Rabies awareness among dog owners and detection of antibody levels against rabies in dogs presented for treatment at selected veterinary clinics in Abeokuta, Ogun State, Nigeria

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Summary
This study was conducted to profile the antibody levels to rabies in dogs presented at veterinary clinics and determine rabies awareness among dog owners in Abeokuta, Nigeria. Records of dogs' rabies vaccination were obtained to determine their vaccination status and number of times they had been vaccinated. Sera from 138 dogs of consenting owners were analysed using indirect ELISA technique to detect rabies antibodies. Structured questionnaire was administered to 138 dog owners to determine their awareness on rabies. Data were analysed using descriptive statistics, Chi-square, ANOVA and t-test at $p \leq 0.05$. Of 138 dogs screened, 114 (82.6%) had history of vaccination against rabies. Of these 114, 87 (76.3%) were seronegative; however, 5 (3.6%) of the 24 unvaccinated dogs were seropositive. Overall, 32 (23.2%) comprising 15 (10.8%) males and 17 (12.3%) females had positive rabies antibodies level. Five (3.6%), 3 (12.1%) and 24 (17.4%) were seropositive among dogs of < 6 months, 6-12 months and > 1 year of age, respectively. Dogs > 1 year had significantly higher antibodies than < 6 months ($p < 0.05$). Most (86.9%) of the dog owners were aware of rabies. The low seroconversion in vaccinated dogs and prevalence of rabies antibodies in unvaccinated dogs are of public health concern. There is need for regular sero-profiling of vaccinated and unvaccinated dogs.

Introduction

Rabies is a zoonotic disease caused by the Rabies virus (RABV) of the *Lyssavirus* genus within the family *Rhabdoviridae* (WHO 2019). It is a typical zoonosis that is considered as the oldest communicable disease of human, existing for more than 4,300 years (Fooks *et al.* 2012, Yibrah and Damtie 2015, Barecha *et al.* 2017). The rabies virus genome consists of a single stranded, non-segmented, negative sense RNA of approximately 12 kb (Saito *et al.* 2013). Rabies transmission is usually via virus-laden saliva of an infected animal through bite or scratch from animal to animal or animal to man. Rabies virus is highly neurotropic; it has high affinity for the central nervous system (Barecha *et al.* 2017).

Rabies remains a zoonotic viral disease that affects human, domestic and wild animals. All mammals are susceptible to rabies, although canine rabies presents the greatest threat to humans, as exposure to rabid dogs contributes up to 99% of all rabies transmissions to humans worldwide (WHO 2019). Otolorin and colleagues (Otolorin *et al.* 2015) reported that 99% of human rabies in Nigeria were due to dog bite. Domestic dogs are the most common reservoir of the virus, with more than 99% of human deaths caused by dog-mediated rabies (WHO 2019). The vast majority of humans, who develop rabies, die because of the infection (Tekki *et al.* 2013, Hurisa *et al.* 2015). About 60,000 people die of rabies every year in over 150 countries, with 95% of cases occurring in Africa and Asia (WHO 2019). Due to widespread underreporting and uncertain estimates, the true burden of disease could even be more; as in the case of Nigeria with only a total of 78 human deaths recorded in health care institutions in various States, spanning from

1980 to 2014 (Richard *et al.* 2015). As much as 99% of rabies cases in developing countries are dog-mediated and the burden of disease is largely borne by the rural poor communities (WHO 2019). While majority of human rabies associated with dogs has reduced in the last two decades in Mexico, South America and the Carribean, dog-associated human rabies has increased in parts of sub-Saharan Africa in the same period (Olugasa *et al.* 2011).

