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Rabies-specific antibodies among confined and free-roaming dogs in three Liberian cities: implications for elimination of human deaths from dog-mediated rabies by 2030

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Abstract

Introduction: *as a crucial step towards achieving zero human rabies deaths by 2030 in post-conflict Liberia, we assessed the levels and distribution of rabies-specific antibodies among different categories of domestic dogs in Buchanan, Gbarnga and Voinjama, Liberia.* ***Methods:*** *in this cross-sectional study, a semi-structured questionnaire was used to obtain relevant information on dogs and dog management practices from 758 consenting dog owners in the study area. Demographic variables were presented using descriptive statistics, and Chi-square test was used to evaluate statistical significance of findings. Values of p<0.05 were considered significant. Domestic dogs in each city were selected using multi-stage, stratified random sampling method, categorized by management system, gender, and age group. Management systems comprised dogs reared with some level of confinement (confined/semi-confined dogs) and free-roaming dogs. Blood specimens were collected, and quantitative indirect ELISA was conducted for detection of rabies-specific antibodies in dog sera. Rabies antibody titres ≥ 0.5eu/mL were considered protective.* ***Results:*** *about 42.0% of the 758 questionnaire respondents reared their dogs with some level of confinement, while only 3.4% of all respondents had vaccinated their dogs. This implied that many confined/semi-confined dogs, as well as many free-roaming dogs, in the three Liberian cities had not been vaccinated against rabies. In all, only 12.9% (n = 210) of domestic dogs tested in the three cities had rabies-specific antibody titres ≥0.5 eu/mL, of which 2.9% and 10.0% were confined/semi-confined and free-roaming, respectively. Among each of the confined/semi-confined and free-roaming groups, 12.0% (n = 50) and 13.1% (n=160) had protective levels of rabies-specific antibodies, while 10.1% (n = 89) and 14.9% (n = 121) of male and female dogs also had antibody titres ≥0.5 eu/mL. In each of the three cities, highest proportions of dogs with rabies-specific antibody levels below 0.5 eu/mL were generally found among age-groups 12-35 and >60 months, and among male dogs.* ***Conclusion:*** *the very poor anti-rabies vaccination rate observed may have largely contributed to the generally low levels of rabies-specific antibodies among domestic dogs in these Liberian cities. This is the first community-based prevalence report on rabies-specific antibody levels among dogs in Liberia. The critically low levels of rabies-specific antibodies among confined/semi-confined and free-roaming domestic dog populations in Liberia emphasize the need for more attention by national, regional, and international organizations for achievement of the global target of zero dog-mediated human rabies death by 2030. Responsible dog ownership, mass antirabies vaccination campaigns, and sustained sensitization of the public on the need for compliance with guidelines on acceptable dog management practices, including timely vaccination, are recommended.*

Introduction

Rabies continues to cause thousands of human deaths every year, with over 95% of these occurring in developing nations of Asia and Africa [1]. Of the known species in genus Lyssavirus, the classical rabies virus is the virus most frequently associated with rabies [2,3]. Dogs are the major sources of human infection, contributing up to 99.0% of all rabies transmissions to humans [1]. In West Africa, urban cycle of rabies transmission, involving humans and domestic dogs, predominates [4]. Consequently, the burden of human rabies remains high in countries in the sub-region partly because of low levels of anti-rabies immunity among domestic dog populations either as a result of poor vaccination coverage or inadequate antibody response after vaccination [5,6]. Assessment of anti-rabies immunity in West African domestic dog populations may therefore represent a very crucial step towards achievement of the "Zero by 2030" target of the global "United Against Rabies" collaboration of the World Health Organization (WHO), the World Organization for Animal Health (OIE), the Food and Agriculture Organization of the United Nations (FAO), and the Global Alliance for

Rabies Control (GARC) [1,7]. Proper dog population management has also been recommended as a useful tool for achievement of this target [8].

