)}$ …….. Equation 25

$C = 2(n + 1.96^2)$ …….. Equation 26

Where: r = number of reactors at the cut-off point of interest

n = number in the sample

P = observed proportion

However, when the sample sizes were small for the investigated parameters (n>30) and distributed normally (eg herd level parameters) the 95% confidence intervals for the true proportions were calculated as follows:

$$\sqrt{\frac{\frac{r}{n}(1-\frac{r}{n})}{n}} \quad \text{or} \quad \sqrt{\frac{P(1-P)}{n}} \qquad \text{……. Equation 27}$$

(r, n and P are as defined above for equations 24 – 26)

This formula was used where the application of Wilson's formula [$(A-B)/C$ and $(A+B)/C$] was inappropriate for interval estimation of the proportions (e.g.: SICCT-AT and SIT-AT results as well as SIT-BT for second TST).

The Beyer's reference tables for values of exact 95% confidence limits for proportions was used to obtained the intervals for proportions of small sized parameters such as the analyses of herd infection rates (Thrusfield 2007).

The agreement between TST and antibovine TB Ab tests at the predefined cut-off points were estimated (Thrusfield 2007). The *kappa* statistics was used to estimate the degree of agreements between both methods in detecting bovine TB reactors in the different variables (Petrie and Watson 1999; Thrusfield 2007).

The McNemar's test approximating Chi-square distribution and normal distribution techniques were applied to compare and determine the level of significant differences between individual and herd prevalence of reactors in the different groups of variables. The true prevalence values obtained in this study

were also compared with data obtained using sensitivity and specificity values reported by Ameni *et al.* (2000) for SICCT-BT and Pollock *et al.* (2003) for SIT-BT (Table 27 – Appendix 6). Furthermore, the TST data of 2,853 cattle tested in the year 2009 (see section 3.3.2.2 above) and 1,381 cattle tested in the year 2010 were re-assessed at the pre-defined cut-off points and estimated test accuracy data of this study.

3.8.3 Questionnaires

The data obtained in this study were the non-parametric category. The responses for each main area of the questionnaires were classified, frequencies estimated and these data were used to explain the variables (risk factors). The data was sorted / categorized using Microsoft excels before exporting to SPSS Statistics 18 (SPSS Inc. Chicago, USA) for further analysis. The degree of association or difference between variables was compared by running the McNemar test (which approximates the chi-square test of significance) on the different classes of data.

Chapter 4

Prevalence of bovine tuberculosis in cattle in the highland regions of Cameroon based on the detection of lesions in slaughtered cattle and tuberculin skin tests of live cattle

4.1 Introduction

Bovine TB is endemic in most of Africa (Ayele et al. 2004) and under investigated. Over 98.9% of reported cases in Africa are in cattle (AU/IBAR 2006). The detection of gross tuberculous lesions at post mortem examination of carcasses including meat inspection of slaughtered animals, which usually indicate advance stages of bovine TB (Corner 1994; Shitaye et al. 2006), is the basis for indicating the occurrence of the disease in Cameroon (Doufissa 1993; Awah-Ndukum et al. 2005). The reported prevalence of bovine TB from various abattoirs in the country range from 0.18% to 4.25% and tuberculous lesions were most commonly encountered 3 – 5 times compared to other pathologies (Awah-Ndukum et al. 2005; Fon-Tebug 2009). However, there are few reports of bacteriological isolation and molecular genotyping of TB in Cameroon (Njanpop-Lafourcade et al. 2001; Niobe-Eyangoh et al. 2003).

Mycobacteriological culture for the diagnosis of bovine TB is rarely done in Cameroon; while culture of human sputa is sporadically performed and mainly for research purposes. These conventional methods are often unsafe in inadequately constructed and poorly equipped laboratories (Igbokwe et al. 2001). The interpretations of results are highly subjective and prone to errors

such as interpreting differences in colony morphology (Strong and Kubica 1985; Grange et al. 1996; Ameni et al. 2010b). Though also prone to inspectors' subjectivities and errors, meat inspection provides very significant insight into the prevalence of many infectious diseases and plays vital roles in both the quality assurance and quality control systems for gross inspection of carcasses (Edwards et al. 1997; Hinton and Green 1997; Asseged et al. 2004). Major improvements in animal and human health within the concept of meat consumer protection and eradication of epizootic TB in many developed countries was achieved when drastic reduction of relevant or suspicious lesions at meat inspection was strictly employed to provide the quality demanded and protection of consumers (Grossklaus 1987; Hinton and Green 1997). The implementation of post mortem detection of TB lesions in carcasses and during meat inspection of slaughtered animals has been proposed (Corner 1994; Edwards et al. 1997; Shitaye et al. 2006); and continues to be the appropriate diagnostic tool in many developing countries with endemic bovine TB.

TST are the best procedures available for international field diagnosis of bovine TB in live animals (de la Rua-Domenech et al. 2006a; de la Rua-Domenech et al. 2006b). The single intradermal comparative cervical tuberculin test (SICCT) involving the intradermal injection of bovine tuberculin (BT) and avian tuberculin (AT) at separate sites in the skin of the neck, gives more specific results than the single intradermal tuberculin test (SIT) which uses only BT (Monaghan et al. 1994). There are recommended cut-off points of the increase in skin thickness for a tuberculin test to be positive (OIE 2009) and are the basis for eliminating positive reactors in eradication programmes (Good 2006). However, political and socio-economic constraints as well as lack of attention in countries like Cameroon are drastically preventing the "test and slaughter" strategy of

eliminating infected cattle which has proved very effective in the developed world (Gilbert et al. 2005; Abernethy et al. 2006; Good 2006). Furthermore, there are scanty and inconsistent reports approximating the prevalence of bovine TB based on TST of live cattle in Cameroon (Merlin and Tsangueu 1985; Tanya et al. 1985; Martrenchar et al. 1993; Nfi and Ndi 1997; Muchaal 2002).

The epidemiology of bovine TB and its implications on livestock productivity and risks to human health are largely unknown; and zoonotic bovine TB is increasingly becoming a source of concern to veterinary and especially human medicine in the country because it is the most opportunistic disease of HIV/AIDS patients in Cameroon (Noeske et al. 2004). The apparent risks of exposure and transmission of bovine TB in cattle and humans warrant comprehensive investigations of the prevalence of bovine TB in cattle, especially in high risk and cattle producing areas.

Improved attention to bovine TB status as well as accurate estimation of the magnitude and distribution of bovine TB in cattle are essential for appropriate intervention strategies in Cameroon. Therefore this study was carried out to ascertain the magnitude and trend of over seventeen years of detection of tuberculous lesions in slaughtered cattle at the Bamenda city abattoir of the Western highlands of Cameroon. The study also determined the prevalence of bovine TB based on TST of live cattle in two wide agro-ecological highlands of Cameroon.

4.2 Results

The materials and methods employed to estimate the prevalence of bovine TB have been described in Chapter 3 (section 3.1 to section 3.3.2.2 and section 3.8 and section 3.8.1).

4.2.1 Prevalence of bovine tuberculosis based on detection of tuberculous lesions in slaughtered cattle

The cattle slaughtered at the Bamenda municipal abattoir were mainly of the Zebu type; originating from within the sudano-guninea Western highland regions of the country. Analysis of 17 years of meat inspection records showed that of 129,165 slaughtered cattle, a total of 599 (0.46%; 95%CI: 0.43%–0.50%) suspect TB lesions among 983 (0.76%; 95%CI: 0.71%–0.81%) pathologies were identified (Tables 27 and 28 – Appendices 6 and 7). Although lesions were not systematically recorded, TB abscesses (with yellowish pus) and firm yellowish nodular lesions (often 'gritty' on cutting) were detected in the lungs and associated lymph nodes (over 60%), lymph nodes of the head, mesenteric lymph nodes and liver.

TB lesions were recorded throughout the study period (Figures 12 and 13) with the monthly prevalence rates ranging from 0.30% (95%CI: 0.2%–0.4) to 0.81% (95%CI: 0.64%–0.98%) and annual rates from 0.04% (95%CI: 0%–0.11%) to 1.46% (95%CI: 1.22%–1.69%). Over 60.94% of all pathologies that warranted partial or whole carcass condemnation were due to TB lesions. The detection rates of TB lesions varied widely between season but there was no significant difference (Mann-Whitney U Statistic = 4588; P = 0.927) between the detection rate for the wet season (0.46%; 95%CI: 0.32%–0.59%) and dry season (0.33%; 95%CI: 0.25%–0.41%). High detection rates in March, April, June and August (Figure 12) and several fluctuating annual peaks (Figure 13). Furthermore, regression modelling over the entire study period revealed a slope with a weak positive gradient ($Y = 0.0529X – 0.0978$; $R^2 = 0.5262$) suggesting stagnation or a mild an increase in trend of TB occurrence in the Bamenda municipal abattoir.

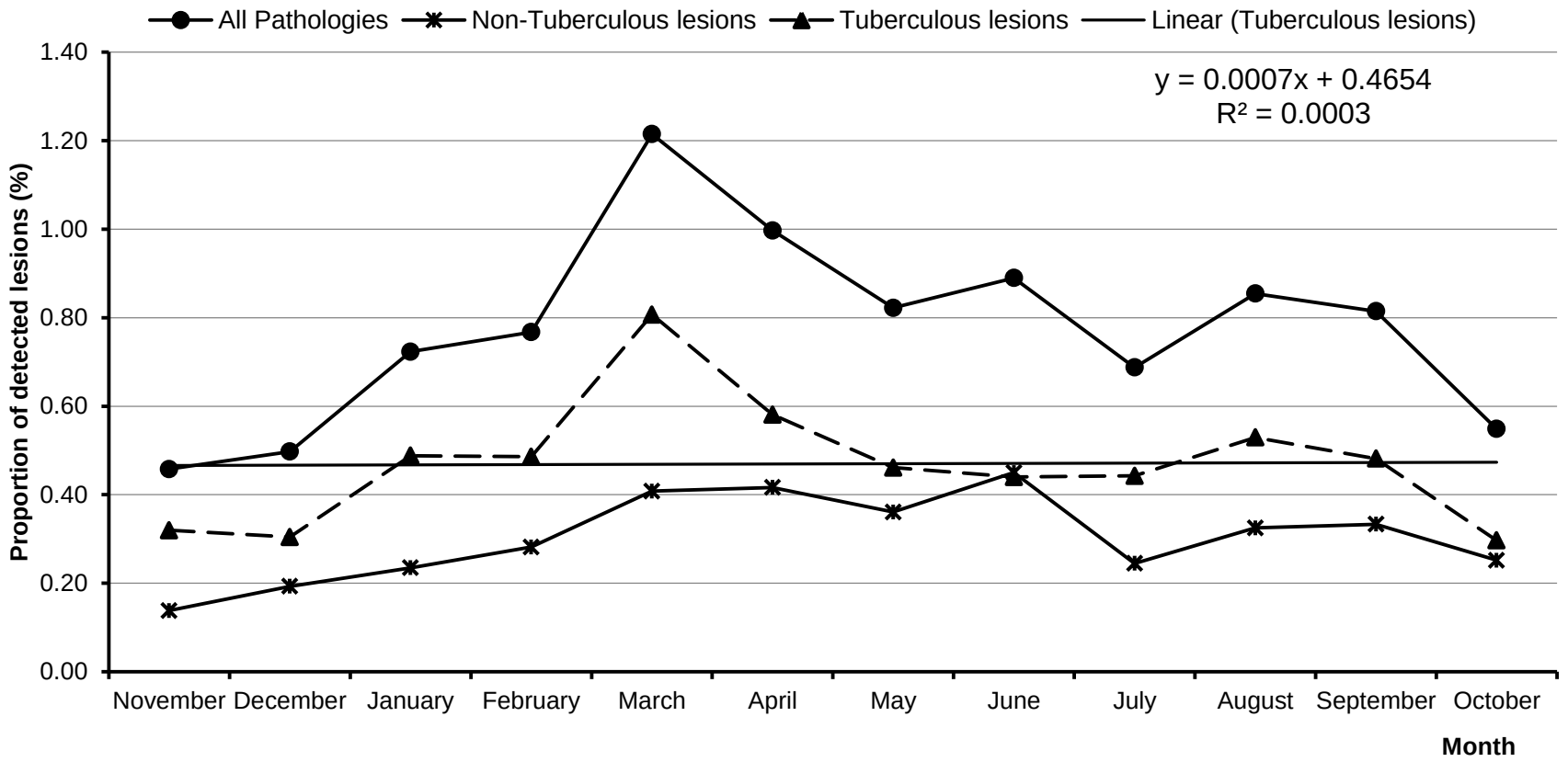


Figure 12: Proportional monthly distribution of bovine tuberculous and non-tuberculous lesions in slaughtered cattle recorded at the Bamenda Municipal abattoir, Cameroon (1994 – 2010).

* : months arranged according to season (Dry season: November – February; Rainy season: March – October)

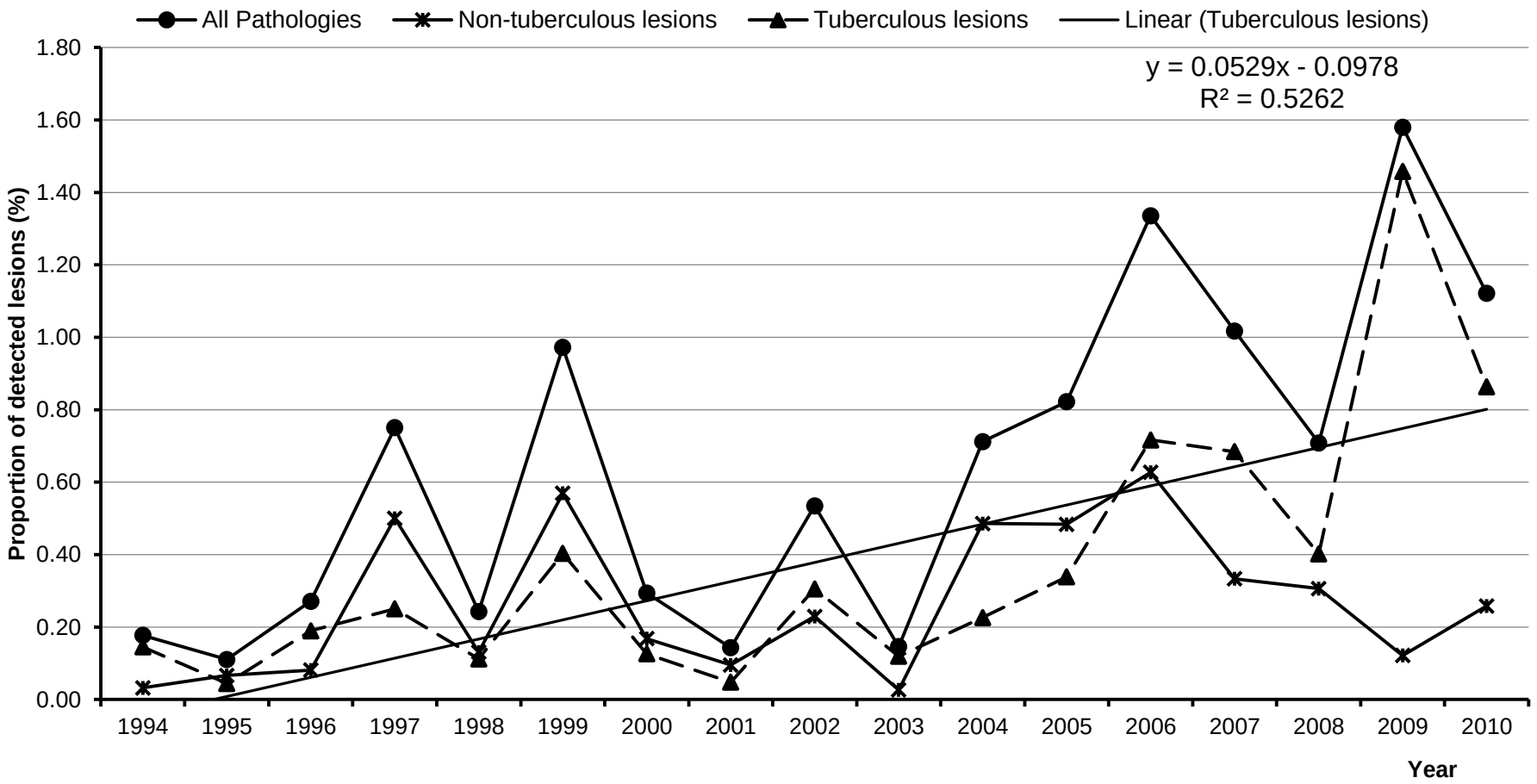


Figure 13: Annual prevalence of tuberculous and non-tuberculous lesions in slaughtered cattle recorded at the Bamenda municipal abattoir, Cameroon

4.2.2 Prevalence of bovine tuberculosis by tuberculin skin tests

4.2.2.1 Single Intradermal comparative cervical tuberculin skin test responses

Adjusted observed prevalence (or true prevalence) rates of animals with SICCT–BT positive responses according to location, breed, sex, age group, management system, and herd sizes are shown in Table 12. While the general SICCT test-positive response patterns using bovine tuberculin and avian tuberculin for the different variables are summarised in Figure 14.

Overall, 4.67% of the 2,853 animals tested were positive for the SICCT-BT and widespread over the entire study regions. The prevalence of positive reactors showed distinct variations between the agro-ecological highland regions and study sites (divisions). There were significantly more (χ^2=17.50, P≤0.001) SICCT-BT reactors in the western highland regions (5.38%; 95%CI: 4.42 – 6.34) than Adamawa plateaux (2.57%; 95%CI: 1.42 – 3.72) with the records ranging from 0.76% in Donga-Mantung to 12.45% in Mezam Division (Table 12). However, the proportion of SICCT-BT doubtful results were highest in Bui (17.16%) followed by Momo (11.32%), Donga-Mantung (9.10%) and Mezam (9.08%) divisions.

SICCT-BT prevalence values were significantly higher (P<0.05) for cattle managed in semi-intensive and beef production systems compared to the other systems, though more cattle in the intensive and dairy production systems showed significantly higher (P<0.05) doubtful reactions (Figure 14D). The animals in small herds (≤ 40 animals) showed higher (χ^2 = 4.283, P = 0.038) bovine TB cases than in large herds. The rates were significantly higher for the upgraded / exotic cattle than the Guadali (χ^2=4.971, P=0.026) and White Fulani

(χ^2=5.6, P=0.018) zebus. However, among the indigenous zebus, the disease was more prevalent in the Red Mbororo breed than the Guadali (χ^2=6.244, P=0.012) and White Fulani (χ^2=6.568, P=0.010). Comparable rates were noted between the upgraded / exotic and local Red Mbororo breeds (Figure 14B). Sex did not seem to influence (χ^2=0.410, P=0.522) prevalence rates of bovine TB (Figure 14C) but there was a higher trend towards diagnosing the presence of disease (X^2= 5.787; P=0.016) and more doubtful cases among adult / older cattle than in younger animals (Figures 14C and 15).

The different predicted variables for SICCT–BT test outcomes (Table 13) showed that sex, age and breed could have served as good indicators of the disease in the agro-ecological regions. Female cattle; adult / older animals; upgraded/exotic and Red Mbororo breeds were more likely to test positive for SICCT–BT than the male cattle; younger animals; Gudali and White Fulani cattle, respectively. Although the prevalence of SICCT–BT positive reactors was significantly higher (χ^2=17.50, P≤0.001) in the Western highland regions compared to the Adamawa plateaux, the different study sites on their own seem to pose little or similar risks (OR=0.0; RR=0.97 (95%CI; 0.96 – 0.98)). Detection of SICCT-AT positive reactors were also not influenced by the difference in regions (OR=0.0; RR=0.92 (95%CI: 0.91 – 0.94); χ^2=0.0145, P=0.904).

Table 12: Prevalence of bovine tuberculosis (SICCT-BT reactors) as influenced by study location, breed, sex, age group, management system and herd sizes

Variable	Label	No animals tested	No positive reactors	Prevalence, %(95% CI)
Total	All animals	2853	121	4.67 (3.89 - 5.44)
Agro-ecological Regions	Western Highlands	2126	104	5.38[a] (4.42 – 6.34)
	Adamawa plateau	727	17	2.57[b] (1.42 – 3.72)
Divisions / Study sites	Boyo	299	28	10.30[e] (6.86 – 13.75)
	Bui	468	22	5.17[f] (3.17 – 7.18)
	Donga-Mantung	145	1	0.76
	Menchum	508	8	1.73[g] (0.60 – 2.87)
	Mezam	327	37	12.45[e] (8.87 – 16.03)
	Momo	379	8	2.32[h] (0.81 – 3.84)
	Vina	727	17	2.57[h] (1.42 – 3.72)
Breed	Graded / exotic	368	26	7.77[i] (5.04 – 10.51)
	Guadali	1317	45	3.76[j] (2.73 – 4.79)
	Namchi	33	1	3.33
	Red Mbororo	487	32	7.23[i] (4.93 – 9.53)
	White Fulani	648	17	2.89[j] (1.60 – 4.18)
Sex	Female	2212	97	4.82[k] (3.93 – 5.72)
	Male	641	24	4.12[k] (2.58 – 5.66)
Age (years)	Age ≤ 2	613	18	3.23[l] (1.83 – 4.63)
	2 < Age ≤ 4	868	31	3.93[lm] (2.64 – 5.22)
	4 < Age ≤ 6	681	38	6.14[n] (4.34 – 7.94)
	Age > 6	691	34	5.41[mn] (3.73 – 7.10)
	Young (Age ≤ 4)	1481	49	3.64[o] (2.69 – 4.59)
	Adult (Age > 4)	1372	72	5.77[p] (4.54 – 7.01)
Management system	Extensive	1510	58	4.23[q] (3.21 – 5.24)
	Intensive	138	5	3.99[q] (0.72 – 7.25)
	Semi-intensive	1205	58	5.30[q] (4.03 – 6.56)
	Beef herds	2357	109	5.09[r] (4.20 – 5.97)
	Dairy herds	496	12	2.66[s] (1.25 – 4.08)
Herd sizes	Animals ≤ 40	1325	69	5.73[t] (4.48 – 6.98)
	Animals > 40	1528	52	3.74[u] (2.79 – 4.70)

a – u: different letters in a column are significant different (P<0.05)

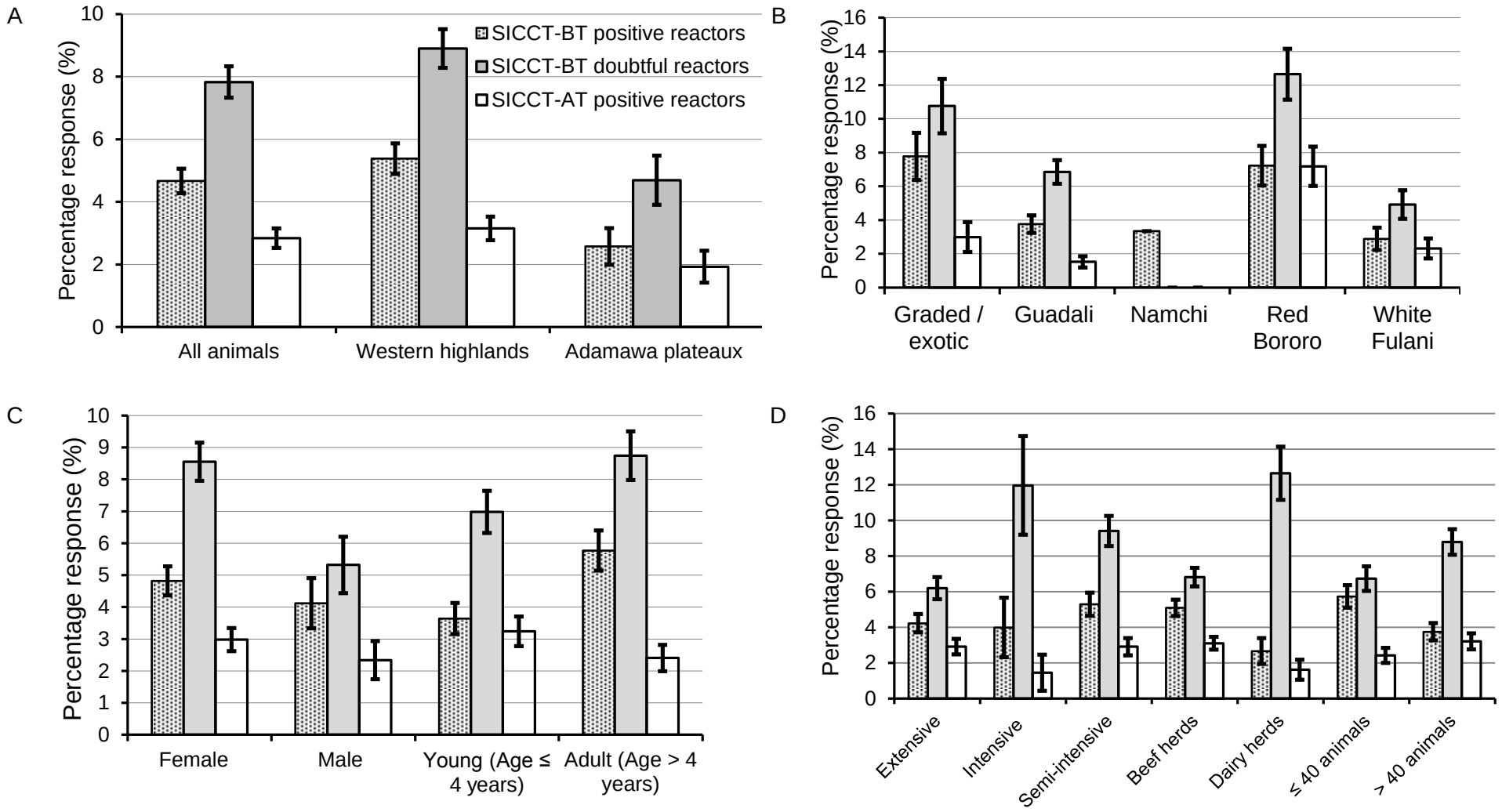


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Figure 14: Frequency distribution of single intradermal comparative cervical tuberculin skin test (SICCT) responses according to: (A) study location, (B) Breed, (C) Sex and Age group, (D) Management systems and Herd sizes.

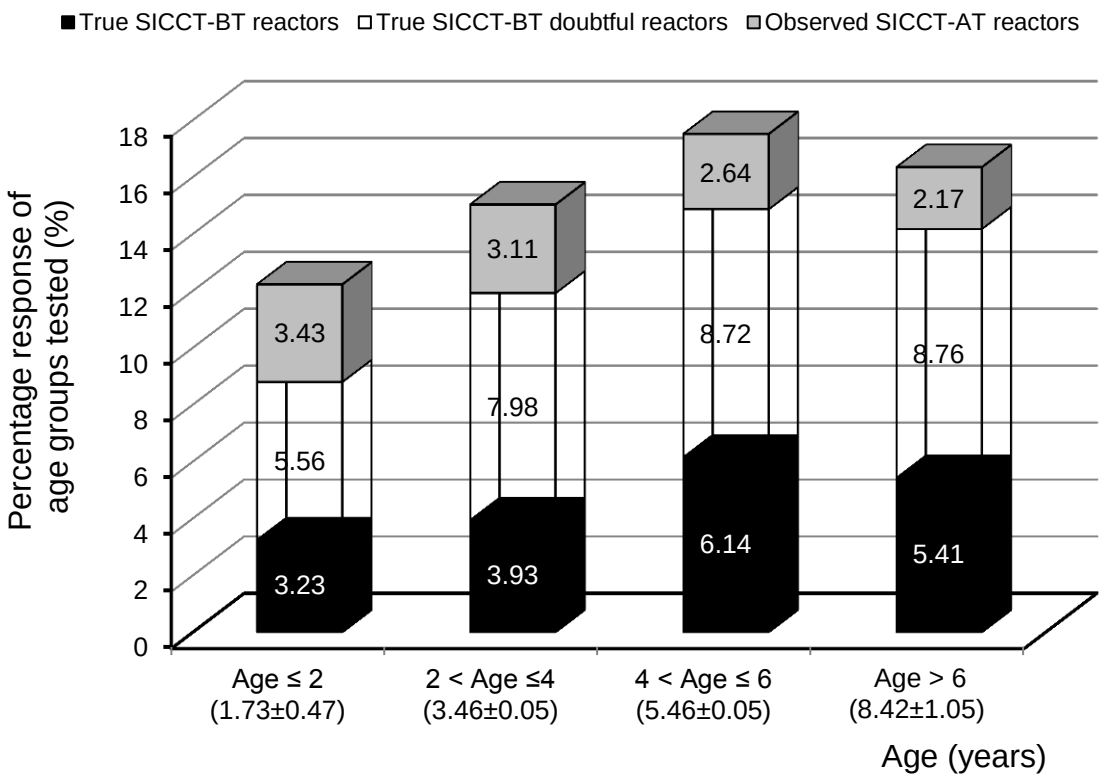

Figure 15: Variations of single intradermal comparative cervical tuberculin skin test (SICCT) responses according to age group

Table 13 : Association between skin response to SICCT-BT and different predicted variables

Variable	Odds ratio (95% CI)	Relative risk (95% CI)	χ^2 (P value)	Pearson's χ^2, (P value)
Guinean savannah plateau (ADP) vs. Sudano-guinea highlands (WHC)	0	0.975 (0.963 – 0.987)	0.487 (0.485)	710 (0.638)
Breed				
Upgraded/Exotic vs. Guadali	2.767 (0.573 – 13.348)	1.119 (0.868 – 1.444)	0.558 (0.455)	368 (0.446)
Upgraded/Exotic vs. White Fulani	1.328 (0.163 – 10.795)	1.023 (0.847 – 1.236)	0.110 (0.741)	368 (0.446)
Upgraded/Exotic vs. Red Mbororo	0	0.925 (0.897 – 0.953)	0.894 (0.344)	345 (0.767)
Guadali vs. Red Mbororo	3.819 (1.020 – 14.292)	1.074 (0.960 – 1.202)	2.570 (0.109)	487 (0.453)
Guadali vs. White Fulani	0	0.975 (0.962 – 0.987)	0.0162 (0.899)	631 (0.646)
White Fulani vs. Red Mbororo	1.097 (0.139 – 8.660)	1.006 (0.869 – 1.166)	0.211 (0.646)	487 (0.453)
Sex and Age				
Female vs. Male	1.234 (0.159 – 9.574)	1.008 (0.926 – 1.097)	0.137 (0.711)	641 (0.459)
Age ≤ 4 years vs. Age > 4 years	2.292 (0.877 – 5.987)	1.041 (0.976 – 1.109)	1.968 (0.161)	1372 (0.472)
Husbandry system and herd size				
Semi-intensive vs. Extensive	0.784 (0.186 – 3.303)	0.989 (0.936 – 1.046)	3.7×10^{-6} (0.998)	1205 (0.470)
Extensive vs. Intensive	0	0.962 (0.931 – 0.995)	0.604 (0.437)	133 (0.533)
Semi-intensive vs. Intensive	0	0.977 (0.953 – 1.003)	1.494 (0.222)	133 (0.533)
Dairy herds vs. Beef herds	0	0.965 (0.949 – 0.981)	0.0203 (0.887)	484 (0.605)
Herds ≤ 40 animals vs. Herds >40 animals	0.396 (0.0537 – 2.914)	0.968 (0.925 – 1.012)	0.366 (0.545)	1325 (0.472)

4.2.2.2 Bovine tuberculosis herd infection rates

Classification of herds with at least one test positive SICCT-BT and SIT-BT animal and showing major differences between agro-ecological zones and husbandry management systems is shown in Table 14. Significantly higher ($P<0.05$) herd prevalence rates for SICCT-BT and SIT-BT reactors were recorded in the sudano-guinean (Western highlands) region (68.53% and 86.89%, respectively) than in the guinea-savannah (Adamawa plateaux) region (38.26% and 73.91%, respectively), respectively. Large herds, extensive and beef production systems showed significant ($P<0.05$) herd risk factors compared to the other measured herd parameters.

Table 14 : Distribution of SICCT-BT positive herds (≥1 positive reactor)

Variable	Label	Herds tested	SICCT-BT Positive		SIT-BT positive	
			No	% (95%CI)	No	%
Total	All herds	84	46	60.24 (50.73 - 69.76)	70	83.33 (75.36-91.30)
Agro-ecological Regions	Sudano-guinea (Western Highlands)	61	38	68.53[a] (57.94 - 79.12)	53	86.89[a] (78.41-95.36)
	Guinean savannah (Adamawa plateau)	23	8	38.26[a] (20.21 - 56.32)	17	73.91[a] (89.80-51.60)
Management system	Extensive	44	30	75.01[a] (63.38 - 86.64)	39	88.64[a] (79.26-98.01)
	Intensive	4	2	55.01[b] (10.69 - 99.32)	3	75.00[b] (19.40-99.40)
	Semi-intensive	36	14	42.78[c] (28.09 - 57.47)	28	77.78[a] (64.20-91.36)
	Beef herds	69	40	63.77[a] (53.47 - 74.08)	58	84.06[a] (75.42-92.70)
	Dairy herds	15	6	44.00[b] (21.17 - 66.84)	12	80.00[b] (95.70-51.90)
Herd sizes	Animals ≤ 40	56	30	58.93[a] (47.22 - 70.65)	45	80.36[a] (69.95-90.76)
	Animals > 40	28	16	62.86[a] (46.60 - 79.13)	25	89.29[b] (99.10-76.50)

a – c: different letters in a class of labels are significantly different (P<0.05)
SICCT-BT: Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis
SIT-BT: Single Intradermal Tuberculin skin test for the diagnosis of bovine tuberculosis

4.2.2.3 Relationship between skin responses to bovine tuberculin and avian tuberculin

Positive skin responses to bovine tuberculin (SIT-BT: 12.21%) and avian tuberculin (SIT-AT: 8.76%) for the tested animals assessed with optimal skin swellings of ≥ 4 mm cut-off at the respective injections sites are shown in Figure 16 and Table 15.

The results showed that the prevalence of SIT-BT (χ^2=34.008, P≤0.001) and SIT-AT (χ^2=15.611, P≤0.001) positive reactors (Figure 15) were significantly higher in the western highlands than the Adamawa plateaux. A strong association was observed between the detection of SIT-BT and SIT-AT positive reactors (Table 15) [OR=121.17 (95%CI: 83.02 – 176.85); RR=4.42 (95%CI: 3.50 – 5.58); X^2=1499.942; P ≤0.001]. The trend of atypical (SIT-AT positives) and typical (SIT-BT positives) mycobacterial infections in cattle seemed to occur together and their prevalence rates also seemed not to be significantly different (X^2= 2.512; P=0.113) in the study regions (Figure 15). Overall, 6.83% of tested animal responded positively to both SIT-BT and SIT-AT. However, the tested animals were approximately four and half times more likely to present positive skin response to SIT-BT than to SIT-AT (RR = 4.416; 95%CI: 3.497–5.578; X^2=1499.942; P<0.001).