Rabies is a 100% vaccine-preventable zoonotic disease and marked reductions, often progressing to the elimination of rabies, have been achieved in countries embarking on rabies elimination programmes (WHO 2019). Prevention or elimination programmes often revolve around mass dog vaccination campaigns using rabies vaccines, where at least 70% of the dog population should be covered in order to prevent the disease or break the cycle of transmission in dogs, and to humans (Fitzpatrick *et al.* 2012, WHO 2019). According to the World Health Organization (WHO), at least 500,000 people are given post-exposure vaccinations every year and mass vaccination of domestic dogs has been the most effective measure in reducing human rabies (WHO 2005, Yang *et al.* 2011). In order to achieve the global target of "zero human rabies deaths by 2030" set by the WHO, World Organisation for Animal Health (OIE), Food and Agriculture Organization of the United Nations (FAO) and Global Alliance for Rabies Control (WHO 2019), there is a need to intensify efforts at vaccinating dog populations with effective vaccines. Dog owners and policy makers should be well informed of vaccination schedule in order to ensure effective vaccination programmes (Olugasa *et al.* 2011). Types of rabies vaccines used for immunisation include modified live vaccine, inactivated rabies vaccine and oral modified live vaccine. Although these have largely proven to be safe and efficacious worldwide, new-generation rabies vaccines including recombinant rabies virus-based vaccines, vectored vaccines, DNA-based vaccines, and plant vaccines have recently been explored to overcome the limitations of the conventional vaccines (Yang *et al.* 2013).

In Nigeria, pre-exposure vaccination of dogs usually involves a primary dose of low egg passage (LEP) Flury strain vaccine produced by the National Veterinary Research Institute, Vom, Nigeria (NVRI), given at 3 months of age. Yearly booster doses are administered to sustain threshold immunity against rabies since rabies is enzootic in the country (Adeyemi *et al.* 2005, Ohore *et al.* 2007, Olugasa *et al.* 2011). In addition to the rabies vaccines produced by NVRI, imported rabies vaccines from other countries of the world are used since the local production by the NVRI cannot meet up with the demand for the vaccine in Nigeria (Awoyomi and Ogundipe 2019). Annual rabies vaccine production for dog was less than 30,000 doses in 2011 (NVRI 2012). The voluntary routine vaccination of dogs against rabies at the veterinary clinics in Nigeria places responsibility on the individual dog owners to bring their dogs to the clinic or to book for ambulatory services at a fee with registration of the dogs and issuance of rabies vaccination license and certificate (Adeyemi *et al.* 2005). Alternatively, there is the mass vaccination campaigns usually sponsored by government and non-government agencies, which are commonly administered free of charge or at subsidised cost; although not carried out regularly (Adeyemi *et al.* 2005).

Enzyme linked immunosorbent assay (ELISA) has been useful as an acceptable method which is fast and practical in the quantitative detection of antibody levels to rabies (Olugasa *et al.* 2010, Moore *et al.* 2017). Several studies have been carried out to detect rabies antibody levels in dogs in Nigeria (Ohore *et al.* 2007, Olugasa *et al.* 2011, Oluwayelu *et al.* 2015, Awoyomi and Ogundipe 2019). Antibody response to rabies vaccine greater than or equal to 0.5 IU/ml serum is considered adequate to protect against the development of the infection (Hurisa *et al.* 2015). According to Olayemi and colleagues (Olayemi *et al.* 2017), 88.6% of the dogs sampled had sufficient antibody levels in Abuja, Nigeria. However, some reports have shown low antibody responses in dogs vaccinated in Nigeria with commercial rabies vaccines, both local and imported (Ohore *et al.* 2007, Awoyomi and Ogundipe 2019, Ishola *et al.* 2019). Majority of such studies have focussed on the general dog population with less emphasis on those presented at veterinary clinics. Furthermore, there is the general believe that all vaccinated dogs are protected; and have adequate antibody levels.

Rabies vaccination failures in dogs, although rare, have been documented while falsification of records has resulted in the entry of canine rabies virus variant infected dogs into rabies-free countries (Rota Nodari *et al.* 2017, Wallace *et al.* 2017). Therefore, in addition to veterinary records, many countries require that dogs seeking entry into rabies-free jurisdictions also provide serologic evidence of vaccination (Wallace *et al.* 2017). This study therefore aimed at determining the antibody levels against rabies in dogs routinely presented for treatments at selected veterinary clinics as well as the dog owners' awareness of rabies in Abeokuta, Ogun State Nigeria.

