

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

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ABSTRACT: Bovine tuberculosis (TB) is an important neglected zoonosis in Cameroon, where many communities depend on their livestock for livelihood and the incidence of human TB and TB-HIV/AIDS co-infection are high and increasing annually. The aim of this study was to estimate the prevalence of bovine TB in cattle in the highlands of Cameroon. The magnitude and trend of detecting TB lesions in slaughtered cattle (1994 to 2010) and tuberculin skin tests (TST) in 2853 cattle (84 herds) of 39 livestock rearing communities were analysed. Of 129 165 slaughtered cattle inspected, 599 (0.46%; 95% CI: 0.43%–0.50%) showed suspected TB lesions among a total of 983 (0.76%; 95% CI: 0.71%–0.81%) identified pathologies. The monthly TB detection rates ranged from 0.30% (95% CI: 0.20%–0.40%) 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%). The rates were not affected ($P < 0.05$) by season and fluctuating peaks were also recorded. The comparative TST revealed that bovine TB was widely distributed in live cattle (4.67%; 95% CI: 3.89%–5.44%) and was higher ($\chi^2 = 17.50$, $P \leq 0.001$) in the Western highlands than Adamawa plateaux. Comparative TST bovine TB reactors were higher ($P < 0.05$) in cattle managed in semi-intensive and beef production systems compared to the others. Animals in small herds showed higher ($\chi^2 = 4.283$, $P = 0.038$) rates than those in large herds. Bovine TB prevalence in exotic/upgraded cattle was comparable to that of the Red Bororo zebu but higher than the rates in Guadali ($\chi^2 = 4.971$, $P = 0.026$) and White Fulani ($\chi^2 = 5.6$, $P = 0.018$) zebras. Among the indigenous zebu, the rate was higher in Red Bororo than the Guadali ($\chi^2 = 6.244$, $P = 0.012$) and White Fulani ($\chi^2 = 6.568$, $P = 0.010$). Sex did not influence ($\chi^2 = 0.410$, $P = 0.522$) bovine TB prevalence in this study but diagnosis of the disease was higher ($\chi^2 = 5.787$; $P = 0.016$) among adult/older cattle than in younger animals. Further analysis of the TST responses revealed that atypical mycobacterial infections was widespread and 6.83% of tested animals showed positive reactions at both bovine and avian tuberculin injection sites and a strong association ($\chi^2 = 2.512$; $P = 0.113$) between skin responses to both tuberculin. The study confirms that bovine TB is prevalent in live cattle and meat production abattoirs in Cameroon and we recommend strict interpretation of TST results for maximum diagnosis of the disease in the local environment. A need for comprehensive investigation of the molecular epidemiology, zoonotic risks and the public health importance of bovine TB in Cameroon cannot be overemphasised.

Keywords: *Mycobacterium bovis*; neglected zoonosis; risks; zebu cattle; Cameroon

Tuberculosis (TB) is an important zoonosis caused by bacteria of the *Mycobacterium tuberculosis* complex. *M. bovis* is virulent for cattle but can infect other animals and humans causing disease and pathology similar to *M. tuberculosis*, which is naturally pathogenic for man (Biet et al. 2005; Kaneene and Pfeiffer 2006; Thoen et al. 2006). The prevalence of human TB in the Central African sub-region is high and increasing rapidly with the spread of the HIV/AIDS infection (WHO 2009, 2010). For example, TB remains a common disease in Cameroon, with a current annual incidence of almost 200 cases per 100 000 of the population (WHO 2009), especially among the economically active 21–40 age group (Noeske et al. 2004; Ane-Anyangwe et al. 2006). Extraordinarily high rates of TB-HIV/AIDS co-infection exist in Cameroon and between 29% and 43% of TB cases of all ages are also positive for HIV/AIDS (Noeske et al. 2004; WHO 2009, 2010). Bovine TB is endemic in most of Africa (Ayele et al. 2004), and is under investigated. Over 98.9% of all reported cases are in cattle which is also a major source of animal protein (AU/IBAR 2006). An increasing human population and food shortage are some evident concerns in Cameroon. The keeping of livestock is integral to the socio-economic, cultural and religious activities of many communities in the country and close human-livestock interactions are common. *M. bovis* have been isolated from humans in parts of Africa including Cameroon (Cosivi et al. 1998; Kazwala et al. 2001a; Niobe-Eyangoh et al. 2003; Cadmus et al. 2006; Zinsstag et al. 2006) and epidemiological associations between bovine TB in cattle and human TB have been suggested (Cook et al. 1996; Regassa et al. 2008).

Post mortem detection of TB lesions in animal carcasses usually indicates the advanced stages of bovine TB (Corner 1994; Shitaye et al. 2006). The detection of lesions during the meat inspection of slaughtered animals is the basis for indicating the occurrence of the disease in Cameroon (Doufissa 1993; Awah-Ndukum et al. 2005). TB lesions are common pathologies identified in slaughtered cattle in the country and rates ranging from 0.18% to 4.25% have been recorded (Awah-Ndukum et al. 2005, 2010). However, there are only a few reports of the bacteriological isolation and molecular genotyping of TB in Cameroon (Njanpop-Lafourcade et al. 2001; Niobe-Eyangoh et al. 2003). Mycobacteriological diagnosis of bovine TB in animals is practically unknown in Cameroon and the

culture of human sputa is only sporadically performed and then mainly for research purposes. Awah-Ndukum et al. (2010) reported acid-fast tubercle bacilli in 51% of suspected TB lesions in cattle based on mycobacterial culture and Ziehl-Neelsen staining with microscopy and in 31% of the specimens based on direct smear microscopy with Ziehl-Neelsen staining without culture.