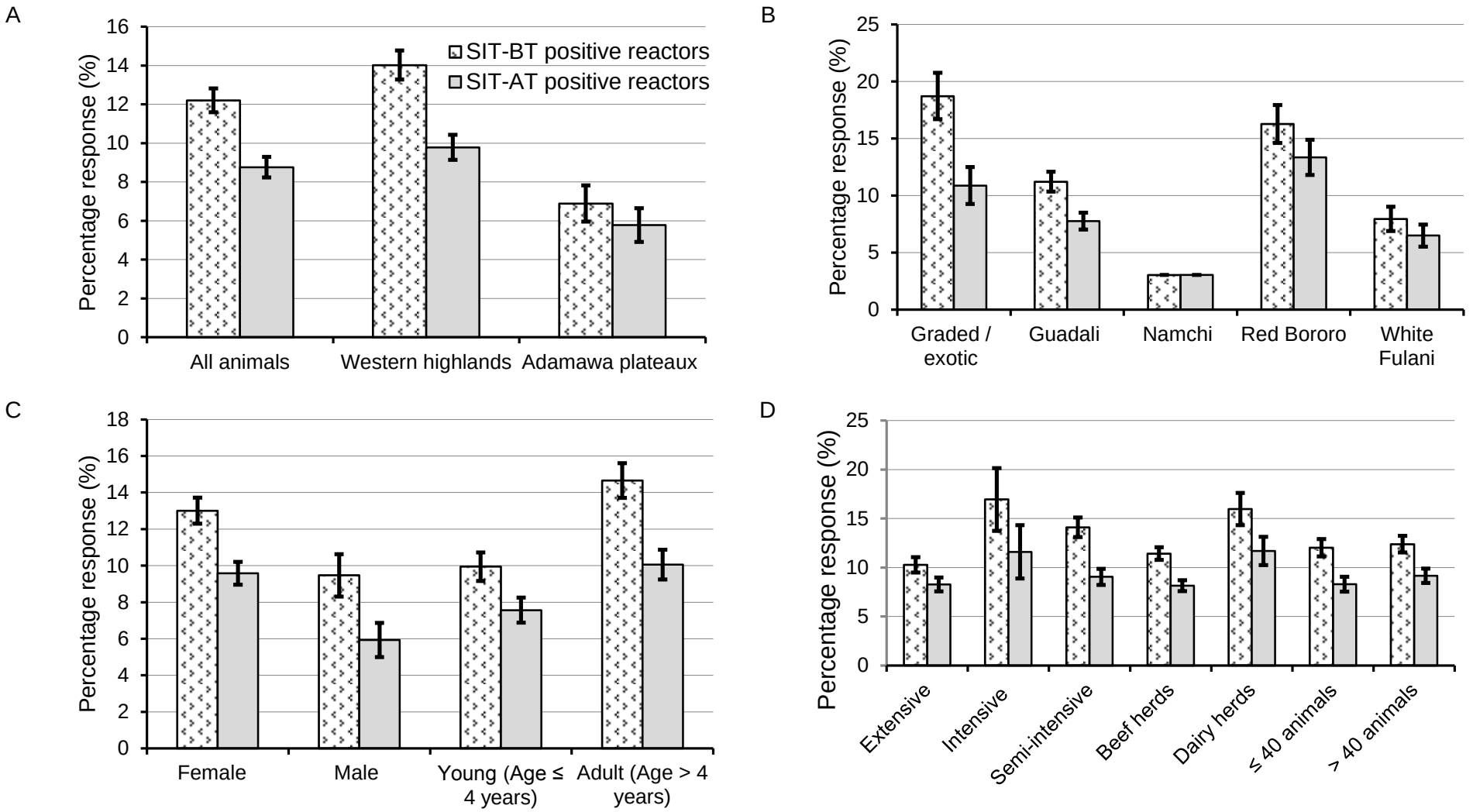

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Figure 16: Frequency distribution of single intradermal tuberculin skin (SIT) test responses according to: (A) study location, (B) Breed, (C) Sex and Age group, (D) Management systems and Herd sizes.

Table 15 : Association between individual responses to avian tuberculin and bovine tuberculin*

Test		Bovine PPD results, No (%)		Total, No (%)
		Positive	Negative	
Avian PPD results, No (%)	Positive	195 (6.83)	55 (1.93)	250 (8.76)
	Negative	74 (2.60)	2529 (88.64)	2603 (91.24)
	Total	269 (9.43)	2584 (90.57)	2853 (100)

* Positive and negative reactions were defined as skin indurations of ≥ 4 mm and < 4 mm, respectively. [OR=121.17 (95%CI: 83.02 – 176.85); RR=4.42 (95%CI: 3.50 – 5.58); X^2 = 1499.942; P ≤0.001]

4.3 Discussion

Livestock keeping was integral to the socio-economic, cultural and religious activities for most of the communities in the study (e.g. Foulbe, Fulani, Mbororo), close human-livestock interactions were also common and cattle owners placed great importance on the number of cattle an individual owned (Gwanfogbe et al. 1983). For example, the economy of the Adamawa region was noted to be almost entirely based on cattle (“Foulbe monopoly”) and this region's vast fields of grass made it ideal for grazing (Gwanfogbe et al. 1983; Pamo 2007).

Herdsmen rotated pastures, and often constructed watering holes or wells for themselves and their animals. Culturally too, the more head of cattle a man had, the wealthier he was considered and this often caused the herders not to exploit their herds, especially when there were disease outbreaks for fear of losing a portion of their net worth. However, many indigenous herders transported cattle to animal markets in big cities of the country (Bamenda, Ngaoundere, Douala, Yaoundé) and also across the borders to neighbouring countries (Nigeria, Central African Republic, Gabon, Chad) for trade and commerce.

4.3.1 Prevalence of bovine tuberculosis based on the detection of lesions in slaughtered cattle

The Bamenda municipal abattoir is the largest in the Northwest region of Cameroon and provides daily beef requirements to about one million inhabitants of the city, peri-urban areas and its neighbouring villages (rural areas). Cattle slaughtered at the abattoir were mainly of the Zebu type and originated from within the sudano-guninea Western highlands. With the exception of few parts

(e.g. horn and hooves), almost all other parts and organs of a carcass are edible in Cameroon if passed at slaughter and meat inspection. Prior to the study period, documented information on bovine TB during abattoir meat inspection in the region was sparse. However, the retrospective analysis showed that more tuberculous lesions than other pathologies were detected; and the main cause of condemnations at the abattoir was due to TB. The findings of this study are lower than those reported in other regions of the country by Awah-Ndukum et al (2005) who recorded the prevalence of bovine TB in slaughtered cattle to range from 0.67% – 4.28% and also observed that tuberculous lesions were 3 – 5 times more frequent than other lesions in slaughtered cattle in the western highland and littoral regions of Cameroon. Bovine TB is therefore endemic in Cameroon since the cattle slaughtered in these abattoirs are from the main cattle producing regions of the country including both highland regions in this study. Compared to data recorded elsewhere in Africa, the abattoir bovine TB prevalence rate recorded in this study was higher than 0.052% (Shitaye et al. 2006) but lower than other findings ranging from 0.49% to 11.3% (Du-Sai and Abdullahi 1994; Ankugah 2002; Ameni and Wudie 2003; Asseged et al. 2004; Diguimbaye-Djaibé et al. 2006; Müller et al. 2008a; Cadmus and Adesokan 2009; Ngandolo et al. 2009). These differences could be explained by many factors including the differences in the disease status in animal populations, level of implementing the disease control programme and various environmental influences.

The association noted between the increase in TB detection rate in recent years (especially from 2002 – 2010) could be associated to the increase in number of slaughter cattle (due to increasing public demands for meat) but it also coincided with additional recruitments of dynamic personnel and enhanced

efficiency of meat inspectors. Thus, the increasing trend recorded could not have been an actual increase of the disease state but probably due to improved diagnostic awareness of the disease and intensification of slaughter / meat inspection procedure. Edwards et al (1997) have argued that, while in need of modification, meat inspections still play an important role in meat safety and quality assurance for the consumer, and should also be based on the identification and analysis of risks. Indeed, limited progress with bovine TB eradication schemes in some industrialised countries and increasing non-tuberculous mycobacteria involvement in mycobacterial illness in animals and humans (Edwards et al. 1997; Biet et al. 2005) continue to justify the maintenance of intensive post mortem examination of carcasses (Edwards et al. 1997).

However, the wide fluctuations and numerous peaks of TB lesions occurrences in this study could be associated to an unchecked course of bovine TB in live cattle in the environment since there is no active control of the disease in Cameroon. Also, animals presenting poor symptoms of health and production characteristics are usually among the first to be removed from herds and slaughtered for meat production. Natural and arbitrary (human) selections of live animals therefore determined the semi-natural course of bovine TB in the herds, rearing communities and the detection rates in slaughtered animals. The improved meat inspection team from 2002 was more thorough and detected cases which otherwise would have been missed or “passed” by the former setup, though evidence associated with under-recording and under-detection could still be suggested. For example, it was not uncommon to note questionable summary meat inspection reports when compared to the crude abattoir registers. Poor clinical meat inspection records, which could not be

relied upon, have been reported elsewhere in Cameroon (Awah-Ndukum et al. 2005). These findings agree with earlier reports that post mortem surveillances for detection of bovine TB lesions in particular depend on the work load, time and diligence of the inspector conducting the examination (Corner et al. 1990; Edwards et al. 1997; Shitaye et al. 2006). The detection of TB lesions in slaughtered cattle can be affected by infections other than *M. bovis,* parasites, non-specific reactions (Corner 1994; Shitaye et al. 2006) and other irregularities of abattoir meat inspections (FAO 1994; Edwards et al. 1997) and lesions may be confused with those caused by *Nocardia*, *Corynebacterium* and other granuloma causing organisms (Blood and Radostits 1989; Gracey and Collins 1992; FAO 1994; Grist 2008). Thus, for meat inspection to offer an effective means of monitoring the level of bovine TB in cattle, all predilection tissues and organs should be thoroughly examined for detection of a single or multiple lesions (Grossklaus 1987; Hinton and Green 1997). The introduction of confirmatory mycobacteriological and other modern diagnostic techniques, along with intensification of meat inspection and tracing back of infected / suspicious cases to the herds of origin would be necessary for effective surveillance of TB in the study regions and whole of Cameroon. However, the importance of this study cannot be underestimated considering the zoonotic implication of bovine TB. Furthermore, most farmers shared the same micro-environments with their animals, thereby increasing the risk of exposure and spread of the disease from infected to uninfected or “clean” herds and people in the community.

4.3.2 Prevalence of bovine tuberculosis based on tuberculin skin tests

The findings of this study suggest high levels of infectiousness of the disease under the common practices of cattle management systems and humid tropical climatic conditions (Inangolet et al. 2008). Beef and small size herds also showed significantly higher SICCT-BT detection rates than dairy and large herds. However, inefficient close contact between diseased and healthy animals in the extensive systems, dairy herds and large herds; and possibly a decreased in virulence and transmission capacity of the causal strains due to adverse weather (Oloya et al. 2006) and low infectiousness of the local zebu cattle (Ameni et al. 2006; Ameni et al. 2007a; Inangolet et al. 2008) were also characteristics in the study environments. A better resistance or adapted tolerance to bovine TB infection of some local breeds have been suggested (O'Reilly and Daborn 1995; Ameni et al. 2008a) and could have been the reason for the lower prevalence rate recorded among the Namchi, Guadali and White Fulani compared to the Red bororo cattle. The difference in TB prevalence between the local breeds in this study was not understood and need further investigation. However, continuous close contacts between animals due to increasing animal and human population densities and limited pasture for grazing played additional roles in the higher prevalence rates recorded in the western highlands. The lower SICCT-BT prevalence rate (2.57%) in the Adamawa plateaux agreed with earlier findings of about 2.7% by Martrenchar et al. (1993) in the neighbouring Northern and Far Northern regions of the country. The Adamawa plateaux and Northern regions of Cameroon are characterised by lower population densities, abundant natural pasture and lower herd-herd (animal-animal) contacts.

Intensive management systems provide favourable conditions for bovine TB transmission by promoting closer and prolonged contacts between animals than the extensive systems (Ayele et al. 2004; Zinsstag et al. 2006; Inangolet et al. 2008). In this work, more positive responses to SICCT-BT were recorded among animals under the semi-intensive system compared to the other systems. In the extensive and semi-extensive systems the spread of the pathogen could have resulted from the closer contacts experienced during periods of drought at shared grazing, watering and salt leak points, as well as “cattle markets”, during veterinary interventions (vaccination campaigns) and other ventures that involved gathering different herds of animals. However, daily gathering of different herds at a site may not necessarily lead to a spread of bovine TB (Tschopp et al. 2009) but a few infective agents can still cause the disease in susceptible cattle (Francis 1971; Goodchild and Clifton-Hadley 2001; Cassidy 2006). The presence of a single or few animals shedding the disease agent (*M. bovis*) in their faeces, milk, discharging lesions, cough and aerosol sprays, saliva and urine would therefore be important sources for contamination and spread (O'Reilly and Daborn 1995; Cook et al. 1996; Asseged et al. 2004; Ayele et al. 2004). During transhumance there is crisscrossing of the regions by animals with implicating situations such as close and repeated animal to animal contacts, environmental stress factors and other conditions suitable for exposure and spread of the disease. It was also not uncommon to find returning *transhumance animals* mixed with the more stationed or semi-intensively reared animals. In this study, animal environments were generally humid or wet and favourable for long survival of TB agents (Goodchild and Clifton-Hadley 2001; Philips et al. 2003). Contrary results of lower bovine TB prevalence in cattle maintained in traditional pastoral systems in Uganda and Ethiopia were

associated with relative dryer conditions of the environments (Oloya et al. 2006; Ameni et al. 2007a; Inangolet et al. 2008).

Ayele et al. (2004), O'Reilly & Daborn (1995) and Omer et al. (2001) had reviewed situations of traditional pastoral systems in the tropics that create risks which are characteristic of intensive farming in relation to the transmission of bovine TB. For example, close contact between animals occurred in shared micro-environments and gathering of animals at common spots. They further noted that nose-to-nose or mouth-to-mouth contacts between animals was high at these points; and animals also tend to concentrate under trees or shaded areas for most parts of the day due to higher ambient temperature in tropical zones, preferring to graze early in the morning and late in the afternoon. In this study, small herds and beef herds were severely affected by bovine TB but all herds were characterised by the above risk factors though at different levels of intensity.

For the single intradermal tuberculin test (SIT) results, the SIT-BT prevalence rate for the Adamawa region in this study (6.89%) was lower than 10.6% reported for the neighbouring Northern and Far Northern regions (Martrenchar et al. 1993). Also, the SIT-BT prevalence rate of 14% recorded for the Northwest region is lower than 26% reported earlier in the peri-urban centre of Bamenda (Muchaal 2002) on a sample of exotic breeds, their crosses and some local zebus with no observed test positive response among the zebus. Furthermore, this study recorded significantly higher SIT-BT rates according to management systems (10.27% for extensive; 16.94% for Intensive and 14.10% for semi-intensive) which were not in agreement with the SIT-BT prevalence rates 3% and 13%in the mid-80s recorded by Merlin & Tsangueu (1985) in the

Northwest region of for animals on extensive and ranch farming, respectively; and of 1.4% by Tanya et al (1985) in the Adamawa region for local cattle (Guadali) on an experimental livestock station and 2.8% for dairy cows composed of Holsteins and their crosses. Contrary to the SICCT-BT results of this study and earlier SIT-BT findings of Merlin & Tsangueu (1985) and Muchaal (2002) in Northwest Cameroon, significantly higher SIT-BT prevalence of positive reactors were recorded in intensive settings followed by semi-intensive and extensive systems in the present study. Unlike using the intensive, semi-intensive and extensive husbandry and management systems as in this study, animals used in earlier studies were those on the extensive system by Martrenchar et al. (1993), extensive and ranching by Merlin & Tsangueu (1985) and “zero grazing” husbandry by Muchaal (2002) as well as animals in experimental livestock stations by Tanya et al (1985) and Nfi & Ndi (1997). Therefore, the wide contrast in bovine TB prevalence rates recorded by various studies was related to the differences in the husbandry and management practices. Due to repeated and close contacts between animals (feeding and drinking spots, shelters and gathering of animals during veterinary manipulations), the transmission of bovine TB between infected and susceptible animals would be expected to be highest in the intensive followed by the semi-intensive, ranching and extensive systems in descending order.

Furthermore, the SIT-BT showed that TB prevalence varied due to sex, age, breed, husbandry systems and study site but not by the herd size. SICCT-BT and SIT-BT test results showed similar trends for study locations, age, sex and breed of cattle. For both tests, the disease was severe in the Western highlands than the Adamawa plateaux and age was a significant risk factor than sex. Also, exotic and Red Mbororo cattle showed the highest prevalence rates. The

observation of age and sex (Ameni et al. 2003; Oloya et al. 2006; Ameni et al. 2007a; Inangolet et al. 2008), herd size and body condition (Ameni et al. 2003), husbandry system and breed (Ameni et al. 2006; Ameni et al. 2007a) as important risk factors influencing individual bovine TB prevalence rates have been reported. However, Ameni et al. (2003) and Oloya et al. (2006) have suggested sex as a weak risk factor as in this study where more female than male animals were non-significantly affected. The chronic nature of bovine TB, low transmissibility of the disease in an extensive / transhumance system, delayed onset of tuberculin positive response in adult and old animals has been attributed to the long incubation period the disease, pre-allergenic status, and acquired and maternal immunity (Kazwala et al. 2001b; Oloya et al. 2006). These factors were also noted as important risk factors in this study. Contrary SICCT-BT and SIT-BT observations were noted for husbandry practices (herd size, management and type of production systems). Significantly higher SICCT-BT rates were recorded in large herds and comparable values in the different husbandries. The introduction and propagation of bovine TB in large herds in the study regions were favoured by potentially more contacts and other conditions for transmission of the disease between the infected and uninfected (susceptible) animals. However, SIT-BT detection rates were high and comparable between small and large herds but dairy animals and animals in intensive and semi-intensive managements were significantly affected than the others. Nonetheless, severe interpretations of the SICCT-BT test could give comparable bovine TB detection rates between small and large herds.

Widespread SICCT-AT and SIT-AT positive responses were recorded in the study with the prevalence rates in some areas and herds being almost equal to those of SICCT-BT and SIT-BT, respectively. The influence of atypical or

environmental mycobacteria and non-specific responses on TST for the diagnosis of bovine TB have been widely reported (Philips et al. 2003; Biet et al. 2005; de la Rua-Domenech et al. 2006a; Oloya et al. 2006; OIE 2009). Indeed, Lesslie et al (1975; 1975a; 1975b) recorded hypersensitivity responses to AT that was equal or higher than responses to BT in cattle naturally infected with *M. bovis* and presenting visible lesions at slaughter. This confirmed that the nature of the tuberculin is vital in non-specific skin test responses for the diagnosis of bovine TB; and the use of *M. bovis* specific purified antigen as the test reagent is imperative for improved test performances (Pollock et al. 2003; de la Rua-Domenech et al. 2006a).

Though not statistically significant, the observed prevalence of SICCT-AT reactors was higher in young cattle but SICCT-BT doubtful reactions increased with age or were higher among the adult and/or old cattle. This finding agrees with that of Chacon et al. (2004) that cattle are very susceptible to *M. avium* infection and the young ones are most affected. The rearing of poultry and small ruminant (sheep and goats), which are the natural and good reservoir hosts of *M. avium* complex (Biet et al. 2005) was common among the farmers. Infected free range poultry could have contaminated the animal/human microenvironments, pastures and watering points by shedding *atypical mycobacterium* in their faeces. Goats and sheep could have also served as additional sources of infection. Large groups of goats and sheep frequently sharing the same grazing and drinking environments with cattle were observed in this study. The bias reactions toward SICCT-AT test was linked to environmental mycobacteria or non-specific immune response to agents in the animal environments (Philips et al. 2003; Biet et al. 2005; OIE 2009). The shift from more positive SICCT-AT in the young animals (or in early years) to more

doubtful SICCT-BT status in adults/old animals (or later years) was therefore in accordance with the theory stated by Lauzi and co-workers (Oloya et al. 2006) that non-specific immune responses induced by atypical mycobacteria towards BT overcame the effect of contamination by *M. avium* complex with increasing age.

Anergy has been reported to cause false negative reactions during TST but the reasons are still poorly understood (Inangolet et al. 2008). However, recently infected cattle, cattle under stress due to malnutrition, gastrointestinal parasitoses, other concurrent infections and cattle with generalized TB would be anergic and fail to react to TST (Ameni and Medhin 2000; Inangolet et al. 2008). The tuberculin skin screening period in this study coincided with the end of the dry season and return of animal from transhumance. Stress due to starvation, long trekking, other environmental stressors and clinical symptoms of ill-health due to trypanosomosis, tick-borne diseases, heavy ectoparasitoses and gastro-intestinal parasitoses were common in the herds of this study. These conditions could have contributed at varying degrees to the observed higher rates of doubtful SICCT-BT reactors; and the widespread SICCT-AT and SIT-AT reactors. Alternatively, lack of SICCT-BT response or SICCT-BT doubtful reactions in old animals could have been due to age related anergy and other conditions that compromised their immune function such as stress (Thoen et al. 2009); and the animal's immune system was not stimulated enough for a positive response to be measured (Ameni and Medhin 2000; Inangolet et al. 2008). However, variation in both incidence and prevalence between geographic regions and herds could also be a consequence of the management system used and the risks these offer for transmission and development of the tubercle bacilli infections (Edwards et al. 1997). In agreement with the report of

Morris et al. (1994), there could be specific environmental and management factors in the herds and regions that also contributed to the variations observed in this study.

4.4 Conclusion

The study reports the first comparative TST in cattle in the highlands of Cameroon and confirmed that bovine TB is an existing livestock health and production problem in Cameroon which needs to be further investigated. Bovine TB prevalence rates based on meat inspection showed that the disease was widespread while TST confirmed high prevalence rates in live cattle in both Western and Adamawa highland regions where cattle population is dense compared to other parts of the country. However, it would be important to investigate the performance of the TST at different cut-off points for maximum and more accurate detection of bovine TB in cattle in the Cameroon environment.

The variable levels of bovine TB infectiousness within and between cattle breeds in the study could be due to innate resistance or adapted tolerance mechanisms in the indigenous Zebu breeds. Further investigations of the relative susceptibility and genetic resistance of indigenous zebus to bovine TB are needed for clarity. The habits and level of awareness of cattle professionals and handlers of cattle products on the significance of zoonotic bovine TB; as well as the hygienic status of cattle farms and abattoir environments as potential risk factors for zoonotic TB disease are not known. A comprehensive research of the molecular epidemiology, risk factors, reservoir and maintenance hosts' status and public implications of zoonotic bovine TB in cattle in Cameroon cannot be overemphasized.

Chapter 5

Comparison of different tuberculin skin test cut-off points and lateral flow assay for the diagnosis of bovine tuberculosis in Cameroonian cattle

5.1 Introduction

The Single Intradermal Tuberculin (SIT) and Single Intradermal Comparative Cervical Tuberculin (SICCT) skin tests are currently the best available techniques for international field diagnosis of bovine TB in live animals (de la Rua-Domenech et al. 2006a; de la Rua-Domenech et al. 2006b). Also, it is based on delayed hypersensitivity reactions (OIE 2009). The SICCT skin test involving the intradermal injection of bovine tuberculin (BT) and avian tuberculin (AT) at separate sites in the skin of the neck, gives more specific results than SIT which uses only BT (Francis et al. 1973; Monaghan et al. 1994). The World Organisation for Animal Health (Office Internationale des Epizooties – OIE) recommended difference between the increase in skin thickness for the test to be positive should be >4mm after 72 hours (OIE 2009). However, the OIE-recommended cut-off value was established mainly in developed countries for *Bos taurus* cattle and different cut-off values are applied according to a particular country's disease status and objective of its disease control programme (Monaghan et al. 1994). For example, the >2 mm, ≥3 mm, >4 mm and ≥4mm cut-off points have been used in Chad, Ethiopia and Tanzania (Monaghan et al. 1994; Kazwala et al. 2001b; Delafosse et al. 2002; Ameni et al. 2008b; Ngandolo et al. 2009). The performance of TST could also be affected

by environmental factors, host factors, (status of immunity, genetics, etc.) and the nature of the tuberculin used (Francis et al. 1973; Monaghan et al. 1994; de la Rua-Domenech et al. 2006b; Ameni et al. 2008b). A perfect cut-off point in a specific geographic area may not be so useful at another environment (Monaghan et al. 1994; de la Rua-Domenech et al. 2006b) and the ability of the test to accurately predict the true positive disease status depends on its sensitivity, specificity and prevalence of the disease in the population tested (de la Rua-Domenech et al. 2006b). Furthermore, severe interpretations were done in regions or herds where *M. bovis* infection had been confirmed based on the discretion of the veterinarian while SIT-BT positive reactors may also be subjected to a SICCT-BT test (Monaghan et al. 1994).

TST together with slaughter of positive reactors to examine for TB lesions; culture of suspected tuberculous specimens and other modern diagnostic techniques (eg: gamma-Interferon, ESAT-6 tests, serologic and fluorescence polarization assays) have been compared and are being validated for maximum diagnosis of bovine TB in cattle in various environmental conditions (Amadori et al. 1998; Pollock et al. 2003; de la Rua-Domenech et al. 2006a; de la Rua-Domenech et al. 2006b; Thom et al. 2006; Buddle et al. 2009). Post mortem detection of TB lesions and other bovine TB diagnostic techniques have been used to determine the performance of TST around the world, including some parts of Africa (Table 16).

Table 16 : Estimates of sensitivity and specificity (and sample sizes) of tuberculin skin tests and reference tests used for the diagnosis of bovine tuberculosis in cattle in some African countries

Bovine tuberculin	Avian tuberculin	Number of animal tested	Observed prevalence of disease (%)	Cut-off point at interpretation	Measure to define disease status used for comparison	Sensitivity (%; 95%CI)	Specificity (%;95%CI)	Reference (country)
2000IU	2500IU	30 zebu oxen	73.3	>4mm	Lesions occurrence and Acid fast staining (mycobacterial culture and histopathology	90.9 (69.4 – 98.4)	100 (59.8 – 100)	Ameni et al (2000) (Ethiopia)
2500IU	2500IU	186 cattle (161 Zebu and 25 Holstein)	No details for test animals (Earlier test in 5424 cattle: 13.5% for >2mm & 16.0% for >4mm)	>4mm >3mm >2mm	Post mortem detection of TB lesions	59.4 (48.9 – 69.3) 64.6 (54.2 – 74.1) 68.8 (58.5 – 77.8)	96.9 (89.3 – 99.6) 96.9 (89.3 – 99.6) 96.9 (89.3 – 99.6)	Ameni et al., (2008b) (Ethiopia)
2000IU	2500IU	100 zebu cattle (*moderate prevalence* group)	Range by veterinary district: 21.0 (0 – 30)	Subjective assessment (palpation of injection sites)	Subjective assessment (palpation of injection sites and observation of clinical signs)	For 2 groups (a, b) of animals destined for slaughter[#]: a : 52.0 b : 80 (44 – 98)	For 2 groups (a, b) of animals destined for slaughter: a : 99.0 b : 100 (74 – 100)	Quirin et al. (2001) (Madagascar)
2000IU	2500IU	22 (*high prevalence* group)	45.5	Subjective assessment (palpation of injection sites)	Subjective assessment (palpation of injection sites and observation of clinical signs)	80.0 (44 – 98)	100 (74 – 100)	Quirin et al. (2001) (Madagascar)
2000IU	Not Used	848 cattle in 58 dairy herds	3.66	≥4mm	Based on Quirin et al. (2001); OIE standard and ENV, Lyon*. Assumes Se and Sp >90%.	94 (90 – 100)	97 (90 – 100)	Delafosse et al (2002) (Chad)
2000IU	2500IU	151 (re-testing of SIT-BT positive & doubtful reactors; n=848)	17.34	≥4mm	Based on Quirin et al. (2001) and ENV, Lyon*. Assumes Se=50% – 80% and Sp >95%	82 (50 – 80)	99 (95 – 100)	Delafosse et al (2002) (Chad)
5000IU	2500IU	930	7.7 (6.2 – 9.6) 15.5 (13.3 – 18.0)	>4mm >2mm	Post mortem detection of TB lesions	20.0 (5.7 – 43.7) 65.0 (43.3 – 81.9)	93.1 (91.1 – 94.6) 86.7 (84.2 – 88.9)	Ngandolo et al., (2009) (Chad)
5000IU	2500IU	930 (929 data available)	7.7 15.5	>4mm >2mm	Bayesian modelling approach	51.1 (42.1 – 60.1) 66.3 – (57.5 – 74.6)	98.6 (97.9 – 99.2) 89.2 (86.6 – 91.5)	Müller et al (2009b) (Chad)
0.1ml (20,000 UCT/ml)	Not Used	14,353 animals in 340 herds	6.0 (5.6 – 6.5) (For range of True prevalence: 10 – 23 8 – 19 5 – 15)	>4mm	Based on Quirin et al. (2001) and consideration of Sensitivity as 100% to calculate true herd prevalence	100 For ranges of true prevalence[§] range of 10 15.8%: 100 – 91	98 For ranges of true prevalence[§] stated: 100 98 95	Bernard et al. (2005) (Uganda)

*: La tuberculose, 1990. Lyon, France, Ecoles nationales vétérinaires françaises, 152 p. ;

# : a = highest probability of non-infected expected (Sensitivity = 52% [n=21] ; specificity = 99% [n=71]) ;
b = animals selected on the basis of apparent ill-health in a high prevalent bovine TB area (Sensitivity = 80% [n=10] ; specificity = 100% [n=12])

§ : For true prevalence : Pt = 10% ; Sensitivity = 100% ; specificity = 95% and Pt = 15.8% ; Sensitivity = 91% ; specificity = 99% (ie for true prevalence range varying from 10 – 15.8% ; Sensitivity = 100 – 91% and specificity = 95 – 99% respectively.)

There is scanty documentation of the prevalence of bovine TB in Cameroon and earlier TST (SIT and SICCT) in cattle have applied various standards (Table 17); and obtained diverse results even for the same sites. While Tanya et al (1985) applied the OIE-recommended optimal 4mm cut-off, modified OIE-standards applicable in France (Martrenchar et al. 1993) and Canada (Muchaal 2002) have also been used to interpret TST results in Cameroon. Furthermore, sensitivity and specificity data for SICCT obtained in Ethiopia (Ameni et al. 2000; Ameni et al. 2008b) and of 100% apparent prevalence rate for SIT (Pollock et al. 2003) have been used to correct observed prevalence rates in the study regions (see Chapter 4: Section: 4.2.2). In all, these results have varied widely and necessitated the reassessment of the performance and accuracy of the tuberculin tests at different cut-off points based on the local environmental and specific animal population context in Cameroon, where the disease is widespread.

TST can effectively detect early stages of *M. bovis* infection in cattle which allows for rapid removal of infected animals, limited transmission of the disease and fast eradication of bovine TB (Buddle et al. 2009). The tests may demand physical exertion in the field but it is also simple, relatively inexpensive and offers reliable means of screening entire cattle populations (Monaghan et al. 1994; Buddle et al. 2009). Ancillary tests are being used, and or currently being validated to provide more accurate diagnosis and reduce the number of false positives following skin-testing (de la Rua-Domenech et al. 2006a; de la Rua-Domenech et al. 2006b; Buddle et al. 2009; Ngandolo et al. 2009).

Table 17 : Current prevalence status of bovine tuberculosis in cattle in Cameroon

Region	Number of animals	Prevalence (%)				Authors (Test interpretation standard used)
		Slaughter / Meat inspection	SIT	SICCT	Acid fast stain and culture of lesions and Antibovine TB Ab*	
North and Far North regions	890		10.6	2.7		Martrenchar et al. (1993) (Ministry of Agriculture; France; Order: 07/11/90)
Northwest region	2,492		3 (extensive farms) 13 (Ranch animals)			Merlin & Tsangueu (1985) (OIE Standard cut-off point)
West Region (Experimental livestock station)	142			14.8 (42% Zebu; 9.02% crosses)		Nfi and Ndi (1997) (OIE Standard cut-off point)
Adamawa region (Experimental livestock station)	1,395		1.4 (Zebu cattle) 2.8 (Holstein and their crosses)			Tanya et al. (1985) (OIE Standard cut-off point)
Northwest region (towns & villages of Bamenda)	166 (48% exotic, 28% Zebu, 20% crosses)		26 (95%CI; 11–41)			Muchaal (2002) (Canadian Food Inspection Agency field protocol)
Douala abattoir	385,784	0.82 [1995-2003]				Awah-Ndukum et al., (2010) (Post mortem detection of suspect TB lesions)
Bamenda abattoir	45,737	0.18 [1995-2003]				
Bamenda abattoir	33,835	0.6 [2006-2008]				
Dschang abattoir	1,460	4.2 [2006-2008]				
Bamenda abattoir	39 (Zebu)				31 (Acid fast staining) 51 (Culture & Acid fast stain)	Awah-Ndukum et al., (2010) (OIE and WHO standards)
Bamenda abattoir	90 (Zebu)				60 (Antibovine TB Ab)	Awah-Ndukum et al., (2010) (Manufacture protocol)
WHC and ADP	2,853	/	12.21 (11.01-13.41)	5.38 (4.42-6.34)		Present study: Earlier screening in 2009 (Chapter 4) – OIE Standard cut-off point
Northwest region	2,126	/	14.03(12.55-15.51)	2.57 (1.42-3.72)		
Adamawa (Vina)	727	0.46 (0.43-0.50) [1994-2010]	6.89 (5.05-8.73)			
Bamenda abattoir	129,165					
					Antibovine TB Ab :	
WHC and ADP	1,381		30.56 (25.80-35.32)	5.91 (4.67-7.15)	/	Present study: Second screening done in 2010 (OIE standard cut-off point and Manufacturer's protocol)
WHC and ADP	807		28.55 (25.43-31.67)	4.98 (3.48-6.48)	37.17 (30.64-43.71)	
Northwest region	444 (Zebu/exotic)		51.32 (46.67-55.97)	9.14(6.46-11.82)	43.24 (34.21-52.28)	
Northwest region	1,018(Zebu/exotic)		41.27 (35.34-47.20)	8.06 (6.39-9.73)	/	
Adamawa (Vina)	363 (Zebu)		0.55 (0-1.31)	0.28 (0-0.81)	29.75 (20.53-38.97)	

*: Antibovine tuberculosis antibody assay; [ ]: duration of prevalence study;
SIT: Single Intradermal Tuberculin skin test;
SICCT: Single Intradermal Comparative Cervical Tuberculin skin test
WHC and ADP = highlands of Cameroon (Western highlands and Adamawa plateaux)

A rapid and simple immune-chromatographic assay for the serodiagnosis of bovine TB has been developed (Lyashchenko et al. 1998; Lyashchenko et al. 2004) and proposed as an additional test to the TST for maximum *ante-mortem* diagnosis (Pollock et al. 2005; de la Rua-Domenech et al. 2006a; Ameni et al. 2010a). These chromatographic immunoassays employ cocktails of selected *M. bovis* antigens immobilized on a test strip as both qualitative capture and detectors of specific antibodies against *M. bovis* in plasma, serum and whole blood. The bound antibodies are visualized with the naked eye as a coloured band in the test device within some minutes of application. The assay requires no specific expertise or equipment and may be kept without the need for refrigeration (Lyashchenko et al. 1998; Lyashchenko et al. 2004; Wernery et al. 2007).

The study was therefore carried out to determine the level of exposure of Cameroonian cattle to bovine TB through antibovine TB antibodies detection assay and re-assess various tuberculin test cut-off points to estimate the status of bovine TB in the highland regions of Cameroon. The diagnostic performance, accuracy and implication for applying the TST at various cut-off points were compared for a predominant Zebu cattle population and are discussed. In addition, the prevalence of SICCT-BT bovine TB in 2,853 previously tested cattle in the year 2009 (Chapter 4 of this work) were reanalysed using the results obtained in this chapter's study.

5.2 Results

The materials and methods employed to perform antibovine TB antibody assay and TST in cattle have been described in Chapter 3 (section 3.1 to section 3.3.3 and section 3.8, section 3.8.1 and section 3.8.2).