Materials and methods

Study site, sampling and sample collection

A cross sectional study was conducted involving collection of blood for serum from confined dogs

presented for various veterinary treatments at three major veterinary clinics in Abeokuta, Ogun State, Nigeria, being the largest city in the State. Consent of the dog owners was obtained after explaining the purpose and benefits of the study to them. Only consenting dog owners thereafter presented their dogs for sampling. A total of 138 dogs were sampled (Thrusfield 2007, Oluwayelu *et al.* 2015); sampling was done weekly for a period of six weeks. The age, breed and sex of each dogs were documented. Records of vaccination history of the dogs were obtained to determine the vaccination status and how many times they had been vaccinated.

A volume of 5 ml of blood was collected using sterile needle and syringe via cephalic venipuncture of sampled dogs and poured into respective glass tubes without anticoagulant. The blood samples were allowed to clot at room temperature for about 5-6 hours and the sera were separated into appropriately labeled Eppendorf tubes and stored at - 20 °C until serological test was performed.

Rabies antibody detection

Rabies antibody was assayed using the quantitative indirect ELISA (i-ELISA, Department of Pathology, University Ibadan, Ibadan, Nigeria) technique as described previously (Ohore *et al.* 2007, Oluwayelu *et al.* 2015). The cut-off sample to positive ratio (SPR) was 0.25 (equivalent of 0.5 IU/ml), which corresponded to twice the optical density (O.D.) value of the negative control serum. The O.D. was read at 450 nm. Results were valid when the difference in the mean O.D. of the positive and negative controls was greater than 0.25, and the mean O.D. negative control was less than or equal to 0.25. Vaccinated dogs under this study with SPR greater than 0.25 were considered to have adequate antibody level antibodies against rabies.

Questionnaire

A structured questionnaire (n = 138) was administered to the dog owners to obtain information about the demography of the dogs (age, breed, sex, purpose of keeping the dogs, source of the dogs and management type). Information about the number of rabies vaccinations received, type and source of given vaccine and the last vaccination date as well as the awareness of the owners on rabies was also assessed.

Data analysis

Data obtained from the questionnaire were analysed using descriptive statistics and Chi square. The mean Optimal Density was analysed with SPSS (Version 20), ANOVA and student t-test. The significance was put at $p < 0.05$.

Results

From the total of 138 dogs sampled, 51.4% (71/138) were male and 48.6% (67/138) female. Proportions of 20.3% (n = 28) were less than six months of age, 9.4% (n = 13) were between 6-12 months of age while 70.3% (n = 97) were greater than one year of age. The breed distribution showed that 45.7% (n = 63) were Alsatian, 13.8% (n = 19) Boerboel, 10.1% (n = 14) Caucasian, 9.4% (n = 13) Mastiff, 7.2% (n = 10) Rottweiler and 13.8% (n = 19) other breeds (Table I). Vaccination records showed that 82.6% (114/138) had been vaccinated; 18.8% (26/138) of the sampled dogs had received only a single dose (primer dose), 63.8% (88/138) had received multiple doses of rabies vaccines while the remaining dogs (n = 24) were unvaccinated (Table I).

At the established SPR cut-off of 0.25 (equivalent of 0.5 IU/ml), only 23.2% (32/138) of the screened dogs had adequate rabies antibodies levels. Absence of detectable seroconversion was observed in 87 (76.3%) of the 114 vaccinated dogs screened. A proportion of 14.5% (20/138) of the seronegative dogs had received single vaccination dose and 48.6% (67/138) multiple vaccination doses. However, 3.6% (5/138) among the unvaccinated dogs were seropositive (Table I).

The study showed that 10.9% (15/138) males and 12.3% (17/138) females were seropositive. Although, more females 17/32 (53.1%) were among the seropositive than males, there was no significant difference between the two sexes ($p > 0.05$). There was an increasing seroconversion rate with age (Table 1). The number of positive dogs > 1 year is significantly higher than those < 6 months ($p < 0.05$) (Table I). Based on breed of dogs, the number of seropositive Alsatian breed dogs were significantly higher than Rottweiler breed ($p < 0.05$; Table I). Seroconversion rate among the dogs that had received multiple doses of rabies vaccines was higher 15.2% (21/138) than in those that received only a single dose 4.4% (6/138); but the difference was not statistically significant ($p > 0.05$, Table I).

