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# A survey of rabies virus antibodies in confined, hunting and roaming dogs in Ogun and Oyo States, Southwestern Nigeria

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## PEER REVIEW

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## Comments

Data presented highlight incessant neglect of rabies vis-a-vis ensuring dogs are immunized to prevent human exposures. Comparative sensitivity of ELISA, fat *etc.* may be useful. Additional references as indicated above will improve the quality of argument. Overall it is an interesting and well written paper.

Details on Page 20

## ABSTRACT

**Objective:** To screen for rabies virus (RABV) antibodies in apparently healthy confined, hunting and roaming dogs by a community-based approach.

**Methods:** Sera from 230 (80 confined, 92 hunting and 58 roaming) dogs in some urban and peri-urban communities in Ogun and Oyo states, Southwestern Nigeria were screened for RABV antibodies using the indirect ELISA method.

**Results:** Analysis of administered questionnaires showed that of 80 confined dog owners, 37 were aware of anti-rabies vaccination (*i.e.* they were informed) while 17 were negligent and 26 uninformed. Of the 230 sera tested, only 13 (5.7%) from vaccinated confined dogs in Oyo state were positive (*i.e.* had optimal RABV antibody titres) (mean 0.54, 95% CI: 0.42–0.67) while all confined dog sera in Ogun state were negative. Eleven (12.0%) and 14 (24.1%) of the hunting and roaming dogs respectively had sub-optimal RABV antibody titres while the rest were negative.

**Conclusions:** Evidently, these groups of dogs are a totally unprotected and susceptible dog population that can serve as potential reservoirs of RABV in the study area. Responsible pet ownership, vaccination of hunting and roaming dogs, and community-based active rabies surveillance are therefore advocated in Nigeria.

## KEYWORDS

Rabies virus, Antibodies, Confined, Hunting and roaming dogs, Southwestern Nigeria

## 1. Introduction

Rabies, an endemic neglected zoonosis that is a re-emerging global health threat, is caused by the rabies virus (RABV) which is the prototype species of the genus *Lyssavirus*, family Rhabdoviridae[1]. As one of the most dreaded zoonoses, rabies satisfies all the World Health Organization criteria for diseases that are a priority for control. The disease has traditionally been associated with dogs more than any other animal and, in parts of the world where domestic animal control and vaccination programmes

are limited, dogs remain the most important reservoir of the disease[2]. Although safe and effective animal and human vaccines are widely available for its prevention and control, rabies remains a neglected disease that is poorly controlled throughout much of the developing world, particularly Africa and Asia, where most human rabies deaths occur[2]. According to the World Health Organisation[3], the annual number of human rabies deaths globally is about 61 000 with the vast majority of deaths (84%) occurring in rural areas. In Africa, rabies incidence is reported to be underestimated by more than 100-fold because most deaths occur in

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communities rather than in hospitals<sup>[4,5]</sup>.

Recent increases in human rabies deaths in parts of Africa, Asia and Latin America suggest that rabies is re-emerging as a serious public health issue<sup>[3]</sup>. With rising urbanization, there is an increase in the presence of traditional pets in households and the licking of humans by dogs that are rabid or suspected to be rabid poses a major risk to human health<sup>[6]</sup>. The rise in pet ownership is reflected in the amount of pet dogs in developed and developing nations. According to one estimate, the current world population of domestic dogs may be as high as 500 million, of which a substantial proportion is poorly supervised or free-roaming<sup>[7]</sup>. Free-roaming dogs have long been considered to be a problem in many countries and regions mainly because dogs are likely to form packs and threaten, injure or kill children or adults, apt to chase or prey on livestock and are of primary importance in rabies control in about half the countries in the world<sup>[3,8]</sup>.

Rabies has been reported in owned confined and free-roaming dogs in several countries<sup>[9–11]</sup>. In Nigeria, there have been reports of clinical rabies with the burden of the disease being worsened by atypical cases which present without specific signs or symptoms of rabies<sup>[12–15]</sup>. However, most of these reports were based on records of animals that visited private and government-owned veterinary clinics and hospitals with paucity of information on immune status of hunting dogs<sup>[16,17]</sup>. In view of the recent upsurge in adoption of exotic and local dogs as pets in Nigeria accompanied by increased contact and bonding between the dogs and their owners<sup>[18]</sup>, and the traditional close association between hunters and their dogs, there is a need for studies to determine the level of protection of these dogs against rabies. In the present study, we have adopted a community-based approach to investigate the presence of RABV antibodies in apparently healthy confined, hunting and roaming dogs in Ogun and Oyo states, Southwestern Nigeria. These two states are noted for influx of people and dogs from all regions in Nigeria since dog trade across the country is common and unregulated<sup>[16]</sup>.

