

# 1 Prevalence and public health significance of Lyssavirus in 2 bats in North region of Cameroon

4 Isaac Dah<sup>1,2\*&</sup>, Rodrigue Simonet Poueme Namegni<sup>2&</sup>, Moctar Mouiche Mouliom Mohamed<sup>1</sup>  
5 Simon Dickmu Jumbo<sup>2</sup>, Ranyl Nguena Guefack Noumedem<sup>1,2</sup>, Isabelle Conclois<sup>4</sup>, Liegeois  
6 Florian<sup>4,5</sup>, Laurent God-Yang<sup>1,2</sup>, Jean Marc Feussom Kameni<sup>3</sup>, Abel Wade<sup>2</sup>, Dorothée Misse<sup>4</sup>,  
7 Julius Awah-Ndukum<sup>6,7</sup>

- 8 1. School of Veterinary Medicine and Sciences, The University of Ngaoundéré, Po Box 454,  
9 Ngaoundéré, Cameroon;
- 10 2. National Veterinary Laboratory, LANAVET, Garoua, Cameroon;
- 11 3. Epidemi-Surveillance Service, Ministry of Livestock, Fisheries and Animal Industries  
12 Yaoundé, Yaoundé, Cameroon;
- 13 4. MIVEGEC, IRD, Univ. Montpellier, CNRS, 34394 Montpellier, France;
- 14 5. University of Zimbabwe, Faculty of Veterinary Science, Harare, Zimbabwe;
- 15 6. Department of Animal Science, Faculty of Agronomy and Agricultural Sciences,  
16 University of Dschang, Dschang, Cameroon;
- 17 7. Department of Animal Production Technology, College of Technology, University of  
18 Bamenda, Bambili, Cameroon.

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20 \*Corresponding author: [dah\\_isaac@yahoo.fr](mailto:dah_isaac@yahoo.fr), +237 690905682

21 &These authors contributed equally to this work.

## 23 Abstract (284 words)

## 24 Background

25 Rabies is a zoonotic disease of all warm-blooded animals including humans. Though, there is little  
26 knowledge of the status of rabies in wild animals in Cameroon, the disease is endemic in the  
27 country with dogs being the main source of transmission. Bat habitats are widespread in  
28 Cameroon, but there is little information on the prevalence of rabies-like viruses in bats, nor the  
29 role of bats as a potential reservoir of rabies.

## 30 Methods

31 A cross sectional study was carried out to determine the prevalence and risk factors of Lyssavirus  
32 in bats in the Northern region of Cameroon. A total of 212 bats belonging to three families

33 (Pteropodidae, Vespertilionidae, Molossidae) and 5 species were randomly sampled in 7 localities  
34 in the North of Cameroon and were tested for Lyssavirus antigen using direct  
35 Immunofluorescence Test (IFA). Overall, 57 (26.89%) of the bats collected showed an IFA  
36 positive reaction. The prevalence was higher ( $P<0.05$ ) in adult bats (33.33%, 95% CI: 25.15 –  
37 42.66) compared to young individuals (20.19%). The main risk factors identified in the study for  
38 human exposure to bats were gender (Male), educational level (tertiary), religion (Christianity),  
39 ethnic group (Matal), the presence of bats in the area, the practice of bat hunting and consumption  
40 and the level of awareness on bat rabies-like viruses.

## 41 Conclusion

42 The study found the first evidence of Lyssavirus in bats in Cameroon. This finding revealed that  
43 bat rabies-like viruses are real and constitutes a potential human health problem in communities  
44 with bat habitats in the North region of Cameroon. Enhancing the level of public awareness and  
45 health education on the potential of bats as reservoirs of Lyssavirus in Cameroon as well as the  
46 integration of the “One Health” approach for effective management of animal and human rabies  
47 should be emphasized.

## 48 Author summary (173 words)

49 Rabies is a zoonotic disease caused by a virus of the genus Lyssavirus. It affects all warm-blooded  
50 animals including humans. Canine and Human rabies are well documented as endemic in  
51 Cameroon, but little is known about this disease in wildlife, in particular among bats, despite their  
52 multiple interactions with the inhabitants of Northern Cameroon. Indeed, bats were hunted, sold  
53 and eaten as bush meat by local populations. We investigated the presence of Lyssavirus in bat  
54 and assessed the risk factors of human exposure to bats in the Northern region of Cameroon. The  
55 study highlights that Lyssavirus is present in bats in this area. The population was aware of human  
56 and canine rabies, however, the presence of the disease in bats was less known. Based on these  
57 findings, investigating bat populations on a large scale, to characterise the Lyssavirus strains

58 circulating in the region, as well as educate the local population on the risks of rabies transmission  
59 from bats to humans and other animals.

## 60 **Introduction**

61 Rabies is a virulent fatal zoonotic disease of major public health concern caused by a Lyssavirus  
62 [1]. The virus is known to affect all warm-blooded animals, including domestic pets, which are  
63 the main vectors. Bat Lyssaviruses are distributed worldwide including canine rabies-free  
64 countries and bats are the primary dispensers of Lyssavirus to other animals and humans over vast  
65 geographical areas through their saliva and urine [2]. Bats have been directly involved in the  
66 transmission of rabies to humans through aerosols and unapparent bites as well as indirectly  
67 through animals they infected. Infections of others animal could be through contact with  
68 contaminated body fluids by scratching of mucous membranes and open skin wounds [3, 4, 5, 6,  
69 7]. Fatalities in humans and other animal species due to bat Lyssavirus [8, 9, 10], as well as human  
70 rabies related to Lyssavirus bat variants, have been reported to be associated with bat biting and  
71 simple contact with bats [11]. Bat Lyssaviruses are more infectious in superficial epidermal  
72 inoculation and multiply faster in non-nerve cells at a lower temperature than the canine rabies  
73 virus [12]. The transmission of animal rabies to humans is higher in areas where animal rabies is  
74 widespread [13], and there are frequent human and animal exposures to bats, including sick and  
75 injured bats with high risk of contamination [14].

