Seroprevalence and predisposing factors of rabies antibodies in unvaccinated dogs in Sierra Leone

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Abstract

Objective: This study determines the seroprevalence and associated factors of rabies antibodies in unvaccinated dogs in Sierra Leone.

Background: Rabies control is poorly coordinated in Sierra Leone which was ranked as the third hungriest country in the world. Due to limited access to rabies vaccines, the need for comprehensive serological data on dogs for control of the disease is expedient.

Methods: A random multistage technique considering high, medium and low incident areas of rabies cases in dog-populated communities was adopted. Samples were collected from dogs with owners which were at least 1-year old. Samples were analysed using a commercial ELISA in accordance to manufacturer’s instructions.

Results: 25.2% of the total 270 samples tested positive for the presence of rabies antibodies. Kenema district had the highest number of positive samples, followed by the Bombali district and Moyamba district the least. Predisposing factors, including sex, the status of castration, the type of settlement, district and the availability of fences at locations where dogs are kept, had significant effects ($p < 0.05$) on the exposure of dogs to rabies. The 25.2% antibody seroprevalence obtained is very low.

Conclusions: Concerted effort should be made to enhance rabies vaccination through an awareness campaign and provision of vaccine to dog owners. Good waste disposal and management practices to reduce open garbage disposal in communities will go a long way to limit the stray-dog population and minimize rabies outbreaks especially in third-world countries where rabies vaccines are not accessible.

Keywords
dogs, rabies antibodies, seroprevalence, Sierra Leone
Rabies in dogs was first diagnosed in Sierra Leone in 1949 from the brain of a rabid dog at the Teko Central Veterinary Laboratory (MANR, 1949) when few anti-rabies vaccines were imported by the government (MANR, 1961). Due to cost constraints, only the colonial masters and a few affluent dog owners had their pets vaccinated. This is occasioned by lack of awareness, limited vaccine supplies and cost constraints thereby causing a steep rise in rabies cases (MANR, 1958). The last national-scale anti-rabies vaccination campaign was in 1974 (MANR, 1964–1974). Ever since, no other country-scale vaccination was done in Sierra Leone until after the Lomé Peace Accord in 2005 when the Sierra Leone Animal Welfare Society started vaccinating dogs in the capital city, Freetown (Ali et al., 2010). The major bottleneck against the anti-rabies campaign includes selective anti-rabies vaccination adopted and a lack of data on the dog population (Nel, 2013), which has impeded the planning of an anti-rabies campaign and the control of this dreadful disease. To fill this gap, the Sierra Leone Animal Health Club was founded in 2009 to raise awareness of rabies through radio phone-in programmes, dramas, quiz competitions, local dialect jingles and vaccinations in regional headquarter towns (World Rabies Day Celebration Report, 2018). This approach helped in raising awareness of rabies to improve the consciousness of the teeming populace about dog care and management. The impact of the club improved the human–dog relationship culminating in responsible dog ownership.

Rabies is a well-known viral infection that affects the central nervous system of all warm-blooded animals, including humans (Moges, 2015; Richard et al., 2015). Rabies is a zoonotic disease that leads to fatal encephalitis in mammals (Chernet & Nejash, 2016). The disease is characterized by the appearance of severe nervous symptoms that result in the death of the victims following paralysis (Abera et al., 2015).

Rabies remains a source of serious public health concern and dogs are the vectors of more than 90% of rabies cases in Africa and Asia (Hampson et al., 2015) transmissible via bites. In 2016, the World Health Organization recorded 213 cases of animal bites (especially dogs, 90%) and four deaths in Moyamba district with a cumulative figure of 122 cases in 2017, of which 17 (13.9%) were children under the age of 5 (WHO, 2017). The few cases of rabies that have attracted national attention in Sierra Leone were that at Sembehun Junction in Moyamba district where a lady who was bitten by a rabid dog died 50 days after exposure due to lack of required medical attention. In another development, nine cows died in Modoya Village in Koinadugu district and post-mortem analysis showed that the deaths were rabies-related. The animals had been prepared for public consumption immediately after the rabid-induced death (Suluku et al., 2017a).

Despite the discovery of the rabies vaccine by the French Biologist, Louis Pasteur, some 134 years ago, Sierra Leone still lacks a well-coordinated approach for the control of this disease. Hence, there is limited access to rabies vaccines, and raising awareness is an increasingly daunting task as people expect such activities to be linked with access to vaccines to save infected dogs.