In many parts of Africa, dog vaccination coverage, which is generally between 30-50%, is considerably lower than the level needed for elimination and effective prevention of human rabies deaths in areas where the disease is enzootic, which is at least 70% for five to seven consecutive years [9,10]. Incidentally, because of the abundance of free-roaming dogs, dog management practices in many countries in Africa also adversely impact rabies elimination programmes. As defined by the OIE and the WHO, the recommended minimum titre of rabies neutralizing antibodies capable of conferring immunity in dog is ≥ 0.5 IU/ml [9,11]. In order to ensure elimination of human deaths due to rabies by 2030 in post-conflict, rabies-endemic Liberia, assessment of levels of anti-rabies immunity among domestic dogs of various categories is therefore very crucial. This study was designed to assess the levels of immunity to rabies among different categories of domestic dogs in three Liberian cities. Specific objectives of the study included investigation of levels of rabies-specific antibodies among dogs reared with some level of confinement (confined/semi-confined dogs) and those that were free-roaming, of different gender and age-groups, in Buchanan, Gbarnga and Voinjama, Liberia, West Africa.

Methods

Ethical approval: ethical approval for this study was provided by the Liberian Biomedical Research Institute (LIBR) under approval number EC/LIBR/014/039. Informed consent was also obtained from respondents before administration of questionnaire.

Study design: this cross-sectional study involved domestic dogs selected from dog populations in three Liberian cities. Individual dogs were randomly selected, using multi-stage stratified sampling technique. In each city, dogs were categorized based on management system, age group, and gender. In the present study, management system was categorized as confined/semi-confined (in which dog rearing involved some level of confinement) and free-roaming. A *confined dog* was identified as one which remained under owner control at all times, often within a walled compound or home, and is walked on a leash or kept under control when outside a home or an enclosed premises. A *semi-confined dog* was that which spends part of its time within the premises of a home or a walled property, but is also sometimes allowed to freely roam in the community. A *free-roaming dog* was one which was never confined to a home or walled property [8,12].

Study location and specimen collection: field work for this study was conducted in Buchanan (Latitude 5.8872°N, Longitude 10.0304°W), capital of Grand Bassa County, Gbarnga (Latitude 7.0024°N, Longitude 9.4728°W), capital of Bong County, and Voinjama (Latitude 8.4202°N, Longitude 9.7539°W), capital of Lofa County, Liberia, West Africa in July and August, 2015. These three Counties (Grand Bassa, Bong, and Lofa) are among the fifteen counties that make up the Republic of Liberia. The three cities were purposively selected based on their geographic location, being longitudinal in distribution along the vertical axis of the country (Figure 1). Moreover, each of the three cities had a major referral hospital that served their respective counties, beside the national referral clinic for dog bite victims (DBVs) located in Monrovia, the country´s capital city. A total of 210 apparently healthy dogs were sampled, comprising 89 male and 121 female dogs. Most dogs encountered were mostly indigenous breeds (204/210), but the few exotic breeds encountered were also included (6/210). The number of dogs sampled in Buchanan, Gbarnga, and Voinjama were 70 (33.3%), 68 (32.4%), and 72 (34.3%), respectively. In all, 17 (8.1%), 33 (15.7%), and 160 (76.2%) were confined, semi-confined and free-roaming, in that order. The low proportions of confined and semi-confined dogs sampled in the three cities were mainly due to the predominance

of free-roaming dogs in post-conflict Liberia, and partly because of restricted access to the few confined and semi-confined dogs. Sample size was determined as previously described [13], with 95.0% confidence interval and desired precision of 0.05. The calculated sample size was 200. The cephalic and/or saphenous veins were used for phlebotomy. With the aid of sterile needles and syringes, 3ml of blood was collected from each dog into plain sample bottles (without anticoagulants) and allowed to clot. Sera were obtained by centrifuging at 3,000 rpm, and temporarily stored at 4oC until tests were conducted. Residence of dog owners or where dogs were most frequently seen (for confined/semi-confined dogs), and locations where free-roaming dogs were captured, were geo-referenced using hand-held global positioning system (GPS) as earlier described [14].