Conventional mycobacteriological methods are often unsafe in inadequately constructed and poorly equipped laboratories (Igbokwe et al. 2001), common in most of Africa. Such methods are also expensive, time consuming, laborious and not practical for surveillance purposes (Strong and Kubica 1985; Grange et al. 1996; Parsons et al. 2002; Warren et al. 2006). Further, the interpretation of results is highly subjective and prone to errors such as those possible when interpreting differences in colony morphology (Strong and Kubica 1985; Grange et al. 1996; Ameni et al. 2010). Though also prone to inspector 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 the gross inspection of carcasses (Edwards et al. 1997; Hinton and Green 1997; Asseged et al. 2004). Major improvements in animal and human health with regard to consumer protection and eradication of epizootic TB in many developed countries was achieved when a drastic reduction in relevant or suspicious lesions at meat inspection was strictly employed to provide the quality and protection demanded for consumers (Grossklaus 1987; Hinton and Green 1997). The implementation of the 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 and surveillance tool in many developing countries where bovine TB is endemic.

TST are the best procedures available internationally for the field diagnosis of bovine TB in live animals (de la Rua-Domenech et al. 2006a, b). There are recommended cut-off points for increases in skin thickness for a tuberculin test to be positive (OIE 2009), which are the basis for eliminating positive reactors in eradication programmes (Good 2006). However, political and socio-economic constraints as well as a lack of attention in developing and poor countries like Cameroon are significantly hampering routine tuberculin testing and the elimi-

nation of infected cattle, which has proved very effective in eradicating bovine TB in most developed countries (Gilbert et al. 2005; Abernethy et al. 2006; Good 2006). There are scant reports approximating the prevalence of bovine TB based on the TST of live cattle in Cameroon (Merlin and Tsanguéu 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. Bovine TB is neglected in Cameroon and zoonotic TB is increasingly becoming a source of concern to practitioners of veterinary and especially human medicine in the country because TB is the most common opportunistic disease affecting 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 investigation of the prevalence of bovine TB in cattle, especially in high risk and cattle-producing areas.

A more serious focus on bovine TB status as well as accurate estimation of the magnitude and distribution of bovine TB in cattle are essential for appropriate intervention strategies. This study was carried out to ascertain the magnitude and trend of detection of TB lesions in slaughtered cattle at the Bamenda city abattoir of the Western highlands of Cameroon over a period of more than seventeen years. The study also reports the estimated prevalence of bovine TB based on the TST of live cattle in the wider agro-ecological highlands of Cameroon.

MATERIAL AND METHODS

Location of the abattoir and post mortem detection of tuberculosis lesions

The present study reports on the prevalence of tuberculosis lesions in cattle in the Bamenda municipal abattoir of the Western highlands of Cameroon (5°–7°N and 10°–11°E) between 1994 and 2010, with reference to the variations which occurred over time. The abattoir is the largest in the region and was selected based on its geographic proximity to the largest cattle markets and main trade routes. The abattoir was under the supervision of MINEPIA (Ministère de l'Élevage, des Pêches et des Industries Animales – Ministry of Livestock, Fisheries and Animal Industries) and qualified veterinarians served as meat inspectors

conducting *ante mortem* of live animals and post mortem examinations of slaughtered cattle.

After obtaining consent from the relevant authorities, members of the research team liaised with the abattoir veterinarians, assisted in meat inspection from March 2008 to December 2010 and also collected the required data. The relevant data (daily, monthly and yearly) retrieved from the records were the number of slaughtered and inspected cattle carcasses, and the number with suspected TB lesions. The slaughter and meat inspections were based on procedures legislated 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 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 the affected lymph nodes and affected organs were condemned (Corner 1994; FAO 1994). There were no pre-slaughter TB testing schemes at the abattoir but ante-mortem inspections for general examination of health were carried out. Animals that showed signs of ill-health were usually not slaughtered but kept for further appraisal before slaughter could be recommended at a later date.

Tuberculin skin tests in cattle: Study area and selection of herds

The cattle populations in the Western highlands (5°–7°N and 10°–11°E) and Adamawa plateaux (6°–7°30'N and 12°30'–14°E) of Cameroon were sampled from March to September 2009 to analyse responses to tuberculin skin tests (TST) using both avian (AT) and bovine (BT) tuberculin purified protein derivatives (Lelystad Biologicals, Lelystad, the Netherlands) as part of a bovine TB prevalence study. A single intradermal TST (SIT) bovine TB prevalence rate of 26% recorded in the peri-urban areas of Bamenda, Northwest Cameroon (Muchaal 2002), was used to estimate the number of cattle required to detect ≥ 1 infected animal with a desired 95% confidence and precision of $\geq 5\%$ as previously described (Thrusfield 2007). The selection of cattle herds was done by the random-number generation method of cattle-

keeping communities, cattle owners and locations of herds from records of annual livestock vaccination campaigns (contagious bovine pleuropneumonia, pasteurellosis, black quarter) at the Regional Delegations of MINEPIA. The selection procedure took into consideration costs, season and road accessibility (including distance and time to trek to herds) and local cultural beliefs because a farmer's willingness to participate was never guaranteed.

All animals within the chosen herds were tested except for recently calved cows (within two months post-partum) and calves less than six months old because of immuno-suppression in lactating cows and high maternal antibodies in calves which desensitise them to tuberculin (Costello et al. 1997; Shirima et al. 2003). Placed ear tags and skin tattoos were used to identify test animals and herdsmen had also memorised features for each animal under their care (e.g., 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. Other information relating to the location, husbandry practices, breed, sex and age of the animal were noted. The ages and breeds of the animals were provided by the farmers, or otherwise were determined as described earlier (Blench 1999; Turton 1999; MINEPIA 2002).