5.2.1 Observed prevalence rates and agreements between lateral flow assay and tuberculin skin tests at various cut-off points

Anti-bovine TB Ab was detected in 37.17% (95%CI: 30.64 – 43.71) of 807 tested animals. About 11.77% (95%CI: 9.55-14.00), 8.92% (95%CI: 6.96-10.88) and 3.59% (95%CI: 2.31-4.88) of tested cattle were SICCT-BT positive at ≥2-mm, ≥3-mm and ≥4-mm cut-off points, respectively. The proportion of SICCT-BT/anti-bovine TB Ab reacting cattle was highest ($P<0.05$) at the ≥2-mm [9.42% (95%CI: 7.40-11.43)] followed by the ≥3-mm [7.93% (95%CI: 6.07-9.79)] and ≥4-mm [3.59% (95%CI: 2.31-4.88)] cut-off groups. Overall, 0.62% (95%CI: 0.08% – 1.16%), 3.47% (95%CI: 2.21% - 4.73%) and 8.80% (95%CI: 6.84% – 10.75%) of the tested cattle showed SICCT-BT doubtful results and 0.62% (95%CI: 0.08% – 1.16%), 2.11% (95%CI: 1.12% – 3.10%) and 7.81% (95%CI: 5.96% – 9.66%) reactors were SICCT-BT doubtful / anti-bovine TB Ab positive at the ≥2-mm, ≥3-mm and ≥4-mm cut-off points, respectively. Also, over 27.14% (95%CI: 24.07% – 30.21%) negative SICCT-BT reactors were also positive for anti-bovine TB Ab.

Furthermore, 13.14% (95%CI: 10.80% – 15.47%) SIT-BT and 10.04% (95%CI: 7.96-12.11) SIT-BT/anti-bovine TB Ab positive reactors were recorded. Among the SIT-BT positive reactors, 76.42% were anit-bovine TB Ab positive reactors and 89.62 ± 2.96%, 67.92 ± 4.53% and 27.36 ± 4.33%, were SICCT-BT reactors while 71.70%, 60.38% and 27.36% were SICCT-BT/anti-bovine TB Ab reactors at the ≥2-mm; ≥3-mm and ≥4-mm cut-off points, respectively. However, analysis of the anti-bovine TB Ab positive reactors (n=300) revealed observed prevalent rates of SICCT-BT positive reactors of 25.33%, 21.33%, 9.67% and

27.00% at the ≥ 2-mm, ≥ 3-mm and ≥ 4-mm cut-off points and SIT-BT, respectively.

The results of observed TST at various cut-off points and anti-bovine TB Ab assay in 807 cattle are summarized in Table 18. Overall, about 3.84% of test animals (n=31) considered as SICCT BT doubtful and SIT BT positive reactors at the ≥ 4-mm cut-off point were also classed as SICCT-BT positive reactors at ≥ 3-mm (2.97%) and ≥ 2-mm (3.84%) cut-off points, respectively.

In all, the concordances (TST positive/antibovine TB Ab positive) were 100%, 88.89%, 80% and 76.42% in positive subjects at SICCT-BT ≥ 4 mm, ≥ 3 mm and ≥ 2 mm cut-off points and SIT-BT, respectively (Table 19). The discordances (TST negative/antibovine TB Ab positive) were 34.83%, 32.11%, 31.46% and 31.24%, at the SICCT-BT ≥ 4 mm, ≥ 3 mm and ≥ 2 mm cut-off points and SIT-BT, respectively. However, the concordances (TST positive/antibovine TB Ab positive) in antibovine TB Ab positive subjects were 9.67%, 21.33%, 25.33% and 27% while the discordances (TST negative/antibovine TB Ab positive) were 94%, 78.67%, 74.67% and 73%, at the SICCT-BT ≥ 4 mm, ≥ 3 mm and ≥ 2 mm cut-off points and SIT-BT, respectively. Thus, the recommended "bench marks[10]" for evaluating points estimates of *kappa* values (Thrusfield 2007) revealed a poor agreement between SICCT-BT test and anti-bovine TB Ab assay at the ≥ 4 mm skin response cut-off point and fair agreements at the other cut-off points (≥ 3 mm & ≥ 2 mm; and SIT-BT) in this study.

[10] >0.80: very good agreement; 0.61 – 0.80: good agreement; 0.41 – 0.60: moderate agreement; 0.21 – 0.40 fair agreement and ≤ 0.20: poor agreement

Table 18 : Apparent proportions of tuberculin skin tests and anti-bovine tuberculosis antibodies assay (according to region, sex, age and herd size) at various cut-off points in cattle in Cameroon

Variable / Label	No of animals tested	SICCT-BT reactors (%)			SIT-BT reactor	D = SICCT BT doubtful response / SIT-BT reactor (%)			d = SICCT BT doubtful / SIT BT doubtful responses (%)			Excess A* (%)	% of SICCT-BT doubtful and classed positive at inferior cut-off points (%)		Anti-bovine TB Ab reactors# (%±SE)
		T4	T3	T2		XD4	XD3	XD2	Xd4	Xd3	Xd2		XD4 at ≥ 3 mm	XD4+XD3 at ≥ 2 mm	
All animals	807	3.59	8.92	11.77	13.14	3.72	0.87	0.50	5.08	2.60	0.12	4.46	2.97	3.84	37.17±3.33
Agro-ecological Regions															
Adamawa plateaux	363	0.28	0.28	0.55	0.55	0.28	0.28	0.00	0.28	0.28	0.28	0.00	0.00	0.28	29.75±4.70
Western Highlands	444	6.31	15.99	20.95	23.42	6.53	1.35	0.90	9.01	4.50	0.00	8.11	5.41	6.76	43.24±4.61
Sex and Age															
Female	647	4.02	8.96	11.44	13.14	4.17	1.08	0.62	4.64	2.16	0.15	4.48	3.25	4.33	36.32±3.71
Male	160	1.88	8.75	13.13	13.13	1.88	0.00	0.00	6.88	4.38	0.00	4.38	1.88	1.88	40.63±7.61
Young (≤ 4 years)	481	3.33	8.32	11.02	11.85	3.33	0.42	0.21	4.99	2.49	0.00	3.53	2.91	3.33	38.46±4.35
Adult (> 4 years)	326	3.99	9.82	12.88	15.03	4.29	1.53	0.92	5.21	2.76	0.31	5.83	3.07	4.60	35.28±5.19
Herd sizes															
Animals ≤ 40	169	5.92	10.06	12.43	13.02	6.51	1.18	0.59	2.96	1.78	0.00	4.14	5.92	7.10	28.99±6.84
Animals > 40	638	2.98	8.62	11.60	13.17	2.98	0.78	0.47	5.64	2.82	0.16	4.55	2.19	2.98	39.34±3.79

T4, T3, T2, XD4, XD3, XD2, Xd4, Xd3, Xd2 and Excess A are defined earlier in Figure 11 (Section: 3.3.3).
SICCT-BT = Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis
SIT-BT = Single Intradermal Tuberculin skin test for the diagnosis of bovine tuberculosis
Anti-bovine TB Ab = Anti-bovine tuberculosis Antibody assay
#: **Breed**: Upgraded/Exotic = 42.03 ± 6.72; Gudali = 31.30 ± 4.10; Namchi = 22.58 ± 14.72; Red Bororo = 67.53 ± 10.46
Management and production system: Extensive = 31.76 ± 4.13; Intensive = 75.00 ± 30.01; Semi-intensive = 44.69 ± 5.53;
Beef herds = 38.07 ± 3.70; Dairy herds = 33.10 ± 7.66

Table 19 : Agreement between tuberculin skin tests and antibovine tuberculosis antibodies assay in detecting bovine tuberculosis according to tuberculin skin response cut-off points

	SICCT-BT cut-off points						SIT-BT	
	≥ 4 mm		≥ 3 mm		≥ 2 mm		≥ 4 mm	
	number	%	number	%	Number	%	number	%
TST positive / Anti-BTB Ab positive	29	3.59	64	7.93	76	9.42	81	10.04
TST negative / Anti-BTB Ab positive	271	34.82	236	29.24	224	27.76	219	27.14
TST positive / Anti-BTB Ab negative	0	0	8	0.99	19	2.35	25	3.10
TST negative / Anti-BTB Ab negative	507	62.83	499	61.83	488	60.47	482	59.73
Total	807		807		807		807	
Agreement	29/807	3.59	64/807	7.93	76/807	9.42	81/807	10.04
Kappa statistics*	0.119		0.234		0.251		0.254	

TST : Tuberculin skin test

Anti-BTB Ab : Antibovine tuberculosis antibody assay

*: *Kappa* ranges from 1 (complete agreement beyond chance) to 0 (agreement is equal to that expected by chance), whereas negative values indicate agreement less than is expected by chance.

5.2.2 Comparison and performance of tuberculin skin tests at various cut-off points and lateral flow assay in detecting bovine tuberculosis

The risk of bovine TB, proportion of exposed cattle and measures of interaction between the risk factors of exposure of cattle to bovine TB and developing (or detecting) the disease in the study at different SICCT-BT cut-off values (≥2 mm, ≥3 mm and ≥4 mm) and SIT-BT are summarised in Table 19. Though the risk of detecting bovine TB in exposed animals increased significantly with increase in SICCT-BT cut-off value (80%, 89% & 100% for ≥2 mm, ≥3 mm & ≥4 mm cut-off points, respectively), the risk of detecting disease among the non-exposed animals was low and comparable at the different cut-off points (31%, 32% & 35% for ≥2 mm, ≥3 mm & ≥4 mm cut-off points, respectively). Also, decreasing the cut-off value revealed significantly higher probability of exposure of the animals to bovine TB (25%, 21% & 10% for ≥2 mm, ≥3 mm & ≥4 mm cut-off points, respectively). Furthermore, the calculated Chi square (χ^2) values were greater than the tabulated statistic (χ^2=3.841, P=0.05) and confirmed that irrespective of the SICCT-BT cut-off value, strong associations exist between exposure of cattle to bovine TB (detection of circulating anti-bovine TB Ab) and the true disease status in the study sites. The ratio of detecting bovine TB in exposed animals to the detection in unexposed animals (relative risk in exposed animals) was comparable between the different TST cut-off points. However, the disease odds ratio (prevalence odds ratios) was significantly greater than 1 at the 5% level for all cut-off values and also increased significantly with increase in cut-off points (Table 20).

Table 20 : Comparison of tuberculin and anti-bovine tuberculosis antibodies test reactors at ≥2-mm, ≥3-mm and ≥4-mm cut-off points for the detection of bovine TB in cattle in the highlands of Cameroon

Cut-off point	Test results	Disease status	Exposure status#	Risk of detecting positive reactors; %(95% CI)		Frequency of exposure; %(95% CI)		Chi (χ) square	OR; (95% CI)	RR; (95% CI)
		SICCT-BT†	Anti-bovine TB Ab assay‡	Non-exposed animals	Exposed animals	Non-diseased animals*	Diseased animals			
≥2	positive	95	300	31.46	80.00	3.75	25.33	82.49	8.71	2.54
	negative	712	507	(28.16 - 34.96)	(70.86 - 86.81)	(2.41 - 5.78)	(20.74 - 30.55)		(5.46 - 13.90)	(2.08 - 3.11)
≥3	positive	72	300	32.11	88.89	1.58	21.33	88.11	16.92	2.77
	negative	735	507	(28.83 - 35.57)	(79.58 - 94.26)	(0.80 - 3.08)	(17.08 - 26.32)		(9.37 - 30.53)	(2.24 - 3.42)
≥4	positive	29	300	34.83	100.00	0.00	9.67	48.08	∞	2.87
	negative	778	507	(31.57 - 38.25)	(88.30 - 100)	(0.00 - 0.75)	(6.81 - 13.54)			(2.13 - 3.87)
		SIT-BT								
≥4	positive	106	300	31.24	76.42	4.93	27.00	78.53	7.13	2.45
	negative	701	507	(27.92 - 34.77)	(67.50 - 83.48)	(3.36 - 7.18)	(22.29 - 32.29)		(4.62 - 11.01)	(2.01 - 2.98)

#: Risk factor of exposure of cattle to bovine TB and developing the disease

†: Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis

‡: Anti-bovine tuberculosis antibody assay

*: Healthy animals or animals with no positive skin response

OR: Disease Odds ratio (Prevalence Odds ratio)

RR: Relative risk in exposed animals

The accuracy of different SICCT-BT cut-off values and SIT-BT to detect bovine TB in cattle based on their exposure to bovine TB as defined by anti-bovine TB Ab assay in the study are shown in Table 20. The maximum sensitivity for the SICCT-BT test was obtained with the ≥4-mm (100%) cut-off point while the highest specificity was realized at the ≥2-mm (68.54%) cut-off point. Reducing the cut-off value resulted in a loss of sensitivity and an increase in specificity for SICCT-BT test against the anti-bovine TB Ab assay. Also, decreasing the cut-off points revealed inverse relationships with positive test predictive values and negative diagnostic likelihood ratios. Therefore, the ability of SICCT-BT to produce no false negative result increased with increase in cut-off point (non-significant differences were observed between the ≥2 mm vs. ≥3 mm and ≥3 mm vs. ≥4 mm cut-off points) while the prediction and detection of true disease status increased with decrease in cut-off point.

However, the contrary was noted for the anti-bovine TB Ab assay against SICCT-BT test indicating that using anti-bovine TB Ab assay as an ancillary diagnostic test to SICCT-BT would detect maximum true positive disease cases especially at severe TST than using SICCT-BT as ancillary to anti-bovine TB Ab assay. Statistically, the best all round SICCT-BT performance and accuracy was realized at the ≥3-mm cut-off point in this study. However, the ≥2-mm cut-off value showed the highest positive predictive value and a positive diagnostic likelihood ratio comparable to the others. Indeed, the predictive values and diagnostic likelihood ratios are comparable between the SICCT-BT ≥3 mm and ≥2 mm cut-off points; and both cut-offs could be applied for improved detection of bovine TB in cattle in the study regions compared with applying the ≥4-mm cut-off point. Furthermore, 100% SICCT-BT and SIT-BT herd level sensitivity was observed in this study irrespective of cut-off point and the 95% CI were

from 60.97% to 100% for the SICCT-BT ≥4-mm and ≥3-mm cut-off points and 64.57% to 100% for the SICCT≥2-mm and SIT-BT positive reactors. Also, the anti-bovine TB Ab assay compared to the TST showed very high herd detection accuracy (Sp=100%; 95%CI; 20.65% – 100%).

The detection of anti-bovine TB Ab positive cattle, prevalence of SICCT-BT reactors and anti-bovine TB Ab/SICCT-BT reactors at the different cut-offs are shown in (Figure 17). Using the sensitivity and specificity obtained in this study, the SICCT-BT ≥2mm cut-off value gave the highest (P<0.05) prevalence rate (23.60%) followed by the ≥3-mm (15.15%) and ≥4-mm (4.98%) cut-off points. Overall, similar trends of significant differences between cut-off groups were observed for SICCT-BT and anti-bovine TB Ab/SICCT-BT reactors for the parameters considered (Figure 17; Table 29 – Appendix 8) in this study.

Anti-bovine TB Ab was detected in 300 (37.17%) of the 807 tested animals with 27.87%, 21.27% and 3.67% of these reactors also being SICCT-BT ≥2-mm, ≥3-mm and ≥4-mm positive, respectively. The proportion of SICCT-BT/anti-Bovine TB Ab reacting cattle was also highest (P<0.05) at the ≥ 2-mm (18.75%) followed by the ≥ 3-mm (13.40%) and ≥ 4-mm (4.98%) cut-off groups. Overall, 0.63% (95%CI: 0.08% – 1.17%), 5.55% (95%CI: 3.97% - 7.12%) and 12.97% (95%CI: 10.65% – 15.28%) of the tested cattle showed SICCT-BT inconclusive results while 0.63% (95%CI: 0.08% – 1.17%), 3.14% (95%CI: 1.94% – 4.35%) and 11.44% (95%CI: 9.25% – 13.64%) reactors were SICCT-BT inconclusive but anti-bovine TB Ab positive at the ≥ 2-mm, ≥ 3-mm and ≥ 4-mm cut-off points, respectively. Also, over 27.14% (95%CI: 24.07% – 30.21%) negative SICCT-BT reactors of the tested animals were also positive for anti-bovine TB Ab.

Table 21 : Sensitivities, specificities, predictive values and likelihood ratios at the ≥2-mm, ≥3-mm and ≥4-mm cut-off points for tuberculin skin tests and anti-bovine tuberculosis antibodies assay for bovine tuberculosis diagnosis in cattle in the highlands of Cameroon

Cut-off point	Test performance; % (95% CI)		Test predictive value; % (95% CI)		Diagnostic likelihood ratio; %(95% CI)	
	Sensitivity	Specificity	Positive result	Negative result	LR+	LR-
a) For SICCT-BT test against anti-bovine TB Ab assay						
≥2 mm	80 (70.86 – 86.81)	68.54 (65.04 – 71.84)	34.05 (29.16 – 38.50)	94.41 (91.66 – 96.41)	2.54 (2.03 – 3.08)	0.29 (0.45 – 0.18)
≥3 mm	88.89 (79.58 – 94.26)	67.89 (64.43 – 71.17)	29.55 (25.32 – 33.13)	97.58 (95.42 – 98.79)	2.77 (2.24 – 3.27)	0.16 (0.32 – 0.08)
≥4mm	100 (88.30 – 100)	65.17 (61.75 – 68.43)	14.67 (12.15 – 15.94)	100 (98.88 – 100)	2.87 (2.31 – 3.17)	0* (0.19 – 0)
b) For anti-bovine TB Ab assay against SICCT-BT test						
≥2 mm	25.33 (20.74 – 30.55)	96.25 (94.22 – 97.59)	57.84 (42.15 – 72.02)	86.39 (85.41 – 87.37)	6.75 (3.59 – 12.68)	0.78 (0.84 – 0.71)
≥3 mm	21.33 (17.08 – 26.32)	98.42 (96.92 – 99.20)	67.17 (45.66 – 83.29)	89.20 (88.20 – 89.88)	13.50 (5.55 – 32.90)	0.80 (0.86 – 0.74)
≥4mm	9.67 (6.81 – 13.54)	100 (99.25 – 100.00)	100.00 (35.22 – 100)	94.87 (94.68 – 95.08)	∞* (9.08 – ∞)	0.90 (0.94 – 0.86)
c) For SIT-BT test against anti-bovine TB Ab assay						
≥4mm	76.42 (67.50 – 83.48)	68.76 (65.23 – 72.08)	33.03 (28.13 – 37.61)	93.53 (90.87 – 95.58)	2.45 (1.94 – 2.99)	0.34 (0.50 – 0.23)
d) For anti-bovine TB Ab assay against SIT-BT test						
≥4mm	27.00 (22.29 – 32.29)	95.07 (92.82 – 96.64)	52.48 (38.50 – 65.96)	86.59 (85.56 – 87.62)	5.48 (3.10 – 9.61)	0.77 (0.84 – 0.70)

SICCT-BT: Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis
SIT-BT: Single Intradermal Tuberculin skin test for the diagnosis of bovine tuberculosis
Anti-bovine TB Ab assay: Anti-bovine tuberculosis antibodies assay

*: the perfect diagnostic test would be expected to have an LR– equal to zero and an LR+ equal to infinity (producing no false negatives, but detecting all true negatives and detecting all true positives, and generating no false positives). The best test therefore for excluding a disease is the one with the lowest LR– and the test with the highest LR+ is the best for accurately detecting a disease (Thrusfield 2007).

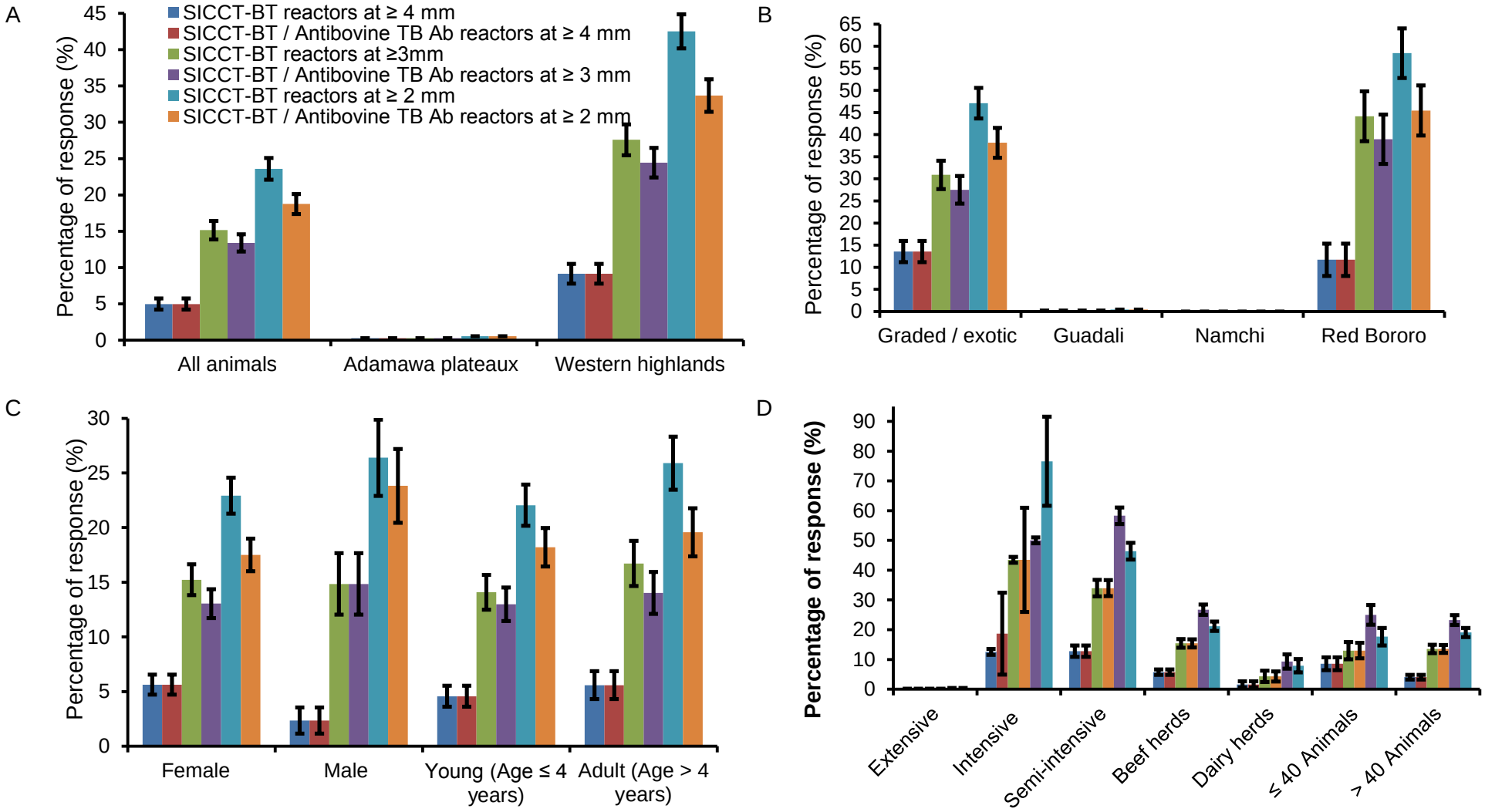

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Figure 17 : Distribution of anti-bovine tuberculosis antibodies and SICCT-BT reactors in 807 tested cattle at the ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points according to: (A) study location, (B) Breed, (C) Sex and Age group, (D) Management systems and Herd sizes

On the whole, 95% (95%CI; 75.1 – 99.9%) of the tested herds had ≥ 1 anti-bovine TB Ab and/or TST / anti-bovine TB Ab positive reactors; SIT-BT and SICCT-BT-≥ 2-mm reactors (36.84%; [95% CI; 16.3 – 61.6%]) gave more SICCT-BT/anti-bovine TB Ab positive herds (n=19) than at the ≥ 3-mm and ≥ 4-mm cut-off points (31.58%; [95% CI; 12.6 – 56.5%]). Indeed, the study showed that the frequency of tested herds (n=20) with ≥ 1 positive reactor was 35%; [95% CI; 15.4 – 59.2%] for SIT-BT and SICCT-BT at ≥ 2 mm cut-off point and 30%; [95% CI; 11.9 – 54.3%] for SICCT-BT at ≥ 3 mm and ≥ 4 mm cut-off values. The detection of circulating anti-bovine TB Ab in cattle and distribution of SIT-BT reactors are shown in Figure 18.

In all, 16.78% SIT-BT and 12.73% SIT-BT/anti-bovine TB Ab positive reactors were noted in the study. Among the SIT-BT positive reactors over 98.59%, 61.23% and 10.38% were SICCT-BT reactors while 78.88%, 60.19% and 10.38% were SICCT-BT/anti-bovine TB Ab reactors at the ≥2-mm; ≥3-mm and ≥4-mm cut-off points, respectively. Also, 84.07% SICCT-BT/anti-bovine TB Ab reactors were noted among the SIT-BT reactors, irrespective of the SICCT-BT cut-off point result.

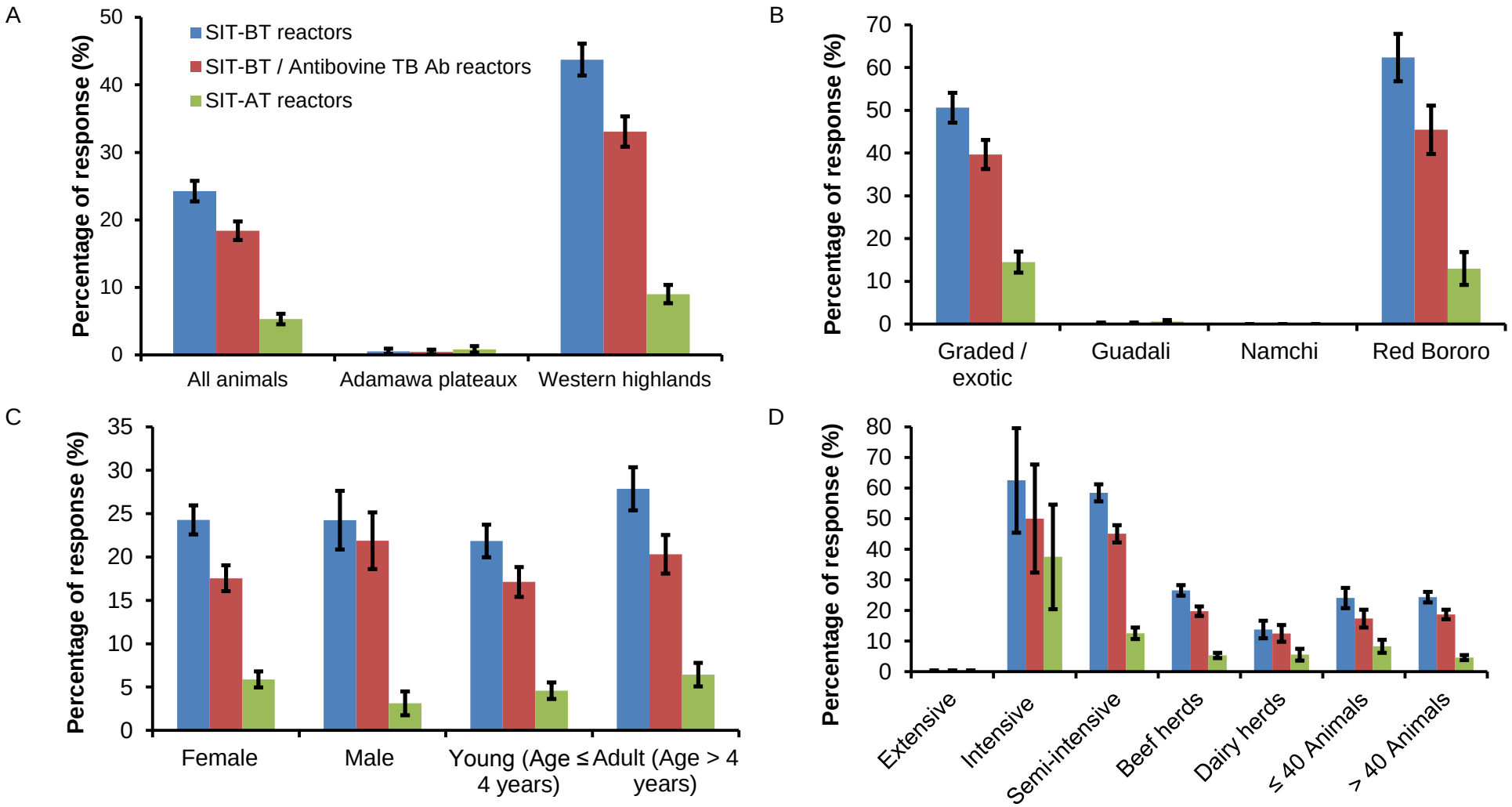

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Figure 18 : Detection of anti-bovine tuberculosis antibodies and distribution of SIT positive reactors according to: (A) study location, (B) Breed, (C) Sex and Age group, (D) Management systems and Herd sizes

5.2.3 True prevalence of bovine tuberculosis in cattle in the study regions at the ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points

The earlier study (Chapter 4) of SICCT skin test prevalence of bovine TB in 2,853 cattle using the OIE-standard cut-off point of ≥4 mm (OIE 2009) consisting of indigenous zebus, upgraded cattle, mixed exotic breeds and their crosses were reassessed. Based on the sensitivity and specificity values obtained in this study, the data for the previous tested cattle (n = 2,853) and the complete data for the second survey (n = 1,381) were reanalysed using the ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points (Tables 22 and 23). Overall, the true prevalence of bovine TB in the earlier SICCT-BT of 2,853 cattle was very similar to that obtained using 1,381 cattle in the present study. The differences in true prevalence of the SICCT-BT were highly significant between the cut-off points (≥4 mm vs. ≥3 mm: χ^2 = 46.021; $P \leq 0.001$; ≥4 mm vs. ≥2 mm: χ^2 = 64.015; $P \leq 0.001$; ≥3 mm vs. ≥2 mm: χ^2 = 16.056; $P \leq 0.001$). Age, sex, breed, animal site and husbandry systems served as significant ($P<0.05$) risk factors to the prevalence and exposure of bovine TB in cattle.

Sensitivity and specificity values obtained in the present study revealed comparable true prevalence rates compared with rates obtained from data reported by Ameni et al. (2000) for SICCT-BT in Ethiopia and significantly higher values compared with data from Delafosse et al. (2002) for SIT-BT in Chad (Table 23). However, the findings were comparable when data obtained by Ameni et al. (2008b) and Pollock et al. (2003) were used to correct the observed SICCT-BT (Table 29 – Appendix 8). In all, re-assessments of SICCT-BT at the ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points showed significant increasing trends in prevalence rates with severe interpretations.

Table 22 : True prevalence of bovine TB in 1,381 cattle based on SICCT-BT tests at ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points and SIT tests at the ≥ 4-mm cut-off point in the highlands of Cameroon (using Se and Sp values obtained in this study)

Variable	No animals tested	SICCT-BT reactors % (95% CI)			SICCT-AT reactors*; %(SE)	SIT-BT reactors; %(95% CI)	SIT-AT reactors*; %(SE)
		≥ 4 mm	≥ 3 mm	≥ 2 mm	≥ 4 mm	≥ 4 mm	≥ 4 mm
All animals	1,381	5.91 (4.67-7.15)	13.85 (12.02-15.67)	22.77 (20.56-24.98)	0.65±0.42	30.56 (25.80-35.32)	7.46±1.39
Agro-ecological location							
ADP	363	0.28[a] (0-0.81)	0.28[a] (0-0.81)	0.55[a] (0-1.31)	0.55±0.76	0.55[a] (0-2.04)	0.83±0.93
WHC	1,018	8.06[b] (6.39-9.73)	18.81[b] (16.41-21.21)	30.72[b] (27.89-33.55)	0.69±0.51	41.27[b] (35.34-47.20)	9.82±1.83
Breed							
Upgraded/Exotic	764	8.91 (6.89-10.92)	16.95[a] (14.29-19.61)	27.94[a] (24.75-31.12)	0.79±0.63	40.16 (33.34-46.97)	11.39±2.25
Guadal	492	0.20 (0-0.60)	0.20 (0-0.60)	0.41 (0-0.97)	0.41±0.56	0.41 (0-1.51)	0.61±0.69
Namchi	31	0	0	0	0	0	0
Red Bororo	94	10.64 (4.41-16.87)	38.30[b] (28.47-48.13)	52.13[b] (42.03-62.23)	1.06±2.07	55.32 (35.62-75.02)	13.83±6.98
White Fulani							
Sex and Age							
Female	1,107	6.67[a] (5.20-8.14)	14.55 (12.47-16.63)	23.36 (20.87-25.85)	0.63±0.47	32.90 (27.47-38.32)	8.49±1.64
Male	274	2.83[b] (0.86-4.79)	11.00 (7.30-14.71)	20.40 (15.63-25.18)	0.73±1.01	21.12 (11.65-30.59)	3.28±2.11
Age ≤ 4 years	716	3.32[c] (2.01-4.64)	10.01[a] (7.81-12.21)	16.33[a] (13.62-19.04)	0.28±0.39	19.71[a] (14.00-25.42)	4.05±1.44
Age > 4 years	665	8.70[d] (6.55-10.84)	17.97[b] (15.05-20.89)	29.71[b] (26.24-33.19)	1.05±0.78	42.24[b] (34.89-49.60)	11.13±2.39
Management system							
Extensive	488	0.20 (0-0.61)	0.20 (0-0.61)	0.41 (0-0.98)	0	0.22 (0-1.02)	0.20±0.40
Intensive / Zero grazing	552	7.25[a] (5.09-9.41)	12.52[a] (9.76-15.28)	21.74[a] (18.30-25.19)	1.09±0.87	34.59[a] (26.82-42.37)	10.87±2.60
Semi-intensive	341	12.52[b] (9.00-16.03)	36.10[b] (31.01-41.20)	56.75[b] (51.49-62.00)	0.88±0.99	67.46[b] (57.72-77.21)	12.32±3.49
Beef herds	692	5.90[c] (4.14-7.65)	17.25[c] (14.43-20.06)	27.34[c] (24.02-30.66)	0.43±0.49	31.61 (24.82-38.40)	5.49±1.70
Dairy herds	689	12.61[d] (10.13-15.08)	10.43[d] (8.14-12.71)	18.19[d] (15.31-21.07)	0.87±0.69	29.51 (22.83-36.18)	9.43±2.18
Herd size (No animals per herd)							
≤ 40 animals	713	7.64[a] (5.69-9.59)	13.27 (10.78-15.76)	21.60 (18.58-24.62)	1.12±0.77	31.90 (25.20-38.61)	9.96±2.20
> 40 animals	668	4.06[b] (2.56-5.56)	14.46 (11.80-17.13)	24.02 (20.78-27.26)	0.15±0.29	29.13 (22.38-35.88)	4.79±1.62

a, b, c, d: label in a category with the different letters in a column are significantly different (P<0.05); *: observed prevalence; ADP: Adamawa plateaux of Cameroon; WHC: Western highlands of Cameroon ; SICCT-BT = Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine TB; SIT-BT = Single Intradermal Tuberculin skin test for the diagnosis of bovine TB

Table 23 : True prevalence of bovine TB in 2,853 cattle based on SICCT-BT test at ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points and SIT-BT in the highlands of Cameroon (using Se and Sp values obtained in this study)

	Animals tested	SICCT-BT reactors; % (95% CI)				SIT-BT reactors; % (95% CI)	
		≥ 4 mm*	≥ 4 mm	≥ 3 mm	≥ 2 mm	≥ 4 mm#	≥ 4 mm
All animals	2,853	4.67 (3.89 - 5.44)	5.97 (5.10-6.84)	11.97 (10.77-13.16)	16.90 (15.52-18.27)	10.33 (9.21 – 11.44)	20.18 (18.71 – 21.65)
Agro-ecological location							
ADP	727	2.57[b] (1.42 – 3.72)	3.05[b] (1.80-4.30)	5.25[b] (3.63-6.87)	8.99[a] (6.91-11.07)	11.86 (10.48 – 13.23)	23.25 (21.46 – 25.05)
WHC	2,126	5.38[a] (4.42 – 6.34)	6.97[a] (5.89-8.05)	14.26[a] (12.78-15.75)	19.60[b] (17.92-21.29)	5.86 (4.15 – 7.57)	11.18 (8.89 – 13.47)
Breed							
Upgraded/Exotic	368	7.77[a] (5.04 – 10.51)	10.31[a] (7.20-13.41)	20.49[a] (16.37-24.62)	27.90[a] (23.32-32.49)	15.79 (12.07 – 19.52)	31.19 (26.45 – 35.92)
Guadali	1,317	3.76[b] (2.73 – 4.79)	4.71[b] (3.56-5.85)	10.67[b] (9.00-12.33)	16.09[b] (14.11-18.07)	9.48 (7.90 – 11.06)	18.47 (16.37 – 20.56)
Namchi	33	3.03	3.03	3.03	3.03	3.30	3.03
Red Bororo	487	7.23[a] (4.93 – 9.53)	9.55[a] (6.94-12.16)	16.43[a] (13.14-19.72)	21.77[a] (18.11-25.44)	13.73 (10.67 – 16.79)	27.03 (23.09 – 30.98)
White Fulani	648	2.89[b] (1.60 – 4.18)	3.49[b] (2.08-4.90)	6.77[b] (4.84-8.71)	9.21[b] (6.98-11.43)	6.75 (4.82 – 8.68)	12.97 (10.38 – 15.56)
Sex and Age							
Female	2,212	4.82 (3.93 – 5.72)	6.19 (5.19-7.20)	12.81[a] (11.42-14.20)	18.26[a] (16.65-19.87)	8.02 (5.92 – 10.13)	21.52 (19.81 – 23.23)
Male	641	4.12 (2.58 – 5.66)	5.21 (3.49-6.93)	9.05[b] (6.83-11.27)	12.21[b] (9.67-14.74)	8.43 (7.01 – 9.84)	15.54 (12.73 – 18.34)
Age ≤ 4 years	1,481	3.64 (2.69 – 4.59)	4.54 (3.48-5.60)	8.59[c] (7.16-10.02)	12.57[c] (10.88-14.26)	12.38 (10.64 – 14.12)	12.67 (10.04 – 15.30)
Age > 4 years	1,372	5.77 (4.54 – 7.01)	7.52 (6.12-8.91)	15.61[d] (13.69-17.53)	21.58[d] (19.40-23.75)	8.70 (7.28 – 10.12)	18.94 (16.34 – 21.55)
Management system							
Extensive	1510	4.23 (3.21 – 5.24)	5.36 (4.22-6.50)	9.58[a] (8.10-11.07)	12.86[a] (11.17-14.55)	12.38 (10.64 – 14.12)	24.33 (21.11 – 27.56)
Intensive	138	3.99 (0.72 – 7.25)	5.03 (1.38-8.67)	18.58[b] (12.09-25.07)	26.22[b] (18.88-33.56)	8.70 (7.28 – 10.12)	24.29 (21.10 – 27.49)
Semi-intensive	1205	5.30 (4.03 – 6.56)	6.85 (5.42-8.28)	14.20[b] (12.23-16.17)	20.89[b] (18.60-23.19)	14.30 (8.46 – 20.14)	16.35 (14.46 – 18.23)
Beef herds	2,357	5.09 (4.20 – 5.97)	6.56[a] (5.56-7.56)	11.17 (9.89-12.44)	15.26[c] (13.81-16.71)	11.91 (10.08 – 13.74)	24.31 (22.04 – 26.58)
Dairy herds	496	2.66 (1.25 – 4.08)	3.18[b] (1.63-4.72)	15.77 (12.56-18.98)	24.69[d] (20.89-28.48)	9.66 (8.47 – 10.86)	16.90 (15.01 – 18.79)
Herd size (No animals per herd)							
≤ 40 animals	1,325	5.73[a] (4.48 – 6.98)	7.46[a] (6.04-8.87)	12.46 (10.68-14.24)	17.70 (15.64-19.75)	10.17 (8.54 – 11.80)	28.18 (20.67 – 35.68)
> 40 animals	1,528	3.74[b] (2.79 – 4.70)	4.69[b] (3.63-5.75)	11.54 (9.94-13.14)	16.21 (14.36-18.05)	10.47 (8.93 – 12.00)	23.37 (20.98 – 25.76)

a, b, c, d: label in a category with the different letters in a column are significantly different (P<0.05);
* : true values obtained using sensitivity and specificity on trials carried out by Ameni et al. (2000) in Ethiopia, Africa.
# : true values obtained using sensitivity and specificity from Delafosse et al. (2002) in Chad, Africa.
SICCT-BT = Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis
SIT-BT = Single Intradermal Tuberculin skin test for the diagnosis of bovine tuberculosis.

