Seroprevalence and risk factors of leptospirosis among slaughtered cattle and abattoir workers in Ngaoundéré, Cameroon.

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ABSTRACT

Aims: This study aims to determine the seroprevalence and risk factors of leptospirosis in slaughtered cattle and abattoir workers at the Ngaoundéré Municipal Abattoir, Adamawa region, Cameroon.

Study Design: 10% of the average number of cattle slaughtered in the abattoir within the one month visitation period was randomly chosen and evaluated. And 96 of 108 human sera were randomly selected and screened for the presence of anti-Leptospira spp antibodies.

Place and Duration of Study: Municipal abattoir Ngaoundéré and Veterinary Research Laboratory of the Institute of Agricultural Research for Development (IRAD), Wakwa Regional Center, Ngaoundéré, Cameroon, between March and June 2018.

Methodology: A total of 172 bovine and 96 human serum samples were screened for the detection of *Leptospira spp* antibodies by Lepto ELISA kit. Structured questionnaires were used to collect data on socio-demographics and risk-factors.

Results: The results showed that 18.02% (95% CI (4.7 – 33.34)) of the animals slaughtered were seropositive to *Leptospira spp hardjo* antibody. Though sex did seem to influence (P>0.05) leptospira seropositivity, age and body condition score were major (P<0.05) risk factors. A seroprevalence of 10.42% CI (4.30-16.52) was observed among the abattoir personnel with the use of personal protective equipment such as gloves significantly (P<0.05) influencing seropositivity.

Conclusion: Antibodies against Leptospira are prevalent among slaughtered cattle and workers in the Ngaoundéré municipal abattoir. This study reports the first evidence of human leptospirosis in Cameroon revealing real public health concern in the country. Public awareness campaigns and health education especially in agropastoral communities based on the One Health approach is essential to disseminate knowledge, associated risk factors and control measures of this occupational disease in Cameroon.

Keywords: *Bovine, Humans, Leptospirosis, Prevalence, Risk factors, Ngaoundéré abattoir-Cameroon*

1. INTRODUCTION

Leptospirosis is one of the most common and widespread bacterial zoonotic infection of economic importance worldwide [1] caused by infection with a pathogenic serovar of *Leptospira* spp. [2]. In humans, it can cause a wide range of symptoms, some of which may be mistaken for other diseases and most cases go undiagnosed and untreated leading to considerable suffering to the affected. Nevertheless, some infected individuals may be asymptomatic [3].

Many aspects of this neglected tropical and zoonotic disease is poorly understood in part because of lack of diagnostic laboratory services, complexity of the host-leptospire relationship, and changing patterns of infection [4, 5]. Leptospirosis has not been widely studied in Africa [6], and in Cameroon, the last focus of the disease in humans was described in 1976 when an epidemic caused 95 cases, including 63 serologically confirmed cases, within two years [7]. Nevertheless, it is worth noting that the first leptospirosis epidemic in Cameroon occurred in 1975 which was initially misdiagnosed as a yellow fever epidemic [8].

In bovine, leptospirosis is noted for the "milk drop syndrome" which occurs in serovar Hardjo-infected lactating cows [9] and enough loss due to abortions and infertility. Despite its global importance, large gaps persist in the understanding of the burden and epidemiology of leptospirosis in Africa [10]. However, Leptospirosis has been classified as the 12^{th} out of 41 major priority zoonotic infections in Cameroon [11].

Although reported seroprevalence data demonstrates widespread exposure to *Leptospira* spp. in humans and animals in Africa, little is known about the extent of the human disease and the epidemiology of *Leptospira* infection in different animal species in the continent [10]. There is dearth of information on leptospirosis in humans and scanty data of the diseases in cattle in Cameroon. A herd seroprevalence rate of 35% reported about a decade ago in the country [12] did not take into consideration infected animals in the food value chain and the public health concern of the disease. In this context, this study was carried out to determine the prevalence and associated risk factors of leptospirosis in slaughtered cattle and personnel of the Ngaoundéré municipal abattoir Cameroon.