## 2. Materials and methods

### 2.1. Study locations

The study was carried out in Abeokuta, Sagamu and Odeda in Ogun state and in Ibadan, the capital of Oyo state. The two states are located in Southwestern Nigeria at 7°00' N 3°35' E with estimated population of 4.1 million people and 8°00' N 4°00' E with estimated population of 5.6 million people, respectively<sup>[19]</sup>.

### 2.2. Sample population and specimen collection

A total of 230 apparently healthy dogs of both sexes (77 males and 153 females) were used for this study. They comprised 80 confined dogs presented for routine clinical examination or vaccination at some of the major government-owned veterinary clinics in Ogun and Oyo states, 92 hunting dogs from Odeda farm (hunting) settlement in Ogun state and 58 roaming dogs from urban and peri-urban areas of Ibadan (including dogs captured by traders and sold to the public for consumption as delicacies). Questionnaires were administered to collect pertinent demographic data including age, sex and breed of dog, ownership type, purpose for keeping the dog, type of management, anti-rabies vaccination history and educational status of owner.

Using sterile syringes and needles, about 2.5 mL of blood was collected from each dog through the cephalic vein into plain sample bottles without anticoagulant. The blood was allowed to clot at room temperature for about 5 to 6 h and sera obtained were stored at –20 °C until tested.

### 2.3. Detection of rabies virus antibodies using ELISA procedure

The indirect ELISA technique used was as described by Ohore *et al*<sup>[17]</sup>. Optimal working dilutions obtained following chequerboard titration were antigen 1:500, sera 1:100 and rabbit anti-dog horse radish peroxidase IgG (Sigma, USA) 1:1000. The cut-off sample to positive (SP) ratio was calculated to be 0.25, which corresponded to twice the optical density (OD) value of the negative control serum. Results were read using the Top-Read Microplate ELISA reader (Axiom, Germany) and were considered valid when the difference between the mean OD of the positive and negative control sera was greater than 0.2 and the mean OD of the negative control serum was less than or equal to 0.25. Samples with SP ratio greater than the cut-off value of 0.25 were considered to have optimal RABV antibody levels (positive), those with SP ratio lower than the cut-off had sub-optimal antibody levels while serologically negative samples were those with zero SP ratio. Data obtained were subjected to One-way ANOVA to determine statistical significance of the findings.

## 3. Results

The demographic data of the screened dogs in Ogun and Oyo states including breed and vaccination status as well as educational status of the dog owners are shown in Table 1. Interviews conducted among the dog owners showed

that 21.3% (17/80) of them were aware of the anti-rabies vaccination programme but refused to vaccinate their dogs (*i.e.* they were negligent), 36.3% (29/80) were misinformed about the vaccination (*i.e.* they were made to believe their dogs had been vaccinated when, in reality, they had not been vaccinated), while the remaining 34 (42.5%) were uninformed (*i.e.* they had no knowledge of anti-rabies vaccination). All the hunting and roaming dogs and some confined dogs were local breeds. The prevalence of RABV antibodies in confined dogs in the two states is shown in Table 2 with none of the vaccinated dogs from Ogun state being positive for RABV antibodies while 54.2% (13/24) from Oyo state were positive. None of the 92 hunting dogs, which were all unvaccinated, was positive for RABV antibodies but 11 (12.0%) had sub-optimal antibody levels while 81 (88.0%) were negative. Similarly, none of the 58 unvaccinated roaming dogs was positive for RABV antibodies but 14 (24.1%) and 44 (75.9%) had sub-optimal and no antibody levels respectively. Overall, the prevalence of RABV antibodies in confined, hunting and roaming dogs was 16.3%, 0% and 0% respectively (Table 3).