5.2.4 Bovine tuberculosis herd infection rates at various cut-off points

Classification of herds with at least one test positive SICCT-BT and SIT-BT positive animals and showing major differences between agro-ecological zones, study sites and husbandry management systems is shown in Table 24. Significantly higher ($P<0.05$) herd prevalence rates for SICCT-BT and SIT-BT reactors were recorded in the sudano-guinean (WHC) region than the guinea-savannah (ADP) region. Also, the ≥ 2-mm SICCT-BT cut-off point showed the same SIT-BT test positive herds; and was significantly more than herd infection rates at the other SICCT-BT cut-off points.

Table 24 : Distribution of single intradermal comparative cervical tuberculin skin test (SICCT) and single intradermal tuberculin skin test (SIT) bovine tuberculosis positive herds (≥1 positive reactor)

Variable	Label	No herds tested	SICCT-BT Positive				SIT-BT positive	
			≥ 4 mm and ≥ 3 mm		≥ 2 mm		≥ 4 mm	
			No	% (95%CI)	No	% (95%CI)	No	% (95%CI)
Total	All herds	40	16	40.00 (24.90-56.7)	18	45.00 (29.3 -61.5)	19	47.50 (33.8 -66.2)
Agro-ecological Regions	ADP	9	1	11.11 (0.3 -48.3)	2	22.22 (2.8 -60.0)	2	22.22 (2.8 -60.0)
	WHC	31	15	48.39 (30.2-66.9)	16	51.61 (33.1 -69.8)	17	54.84 (36.0 -72.7)
Management system	Extensive	12	1	8.33 (0.2-38.5)	2	16.67 (2.1 -48.4)	2	16.67 (2.1 -48.4)
	Intensive	21	11	52.38 (29.8-74.3)	12	57.14 (34.0 -78.2)	13	61.90 (38.4 -81.9)
	Semi-intensive	7	4	57.14 (18.4-90.1)	4	57.14 (18.4 -90.1)	4	57.14 (18.4 -90.1)
	Beef herds	16	4	25.00 (7.3-52.4)	5	31.25 (11.0 -58.7)	5	31.25 (11.0 -58.7)
	Dairy herds	24	12	50.00 (29.1-70.9)	13	54.17 (32.8 -74.4)	14	58.33 (36.6 -77.9)
Herd sizes	Animals ≤ 40	27	12	44.44 (25.5-64.7)	13	48.15 (29.9 -70.1)	14	51.85 (32.0 -71.3)
	Animals > 40	13	4	30.77 (9.1-61.4)	5	38.46 (13.9 -68.4)	5	38.46 (13.9 -68.4)

SICCT-BT: Single Intradermal Comparative Cervical Tuberculin skin test for the diagnosis of bovine tuberculosis
SIT-BT: Single Intradermal Tuberculin skin test for the diagnosis of bovine tuberculosis
ADP = Guinean savannah (Adamawa plateau); WHC = Sudano-guinea (Western Highlands)

5.3 Discussion

The TST are not routinely done in Cameroon and interpretations of tests results have always relied on standards established elsewhere in the world. Thus, the real epidemiology and zoonotic potential (in magnitude and distribution) of bovine TB in the country is largely unknown. Affordable methods for accurate and maximum detection of the disease in the cattle populations would greatly contribute to improving the understanding of its epidemiology and control.

Few tuberculin tests have been carried out in Cameroon applying various standards and obtaining varied results even for the same study sites. For example, the OIE-recommended cut-off and other standards applicable in France by Martrenchar et al. (1993) and in Canada by (Muchaal 2002) while various data recorded elsewhere (Ameni et al. 2000; Pollock et al. 2003; Ameni et al. 2008b; OIE 2009) to assess test performances in bovine TB surveys have been used in the country. However, test performance assessments done using the findings at the SICCT-BT ≥ 3-mm and ≥ 4-mm cut-off point of the present study agrees with data of Ameni et al., (2000; 2008b) in Ethiopia but the SIT-BT results disagreed with data of Delafosse et al. (2002) in Chad which are also tropical environments with widespread bovine TB in their cattle. The disease status in the tested animals, prevailing environmental factors and different diagnostic tests used to determine the TST accuracy could have contributed to the differences noted.

The findings of this study suggest that any cut-off point could be used for detecting bovine TB in cattle, though the test performance increased with increase in cut-off value. Cattle presenting differential SICCT-BT skin thickness of ≤4 mm should not be excluded that they are not affected by bovine TB,

especially animals in highly endemic areas or animals sensitized to environmental mycobacteria. These animals could actually be infected but low reacting or not reacting at all because their immune systems may not be stimulated enough for a positive response at the ≥ 4 mm cut-off point (Ameni and Medhin 2000; Inangolet et al. 2008), conditions such as stress may compromised their immune function (Thoen et al. 2009) or the animals may be sensitized to environmental mycobacteria (Palmer et al. 2006). The prevalence of SICCT-BT positive reactors at the ≥ 2-mm cut-off point was significantly higher than at the ≥ 3-mm and ≥ 4-mm cut-off points but was comparable to that of SIT-BT positive reactors. The study also showed that over 3.72% of the tested animals could have been misdiagnosed or considered as SICCT-BT doubtful reactors at ≥ 4-mm cut-off point and suggested that classifying them as positive for bovine TB at the ≥ 3-mm and ≥ 2-mm cut-off points would reflect the true diagnosis of their disease status.

The tuberculin prevalence rates recorded in this study are comparable (but lower) with the findings when sensitivity and specificity values obtained during the trials of Ameni et al. (2008b) were used to correct the observed SICCT-BT and significantly higher values than values obtained with data from Pollock et al. (2003) for SIT-BT prevalence rates to the true rates (Table 29 – Appendix 8). Also, wide variations were observed when other sensitivity and specificity data obtained for SICCT-BT in Ethiopia (Ameni et al. 2000) and SIT-BT in Chad (Delafosse et al. 2002) and the present study were used to determine true prevalence rates. Overall, maximum specificity at the SICCT-BT ≥ 2-mm cut-off point and maximum sensitivity at the ≥ 4-mm cut-off point was observed. These findings are contrary to those of Ameni et al (2008b) who observed maximum sensitivity in central Ethiopia using a 2-mm cut-off without affecting the

specificity. Delafosse et al. (2002) estimations were based on the subjective assessments of Quirin et al. (2001) and OIE-standards as it is used in France. Ameni et al. (2000; 2008b) used post mortem examination to define the disease status and mycobacterium culture method as the gold standard while anti-bovine TB antibodies assay as a measure of exposure to the disease was used in this work to estimate these performance criteria values. However, the accuracy (sensitivity and specificity) of culture, post mortem examinations and antibodies detection vary significantly. While antibodies assay has significantly higher specificity, post mortem examination has a high sensitivity while mycobacterium culture is the widely accepted referral method. The variations observed for assessing the cut-off points of TST for the diagnosis of bovine TB between the data of the present study and those of Ameni et al., (2000; 2008b) could also be linked to the techniques used. Reference diagnostic techniques for comparison, bovine TB disease status in the tested animal populations and environmental variations therefore play important roles in the observed differences.

The poor to fair agreements recorded in this study suggested that severe interpretation of TST (ie decreasing skin response cut-off values) improved the agreement between TST and the lateral flow assay to detect natural bovine TB infection in cattle. The prevalence rates at the different cut-off points could have influenced the estimated *Kappa* values in the study and the low values could be explained by the fact that both tests were good but negatively correlated (Thrusfield 2007). The variations observed between TST at the predefined cut-off points in this study and other regions of Cameroon (Merlin and Tsangueu 1985; Tanya et al. 1985; Martrenchar et al. 1993; Nfi and Ndi 1997; Muchaal 2002) and parts of Africa (Ameni et al. 2000; Ameni et al. 2008b) could be

linked to the reference diagnostic techniques, bovine TB disease status in the tested animal populations and environmental variations (Monaghan et al. 1994; de la Rua-Domenech et al. 2006b). Thus, severe interpretations of TST based on reference performance and accuracy data from regions with similar environmental conditions have been propsed in parts of Africa (Kazwala et al. 2001b; Tschopp et al. 2009) and should be extended to the entire continent for maximum detection of true bovine TB cases. Nonetheless, the importance of determining appropriate localised values for significant reduction of the disease in Cameroon cannot be overemphasized.

The prevalent of bovine TB in cattle were confirmed to be high by TST and atypical mycobacteria infections was also widespread. However, non-specific responses of TST due to atypical or environmental mycobacteria have been widely reported (Philips et al. 2003; Biet et al. 2005; de la Rua-Domenech et al. 2006a; Oloya et al. 2006; OIE 2009). Indeed, Lesslie et al. (1975; 1975a; 1975b) recorded hypersensitivity responses to avian tuberculin that was equal or higher than responses to bovine tuberculin in cattle naturally infected with *M. bovis* and presenting visible lesions at slaughter. SICCT-BT doubtful reactions were higher among adult / old cattle and increased with age while widespread atypical mycobacteria with high proportions (SIT-AT and SICCT-AT) in some sites were recorded in both surveys. These findings agree with Martrenchar et al. (1993) who reported high frequency of atypical mycobacteria that severely limited the reliability of SIT-BT and produced large numbers of uncertain SICCT-BT reactors in Northern Cameroon. Severe interpretations of test results in the present study revealed that SIT-BT positive and SICCT-BT doubtful reactors at ≥ 4-mm cut-off point were accurately diagnosed as bovine TB cases at the SICCT-BT ≥ 3-mm and ≥ 2-mm cut-off points [i.e. the proportion of

(Excess D4 and ExcessD3) reactors that are true bovine TB disease cases (Figure 11 – Section: 3.3.3)]. The absolute detection (100% sensitivity) of bovine TB affected herds irrespective of TST cut-off point and findings of circulating anti-bovine TB antibodies, further confirmed that Cameroonian cattle are widely exposed to bovine TB.

Reducing the cut-off point from ≥4-mm maximised the diagnostic performance and accuracy for *ante mortem* diagnosis of bovine TB using SICCT-BT and anti-bovine TB Ab tests. The study showed that maximum test performance and accuracy were realized at the ≥3 mm cut-off point and the best SICCT-BT positive predictive value was obtained at the ≥2 mm cut-off point. The negative predictive value and diagnostic likelihood ratio at the ≥ 2-mm cut-off point were also comparable to those of the other cut-off values. These findings revealed that interpreting SICCT-BT at the ≥ 2-mm cut-off point, and not at the ≥ 3-mm or ≥ 4-mm cut-off points, was beneficial from a public health perspective since more disease cases would be predicted accurately.

True bovine TB infected animals which maybe anergic due to age, malnutrition or suffering from concurrent diseases related to internal and external parasitosis (common scenarios in the study regions) could be diagnosed at severe SICCT-BT interpretation at the ≥ 2-mm cut-off point; since their delayed hypersensitivity responses to the tuberculins would be limited and cannot express full OIE-recommended ≥ 4-mm cut-off point. Furthermore, the accuracy of SICCT-BT at ≥2 mm cut-off point was comparable to that of SIT-BT that employed the OIE-recommended cut-off point. These results suggest that the application of the SICCT-BT ≥2-mm cut-off point in cattle in the agro-ecological highland environments of Cameroon would maximise the true detection of bovine TB in

cattle. Maximum detection of bovine TB is key to the effective control of the disease and reduction of the zoonotic risks to public health and safety in the country.

5.4 Conclusion

There is gross inadequacy in the implementation of the existing bovine TB control policy, including the lack of routine TSTing in Cameroon. Accurate and maximum detection of diseased animals would therefore be the important preliminary step toward achieving a significant progressive reduction and control of bovine TB in livestock. Presently in Cameroon, detailed post-mortem examination for the detection of TB lesions is used to define disease status and is only done on dead animals (if cause is unknown) or during slaughter / meat inspection for meat production in the abattoir. Culling of positive reactors to TST as part of a strict national animal disease control policy is not yet practical due to political, economic and social limitations. However, bovine TB lesions have been found in SICCT-BT reactors at cut-off points less than the OIE-recommended >4-mm cut-off (Ameni et al. 2000; Kazwala et al. 2001b; Ameni et al. 2008b), as well as in cases with doubtful (irrespective of cut-off) and negative SICCT-BT response (a farm in Mezam Division in this study). While veterinarians continue to detect TB lesions in slaughtered cattle across the country, lack of knowledge on the actual magnitude and distribution of the disease, inadequate laboratories and field expertises, and politico-economic deficiencies are some of the factors that limit bovine TB control. The current control approach is based on controlling animal movements, culling suspected bovine TB cases and condemnation (partial or whole) of affected carcasses at meat inspection. Apparently, these strategies were not designed to eradicate

but reduce the general prevalence and monitor the spread of the disease especially in livestock. The TST are presently passive components of the Cameroon's government strategy to control bovine TB but reformation of the country's strategy is imminent. Appropriate cut-off points for the tests in Cameroonian cattle and environmental conditions need to be defined for maximum detection of bovine TB. Furthermore, the segregation and phase slaughter of infected cattle (WHO 1994b) has been proposed as an achievable method to adopt in Africa, which if based on local relevant TST cut-off points, would greatly reduce bovine TB in cattle as well as eliminate the risks to human health, food security and food safety.

The study showed that irrespective of the SICCT-BT cut-off value there were strong associations between exposure of cattle to bovine TB (detection of circulating anti-bovine TB Ab) and the true disease status in cattle (TST results) in the highlands of Cameroon. The TST and antibovine TB antibody assay when used in parallel offered higher sensitivity compared to individual tests. Also, bovine TB was detected at all the cut-off points, though the maximum test performance was realized at the ≥3-mm cut-off point. Interpreting SICCT-BT at the ≥ 2-mm cut-off point was more strategic from a public health context since more disease cases would be predicted accurately; and the positive likelihood ratio at the ≥ 2-mm cut-off point was comparable to the others. However, it is important to investigate the performance of the TST cut-off points against defined bovine TB status based on detailed post-mortem examination for the presence of TB lesions for mycobacteriological culture and molecular characterisatiomn of the isolated tubercle bacilli in reacting animals in the Cameroon environment.

Chapter 6

Identification of *Mycobacterium tuberculosis* complex isolates from tuberculous cattle tissues and human sputa in Cameroon by PCR-based genomic analysis

6.1 Introduction

The *Mycobacterium tuberculosis* complex (MTC) of bacterial strains include at least seven species and subspecies (*M. tuberculosis, M. bovis, M. microti, M. africanum, M. cannetti, M. caprae, M. pinnipedii*) which cause similar disease in mammalian hosts (Thoen et al. 2009; Parry 2010). *M. tuberculosis* is specifically adapted to humans but it has occasionally been isolated from other mammals (Smith et al. 2006a; Berg et al. 2009). *M. bovis* is the natural pathogen of cattle and it can cause essentially the same symptoms and pathology as *M. tuberculosis* in humans (Gupta and Katoch 2005; Cassidy 2006; de la Rua-Domenech 2006b; Palmer and Waters 2006; Pollock et al. 2006); the distinction being made only by bacterial culture or molecular characterization of the isolated microorganism (Strong and Kubica 1985; Collins et al. 1994; WHO 1998b; Brosch et al. 2001; Brosch et al. 2002; Smith et al. 2006a). *M. bovis* is estimated to account for 0.5 to 7.2% of human TB cases in industrialized nations and 10 to 15% of new cases in developing countries (Cosivi et al. 1998; Ashford et al. 2001; de la Rua-Domenech 2006b).

Conventional laboratory diagnosis of TB relying mainly on culture and acid-fast staining / microscopy, is low in sensitivity and does not identify the species of

mycobacterial causing the disease (Wilton and Cousins 1992; Walker et al. 1994; Parsons et al. 2002).

Differentiation of MTC members by culture and biotyping procedures (Oxygen preference (aerophilic vs microaerophilic), niacin accumulation, nitrate reductase activity, colony morphology, and resistance to two compounds, thiophen-2-carboxylic acid hydrazide (TCH) and pyrazinamide) are time consuming and laborious usually taking several weeks to obtain results; as well as they are not practical for surveillance purposes (Strong and Kubica 1985; Grange et al. 1996; Parsons et al. 2002; Warren et al. 2006). These conventional methods were very useful in the past in industrialised countries (Parsons et al. 2002) and are more affordable to some developing countries compared to modern and high performing techniques where further differentiation of MTC is desired. However, interpretations of the results of conventional methods are highly subjective and prone to errors; such as interpretations of differences in colony morphology after culture and identifying acid fast bacilli in staining / microscopy (Strong and Kubica 1985; Grange et al. 1996; Ameni et al. 2010b). Nonetheless, the differentiation of MTC members is important for accurate diagnosis of the mycobacterial agents, epidemiologic and public health surveillance and appropriate case management (O'Reilly and Daborn 1995; de la Rua-Domenech 2006b; Warren et al. 2006).

Molecular epidemiologic techniques such as genomic deletion typing, *IS6110*-restriction fragnment length polymorphism (RFLP), spoligotyping and variable number tandem repeat (VNTR) have been used for detail characterization of MTC and geographical mapping of the spread of mycobacterial diseases in animals and humans (Smith et al. 2006a; Warren et al. 2006; Pinsky and

Banaei 2008; Müller et al. 2009a). Some of these techniques have been simplified and are used in routine diagnostic laboratories for disease surveillance purposes (Smith et al. 2006a; Warren et al. 2006). MTC are 99.9% similar at the nucleotide level but comparative genomic analysis have showed that all of them evolved from a common ancestor (Figure 19) through sequential DNA deletions of precise genomic locations (Brosch et al. 2002; Smith et al. 2006a; Smith et al. 2006b; Pinsky and Banaei 2008).

Rapid and robust PCR assays based on genomic deletion analysis can distinguish between members of the MTC by detecting the presence or absence of regions of difference and using multiplexed primer approaches has been proposed (Brosch et al. 2002; Smith et al. 2006a; Warren et al. 2006; Pinsky and Banaei 2008). However, lack of resources and lack of expertise limits the use of these molecular techniques in most African countries including Cameroon where TB is widespread in humans and livestock populations. There are few laboratories that sporadically do mycobacterial culture from humans and the procedures employed are not detailed to differentiate between MTC members. The situation is even worse because of the inadequacies to investigate the causal agent and control against TB in animals. Bovine TB is widespread in cattle in Cameroon, but the extent of the epidemiological link between *M. bovis* infection in cattle and humans is not clear. There is lack of quarantine and mass transboundary movements of cattle between Cameroon, Nigeria and Chad; where endemic bovine TB and *M. bovis* infections in humans have been reported (Diguimbaye et al. 2004; Cadmus et al. 2006; Diguimbaye-Djaibé et al. 2006; Cadmus et al. 2010a; Cadmus et al. 2010b). The impact of human TB due to *M. bovis* could therefore be very significant.

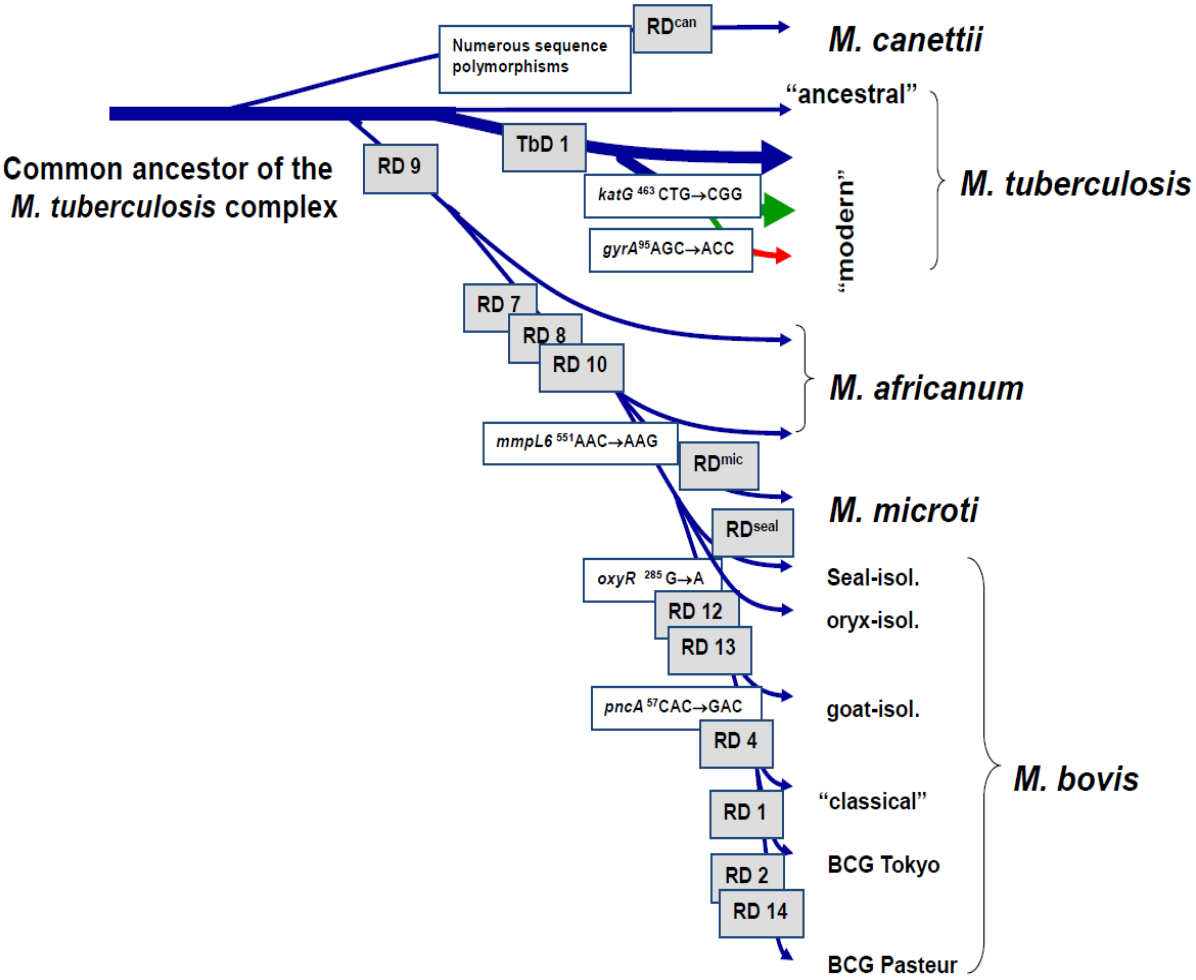


Figure 19: Proposed evolution of *Mycobacterium tuberculosis* complex from a common ancestral tubercle bacillus illustrating successive loss of DNA in certain lineages

The scheme is proposed by Brosch *et al.* (2002) and is based on the presence or absence of conserved deleted regions and on sequence polymorphisms in five selected genes. The distances between certain branches may not correspond to actual phylogenetic differences calculated by other methods. Blue arrows indicate that strains are characterized by *katG*463. CTG (Leu), *gyrA*95 ACC (Thr), typical for group 1 organisms. Green arrows indicate that strains belong to group 2 characterized by *katG*463 CGG (Arg), *gyrA*95 ACC (Thr). The red arrow indicates that strains belong to group 3, characterized by *katG*463 CGG (Arg), *gyrA*95 AGC (Ser), as defined by Sreevatsan *et al*. (1997).

Controlling TB in humans is currently fully geared in Cameroon, supported by the government as well as other national and international institutions. With optimism and the eminent increasing attentions for better understanding the epidemiology of bovine TB in cattle, generating molecular epidemiological data on bovine TB and assessing the degree of human TB due to *M. bovis* and various impacts of zoonotic bovine TB would play a major role in modelling and managing the distribution of the strains of *M. bovis* in the country.

This study was therefore performed to characterise mycobacteria isolated from tuberculous lesions from cattle and infected human sputa from the highlands of Cameroon using the multiplex PCR-based genomic deletion analysis of RD9, RD4, and also African 1 (a clonal complex of *M. bovis* dominant in parts of Africa (Müller et al. 2009a)) to definitively separate *M. tuberculosis* and *M. bovis*. The study also describes further differentiation of isolates from cattle identified as *M. bovis* strains by deletion of both RD9 and RD4 by spoligotyping method.

6.2 Results

The materials and methods to culture of human and cattle TB speciemens and PCR-based genomic analyses of mycobacteria isolates have been described in Chapter 3 (section 3.1 and section 3.5).

6.2.1 Frequency of suspected tuberculous pathology identified during inspection in slaughtered cattle and mycobacterial growth

During March 2009 to April 2010 characteristic gross TB lesions were detected in 178 of 11, 231 (1.58 %) of cattle and in different tissues. Over 92.7% of cattle

(178/192) that demonstrated any form of pathology at inspection also showed suspected lesions. Most (67.14%) of the affected animals were female and adult or old animals with an average age of 61.8 (± 18.3) months. Also, the cattle slaughtered at the study abattoir were predominantly the native Zebus (White Fulani 34.5% and Red Bororo 63%, Guadali 1.5%, Exotic and their crosses 1%) originating from within the Western highland regions of the country.

Tuberculous lesions were severe and more frequent in lymph nodes associated with virtually all the lobes of both lungs. Lymph nodes of the fore quarter of the carcass as well as the liver, mesenteric and mammary associated lymph nodes were also affected. Yellowish granulomatous lesions and abscesses (containing yellowish pus) were detected in the mediasternal (30%), bronchial (12%), retropharyngeal (24%), pre-scapular (5%), parotid (7%), submandibular (8%) and supramammary (10%) lymph nodes as well as the liver, lungs and other tissues (4%).

About 77.17%[11] (169/219) suspected tuberculous lesions from 125/168 (74.4%) cattle showed evidence of growth with further acid-fast staining (ZN) and microscopy, morphological features of colonies and biochemical characterisation (Niacin production, Nitrate reduction and catalase tests). However, 23 cattle samples were not cultured due to poor storage and spoilage. Growth was recorded in all the media used though at different rates and was fastest in the modified Middlebrook 7H9 broth base, followed by Lowentein-Jesen (LJ) slants enriched with pyruvate and glycerol for *M. bovis* (based on

[11] Media inoculation for each sample was as follows: 2 LJ glycerol enriched slants, 2 LJ Pyruvate enriched slants and for some samples 1 modified Middlebrook 7H9 broth base (Becton Dickinson Diagnostics, New York, USA). Overall, 219 samples were inoculated on media. Incubation was at 37°C for up to 12 weeks (depending on media) with weekly observation for growth of colonies.

observation of the interval range from inoculation of media to indication of first growth).

6.2.2 Frequency of mycobacterial growth from human sputa

About 84.78%[12] (78/92) sputa from human TB patients showed growth of mycobacterial organisms in LJ glycerol enriched slants. *M. tuberculosis* was mainly identified after further acid-fast staining (ZN) and microscopy, morphological features of colonies and biochemical characterisation (Niacin production, Nitrate reduction and Catalase tests).

6.2.3 Molecular characterisation of mycobacterial isolates from cattle and human speciemens

6.2.3.1 Genomic deletion typing of Mycobacterial isolates from cattle tissues and human sputa

No growth was observed when samples from heat treated suspensions (100°C for 20 minutes) of 247 isolates (169 cattle and 78 human) were inoculated on LJ slants (enriched with glycerol or pyruvate) and also modified Middlebrook 7H9 broth base as previously described (Chapter 3; Section: 3.5.3). RD9, RD4 and African 1 deletion analysis to identify *M. tuberculosis* and *M. bovis* isolates from cattle and humans in the study are shown in Table 25. A total of 174 isolates in this study (108 cattle and 66 humans) showed results for RD9 and or RD4 deletion typing. However, deletion analysis of RD4 revealed that 81 (55 isolates

[12] Media inoculation for each sample was as follows: 2 LJ glycerol enriched slants. Overall, 92 inoculations were done. Incubation was at 37°C for up to 8 weeks with weekly observations.

showed strict deletion for RD4 and RD9) isolates from cattle were *M. bovis* and approximately 22 (3 isolates showed strict presence of RD9 and RD4 suggesting *M. tuberculosis*) were not *M. bovis* but other mycobacteria isolates. RD9 deletion typing showed that approximately 55 (41 isolates showed strict presence of RD9) human isolates were *M. tuberculosis* but 14 (3 isolates showed strict deletion for RD9) were suggestive of non-human strains of *Mycobacterium tuberculosis* complex including *M. bovis.* Further deletion typing analysis for African 1 [a clonal complex of *M. bovis* dominant in parts Africa (Müller et al. 2009a)] confirmed its distribution in the study areas. Thirteen of fifty five (23.64%) RD4 and RD9 deleted isolates also showed deletion for African 1. The deletion typing results for RD9; RD4 and African 1 of some mycobacterial isolates (Flanking and Internal primers sets used on the same batch of isolates) from suspected TB lesions in cattle and infected human sputa are shown in Figure 20.

Overall, the RD deletion analysis of Mycobacteria isolated from suspected TB lesions in cattle and infected human sputa in this study showed inconclusive findings (Table 24). PCR products were obtained with both internal and flanking primers for some isolates, suggesting that the isolates have both intact and deleted RD for the category being assessed.

Table 25: Genomic deletion analysis of tubercle bacilli strains isolated in cattle tissues and human sputa in Cameroon[13]

Region of difference and clonal complex	Cattle			Humans		
	Present (Intact)	Absent (deleted)	Inconclusive*	Present (Intact)	Absent (deleted)	Inconclusive*
RD9 (n_c=169; n_h=78)	3[#] (13)	103 (113)	10	41 (55)	3 (14)	11
RD4 (n_c=162; n_h=78)	4 (22)	55 (81)	17	21 (45)	2 (19)	17
African 1 (n_c=95; n_h=29)	5 (41)	13 (62)	35	0 (27)	0 (27)	27
RD9 + RD4 (n_c=162; n_h=78)	1	**55**	/	(2)	**(2)**	/

n_c : number of isolates from cattle;
n_h : number of isolates from humans
: values represent strict assessment for the category (ie isolates that have strictly intact or deleted RD)
* : PCR product obtained with both Internal and Flanking primers
( ): values in parenthesis represent isolates that have intact and or deleted RD

[13] African 1 deletion typing was carried out on all isolates from cattle and humans that showed evidence (slight or strong) of RD4 being absent.