2. MATERIAL AND METHODS

2.1 Study area

This study was carried out from March to June 2018 in the Ngaoundéré municipal abattoir of the Adamawa Region of Cameroon (7°- 8° N and 13°- 14°E) (Figure 1) where about 55 cattle are slaughtered daily [MINEPIA (Ministry of Livestock, Fisheries, and Animal Industries) abattoir records, 2018].

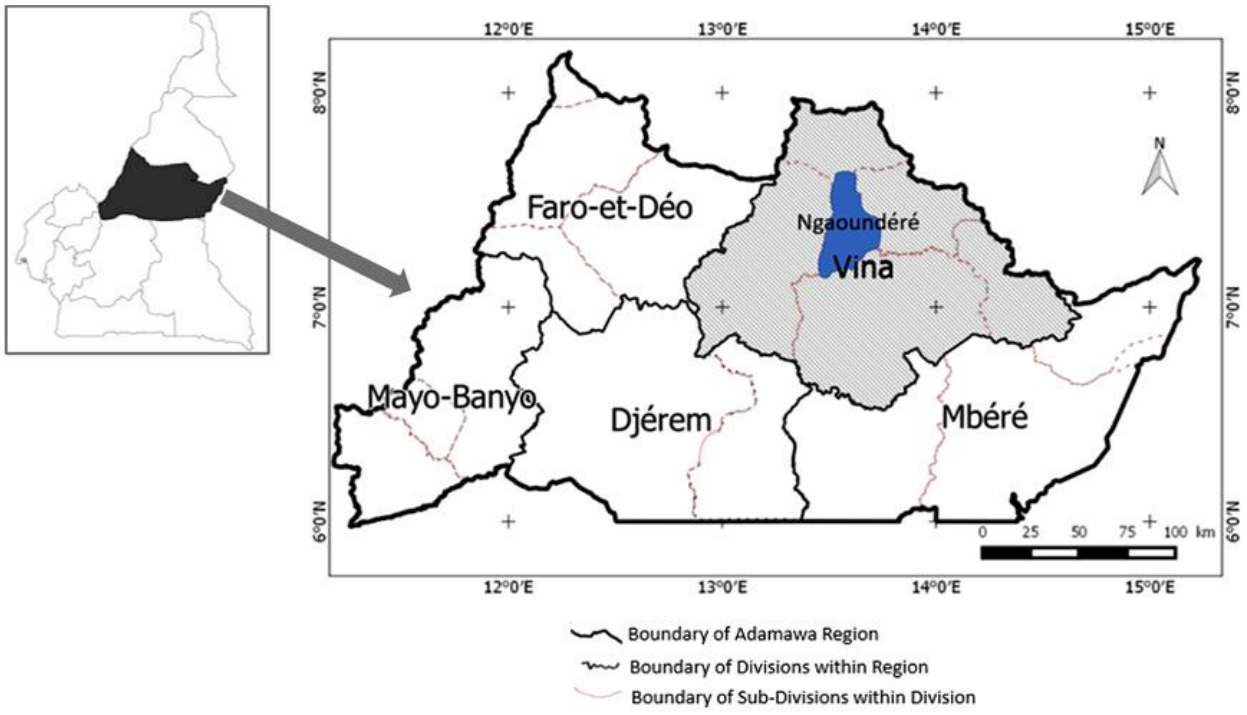

Figure 1: Map showing study area (Ngaoundéré) in Vina Division of the Adamawa Region in Cameroon. (Source: Ngaoundéré City Council for map of Adamawa Region, Cameroon. Map of Cameroon was adapted from Wikimedia Commons: https://commons.wikimedia.org/wiki/Maps_of_Cameroon)