The single intradermal comparative TST (SICCT) and single intradermal TST (SIT) were performed on a total of 2853 cattle from 61 herds (33 village communities) in the Sudano-guinea Western highlands and 23 herds (seven village communities) in the Vina area of the Guinean savannah Adamawa plateaux. TST were carried out in the selected cattle by intradermal injections of 0.1ml each of AT (2500 IU/dose) and BT (3000 IU/dose) in two sites, at 12 cm apart in the right neck region. A correct intradermal injection was confirmed by palpating a small grain-like swelling at each injection site. Skin thickness was measured prior to and 72 h after injecting the tuberculins using a digital calliper. The OIE-recommended ≥ 4 -mm cut-off point of increase in skin fold thickness (OIE 2009) was assessed for TST reactor status. Briefly, for the 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

the increase in the skin thickness at the AT site of injection. Thus, the skin response for SICCT-BT was given by $[(BT_{72}-BT_0) - (AT_{72}-AT_0)]$ and SICCT-AT by $[(AT_{72}-AT_0) - (BT_{72}-BT_0)]$. BT_0 and AT_0 are the measures of skin fold thickness prior to injecting BT and AT. BT_{72} and AT_{72} are the measures 72 h after injecting BT and AT. For SIT-BT and SIT-AT, a reaction was considered positive if the increase in skin-fold thickness at the specified tuberculin site of injection $[(BT_{72}-BT_0)$ or $(AT_{72}-AT_0)]$ was ≥ 4 mm, negative if the increase was ≤ 2 mm, and inconclusive if the increase was between > 2 mm and < 4 mm.

The animals in this study were reared traditionally with or without transhumance, as well as in semi-intensive and intensive systems. They were composed of the indigenous zebu, upgraded and exotic breeds. The purpose of the study was explained to the farmers with the assistance of local veterinarians, community leaders and trusted intermediaries. A herd was tested after an informed consent was given by the owner. Apart from the intradermal injections of AT and BT and procedural restraining manipulations for safety purposes, the animals were not subjected to suffering.

Management of data and statistical analysis

Slaughter and meat inspection records of approximately 129 165 mostly Zebu cattle originating from the Western highland regions were scrutinised. The proportion of carcasses with suspected TB lesions of the total slaughtered cattle and the standard error were calculated and the Mann-Whitney test was used to compare seasonal observations as previously described (Greiner and Gardner 2000; Thrusfield 2007).

The existence of bovine TB had been established in the country (Doufissa 1993; Martrenchar et al. 1993; Muchaal 2002; Awah-Ndukum et al. 2005), and the diagnostic accuracy of the tuberculin tests was assumed to be constant across the regions. However, due to the lack of TST accuracy data for the exact Cameroon environments, the sensitivity [100% (95% CI: 59.8–100%)] and specificity [90.9% (95% CI: 69.4–98.4%)] of the findings obtained by Ameni et al. (2000) in similar tropical conditions in Ethiopia were used to correct the observed rates of SICCT-BT reactors. The data (90% for specificity and 86.4% for sensitivity) obtained by Pollock et al (2003) was also used to adjust the observed rates of

SIT-BT reactors. These rates were corrected using the Rogan-and-Gladen formula and the 95% confidence intervals calculated (Greiner and Gardner 2000; Thrusfield 2007).

All observed data were initially entered into Excel 2007 (Microsoft Corporation, USA) and exported to the SigmaPlot 11.0 (Systat Software Inc., Richmond, USA) for further analysis. The Chi-square test was applied to compare rates of individual and herd TST reactors in the groups of variables studied. The odds ratio, relative risks and regression analysis were used to assess the strength of association of different factors with the prevalence of bovine TB (Thrusfield 2007).

RESULTS

Prevalence of bovine tuberculosis based on post mortem examination

Analysis of meat inspection records over 17 years showed that of the 129,165 slaughtered cattle, a total of 599 (0.46%; 95% CI: 0.43%–0.50%) suspected TB lesions (Figure 1) among 983 (0.76%; 95% CI: 0.71%–0.81%) pathologies were identified. TB abscesses (with yellowish pus) and firm nodular lesions (often 'gritty' on cutting) were detected in the lungs and associated lymph nodes (over 60% – data not shown), lymph nodes of the head, mesenteric lymph nodes and liver.

TB lesions were recorded throughout the study period with monthly detection 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. High detection rates of TB lesions in March, April, June and August and several fluctuating annual peaks (Figure 2) were recorded. However, no difference (Mann-Whitney Statistic = 4588; $P = 0.927$), was observed between the rates for wet season (0.46%; 95% CI: 0.32%–0.59%), and dry season (0.33%; 95% CI: 0.25%–0.41%). The regression model revealed a weak positive gradient ($Y = 0.0529X - 0.0978$; $R^2 = 0.5262$), suggesting stagnation or a mild increase in the trend of TB lesion occurrence in the Bamenda municipal abattoir.

Prevalence of bovine tuberculosis by Tuberculin skin tests

The prevalence rates of SICCT reactors according to study site, breed, sex, age, management system, and herd sizes are shown in Table 1. Overall, 4.67% of the 2853 animals tested were positive for SICCT-BT and these were widely distributed in the study regions. There were significantly more reactors in the Western highlands ($\chi^2 = 17.50$, $P \leq 0.001$), than in the Adamawa plateaux. SICCT-AT positive reactors (2.84%) were widespread in the study and the rates in both highland regions were comparable ($\chi^2 = 1.361$, $P > 0.243$). The rates of SIT-BT ($\chi^2 = 34.008$, $P \leq 0.001$), and SIT-AT ($\chi^2 = 15.611$, $P \leq 0.001$) positive reactors (Table 2), were significantly higher in the western highlands than in the Adamawa plateaux. A strong association was noted between the detection of SIT-BT and SIT-AT