**FIGURE 1** A map of Sierra Leone depicting the provinces, districts and chiefdoms covered in the study

Rabies cases remain relatively high in Sierra Leone due to a dearth of rabies vaccines and selected vaccinations have only been conducted in some communities. Based on the global hunger index, Sierra Leone is ranked as the third hungriest country in the world (Sandi, 2017), with inadequate health facilities and services across the country, hence the need to understand that this disease requires comprehensive serological data on dogs. This study, therefore, determines the seroprevalence of rabies and associated factors in unvaccinated dogs in Sierra Leone.

**2 | METHODS AND MATERIALS**

**2.1 | Study area and sampling technique**

Three provinces were selected for sampling (East, South and Northern) of Sierra Leone (Figure 1). A multistage sampling technique was adopted wherein a province, dog blood samples were collected in a district, chiefdom and a section. At the district level, the district headquarter town was selected, then at the chiefdom level, the chiefdom headquarter town was selected for data collection. Finally, at the section level, any village or town within the section with at least 30 dog owners was selected for the data collection.

**2.1.1 | Kenema**

A district in the Eastern province which has a land area of 7985 km² and a population of 606,544 people. Rabies cases were rated as high as the district has consistently reported rabies cases since 1953. The
TABLE 1  The table shows the selected chiefdom and district-level towns for sample collection in the study area

| Region          | District          | Township       |
|-----------------|-------------------|----------------|
| Southern region | Moyamba district  | District level |
|                 |                   | Chiefdom level |
|                 |                   | Section level  |
| Eastern region  | Kenema district   | District level |
|                 |                   | Chiefdom level |
|                 |                   | Section level  |
| Northern region | Bombali district  | District level |
|                 |                   | Chiefdom level |
|                 |                   | Section level  |

- Moyamba district has 9 months of rainfall and is predominantly forest vegetation with limited secondary forest and grassland vegetation on hills and mountain tops.
- Bombali district has 6 months of rainfall and is predominantly grassland with patches of forest.
- A total of 270 blood samples in all (30 per town) were collected from one dog per dog-owning person/family in each town (district, chiefdom and section) as shown in Table 1.

2.1.2  | Moyamba

A district in the southern province with a land area of 6902 km² and a population of 318,588 people. Rabies cases were rated medium as the district has reported one rabies death since 2017. It has 8 months of rainfall and is predominantly secondary forest and coastal mangroves.

2.1.3  | Bombali

A district in the northern province with a land area of 6053 km² and a population of 609,891 people (CSO-SL, 2015). Although the first dog rabies in Sierra Leone was diagnosed at the Teko Laboratory in 1949 in Makeni Town, Bombali district (MANR, 1949), the risk of disease is low. It has 6 months of rainfall; the natural vegetation cover is grassland that is spotted with patches of forest. Lumbering, slash and burn agriculture, and charcoal burning have reduced primary forest vegetation in some of the districts to grassland or secondary forest, particularly Moyamba and Bombali districts. Agricultural (livestock and crop) and mining activities (iron ore, diamond and gold are mined in Bombali and Kenema districts, while rutile, zircon and bauxite are mined in Moyamba district) in the districts create a sense of insecurity, driving people to keep dogs to provide security against intruders at night.

2.2  | Sample size determination

The required sample size (n) to estimate seroprevalence with 95% confidence interval was calculated based on Equation (1) (Charani et al., 2010):

\[
1.962 \times \sqrt{p(1-p)/d^2},
\]

where \( p \) is the expected seroprevalence (10%) (based on data from the Ministry of Agriculture, Sierra Leone) and \( d \) is the desired precision (5%). We calculated a minimum required sample size of 138.

2.3  | Sampling method

The data collected in the district, chiefdom and section towns were purposive and multistage considering high, medium and low incident areas of rabies cases in dog-populated communities.

2.3.1  | Dogs sampled

A well-structured questionnaire with information on age, sex, vaccination status, owner’s name, district and chiefdom was used to collect relevant information. Local, unvaccinated, owned and 1-year-old dogs that were commonly used as guard or hunting dogs in selected communities were enrolled for the study. Randomly, blood was collected from the dogs using a 21-gauge sterilized needle and syringe. Three millilitres of whole blood was collected through the cephalic vein and aspirated into a 5 ml vacutainer without anticoagulant. The blood was centrifuged at 2500 rpm for 15 min and the sera were collected in a cryovial. The sera samples were transported via the cold box to the Serological laboratory at Njala University where testing was carried out.