2.2 Selection of animals and blood sampling

Selection of individual cattle for the study was done in the Ngaoundéré municipal abattoir during the study period using previously described systematic random sampling technique [13], and the sample size estimated as described by Thrusfield [14]. Briefly, about 20 % of the 40 – 60 cattle slaughtered daily in the abattoir were randomly selected and sampled each day, uninterruptedly within a two-week period in the rainy and dry seasons, were included in the study. Based on a calculated sampling fraction of five (every fifth animal was sampled) for daily use, the first animal selected by picking one animal by random generation method of the first five animals on the slaughter chain. Thereafter, every fifth animal (adding 5 to previous picked number) was chosen till the sample size was achieved. Apart from procedural restraining manipulations for safety purposes, 5ml of blood was collected from the jugular vein using VenojectND needle into sterile dry tubes. All samples collected were labelled, stored in ice pack container and carried to the Veterinary Research Laboratory of the Institute of Agricultural Research for Development (IRAD), Wakwa, Ngaoundéré, Cameroon, where they were centrifuged at 4000 rpm for 5 minutes, aliquoted into cryotubes, stored at –20 ^{0}C until serological analysis.

2.3 Human sera samples

The human sera samples used for this investigation were originally collected from the same abattoir workers during the period of August 2015 to March 2016 in Ngaoundéré as part of a study of brucellosis at the Ngaoundéré Municipal Abattoir [13] and stored at –20^{0}C. Briefly, 96 (representing the number of ELISA plate wells) of 108 human sera randomly selected by drawing out the cryotubes without replacement were screened for the presence of anti-Leptospira spp antibodies.

2.4 *Leptospira* ELISA analysis

2.4.1 Animal sample: The Linnodee Lepto Kit (Linnodee Animal Care, Ballyclare, UK) was used to screen the cattle sera for anti-Leptospira hardjo antibodies. This ELISA Kit is a double sandwich ELISA for the swift and safe detection of anti-*L. hardjo*-specific antibodies in serum with a sensitivity of 94.1% and specificity of 94.8%. The procedure was performed according to the manufacturer's instructions and essentially as described by Yan et al [15]

2.4.2 Human sample: The commercial Leptospira IgG (Lep IgG) ELISA Kit' (Schloss-Rahe-Str. 15, 52072 Aachen, Germany) was used to screen the human sera for anti-Leptospiral antibodies according to the manufacturer's instructions.

2.5 Risk factor analysis

Information on risk factors for bovine and human leptospirosis was obtained by examination of individual cattle as well as questionnaire interview of personnel at the Ngaoundéré abattoir. The questionnaires were structured to collect information on a range of variables including lifestyle, socio-demographic data and risk factors related to occurrence of leptospirosis.

2.6 Data analysis

The data obtained were entered into Microsoft Office Excel 2013 to obtain descriptive statistics. Data analyses were done using SPSS Version 23. The chi-square test was used to test significant levels within factors on seroprevalence rates and odds-ratios were determined for associated risk factors along 95% confidence intervals and statistical significance set at $P<0.05$.

3. RESULTS

3.1 Prevalence of anti-*Leptospira spp* serovar hardjo antibodies in cattle

Overall, 31 of 172 tested bovine sera with sandwich ELISA were positive to anti-*Leptospira* serovar *Hardjo* antibodies corresponding to a prevalence of 18.02% [95% CI (12.28 – 23.76)].

3.2 Prevalence of leptospirosis in cattle with respect to risk factors

Table 1 presents the seroprevalence of bovine leptospirosis according to endogenous and exogenous risk factors. Over 82.6% (142/172) of the cattle sampled were females whilst 17.4% (30/172) were males; and of these, 23 (74.2%) females and 8 (25.8%) males were seropositive. There was no significant difference in seropositivity between the sexes (P=0.175). Of the seropositive animals, 5 (16.1%) were in the age group <4years, 20 (64.5%) in the age group 4-8 years and 6 (19.4%) in the age group >8years. There was a high significant difference in the seropositivity due to age (P< 0.000063).

Out of the 172 animals sampled, 131 (76.16%) registered a poor body condition score (BCS of 1 or 2)) and 41 (23.83%) were average (BCS=3). Of these, 31 poor or emaciated animals were seropositive for anti-*Leptospira* antibody while all the average score animals were seronegative. There was a high significant difference (P<0 .001) in seropositivity due to BCS.