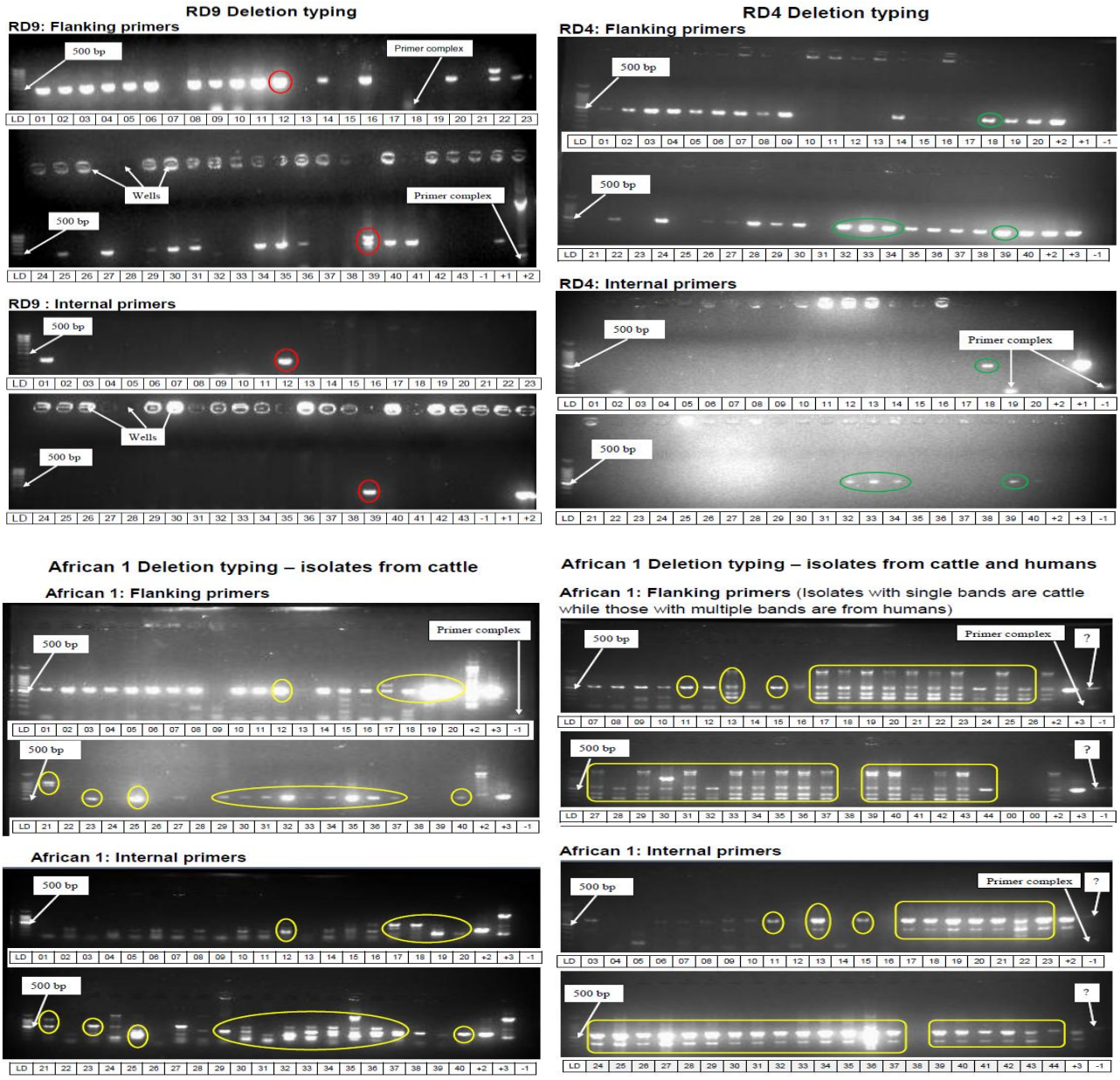


Figure 20 : Electrophoretic separations of PCR products by RD9, RD4 and African 1 deletion typing of mycobacterial isolates from slaughtered cattle tissues and human sputa

Legend: LD = 100bp DNA ladder (Promega Corporation, USA);

RD9: – 1= negative control (water); +1 = heat killed cells of *M. bovis* (CHAD491); +2 = *M. tuberculosis* ($H37R_v$) strain

RD4: – 1= negative control (water); +1 = *M. tuberculosis* ($H37R_v$) strain; +2 = heat killed cells of *M. bovis* (AF2122/97); +3 = heat killed cells of *M. bovis* (CHAD491)

African 1: – 1= negative control (water); +2 = heat killed cells of *M. bovis* (AF2122/97); +3 = heat killed cells of *M. bovis* (CHAD491)

*: images for Flanking primers and Internal primers for an RD (RD9, RD4 or African 1) analysis are for the same batch of isolates. Inconclusive results (PCR products obtained with both Internal and Flanking primers) for some isolates for the RD category being assessed are also shown in oval or rectangular shapes

6.2.3.2 Spoligotyping of *Mycobacterium bovis* isolates from cattle tissues

Spoligotyping was performed after genomic typing (deletion analysis of both RD9 and RD4) of TB lesions of Red Bororo and White Fulani cattle and was done at the Veterinary Laboratory Agency – Weybridge, UK (Table 26). Five spoligotypes, which showed consistent absence of spacer 30 and also spacers 3, 9, 10, 14 – 16 & 39 – 43 in the direct repeat (DR) locus, were obtained from 16 of the 24 isolates tested. Spoligotype patterns were successfully identified in 16 (66.67%) while 8 (33.33%) isolates failed to show any pattern probably due to lack of enough DNA or test limitations. Of the spoligotype pattern, 4 (25%) of the isolates showed unique patterns (new spoligotypes). The remaining 12 (75%) isolates were clustered into one group and showed the similar spoligotype patterns of SB0953 (Mbovis.org) and also identified as dominant in the study.

However, the four new sopilogtype patterns were similar to the dominant type with the only differences being: the deletion of spacer 36 for NT1; deletion of spacer 27 & 28 for NT2; deletion of spacer 5 & 6 and presence of spacer 8, 11 – 13 & 17 for NT3 and deletion of spacer 29 & 31 through 34 for NT4. The results indicated a high degree of homogeneity among the *M. bovis* isolated from cattle in the Western highlands of Cameroon.

Due to lack of resources spoligotyping was not carried out on all the isolates including those from infected human sputa.

Table 26 : Spoligotype patterns of *Mycobacterium bovis* strains isolated from lesions in slaughtered cattle in the Bamenda municipal abattoir of the western highlands of Cameroon

	Breed	Sex	Age* (Months)	Tissue (Lymph node)	Spoligotype pattern	Name	RD Af1
H37Rv					1111111111111111111001111111111100001111111	*M. tuberculosis*	**Intact**
H_2O					0000000000000000000000000000000000000000000		
2122/97					1101101000001110111111111111111111111100000	*M. bovis*	**Deleted**
1	RB	Female	42	Submammarian	Fail		
2	Zebus#	Female	76	Retropharyngeal	1101111000000000011111111111101111101100000	NT1	
3	RB	Female	82	Retropharyngeal	1101111000000000011111111111101111111100000	SB0953	Deleted
4	RB	Female	76	Mediastinal	1101111000000000011111111111101111111100000	SB0953	Deleted
5	WF	Female	82	Apical	1101111000000000011111111111101111111100000	SB0953	Deleted
6	WF	Male	12	Mediastinal	Fail		
7	WF	Female	54	Retropharygneal	Fail		
8	WF	Female	36	Apical	Fail		
9	WF	Male	30	Retropharyngeal	Fail		
10	WF	Female	60	Apical	Fail		
11	WF	Female	76	Mediastinal	1101111000000000011111111111101111111100000	SB0953	Deleted
12	WF	Female	76	Mediastinal	1101111000000000011111111111101111111100000	SB0953	Deleted
13	WF	Female	76	Bronchial	Fail		
14	RB	Female	82	Retropharyngeal	1101111000000000011111111111101111111100000	SB0953	Deleted
15	WF	Male	82	Mediastinal	1101111000000000011111111111**00000**111100000	NT4	**Deleted**
16	RB	Female	76	Mediastinal	1101111000000000011111111111101111111100000	SB0953	Deleted
17	RB	Female	76	Mediastinal	1101111000000000011111111111101111111100000	SB0953	Deleted
18	RB	Female	82	Apical	1101111000000000011111111111101111111100000	SB0953	Deleted
19	RB	Female	82	Apical	1101111000000000011111111111101111111100000	SB0953	Deleted
20	WF	Male	82	Bronchial	1101111000000000011111111111101111111100000	SB0953	Deleted
21	RB	Female	82	Mediastinal	1101111000000000011111111111101111111100000	SB0953	Deleted
22	RB	Female	60	Mediastinal	1101111000000000011111111**00**101111111100000	NT2	
23	WF	Female	70	Submammarian	1101**00**1**1**00**111**000**1**11111111111101111111100000	NT3	
24	WF	Female	70	Submammarian	Fail		

*: Average age = 67.58 ± 19.36 months; #: local cattle (exact breed not determined)
RD Af1: region of difference African 1
RB: Red Bororo; WF: White Fulani
Spoligotype pattern: 1 indicates the presence of a spacer and 0 represents the loss of a spacer
NT = New spoligotype not yet described in the *M. bovis* spoligotype database (Mbovis.org). Yellow colour shows difference between NT and SB0953 patterns

6.3 Discussion

The frequency and severity of lesions in the lymph nodes associated with the thoracic (mediasternal and bronchial) and fore-quarter (retropharyngeal, submandibular and parotid) agrees with previous reports (Hojle 1990; Herenda et al. 1994; Whipple et al. 1996; Headley 2002) and suggests that the respiratory route was the main mode of animal to animal transmission bovine TB (Francis 1971; Pollock and Neill 2002; Pollock et al. 2006) and primary foci for excretion of infective *M. bovis* (Corner 1994; Neill et al. 1994) in this study.

The PCR-based genomic deletion analysis used in this study enabled the rapid and accurate differentiation of *M. bovis* and *M. tuberculosis* from up to 96 MTC isolates within 24 – 48 hours. The rapidity and efficiency of the technique had been stated earlier by Warren et al., (2006) who also described its fastness and simplistic approach as well as its benefits to the veterinary and human health professions, compared to conventional mycobacteriological methods which would have taken weeks and probably not achieve the same level of classification. The method has been widely applied in surveillance studies and for the assessment of high-risk cases to differentiate MTC members and detect transmission in animal populations (Parsons et al. 2002; Warren et al. 2006; Pinsky and Banaei 2008; Cadmus et al. 2009; Ameni et al. 2010b).

In this study there was evidence of *M. bovis* from humans and *M. tuberculosis* from cattle suggesting possible animal to human and human to animal cyclic transmission patterns that need to be further investigated. Also, some cattle isolates showed strict evidence of being *M. tuberculosis* strains (3 isolates showed strictly intact RD9 and 4 intact for RD4) while strict *M. bovis* strains in humans could also be suggested (3 isolates showed strictly deleted of RD9 and

2 deleted of RD4). Inconclusive results were also noted but the reasons were not clear. The use of high titre levels of primer sets in PCR reaction volumes (1 µl each of 10 pmol / µl Flanking and Internal primers) could be contributing factors. Various complexes approximating the expected product weights could have been generated. However, *strangeness* of isolates and the possibility of dual or multiple mycobacterial infections could also be considered. Dual infections of *M. tuberculosis* and *M. gordonae* of cattle (Berg et al. 2009) and confirmation of *M. tuberculosis* infection among *M. bovi*s infected cattle (Tsegaye et al. 2010) have been reported in Ethiopia. African 1, a clonal complex of *M. bovis* dominant in parts of Mali, Nigeria, Cameroon (North) and Chad (Müller et al. 2009a) where bovine TB (*M. bovis* infections) is endemic in cattle has also been recently described.

Among mycobacteria species, *M. paratuberculosis* and *M. avium* are closely related and have identical sequence at the DNA level in the specific priming region (Wilton and Cousins 1992). Nonetheless, primers designed to differentiate *M. avium* from *M. intracellulare* by generating appropriate sized products in the presence of specific targets *unexpectedly* generated another product (180bp) with *M. paratuberculosis* (Walker et al. 1992; Wilton and Cousins 1992). Furthermore, Walker et al. (1992; 1994) have reported that background amplification reactions competing with target specific amplifications in Strand Displacement DNA Amplification (SDA) techniques were more prevalent at higher non-target DNA concentrations. Walker et al. (1992) actually observed that initial priming events with non-target DNA would involve imperfectly paired sequences that occurred less efficiently than carefully designed priming – extension reactions for the true target generation. They also stated that once a non-target sequence was attached to two primers, it

underwent amplification as effectively as the genuine target sequence. Cross reactions or non-target DNA amplification between species specific primers and other mycobacterial species (not investigated but present in some isolates) may therefore be associated to the inconclusive findings recorded in this study.

It is also essential to assess if the PCR-based RD deletion typing assay was amplifying DNA from closely related non-mycobacteria competing side reactions (thus lowering the mycobacterium sensitivity) and the inconclusive results in this study. However, species of the *M. tuberculosis* complex and their strains may also differ in key biological properties, such as virulence, transmissibility, stability and antigenic variation, which may help to explain field observations (Skuce and Neill 2001). Additional molecular analysis (other RD deletion typing as well as other techniques) and immunological analysis would be needed to provide more precise data on the issues of the observed doubtful RD deletion analysis.

Spoligotyping of some of the isolates with RD9 and RD4 deletion in this study (Kamerbeek et al. 1997) have revealed five distinct patterns. Spoligotyping is a rapid way of typing MTC strains with similar direct repeat (DR) spacer markers (van Soolingen et al. 1995; Kamerbeek et al. 1997). Five closely related spoligotype patterns were identified but only the dominant spoligotype pattern SB0953, has been previously described and only from the Adamawa region of Cameroon (Njanpop-Lafourcade et al. 2001); suggesting that five strains of *M. bovis* were involved in bovine TB in cattle in the study. This also implied that the majority of bovine TB in cattle was caused by the SB0953 dominant type.

The present results also suggest a high degree of homogeneity among the *M. bovis* isolates in the highlands regions of Cameroon (Western highlands and

Adamawa plateaux). Indeed, Njanpop-Lafourcade et al. (2001) had identified related groups of *M. bovis* strains in Northern Cameroon (including the spoligotype SB0944 which was dominant followed by SB0953 and others) and proposed Cameroon to belong to the group of countries (e.g. Australia and Tanzania) in which there is a low level of heterogeneity among *M. bovis* isolates based on a high level of homogeneity that they observed.

The *M. bovis* strains in this study have different spoligotype patterns from previously reported patterns from other parts of Africa (Ameni et al. 2007b; Müller et al. 2008a; Müller et al. 2009a; Ameni et al. 2010b). In fact, the dominant spoligotype SB0953 was not identified in neighbouring regions Nigeria (southern), Mali, Chad and Cameroon (northern) where the SB0944 was the dominant spoligotype (Cadmus et al. 2004; Cadmus et al. 2006; Diguimbaye-Djaibé et al. 2006; Müller et al. 2009a). However, the consistent loss of spacer 30 and close similarities of the spoligotype patterns of the *M. bovis* strains isolated in the present study and earlier findings in neighbouring regions and other parts of Sub-Sahara Africa (Njanpop-Lafourcade et al. 2001; Cadmus et al. 2004; Cadmus et al. 2006; Diguimbaye-Djaibé et al. 2006; Müller et al. 2008a; Müller et al. 2009a) confirmed the common evolutionary descent of the *M. bovis* strains in Cameroon as proposed earlier (Njanpop-Lafourcade et al. 2001; Müller et al. 2009a).

The presence of 4 unique (new) spoligotypes and wide dissemination of the spoligotype SB0953 in this study may be explained by the extensive movement of cattle, both for commercial and transhumance purposes (Müller et al. 2009a), which was common within the highland regions and occurs in over 60% of cattle in Cameroon. The epidemiological implications of the strains detected in this

study could therefore be that new *M. bovis* strains are evolving from older strains (evident by their closeness to previously described strains); while strains of *M. bovis* are also being introduced into uninfected communities and naïve animals, where they are maintained and multiplied to become the predominant strains. The public health impact would be the potential zoonotic consequences and multiple infections by *M. bovis* strains. However, the study provides additional information on the identity and distribution of *M. bovis* strains which would be useful for modifying and implementing TB control measures in Cameroon.

Njanpop-Lafourcade et al. (2001) had reported that some spoligotypes including SB0953 were unique to the Adamawa region than the further northern (North and Far North) regions of Cameroon. These workers supported their finding with the introduction of measures, in 1976, to prevent the circulation of cattle between the Adamawa region and other parts of the country, which was aimed at protecting the health status of the better performing Adamawa Gudali Zebus. The dominance of the spoligotype SB0953 in this study could be associated to the Northwest region (of the Western highlands) bordering the Adamawa region and also consistent with the recent lifting of their restrictive location in Adamawa and the widespread distribution in the country of Gudali zebus. However, earlier works did not identify the spoligotype SB0953 in *M. bovis* strains isolated in the further northern parts of Cameroon (Njanpop-Lafourcade et al. 2001; Müller et al. 2009a). Nonetheless, further work is required to better understand the distribution of *M. bovis* strains within the study regions and the whole of Cameroon. It would be interesting to compare spoligotypes of *M. bovis* isolates from the shared frontier areas of the Western and Adamawa highland regions of Cameroon.

It is important to note that several *M. bovis* strains have been identified from specimens of humans living in the United Kingdom (one originally from Nigeria and had a unique spoligotype pattern); most of whom had some contact or interaction with a dairy farm and the isolates showed typical *M. bovis* characteristics including drug resistance (Gibson et al. 2004). *M. bovis* has been isolated from human TB cases with cervical adenitis (Kazwala et al. 2001a) and spoligotype patterns shared by strains of *M. bovis* in man and cattle have been reported in Tanzania (Kazwala et al. 2006) and Nigeria (Cadmus et al. 2006). Also, Meyer et al. (2008) as reported by Müller et al. (2009a) identified a strain of *M. bovis*, with loss of the spacer 30 in the spoligotype pattern of isolates from a human case with pulmonary TB in Ghana. However, the spoligotype patterns identified in this study are different from previously reported patterns of *M. tuberculosis* complex isolates from infected humans in Cameroon (Niobe-Eyangoh et al. 2003).

However, Müller et al. (2009a) has described at high frequency in a population samples of *M. bovis* from cattle in several sub-Saharan west-central African countries (Mali, Southern Chad, Northern Cameroon and Southern Nigeria) an African 1 clonal complex or closely related group of bacteria defined by a specific chromosomal deletion and identified by the absence of spacer 30 in the standard spoligotype typing scheme. The population of *M. bovis* in each of these countries is distinct, suggesting that the African 1 clonal complex is geographically localized (with dominance of the clonal complex resulting from an original introduction into cows naïve to bovine TB) and the mixing of strains between countries was not common in this part of Africa (Müller et al. 2009a).

6.4 Conclusion

M. bovis can be transmitted to humans through the consumption of contaminated unpasturised milk, eating raw meat and inhalation of aerosol from infected cattle (Cosivi et al. 1998; Morrison et al. 2000; Biet et al. 2005; Etter et al. 2006; Shitaye et al. 2007; Anaelom et al. 2010). Since most of the diseased animals were adult / old (more than 5 years old) and female in this study, it can be inferred that there are many infected adult and aging females maintaining and acting as source of *M. bovis* infections in various production systems across the region and may transmit the disease to their offsprings, clean susceptible and humans.

M. tuberculosis has been reported in other animal species living in close and prolonged contact with humans (Chandrasekharan and Ramakrishnan 1969; Ocepek et al. 2005; Pavlik et al. 2005; Cadmus et al. 2006; Abubakar 2007; Thoen et al. 2009). Cattle exposed to tuberculous herdsmen or *care-takers* are often positive to TST (Regassa et al. 2008; Munyeme et al. 2009; Thoen et al. 2009). Also, *M. tuberculosis* have been isolated from milk of TST positive cows (Ameni and Erkihun 2007; Regassa et al. 2008) though lesions of *M. tuberculosis* infected cattle were generally limited to lymph nodes of the head (Thoen et al. 2009). The incidence of human TB in Cameroon is high and increasing (Kuaban et al. 2000a; Kuaban et al. 2000b; Noeske et al. 2004; Ane-Anyangwe et al. 2006; WHO 2009; 2010; 2011). Similarly, bovine TB is widespread but neglected in cattle in the country, most cattle professionals have very close contacts with the disease and many suspected TB lesions have been identified in the head and fore-quarters of carcasses during inspection. *M. tuberculosis* a natural pathogen of humans was evident from cattle in this work.

Its presence in cattle may have been caused by direct transmission from infected humans, particularly infected traditional cattle owners and herdsmen as they are known to have intimate and repeated contacts with their cattle.

Therefore, a possible interface between bovine TB and human TB could be implied in Cameroon, given the opportunities for repetitive close human-livestock contacts, existence of other opportunities for transmission and the important socioeconomic role cattle keeping has in many communities. These findings provide overwhelming evidence for further investigation of the incidence of human TB due to *M. bovis*, useful hints on high zoonotic risks, drug resistant TB cases (multi-drug resistant and extreme drug resistant strains) and importance of bovine TB in Cameroon. The cattle slaughtered at the Bamenda city abattoir are for consumption by inhabitants of the city, peri-urban areas and neighbouring villages. An appropriated control at this level would be to trace bovine TB lesions in carcasses back to their origin, restrictive movement of affected and in-contact herds, quarantine measures, regularly screen cattle using TST at a relevant cut-off point for maximum and accurate diagnosis in the given environment and eliminate TST positive reactors by segregation and phasing out of infected animals. The Cameroon's government legislature of routine testing of butchers and other cattle professionals for TB should be reinforced and tuberculous individuals prevented from handling livestock. Furthermore, veterinary and medical investigations into the epidemiology and control of TB in cattle and humans in Cameroon; continuous education of targeted communities and other collaborative research involving public health experts, biomedical and social scientists cannot be overemphasised.

This study presents preliminary mycobacteriological laboratory confirmation of bovine TB in cattle by culturing and molecular characterisation of the *M. bovis* strains in the highlands of Cameroon. A high level of transmission of *M. bovis* was suspected since it is common to have extensive movements of cattle within Cameroon and between countries in the West and Central African sub-regions. However, detailed molecular epidemiology and immunological studies are needed to investigate the modes of transmission, risk factors of the disease, geographic distribution of *M. bovis* strains and differential susceptibility among the indigenous zebus to *M. bovis.*

The five spoligotypes identified in this study are suggestive of new *M. bovis* strains possibly evolving from older related strains predominant in the environment and neighbouring sub-regions. *M. bovis* strains (new and old) were introduced into the uninfected communities and naïve animals, where they became established as predominant or emerging strains with specific geographic distributions. Though the potential zoonotic consequences and multiple infections of these *M. bovis* strains cannot be overstated, the study provides additional knowledge on the strains of *M. bovis* which are vital for the control TB in humans and cattle in Cameroon. A further sampling and comprehensive investigation of the public health implication of *M. bovis* infection is warranted based on the evidence of *M. bovis* from infected human sputa in this study.

Chapter 7

Zoonotic bovine tuberculosis in the highlands of Cameroon: Risk factor analysis, implications for public health and control strategy in Cameroon

7.1 Introduction

Increasing human population and food shortages are of some concern in Cameroon. The devaluation of the Central African CFA franc[14], in 1994, has caused food animal production to become a strategic sub-sector for diversification of income and the fight against malnutrition and unemployment in the country (Tanya 2004). However, many diseases affecting livestock and humans (including zoonoses) have huge negative impact on animal productivity and threaten public health, with the poor being particularly vulnerable. Therefore, improved attention to bovine TB status, accurate estimation of the magnitude and distribution of bovine TB in cattle are essential for appropriate intervention strategies and poverty alleviation through improved livestock productivity in Cameroon.

The use of TST to diagnose bovine TB in cattle is a non-implemented control policy in Cameroon but the existence of bovine TB in livestock has since been considered based on the identification of tuberculous lesions during slaughter / meat inspections (Doufissa 1993; MINEPIA 2002; Awah-Ndukum et al. 2005). The zoonotic implications of bovine TB are neglected in Cameroon and scanty

[14] CFA franc is the currency used in Cameroon and other formerly French ruled countries in Africa

attentions are given to the implementation of existing laws on its control and reduction. Information on the disease epidemiology and interface in animals and humans is largely unknown. There is dearth of reliable national data on the magnitude and distribution of bovine TB in Cameroon but many habits such as the consumption of unpasteurised fresh cow's milk and milk products, the consumption of raw meat and close human – livestock contact (Fon-Tebug 2009) have been cited in many communities. These factors favour the emergence and transmission of zoonotic TB due to *M. bovis* in animals and humans (Cosivi et al. 1998; Biet et al. 2005; Etter et al. 2006; Shitaye et al. 2007). Furthermore, there is evidence of other conditions also existing in different husbandry systems that promote the transmission of *M. bovis* between infected cattle and "clean" cattle and / or humans. Long trekking, large herds and frequent overcrowding of cattle (O'Reilly and Daborn 1995; Omer et al. 2001; Ayele et al. 2004; Neill et al. 2005) often associated with transhumance usually create ideal environments for increase herd-to-herd, and thus animal-to-animal contacts from different areas.

The lack of active bovine TB surveillance in Cameroonian livestock, close human – animal interactions in the management of herds and a culture of keeping animals until they die of disease or old age in traditional pastoral systems provide suitable conditions for the emergence and transmission of animal diseases including Zoonosis (e.g. bovine TB). The potential threat of zoonotic TB due to *M. bovis* to human health, even at a low prevalence cannot be overemphasized. In fact little is also known of the extent of bovine TB in Cameroon and the different farming systems despite risky practises. The prevalence of bovine TB in Cameroon is under estimated and under investigated and the threat of human *M. bovis* infection has not been

investigated in the country but zoonotic bovine TB is increasing becoming a major concern to the veterinary and medical services. In order to determine the involvement of bovine TB in the morbidity and mortality of TB in Cameroon, broad multidisciplinary investigations on the sources and identification of TB causing agents, routes of transmission, associated risk factors and epidemiology of TB among humans and animals need to be conducted.

The highland regions as a whole are among the top populated areas in Cameroon with average population densities of over 100 humans and 20 cattle per Km^2 and also bordered by bovine TB endemic countries (Nigeria, Chad and Central African Republic). Based on the comprehensive investigation of the prevalence and tubercle bacilli strains of bovine TB in cattle in the highlands of Cameroon (chapter 4, 5 and 6), studying the public health implications of TB in cattle as major keys to modelling control in livestock and humans in Cameroon is therefore fundamental.

In this context, this study built on records of high prevalence of bovine TB in cattle, various tubercle bacilli strains isolated in cattle and the very closed human-livestock contacts in the highland areas of Cameroon to review risk factors for exposure and transmission of zoonotic bovine TB infection of cattle and cattle professionals, and its public health significance.

7.2 Results

The questionnaire survey and the main areas of animal management and practices, habits and awareness of respondents have been described in Chapter 3 (section 3.4 and section 3.8).

The respondents were catergorised and their responses have been presented according to the following variables:

I. Study area

a. Western Highland region (WHC)

b. Adamawa Plateaux (ADP)

II. Occupation of the cattle handlers

a. Cattle breeder

b. Butcher

c. “Buyem sellem”

d. Herdsmen

III. Level of education of the cattle handlers (School level)

a. None

b. Primary school

c. Secondary school

d. Post secondary

e. Not indicated

IV. Duration of the cattle handlers in cattle business (X)

a. $X \leq 10$ years

b. $10 < X \leq 20$ years

c. $20 < X \leq 30$ years

d. $30 < X \leq 40$ years

e. $X > 40$ years

f. Not indicated

7.2.1 Prevalence of bovine tuberculosis in cattle

The trend and prevalence of bovine TB in the highland regions of Cameroon has been described earlier in Chapters 4 and 5. Molecular characterisation of tubercle bacilli strains isolated from suspected cattle tissues and infected human sputa has been described in chapter 6.

7.2.2 Overview of questionnaire surveys and responses

Overall, 64.5% of 1000 questionnaires issued in the survey were filled and returned for analysis. Over 75.3% of 650 cattle professionals, 28.8% of 250 animal health and production technicians and 56% of 150 human TB patients provided data to investigate risk factor analyses for zoonotic bovine TB in cattle and humans by way of questionnaires (Table 9: Chapter 3; section 3.4). The responses showed that cattle business in Cameroon was mainly carried out by men aged between 15 and 80 years (average age: 41.67±12.19); 10.5% of them were single and 89.5% married (monogamy: 61.3%; polygamy: 38.7%). Respondents had been in cattle business for an average period of 23.7 years (±15.7years) and less than 12% of them had post-primary educational levels. The primary occupations of the respondents were mainly related to cattle rearing, cattle production and handling of fresh cattle products (Figure 21; Table 30 – Appendix 9).

Evidence of closed and repeated interactions between respondents and their cattle (and other livestock) as well as over 10 years involvement in cattle business and limited post-primary educational level were obtained from the general responses. Also, most cattle were managed in extensive and traditional

pastoral husbandry systems in moderately sized herds (40 < herd ≤ 80 animals). The animals usually lived to old age mixed often with other herds and depended on natural pasture and water points (Figures 21 and 22; Tables 30 and 31 – Appendices 9 and 10). Fresh milk and meat consumption habits of cattle professionals, their level of awareness and knowledge of the modes of transmission of TB including zoonotic bovine TB, their awareness and practices of Cameroon's bovine TB control policy as well as the impact of TB on the cattle industries are shown in Figures 23 – 26 (Tables 32 – 35 in Appendices 11 – 14). Chi square test and significant levels between variables with differences are summarised in Table 36 (Appendix 15).

7.2.3 Risk factors of zoonotic bovine tuberculosis to humans

7.2.3.1 Responses of handlers of cattle and cattle products

The interviewed groups were predominantly ethnic (Fulani, Bororo, Foulbe) and non-ethnic groups with a passion for animal rearing composed of cattle breeders, herdsmen (employed by cattle breeders but may also own cattle with the herds in their care), handlers of fresh animals products (Bucthers, "Buyem Sellems"). All respondents knew about human TB and usually referred to it simply as "strong cough". Many respondents (55.6%) agreed that bovine TB can be transmitted from animals to humans, acquiring the knowledge from elders, personal observations and previous encounters, "Njangies [15] " and various communal socio-cultural meetings, formal and informal communications (radios, televisions, NGOs), extension workers as well as contacts with

[15] Group of persons with common objectives and meeting regularly to improve targeted social, economic, cultural and or religious goals

veterinary staff and hospital consultations. Most respondents had close and repeated interactions with animals for very long durations (Figure 21; Table 30 – Appendix 9) and generally consumed cooked meat and boiled milk to minimize disease transmission (Figure 23; Table 32 – Appendix 11). However, many of them admitted to have consumed or still consume fresh raw meat (16%) and milk (61%). “Suya” and “Kilishi”[16] consumption which might have been poorly prepared due demand pressures were also common (80%) especially in cattle market and other cattle gatheaaaring points such as “stops” during transit. Many cattle professionals knew at least one (>52%) mode of the common vehicles of transmitting bovine TB to humans (Figure 24; Table 33 – Appendix 12): raw fresh milk (14.3%), raw meat (44.7%) and aerosol (14.5%). Over 93% of respondents accepted milking their cows after every calving for home consumption or sell the milk. Furthermore, over 87% of respondents reported daily practices of at least one activity that favours the transmission of zoonotic bovine TB to humans. These activities were mainly related to handling cattle and their products such as directing the animals to pasture, water points and other animal gathering centres (cattle markets, vaccinations, dipping), restraining for routine and clinical manipulations, milking, slaughtering and dressing of carcasses (Figure 21 – 23; Table 30 – 32 in Appendices 9 – 11).

As for action taken on sick and dead animals due bovine TB (Figure 24; Table 33 – Appendix 12), many respondents (up to 28%) reported consuming meat from sick or recently dead animals (cause not usually known) and or passing the meat into the human / public food chain (sell, share). They were also ignorant of the risks of exposure, transmission and potential hazards of zoonotic

[16] “Suya” is meat briefly roasted over hot charcoal. Kilishi is a traditional sun dried (sometimes briefly roasted) meat. The products are not standardized and are prepared by marinating thin sheets (kilishi) or small bundles (suya) of meat in slurry of mixed local ingredients.

diseases including bovine TB. However, many respondents were not aware of what action to take if TB was suspected in their herds (60%) or if animal death (30%) was associated to bovine TB. Nonetheless, over 80% of respondents had encountered of human TB (family members, friends, colleagues) and over 44% of bovine TB in owned herd(s) or adjacent herds. Indeed, about 30% of respondents claimed to have noticed animals in their herds or adjacent herds with symptoms characteristic of bovine TB namely chronic cough (long lasting cough or strong cough as it was commonly described) associated with fever, weight loss / emaciation, lethargy and also death.

Most cattle professionals (over 80%) did not enforce or apparenetly did not know how to enforce the known bovine TB control measures in their animals' or herds' "semi-natural" environments (Figure 25; Table 34 – Appendix 13). Some respondents attempted any practice (chemotherapy, traditional pastoral husbandry and ethno-Veterinary interventions) to keep sick animals alive (Figure 25; Table 34 – Appendix 13). Negative impact of bovine TB on cattle business was a common response (81%) and condemnations (95%) at slaughter / meat inspections was viewed as the main bovine TB control measure in the country. Lack of knowledge of reporting suspected bovine TB cases to the veterinary service (≈ 87%) were also noted (Figure 26; Table 35 – Appendix 14). Loss of animals ("bankruptcy of living banks") was the most important aspect cited as impact to cattle production and health. Overall, the ethnicity and characteristics of communities in the highlands, primary occupation, educational level and duration in cattle business of the respondents were important determining risks factors for potential exposure and transmission of bovine TB from cattle to cattle professionals.

7.2.3.2 Responses of animal health technicians

The response of Animal health technicians (Veterinary Doctors and Nurses) in this survey showed that all of them were aware of the legislature governing bovine TB control in animals. All of them confirmed that strict control were not implemented, citing free and unchecked movements of cattle and lack of routine TST in the country. The responses of most veterinarians across the study confirmed frequently identifying TB lesions (>70.4%) during inspection of slaughtered cattle; upward trends (52.1%) of TB lesions in abattoirs and limited (43%) collaborations with Medics in the control of TB (Zoonotic TB). However, less than 45% of them agreed that the slaughter / meat inspection practices were satisfactory for the monitoring of bovine TB in the country. Partial condemnation of affected carcass was common. Whole carcass condemnations were also done if TB lesions were in multiple organs / tissues though with difficulties due to lack of resources to compensate cattle professionals (butchers and cattle breeder). The meat inspectors reported that lack of cooperation from the butchers or animals owner was common and often required forced seizures of condemned carcasses (partial or whole), such as involving the police and local adminstrations.