2.3.2  | Data on predisposing factors

Questionnaires were designed to obtain information on sex, the status of castration, type of settlement, district and the availability of fences at locations where dogs are kept from every dog sampled. In order to minimize possible injury to the animals, dogs that were at least 1 year of age were enrolled in the study.

2.4  | Serologic assay for rabies antibodies

Sera obtained from sampled dogs were analysed using the Indirect Enzyme-linked Immunosorbent Assay (Platelia™ Rabies II Kit
TABLE 2  Effects of predisposing factors of exposure of dogs to rabies

| Variable   | Categories | Sero-positive | Significance (p-value) |
|------------|------------|---------------|------------------------|
| Sex        | Male       | 46/68 (67.75%) | 0.040*                 |
|            | Female     | 22/68 (32.25%) |                        |
| Castration status | Castrated | 18/68 (26.47%) | 0.01*                 |
|            | Uncastrated| 50/68 (73.53%) |                        |
| Fencing    | Fenced     | 19/68 (27.94%) | 0.032*                 |
|            | Unfenced   | 49/68 (72.06%) |                        |
| Settlement type | Urban    | 21/68 (30.88%) | 0.049*                 |
|            | Rural      | 47/68 (69.12%) |                        |
| District   | Kenema     | 27/68 (39.71%) | 0.013*                 |
|            | Bombali    | 15/69 (22.06%) |                        |
|            | Moyamba    | 26/28 (38.23%) |                        |

* Significant differences exist between variable categories at 5% significant level.

[Bio-Rad]) to detect IgG antibodies against rabies virus glycoprotein (R1) using the manufacturer's instructions. The optical density (OD) was read using ELISA Reader (LEDETECT 96). The sensitivity of the kit as stated by the manufacturer was 98.6%, while the specificity was 99.4%. The OD of the positive control and antibody titers (expressed as equivalent unit per millilitre – EU/ml) were obtained from a standard OD-anti-titer curve. The subject was considered to have a threshold of antibodies against rabies virus infection if the periodical ELISA titers were greater than 0.5 EU/ml as recommended by the manufacturer.

### 2.5 Statistical analyses

The data gathered were analysed using Microsoft Excel and Statistical Package for Social Sciences (SPSS) Version 25.0. Descriptive statistics were used to analyse the presence of rabies antibodies in dogs in the form of frequencies and percentages. Pearson Chi-square test was used to determine the significance and relatedness of the associated factors (sex, castration, fencing, settlement and district) of rabies prevalence in this study. The statistical significance between the categorical variables was tested at a 5% significance level (p-value < 0.05).

### 3 RESULTS

#### 3.1 Presence of rabies antibodies in dog sera from the study area

The presence of rabies antibodies in serum from blood samples collected from unvaccinated dogs from the three districts was shown using an ELISA kit. Out of the samples analysed in the study, 25.2% of the total 270 tested positive for the presence of rabies antibodies, while 77.04% of the samples tested negative. Kenema district had the highest number of positive samples, followed by the Bombali district and Moyamba district the least (Table 2).

#### 3.2 Effect of castration, sex, settlement, district and fencing on rabies exposure

The findings on the factors associated with the presence of rabies antibodies in dogs indicated that the sex, the reproductive status (castrated or uncastrated), the type of settlement and the availability of fence at locations where dogs are kept had significant effects (p<0.05) on the exposure of dogs to rabies in Sierra Leone (Table 2).

Male dogs (67.75%) were more exposed to rabies as compared to females (32.25%), also uncastrated males (73.53%) were more exposed to rabies compared to their castrated counterparts (26.47%). There were more rabies seropositive dogs in unfenced houses (72.06%) that kept dogs as compared to the dogs kept in fenced houses (27.94%). Rural areas recorded more seropositive rabies dogs (69.12%) as compared to the urban areas (30.88%) in Sierra Leone. More of the dogs sampled from Kenema (39.71%) recorded relatively higher levels of rabies glycoprotein in their serum as compared to those from Moyamba (38.23%) and Bombali (22.06%) as shown in Table 2.

### 4 DISCUSSION

Serological detection had been one of the major approaches used to measure the exposure and protective levels of the dog population to rabies, especially in resource-poor communities. This study adopted this approach to assess the level of exposure of unvaccinated dogs in Sierra Leone to rabies. This study showed a 25.2% (68 out of 270 samples) prevalence of rabies antibodies among unvaccinated dogs in Sierra Leone. The percentage prevalence could have been higher than 25.2% if the subject was considered to have a threshold of antibodies
against rabies virus infection when ELISA titers were greater than 0.3 EU/ml as recommended by Wasniewski et al. (2014).