Figure 1. Cattle tissues showing incised tuberculous lesions detected at slaughter/meat inspection in the Bamenda city abattoir; (a) lung and lung lymph nodes; (b) various lymph nodes (and enlarged kidney)

64 Table 1. Prevalence of single intradermal comparative cervical tuberculin skin test (SICCT) responses in cattle according to location, breed, sex, age group, management system and herd sizes in the highland regions of Cameroon

Variable	Label	Animals tested (n)	SICCT-AT positive reactors*		Doubtful SICCT-BT reactors		SICCT-BT positive reactors	
			n	% (95%CI)	n	% (95%CI)	n	% (95%CI)
Total	all animals	2853	81	2.84 (2.23–3.45)	203	7.83 (6.84–8.81)	121	4.67 (3.89–5.44)
Agro-ecological Regions	Sudano-Guinea (Western Highlands)	2126	67	3.15 ^a (2.41–3.89)	172	8.90 ^a (7.69–10.11)	104	5.38 ^a (4.42–6.34)
	Guinean savannah (Adamawa plateaux)	727	14	1.93 ^a (0.93–2.92)	31	4.69 ^b (3.15–6.23)	17	2.57 ^b (1.42–3.72)
Breed	Graded/Exotic	368	11	2.99 ^a (1.25–4.73)	36	10.76 ^a (7.60–13.93)	26	7.77 ^a (5.04–10.51)
	Guadali	1317	20	1.52 ^a (0.86–2.18)	82	6.85 ^b (5.49–8.21)	45	3.76 ^b (2.73–4.79)
	Namchi	33	0	0	0	0	1	3.03
	Red Mbororo	487	35	7.19 ^b (4.89–9.48)	56	12.65 ^a (9.70–15.60)	32	7.23 ^a (4.93–9.53)
	White Fulani	648	15	2.31 ^a (1.16–3.47)	29	4.92 ^b (3.26–6.59)	17	2.89 ^b (1.60–4.18)
Sex	female	2212	66	2.98 ^a (2.27–3.69)	172	8.55 ^a (7.39–9.72)	97	4.82 ^a (3.93–5.72)
	male	641	15	2.34 ^a (1.17–3.51)	31	5.32 ^a (3.58–7.06)	24	4.12 ^a (2.58–5.66)
Age (years)	young (age ≤ 4)	1481	48	3.24 ^a (2.34–4.14)	94	6.98 ^a (5.68–8.28)	49	3.64 ^a (2.69–4.59)
	adult (age > 6)	1372	33	2.41 ^a (1.59–3.22)	109	8.74 ^a (7.25–10.23)	72	5.77 ^b (4.54–7.01)
Management system	extensive	1510	44	2.91 ^a (2.07–3.76)	85	6.19 ^a (4.98–7.41)	58	4.23 ^a (3.21–5.24)
	intensive	138	2	1.45 ^{ac} (–0.54–3.44)	15	11.96 ^b (6.54–17.37)	5	3.99 ^a (0.72–7.25)
	semi-intensive	1205	35	2.90 ^{bc} (1.96–3.85)	103	9.40 ^b (7.76–11.05)	58	5.30 ^a (4.03–6.56)
	beef herds	2357	73	3.10 ^a (2.40–3.80)	146	6.81 ^a (5.80–7.83)	109	5.09 ^a (4.20–5.97)
	dairy herds	496	8	1.61 ^a (0.50–2.72)	57	12.64 ^b (9.72–15.57)	12	2.66 ^a (1.25–4.08)
Herd sizes (animals per herd)	animals ≤ 40	1325	32	2.42 ^a (1.59–3.24)	81	6.73 ^a (5.38–8.07)	69	5.73 ^a (4.48–6.98)
	animals > 40	1528	49	3.21 ^a (2.32–4.09)	122	8.78 ^b (7.36–10.20)	52	3.74 ^b (2.79–4.70)

AT and BT = avian tuberculin and bovine tuberculin, respectively

*observed prevalence values analysed

^{a-c}different letters in a class of labels are significantly different ($P < 0.05$)

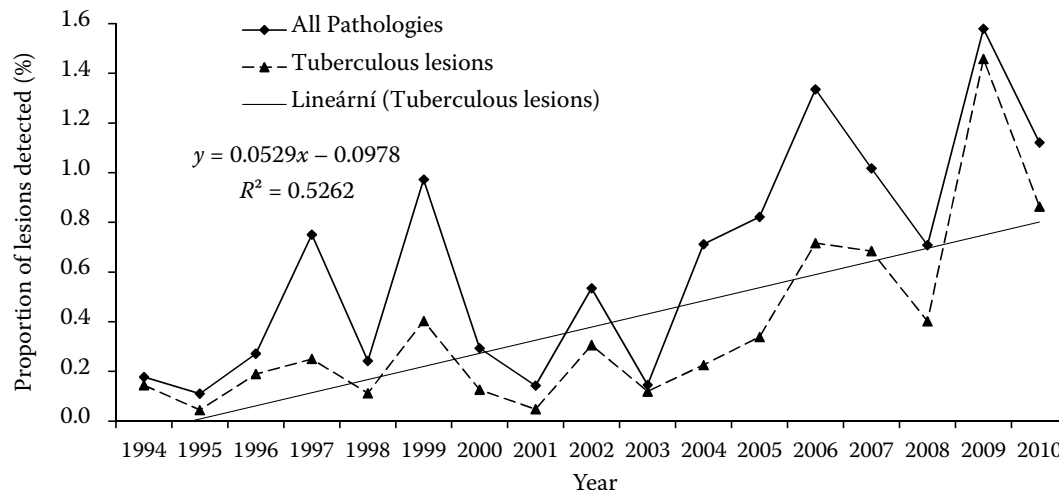


Figure 2. Annual prevalence of tuberculous lesions in slaughtered cattle recorded at the Bamenda municipal abattoir, Cameroon

positive reactors (Table 3) [OR = 121.17 (95%CI: 83.02–176.85); RR = 4.42 (95%CI: 3.50–5.58); $\chi^2 = 1499.942$; $P \leq 0.001$]. The trend of typical and atypical mycobacterial infection in cattle was of co-occurrence and their rates were not significantly different ($\chi^2 = 2.512$; $P = 0.113$) in the studied regions. Overall, 6.83% of tested animals responded positively to both SIT-BT and SIT-AT (Figure 3).