Questionnaire survey and key informant interview: a questionnaire survey was conducted to obtain relevant information for the study in Buchanan, Gbarnga and Voinjama cities, using a semi-structured questionnaire. Street-by-street visits were also conducted by a team of trained personnel to interview key informants such as dog breeders, animal scientists and veterinarians. The questionnaire survey was conducted purposively, with dog ownership in the study locations being the key inclusion criterion. A dog owner, or a consenting member of households where dogs were found, was interviewed using the semi-structured questionnaire. Where necessary, questions were read and translated into the local language and the answers were captured by trained personnel. Through the questionnaire survey and key informant interview, information was obtained on age, gender, breed, and vaccination history of dogs currently owned, number of dogs that died in the past two years, reasons for keeping dogs, dog management system practiced, and previous mass vaccination programmes in the study areas. Respondents were asked to produce dog anti-rabies vaccination certificates as proof of vaccination. In all, 758 consenting dog owners participated in the survey.

Detection of rabies antibodies: detection and measurement of rabies-specific antibodies is carried out using either antigen-binding assays or virus-neutralization assays. Antigen-binding assays detect and quantify antibodies in serum or cerebrospinal fluid (CSF) by their ability to bind to various rabies virus antigens, and they usually involve fixing the antigen to a solid surface, such as in enzyme linked immunosorbent assay (ELISA). On the other hand, virus neutralization assays are cell-based assays that detect the functional activity of antibodies in the serum or CSF against live virus [15]. The outcome of a virus-neutralization assay is determined by ability of virus to escape neutralization and grow in cell culture. Binding assays, on the contrary, measure a different set of characteristics of the rabies-specific antibody response. Consequently, while a measure of immunity against rabies virus infection is best determined by virus neutralization test, with appropriate validation, binding assays can be used to determine the absence or presence of rabies-specific antibodies that could be used as approximation or confirmation of the virus neutralizing antibody response [16]. In the present study, quantitative indirect ELISA was conducted for detection of rabies virus anti-glycoprotein antibodies, using the Platelia™ Rabies II kit (Bio-Rad, Marnes la-Coquette) [17]. The kit included a microplate that was pre-coated with rabies glycoprotein extracted from the inactivated and purified virus membrane. The Platelia Rabies ELISA has been tested and validated to be of high sensitivity (98.6%) and specificity (99.4%) for virus neutralising antibody detection. Platelia Rabies ELISA is considered to be as sensitive and specific as the current standard reference method for rabies neutralization tests, and the method is simple, safe, rapid and can be considered a useful alternative for the rapid fluorescent focus inhibition assay [17]. The Platelia Rabies is used in this study in view of its relevance and relative advantages, and due to limited laboratory facilities and technology. Negative and positive controls were included for each test. All steps were conducted in accordance with manufacturer´s instructions, and results were

read with the aid of an ELISA Reader (IRE 96 , Saint Jean d´Illac) at wavelength of 450-620nm. The optical density (OD) values for the test specimens were compared with the OD value of positive controls, and antibody titres were obtained from a standard OD antibody titres curve. Dogs were considered to have protective levels of antibody against rabies virus if they produced antibody titres of ≥0.5 eu/Ml [9,11].

Data analysis and interpretation of results: data obtained were entered into a computer using Microsoft Excel 2007 spreadsheet, and analyzed using SPSS (IBM SPSS Statistics version 20). Demographic variables were presented using descriptive statistics, and chi-square test was used to evaluate statistical significance of findings. Values of $p<0.05$ were considered significant. Sera sample of ≥0.5 eu/mL antibody levels were considered immune to rabies virus infection [18]. The threshold value was equal to the mean of the two standard positive control OD values and corresponds to the threshold value at 0.5eu/mL. High titre value was equal to the mean of the two hyper-immune positive values (OD R4b). The quantity of rabies-specific antibodies in a sample was determined by comparing the OD of the sample to a standard curve. Sera titres were expressed as equivalent units per ml (eu/mL), which correlates well with the international units defined for sero-neutralization (IU/ml).