The data from questionnaire respondents revealed that 121 (87.7%) of the screened 138 dogs were kept for guard duties while 17 (12.3%) were owned as pets. A total of 113 (81.9%) of the dogs were acquired through purchases and 18 (13.0%) as gifts. Majority of the vaccines used were imported from USA (n = 103; 90.3%) followed by Korea (n = 6; 5.3%), France (n = 3; 2.6%), Czech (n = 1; 0.9%) with only (n = 1; 0.9%) produced in Nigeria. There was no significant difference in the seroconversion rate based on the types of vaccines used. Among the dog owner

Table I. *Prevalence of rabies antibodies based on sex, age, breed and vaccination status of dogs in Abeokuta.*

Variable	Total sample collected	Positive (%)	Negative (%)	p Value
Sex				
Male	71	15 (10.9)	56 (40.6)	
Female	67	17 (12.3)	50 (36.2)	0.15
Total	**138**	**32 (23.2)**	**106 (76.8)**	
Age				
< 6 months	28	5(3.6)	23(16.7)	
6-12 months	13	3(2.2)	10(7.3)	0.80
> 1 year	97	24(17.4)	73(52.8)	0.04*
Total	**138**	**32 (23.2)**	**106 (76.8)**	
Breed				
Alsatian	63	14 (10.1)	49 (35.5)	0.02*
Boerboel	19	3 (2.2)	16 (11.6)	0.14
Mastiff	13	4 (2.9)	9 (6.5)	0.27
Caucasian	14	3 (2.2)	11 (7.9)	0.21
Rottweiler	10	3 (2.2)	7 (5.07)	
Others	19	5(3.6)	14(10.1)	0.07
Total	**138**	**32 (23.2)**	**106 (76.8)**	
Dose of rabies vaccine				
Single dose	26	6 (4.4)	20 (14.5)	0.89
Multiple dose	88	21 (15.2)	67 (48.6)	0.07
Unvaccinated	24	5 (3.6)	19 (13.7)	
Total	**138**	**32 (23.2)**	**106 (76.8)**	

*Significant at $p < 0.05$

Table II. *Demographics and awareness of dog owners on rabies and related epidemiological data on dog vaccination at selected veterinary clinics in Abeokuta, Ogun State, Nigeria.*

Variable/Category	Frequency (n)	Percentage (%)
Purpose of keeping dogs		
Pets	17	12.3
Guards	121	87.7
Total	**138**	**100.0**
Source of dogs		
Local	7	5.1
Bought	113	81.9
Gift	18	13.0
Total	**138**	**100.0**
Vaccination status		
Vaccinated	114	82.6
Unvaccinated	24	17.4
Total	**138**	**100.0**
Source of vaccine used		
USA	103	90.3
Korea	6	5.3
France	3	2.6
Czech	1	0.9
Nigeria	1	0.9
Total	**114**	**100.0**
Awareness of dog owners on rabies transmission		
Yes	120	86.9
No	18	13.1
Total	**138**	**100.0**

respondents, 120 (86.9%) were aware of how rabies could be contracted (Table II).

Discussion

Although, vaccination of dogs has been shown to be the most effective method of prevention of rabies in humans, cases of human rabies remain a significant problem in many developing countries including Nigeria. In this study, only 23.2% of the sampled dog population had the expected protective level of antibodies against rabies. This percentage is higher than that found in confined, hunting and roaming dogs in Ogun and Oyo States, Nigeria (Oluwayelu *et al.* 2015) but lower than that found in non-hunting confined dogs in Ogun State, Nigeria (Awoyomi and Ogundipe 2019). The result of this study also agrees with the report of seroconversion in only 32.6% of dogs presented for rabies vaccination at veterinary clinics in Lagos State, Nigeria, while majority (68.4%) of the dogs had low rabies seroconversion despite the fact that > 90% of the dogs had been vaccinated (Ishola *et al.* 2019). These indicate a consistently low population of protected animals' consequent to inadequate immune response despite high (82.6%) level of vaccination. This phenomenon could be responsible for the significant occurrence of rabies infection in dogs and subsequently in humans despite the vaccination coverage of over 70% as recommended by WHO for the control of rabies transmission (WHO 2019). The low antibody level in the vaccinated dogs may be due to vaccine failure arising from poor cold-chain storage that leads to poor vaccine quality (Dairo and Osizimete 2016). In a study of factors influencing the antibody response of dogs vaccinated against rabies in the UK, Kennedy and colleagues (Kennedy *et al.* 2007) observed considerably high failure rates for different vaccines tested.