The rates of SICCT-BT reactors were significantly higher in cattle managed in semi-intensive and beef production systems compared to the other systems ($P < 0.05$). However, more cattle in the intensive and dairy production systems showed significantly higher doubtful reactions ($P < 0.05$). The animals in small herds (≤ 40 animals), showed higher ($\chi^2 = 4.283$, $P = 0.038$) bovine TB cases than

those in large herds. The rates were significantly higher for the upgraded and exotic cattle than the Guadali ($\chi^2 = 4.971$, $P = 0.026$), and White Fulani ($\chi^2 = 5.6$, $P = 0.018$) zebus. However, among the indigenous zebus, the disease was more prevalent in the Red Mbororo breed than the Guadali ($\chi^2 = 6.244$, $P = 0.012$), and White Fulani ($\chi^2 = 6.568$, $P = 0.010$). Comparable rates were noted between the upgraded / exotic and local Red Mbororo breeds. Sex did not seem to influence the prevalence rate of bovine TB ($\chi^2 = 0.410$, $P = 0.522$). The trend was towards a higher detection of positive reactors ($\chi^2 = 5.787$; $P = 0.016$), and more doubtful cases among adult/older cattle than in younger animals (Figure 4).

The different predicted variables for SICCT-BT outcomes (Table 4) showed that sex, age and breed served as good indicators of bovine TB in the study. Female cattle, adult/older animals, upgraded/exotic and Red Mbororo breeds were more likely to test positive for SICCT-BT than male cattle, younger animals, Gudali and White Fulani cattle, respectively. Although the rates of SICCT-BT positive reactors was significantly higher in the Western highland regions compared to the Adamawa plateaux ($\chi^2 = 17.50$, $P \leq 0.001$), 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)]. The detection of SICCT-AT positive reactors was also not influenced by the difference in regions [OR = 0.0; RR = 0.92 (95%CI: 0.91–0.94); $\chi^2 = 0.0145$, $P = 0.904$]. Classification of herds with at least one test positive SICCT-BT and SIT-BT reactor and showing major differences between study sites and husbandry manage-



Figure 3. Neck of cattle showing Tuberculin skin test response to bovine tuberculin (red arrow) and avian tuberculin (orange arrow) purified protein derivatives

Table 2. Distribution of single intradermal tuberculin skin test (SIT) reactors according to location, breed, sex, age group, management system and herd size

Variable	Label	Animals tested (<i>n</i>)	SIT-AT reactors		SIT-BT reactors	
			<i>n</i>	% (95%CI)	<i>n</i>	% (95%CI)
Total	all animals	2853	250	8.76 (7.73–9.80)	269	12.21 (11.01–13.41)
Agro-ecological Regions	Sudano-Guinea (WHC)	2126	208	9.78 ^a (8.52–11.05)	230	14.03 ^a (12.55–15.51)
	Guinean savannah (ADP)	727	42	5.78 ^b (4.08–7.47)	39	6.89 ^b (5.05–8.73)
Breed	Graded/Exotic	368	40	10.87 ^a (7.69–14.05)	53	18.72 ^a (14.73–22.71)
	Guadali	1317	102	7.74 ^b (6.30–9.19)	114	11.20 ^b (9.50–12.90)
	Namchi	33	1	3.03	1	3.03
	Red Mbororo	487	65	13.35 ^a (10.33–16.37)	61	16.26 ^{ab} (12.99–19.54)
	White Fulani	648	42	6.48 ^b (4.59–8.38)	40	7.95 ^b (5.87–10.03)
Sex	female	2212	212	9.58 ^a (8.36–10.81)	222	13.01 ^a (11.60–14.41)
	male	641	38	5.93 ^a (4.10–7.76)	47	9.47 ^a (7.20–11.73)
Age	young	1481	112	7.56 ^a (6.22–8.91)	114	9.94 ^a (8.42–11.47)
	adult	1372	138	10.06 ^a (8.47–11.65)	155	14.66 ^b (12.78–16.53)
Management system	extensive	1510	125	8.28 ^a (6.89–9.67)	120	10.27 ^a (8.74–11.80)
	intensive	138	16	11.59 ^{bc} (6.25–16.94)	18	16.94 ^{ab} (10.68–23.20)
	semi-intensive	1205	109	9.05 ^c (7.43–10.67)	131	14.10 ^b (12.13–16.06)
	beef herds	2357	192	8.15 ^a (7.04–9.25)	208	11.42 ^a (10.14–12.70)
	dairy herds	496	58	11.69 ^b (8.87–14.52)	61	15.97 ^b (12.74–19.19)
Herd sizes	animals ≤ 40	1325	110	8.30 ^a (6.82–9.79)	123	12.02 ^a (10.27–13.77)
	animals > 40	1528	140	9.16 ^a (7.72–10.61)	146	12.38 ^a (10.72–14.03)

AT and BT = avian tuberculin and bovine tuberculin, respectively

^{a–c}different letters in a class of labels are significantly different ($P < 0.05$)

ment systems is shown in Table 5. A significantly higher herd prevalence rate for SICCT-BT and SIT-BT reactors ($P < 0.05$), was recorded in the western highlands (68.53% and 86.89%), than in the Adamawa plateaux (38.26% and 73.91%), respectively. Large herds, extensive and beef production systems were shown to be significant herd risk factors ($P < 0.05$), compared to the other measured herd parameters.