Results

Findings from the questionnaire survey revealed that 63.8%, 53.8%, and 56.6% of dog owners in Buchanan, Gbarnga, and Voinjama, respectively practiced free-roaming system, and more than 94.0% of dog owners in each of these cities had not vaccinated their dogs. In all, about 42.0% of the 758 questionnaire respondents reared their dogs with some level of confinement, while only 3.4% of all respondents had vaccinated their dogs. A summary of responses from the survey is shown in Table 1. The levels and distribution of rabies-specific antibodies among domestic dogs in the three Liberian cities are shown in Table 2, while Figure 2 shows these results based on their confinement status. In all, 12.9% (n = 210) of dogs tested had antibody titres of ≥0.5 eu/ml, of which 2.9% and 10.0% were confined/semi-confined and free-roaming, respectively. In Buchanan, Gbarnga, and Voinjama, 12.9% (n = 70), 11.8% (n = 68), and 13.9% (n = 72) of domestic dogs tested had protective rabies-specific antibody levels. Among the confined/semi-confined and free-roaming groups, 12.0% (n = 50) and 13.1% (n=160) had protective levels of rabies-specific antibodies, respectively, while 10.1% (n=89) and 14.9% (n=121) of male and female dogs also had antibody titres ≥ 0.5 eu/mL, in that order. In each of the three cities, highest proportions of dogs with rabies-specific antibody levels below 0.5 eu/mL were generally found among age-groups 12-35 and >60 months, and among male dogs. Results on levels of rabies-specific antibodies based on age and gender are shown in Table 3, which revealed that 14.9% (n = 121) of female dogs and 10.1% (n = 89) of male dogs had protective antibody levels.

Discussion

Findings from this study revealed the levels and distribution of rabies-specific antibodies among different categories of domestic dogs in Buchanan, Gbarnga and Voinjama, Liberia. To the best of our knowledge, this is the first community-based report on rabies-specific antibody levels among domestic dogs in Liberia. In all, 12.9% of dogs tested across the three cities had protective antibody levels. While there are no previous studies on levels of anti-rabies immunity among dogs in Liberia, a similar community-based report in Ilorin, Nigeria indicated that 42.6% of domestic dogs had protective levels of rabies-specific antibodies [19]. Findings from the present study therefore indicated much lower immunity coverage among dog populations in Buchanan, Gbarnga and Voinjama, Liberia (12.9%, 11.8%, and 13.9%, respectively), in comparison to the 70.0% recommended coverage for achievement of herd immunity in such high-risk areas [9,11]. Findings from this study also revealed

that 12.0% of confined/semi-confined dogs and 13.1% of free-roaming dogs tested had protective levels of rabies-specific antibodies, with the levels being consistently higher in the free-roaming group in each of the three cities. The relatively lower number of confined/semi-confined dogs tested (which actually corresponded to their relatively fewer populations in the study area) may have had some effect on these results. Future studies on rabies antibody levels in domestic dogs in Liberia should involve larger sample sizes for all categories of dogs tested, with inclusion of more cities in other counties in the country. Nonetheless, results from this pioneering study have shown that rearing of dogs under full or partial confinement may not necessarily translate into adequate antirabies vaccination of such dogs, and many confined/semi-confined dogs during the study were likely unvaccinated. This view is supported by results obtained from the questionnaire survey, which revealed that while 41.8% of respondents in the three cities reared their dogs under some level of confinement (that is, confined/semi-confined), only 3.4% of them had vaccinated their dogs. This implied that many confined/semi-confined dogs, as well as many free-roaming dogs, in the three Liberian cities had not been vaccinated against rabies.

In addition, some previous reports have suggested that nonlethal rabies exposure of healthy, unvaccinated individuals (including humans, bats and dogs) could occur, leading to the development of rabies-specific antibodies [16,20-23]. Such nonlethal rabies exposure was considered to be the result of one (or more) of four alternative courses of rabies exposure. These alternative courses are subclinical infection, a carrier state, recovery from clinical infection, and an extended latent period, with subclinical infection thought to be the most likely alternative course of rabies exposure that could lead to development of rabies-specific antibodies in dogs under natural conditions [23]. This possibility, of acquiring some levels of rabies-specific immunity through subclinical infection, and the inferred low vaccination rates among confined/semi-confined dogs, could therefore partly explain the relatively higher number of dogs with protective levels of rabies-specific antibodies among the free-roaming population in Liberia.