Similar studies showed that 37.5% of unvaccinated dogs in Ibadan and 71.4% in Southwest Nigeria tested seropositive for rabies antibodies (Olugasa et al., 2011). In Santa Maria in Brazil, 42.3% (127 out of 300) of dogs with unknown history of vaccination tested positive for rabies antibody titers (Karina et al., 2017).

The high number of positive cases indicates the possibility of the existence of the non-lethal rabies infection in the regions sampled or the possible existence of a host-adapted strain with low pathogenicity in Sierra Leone. This study represents the first report of the presence of rabies antibodies in unvaccinated dogs in Sierra Leone. The presence of rabies antibodies in the unvaccinated dogs may be connected with their use for hunting which indicates possible contact with rabies virus from wildlife as reported by Gilbert et al. (2012) in the Peruvian Amazon.

With the available evidence gathered in this study, sex had a significant influence on exposure to rabies with male animals being more predisposed to rabies compared to females. The aggressive nature of male dogs and their quest to dominate their territories often leads to physical combat with intruding dogs. This combat may expose both parties to physical injuries, which can cause the exchange of body fluids. When the body fluid of a seropositive rabies dog comes into contact with that of a seronegative rabies dog, transmission is established. In addition to this, castrated dogs were less exposed to rabies virus as compared to uncastrated dogs. Uncastrated dogs have the urge and the instincts to search for bitches to mate. This innate characteristic of such uncastrated male dogs causes them to invade new dog territories which easily leads to wrestling with resident male dogs to find mating partners. As a result of this behaviour, uncastrated dogs may become more likely to be exposed to the rabies virus.

Furthermore, this study reported more dogs in unfenced houses were seropositive for rabies antibodies. Unfenced dogs are at liberty to move about at all times to any place of their choice in the search of food, especially at bushes, garbage and waste dump sites. According to Teampanpong (2021), waste food at garbage and dumpsites attracts more wildlife vertebrates into the human community. The interaction of dogs with wild animals (which are carriers of rabies virus) at garbage and dumpsites could be a potential source of infection of the virus in domesticated dogs. Again, unfenced dogs can easily come into contact with stray dogs which are carriers of the rabies virus and move about in search of food.

This study also showed that dogs in rural areas have higher levels of rabies antibodies as compared to urban area dogs. This observation could be due to the exposure of dogs raised in rural areas to more stray dogs as well as wild animals during their search for food.

In this study, dogs sampled within districts characterized by high rainfall and huge forest vegetation, such as Kenema, were more exposed to rabies virus possibly due to exposure to wildlife which were residents in the forest vegetation and were reservoirs of the rabies virus.

### 5 CONCLUSIONS

Based on the results from this study, the level of seropositivity is very low. This indicates the need for concerted efforts should be geared towards enhancing anti-rabies vaccination through an awareness campaign and provision of vaccines to dog owners in order to achieve WHO standards of 70–80% baseline needed for herd immunity when vaccinated. More importantly, a one-health approach where dog owners work closely with veterinarians to learn about dog care and best management skills should be encouraged. Also, environmentalists should teach dog owners about good waste disposal and management practices to reduce open garbage disposal in communities. This approach will go a long way to limit the stray-dog population and minimize rabies outbreaks. Hence, good dog care and waste disposal management could be sound approaches to be adopted in third-world countries to control the stray dog population (Teampanpong, 2021), especially where rabies vaccines are not affordable to an average dog owner.

### AUTHOR CONTRIBUTIONS

Suluku Roland: Conceptualization; investigation; methodology; project administration; writing – original draft. Emikpe Benjamin: Conceptualization; methodology; supervision; writing – review and editing. Bagla Victor Patrick: Investigation; methodology; validation; writing – original draft. Moiwo Juana: Investigation; methodology; project administration. Asare Derrick: Formal analysis; methodology; validation.

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### CONFLICT OF INTEREST

The authors of this current study declare no conflict of interest whatsoever.

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### DATA AVAILABILITY STATEMENT

Data that support the findings will be available on request from the corresponding author.

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The authors confirm that the ethical policies of the journal, as noted on the journal’s author guidelines page, have been adhered to and the appropriate ethical review committee approval has been received. The US National Research Council’s guidelines for the Care and Use of Laboratory Animals were followed.

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