**Table 1**

Vaccination status of confined dogs and educational status of their owners in Ogun and Oyo states.

| State | Dog breed  | Number | Educated   |            | Uneducated |            | Vaccinated |            | Unvaccinated |      |
|-------|------------|--------|------------|------------|------------|------------|------------|------------|--------------|------|
|       |            |        | owners     | owners     | owners     | owners     | dogs       | dogs       | dogs         | dogs |
| Ogun  | Alsation   | 19     | 14         | 5          |            | 10         |            | 9          |              |      |
|       | Rottweiler | 5      | 5          | 0          |            | 1          |            | 4          |              |      |
|       | Local      | 8      | 4          | 4          |            | 2          |            | 6          |              |      |
|       | Others     | 2      | 2          | 0          |            | 1          |            | 1          |              |      |
|       | Total      | 34     | 25 (73.5%) | 9 (26.5%)  |            | 14 (41.2%) |            | 20 (58.8%) |              |      |
| Oyo   | Alsation   | 24     | 18         | 6          |            | 14         |            | 10         |              |      |
|       | Rottweiler | 7      | 4          | 3          |            | 4          |            | 3          |              |      |
|       | Local      | 8      | 4          | 4          |            | 2          |            | 6          |              |      |
|       | Others     | 7      | 7          | 0          |            | 4          |            | 3          |              |      |
|       | Total      | 46     | 33 (71.7%) | 13 (28.3%) |            | 24 (52.2%) |            | 22 (47.8%) |              |      |

**Table 2**

Prevalence of rabies virus antibodies in confined dogs in Ogun and Oyo states.

| Confined dog | No. dogs |     | No. positive (%) |           | No. sub-optimal (%) |           | No. negative (%) |           |
|--------------|----------|-----|------------------|-----------|---------------------|-----------|------------------|-----------|
|              | Ogun     | Oyo | Ogun             | Oyo       | Ogun                | Oyo       | Ogun             | Oyo       |
| Vaccinated   | 13       | 24  | 0 (0.0)          | 13 (54.2) | 3 (23.1)            | 9 (37.5)  | 10 (76.9)        | 2 (8.3)   |
| Unvaccinated | 21       | 22  | 0 (0.0)          | 0 (0.0)   | 3 (14.3)            | 9 (40.9)  | 18 (85.7)        | 13 (59.1) |
| Total        | 34       | 46  | 0 (0.0)          | 13 (54.2) | 6 (17.6)            | 18 (39.1) | 28 (82.4)        | 15 (32.6) |

**Table 3**

Overall prevalence of rabies virus antibodies in confined, hunting and roaming dogs in the study locations.

| Dog      | Number | Positive (optimal) | Sub-optimal | Negative    |
|----------|--------|--------------------|-------------|-------------|
| Confined | 80     | 13 (16.3%)         | 24 (30.0%)  | 43 (53.8%)  |
| Hunting  | 92     | 0 (0.0%)           | 11 (12.0%)  | 81 (88.0%)  |
| Roaming  | 58     | 0 (0.0%)           | 14 (24.1%)  | 44 (75.9%)  |
| Total    | 230    | 13 (5.7%)          | 49 (21.3%)  | 168 (73.0%) |

## 4. Discussion

Rabies is endemic in settings where large groups of

unvaccinated and unconfined domestic dogs are present and the most cost-effective strategy for preventing the disease in people is by eliminating it in dogs through vaccination<sup>[20]</sup>. Indeed, transmission of rabies has been reported to effectively stop when over 70% of the canine population is immune<sup>[10]</sup>. In the present study, we provide the rabies antibody profile of confined, hunting and roaming dogs in two states in Southwestern Nigeria. Compared to previous studies which reported higher rabies antibody prevalence rates of 71.4% and 42.6% in confined dogs in Ibadan, Oyo state and Ilorin, Kwara state respectively, the overall low (16.3%) rabies seroprevalence rate obtained for confined, hunting and roaming dogs in this study suggests that only a small proportion of the dog population in the study locations received anti-rabies vaccination. This observation suggests a poor attitude/response of the dog owners to anti-rabies vaccination and is corroborated by the results of questionnaire analysis which showed that a substantial percentage of dog owners in this study did not vaccinate their dogs against rabies, even when they were educated. Thus, the low rabies seroprevalence rate obtained in this study could be attributed to the fact that the dog owners were negligent, misinformed or totally uninformed about dog rabies vaccination.

The non-detection of RABV antibodies in some vaccinated confined dogs suggests poor vaccination responses or decayed rabies antibody levels as at the time of blood sample collection. These poor vaccination responses observed may be due to factors such as antigenic differences between the rabies virus strain used for vaccine production and the circulating wild rabies virus strains<sup>[4]</sup>, administration of incorrect or sub-optimal doses of vaccine, failure of some dogs to seroconvert even with repeated doses of vaccine<sup>[10]</sup>, and poor immunogenicity of the vaccine, probably as a result of fluctuation in storage temperature. In such circumstances, although dogs may have been correctly vaccinated, their immune response and the quality of immunity may be inadequate. In tropical countries like Nigeria, vaccine virus titres may rapidly wane if proper cold storage is not maintained. Moreover, the fact that a large proportion (83.8%) of confined dogs studied was unprotected against rabies indicates that they are a highly susceptible dog population which constitutes a threat to public health.