7.2.3.3 Responses of human tuberculosis patients

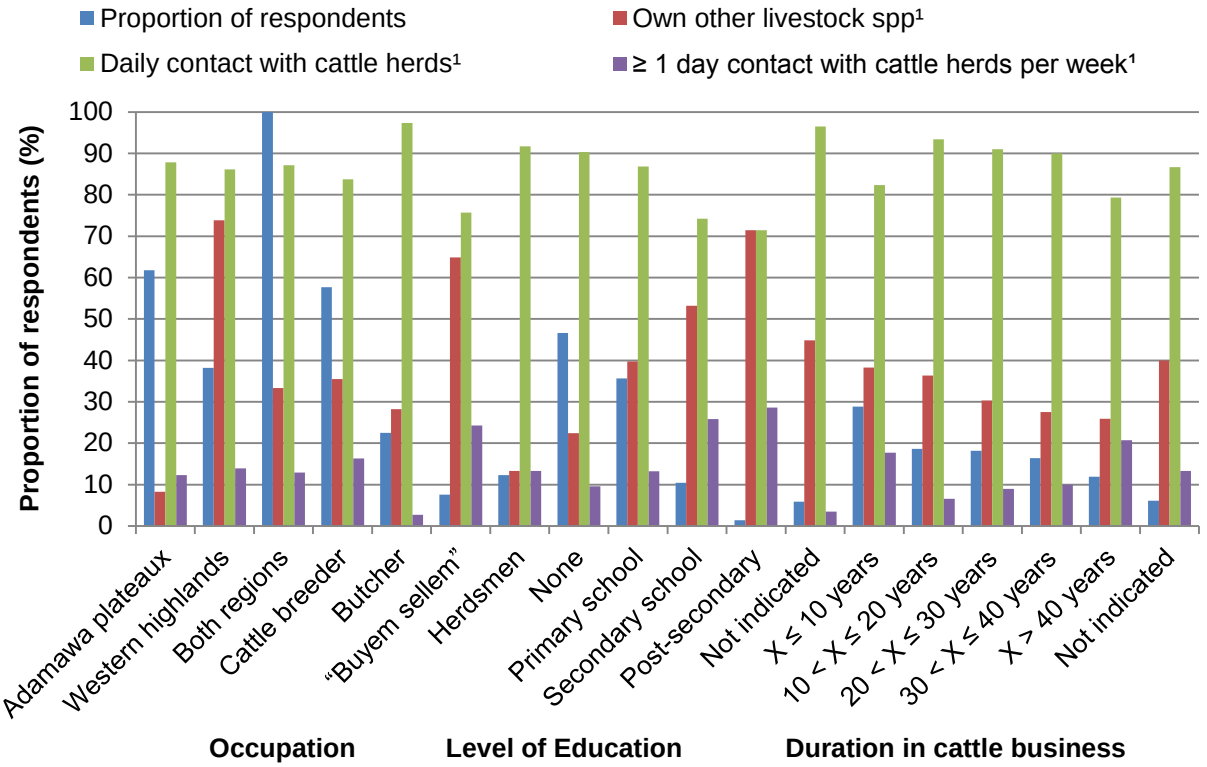
The interviewed human TB patients reported that they frequently drank fresh milk boiled and or not boiled (32.1%), not boiled (19.8%), ate raw meat (2.5%) and ate “Suya” (61.3%). Some (17.3%) were aware of zoonotic bovine TB while

others (82.7%) claimed that they were ignorant and many of them were having the information for the first time during this study.

7.2.4 Risk factors for bovine tuberculosis in cattle

The responses cattle handlers showed that over 70% of cattle were kept in moderate to large herds (Figure 23; Table 32 – Appendix 11). Generally, many cattle lived to very old age (>84%) and in traditional extensive (38%) and semi-extensive (58%) systems of (Figure 22; Table 31 – Appendix 10). Many cattle trekked at least 5 km daily for grazing and drinking (60%) and there were plenty of herd / herd mixing and animal / animal contacts (99%) when different herds of same or different owners meet (Figure 22; Table 31 – Appendix 10) during grazing, at drinking points and other animals gathering centres. Although many respondents (≈30%) recognised bovine TB in their herds or adjacent herds, most cattle professionals (>86%) reported that they did not implement the known control measures in their communities which were predominantly rural and in the animals' "semi-natural" or "semi-wild" environments (Figures 25 and 26; Tables 34 and 35 in Appendices 13 and 14). Many respondents were interested in more animals (increasing the size of their "living banks") but were not aware of the negative impact of bovine TB on their animals' production and health (Figure 26; Table 35 – Appendix 14).

a) Contact between cattle handlers and animals[1]



b) Herd size[2] and Number of herds[3]

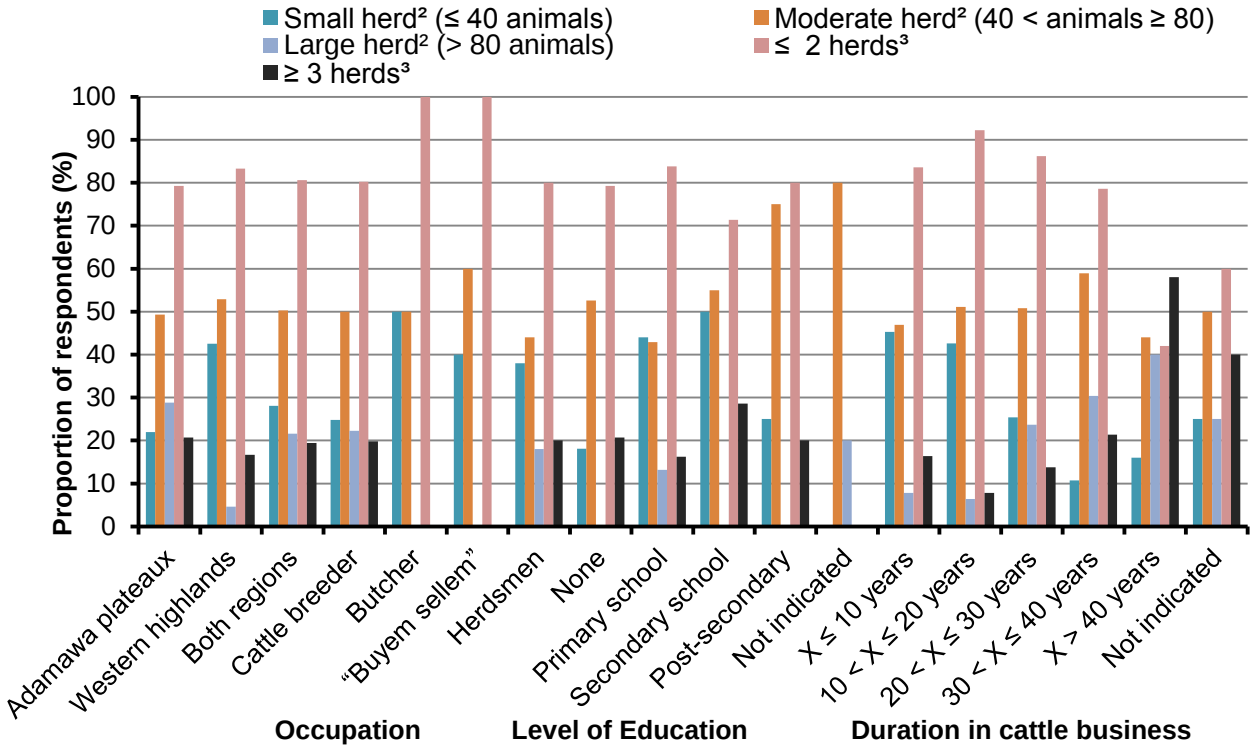
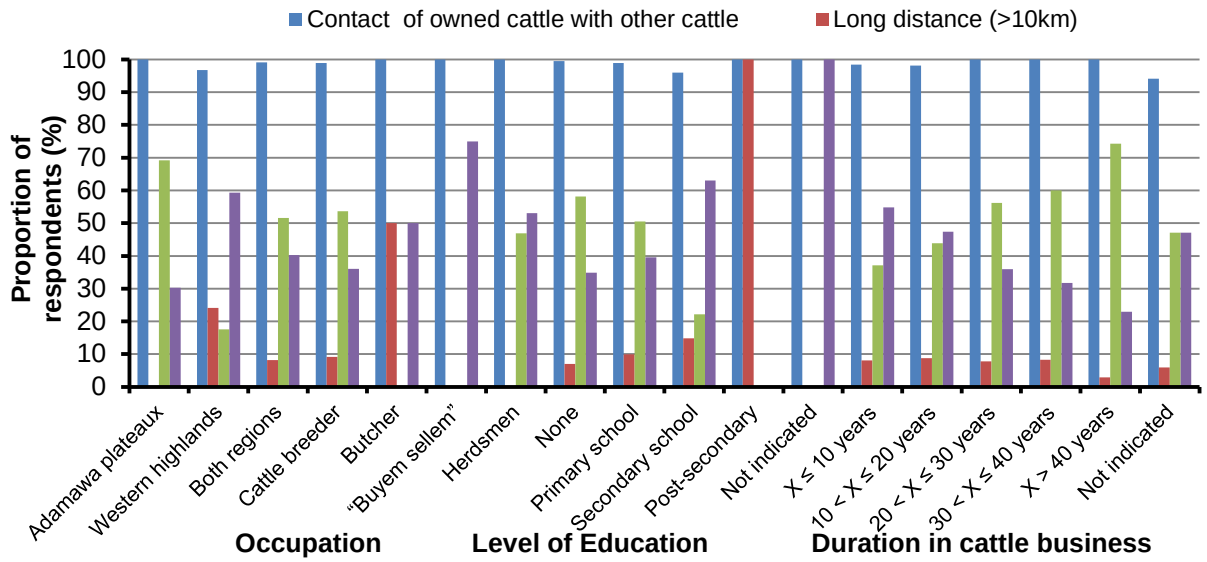
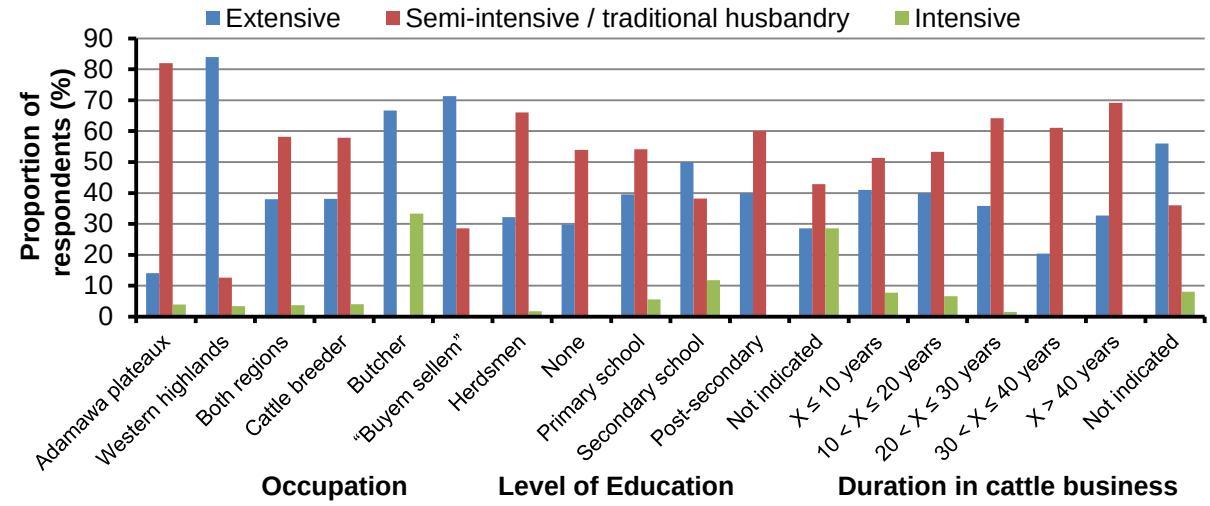


Figure 21 : Degree of interaction of cattle handlers with their cattle and other animals – human risk factors

a) Average daily trekking distance for grazing



b) Husbandry system and practice



c) Reasons for exploiting cattle herds

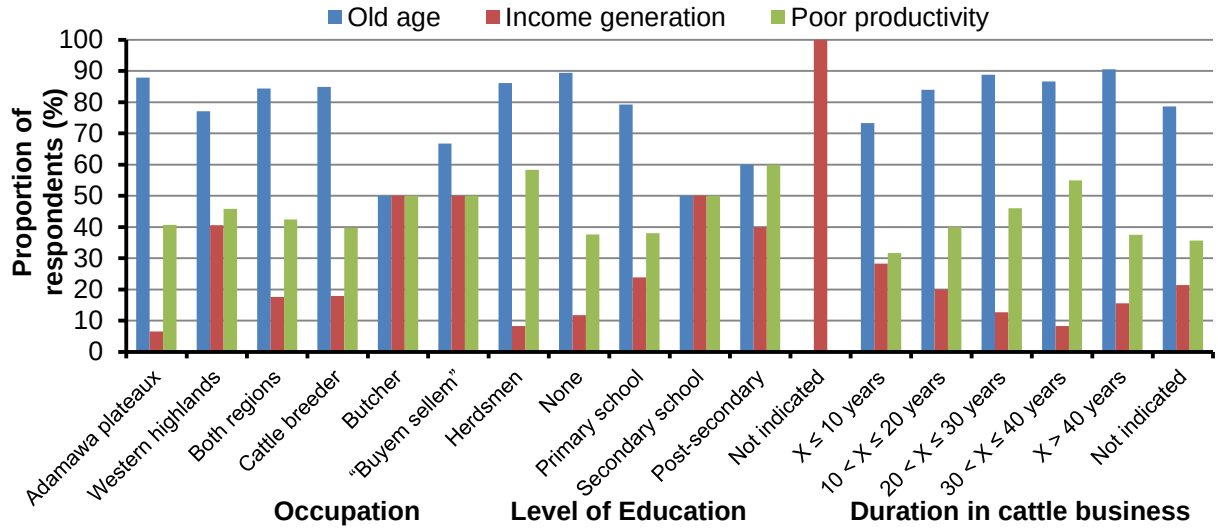
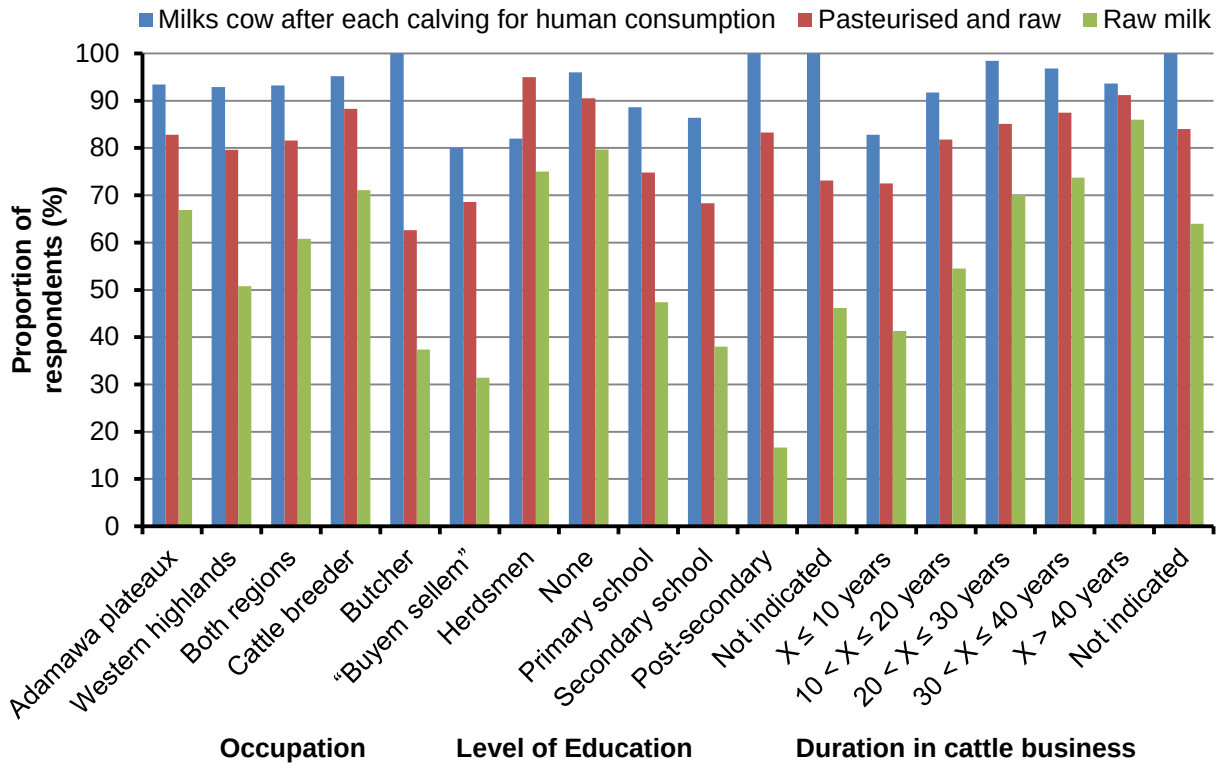
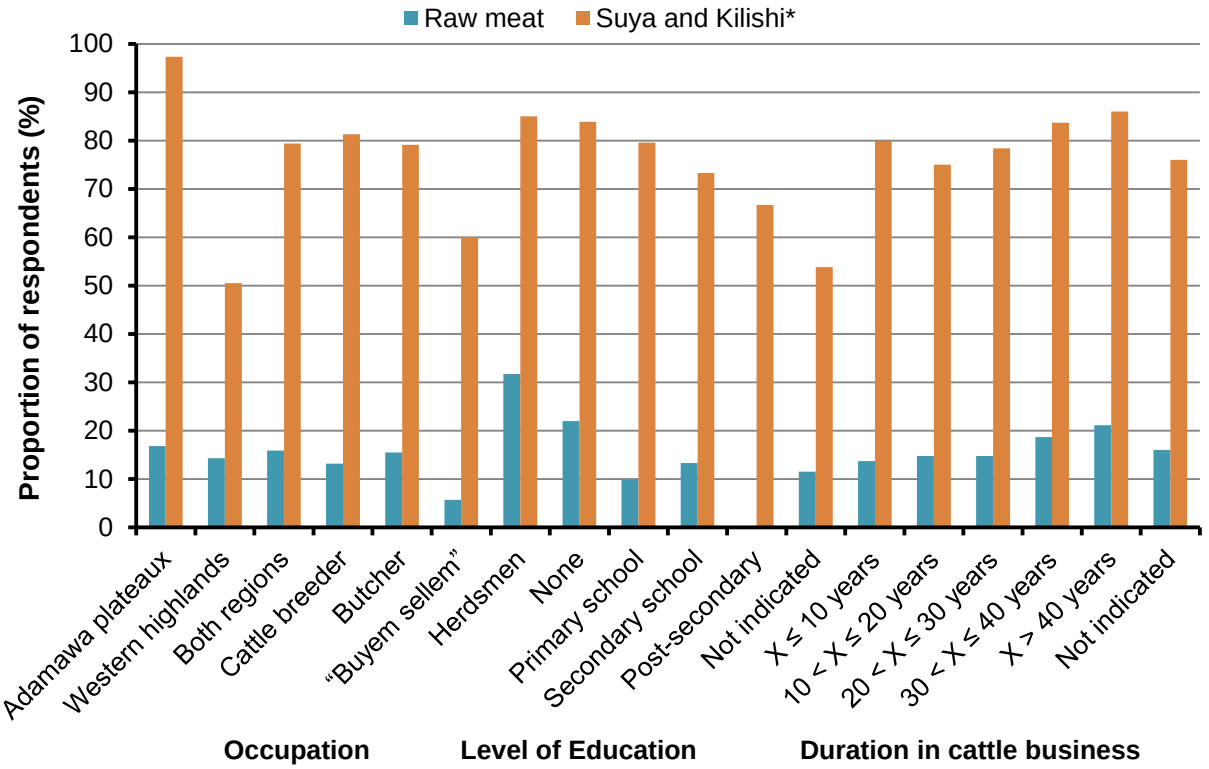


Figure 22 : Animal management and practices of cattle professionals – animal risk factors

a) Fresh milk consumption



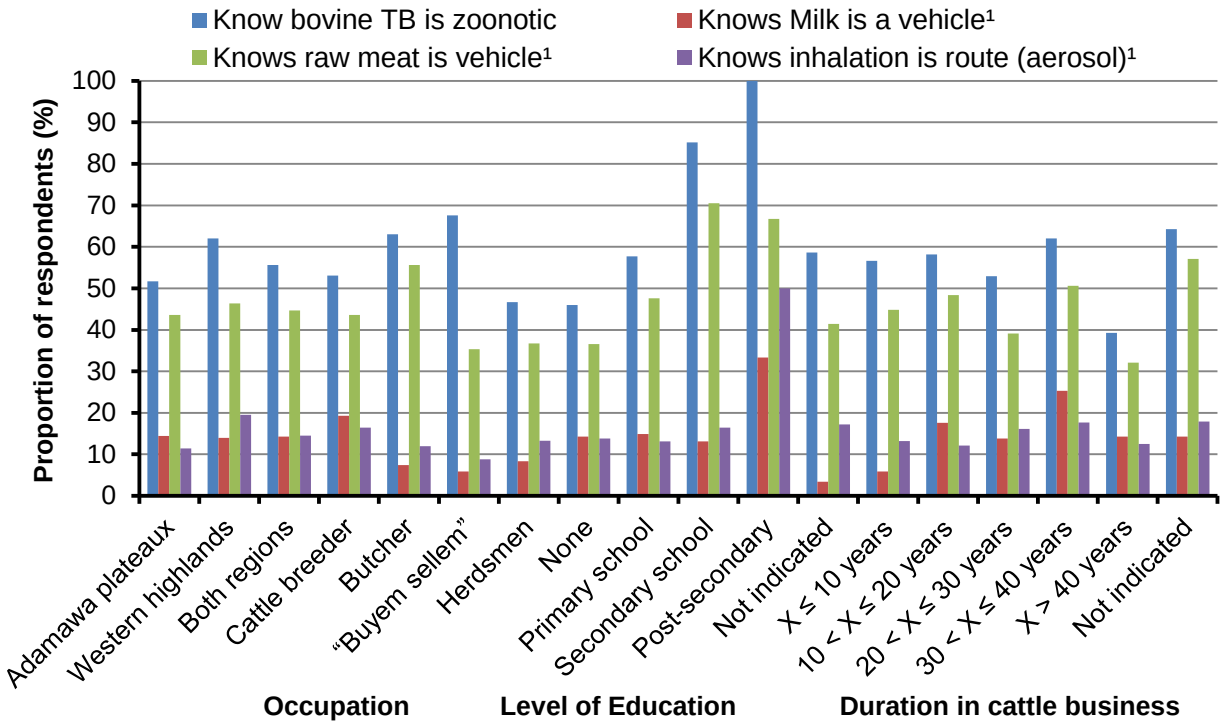
b) Meat consumption



*: "Suya" is meat briefly roasted over hot charcoal or fire. "Kilishi" is a traditional Cameroonian sun dried (and sometimes briefly roasted) meat.

Figure 23 : Factors affecting meat / milk consumption habit of cattle owners – human risk factors

a) Mode of transmission of bovine TB to humans[1]



b) Knowledge of number of modes of transmission

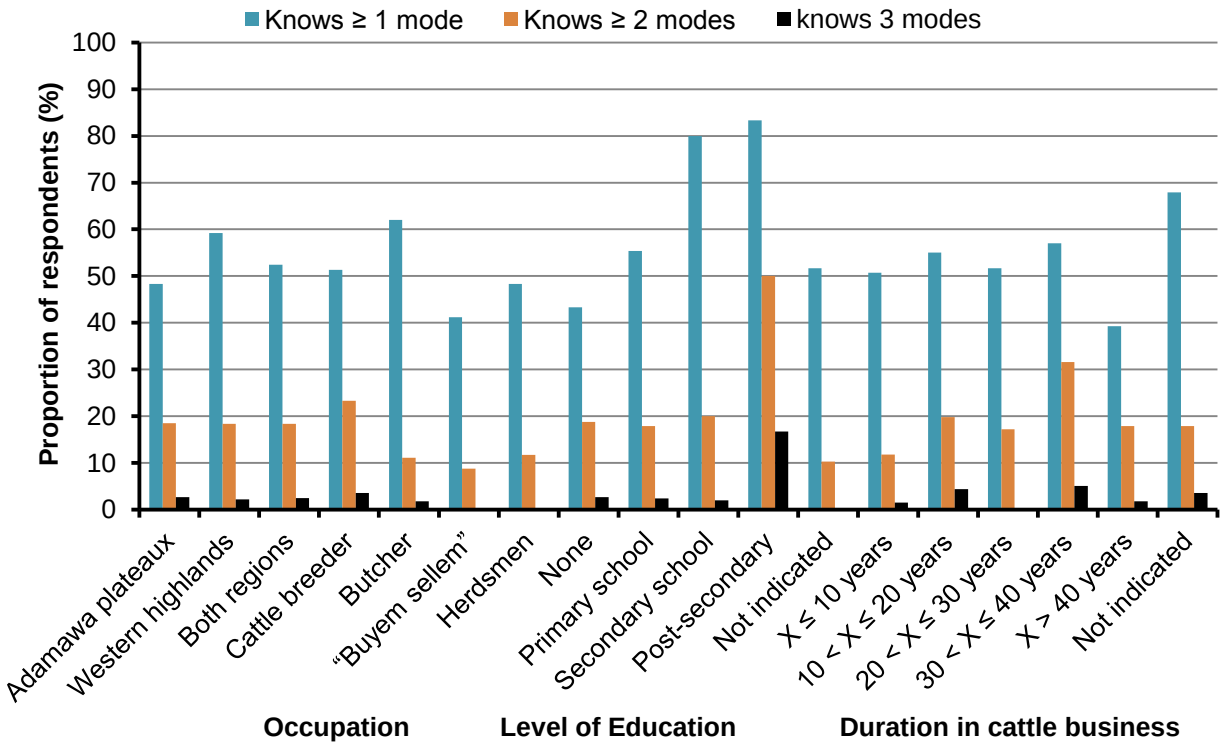
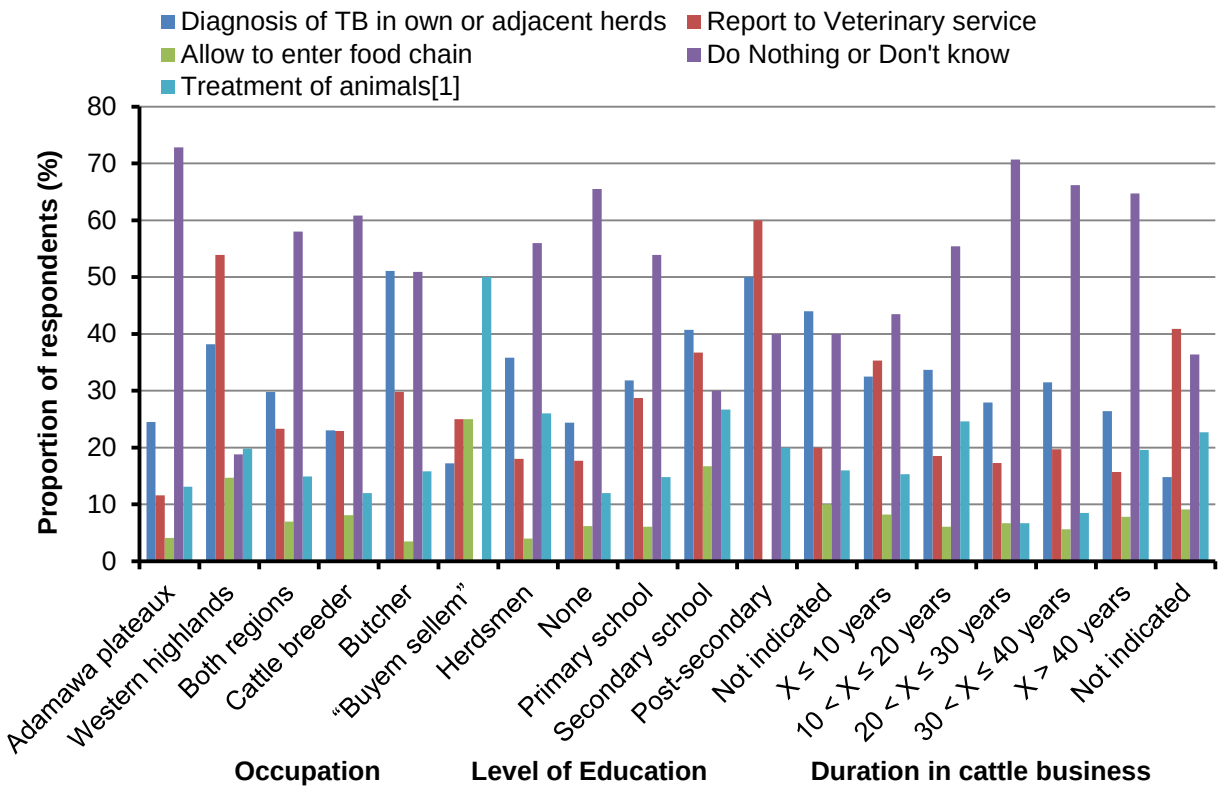


Figure 24 : Knowledge of cattle handlers about zoonotic bovine tuberculosis and its modes of transmission – Animal to humans risks and vice versa

a) Action taken if bovine TB is suspected in animal



[1] In Cameroon, animals suspected or diagnosed with bovine TB should be immediatedly removed from the herd and culled. However, some respondentsstill attempt to treat.

b) Action if animal dies of suspected bovine TB

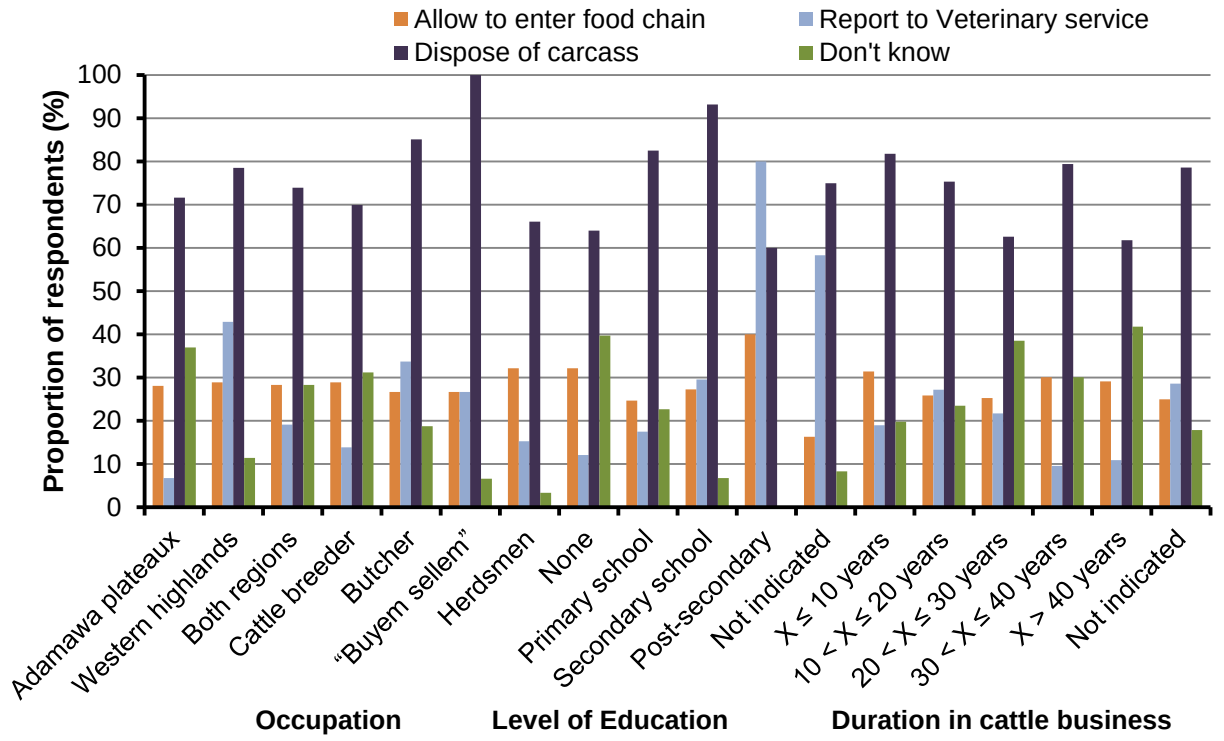
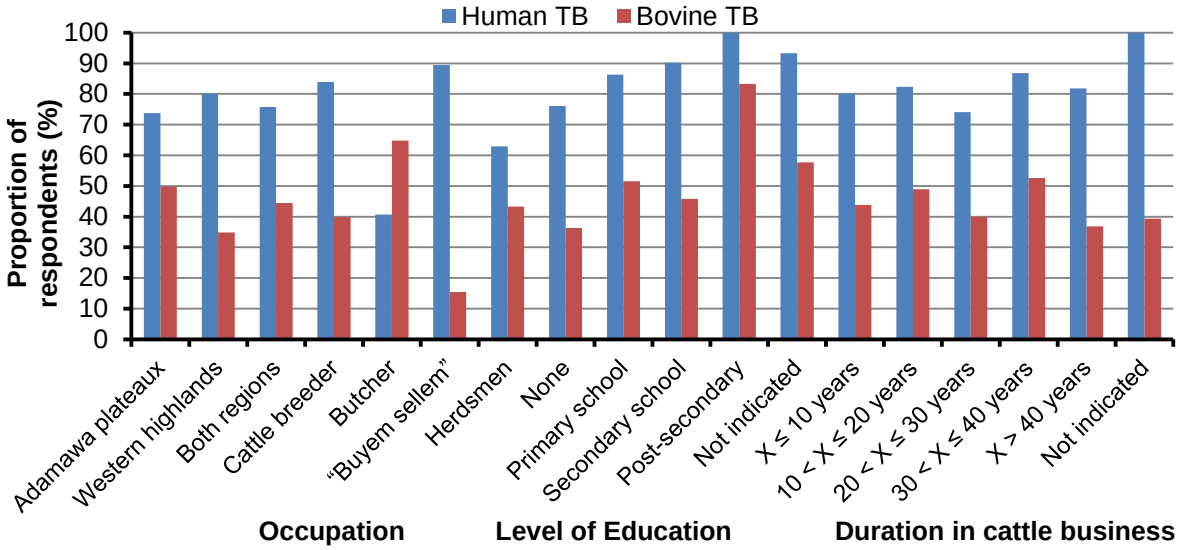
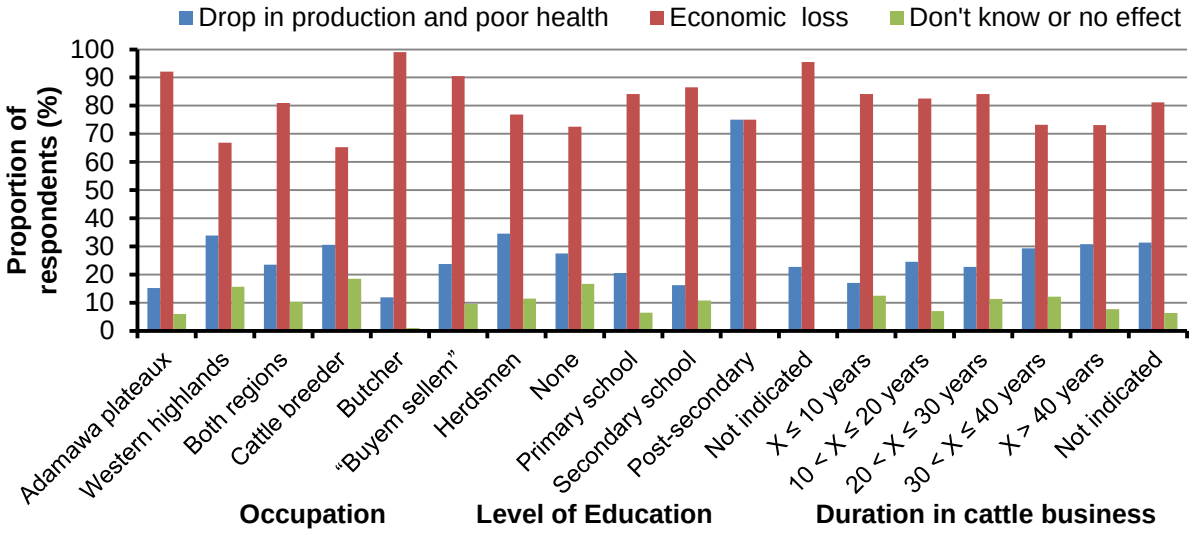


Figure 25 : Knowledge of cattle handlers about management

a) Previous contact with TB



b) Impact of bovine TB to cattle business



c) Awareness and implementation of bovine TB control

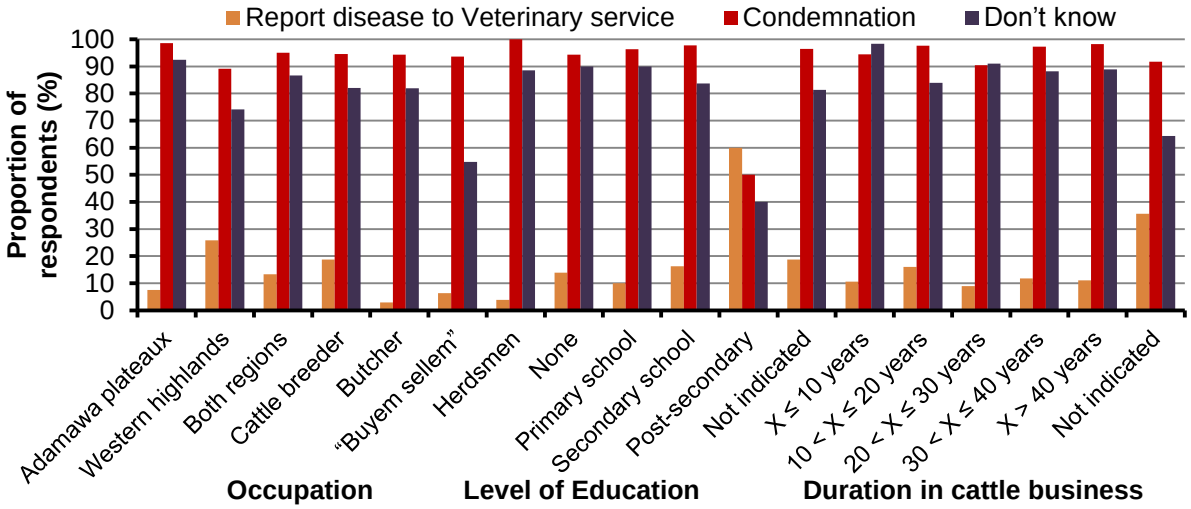


Figure 26 : Impact of bovine tuberculosis on cattle business and knowledge of cattle handlers about control of bovine tuberculosis

7.3 Discussion

7.3.1 Risk factors for bovine tuberculosis in cattle

Bovine TB is widespread in cattle herds in the highlands of Cameroon. The numbers of TST positive reactors are indeed high in some areas (Chapter 4 and 5). Very high circulating levels of anti-bovine TB antibodies in cattle have also been detected in the regions indicating that cattle are widely exposed to bovine TB. Using the relevant tuberculin cut-off point of ≥ 2 mm compared to the OIE recommended ≥ 4-mm value for skin responses in the Cameroonian environment revealed significantly higher prevalence rates of 16.90% vs. 5.97% and 22.77% vs. 5.91% for two separate comparative TST carried out at 12 months interval of each other (Chapter 5). TST positive cattle may be considered and treated as “open” cases of TB and potentially transmission sources of the infection to other animals and humans (O'Reilly and Daborn 1995). Therefore, the animals and communities in these study areas were and are at risk of infection with *M. bovis*.