Nonetheless, due to the large populations of unvaccinated free-roaming dogs in these Liberian cities, with only few having protective levels of rabies-specific antibodies (13.1%), attainment of herd immunity among Liberian domestic dog populations for the purpose of rabies control would be practically impossible without well-organized and properly executed mass vaccination programmes. This is because dog vaccination coverage of at least 70% for five to seven consecutive years is recommended for achievement of herd immunity for breaking rabies transmission cycle in high-risk areas, control of canine rabies, and elimination of dog-mediated human rabies death by 2030 [9,10,24-26]. Furthermore, the number of free-roaming dogs (which are mostly unvaccinated in many resource-limited settings) in a population where rabies is enzootic, such as Liberia, is essentially associated with rabies transmission, and large populations of free-roaming dogs could clog the wheels of rabies control programmes [8]. These findings therefore highlight the need for responsible dog ownership and enforcement of regulations on dog management, including vaccination. The first antirabies vaccine for dogs is recommended to be administered not earlier than three months, followed by annual booster doses [27]. During this study, the low proportions of dogs with protective levels of rabies-specific antibodies among all age groups (with dogs <12 months generally having the highest proportions) suggest that at best, majority of the few vaccinated dogs in these Liberian cities only received the primary dose of antirabies vaccine, indicating poor compliance with extant recommendations on antirabies vaccination [28]. In post-conflict Liberia, where veterinary personnel and infrastructures were very few, and adherence to relevant regulations was poor [29,30], vaccine failure may also have occurred due

to poor injection techniques and/or storage conditions [31].

Analysis of responses obtained from key informant interview also revealed that the few dogs with protective levels of rabies-specific antibodies mostly received vaccination during some humanitarian mass vaccination exercises conducted by Veterinarians Without Borders, Samaritan Purse, or by the Centre for Control and Prevention of Zoonoses (CCPZ), University of Ibadan, Nigeria, in collaboration with the Ministry of Agriculture of Liberia, during outreach programmes in the country. As observed in the present study, the critically low anti-rabies immunity among Liberian dog populations could be due to the humanitarian crisis following the prolonged civil war in Liberia [28-30]. In post-civil war Liberia, human health services were at an all-time low, while structures and personnel for animal healthcare were virtually non-existent. Amelioration of the persistent humanitarian situation of rabies endemicity and generally low immunity against rabies among domestic dogs in the country may therefore require more regional and international assistance. In order to build manpower for the much-needed, and recommended, One Health approach for dealing with these challenges, the manpower for human-animal disease surveillance and control in the country must be developed. Thus, in addition to the efforts mustered by the organizations previously listed, and those being mobilized by other organizations such as the Global Alliance for Rabies Control (GARC) (through the Stepwise Approach towards Rabies Elimination, SARE), the critical humanitarian gaps which exist in post-conflict Liberia necessitate more attention from local, regional, and international organizations for achievement of the global target to eliminate dog-mediated human rabies deaths by 2030 (which is abbreviated as “Zero by 30”) [1,7,29,30].