76 Rabies has induced over 3.7 million (95% CI: 1.6 – 10.4 million) disability-adjusted life years  
77 (DALYs), 8.6 billion USD (95% CI: 2.9 – 21.5 billion) economic losses annually and 59,000  
78 human deaths per year worldwide [15]. Bat Lyssavirus could be underestimated in Africa, due to  
79 insufficient surveillance programs [16]. The disease has been reported in bat populations in parts  
80 of the continent with prevalence ranging from 29 to 67% in Kenya [17], 38% in Ghana [18], 19%  
81 in Nigeria [19] and 5.5% in the Democratic Republic of Congo [20]. In Cameroon, over US \$  
82 576,232.88 was estimated as direct financial losses linked to rabies prevention measures and post-  
83 exposure treatments between 2004 and 2013 in three cities (Garoua, Yaoundé and Ngaoundéré)

84 of Cameroon [21]. Urban rabies is widespread in dogs, which are considered as the main source  
85 of animal and human rabies in Cameroon [21, 22, 23, 24, 25] and canine and human rabies are  
86 endemic in the Northern region of the country [21, 23, 26]. There are many bat colonies and a  
87 majority of bat roosting sites are located adjacent to human communities where the level of  
88 physical interactions between bats and humans is high in many parts of the country. However,  
89 there is little or no attention on the epidemiology of bat Lyssaviruses. Insectivorous bats also  
90 frequently flock and inhabit roof-tops and homes while hunting of frugivorous bats for food are  
91 common in Cameroon. According to Mickleburgh *et al.* [27], the consumption of these animals  
92 by local populations could endanger the long-term survival of these species (*Eidolon helvum*).  
93 Indeed, *E. helvum* is consumed and marketed locally in the Bomboko area (South-West) and  
94 elsewhere in Cameroon, where they constitute an important source of income for local hunters  
95 [27]. Meanwhile, human–wildlife interactions that increase the risk of transmission are frequent  
96 and various, namely hunting, butchering and consuming wild animals, including bats, are common  
97 in Cameroon [28]. Additionally, subsistence activities and large-scale agriculture expose people  
98 not only to bat bites, but to potential infection through scratching due to the presence of urine and  
99 droppings of bats [28]. In Northern Cameroon, bats usually form colonies in the roofs, trees and  
100 abandoned tall structures in human communities and are also hunted as a source of animal protein  
101 and guano, which includes bat droppings, are widely collected as fertilizer [29]. In this context,  
102 the present study was carried out to determine the prevalence of rabies related-virus in bats and  
103 associated risk factors to human health in the Northern region of Cameroon.

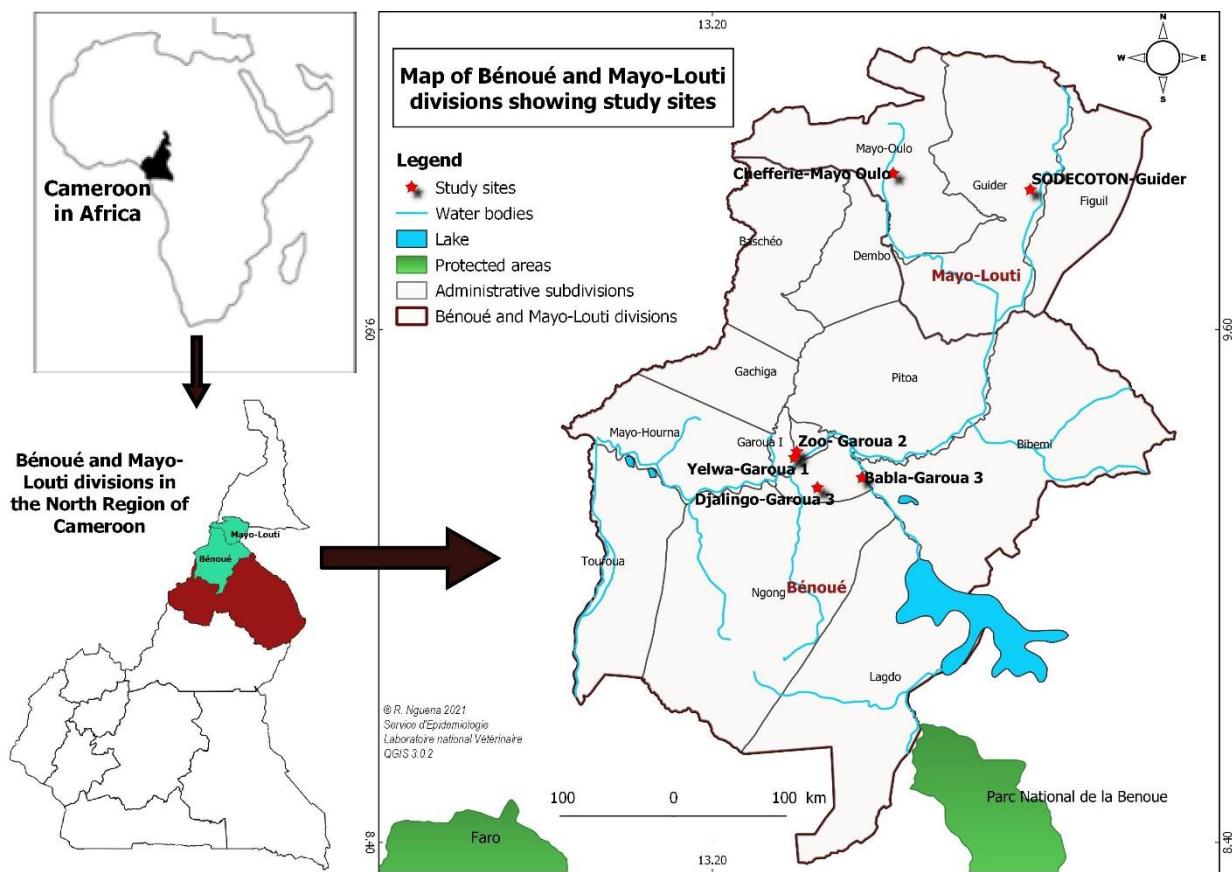
104

## 105 **Material and Methods**

### 106 **Description of study areas**

107 This study was carried out in 7 localities (Babla, Djalingo, Lagdo, Guider, Mayo Oulo, Yelwa and  
108 Garoua II) in two Administrative Divisions (Mayo-Louti and Bénoué) of the North region of  
109 Cameroon (6° - 10° N and 12° - 16°E) (Fig 1) based on the available information on bat roosts,

110 bat colonies and field observations of flying and foraging bats. The North region is situated in the  
111 Sudano-sahelian region with an average altitude of 249 m, a short rainy season from mid-March  
112 to October of 1200 – 1600 mm per annum and an ambient temperature range from 21° to 36°C.