Out of the 172 animals sampled, 99 (57.55%) were of the Gudali breed, 39 (22.67%) White Fulani, 20 (11.63%) Red Fulani, 3 (1.74%) Bokolo, 3(1.74%) Bokolo-Charolais Hybrid and 8 (4.65%) Gudali-White Fulani hybrid. Of these, 17 (54.8%) Gudali, 11 (35.5%) White Fulani and 3(9.7%) Bokolo-Charolais hybrids were positive for anti-*Leptospira spp.* Serovar *Hardjo* antibodies. There was no significant difference (P=0.147) in seropositivity due to breeds.

Overall, 62 (36.04%) animals were from Mayo Rey Administrative division in the North Region and 110 (63.95%) animals from Vina Administrative division of the Adamawa Region were used in the study. Also, *Leptospira* seropositive reactions were observed in 11 (35.5%) cattle from Mayo Rey and 20 (64.5%) from Vina. There was no significant difference (P= .9) for seropositivity due to origins of the slaughtered cattle.

As for season, 86 (50%) cattle were sampled during the dry and 86 (50%) during the rainy season. Of these, 11 (12.8%) animals in the dry season and 20 (23.25%) in the rainy season were seropositive for anti-*Leptospira spp* serovar *hardjo* antibodies. However, there was no significant difference (P>0.05) due to season.

Table 1. Seroprevalence of leptospirosis in cattle of the Ngaoundéré abattoir according to endogenous and exogenous risk factors (N=172).

Factors	Variables	Positive ELISA	N	Prevalence % (95% CI)	OR (95% CI)	
Breed	Bokolo	0	3	0.0	-	
	Red Fulani	0	20	0.0	-	
	Gudali*White Fulani cross	0	8	0.0	-	
	Bokolo*Charolais cross	3	3	100	-	
	White Fulani	11	39	28.2 [14.08-42.32]	/	
	Gudali	17	99	17.7 [10.18-25.22]	1.059(0.336-3.338)	
Sex	Female	23	142	16.2 [10.14-22.26]	0.173(0.04-0.747)	
	Male	8	30	26.6 [4.93-52.27]	/	0.1
Age (Years)	Adult [4 ≤ x ≤ 8]	6	88	6.8 [1.54-12.06]	0.329(0.075-1.45)	
	Old [x > 8]	20	56	35.71 [23.16-48.26]	2.50(0.55-11.34)	

	Young [x <4]	5	28	17.85 [3.32-31.38]	/	
Body Condition Score	Average (3)	0	41	0.0	-	
	Poor (1 and 2)	31	131	23.7 [16.42-30.98]	-	
Season	Dry	11	86	12.8 [10.05-26.35]	0.760(0.250-2.313	
	Rainy	20	86	23.25 [14.32-32.18]	/	
Origin of animals	Vina	20	110	18.2 [10.9-25.41]	-	0.9
	Mayo Rey	11	62	17.8 [8.28-27.32]	-	

N=number of animals; BCS=body condition score (Significant if $P<0.05$)

The influence of endogenous risks factors (breed, sex, BCS and age) on the seroprevalence of Leptospirosis are presented in Table 1.

The White Fulani was more likely of getting leptospirosis compared to the Gudali though OR<1. Age was a significant risk factor with the animals in the old age group (>8 years) being more less and the young groups (<4years) being more likely of getting bovine leptospirosis than the others

3.3 Prevalence of leptospirosis among abattoir workers

The seroprevalence of leptospirosis among the abattoir workers was recorded as 10.41% at 95% CI (4.30-16.52). Overall, of the 16 (16.66%) females and 80 (83.34%) males tested, 4 (25.0%) females and 6(75%) males were seropositive for anti-*Leptospira* antibodies. The study showed that sex (P=0.06) and age (*P=0.187*) (Table *2*) did not significantly affect *Leptospira* seropositivity. However, the use of personal protective wares such as gloves significantly influences (P< 0.001) Leptospira seropositivity amongst abattoir workers (Table 3).