In the study regions, higher herd infection rates (≥1 TST positive cattle) were recorded in large herds compared to small herds (Chapter 4 and 5), suggesting that the risk of bovine TB infection in a herd of cattle increases with increase in herd size. Infected new animals introduced into the herd may contaminate in-contact animaks followed by a lateral spread within the herd and also to other herds at “meeting” points. The increase in risk of cattle being infected with bovine TB with increase in herd size has been reported earlier (Cook et al. 1996; Ameni et al. 2003; Asseged et al. 2004). The prevalence of bovine TB in cattle in most of Africa is influenced by cattle breed, housing and gathering of animals at grazing, watering and other sites (Cosivi et al. 1998; Ayele et al.

2004; Ameni and Erkihun 2007). In this study many cattle professionals kept their animals in open pasture but they also reported criss-crossing the regions with their animals for grazing and watering; and confirmed that there were / are several occasions for close and repeated contacts between different herds. Therefore, the potential for maximum transmission and prevalence of bovine TB is high and hence the high prevalence rates of TST and anti-bovine TB antibodies reactors recorded in cattle in the study regions (Chapter 4 and 5).

Adult and old cattle have been reported to be most affected and at higher risks of bovine TB infection (Philips et al. 2003; Cleaveland et al. 2007; Tschopp et al. 2009). It has also been suggested that very little or no transmission during extensive communal grazing, even on crowded pastures and spread of the disease may occur during daily gathering of many animals from different herds at one site (Tschopp et al. 2009). However, gatherings of cattle at drinking points, vaccination centres, communal night enclosures and cattle markets among others have been found to positively influence transmission of bovine TB (O'Reilly and Daborn 1995; Cosivi et al. 1998; Ayele et al. 2004). Also, young animals could be infected if grazed with heavily infected older animals (Francis 1971) and infected cows shedding mycobacteria in their milk could be sources of early infection of young animals (Hojle 1990; Tschopp et al. 2009). In this study, traditional extensive and semi-extensive animal husbandries were the common animal management practices reported by the respondents, where they keep all animals together irrespective of age and sex. This agrees with earlier findings (Chapter 4; section: 4.2.2) which recorded higher bovine TB prevalence rates in cattle and herd infection rates in extensive and semi-extensive management systems; irrespective of the herd size. Also, anti-bovine TB antibodies have been observed in about 95% of tested herds and in

significant proportions of tested cattle irrespective of sex, age, breed, herd size, husbandry practices and health status (Chapter 5; sections: 5.2.1 & 5.2.2), suggesting that all class of animals were highly exposed to *M. bovis* and the risk of developing bovine TB could be very high with serious public health implications. Keeping other livestock in close contact with cattle could increase the risk of positive tuberculin reactions in cattle (Etter et al. 2006; Tschopp et al. 2009) and over a third of cattle professionals in this survey kept livestock other than cattle (fowls, sheep, goats) in mixed herding with their cattle. A strong association between typical and atypical mycobacterial prevalence rates in cattle in the study regions has been described earlier (Chapter 4; section: 4.2.2.3). This suggests high risks of exposure and transmission of multiple mycobacteria infections.

Widespread bovine TB, large herds, gathering of animals in common spots, adult and aging animals, stressors such as environmental factors, drought, long trekking to grazing and drinking spots are the major factors influencing bovine TB infection in cattle reported in the study.

7.3.2 Public health significance of bovine tuberculosis

Human TB is high and increasing in Cameroon (Noeske et al. 2004; Ane-Anyangwe et al. 2006) but investigation of *M. bovis* infection in humans is sparse. Bovine TB and zoonotic TB due to *M. bovis* are poorly investigated and controlled in most of Africa including Cameroon. However, multiplex PCR based deletion typing of RD9 and RD4 showed evidence of *M. bovis* from infected human sputa and *M. tuberculosis* from cattle tissues (Chapter 6; section: 6.2.3)

suggesting a possible interaction and transmission between TB in cattle and humans which needs detailed investigation. In fact *M. bovis* has been reported in one human TB subject in West Cameroon (Niobe-Eyangoh et al. 2003) further indicating that zoonotic bovine TB is a real public health problem that is under estimated and not investigated. One of the five spoligotypes of *M. bovis* isolated from cattle tissue (Chapter 6; section: 6.2.3.2) was widely distributed in the Western highlands; while the other four patterns have not been previously detected. The potential implication for drug resistance of human TB due to these *M. bovis* strains in Cameroon cannot be overemphasised. Indeed, cattle to human and human to human transmission of *M. bovis* infection (Gibson et al. 2004) and drug resistant of human TB cases related to several strains of *M. bovis* isolates have been reported (Gibson et al. 2004; Diguimbaye et al. 2006). A possible interface between bovine TB and human TB could therefore be implied given that there are also many opportunities for close and repeated human-livestock interactions and cattle keeping play important socio-economic role in many communities in the country.

Tuberculous lesions in cattle carcasses have been widely recorded during meat inspection in abattoirs in Cameroon (Doufissa 1993; Awah-Ndukum et al. 2005; Fon-Tebug 2009) including the study regions. Most cattle handlers were aware of bovine TB, its zoonotic nature and public health implications but many of them were also not informed about the modes of transmission of the disease. Butchers and other cattle professionals with low level of education were least knowledgeable and most at risk of exposure to zoonotic bovine TB. The populations' demands for meat supply from the Bamenda city abattoir are high and continually increasing and the public health threats of zoonotic bovine TB infection are very real. Consumption of unpasteurised milk was common in this

study but the proportion is expected to be even higher in further rural areas where poverty levels are high, literacy levels are low, and livestock keeping is high.

Inhalation of cough spray from infected animals and ingestion of infected animal products are the main routes *M. bovis* can be transmitted to humans (Francis 1971; Goodchild and Clifton-Hadley 2001; Cassidy 2006). However, a cow with tuberculous mastitis can shed viable tubercle bacilli to contaminate milk from up to 100 clean cows when milk pooling and bulk transportation is used (Hassanain et al. 2009). The presence of bovine TB and *M. bovis* in milk therefore represents major sources of infection to human and cattle (old and young). Most cattle professionals milked their cows and pooled the milk in units for home consumption as well as sell to local people or process locally to various products (eg: sour milk, nounou, kounou)[17] usually without sufficient initial heat treatment. Due to cattle professionals' poor comprehension of the hazards of zoonotic bovine TB the risks of contamination are high and there are real potential health hazards to consumers. Furthermore, the study regions were mainly rural and the questionnaire survey showed poor levels of awareness of zoonotic bovine TB, poor understanding of the modes of transmission of the disease from cattle to humans, close and repeated human-cattle interactions, and the consumption of raw milk and raw meat. Approximately 85% of cattle and 82% of human populations in Africa have been estimated to live in areas where animal TB is either partially controlled or uncontrolled (Ayele et al. 2004; Shitaye et al. 2006). Also, isolated detection of *M. bovis* from patients with pulmonary TB has been reported in other parts of Africa including Egypt, Nigeria, Democratic Republic of Congo, and Tanzania

[17] Locally processed milk products such as yogurt, cheese, butter

(Cook et al. 1996; Cosivi et al. 1998; Kazwala et al. 2001a; Cadmus et al. 2006; Zinsstag et al. 2006; Regassa et al. 2008) while epidemiologic associations between tuberculin-positive cattle and human TB have been reported in Zambia (Cook et al. 1996; Regassa et al. 2008). Elsewhere in the world, human TB due to *M. bovis* and the transmission of *M. bovis* from animals to man and back to animals have been reported (Fritsche et al. 2004; Thoen and LoBue 2007; Hlavsa et al. 2008; Tsegaye et al. 2010). There are therefore lots of circumstantial and real evidence for transboundary transmission of bovine TB as well as threats and hazards of zoonotic TB due to *M. bovis* to human health in most of Africa including Cameroon.

Bovine TB has severe public health significance but it is neglected in Cameroon. The inadequacies of control measures and poor understanding of the epidemiology of TB in cattle and humans in Cameroon poses additional risks for humans particularly because of high HIV/AIDS prevalence rates (Noeske et al. 2004; WHO 2011) and for cattle if the caretakers are infected (Ocepek et al. 2005; Berg et al. 2009). Also, the risk of multiple strains and or dual *M. bovis* and *M. tuberculosis* infections in cattle and humans cannot be ruled out. HIV/AIDS is the greatest single risk factor for developing active tuberculosis (Raviglione et al. 1993; Fätkenheuer et al. 1999; Pešut et al. 2008), due to decrease immunity while other tuberculosis risk factors (poverty, malnutrition, stress and smoking) become more pronounced and even multiplied in patients in TB risk groups (Pešut et al. 2008). The poor implementation of existing legislatures governing bovine TB control and neglect of a broad approach in the control of TB in animals and humans particularly the lack of collaboration between veterinary and medical professionals was widely reported in the survey.

A multidisciplinary approach mimicking the One Health Initiative approach, (Kahn et al. 2007; Anonymous 2009; Vallat 2009) where people's awareness is enhanced through continuous education of cattle professionals and the general public on hazards of TB and the potential risk of bovine TB, proper food (animal products) handling, good animal husbandry, personal hygiene and maintaining a healthy environmental is urgently needed in Cameroon to control TB. Targeted controlled movements of infected animal populations, concerted veterinary and medical efforts to maximise TB detection rates, active involvement of the populations at risk, and good health systems are essential for effective control of the disease in animals and humans. Biomedical education of people on TB symptoms in animals and humans may greatly contribute to the prevention of TB (animal TB and human TB) spread within the community.

7.3.3 Limitations to bovine tuberculosis control in Cameroon

Although poorly implemented, the control of animal TB in Cameroon is mainly through the regulation of animal movements, slaughter / meat inspection and post mortem examination of carcasses. TSTing and elimination of infected animals (test-and-slaughter policy) which have been used effectively in other parts of the world (Good 2006; Pavlik 2006a; Pavlik 2006b; OIE 2009) are not practicable in the country due to lack of compensatory policy if infected animals are eliminated. However, testing and segregating with phase slaughtering of infected animals could be economically and technically achievable as alternative to the direct test and slaughter method (WHO 1994b). Meanwhile, the need for intensification of meat inspection, good reliable abattoir records,

and validation of various diagnostic tests under the Cameroon environment for direct screening of live animals for bovine TB; and real epidemiologic status cannot be overemphasised.

Animal TB and human TB affects all sectors of the community but the poor are most vulnerable (Larson 2000b; a). Also, the impact of the interrelationships between human / animal / environment / disease factors and the interplay between them are not quite understood. Government resources for monitoring animal diseases including zoonoses are poor and the capacity of the private sector to assume the responsibility is also very lacking. Tackling the problems of monitoring animal diseases and impact of animal / human interactions such as zoonotic bovine TB on human health can be achieved through collaborative veterinary and medical programmes involving policy makers, animal and human populations at risk of exposure and transmission. Furthermore, urban and peri-urban (compared to rural) livestock farming is fast growing and most livestock professionals and handlers in Cameroon are small-scale farmers, nomads, herders, wage labourers, and unemployed youths who are also poor and uneducated. Supported development, education, capacity enforcements, and constant reassessment of cattle handlers' / professionals' level of awareness are therefore critical to good human health, improving animal health and productivity through good husbandry practices as well as poverty alleviation in Cameroon. Further investigations are needed to assess and evaluate the extent of the problem and design feasible cost-effective control methods.

Chapter 8

General discussion, conclusion and recommendations

There is sparse information on magnitude and distribution, risk of exposure and transmission, and public health significance of bovine TB in cattle in Cameroon. However, the occurrence of the disease had long been established based on post mortem finding of suspected TB lesions during meat inspections (Doufissa 1993; Awah-Ndukum et al. 2005). Analysis of 17 years slaughter / meat inspection records showed that more TB lesions than other pathologies were detected among the inspected animals. The increase in TB lesions detection rates in recent years in this study was explained by the correct detection of cases which could have been misdiagnosed due to enhanced efficiency of the meat inspectors.

Improved diagnostic awareness of bovine TB by inspectors and intensification of meat inspection procedures was responsible for the increasing trend recorded and not the actual disease state. However, the fact that bovine TB was unchecked in live cattle in the environment could have contributed to the wide fluctuations and numerous peaks of TB lesions in the Bamenda abattoir; which also poses the question of its real zoonotic threat and public health importance in Cameroon. It is important to note that, for meat inspection to offer effective means of monitoring bovine TB, all predilection tissues and organs should be thoroughly examined for detection of lesions (Grossklaus 1987; Hinton and Green 1997). Also, confirmation of the mycobacteria strains causing

the disease by conventional and other modern methods, including molecular techniques would be necessary for effective surveillance and control of zoonotic bovine TB in the country.

The high detection rates of circulating anti-bovine TB antibodies in cattle in the study revealed that cattle were highly exposed to bovine TB and the risk of developing the disease was high in the highlands of Cameroon. TSTing of cattle further confirmed that bovine TB was widespread and the number of positive reactors was actually very high in some areas. Also, response frequencies of the breeds to tuberculin skin and antibovine TB antibodies tests suggest differential susceptibility levels among the indigenous zebus to natural bovine TB; warranting further immunological investigations. For example, the Red Bororo consistently showed the highest levels of tuberculin skin and antibovine TB antibodies positive responses compared to the other local breeds. Atypical mycobacteria infections were also prevalent in the tested animals.

The TST are currently the techniques of choice for field diagnosis of bovine TB worldwide (de la Rua-Domenech et al. 2006a; de la Rua-Domenech et al. 2006b). Nonetheless, the ability of the tests to accurately diagnose the disease in live animals is affected by many factors including the country's disease status and control programme, environmental and host factors, prevalence of bovine TB in the tested population and nature of the tuberculin; and a perfect cut-off point in a specific geographic area may not be useful in another environment (Monaghan et al. 1994; de la Rua-Domenech et al. 2006b). The OIE-recommended cut-off of an optimal value of 4 mm is unsatisfactory in many countries and regions (Monaghan et al. 1994; Kazwala et al. 2001b; Ameni et al. 2008b). Severe interpretations have been employed in regions where the

disease is known to occur or in herds where *M. bovis* infection has been confirmed; while positive reactors to the single tuberculin test may also be subjected to a comparative TST (Monaghan et al. 1994). The comparative TST using bovine tuberculin and avian tuberculin injections at separate sites in the skin of the neck, gives more specific results than the single TST which uses only bovine tuberculin (Francis et al. 1973; Monaghan et al. 1994).

A comprehensive investigation of the performance of the TST at different cut-off points using anti-bovine TB antibodies assay in the Cameroon environment revealed that, irrespective of the cut-off value, there were strong associations between exposure of cattle to bovine TB (detection of circulating anti-bovine TB Ab) and the true disease status in cattle (TST results) in the highlands of Cameroon. Bovine TB was detected at all the cut-off points, though the best test performance was realized at the ≥3-mm cut-off point. However, interpreting the results of comparative TST for the detection of bovine TB at the ≥ 2-mm cut-off point was more strategic from a public health context since more disease cases would be predicted accurately. Non-significant difference in performance and accuracy values of TST at ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off values were recorded in this study compared to those reported in Ethiopia (Ameni et al. 2000; Ameni et al. 2008b) where post mortem examination of TB lesions and mycobacterial culture were used as the elective diagnostic techniques. These findings therefore confirmed the importance of defining relevant TST cut-off values to maximize the diagnosis of bovine TB in different environments. Also, application of the ≥2-mm cut-off point would be best to rapidly and effectively manage bovine TB in cattle; and reduce risks to public health and food safety in Cameroon. However, it is important to investigate the performance of TST cut-off points in the Cameroon environment against defined bovine TB status based

on detailed post-mortem examination for the presence of TB lesions in reacting animals.

TST positive cattle may be considered and treated as “open” cases of TB and potentially transmission sources of the infection to other animals and humans (O'Reilly and Daborn 1995). Therefore, the animals and communities in these study areas were at risk of infections with *M. bovis*. Very closed and repetitive human-livestock interactions were noted in the regions which poses serious risks for exposure and transmission of zoonotic bovine TB infection between cattle and from cattle to cattle professionals and the general public. Cattle keeping played important socio-economic roles in the study communities. A possible interface between bovine TB and human TB and threats of zoonotic bovine TB infection in the regions were also indicated by the populations’ continual and increasing demands for meat supply from the Bamenda city abattoir. The risk of bovine TB infection in a herd of cattle was influenced by large sizes of herds, high prevalence of the disease, high proportions of aging animals, gathering of animals, uncontrolled movements of animals, drought and various stressors for example long trekking to grazing and watering points and anergic states of the animals. However, most cattle handlers were aware of bovine TB, its zoonotic nature and public health implications but many of them were also not informed about the modes of transmission of the disease. Butchers and other cattle professionals with low level of education were least knowledgeable and most at risk of exposure to zoonotic bovine TB. Inhalation of cough spray from infected animals and ingestion of infected animal products (raw milk and raw meat) which are important routes for *M. bovis* spread to humans (Francis 1971; Goodchild and Clifton-Hadley 2001; Cassidy 2006) were commonly reported by cattle professionals. Furthermore, high levels of

awareness but poor implementation of existing legislatures governing bovine TB control and lack of collaboration between the veterinary and medical services were widely noted.

Bovine TB has severe public health significance but it is neglected in Cameroon. The inadequacies of control measures and poor understanding of the epidemiology of TB in cattle and humans in Cameroon poses additional risks, for humans particularly because of high HIV/AIDS prevalence rates (Noeske et al. 2004; WHO 2011) and risk for cattle if the caretakers are infected (Ocepek et al. 2005; Berg et al. 2009). HIV/AIDS is the greatest single risk factor for developing active tuberculosis (Raviglione et al. 1993; Fätkenheuer et al. 1999; Pešut et al. 2008), due to decrease immunity while other TB risk factors (poverty, malnutrition, stress and smoking) become more pronounced and even multiplied in patients in TB risk groups (Pešut et al. 2008).

TB is prevalent in cattle destined for human consumption in Cameroon with serious public health implications. The general public is at risk, and infected individuals can serve as sources of infection. Many opportunities exist for the emergence of zoonotic bovine TB in Cameroon and necessitate further study into the modes of transmission and link between human TB and bovine TB through molecular techniques. Targeted controlled movements of infected animal populations, concerted veterinary and medical efforts to increase TB detection, active involvement of the populations at risk of exposure and transmission, and good health systems are essential for effective control of the disease in animals and humans. These would further facilitate effective tackling the problems of monitoring zoonotic bovine TB in animals and impact of animal / human interactions on human health to be achieved. Continuous biomedical

education of people on TB symptoms in animals and humans, capacity enforcements, and constant reassessment of cattle handlers' / professionals' level of awareness are therefore critical to eliminating the risk of bovine TB to human health, great reduction of TB (animal TB and human TB) spread within communities as well as improvement of animal health and productivity in Cameroon.

The test-and-slaughter policy has been used effectively in many developed countries (Good 2006; Pavlik 2006a; Pavlik 2006b; OIE 2009). However, its application is yet not practicable in developing countries including Cameroon due to lack of compensatory policy if infected animals are eliminated. Testing and phase slaughtering of infected animals would be an accepted alternative as it is economically and technically achievable (WHO 1994b).

PCR-based genomic deletion analysis used in this study enabled the rapid and accurate differentiation of *M. bovis* and *M. tuberculosis* from cattle and human isolates within 24 – 48 hours (Warren et al. 2006). The analysis showed evidence of *M. bovis* from humans and *M. tuberculosis* from cattle suggesting possible animal to human and human to animal cyclic transmission patterns that need to be further investigated.

Spoligotyping is a rapid way of typing *M. tuberculosis* complex strains with direct repeat (DR) spacer markers (van Soolingen et al. 1995; Kamerbeek et al. 1997). Five closely related spoligotype patterns were identified in this study but only the dominant spoligotype pattern SB0953, has been previously described and from the Adamawa region of Cameroon (Njanpop-Lafourcade et al. 2001); suggesting that five strains of *M. bovis* were involved in causing bovine TB in cattle in the regions. The presence of 4 unique (new) spoligotypes and wide

dissemination of the spoligotype SB0953 was associated with extensive movement of cattle, both for commercial and for transhumance purposes (Müller et al. 2009a), which was very common within the highland regions. The spoligotype patterns identified in this study are different from previously reported patterns of *M. tuberculosis* complex strains shown by isolates from infected humans in Cameroon (Niobe-Eyangoh et al. 2003). The epidemiological implications of these findings are that new *M. bovis* strains maybe evolving from older strains (evident by their closeness to previously described strains). *M. bovis* strains (new and old) were introduced into the uninfected communities and naïve animals, where they became established as predominant or emerging strains with specific geographic distributions. Though the potential zoonotic consequences and multiple infections of these *M. bovis* strains cannot be overstated, the study provides additional knowledge of *M. bovis* strains in Cameroon which is vital for the control TB in humans and cattle.

However, the study presents preliminary mycobacteriological laboratory confirmation of bovine TB in cattle by culturing and characterisation of the *M. bovis* strains in the highlands of Cameroon. Further investigation is required to better understand the distribution of *M. bovis* strains within the study regions and also compare spoligotypes of *M. bovis* isolates from the shared frontier areas with the Western highland and Adamawa plateau regions of Cameroon and other neighbouring countries. Molecular epidemiology and immunological studies are also needed to investigate the modes of transmission, risk factors of the disease, geographic distribution of the *M. bovis* strains and differential susceptibility among the indigenous zebus to *M. bovis.* The variable levels of bovine TB infectiousness within and between the cattle breeds also need to be

further investigated in relation to susceptibility and genetic resistance of the indigenous zebus to bovine TB.

The high prevalence of bovine TB, the risks for zoonotic bovine TB in cattle and humans as well as the evidence of relationship between human TB and bovine TB by molecular typing presents the need for a national survey of the impact of TB and a reformulation of the TB control policy. Further studies on the burden of TB in cattle and humans, and human TB due to *M. bovis* would be needed to improve understand of the true epidemiological picture. Also, relating the disease burdens with its economic costs and costs/benefits analysis of controlling the disease in both humans and cattle would be very important for further assessment of the impact of TB in Cameroon. Additional research of all-inclusive lists of risk factors and public health implications of zoonotic bovine TB in cattle from a Cameroon context cannot be overemphasized. Comprehensive molecular epidemiological studies would be powerful tools to provide more precise epidemiology data on the issues of inter bovine transmission, the role of wildlife reservoirs and other domestic animals in maintenance and transmission of TB in animals and humans.

The following considerations are required to improve the understanding of the epidemiology and public health significance of zoonotic bovine TB in Cameroon and are recommended for prompt and decisive actions to control the disease in both humans and animals;

- The intensification of meat inspection procedure of slaughtered cattle and tracing back of infected / suspicious TB cases to the herds and region of orgin for monitoring bovine TB at the level of abattoirs. The introduction of confirmatory mycobacteriological diagnostic techniques

(e.g. culturing, molecular differentiation and characterisation of isolates from suspected TB lesions) would be important for effective surveillance.

- Severe interpretation SICCT-BT (such as at the ≥ 2-mm cut-off point) should be applied for the detection of bovine TB in cattle in Cameroon since more disease cases would be accurately diagnosed.
- However, the use of more than one test (such as using the lateral flow assays for antibovine TB antibodies detection together with the TST in this study) lead to the detection of the maximum number of bovine TB infected animals. Nonetheless, it is important to further investigate the performance of various TST cut-off points against defined bovine TB disease status based on other reference diagnostic techniques such as detailed post-mortem examination of TB lesions in TST reactors, mycobacteriological culture of TB lesions and gamma interferon assays in the local environment.
- Four of five spoilgotype patterns detected in the western highlands of Cameroon have not been previously identified elsewhere. It would be very interesting to identify, compare and geographical map the *M. bovis* strains in Cameroon, the shared frontier areas with neighbouring countries and the whole central Africa sub-region through extensive sampling and molecular characterisations using methods such as the spoligotyping and variable number tandem repeat (VNTR).
- Comprehensive molecular epidemiology and immunological studies are needed in Cameroon to further investigate the modes of transmission, risk factors, reservoir and maintenance hosts' status, and impact to human health of zoonotic bovine TB as well as the differential susceptibility among the indigenous zebus to *M. bovis.*

- Continuous education and capacity enhancements of cattle handlers, butchers, “buyem sellams” and the general public about the hazards of zoonotic bovine TB, proper food handling, good personal hygiene habits, healthy cattle husbandry practices and maintenance of the environments are indispensable for good human health and improving cattle health and productivity in Cameroon.
- In view of the findings of the present study (widespread bovine TB, favourable lifestyle of cattle professionals for occurrence and spread of zoonotic bovine TB, close cattle human interactions, identification of several spoligotypes of *M. bovis* isolates), the risk of bovine TB as a livestock health and production problem as well as a public health threat are real and good health systems are essential for effective control. Establishing a multidisciplinary body of investigators involving veterinary and medical professionals, public health workers, affected animals and human populations, socio-economic and biomedical scientists is needed to further assess and evaluate the extent of the problems of zoonotic bovine TB in Cameroon and propose feasible cost-effective control methods.

Appendices

Appendix 1

Making of Lowentein-Jesen (LJ) slants

The LJ medium used were prepared and enriched with Glycerol or pyruvate as recommended by the manufacturer and as previously described (Strong and Kubica 1985; WHO 1998b).

1) *Lowenstein*-Jesen *Medium Base* *37.3g*
   *(*Potassium dihydrogen phosphate anhydrous (KH_2PO_4) 2.4g
   Magnesium sulphate ($MgSO_4$. $7H_2O$) 0.24g
   Magnesium citrate 0.6g
   Malachite green *0.4g*
   Asparagine 3.6g
   Potato flour 30.0g)
2) *Bacto* Glycerol (reagent grade) 12ml
   (For pyruvate LJ meduium: glycerol is omitted and 8.0g sodium pyruvate is added)
3) Distilled water 600ml
   Dissolve the LJ medium base by heating to boiling with constant agitation. The final pH was ~ 7.0 at room temperature (20º – 25ºC).
4) Sterilise the solution by autoclaving at 121°C for 30 minutes and allowed to cool to 45º – 60ºC.
5) Add homogenised fresh and cleaned hen's whole eggs 1000ml
   The uniform suspension was prepared under aseptic conditions, avoiding air bubbles and sedimentations.
6) Thoroughly mix egg suspension with the sterile LJ Medium base and pour ~ 10 ml of the complete medium into 20ml sterile screw-capped test tubes, and the tops fastened securely.
7) Slant tubes to coagulate by inspissation at 85°C for 45 minutes.
   Before loading, heat the inspissator to 80°C to quicken the build-up of the temperature.
8) Incubate at 37°C for 24 – 48 hours as a check of sterility
9) Ensure tube caps are tightly closed to prevent evaporation or drying out of the LJ medium and store in the refrigerator (2° – 8°C) and used within 4 weeks.

Appendix 2

Reagents for Ziehl-Neelsen (ZN) staining

Based on Strong & Kubica (1985) and WHO (1998a)

1. *Stock solutions*
    a. *Fuchsin*

Basic fuchsin	3.0g
95% ethanol (technical grade)	100ml

Dissolve basic fuchsin in ethanol.....Solution 1

    b. *Phenol*

Phenol crystals 5g
Distilled water 100ml
Dissolve phenol crystals in distilled water with gentle heating .....Solution 2

2. *Working solution*

10ml of solution 1 was combined with 90ml of solution 2 and stored in an amber bottle – preparation and expiry dates labelled on the bottle (stored at room temperature for six to twelve months) and filter before use.

3. *Decolourising agent: 3% acid-alcohol*

Concentrated hydrochloric acid (technical grade)	3ml
95% ethanol (technical grade)	97ml

Concentrated hydrochloric acid was c*arefully* added to 95% ethanol and stored in an amber bottle – preparation and expiry dates labelled on the bottle (stored at room temperature for six to twelve months).

4. *Counterstain: Methylene blue*

Methylene blue chloride	0.3g
Distilled water	100ml

Methylene blue chloride was dissolve in distilled water and stored in an amber bottle – preparation and expiry dates labelled on the bottle (stored at room temperature for six to twelve months).

Appendix 3

Niacin paper strip test for the identification of *M. tuberculosis*

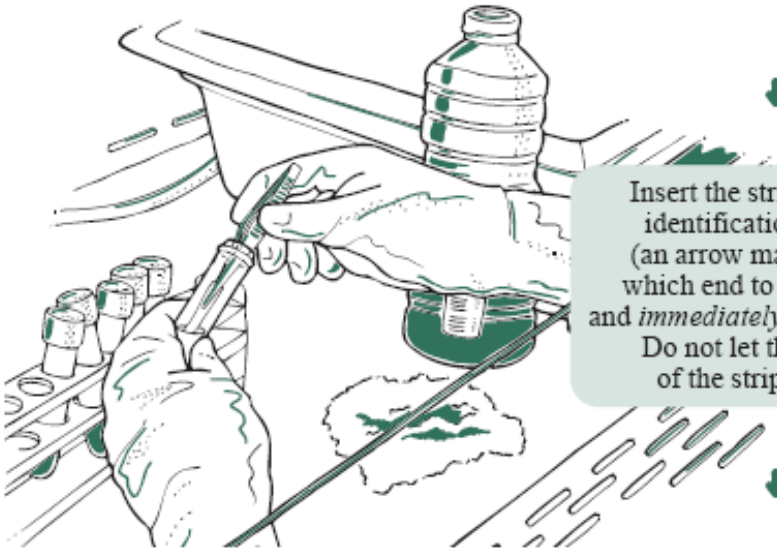
PROCEDURE

Add 1ml of sterile saline to the culture slant. If growth is confluent, puncture the medium with a Pasteur pipette to allow contact of the saline with the medium

Place the tube horizontally so the fluid covers the entire surface of the medium

Allow 30 minutes for the extraction of niacin. The extraction time may be longer if the culture has few colonies

Raise the slant upright for 5 minutes to allow the fluid to drain to the bottom. Remove 0.5ml of the fluid extract to a clean screwcap tube



Insert the strip with the identification end up (an arrow may indicate which end to insert first) and *immediately* seal the tube. Do not let the middle of the strip get wet

Leave at room temperature for 15-20 minutes. Occasionally agitate the tube without tilting it

Observe the colour of the liquid in the bottom of the tube against a white background (yellow = positive). Discard any colour on the strip itself; this may occur because of oxidation of chemicals, especially at the top of the strip

Neutralise the strips with 10% sodium hydroxide or discard them into alkaline disinfectant

Procedure as described by WHO (1998b).

Appendix 4

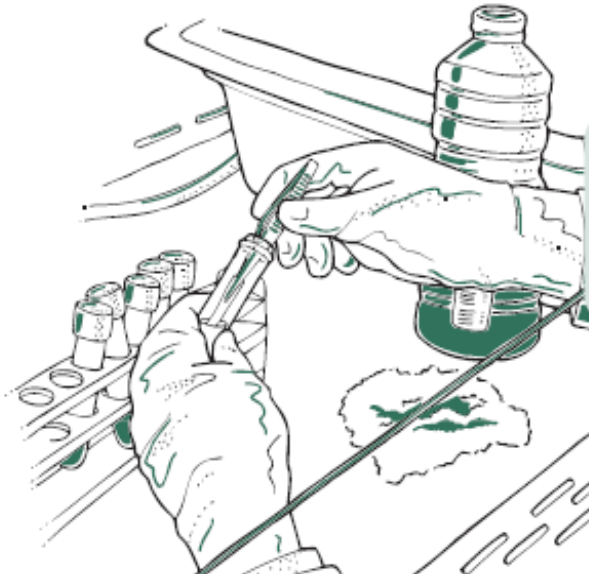
Nitrate paper strip test for confirmation of *M. tuberculosis*

PROCEDURE

Add 1ml sterile saline to a sterile screwcap test tube

↓

Use a sterile spade/applicator stick to emulsify in the saline two spadefuls of growth from a four-week-old culture



Use sterilised forceps and carefully insert a nitrate test strip (arrow indicates which end to insert first); do not let the strip contact any fluid on the side of the tube

Cap the tube tightly and incubate in a vertical position at 37°C for two hours

↓

After one hour of incubation, shake the tube gently without tilting

↓

After two hours of incubation, tilt the tube six times to wet the entire strip

↓

Allow the tube to remain slanted for 10 minutes with the liquid covering the strip

↓

Examine the top portion of the strip for changes to light or dark blue (= positive)

Procedure as described by WHO (1998b).

Appendix 5

Catalase test

Catalase is an intracellular, soluble enzyme capable of splitting hydrogen peroxide into water and oxygen. Thus, oxygen bubbles into the reaction mixture indicate catalase activity. Nearly all mycobacteria possess catalase enzymes that vary in heat stability. Quantitative differences in catalase activity were demonstrated by the following tests according to WHO (1998b) :

- Room temperature or drop method (indicates the presence of catalyse)
- 68°C test at pH7 (indicates loss of catalyse activity due to heat)

For these tests the media tubes were placed in an upright position and not slanted. The tubes were also capped loosely to permit exchange of air.