113  
114 **Fig 1 :** Map showing study sites. Bats specimen were collected in several study sites (red stars)  
115 in Mayo-Louti and Bénoué administrative divisions of the North Cameroon region.  
116

## 117 Sampling of bats for the study

118 A cross sectional study was conducted in the North Region of Cameroon from February to May  
119 2017 following identification and listing of communities and geographical areas with bats roosts  
120 and bat activities. Information on bats roosts was obtained through the aid of local communities'  
121 leaders. All identified localities with bat activities (hunting, selling of bat as bush meat etc.) were  
122 included and visited for sample collection in the study. Insectivorous bats and Fruit bats were  
123 collected twice weekly from the hunters in exchange of money before delivery to their clients.  
124 Indeed, there are vendors who bought bats and resold the meat to the consumers in these localities.  
125 Also, 7 dead bats including 6 *Eidolon helvum* at the Garoua zoological garden and 1 *Chaerephon*

126 *pumilus* at Lagdo were also collected during the study period. Whole animals were shipped to the  
127 National Veterinary Laboratory (LANAVET) Garoua in an individual ziplock bag placed in a  
128 cooler with frozen ice pack [30]. The sample size was determined according to Thrusfield [31]  
129 based on a bat Lyssavirus prevalence of 19% obtained in Nigeria [19]. Overall, 212 collected from  
130 hunters and dead bats made up of 76 frugivorous bats (*Eidolon helvum*) and 136 insectivorous  
131 bats (10 *Chaerephon chapini*; 10 *Chaerephon leucogaster*; 96 *Chaerephon pumilus* and 20  
132 *Scotophilus leucogaster*) were collected in 7 localities of the North region.

133

## 134 **Laboratory analysis**

135 Laboratory analysis was done at LANAVET Garoua, in the North Region of Cameroon. The bat  
136 species was identified based on biomorphometric measurements with calliper using dichotomous  
137 keys [29]. The sex of bats was determined based on the observation of external genital organs and  
138 stage of growth (whether the bats were juvenile or adult) was through appraisal of body  
139 development and pelage coloration as previously described [19, 32].

140 A cross-section of the brain (including cerebral cortex and cerebellum) of each bats collected (212)  
141 in the study was taken after skull dissection in a certified biosafety cabinet [33]. An equally portion  
142 of each brain part collected was mixed and a thin smear spread on a slide was made. The slides  
143 were fixed in cold acetone at -20°C for one hour and then dried for 30 min at room temperature.

144 The Lyssavirus ribo-nucleoprotein complex was detected using Rabies specific labelled  
145 polyclonal antibodies with Evans blue (1/2000 final dilution) as counterstain to denote stained  
146 areas and incubated at 37°C in a humidified chamber according to the manufacturer's instructions  
147 (Bio-Rad kit 357-2112, Marnes-La-Coquette, France). The direct Immunofluorescence Assay  
148 (IFA) was used to detect Lyssavirus antigen in brain tissue, in accordance with WOHA (World  
149 Organization for Animal Health) and WHO (World Health Organisation) recommendations [34].  
150 Briefly, positive and negative canine rabies controls available at the National Veterinary  
151 Laboratory in Garoua were used to validate the test results. A test was validated when there was

152 fluorescence in the positive control and no fluorescence in the negative control. Three experienced  
153 staff members read the test slides before validating the results.

154

## 155 **Analysis of bat rabies exposure risk factors**

156 For risk factors to determine human exposure, a structured questionnaire was issued to 535 willing  
157 inhabitants around the bat collection sites. Briefly, households within 5km radius of the bats  
158 collection sites and present during a visit were randomly surveyed by simple number generation  
159 without replacement. An oral consent of each respondent was obtained in advance to participate  
160 in the study. Participants ( $\geq 15$  years old) were interviewed individually to avoid communication  
161 bias during the survey. The questionnaires were structured to collect information on a range of  
162 variables including lifestyle, socio-demographic data, knowledge about rabies, bat activities and  
163 human – bat interactions.

## 164 **Statistics**

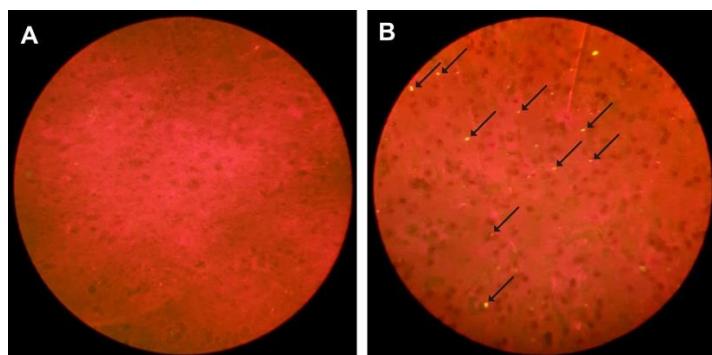
165 Data obtained in the study was entered into Microsoft Excel (Microsoft, PC/windows XP, 2010,  
166 Redmond WA, USA) for descriptive statistics and transferred to IBM® Statistical Package for  
167 Social Sciences Software (SPSS Inc., Chicago IL, USA) version 21 for further analysis. Variables  
168 of interest were compared between exposed and non-exposed persons using odds ratios (OR) with  
169 95% confidence intervals (CI) and p-values were calculated with the chi square test or Fisher's  
170 exact test where appropriate [35]. Exposure to bat Lyssavirus was defined as having been bitten  
171 by a bat, scratched by a bat or touched a bat with bare hands [36, 35] as well as having hunted  
172 bats, prepared and / or consumed bat meat. The presence of bat in living space (home, roofs and  
173 trees in vicinity of home) and / or contact with bat wastes (body fluid, faeces, urine) was also  
174 considered as risk exposure to bats.