Table 2. Seroprevalence of leptospirosis in abattoir workers in Ngaoundéré according to potential intrinsic risk factors (N=96)

Category	Variable	Total	Positive ELISA N (%)	95%CI of positive	OR (95% CI)	*P*-value (χ^2)
Sex	Female	16	4 (25.0%)	[3.78-46.22]	4.111(1.009-16.747)	0.06 (5.987)
	Male	80	6 (7.5%)	[1.73-13.27]	/	
	Total	96	10 (10.4%)	[4.30-16.52]	-	
Age	[15-25[	26	4 (15.4%)	[1.53-29.27]	0.545[0.08-3.73]	0.187 (4.447)
	[25-35[	43	2 (4.7%)	[0-11.03]	0.146[0.017-1.243]	
	[35-45[	19	2 (10.5%)	[0-14.22]	0.353[0.04-3.09]	
	[45-65]	8	2 (25.0%)	[0-55.01]	/	
	Total	96 (100.0%)	10 (10.4%)	[4.30-16.52]	-	

Significant if $P < 0.05$

Table 3. Seroprevalence of leptospirosis in abattoir workers in Ngaoundéré with respect to potential extrinsic risk factors (N=96)

Variable	N	Positive ELISA	95%CI of positive	OR (95% CI)	*P*-value (χ^2)

			N (%)			
Longevity in service	<1year	6	0 (0.0%)	-		
	[1-5]	32	4 (12.5%)	[2.42-22.58]	0.90 (0.21 – 3.96)	
	[6-10]	14	2 (14.3%)	[4.22-24.38]	0.77 (0.13 – 4.80)	0.962
	[11-20]	35	4 (11.4%)	[1.32-21.48]	/	(0.076)
	>20	9	0 (0.0%)	-		
Use of personal protective equipment	No	20	0 (0.0%)	-		
	Yes	76	10 (13.2%)	[3.12-23.28]		
Use of hand gloves	No	86	6 (7.0%)	[-3.08-17.08]	/	0.001
	Yes	10	4 (40.0%)	[29.92-50.08]	0.11 (0.02 – 0.51)	(10.469)
Boots and Water proof overalls	No	22	2 (9.1%)	[-0.98-19.18]	/	0.817
	Yes	74	8 (10.8%)	[0.72-20.88]	0.83 (0.16 – 4.20)	(0.054)
Contact with aborted fetus	No	76	8 (10.5%)	[0.42-20.58]	/	0.945
	Yes	20	2 (10.0%)	[-0.08-20.08]	1.06 (0.21 – 5.42)	(0.005)
Consumption of unpasteurized milk	No	56	8 (14.3%)	[4.22-24.38]	0.38 (0.08 – 1.89)	0.142
	Yes	40	2 (5.0%)	[-8.08-12.08]	/	(2.156)
Consumption of poorly cooked meat	No	22	0 (0.0%)	-	-	
	Yes	74	10 (13.5%)	[3.42-23.58]	-	

Significant if $P<0.05$

3.4 Association between Seroprevalence and risk factors in abattoir worker population.

As concerns the level of association between the observed prevalence in the worker population and identified risk factors ; it was noticed that there was a very strong association between the use of boots and waterproof overalls and the minimal use of personnel protective equipment (shoes, slippers etc.) with R=0.941. There equally existed a correlation between thee sex and the longevity of service (R=0.530), the sex and the working post (R=0.637), the sex and the use of boots and waterproof overalls (R=0.616) and finally the sex and the use of personal protective equipment (R=0.513) (Table 4).