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Conclusion

This study revealed that the levels of rabies-specific antibodies among different categories of domestic dogs in Buchanan, Gbharnga and Voinjama, Liberia were generally very low, indicating poor population immunity against rabies among domestic dogs in these rabies-endemic areas. The very poor compliance, of dog owners in these cities, with guidelines on anti-rabies vaccination of domestic dogs may have largely contributed to the generally low levels of rabies-specific antibodies among domestic dogs in these cities. This is the first community-based prevalence report on rabies-specific antibody levels among dogs in Liberia. The critically low levels of rabies-specific antibodies among confined/semi-confined and free-roaming domestic dog populations in Liberia emphasize the need for more attention by national, regional, and international organizations for achievement of the global target of zero dog-mediated human rabies death by 2030. Responsible dog ownership, mass antirabies vaccination campaigns, and sustained sensitization of the public on the need for compliance with guidelines on acceptable dog management practices, including timely vaccination, are recommended

What is known about this topic

- *Rabies is a vaccine-preventable disease that is almost always fatal following the onset of clinical symptoms;*
- *In many developing countries, including those in West Africa, domestic dogs are important for transmission of rabies;*
- *Prevention of rabies in dogs is an effective strategy for preventing rabies in humans.*

What this study adds

- *The first community-based report on levels of rabies-specific antibody among domestic dogs in Liberia, West Africa;*

- *Generally low levels of rabies-specific antibodies among different categories of domestic dogs in three selected Liberian cities, indicating poor compliance with regulations on antirabies vaccination;*
- *High population of rabies-susceptible free-roaming dogs encountered may clog the wheels of rabies control programmes in rabies-endemic, post-conflict Liberia.*

Competing interests

The authors declare no competing interests.

Authors' contributions

NDJ and BOO conceptualized the study; OOI and BOO supervised the study; NDJ, OOI and BOO designed the study; NDJ and BOO were involved in field investigation; NDJ, OAA and BOO participated in laboratory analysis; NDJ and BOO analyzed the data; NDJ, OAA, OOI and BOO wrote and revised the first draft of the manuscript; OAA and BOO revised the manuscript. All authors read and approved the final version of the manuscript.

Tables and figures

Table 1: summary of findings from questionnaire survey

Table 2: levels and distribution of rabies antibodies among domestic dogs in Buchanan, Gbarnga, and Voinjama, Liberia based on dog management practices

Table 3: levels and distribution of rabies antibodies among domestic dogs in Buchanan, Gbarnga, and Voinjama, Liberia based on age and gender

Figure 1: map of the study areas showing Buchanan (Grand Bassa County), Gbarnga (Bong County) and Voinjama (Lofa County), Liberia

Figure 2: bar chart showing the distribution of dogs based on confinement status

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Table 1: summary of findings from questionnaire survey

Questionnaire item	**Number of respondents***			
	Buchanan (n=265)	**Gbarnga (n=251)**	**Voinjama (n=242)**	**Total (n=758)**
Purpose for keeping dogs				
Security	159	212	119	490 (64.6)
Hunting	2	9	20	31 (4.1)
Companion	3	20	9	32 (4.2)
Breeding	0	0	2	2 (0.3)
Consumption	0	0	1	1 (0.1)
Sale	0	0	0	0 (0.0)
Prestige	0	0	0	0 (0.0)
Multi-purpose	101	10	91	202 (26.6)
Management system				
Confined	16 (6.0)	57 (22.7)	15 (6.2)	88 (11.6)
Semi-confined	80 (30.2)	59 (23.5)	90 (37.2)	229 (30.2)
Free-roaming	169 (63.8)	135 (53.8)	137 (56.6)	441 (58.2)
Dog vaccination status				
Vaccinated	15 (5.7)	9 (3.6)	2 (0.8)	26 (3.4)
Unvaccinated	250 (94.3)	242 (96.4)	240 (99.2)	732 (96.6)
*Numbers in parentheses represent percentages				

Table 2: levels and distribution of rabies antibodies among domestic dogs in Buchanan, Gbarnga, and Voinjama, Liberia based on dog management practices