## 175 Results

### 176 Prevalence of rabies in bats

177 Between February to May 2017, brain tissues samples were taken from 212 bats belonging to 3  
178 families (Vespertilionidae, Molossidae, Pteropodidae), including 5 species (*Chaerephon chapini*,  
179 *Chaerephon leucogaster*, *Chaerephon pumilus*, *Eidolon helvum*, *Scotophilus leucogaster*). The  
180 tissue samples were tested using direct Immunofluorescence Assay (IFA) to detect the Lyssavirus  
181 ribo-nucleoprotein complex (Fig. 2 and Table 1).

182 Out of 212 (26.89% (95% CI: 21.37 – 33.23) bat brain samples, 57 tested positive for Lyssavirus  
183 antigen using direct Immunofluorescence Assay (IFA) (Table 1).



184  
185 **Fig 2. Detection of Rabies' ribo-nucleoprotein complex using Direct Immunofluorescence**  
186 **Assay.** Negative (A) and Positive (B) brain samples were fixed, analysed for the presence of the  
187 Rabies virus antigen and visualized under fluorescence microscope (100X objective). Arrows  
188 indicate viral ribo-nucleoprotein complex. Evans blue was added to the conjugate as a  
189 counterstain, which turned the tissue noticeably red to denote the green fluorescence.

190  
191 The prevalence of Lyssavirus in bats in North Cameroon according to endogenous and exogenous  
192 factors is detailed in Table 1. The positivity rate was significantly higher in adult compared to  
193 young bats (p-value= 0.03002). The Lyssavirus prevalence in this study was not associated with  
194 bat families (p-value= 0.9130), bat species (p-value = 0.9625), sex (p-value =0.4620), body  
195 condition score (p-value =3002), trophic diet (p-value =0.8549) and collection sites (p-value

196 =0.6020). There was at least one positive case of Lyssavirus in each of the bat families, each bat  
197 species tested and each collection site suggesting that the virus is circulating in bats in the region.

198

199 **Table 1. Prevalence of Lyssavirus in bats by Immunofluorescence Assay in North Cameroon**  
200 **according to endogenous and exogenous factors**

Parameter	Variable	N	N+	Prevalence (95% CI)	P-value (X <sup>2</sup> value)
Families	<i>Vespertilionidae</i>	20	6	30 (14.55 – 51.90)	0.9130 (0.1820)
	<i>Molossidae</i>	116	30	25.86 (18.76 – 34.51)	
	<i>Pteropodidae</i>	76	21	27.63 (18.84 – 38.57)	
Species	<i>Chaerephon chapini</i>	10	2	20 (5.67 – 50.98)	0.9625 (0.604)
	<i>Chaerephon leucogaster</i>	10	2	20 (5.67 – 50.98)	
	<i>Chaerephon pumilus</i>	96	26	27.08 (19.20 – 36.72)	
	<i>Eidolon helvum</i>	76	21	27.63 (18.84 – 38.57)	
	<i>Scotophilus leucogaster</i>	20	6	30 (14.55 – 51.90)	
Sex	Male	88	26	29.54 (21.03 – 39.78)	0.4620 (0.54099)
	Female	124	31	25.00 (18.21 – 33.29)	
Age	Adult	108	36	33.33 (25.15 – 42.66)	0.03098* (4.654)
	Juvenile	104	21	20.19 (13.60 – 28.90)	
Body condition score	1 (good)	104	32	30.77 (22.72 – 40.19)	0.3002 (2.4065)
	2 (medium)	105	25	23.81 (16.68 – 32.79)	
	3 (thin)	3	0	0	
Trophic diet	Frugivorous	76	21	27.63 (18.84 – 38.57)	0.8549 (0.0334)
	Insectivores	136	36	26.47 (19.78 – 34.46)	
Samples collection site	Babla (church)	20	5	25 (11.19 – 46.87)	0.6020 (4.5550)
	Djalingo (church)	45	9	20 (10.90 – 33.82)	
	Guider/Mayo Oulo	6	3	50 (18.76 – 81.24)	
	Lagdo (Integrated Health centre of Plateau)	55	17	30.82 (18.68 – 43.11)	
	Yelwa (primary school)	16	3	18.75 (6.59 – 43.01)	
	Zoological garden of Garoua	70	20	28.57 (17.98 – 39.15)	
	Global prevalence			26.89 (21.37 – 33.23)	

201 \* : P < 0,05 ; N: Number of samples tested, N+: Number of positive samples; () : Chi-2 value, IFA :

202 Immunofluorescence Assay;

203

## 204 **Risk factors to human health of rabies**

205 There were 535 volunteers of different religions, genders, and educational backgrounds that were  
206 interviewed. Data on a range of variables, including lifestyle, socio-demographics, and  
207 knowledge of rabies, bat activities, and human-bat interactions were collected.

208

209 The study showed more respondents were aware of canine rabies (74.57% (95%CI: 70.72 –  
 210 78.09)) than of bat rabies related viruses (4.67% (95%CI: 3.18 – 6.80)) and their zoonotic risks  
 211 (Table 2). The proportion of respondents with knowledge of rabies was significantly influenced  
 212 (P<0.05) by gender and level of education for canine rabies compared to level of education for bat  
 213 rabies related virus. Overall, more male than female respondents showed higher (P<0.05) levels  
 214 of awareness of canine rabies. Additionally, more literate respondents showed higher (P<0.05)  
 215 levels of awareness of bat rabies than illiterate respondents (Table 2).