It is noteworthy that all hunting and roaming dogs screened did not possess protective rabies antibody levels. These dogs, which were local breeds, were all unvaccinated. This finding corroborates the reports of Awoyomi *et al.* who noted that local dogs have a high probability of being unvaccinated and Adeyemi *et al.* who observed that there is inadequate rabies vaccination coverage in Nigeria, which increases public health risk<sup>[21,22]</sup>. It is known that behavioural factors

such as mating in these hunting and roaming dogs are indiscriminate and uncontrolled, and predisposes them to occasional fights over mating partners. Therefore, this unprotected group stand a great risk of rabies exposure which may occur when saliva of an infected animal gains entry into the host through any skin abrasion or a fresh wound, or through virus splashing into the conjunctiva<sup>[2,11]</sup>.

The detection of sub-optimal rabies antibody levels in unvaccinated hunting and roaming dogs suggests they may have had field exposure to rabies or rabies-related viruses. These hunting and roaming dogs could therefore serve as canid reservoirs which harbour inapparent infection and shed virus in their saliva in the absence of clinical disease<sup>[10]</sup>. Thus, they constitute a potential high risk dog population that may serve as exposure points for rabies in the community. Furthermore, there is possibility of exposure of the unprotected hunting dogs to sylvatic rabies during hunting expeditions. For instance, Radostits *et al.* noted that it is likely that outbreaks occurring naturally amongst carnivores may originate by them eating bats which have died of rabies<sup>[23]</sup>. Also, Badrane and Tordo reported that all rabies virus variants of terrestrial carnivores are believed to have their origin from variants of rabies virus associated with bats<sup>[24]</sup>. The occasional cross-species transmission (*i.e.* spill-over) of rabies virus variants from a reservoir host to a secondary species in which the original variant subsequently becomes adapted could lead to its emergence as a novel or unique sub-variant of rabies virus<sup>[3]</sup>. Therefore, the detection of sub-optimal RABV antibody levels in unvaccinated hunting dogs in this study indicates that they might have had subclinical exposure to rabies or rabies-related viruses from wildlife. Consequently, they pose a threat to public health since they may serve as reservoirs of both urban and sylvatic rabies and ultimately, as vectors transmitting rabies to humans and other animals<sup>[3]</sup>.

In conclusion, this study has revealed considerable indifference to vaccination of confined dogs even among educated dog owners, and shown that hunting and roaming dogs in the study areas are not protected against rabies. These three categories of domestic dogs thus constitute a threat to public health as it has been reported that human exposure is more likely to occur through bites from owned unvaccinated dogs than stray dogs<sup>[25]</sup>. There is therefore a need for promotion of responsible pet ownership in Nigeria through extensive public education. In addition, the study has not only underscored the fact that hunting and roaming dogs should be included in future rabies vaccination campaigns, it has also highlighted the need for more studies on the epidemiology of rabies and rabies-related viruses in Nigeria. Furthermore, intervention strategies which

have been used in successful rabies control programmes elsewhere such as community-based active surveillance and integration of veterinary and public health services in implementing mass dog vaccination programmes, are advocated<sup>[9,26]</sup>.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

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### Comments

#### Background

Rabies is a dreadful zoonotic disease of high control priority. Dogs being primary reservoir require control by vaccination. Data are however limited in both free roaming and confined dogs. The study was designed to determine rabies protection by measuring antibody level to rabies in dogs in the study area.

#### Research frontiers

Rabies virus antibody level in blood was measured by ELISA test in populations of vaccinated, unvaccinated, confined and free roaming dogs. Lack of or sub-optimal levels were observed.

#### Related reports

Data presented showed poor serum antibody detection in dogs in contrast to previous studies. This was alluded to lack of active vaccination due to negligence and poor attitude. Failure to seroconvert according to the authors may also be due to antigenic variation, vaccine failure due to poor storage.

#### Innovations & breakthroughs

A total of 83.8% of confined or roaming dogs were unprotected against rabies and as such are highly susceptible, thereby constituting public health risk.

#### Applications

Community-based rabies surveillance and mass

vaccination incorporating veterinary and public health services are advocated.

### Peer review

Data presented highlight incessant neglect of rabies vis-a-vis ensuring dogs are immunized to prevent human exposures. Comparative sensitivity of ELISA, fat *etc.* may be useful. Additional references as indicated above will improve the quality of argument. Overall it is an interesting and well written paper.

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