Reagents

1) 0.067M phosphate buffer solution, pH 7.0

Na_2HPO_4 anhydrous	9.47g
Distilled water	1000ml

Dissolve disodium phosphate in distilled water to provide a 0.067M solution .....Solution 1

KH2PO4	9.07g
Distilled water	1000ml

Dissolve monopotassium phosphate in distilled water to provide a 0.067M solution .....Solution 2

2) 30% Hydrogen peroxide (H_2O_2) – stored in the refrigerator
3) 10% Tween 80

Tween 80	10ml
Distilled water	90ml

Mix Tween 80 with distilled water and autoclave at 121°C for 10 minutes. If Tween 80 settled, it was re-suspended by swirling immediately after autoclaving and during cooling. Store in the refrigerator

4) Complete catalase reagent (Tween-peroxide mixture)

   Immediately before use, mix equal parts of 10% Tween 80 and 30% hydrogen peroxide. Allow 0.5ml reagent for each strain to be tested.

5) For Controls

   a. Drop method: Use an uninoculated tube of medium as negative control and M. tuberculosis H37Rv as positive control.
   b. Semiquantitative and 68°C tests: Use an uninoculated tube of medium as negative control and M. terrae as positive control.

Procedures

6) Drop method: Examine LJ slant to ascertain that growth has occurred. Add one to two drops of the freshly-prepared Tween-peroxide mixture to the slant with the culture growth. Observe for a period of 5 minutes for the formation of bubbles.

Results and interpretation:

Negative	No bubbles formed
Positive (slow)	Few slowly forming bubbles
Positive (rapid)	Immediate copious formation of bubbles

7) 68°C, pH7.0 test : See the diagram below for the procedure of heat labile catalase test (68°C, pH 7.0) for identification of M. tuberculosis

   Results and interpretation:

Positive	Bubbles
Negative	No bubbles

Reactions were still recorded as positive when only small quantities of bubbles were seen rising from the bottom of the tube (sedimental cells) with no foam formed at the surface of the fluid.

Procedure of heat labile catalase test (68°C, pH 7.0) for identification of M. tuberculosis

With a sterile pipette, aseptically add 0.5ml of 0.067M phosphate buffer, pH 7.0 to 16x125mm screw-cap tubes

↓

Suspend several loopfulls of test cultures in the buffer solution, using sterile loops

↓

Place the tubes containing the emulsified cultures in a previously heated water bath at 68°C for 20 minutes. *Time and temperature are critical*

↓

Remove the tubes from heat and allow to cool to room temperature

↓

Add 0.5ml of the freshly-prepared Tween-peroxide mixture to each tube and replace caps loosely

↓

Observe the formation of bubbles appearing on the surface of the liquid. *Do not shake the tubes* because Tween 80 also may form bubbles when shaken, resulting in false positive results

↓

Hold negative tubes for 20 minutes before discarding

SUMMARY

IDENTIFICATION OF M. tuberculosis

- Growth rate slow
- Growth temperature 35°-37°C only
- No pigmentation
- Niacin positive
- Nitrate positive
- Catalase negative at 68°C
- No growth on LJ medium containing p-nitrobenzoic acid

Appendix 6

Table 27 : Monthly frequency distribution of tuberculous and other non-tuberculous pathologies in slaughtered cattle recorded at the Bamenda municipal abattoir, Cameroon (1994 – 2010)

Month	**Total slaughtered**	All Pathologies		Non- Tuberculous lesions		Tuberculous lesions	
		No	% (95% CI)	No	% (95% CI)	No	% (95% CI)
January	10647	25	0.72 (0.56 – 0.88)	52	0.23 (0.14 – 0.33)	77	0.49 (0.36 – 0.62)
February	10293	29	0.77 (0.60 – 0.94)	50	0.28 (0.18 – 0.38)	79	0.49 (0.35 – 0.62)
March	10782	44	1.21 (1.01 – 1.42)	87	0.41 (0.29 – 0.53)	131	0.81 (0.64 – 0.98)
April	9124	38	1.00 (0.79 – 1.20)	53	0.42 (0.28 – 0.55)	91	0.58 (0.42 – 0.74)
May	9973	36	0.82 (0.64 – 1.00)	46	0.36 (0.24 – 0.48)	82	0.46 (0.33 – 0.59)
June	10001	39	0.89 (0.71 – 1.07)	44	0.45 (0.32 – 0.58)	89	0.44 (0.31 – 0.57)
July	10616	26	0.69 (0.53 – 0.84)	47	0.24 (0.15 – 0.34)	73	0.44 (0.32 – 0.57)
August	10766	35	0.85 (0.68 – 1.03)	57	0.33 (0.22 – 0.43)	92	0.53 (0.39 – 0.67)
September	10800	36	0.81 (0.65 – 0.98)	52	0.33 (0.22 – 0.44)	88	0.48 (0.35 – 0.61)
October	11119	28	0.55 (0.41 – 0.69)	33	0.25 (0.16 – 0.34)	61	0.30 (0.20 – 0.40)
November	11574	16	0.46 (0.33 – 0.58)	37	0.14 (0.07 – 0.21)	53	0.32 (0.22 – 0.42)
December	13470	26	0.50 (0.38 – 0.62)	41	0.19 (0.12 – 0.27)	67	0.30 (0.21 – 0.40)
Total	129165	381	0.76 (0.71 – 0.81)	599	0.30 (0.27 – 0.33)	983	0.46 (0.43 – 0.50)
Dry season (Nov - Feb)	45984	136	**0.54**[a] (0.42 – 0.66)	242	**0.21**[a] (0.15 – 0.27)	378	**0.33**[a] (0.25 – 0.41)
Rainy season (Mar - Oct)	83181	245	**0.77**[a] (0.61 – 0.93)	357	**0.31**[a] (0.25 – 0.37)	605	**0.46**[a] (0.32 – 0.59)

a, b: different letters in a column are significant different (P<0.05)

Appendix 7

Table 28: Annual prevalence of tuberculous and non-tuberculous lesions in slaughtered cattle recorded at the Bamenda municipal abattoir, Cameroon

Year	Total slaughtered	All Pathologies		Non-Tuberculous lesions		Tuberculous lesions	
		No	% (95% CI)	No	% (95% CI)	No	% (95% CI)
1994	6205	11	0.18 (0.07 - 0.28)	2	0.03 (0 - 0.08)	9	0.15 (0.05 - 0.24)
1995	4519	5	0.11 (0.01 - 0.21)	3	0.07 (0 - 0.14)	2	0.04 (0 - 0.11)
1996	3687	10	0.27 (0.10 - 0.44)	3	0.08 (0 - 0.17)	7	0.19 (0.05 - 0.33)
1997	3598	27	0.75 (0.47 - 1.03)	18	0.50 (0.27 - 0.73)	9	0.25 (0.09 - 0.41)
1998	5358	13	0.24 (0.11 - 0.37)	7	0.13 (0.03 - 0.23)	6	0.11 (0.02 - 0.20)
1999	4216	41	0.97 (0.68 - 1.27)	24	0.57 (0.34 - 0.80)	17	0.40 (0.21 - 0.59)
2000	4764	14	0.29 (0.14 - 0.45)	8	0.17 (0.05 - 0.28)	6	0.13 (0.03 - 0.23)
2001	4202	6	0.14 (0.03 - 0.26)	4	0.10 (0 - 0.19)	2	0.05 (0 - 0.11)
2002	7856	42	0.53 (0.37 - 0.70)	18	0.23 (0.12 - 0.33)	24	0.31 (0.18 - 0.43)
2003	7537	11	0.15 (0.06 - 0.23)	2	0.03 (0 - 0.06)	9	0.12 (0.04 - 0.20)
2004	11940	85	0.71 (0.56 - 0.86)	58	0.49 (0.36 - 0.61)	27	0.23 (0.14 - 0.31)
2005	10339	85	0.82 (0.65 - 1.00)	50	0.48 (0.35 - 0.62)	35	0.34 (0.23 - 0.45)
2006	12280	164	1.34 (1.13 - 1.54)	77	0.63 (0.49 - 0.77)	87	0.72 (0.57 - 0.87)
2007	11105	113	1.02 (0.83 - 1.20)	37	0.33 (0.23 - 0.44)	76	0.68 (0.53 - 0.84)
2008	10450	74	0.71 (0.55 - 0.87)	32	0.31 (0.20 - 0.41)	42	0.40 (0.28 - 0.52)
2009	9874	156	1.58 (1.33 - 1.83)	12	0.12 (0.05 - 0.19)	144	1.46 (1.22 – 1.69)
2010	11235	126	1.12 (0.93 – 1.32)	29	0.26 (0.16 - 0.35)	97	0.86 (0.69 – 1.03)
Total	**129165**	**983**	**0.76 (0.71 - 0.81)**	**384**	**0.30 (0.27 - 0.33)**	**599**	**0.46 (0.43 - 0.50)**

Appendix 8

Table 29 : Distribution of SICCT-BT positive reactors at the ≥ 4-mm, ≥ 3-mm and ≥ 2-mm cut-off points in 1,381 cattle in the highlands of Cameroon (using Se and Sp values observed by Ameni et al. (2008) for SICCT-BT and Pollock et al. (2003) for SIT-BT).

Variable	Animals tested	SICCT-BT reactors, %(95%CI)			SIT-BT reactors, %(95%CI)
		≥ 4 mm	≥ 3 mm	≥ 2 mm	≥ 4 mm
All animals	1,381	7.40 (6.02 – 8.79)	13.25 (11.47 – 15.04)	17.26 (15.26 – 19.25)	18.35 (14.35 – 22.35)
Agro-ecological location					
Adamawa plateaux	363	0.43 (0 – 1.11)	0.40 (0 – 1.04)	0.79 (0 – 1.70)	0.59 (0 – 2.13)
Western highlands	1,018	9.89 (8.06 – 11.72)	17.84 (15.49 – 20.19)	23.13 (20.54 – 25.72)	24.68 (19.49 – 29.88)
Breed					
Upgraded/Exotic	764	10.87 (8.66 – 13.08)	16.12 (13.52 – 18.73)	21.07 (18.18 – 23.96)	24.03 (18.09 – 29.96)
Guadali	492	0.31 (0 – 0.79)	0.28 (0 – 0.75)	0.57 (0 – 1.24)	0.40 (0 – 1.50)
Namchi	31	0	0	0	0
Red Bororo	94	18.84 (10.94 – 26.75)	62.22 (52.42 – 72.02)	79.29 (71.10 – 87.49)	72.28 (54.54 – 90.01)
Sex and Age					
Female	1,107	8.29 (6.66 – 9.91)	13.90 (11.87 – 15.94)	17.69 (15.44 – 19.94)	19.73 (15.14 – 24.33)
Male	274	3.83 (1.56 – 6.11)	10.63 (6.98 – 14.28)	15.51 (11.22 – 19.79)	12.77 (5.02 – 20.51)
Age ≤ 4 years	716	4.41 (2.91 – 5.91)	9.71 (7.55 – 11.88)	12.50 (10.07 – 14.92)	11.93 (7.28 – 16.59)
Age > 4 years	665	10.63 (8.29 – 12.97)	17.07 (14.21 – 19.92)	22.38 (19.22 – 25.55)	25.26 (18.79 – 31.73)
Management system					
Extensive	488	0.31 (0 – 0.80)	0.28 (0 – 0.75)	0.58 (0 – 1.25)	0.41 (0 – 1.51)
Intensive	552	8.95 (6.57 – 11.34)	12.03 (9.31 – 14.74)	16.50 (13.40 – 19.59)	20.74 (14.11 – 27.36)
Semi-intensive	341	15.05 (11.26 – 18.85)	33.81 (28.78 – 38.83)	42.36 (37.11 – 47.60)	40.17 (29.97 – 50.37)
Beef herds	692	7.39 (5.44 – 9.34)	16.40 (13.64 – 19.16)	20.63 (17.61 – 23.64)	18.97 (13.25 – 24.70)
Dairy herds	689	15.15 (12.48 – 17.83)	10.10 (7.85 – 12.35)	13.87 (11.29 – 16.45)	17.73 (12.14 – 23.32)
Herd size (No animals per herd)					
≤ 40 animals	713	9.41 (7.27 – 11.55)	12.72 (10.27 – 15.17)	16.39 (13.67 – 19.11)	19.14 (13.48 – 24.81)
> 40 animals	668	5.26 (3.57 – 6.96)	13.82 (11.21 – 16.44)	18.18 (15.26 – 21.11)	17.50 (11.86 – 23.15)

Appendix 9

Table 30: Degree of interaction of cattle handlers with their cattle and other animals – human risk factors

Variable	Proportion (%) of respondents (n = 489)	Contact between cattle handlers and animals (n=489: ADP=302, WHC=187)			Herd size (n=292: ADP=205, WHC=87)			Number of herds (n=304: ADP=208, WHC=96)	
		Own other livestock spp	Daily contact with cattle herds	≥ 1 day contact with cattle herds per week	Small herd (≤ 40 animals)	Moderate herd (40<animals≥80)	Large herd (> 80 animals)	≤ 2 herds	≥ 3 herds
Highland region									
ADP	61.8	8.3[a]	87.8[a]	12.3[a]	22.0	49.3	28.8	79.3	20.7
WHC	38.2	73.8[b]	86.1[a]	13.9[a]	42.5	52.9	4.6	83.3	16.7
Total	100	33.3	87.1	12.9	28.1	50.3	21.6	80.6	19.4
Occupation									
Cattle breeder	57.7	35.5[c]	83.7[b]	16.3[bd]	24.8	50.0	22.3	80.2	19.8
Butcher	22.5	28.2[d]	97.3[c]	2.7[c]	50.0	50.0	0.0	100.0	0.0
“Buyem sellem”	7.6	64.9[e]	75.7[b]	24.3[b]	40.0	60.0	0.0	100.0	0.0
Herdsmen	12.3	13.3[d]	91.7[c]	13.3[d]	38.0	44.0	18.0	80.0	20.0
Education (school level)									
None	46.6	22.4[f]	90.3[df]	9.6[e]	18.1	52.6	0.0	79.3	20.7
Primary school	35.6	39.7[g]	86.8[df]	13.2[e]	44.0	42.9	13.2	83.8	16.2
Secondary school	10.4	53.2[h]	74.2[e]	25.8[f]	50.0	55.0	0.0	71.4	28.6
Post-secondary	1.4	71.4	71.4	28.6	25.0	75.0	0.0	80.0	20.0
Not indicated	5.9	44.8[h]	96.5[f]	3.5[g]	0.00	80.0	20.0	0.0	0.0
Duration in cattle business									
≤ 10 years	28.8	38.3[jk]	82.3[gj]	17.7[hk]	45.3	46.9	7.8	83.6	16.4
10 < X ≤ 20 years	18.6	36.3[jk]	93.4[h]	6.6[j]	42.6	51.1	6.4	92.2	7.8
20 < X ≤ 30 years	18.2	30.3[jk]	91.0[h]	9.0[j]	25.4	50.8	23.7	86.2	13.8
30 < X ≤ 40 years	16.4	27.5[jk]	90.0[hjk]	10.0[hjk]	10.7	58.9	30.4	78.6	21.4
X > 40 years	11.9	25.9[j]	79.3[jk]	20.7[jk]	16.0	44.0	40.0	42.0	58.0
Not indicated	6.1	40.0[k]	86.7[hjk]	13.3[hjk]	25.0	50.0	25.0	60.0	40.0

n = total number of respondents, ADP = Adamawa Plateau, WHC= Western highlands

a-k: different letters in same column are significantly different (P<0.05)

Appendix 10

Table 31 : Animal management and practices of cattle professionals – animal risk factors

Variable	Contact of owned cattle with other cattle (n=318: Yes =315, No=3)	Average daily trekking distance for grazing (n=316: ADP=208, WHC=108)			Husbandry system and practice (n=347: AD=228, WHC=119)			Reasons for exploiting cattle herds (n=295: ADP=199, WHC=96)		
		Long distance (>10km)	Moderate distance (5km < X ≥ 10km)	Short distance (≤ 5 Km)	Extensive	Semi-intensive / traditional husbandry	Intensive	Old age	Income generation	Poor productivity[18]
Highland region										
ADP	100.0	0.0	69.2	30.3	14.0	82.0	3.9	87.9	6.5	40.7
WHC	96.8	24.1	17.6	59.3	84.0	12.6	3.4	77.1	40.6	45.8
Total	99.1	8.2	51.58	40.2	38.0	58.2	3.7	84.4	17.6	42.4
Occupation										
Cattle breeder	98.9	9.2	53.6	36.0	38.1	57.9	4.0	84.9	17.9	39.8
Butcher	100	50.0	0.0	50.0	66.7	0.0	33.3	50.0	50.0	50.0
“Buyem sellem”	100	0.0	0.0	75.0	71.3	28.6	0.0	66.7	50.0	50.0
Herdsmen	100	0.0	46.9	53.1	32.2	66.1	1.7	86.1	8.3	58.3
Education (school level)										
None	99.5	7.0	58.1	34.9	29.8	53.9	0.4	89.4	11.8	37.6
Primary school	98.9	9.9	50.5	39.6	39.5	54.1	5.5	79.3	23.9	38.0
Secondary school	96.0	14.8	22.2	63.0	50.0	38.2	11.8	50.0	50.0	50.0
Post-secondary	100	100	0.0	0.0	40.0	60.0	0.0	60.0	40.0	60.0
Not indicated	100	0.0	0.0	100	28.6	42.9	28.6	0.0	100.0	0.0
Duration in cattle business										
X ≤ 10 years	98.4	8.1	37.1	54.8	41.0	51.3	7.7	73.3	28.3	31.7
10 < X ≤ 20 years	98.1	8.8	43.9	47.4	40.0	53.3	6.6	84.0	20.0	40.0
20 < X ≤ 30 years	100	7.8	56.2	35.9	35.8	64.2	1.5	88.8	12.7	46.0
30 < X ≤ 40 years	100	8.3	60.0	31.7	20.4	61.1	0.0	86.7	8.3	55.0
X > 40 years	100	2.9	74.3	22.9	32.7	69.2	0.0	90.6	15.6	37.5
Not indicated	94.1	5.9	47.1	47.1	56.0	36.0	8.0	78.6	21.4	35.7

n = total number of respondents, ADP = Adamawa Plateau, WHC= Western highlands

[18] Poor health and production

Appendix 11

Table 32 : Factors affecting meat / milk consumption habit of cattle owners – human risk factors

Variable	Milks cow after each calving for human consumption (n=295: ADP=196, WHC=99)	Fresh milk consumption (n=475: ADP=296, WHC=179)			Meat consumption (n=477: ADP=297, WHC=180)	
		Boiled or raw	Raw milk[19]	Drinking fresh milk since birth	Raw meat	Suya and Kilishi[20]
Highland region						
ADP	93.4	82.8^{a}	66.9^{a}	65.2^{a}	16.8^{a}	97.3^{a}
WHC	92.9	79.6^{b}	50.8^{b}	65.7^{a}	14.3^{a}	50.5^{b}
Total	93.2	81.6	60.8	65.4	15.9	79.4
Occupation						
Cattle breeder	95.2	88.3^{c}	71.1^{c}	85.0^{b}	13.2^{b}	81.3^{c}
Butcher	100.0	62.6^{d}	37.4^{d}	55.1^{c}	15.5^{b}	79.1^{de}
“Buyem sellem”	80.0	68.6^{d}	31.4^{d}	57.1^{c}	5.7^{b}	60.0^{e}
Herdsmen	82.0	95.0^{ce}	75.0^{c}	85.0^{b}	31.7^{c}	85.0^{d}
Education (school level)						
None	96.0	90.5^{f}	79.7^{e}	88.7^{d}	22.0^{d}	83.9^{d}
Primary school	88.6	74.8^{g}	47.4^{f}	68.4^{e}	9.9^{e}	79.6^{g}
Secondary school	86.4	68.3^{g}	38.0^{g}	58.3^{f}	13.3^{e}	73.3^{h}
Post-secondary	100	83.3	16.7	66.7	0.0	66.7
Not indicated	100	73.1^{g}	46.2^{f}	46.1^{g}	11.5^{e}	53.8^{j}
Duration in cattle business						
≤ 10 years	82.8	72.5^{h}	41.3^{h}	62.3^{h}	13.7^{f}	79.9^{k}
10 < X ≤ 20 years	91.7	81.8^{hj}	54.5^{h}	76.1^{jm}	14.8^{f}	75.0^{k}
20 < X ≤ 30 years	98.4	85.1^{j}	70.1^{jm}	78.2^{jm}	14.8^{f}	78.4^{k}
30 < X ≤ 40 years	96.8	87.5^{j}	73.7^{jk}	85.0^{jk}	18.7^{f}	83.7^{k}
X > 40 years	93.6	91.2^{j}	86.0^{k}	91.2^{k}	21.1^{f}	86.0^{k}
Not indicated	100	84.0^{hj}	64.0^{hm}	80.0^{hm}	16.0^{f}	76.0^{k}

n = total number of respondents, ADP = Adamawa Plateau, WHC= Western highlands
a-k: different letters in same column are significantly different (P<0.05)

[19] Almost everybody who drank raw milk also drank boiled milk. But still prefer raw milk and sometimes drinks directly from the cow’s udder
[20] “Suya” is meat briefly roasted over hot charcoal or fire. Kilishi is a traditional Cameroonian sun dried (and sometimes briefly roasted) meat. The products are not standardized and are prepared by marinating thin sheets (kilishi) or small bundles (suya) of meat in slurry of mixed local ingredients.

Appendix 12

Table 33 : Knowledge of cattle handlers about zoonotic bovine tuberculosis and its modes of transmission – Animal to humans risks and vice versa

Variable	Know bovine TB is zoonotic	Mode of transmission of bovine TB to humans					
		Knows Milk is a vehicle	Knows raw meat is vehicle	Knows inhalation is route (aerosol)	Knowledge of number of modes of transmission		
					≥ 1 mode	≥ 2 modes	3 modes
Highland region							
ADP	51.7[a]	14.4[a]	43.6[a]	11.4[a]	48.3	18.5	2.7
WHC	62.0[b]	14.0[a]	46.4[a]	19.5[b]	59.2	18.4	2.2
Total	55.6	14.3	44.7	14.5	52.4	18.4	2.5
Occupation							
Cattle breeder	53.1[cd]	19.3[b]	43.6[bc]	16.4[c]	51.3	23.3	3.6
Butcher	63.0[c]	7.4[c]	55.6[b]	12.0[d]	62.0	11.1	1.8
"Buyem sellem"	67.6[c]	5.9[c]	35.3[c]	8.8[d]	41.2	8.8	0.0
Herdsmen	46.7[d]	8.3[c]	36.7[c]	13.3[d]	48.3	11.7	0.0
Education (school level)							
None	46.0[e]	14.3	36.6[d]	13.8[e]	43.3	18.8	2.7
Primary school	57.7[f]	14.9	47.6[e]	13.1[e]	55.4	17.9	2.4
Secondary school	85.2[g]	13.1	70.5[f]	16.4[e]	80.0	20.0	2.0
Post-secondary	100	33.3	66.7	50.0	83.3	50.0	16.7
Not indicated	58.6[f]	3.4	41.4[e]	17.2[e]	51.7	10.3	0.0
Duration in cattle business							
≤ 10 years	56.6	5.9[d]	44.8	13.2	50.7	11.8	1.5
10 < X ≤ 20 years	58.2	17.6[f]	48.4	12.1	55.0	19.8	4.4
20 < X ≤ 30 years	52.9	13.8[e]	39.1	16.1	51.7	17.2	0.0
30 < X ≤ 40 years	62.0	25.3[g]	50.6	17.7	57.0	31.6	5.1
X > 40 years	39.3	14.3[e]	32.1	12.5	39.3	17.9	1.8
Not indicated	64.3	14.3[e]	57.1	17.9	67.9	17.9	3.6

n = total number of respondents, ADP = Adamawa Plateau, WHC= Western highlands {(n=477: ADP=298, WHC=179)}

a-e: different letters in same column are significantly different (P<0.05)

Appendix 13

Table 34 : Knowledge of cattle handlers about management of bovine tuberculosis in their cattle – Animal risk factors

Variable	Diagnosis of TB in own or adjacent herds (n=439: ADP=269, WHC=170)	Action taken if bovine TB is suspected in animal (n=369: ADP=268, WHC=101)				Action if animal dies of suspected bovine TB (n=441: ADP=292, WHC=149)			
		Report to Veterinary service	Allow to enter food chain	Do Nothing or Don't know	Treatment of animals[21]	Allow to enter food chain	Report to Veterinary service	Dispose of carcass	Don't know
Highland region									
ADP	24.5^{a}	11.6^{a}	4.1^{a}	72.8^{a}	13.1	28.1^{a}	6.8^{a}	71.6^{a}	37.0^{a}
WHC	38.2^{b}	53.9^{b}	14.7^{b}	18.8^{b}	19.8	28.9^{b}	42.9^{b}	78.5^{a}	11.4^{b}
Total	29.8	23.3	7.0	58.0	14.9	28.3	19.1	73.9	28.3
Occupation									
Cattle breeder	23.0^{ce}	22.9^{c}	8.1^{c}	60.8^{c}	12.0	28.9^{c}	13.9^{c}	69.9^{b}	31.2^{c}
Butcher	51.1^{d}	29.8^{c}	3.5^{d}	50.9^{d}	15.8	26.7^{c}	33.7^{d}	85.1^{bc}	18.8^{d}
“Buyem sellem”	17.2^{e}	25.0^{c}	25.0^{e}	0.0	50.0	26.7^{c}	26.7^{d}	100^{bc}	6.6^{e}
Herdsmen	35.8^{c}	18.0^{d}	4.0^{d}	56.0^{d}	26.0	32.2^{d}	15.3^{c}	66.1^{d}	3.4^{e}
Education (school level)									
None	24.4^{f}	17.7^{e}	6.2^{f}	65.5^{e}	12.0	32.2^{e}	12.1^{e}	64.0^{e}	39.7^{f}
Primary school	31.8^{f}	28.7^{f}	6.1^{f}	53.9^{f}	14.8	24.7^{f}	17.5^{f}	82.5^{f}	22.7^{g}
Secondary school	40.7^{g}	36.7^{g}	16.7^{f}	30.0^{g}	26.7	27.3^{g}	29.6^{g}	93.2^{f}	6.8^{h}
Post-secondary	50.0	60.0	0.0	40.0	20.0	40.0	80.0	60.0	0.0
Not indicated	44.0^{g}	20.0^{f}	10.0^{f}	40.0^{g}	16.0	16.3^{h}	58.3^{h}	75.0^{fg}	8.3^{h}
Duration in cattle business									
≤ 10 years	32.5^{hj}	35.3^{h}	8.2^{gh}	43.5^{hl}	15.3	31.4^{j}	19.0^{j}	81.8^{h}	19.8^{m}
10 < X ≤ 20 years	33.7^{hj}	18.5^{j}	6.1^{gh}	55.4^{jl}	24.6	25.9^{j}	27.2^{j}	75.3^{hjk}	23.5^{jm}
20 < X ≤ 30 years	27.9^{k}	17.3^{j}	6.7^{gh}	70.7^{jm}	6.7	25.3^{j}	21.7^{j}	62.6^{jkm}	38.5^{jk}
30 < X ≤ 40 years	31.5^{jk}	19.7^{jk}	5.6^{g}	66.2^{jkm}	8.5	30.1^{j}	9.6^{j}	79.4^{hk}	30.1^{jm}
X > 40 years	26.4^{k}	15.7^{j}	7.8^{gh}	64.7^{hjm}	19.6	29.1^{j}	10.9^{j}	61.8^{km}	41.8^{j}
Not indicated	14.8^{j}	40.9^{hk}	9.1^{h}	36.4^{kl}	22.7	25.0^{j}	28.6^{j}	78.6^{hjk}	17.9^{km}

n = total number of respondents, ADP = Adamawa Plateau, WHC= Western highlands;
a – m: different letters in same column are significantly different (P<0.05); Bovine TB in herd = 30 < X ≤ 40 years Vs. Not indicated: X^2=4.000; P=0.039 and If sick Bovine TB report to vet: ≤ 10 years Vs. 10 < X ≤ 20 years: X^2=5.062; P=0.021

[21] In Cameroon, animals suspected or diagnosed with bovine TB should be immediatedly removed from the herd and culled. The veterinarian carries out post mortem examinations to detect TB lesions. However, some respondentsstill attempt to treat with drugs (usually mixed regimens) including ethno-veterinary drugs and customary practices methods.

Appendix 14

Table 35 : Impact of bovine tuberculosis on cattle business and knowledge of cattle handlers about control of bovine tuberculosis

Variable	Previous contact with TB[22]		Impact of bovine TB to cattle business (n=272: ADP=151, WHC=121)			Awareness and implementation of bovine TB control[23] (n=449: ADP=282, WHC=167)		
	Human TB (n=425: ADP=292, WHC=133)	Bovine TB (n=466: ADP=297, WHC=169)	Drop in production[24] and poor health	Economic loss	Don't know or no effect	Report disease to Veterinary service	Condemnation	Don't know
Highland region								
ADP	73.8[a]	49.8[a]	15.2[a]	92.1[a]	6.0	7.5[a]	98.6[a]	92.5[a]
WHC	80.0[b]	34.9[b]	33.9[b]	66.9[b]	15.7	25.9[b]	89.2[b]	74.1[b]
Total	75.8	44.4	23.5	80.9	10.3	13.3	95.1	86.7
Occupation								
Cattle breeder	83.91[c]	39.9[c]	30.6[ce]	65.3[c]	18.5[ac]	18.7[c]	94.6[c]	82.0[c]
Butcher	40.74[d]	64.8[d]	11.9[d]	99.0[d]	1.0[b]	2.9[d]	94.3[c]	81.9[c]
"Buyem sellem"	89.47[c]	15.4[e]	23.8[e]	90.5[d]	9.5[c]	6.4[e]	93.6[c]	54.8[b]
Herdsmen	62.96[e]	43.3[c]	34.6[c]	76.9[e]	11.5[c]	3.9[de]	100[d]	88.5[d]
Education (school level)								
None	76.1[f]	36.3[f]	27.5	72.5	16.7	13.9	94.3	90.0
Primary school	86.3[fg]	51.5[g]	20.6	84.1	6.5	10.0	96.3	90.0
Secondary school	90.2[g]	45.8[g]	16.2	86.5	10.8	16.3	97.8	83.7
Post-secondary	100	83.3	75.0	75.0	0.0	60.0	50.0	40.0
Not indicated	93.3[g]	57.7[g]	22.7	95.5	0.0	18.8	96.5	81.3
Duration in cattle business								
≤ 10 years	80.0	43.8	17.1	84.1	12.5	10.6	94.4	98.4
10 < X ≤ 20 years	82.3	48.9	24.6	82.5	7.0	16.0	97.7	84.0
20 < X ≤ 30 years	74.1	40.0	22.7	84.1	11.4	9.0	90.5	91.0
30 < X ≤ 40 years	86.8	52.6	29.3	73.2	12.2	11.8	97.3	88.2
X > 40 years	81.8	36.8	30.8	73.1	7.7	11.1	98.2	88.9
Not indicated	100	39.3	31.4	81.2	6.3	35.7	91.7	64.3

n = total number of respondents, ADP = Adamawa Plateau, WHC= Western highlands

a-d: different letters in same column are significantly different (P<0.05)

[22] Respondents with previous contact and knowledge of bovine TB also had previous knowledge of human TB

[23] In Cameroon: all animal disease cases should be reported to the Veterinary services but condemnation during slaughter / meat inspections is generally considered by cattle handlers as the main Government policy to remove affected animals and products from the food chain. Also, these control measures are not enforced in the animals' and rural environments but attempts are made at treating all animal diseases including tuberculosis (by any means possible). Thus this category also includes respondents who consider treatment as a control

[24] Loosing animals (drop in total number of animals owned) was the most important factor in the category.

Appendix 15

Table 36 : Association between different risks factors (χ^2 and P value of significance)

Variable	Consume raw meat	Previous contact with TB		Awareness of zoonotic bovine TB and mode of transmission to humans				Awareness and implementation of bovine TB control		
		Previous contact with human TB	Previous contact with bovine TB	Bovine TB is zoonotic	Milk as a vehicle	Meat as a vehicle	Aerosol as a vehicle	Report disease to veterinary service	Condemnations	Don't know
ADP vs WHC	24.506 (0.000)	3.843 (0.050)	8.679 (0.003)	11.770 (0.001)	NS	NS	6.814 (0.009)	14.694 (0.000)	12.500 (0.000)	14.694 (0.000)

Variable	Fresh milk and meat consumption				Previous contact with TB		Awareness of zoonotic bovine TB and mode of transmission to humans					
	Raw milk			Raw meat	Human TB	Bovine TB		Bovine TB is zoonotic	Milk as a vehicle		Meat as a vehicle		Aerosol as a vehicle
Occupation	Butcher	Buyem Sellem	Shepherd	Shepherd	Shepherd	Butcher	Buyem Sellem	Shepherd	Butcher	Shepherd	Buyem Sellem	Shepherd	Butcher
Breeder	7.113 (0.008)	5.263 (0.019)				8.820 (0.003)			5.633 (0.018)	5.882 (0.013)			3.892 (0.049)
Butcher			19.531 (0.000)	6.857 (0.007)	8.471 (0.002)		4.000 (0.039)	4.971 (0.026)			4.762 (0.027)	6.250 (0.012)	
"Buyem sellem"			5.500 (0.017)	6.750 (0.006)	4.167 (0.031)			4.500 (0.031)					

Variable	Report disease to veterinary service		Condemnations	Don't know	
Occupation	Butcher	Shepherd	Shepherd	Butcher	Shepherd
Breeder	15.750 (0.000)	8.471 (0.002)	5.143 (0.016)	21.441 (0.000)	8.471 (0.002)

Variable	Fresh milk and meat consumption				Previous contact with TB	Awareness of zoonotic bovine TB and mode of transmission to humans								
	Raw milk		Raw meat		Bovine TB	Bovine TB is zoonotic				Meat as a vehicle			Aerosol as a vehicle	
Education	Primary school	Secondary school	Primary school	Not indicated	Primary school	Primary school	Secondary school	Tertiary	Not indicated	Primary school	Secondary school	Not indicated	Secondary school	Not indicated
None	39.622 (0.000)	17.455 (0.000)	8.820 (0.003)	4.167 (0.031)	5.195 (0.023)	4.320 (0.038)	34.568 (0.000)	4.167 (0.031)	12.071 (0.000)	5.157 (0.023)	24.324 (0.000)	5.818 (0.012)		
Primary school		4.321 (0.038)					13.885 (0.000)						6.323 (0.012)	
Secondary school									5.143 (0.016)					4.923 (0.022)

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Published article

- **J. Awah-Ndukum, A. C. Kudi, G. Bradley, I. N. Ane-Anyangwe, S. Fon-Tebug, and J. Tchoumboue, (2010):** Prevalence of bovine tuberculosis in abattoirs of the Littoral and Western highland regions of Cameroon: a cause for public health concern. *Veterinary Medicine International* **2010** (495015), 8 pages; doi:10.4061/2010/495015.

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- **J. Awah-Ndukum, A. C. Kudi, G. S. Bah, G. Bradley, S. Fon-Tebug, P. L. Dickmu, H. N Njakoi, and W. N. Agharih :** Bovine tuberculosis in cattle in the highlands of Cameroon: seroprevalence estimates and rates of tuberculin skin test reactors at modified cut-offs. *Veterinary Medicine International (In Press).* Received 19 October 2011; Revised 10 January 2012; Accepted 22 January 2012.

- **J. Awah-Ndukum, A. C. Kudi, G. Bradley, I. Ane-Anyangwe, V. P. K. Titanji, S. Fon-Tebug and J. Tchoumboue :** 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. *Veterinarni Medicina (In Press).* Resubmitted 24 January 2012; Accepted 30 January 2012.