216 **Table 2. Level of canine and bat rabies awareness of respondents according to socio-  
 217 demographic characteristics in the Northern Region of Cameroon (n=535).**

Parameter / variable	N	Canine Rabies			Bat Lyassavirus		
		Yes (n)	Proportion (% [CI 95%])	P-value (X <sup>2</sup> )	Yes (n)	Proportion (% [CI 95%])	P-value (X <sup>2</sup> )
<b>Localities</b>							
Babla	87	63	72.41 (62.22 – 72.41)	0.85 (2.63)	6	6.90 (3.20 – 14.24)	0.69 (3.92)
Djalingo	62	46	74.19 (62.12 – 83.44)		4	6.45 (2.54 – 15.45)	
Garoua II	70	53	75.71 (64.50 – 84.24)		2	2.86 (0.79 – 9.84)	
Guider	107	77	71.96 (62.80 – 79.60)		6	5.61 (2.60 – 11.70)	
Lagdo	70	55	78.57 (67.61 – 86.56)		1	1.43 (0.25 – 7.66)	
Mayo Oulo	78	56	71.79 (60.96 – 80.57)		3	3.85 (1.32 – 10.71)	
Yelwa	61	49	80.33 (68.69 – 88.37)		3	4.92 (1.69 – 13.49)	
<b>Age (years)</b>							
[15 – 25]	203	138	67.98 (61.28 – 74.01)	0.07 (8.70)	7	3.45 (1.68 – 6.95)	0.42 (3.88)
[26 – 35]	134	102	76.12 (68.24 – 82.55)		8	5.97 (3.06 – 11.34)	
[36 – 45]	92	74	80.43 (71.17 – 87.25)		5	5.43 (2.34 – 12.09)	
[46 – 55]	48	37	77.08 (63.46 – 86.69)		4	8.33 (3.29 – 19.55)	
[56 – 65]	58	48	82.76 (71.09 – 90.36)		1	1.72 (0.30 – 9.13)	
<b>Religion</b>							
Christians	348	264	75.86 (71.10 – 80.06)	0.59 (1.05)	21	6.03 (3.98 – 9.04)	0.05 (5.89)
Muslims	158	115	72.78 (65.36 – 79.12)		2	1.27 (0.35 – 4.50)	
Without religion	29	20	68.97 (50.77 – 82.73)		2	6.90 (1.91 – 21.97)	
<b>Gender</b>							
Female	209	146	69.86 (63.33 – 75.68)	0.045* (4.04)	6	2.87 (1.32 – 6.12)	0.11 (2.50)
Male	326	253	77.61 (72.78 – 81.80)		19	5.83 (3.76 – 8.92)	
<b>Educational level</b>							
None	136	91	66.91 (58.63 – 74.26)	<0.01* (12.74)	2	1.47 (0.40 – 5.20)	<0.001* (17.65)
Primary	151	111	73.51 (65.95 – 79.90)		3	1.99 (0.60 – 5.68)	
Secondary	218	168	77.06 (71.04 – 82.14)		15	6.88 (4.21 – 11.04)	
Tertiary	30	29	96.67 (83.33 – 99.41)		5	16.67 (7.34 – 33.57)	
<b>Total</b>	<b>535</b>	<b>399</b>	<b>74.57 (70.72 – 78.09)</b>		<b>25</b>	<b>4.67 (3.18 – 6.80)</b>	

218 N: number of participants, Yes: number of respondents who are aware of canine and bat  
 219 rabies; \*: p<0.05; (): value of Khi-2.

220  
 221 Respondents who were aware of canine rabies cited dogs (73.64%) and cats (19.81%) as the  
 222 principal sources of rabies infection in humans (63.73%) and other animals. The cited modes of

223 transmission were through bites (92.14%) and scratches (4%) of rabid animals as well as food  
 224 (4%) and water (1.20%) contaminated by fluid and wastes from rabid animals. Goats (10.65%),  
 225 cattle (7.85%), horses (7.1%), sheep (6.91%), pigs (5.42), fowl (4.48%), donkeys (0.25%),  
 226 monkeys (0.25%), gorillas (0.25%) and mice (0.002) were also listed as susceptible animals and  
 227 sources of rabies for humans and other animals (dogs, cats, cattle, sheep, goat, horse, donkey etc.)

228 Gender, educational level, religion and ethnicity of respondents significantly influenced (P<0.05)  
 229 the level of exposure to bats and bat products in the Northern Region of Cameroon (Table 3).  
 230 Similar to the level of rabies awareness among the respondents, more male and literate respondents  
 231 and Christian respondents showed more (P<0.05) interaction with bats and bat products than  
 232 female, illiterate and non-Christian respondents. The highest level of exposure was reported  
 233 among the Matal community (83.33%; 95% CI: 55-19 – 95.30) while the lowest was reported  
 234 among the Peulh community (18.18%; 95% CI: 7.31 – 38.51)) (Table 3).

235 **Table 3: Risk factors associated with human exposure to bats and bat products in the North  
 236 Region of Cameroon (n=535)**