City	Confined/semi-confined			Free-roaming			Total		
	Total number of dogs	Dogs with protective antibody Levels		Total number of dogs	Dogs with protective antibody levels		Total number of dogs	Dogs with protective antibody levels	
		Number (%)	Rabies antibody titre (mean ± SD,eu/ml)		Number (%)	Rabies antibody title (mean ± SD, eu/ml)		Number (%)	Rabies antibody title (mean ±SD, eu/ml)
Buchanan	18	2 (11.1)	0.5 ±0.0	52	7 (13.5)	0.6 ±0.1	70	9 (12.9)	0.6 ±0.1
Gbarnga	9	1 (11.1)	0.5 ±0.0	59	7 (11.9)	0.5± 0.0	68	8 (11.8)	0.5 ±0.0
Voinjama	23	3 (13.0)	1.3 ±0.5	49	7 (14.3)	0.7±0.2	72	10 (13.9)	0.9 ±0.5
Total	50	6 (12.0)	0.9 ±0.5	160	21 (13.1)	0.6±0.2	210	27 (12.9)	0.7 ±0.5

Table 3: levels and distribution of rabies antibodies among domestic dogs in Buchanan, Gbarnga, and Voinjama, Liberia based on age and gender

City	Age groups (months)	Total number of Dogs	Female dogs			Male dogs			Total number of dogs with antibody levels ≥ 0.5 eu/mL (%)
			Number of female Dogs	Antibody title of Female Dogs (mean ± SD), eu/mL	Number of female dogs with antibody levels ≥ 0.5 eu/mL (%)	Number of male dogs	Antibody title of male dogs (mean ± SD), eu/mL	Number of male dogs with antibody levels ≥ 0.5 eu/mL (%)	
Buchanan	<12	16	8	0.40±0.89	3 (37.5)	8	0.00±0.00	1 (12.5)	4 (1.9)
	12-35	14	8	0.00±0.00	0 (0.0)	6	0.00±0.00	0 (0.0)	0 (0.0)
	36-60	27	15	0.29±0.73	2 (13.3)	12	0.00±0.00	1 (8.3)	3 (1.4)
	>60	13	5	0.00±0.00	0 (0.0)	8	0.29±0.76	2 (25.0)	2 (1.0)
Gbarnga	<12	18	14	1.11±0.33	2 (14.3)	4	1.00±0.00	0 (0.0)	2 (1.0)
	12-35	17	9	1.00±0.00	0 (0.0)	8	1.00±0.00	0 (0.0)	0 (0.0)
	36-60	20	11	1.29±0.49	3 (27.3)	9	1.13±0.35	1 (11.1)	4 (1.9)
	>60	13	7	1.17±0.41	1 (14.3)	6	1.00±0.00	1 (16.7)	2 (1.0)
Voinjama	<12	23	12	1.25±0.45	3 (25.0)	11	1.09±0.30	1 (9.1)	4 (1.9)
	12-35	23	16	1.07±0.26	2 (12.5)	7	1.00±0.00	0 (0.0)	2 (1.0)
	36-60	19	9	1.11±0.33	1 (11.1)	10	1.20±0.42	2 (20.0)	3 (1.4)
	>60	7	7	1.14±0.38	1 (14.3)	0		0	1 (0.5)
Total		210	121		18 (14.9)	89		9 (10.1)	27 (12.9)