DISCUSSION

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

The Bamenda municipal abattoir is the largest in the Northwest region of Cameroon and fulfils the daily beef requirements of the approximately

Table 3. Association between individual responses to Avian PPD and Bovine PPD*

Test		Bovine PPD results <i>n</i> (%)		Total <i>n</i> (%)
		positive	negative	
Avian PPD results	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); $\chi^2 = 1499.942$; $P \leq 0.001$

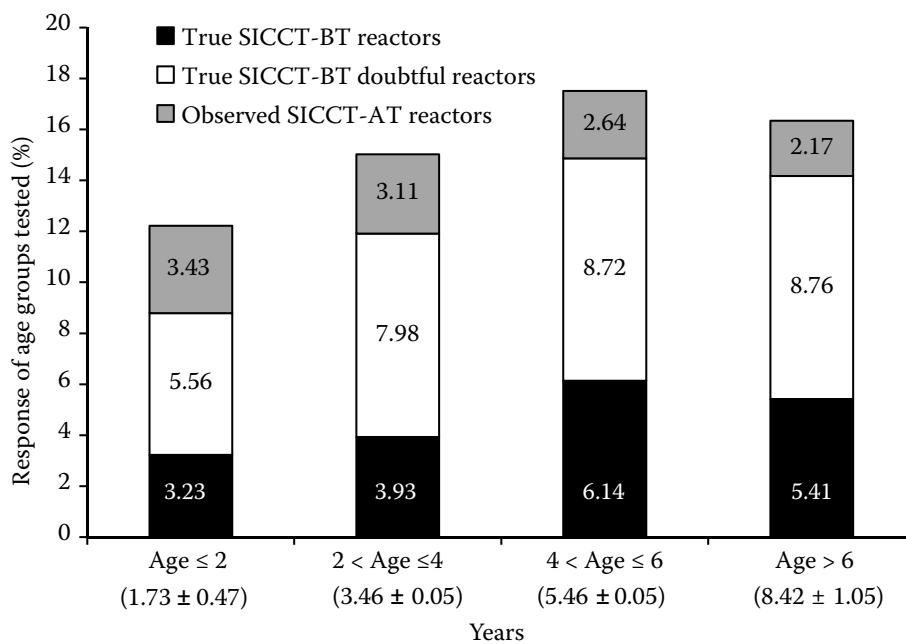


Figure 4. Variation in skin responses to individual bovine and avian tuberculin testing according to age group

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 Western highlands. With the exception of

a few parts (e.g., horn and hooves), almost all the other parts and organs of a carcass are edible in Cameroon if passed at slaughter and meat inspection. Retrospective analysis showed that tuberculous lesions were the most often detected

Table 4. Association between SICCT-BT* response and different predicted variables

Variable	Odds ratio (95%CI)	Relative risk (95%CI)	χ^2 (P-value)
Adamawa Plateaux vs. Western highlands	0	0.975 (0.963–0.987)	0.487 (0.485)
Breed			
Upgraded/Exotic vs. Guadali	2.767 (0.573–13.348)	1.119 (0.868–1.444)	0.558 (0.455)
Upgraded/Exotic vs. White Fulani	1.328 (0.163–10.795)	1.023 (0.847–1.236)	0.110 (0.741)
Upgraded/Exotic vs. Red Mbororo	0	0.925 (0.897–0.953)	0.894 (0.344)
Guadali vs. Red Mbororo	3.819 (1.020–14.292)	1.074 (0.960–1.202)	2.570 (0.109)
Guadali vs. White Fulani	0	0.975 (0.962–0.987)	0.0162 (0.899)
White Fulani vs. Red Mbororo	1.097 (0.139–8.660)	1.006 (0.869–1.166)	0.211 (0.646)
Sex and Age			
Female vs. male	1.234 (0.159–9.574)	1.008 (0.926–1.097)	0.137 (0.711)
Age ≤ 4 years vs. age > 4 years	2.292 (0.877–5.987)	1.041 (0.976–1.109)	1.968 (0.161)
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)
Extensive vs. intensive	0	0.962 (0.931–0.995)	0.604 (0.437)
Semi-intensive vs. intensive	0	0.977 (0.953–1.003)	1.494 (0.222)
Dairy herds vs. beef herds	0	0.965 (0.949–0.981)	0.0203 (0.887)
Herds ≤ 40 animals vs. Herds > 40 animals	0.396 (0.0537–2.914)	0.968 (0.925–1.012)	0.366 (0.545)

*single intradermal comparative cervical tuberculin skin test to detect bovine TB

Table 5. Distribution of tuberculin skin tests positive herds (≥ 1 positive reactor)

Variable	Label	Herds tested	Positive herds			
			SICCT-BT		SIT-BT	
			<i>n</i>	% (95%CI)	<i>n</i>	% (95%CI)
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)

SICCT-BT = single intradermal comparative cervical tuberculin skin test to detect bovine TB