Parameter / variable	Total (N)	Exposed (n)	Proportion [(% 95%CI)]	P ( $\chi^2$ )	OR (95% CI)
<b>Gender</b>					
Male	326	183	56.13 (50.70 - 61.41)	<0.001*	2.71 (1.88 – 3.90)
Female	209	67	32.06 (26.11 - 38.66)	(29.66)	1
<b>Age (years)</b>					
[15 - 25]	203	103	50.74 (43.91 - 57.54)		1.50 (0.94 – 2.47)
[26 - 35]	134	62	46.26 (38.05 – 54.70)		1.28 (0.74 – 2.19)
Above 56	58	27	46.55 (34.33 – 59.20)		1.29 (0.67 – 2.51)
[46 - 55]	48	21	43.75 (30.70 – 57.72)		1.15 (0.57 – 2.34)
[36 - 45]	92	37	40.22 (30.79 – 50.44)		1
<b>Educational level</b>					
Secondary	218	118	54.13 (47.50 – 60.62)		1.97 (1.27 – 3.05)
Tertiary	30	15	50.00 (33.15 – 66.85)	0.02*	1.67 (0.75 – 3.69)
Primary	151	66	43.71 (36.05 – 51.68)	(10.13)	1.29 (0.81 – 2.08)
None	136	51	37.50 (29.81 – 45.87)		1
<b>Religion</b>					
Christianity	348	194	55.75 (50.50 – 60.88)	<0.001*	2.97 (1.99 – 4.44)
Atheists	29	9	31.03 (17.27 – 49.23)	(32.54)	1.06 (0.45 – 2.51)
Islam	158	47	29.75 (23.88 – 37.62)		1
<b>Ethnic groups</b>					
Matal	12	10	83.33 (55-19 – 95.30)		22.5 (3.48 – 145.28)
Massa	57	35	61.40 (48.43 – 72.93)		7.16 (2.14 – 23.95)
Toupouri	56	34	60.71 (47.63 – 72.42)	<0.001*	6.95 (2.07 – 23.30)
Kapsiki	33	19	57.58 (40.81 – 72.77)	(36.46)	6.11 (1.69 – 22.07)
Mousgoum	48	26	54.17 (40.29 – 67.43)		5.32 (1.56 – 18.07)

Moundang	31	15	48.39 (31.97 – 65.16)	4.22 (1.16 – 15.36) 3.94 (1.15 – 13.49) 3.94 (1.07 – 14.44) 3.15 (0.74 – 13.45) 2.84 (0.88 – 9.20) 2.47 (0.72 – 8.48) 2.03 (0.58 – 7.12) 1.69 (0.30 – 9.36) 1
Guiziga	45	21	46.67 (32.94 – 61.97)	
Guidar	30	14	46.67(30.24 – 63.86)	
Mafa	17	7	41.18 (21.61 – 64.00)	
Others	80	31	38.75 (28.26 – 50.33)	
Fali	48	17	35.42 (23.44 – 49.56)	
Daba	45	14	31.11 (19.53 – 45.66)	
Bainawa	11	3	27.27 (7.33 – 56.56)	
Peulh	22	4	18.18 (7.31 – 38.51)	

237 N: number of participants, \*: p<0.05;  $\chi^2$ : value of Khi-2; N: number of responders per specific  
238 question.

239  
240 Though the respondents who were aware of canine rabies were significantly (p<0.05) more  
241 exposed to bat Lyssavirus than those who were less aware of the disease, there was no difference  
242 (P>0.05) in terms of exposure to bat Lyssavirus between respondents who were aware of zoonotic  
243 rabies and, in particular, bat rabies (Table 4). The presence of bats within a locality and homes,  
244 bat consumption, bat hunting and other interactions with bats significantly (P<0.05) exposed  
245 respondents to zoonotic rabies. The proportion of exposed respondents varied significantly  
246 (p<0.05) according to locality.

247 Additionally, respondents with bats in their homes (57.27%) also reported bats in different places  
248 including in the roofs of bedrooms (24.1%), trees (14.2%), abandoned buildings (2.2%) and  
249 warehouses (2.1%). The following tools were mainly used for bat hunting: sticks (66%), chemical  
250 products (15.33%), bare hands (9.33%) and other tools (9.33%) include nets, sling, thorns and  
251 clothes. The reasons for hunting the bats were due to their nuisance (69.04%), for consumption  
252 (12.5%), playing with bats (10.12%), sociocultural practice (2.97%), leisure (3.57%) and research  
253 (1.78%). Some of the respondents (17.60%) reported being bitten and scratched by bats when  
254 hunting the animals, however, none of them were vaccinated against rabies and none sought rabies  
255 post exposure prophylactic measures.

256

257

258

259 **Table 4:** Risks associated to exposure to bat rabies among respondents in the Northern Region of  
260 Cameroon

Parameter / variable	Total N	Exposed (n)	Proportion % (95%CI)	P-value ( $\chi^2$ )	OR (95%CI)
<b>Awareness of canine rabies</b>					
Yes	399	198	49.62 (44.74 – 54.51)	0.02* (5.28)	1.59 (1.07 – 2.37)
No	136	52	38.24 (30.50 – 46.62)		1
<b>Aware about risk of zoonotic rabies</b>					
No	56	28	50.00 (37.33 – 62.67)	0.95 (0.004)	1.02 (0.58 – 1.79)
Yes	341	169	49.56 (44.29 – 54.99)		1
<b>Aware about risk of bat rabies</b>					
Yes	25	16	64.00 (44.52 – 79.75)	0.16 (1.98)	1.82 (0.78 – 4.21)
No	372	184	49.46 (44.41 – 54.52)		1
<b>Presence of bats in the locality</b>					
Yes	490	240	48.98 (44.58 – 53.40)	<0.001* (11.85)	3.36 (1.63 – 6.94)
No	45	10	22.22 (12.54 – 36.27)		1
<b>Presence of bats in home</b>					
Yes	227	129	56.83 (50.33 - 63.11)	<0.001* (16.16)	2.03 (1.43 – 2.88)
No	308	121	39.29 (34.00 – 44.84)		1
<b>Bat hunting</b>					
Yes	150	107	71.33 (63.63 – 77.96)	<0.001* (50.69)	4.21 (2.79 - 6.34)
No	385	143	37.14 (32.46 – 42.20)		1
<b>Bat consumption</b>					
Yes	53	53	100 (93.24 – 100)	<0.001 (67.06)	$\infty$
No	482	197	40.87 (36.57 – 45.31)		1
<b>Tools for bat hunting</b>					
Hands	14	14	100 (78.24 – 100)	0.07 (7.05)	$\infty$
Stick	99	67	67.67 (57.96 – 76.08)		1.12 (0.43 – 2.90)
Others**	14	11	78.57 (52.41 – 92.43)		1.96 (0.42 – 9.11)
Chemicals	23	15	65.21 (44.89 – 81.19)		1
<b>Localities</b>					
Djalingo	62	40	64.52 (52.09 – 75.26)	<0.001 (47.74)	7.42 (3.28 – 16.82)
Lagdo	70	44	62.86 (51.15 – 73.23)		6.91 (3.12 – 15.32)
Babla	87	51	58.62 (48.12 – 68.39)		5.78 (2.70 – 12.39)
Garoua II	70	35	50.00 (38.60 – 61.40)		4.08 (1.86 – 8.96)
Mayo Oulo	77	33	42.86 (32.41 – 53.99)		3.06 (1.41 – 6.65)
Guider	108	35	32.41 (24.32 – 41.71)		1.96 (0.93 – 4.14)
Yelwa	61	12	19.67 (11.63 – 31.31)		1