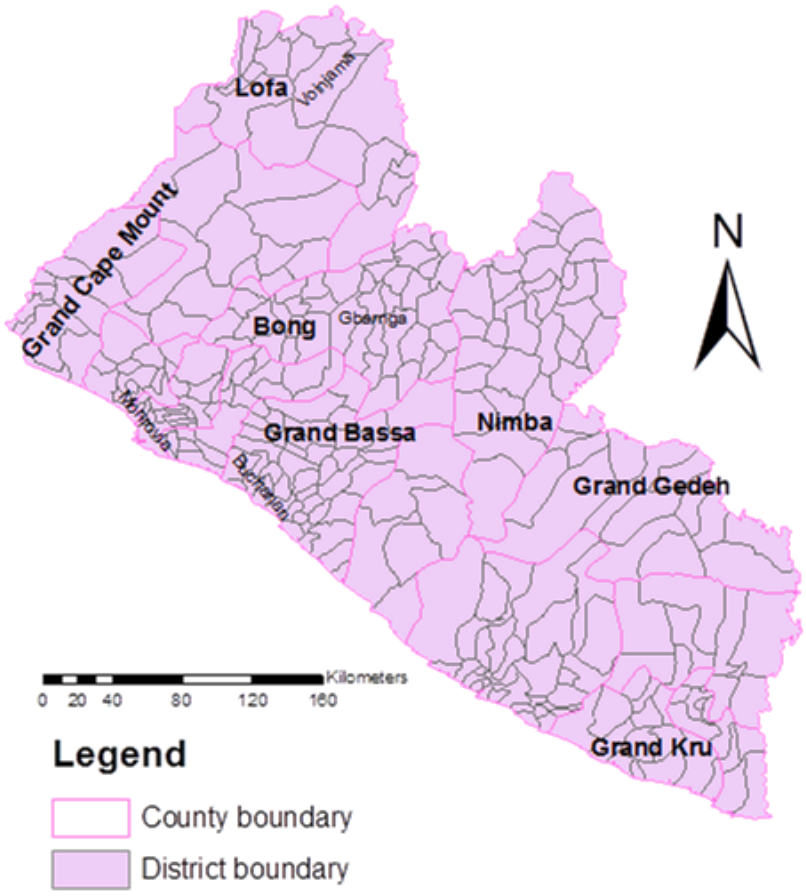

Figure 1: map of the study areas showing Buchanan (Grand Bassa County), Gbarnga (Bong County) and Voinjama (Lofa County), Liberia

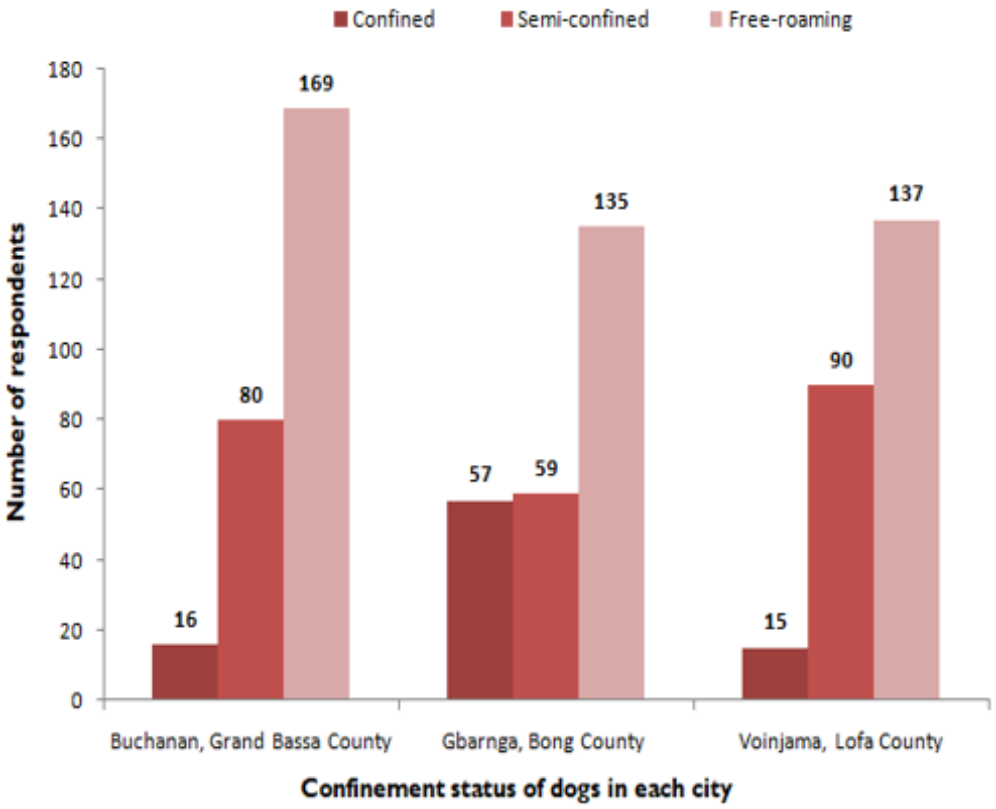

Figure 2: bar chart showing the distribution of dogs based on confinement status