SIT-BT = single intradermal tuberculin skin test to detect bovine TB

^{a–c}different letters in a class of labels are significantly different ($P < 0.05$)

pathologies and the main cause of condemnations at the abattoir. Awah-Ndukum et al (2005, 2010) had recorded higher rates of bovine TB in slaughtered cattle in other parts of the country ranging from 0.67%–4.28%. They also observed that tuberculous lesions were 3–5 times more frequent than other lesions in abattoirs 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 rate recorded in this study was higher than 0.052% reported by 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-Djaibe et al. 2006; Muller et al. 2008; Biffa et al. 2009; Cadmus and Adesokan 2009; Ngandolo et al. 2009). These

differences could be explained by many factors including differences in the disease status in the animal populations, the degree of implementation of the disease control programme and various environmental influences. The association noted between the increase in TB lesion detection rates in recent years (especially from 2002–2010), could be associated with an increase in the number of slaughtered cattle (due to increasing public demands for meat), but it coincided with the additional recruitment of personnel and enhanced efficiency of meat inspectors. Thus, the increasing trend recorded may not have been an actual increase of the disease state but is probably due to improved diagnostic awareness of the disease and intensification of the slaughter/meat inspection procedure. Edwards et al (1997) have stated that while in need of modification, meat inspections still play an important role in meat safety and quality assurance for consumers, 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 the increasing involvement of non-tuberculous mycobacteria in mycobacterial illnesses in animals and humans (Edwards et al. 1997; Biet et al. 2005), justify the continued maintenance of intensive post mortem examination of carcasses (Edwards et al. 1997).

The wide fluctuations and numerous peaks of TB lesion occurrence in this study could be associated with an unchecked course of bovine TB in live cattle in the environment. There is no active control of the disease in animals 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 could have determined the detection rates of bovine TB in slaughtered animals and its semi-natural course in herds and rearing communities in the studied regions. The improved meat inspection team from 2002 was more thorough and detected cases which otherwise would have been missed by the former setup. However, the possibility of under-recording and under-detection remains. 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 in Cameroon (Awah-Ndukum et al. 2005; Awah-Ndukum et al. 2010). The findings of this study agree with earlier reports that post mortem surveillance through the detection of bovine TB lesions 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 the intensification of meat inspection and tracing of infected/suspicious cases to the herds of origin would be necessary for effective surveillance of TB in the studied regions and the whole of Cameroon. However, the importance of this study cannot be underestimated considering the zoonotic implications 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.

Prevalence of bovine tuberculosis based on tuberculin skin tests

The TST showed that bovine TB was widespread in cattle in the studied regions and the prevalence rates were comparable irrespective of the management system employed. This finding is in agreement with Inangolet et al. (2008), who suggested high levels of infectiousness of bovine TB under the common cattle management practices and humid tropical conditions. However, inefficient close contact between diseased and healthy animals in extensive systems, dairy herds and large herds, and possibly a decrease 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, 2007; 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. 2008), and could have been the reasons for the lower prevalence rates recorded among the Namchi, Guadali and White Fulani compared to the Red Mbororo cattle. The reasons underlying the differences in bovine TB prevalence between the local breeds in this study remain unclear and require further investigation. However, continuous close contact between animals (due to increasing animal and human population densities and limited pasture for grazing), also played roles in the higher prevalence rates recorded in the western highlands. The lower SICCT-BT prevalence rate in the Adamawa plateaux (2.57%) is in agreement with Martrenchar et al. (1993) who recorded 2.7% in a trial in the neighbouring Northern and Far Northern regions of Cameroon. 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 more prolonged contacts between animals than in extensive systems (Ayele et al. 2004; Zinsstag et al. 2006; Inangolet et al. 2008). In this work, the spread of the pathogen in the extensive and semi-extensive systems could have resulted from the closer contact experienced during periods of drought at shared grazing, watering and salt leak points, “cattle markets”, during veterinary interventions (vaccination campaigns), and other ventures that involved the gathering together of different herds of animals. While the daily gathering of different herds at a site may not necessarily lead to the spread of bovine TB (Tschopp et al. 2009), a small number of infective agents can still cause disease in susceptible cattle (Francis 1971; Goodchild and Clifton-Hadley 2001; Cassidy 2006). The presence of a single or small number of 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 a crisscrossing of the regions by animals with risky situations such as close and repeated animal to animal contacts, environmental stress factors and other conditions suitable for the exposure and spread of the disease. It was not uncommon to find returning *transhumance animals* mixed with the more stationed and 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). However, contradictory results of lower bovine TB prevalence in cattle maintained in traditional pastoral systems in Uganda and Ethiopia were associated with the relatively dryer conditions of the environments (Oloya et al. 2006; Ameni et al. 2007; Inangolet et al. 2008). Traditional pastoral systems in the tropics that create risks characteristic of intensive farming in relation to the transmission of bovine TB have been well documented (O'Reilly and Daborn 1995; Omer et al. 2001; Ayele et al. 2004). For example, close contact between animals occurs in shared micro-environments and when the animals gather together at common spots. It was 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 and shaded areas when the ambient temperature is high, preferring to graze when it is cooler. In this study, small herds and beef herds were severely affected by bovine TB but all herds were characterised by the above risk factors, albeit at different levels of intensity.

Also, the SIT-BT prevalence rate recorded in the Adamawa region in this study (6.89%), was lower than the 10.6% reported in the neighbouring Northern and Far Northern regions (Martrenchar et al. 1993). As for the Northwest region, the SIT-BT prevalence rate of 14% was lower than the 26% reported earlier in the peri-urban centre of Bamenda on a sample of exotic breeds (Muchaal 2002), their crosses and some local zebus with no observed positive test responses among the zebus. The higher SIT-BT rates according to management systems (10.27% for extensive; 16.94% for Intensive and 14.10% for semi-intensive), in this study were not in agreement with earlier records of 3% for animals on extensive, 13% for ranch farming in the Northwest region (Merlin and Tsangueu 1985), 1.4% for local cattle (Guadali) on an experimental livestock station, and 2.8% for dairy cows composed of Holsteins and their crosses in the Adamawa region (Tanya et al. 1985). In contrast to the SICCT-BT results of this study and earlier SIT-BT findings in Northwest Cameroon (Merlin and Tsangueu 1985; Muchaal 2002), significantly higher SIT-BT positive reactors were recorded in intensive settings followed by semi-intensive and extensive systems in the present study. Unlike the intensive, semi-intensive and extensive husbandry and management systems studied in this work, the earlier studies used animals in extensive systems (Martrenchar et al. 1993), extensive and ranching (Merlin and Tsangueu 1985), “zero grazing” husbandry (Muchaal 2002), and animals in experimental livestock stations (Tanya et al. 1985; Nfi and Ndi 1997). Differences in husbandry practices and management systems could have contributed in the inconsistent rates recorded in these studies. Due to possible repeated and close contacts between animals in these studies (feeding and drinking spots, shelters and gathering of animals during veterinary manipulations), the transmission of bovine TB would be expected to be highest in the intensive followed by the semi-intensive, ranching and extensive systems.