Furthermore, vaccination of diseased or infected dogs coupled with non administration of booster doses, as at when recommended, could be contributory factors to the low seroconversion in vaccinated animals (Olugasa *et al.* 2011). The low level of antibody response could be associated with age, route of vaccination, breed of the dog, haplotype of specific breeds of dogs and sex

hormones (Coyne *et al.* 2001, Mansfield *et al.* 2004, Kennedy *et al.* 2007). This low level of antibody might thus be due to the type of vaccine used and mode of vaccine storage which is mostly challenging in the country due to electric power fluctuations (Ohore *et al.* 2007) since most of the rabies vaccines given to these dogs were imported. This study showed that majority of the vaccines were imported from USA and Korea while few were from France and Czech. Only 0.9% of the vaccines used was made in Nigeria. The findings of this study agree with Awoyomi and Ogundipe (Awoyomi and Ogundipe 2019) who reported that apart from the rabies vaccines produced by NVRI in Nigeria, rabies vaccines are imported from other countries of the world since the local production by the NVRI could not meet up with the demand of the country. Ishola and colleagues (Ishola *et al.* 2019) reported that more than two-thirds (77.6%) of dogs vaccinated against rabies at veterinary clinics in Lagos State, Nigeria were vaccinated with imported rabies vaccines, only few (13.2%) were vaccinated with the local NVRI rabies vaccine; majority (51.3%) of these imported rabies vaccines originated from Asia.

The absence of significant difference in seroconversion with the types of vaccines used found in this study remains consistent with earlier report of Ohore and colleagues (Ohore *et al.* 2007) who observed that there was no significant difference between the various types of rabies vaccines given. They concluded that there was a relative uniform but low potency among the commonly available rabies vaccines in use in Nigeria (Ohore *et al.* 2007). Incorrect or sub-optimal doses of vaccine used, inability of some dogs to seroconvert even with repeated doses of vaccine and poor immunogenicity of the vaccine, probably due to poor storage conditions or break in the cold chain could cause low immunogenicity of the vaccines (Ohore *et al.* 2007, Awoyomi and Ogundipe 2019). The success of efforts against vaccine-preventable diseases is attributable in part to proper storage and handling of vaccines; vaccines exposed to temperatures outside the recommended ranges could have reduced potency and protection (CDC 2019). Vaccines have to be protected from heat and light and should be stored as recommended by manufacturers and national immunization program. Irreversible and permanent loss of vaccine potency may occur by exposure to heat and/or light. Nearly all the inactivated and live attenuated vaccines require refrigerator storage temperatures between 35 °F to 46 °F (2 °C to 8 °C), with a desired average temperature of 40 °F (5 °C) (Arsalan 2014). Vaccine quality is maintained using a cold chain that meets specific temperature requirements.

The findings of young dogs of less than 6 months of age showing significantly lower seroconversion than adults agree with Gunatilake and colleagues and Awoyomi and Ogundipe (Gunatilake *et al.* 2003, Awoyomi and Ogundipe 2019); it may be due to poor immunological development in the young dogs. Mansfield and colleagues (Mansfield *et al.* 2004) reported that dogs less than a year old had a slightly higher probability of responding poorly than those more than one year old. Kennedy and colleagues (Kennedy *et al.* 2007) similarly observed that dogs less than one year of age elicited lower antibody response than adults. Interference of early postnatal vaccination before the recommended period in dogs possessing maternal immunity may be partly responsible for the poor seroconversion in the vaccinated puppies (Ohore *et al.* 2007).