261 \*\* includes the net, sling, clothes, branch of thorn. OR: Odds ratio; CI: confidence interval; \*: p

262 <0.05. N: number of answers to specific question

## 263 Discussion

264 The present study reports the first serological evidence of Lyssavirus in bats in Cameroon and  
265 revealed rabies antigens in bats that appeared healthy. This observation underlines a potential risk  
266 for human communities living in contact with bats.

267 The prevalence of Lyssavirus reported in the present study is lower than the serological prevalence  
268 reported in other studies. A prevalence of 59.3% was reported in bat colonies in Spain [37], a  
269 prevalence of 50.34% of the virus in vampires bats (*Desmodus rotundus*) in Brazil [38]; and in  
270 Kenya a prevalence 40 to 67% was reported in colonies of *Eidolon helvum* and 29 to 46% in  
271 *Rousettus aegyptiacus* [17].

272 The findings of the present study are similar to those observed in moribund vampire bats  
273 (*Desmodus rotundus*) (27.6%) tested by IFA and PCR in northern Brazil [39]. Active infection  
274 has been reported in apparently healthy (0.1%) and moribund bats in Kenya (9%) [17, 40]. The  
275 prevalence of Lyssavirus in this study is also higher than values reported among bat species in the  
276 United States (9% to 10%) [40] Canada (7%) [36] Spain (3.3 to 10%) [38], Nigeria (19%) [19]  
277 and Kenya (9%) [17]. The difference in infection rates could be associated with the method of  
278 collection and the period of sample collection, which was conducted during the dry season. This  
279 period is characterized by food scarcity and elevated temperatures as well as a peak of rabies  
280 infection in bat populations in the study sites. The influence of the season on the occurrence of  
281 rabies in bats such as higher rates during the dry season [41] and fluctuating and cyclical rabies  
282 like infection in bat populations [40] were reported in other studies. The immigration of rabid bats  
283 into a colony can also cause a rapid spread of the infection through bites of infected bats during  
284 mutual grooming, mating and aggressive behaviour such as protection of the territory [42].  
285 Unfavourable living conditions (climate, food source, immunity, density of the colony, parasite  
286 load among others), sex, age and physical depletion caused by stress, co-infections, sexual  
287 exhaustion and migration are also major risk factors for rabies infection [10, 17, 38, 40]. However,

288 aggressive behaviour has been associated with a higher prevalence of rabies. Salmon-Mulanovich  
289 et al [43] reported a higher prevalence of rabies in male bats with more aggressive behaviour such  
290 as territory protection, defence against intrusion, fighting, and licking of body fluids during the  
291 breeding season. There was also a high or similar prevalence of Lyssavirus in female bats  
292 compared to males, which was associated with gregarious maternity behaviour during hibernation  
293 [44]. In the present study, young bats were less likely to be infected with rabies related virus than  
294 adults and older bats. This could be due to the fact that adult and older bats exhibited usual  
295 aggressiveness and adventurous behaviour for longer periods of time in the colonies interacting  
296 (continuous or intermittent) with other congeners in the same and other colonies (some of which  
297 may be infected) compared to young and juvenile bats. . In addition, serological prevalence can  
298 be influenced by antibodies of maternal origin and confer lasting immunity (6 to 8 weeks) to their  
299 offspring through placental and mammary routes [40], which could provide further protection to  
300 the young and juvenile bats.

301 Overall, the findings of this study agree with those of Salmon – Mulanovich *et al.* [43] in Peru,  
302 Kuzmin *et al.* [17] in Kenya and Dzikwi *et al.* [19] in Nigeria who reported that the location of  
303 the bats does not influence rabies infection rates in bats. This is in contrast to the findings of Costa  
304 *et al.* [45] who observed significant variation of bat Lyssavirus rates according to location. This  
305 variation could be associated to sampling biases such as disparities in distances between location  
306 sites of bat populations and the number of bats sampled at the different study sites [45]. Given the  
307 vast spatio-temporal spread of the activities of bat colonies, bat populations in close locations (less  
308 than 100km apart) will have common features since they frequently mix, interact together and  
309 could be considered as the same colony in this study.

310 The zoonotic transmission of bat Lyssavirus is well documented. Bat rabies can be transmitted to  
311 humans and other mammals over long periods and vast geographical areas through various routes  
312 including aerosols, unapparent bites, and contamination of nerve tissue with saliva, urine and other  
313 body fluids of infected bats [2, 3, 4, 5, 6, 7]. Based on the public health perception of bat rabies

314 related virus, many respondents in rural localities in Garoua who shared the same environments  
315 with bat colonies were aware of canine and human rabies. This finding was significantly less than  
316 that observed by Bouli *et al.* [21] who reported over 88.73% awareness level of canine rabies in  
317 urban Garoua areas among respondents who were more literate and with better educational levels  
318 than in rural areas, as was the case in the present study. This is also in line with Moran *et al.* [35]  
319 who reported that over 91% of the respondents living in the rural areas harbouring bat populations  
320 in Guatemala had little or no knowledge of rabies. Costa and Fernandes [46] had also observed a  
321 strong positive correlation between educational levels and knowledge about rabies. Rural  
322 respondents with higher levels of education showed better knowledge of rabies in the present  
323 study.