Table 4. Association between seroprevalence and the different risk factors in abattoir workers

	Age	**Sex**	**Longevity of service**	**Working post**	**Use of PPE**	**Use of gloves,**	**Boots, waterproof overalls**	**Contact with aborted fetus**	**Consu poorly c**
Age	1.000	.315	.371	.328	.295	.048	.346	.051	-
Sex	.315	1.000	.530	.637	.513	.246	.616	.066	
Longevity of service	.371	.530	1.000	.426	.425	.107	.448	.144	
Working post	.328	.637	.426	1.000	.469	.223	.537	.208	
Use of Personal protective equipment	.295	.513	.425	.469	1.000	-.175	.941	.137	
Use of glove	.048	.246	.107	.223	-.175	1.000	-.024	.007	-
Boots and waterproof overalls	.346	.616	.448	.537	.941	-.024	1.000	.158	

Contact with aborted fetus	.051	.066	.144	.208	.137	.007	.158	1.000	
Consumption of poorly cooked meat	-.075	.054	.334	.316	.209	-.186	.174	.280	1
Consumption of unpasteurized milk	-.064	.348	.213	.422	.017	.288	.059	.191	
Proper Value	5.385	2.692	2.178	1.931	.625	.593	.542	.348	

NB: Association if R>0.5.

4. DISCUSSION

4.1 Bovine leptospirosis seroprevalence

The bovine leptospirosis seroprevalence of 18.02% recorded in the present study is higher than the 3.5% obtained by Ngbede et al [16] in the Zango abattoir, Nigeria but less than the 35% recorded by Scolamacchia et al [12] in the Adamawa plateau of Cameroon. Nevertheless, the result is similar to the findings recorded in the Dakar abattoir in Senegal (20.8%) [17] and KwaZulu-Natal, South Africa (19.4%) [18]. The differences in prevalence rates reported in Cameroon and other parts of Africa could be associated with the evolution of the disease, agro-ecological location, sample size, study frame as well as the protocol adopted such as the type and number of diagnostic tests used.

The study observed that sex of the animal was a major risk factor for the occurrence of the disease as more bulls were found to be seropositve than cows. This finding agrees with those recorded by Ngbede et al [16] (42. 94% in cows and 57.04% in bulls) and Ramin et al [19] (10.9% in bulls and 4.8% in cows).

Furthermore, the study revealed that age was a major risk factor for higher leptospiral seropositivity which agrees with the findings of Ngbede et al [16] who reported higher seropositive reactors (35.71%) among animals aged more than 8 years compared to the other age groups in the Zango Abattoir Nigeria. This could be attributed to the duration of exposure and persistence of the antibodies in the aged animals to the pathogen [16]. This finding is in accordance with those reported in Iran and other countries where increase in leptospirosis seropositivity were observed in adult animals than in the young [20, 21].

The body condition score (BSC) was also observed to be a major factor influencing leptospiral seropositivity in this study with the animal of poor BSC being the most affected. Chronic leptospirosis are usually caused by host-adapted Leptospira serovars and *Leptospira* serovar *hardj*o has been observed to cause several clinical manifestations amongst individual with poor growths [22]. However, animals with poor BSC are usually associated with malnutrition, compromised defense system and increased susceptibility to stress and higher risks of being infected. Also, high rates concomitant infections are widespread in livestock in poor African countries. These reasons could have been responsible for the higher seropositivity recorded among animals with poor BCS.

No significant difference ($P<0.05$) in seropositivity between the different cattle breeds was observed in this study. The finding concur with Ngbede et al [16] who however reported a high prevalence in zebu breed of cattle based on origin of the cattle and not as a significant factor of *Leptospira* infection which is contrary to other reports [23, 24, 25]. Seroprevalence difference due to region or locality could be due to many factors including soil type, mean temperature, herd management practices and presence of wildlife. However, due to the

heterogeneous nature of these breeds, care should be cautioned not to over-interpret these findings [23].

4.2 Human leptospirosis seroprevalence

The detection of anti-*Leptospira spp.* antibodies in the abattoir worker – slaughtered animal interface could indicate a continual source of human leptospires which poses problems to public health. The abattoir workers were at high risk of contacting the zoonoses owing to the fact that they were always in contact with live animals and animal products during the meat processing phase. The majority of leptospirosis cases usually show mild symptoms which do not often require medical intervention [10]. However, a diverse range of severe clinical manifestations including jaundice, aseptic meningitis, renal failure and rarely, lethal pulmonary hemorrhage and cardiac involvement, have been reported in infections with *Leptospira* spp. [26]. These non-specific symptoms make diagnosis of leptospirosis difficult [16] as well as reduce the level of awareness of the disease [27].