The rates of SICCT-BT and SIT-BT reactors showed similar trends and also varied based on

study location, age and breed of cattle. For both tests, the disease was more severe in the Western highlands compared to the Adamawa plateaux; age was a more significant risk factor than sex, and upgraded / exotic and Red Mbororo cattle showed the highest prevalence rates. Age and sex (Ameni et al. 2003, 2007; Oloya et al. 2006; Inangolet et al. 2008), herd size and body condition (Ameni et al. 2003), husbandry system and breed (Ameni et al. 2006, 2007), have been extensively reported as important risk factors influencing individual bovine TB prevalence rates. However, Ameni et al. (2003) and Oloya et al. (2006) suggested that sex was a weak risk factor similarly to this study where non-significantly more female than male animals were affected. The chronic nature of bovine TB, low transmissibility of the disease in an extensive/transhumance system and delayed onset of tuberculin positive response in adult and old animals has been attributed to the long incubation period of 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. Differing SICCT-BT and SIT-BT observations were noted for husbandry practices (herd size, management and type of production systems). The introduction and propagation of bovine TB in large herds in the studied regions were favoured by the potential for more contacts and other conditions favourable for the transmission of the disease between infected and uninfected (susceptible) animals.

In this study, widespread SICCT-AT and SIT-AT reactors were recorded and the proportions in some areas and herds were comparable to those of SICCT-BT and SIT-BT, respectively. The influence of environmental mycobacteria and non-specific responses on TST in 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 and Herbert (1975) and Lesslie et al (1975a, b), recorded hypersensitivity responses to AT that were equal to or higher than responses to BT in cattle naturally infected with *M. bovis* and presenting visible lesions at slaughter. 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). The SIT-BT detection rates were not affected by herd size and animals in intensive (and dairy) and semi-

intensive managements were significantly affected. However, SICCT-BT involving the injection of BT and AT at separate sites in the skin of the neck would give more specific results than SIT-BT using only BT (Monaghan et al. 1994), and interpreting SICCT-BT at a cut-off point of less than 4mm could have improved the bovine TB detection rates in this study.

Though not statistically significant, the observed prevalence of SICCT-AT reactors was higher in young cattle and SICCT-BT doubtful reactors increased with age and were higher among adult and/or old cattle. This finding is in agreement with Chacon et al. (2004) who reported that cattle were very susceptible to *M. avium* infection and that the young were most affected. The rearing of poultry and small ruminants (sheep and goats) which are the natural and suitable reservoir hosts of the *M. avium* complex (Biet et al. 2005), was common among the farmers. Infected free range poultry could have contaminated the animal and human microenvironments, pastures and watering points by shedding atypical mycobacteria in their faeces. Large groups of goats and sheep frequently shared the same grazing and drinking environments with cattle and could have also served as additional sources of infection. Bias in the SICCT-AT test was linked to environmental mycobacteria and non-specific immune responses to other mycobacterial agents in the animal environments (Philips et al. 2003; Biet et al. 2005; OIE 2009). The shift from the more positive SICCT-AT in the young animals (or in early years) to the more doubtful SICCT-BT status in adults and old animals (or later years) was also in accordance with Lauzi et al (2000; cited by Oloya et al. 2006) who reported that non-specific immune responses induced by atypical mycobacteria towards BT overcame the effect of contamination by the *M. avium* complex with an increase in age.

Anergy has been reported to cause false TST negative reactions but the reasons are still poorly understood (Inangolet et al. 2008). However, recently infected cattle, cattle under stress due to malnutrition, gastrointestinal parasitoses and 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 in this study coincided with the end of the dry season (period of drought), and the return of animals from transhumance. Stress due to starvation, long trekking, other environmental stressors and clinical symptoms of ill-health due to trypanosomosis, tick-borne diseases, heavy ec-

toparasitoses and gastro-intestinal parasitoses were common in the herds in 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, a lack of a positive SICCT-BT response and doubtful SICCT-BT reactions in old animals could have been due to age related anergy and other conditions that compromised their immune function (Thoen et al. 2009). The animal's immune system would not have been stimulated enough for a positive response to be measured (Ameni and Medhin 2000; Inangolet et al. 2008). However, the variation in bovine TB detection rates observed in this study between geographic regions and herds could have been due to the consequences of environmental and management factors and the risks these pose for the transmission and development of tubercle bacilli infections (Morris et al. 1994; Edwards et al. 1997).

This study reports the first comparative TST in cattle in the highlands of Cameroon and confirms that bovine TB is an current livestock health and production problem in Cameroon which needs to be further investigated. Analyses of the relative susceptibility and genetic resistance of indigenous zebu to bovine TB are needed to clarify the situation. However, the habits and level of awareness of cattle professionals and handlers of cattle products of the significance of zoonotic bovine TB, and the hygienic status of cattle farms and abattoir environments as potential risk factors for zoonotic TB disease are not known. The need for comprehensive research into the molecular epidemiology, risk factors, reservoir and maintenance host status and the public implications of zoonotic bovine TB in cattle in Cameroon cannot be overemphasised.

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