324 Though the awareness about canine rabies was significantly relevant, most respondents seemed  
325 to have little knowledge of the potential zoonotic transmission of bat Lyssavirus. Irrespective of  
326 the respondents' level of knowledge of canine rabies and bat Lyssavirus, the results showed that  
327 respondents who were aware of canine rabies and had bats in their homes and localities were more  
328 involved in bat hunting, and also interacted more with bats and bat products, compared to  
329 respondents who consume bats. Bouli *et al.*, [21] reported a lower awareness level of bat rabies  
330 (0.3%) in communities of Garoua-Cameroon, while higher levels ranging from 10% – 42% were  
331 reported in Thailand and Guatemala due to widespread public awareness campaigns against rabies  
332 in these countries [35, 47]. Similar to the level of knowledge about canine rabies found in the  
333 present study, the levels of awareness about bat Lyssavirus was significantly associated with  
334 literacy and educational levels and the age of the respondents. This finding highlights the lack of  
335 knowledge of the communities regarding the potential risk of bats Lyssavirus and its transmission  
336 to other species and humans. Awah-Ndukum *et al.*, [23] reported that dogs and cats were the main  
337 source and transmitters of rabies in Cameroon, most likely due to the common and visible  
338 manifestations of the disease in these species and not in other species in the country.

339 The clinical manifestations of rabies are more furious and lethal in dogs [3] and non-lethal in bats  
340 [14]. The furious forms of rabies in dog usually constitute a canine rabies outbreak alert for human  
341 communities. Canine and human rabies is endemic in Cameroon, [21, 23]. In the present study,  
342 locality, age and educational level significantly influenced the levels of awareness of bat  
343 Lyssavirus and its zoonotic risk. Also, the presence of bats in homes, bat hunting and type of tools  
344 used for bat hunting as well as gender, religion and ethnicity of respondents played a major role  
345 on the level of human exposure to bats. Consumption of bats was frequent in the study sites, which  
346 is in agreement with Kamins *et al.* [48] who reported that bat consumption was cultural and a food  
347 habit in Ghana. This habit to hunt and consume bats as meat and source of animal protein among  
348 communities explains the difference in level of exposure observed in the present study between  
349 ethnic and religious groups. Indeed, according to the respondents in this study, Islam forbid eating  
350 some animals including bats while the other religions have not diet restriction.

351 Due to a technical problem impacting samples conservation (RNA degradation) the molecular part  
352 of this work could not be performed. It would be important to carry out an in-depth molecular  
353 study to accurately identify the type of Lyssavirus circulating in the region. This could help to  
354 understand the epidemiological pattern of Lyssavirus and identify potential reservoirs in  
355 Cameroon.

356

## 357 Conclusion

358 Rabies is a zoonotic disease of all warm-blooded animals including man. Though the disease is  
359 endemic in Cameroon, with dogs being the main source and transmitters, bat habitats are  
360 widespread in the many Cameroon environments. Bats usually form colonies in the roofs, trees  
361 and abandoned tall structures in human communities and they are hunted as a source of animal  
362 protein in the the Northern region of Cameroon. The present study has shown a 26.89% prevalence

363 of Lyssavirus. . This study is the first to report evidence of Lyssavirus in bats in Cameroon and  
364 revealed that rabies exists in apparently healthy bats and constitutes a human health problem in  
365 communities with bat habitats in the Northern region of the country. In order to isolate and  
366 determine the Lyssavirus genotype circulating in the bat population in Cameroon, future large-  
367 scale studies on bats should continue. Public awareness campaigns and health education are also  
368 essential to evaluate risk factors, develop protective measures against rabies and understand the  
369 potential role of bats as reservoirs of rabies especially in human communities where bat colonies  
370 are found. This study highlights the importance of investigating the dynamics of rabies virus  
371 transmission among animals and humans in this region and other parts of the country and to  
372 characterise the Lyssavirus type circulating among these flying mammals in Cameroon. Multi-  
373 sectorial sensitization of communities in Cameroon to improve their level of awareness on bat  
374 rabies and integration of the “One Health” approach for effective management of rabies in  
375 Cameroon should be emphasized.

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## 379 **Author's contribution**

380 **Conceptualization:** Rodrigue Simonet Poueme Namegni, Isaac Dah, Julius Awah-Ndukum

381 **Data curation:** Moctar Mouiche Mouliom Mohamed, Julius Awah-Ndukum Ranyl Nguena  
382 Guefack Noumedem, Isaac Dah,

383 **Formal analysis:** Julius Awah-Ndukum, Ranyl Nguena Guefack Noumedem, Isaac Dah,

384 **Investigation:** Isaac Dah, Laurent God-Yang, Rodrigue Simonet Poueme Namegni

385 **Methodology:** Isaac Dah, Rodrigue Simonet Poueme Namegni, Moctar Mouiche Mouliom  
386 Mohamed, Simon Dickmu Jumbo, Isabelle Conclos.

387 **Resources:** Rodrigue Simonet Poueme Namegni, Simon Dickmu Jumbo, Abel Wade.

388 **Software:** Isaac Dah, Ranyl Nguena Guefack Noumedem, Laurent God-Yang, Jean Marc  
389 Feussom Kameni

390 **Supervision:** Dorothee Missé, Liegeois Florian, Rodrigue Simonet Poueme Namegni, Abel  
391 Wade, Jean Marc Feussom Kameni, Julius Awah-Ndukum  
392 **Validation:** Liegeois Florian, Dorothee Missoe, Abel Wade, Julius Awah-Ndukum  
393 **Writing original draft preparation:** Isaac Dah, Rodrigue Simonet Poueme Namegni, Julius  
394 Awah-Ndukum  
395 **Writing - review & editing:** Isaac Dah, Rodrigue Simonet Poueme Namegni, Moctar Mouiche  
396 Mouliom Mohamed, Liegeois Florian, Abel Wade, Dorothee Missé, Julius Awah-Ndukum

397 All the authors have approved the final version of manuscript and agreed for submission

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399 The authors have declared that no competing interests exist.

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