This study presents the first of human leptospirosis seroprevalence report from Cameroon and the infected animals in the study area probably serve as reservoirs and sources for the human leptospirosis recorded. The leptospirosis seroprevalence (10.4%) among abattoir personnel recorded in this study is similar to the 11% prevalence obtained by Dreyfus et al [28] in New Zealand but lower than the rates ranging from 15.2 to 81% recorded in some studies carried out in Africa and Asia [27, 29, 30]. The observed differences could be associated to detection of antibodies against more than one serovar, infections by mixed serovars and cross-reactivity among serovars at different titres [27]. Sex, age and longevity in service were observed not to be significant risk factors to *Leptospira* infection among the abattoir personnel.

Although no significant difference between prevalence and sex was noticed, a high association between sex and duty post, years of working, use of boots and waterproof overalls and other Personal protective equipment (PPE) was observed among the abattoir personnel. Several other studies have shown that there is an association between leptospirosis and gender [31]. Leal – Castellanos et al [32] concluded in a study that the risk factors with the highest strength of association was occupational contact with animal excreta and this risk was higher if subjects had no protection against contact and the presence of cut/abrasion of the skin. The above observed associations are an indication that preventive measures need to be implored in other to curb the spread and dissemination of leptospirosis among the risk group.

The presence of Leptospira-agglutinating antibodies among abattoir workers was attributed to constant exposure of these workers to fluids of slaughtered animals and contaminated abattoir environment [29]. Also, minor cuts and injuries sustained during work in the abattoirs did not serve as deterrent from handling animal tissues, which may have been infected [27]. This explained the reason why PPE such as the use of gloves was a major significant risk factor for seropositivity observed amongst the abattoir personnel in this study.

5. CONCLUSION

The study reports the first evidence of human leptospirosis in Cameroon and revealed that leptospiral infection is an important public health problem among abattoir personnel in Ngaoundéré Cameroon. A seroprevalence of 18.02% for bovine leptospirosis and 10.41% for human leptospirosis was recorded in this study. Sex, body condition score, age and breed were the major risk factors observed to be associated with bovine leptospirosis. The risk of transmission to humans was aggravated by not using protective equipment at work and handling of carcasses. Public awareness campaigns and health education especially among livestock

professional and in agropastoral communities should be highlighted to disseminate knowledge, associated risk factors and control measures of leptospirosis. The need for intensification of the integrated "One Health" approach and multi-sectoral policies including interdisciplinary strategies between animal and human health experts, concerned target stakeholders and affected communities about the need for detailed information on animal and human leptospirosis for effective management in the country cannot be overemphasized. In perspective, a bacteriological study of leptospirosis would be necessary to determine circulating serotypes in the Ngaoundéré area and beyond.

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Permission for the study was obtained from the required authorities and Local Ethical Committees in Adamawa Region, Cameroon including the Regional delegation of Livestock, Fisheries and Animal Industries, Regional Delegation of Public Health, School of Veterinary Medicine and Sciences of the University of Ngaoundéré and Ngaoundéré Regional Hospital. The purpose of the study was explained (with the assistance of local veterinary and medical practitioners, community leaders and trusted intermediaries) to personnel at the Ngaoundéré abattoir. Cattle professional including butchers and personnel at the Ngaoundéré abattoir were used in the study after giving their written informed consent.

COMPETING INTERESTS

The authors declare that they have no competing interests.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Author VNN designed the study, coordinated sample collection and wrote the manuscript. Author BTNA coordinated sample collection in the field and processing and performed statistical analysis. Author JAN wrote the manuscript and performed statistical analysis. All authors read and approved the final manuscript.

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