The mean antibody titre of dogs over one year of age which was significantly higher than those of less than six months of age could therefore be as a result of immature immune system and/or maternal antibodies interference in the young dogs. This may be evidence of impaired serocoversion to rabies vaccine among puppies less than 3 months of age, as earlier documented (Morters *et al.* 2015). The higher prevalence of seropositivity observed in the female dogs may be due to better care received by females kept for breeding purpose in order to confer a greater maternal immunologic response on the puppies (Ohore *et al.* 2007). Anecdotal evidence shows that dog owners who are aware of rabies, more often ensure rabies vaccination of their dogs prior to breeding for the protection of the puppies.

The significantly higher ($p < 0.05$) number of Alsatian breed seropositive dogs compared to the Rottweiler may be due to acquired innate properties that confer a higher immune response and a more lasting immune-protection than in Rottweiler. This finding supports the observations of Kennedy and colleagues (Kennedy *et al.* 2007) that larger breeds of dog show lower immune response than smaller breeds. While the precise factors responsible for this appears unclear, the phenomenon confers a shorter immune-protection in the larger breed Rottweiler compared to other smaller dog breeds (Kennedy *et al.* 2007). Dogs which had multiple (booster) doses of the vaccine however, had higher antibody levels and prevalence 21 (15.2%) compared to dogs that had received a single (primer) dose. This is expected as most dogs achieve adequate amnestic seroconversion after booster vaccination especially when the vaccines are highly immunogenic. It is supposed that immunization of over 70% of dog population in rabies endemic regions prevent transmission of the disease (WHO 2019). It is worrisome however that the low seroconversion rate of only 23.2% found in 76.3% of the 114 vaccinated dogs investigated (which represented 82.6% of the total population sampled) would portend failure in arresting rabies transmission within the dog population and consequently, human/public health

hazard in rabies epidemiology in this ecological region. While this study is limited to dogs presented to veterinary clinics, the overall consequence when the total dog population is considered could be worse if rural (less cared for) dogs are included. This strongly identifies this phenomenon as a challenge in the control of rabies in Ogun State, Nigeria.

The higher presence of Alsatian breed among the screened dogs is attributed to the great popularity of this breed and the common use of these dogs for security guard purposes as indicated by owners. The high percentage of the dog owners indicating knowledge of how rabies could be transmitted, suggests considerable high level of awareness of the disease. This could have contributed to the high percentage of the vaccinated dogs as observed in the study in Abeokuta metropolis, Ogun State, Nigeria. The same may not be the case with the dog population (mainly local breeds) in rural areas whose management is mainly free-roaming and where veterinary services are less available (Oluwayelu *et al.* 2015).

Analysis of the post vaccination period in this study showed no significant difference in the antibody levels although there was a slightly higher prevalence at 6-12 months period. It is noteworthy that 3.6% of screened dogs that were unvaccinated showed seroconversion. While this could be due to passive maternal antibody in some of these unvaccinated dogs that were young puppies of less than 6 months of age, there should be caution with regards to occurrence of antibody against rabies in unvaccinated adult dogs. Since this may indicate field exposure and possible latent infection in such dogs, as rabies virus has been recovered from apparently healthy dogs (Aghomo *et al.* 1986). Taiwo and colleagues (Taiwo *et al.* 1998) had reported 4 out of 11 puppies less than 3 months of age, that died of rabies as confirmed by histopathology. A similar finding was reported earlier (Ezebuiro *et al.* 1980). These findings are of public health significance.

Despite the sampling limited to veterinary hospital visiting dogs only, which represent dogs that are receiving better veterinary health care and attention from their owners, the low antibody levels against rabies observed in this study suggest that the antibody levels against rabies among the entire dog population in Abeokuta, Ogun State could even be lower. There is therefore an urgent need for more effective and efficient strategy for rabies vaccination that would attain 70% to 80% of dog population coverage. A radical approach to rabies vaccination programmes is therefore necessary, as there should be sustained increase in mass vaccination. Vaccinations should involve the use of potent rabies vaccines that are handled and stored under cold-chain. More importantly, the antibody responses of dogs to rabies vaccination should be routinely monitored through consistent sero-profiling at a regular post-vaccination interval to ensure successful vaccination programmes.

Conclusion

Dog owners in Abeokuta had high awareness level of rabies. Dogs vaccinated against rabies had low seroconversion while unvaccinated dogs had prevalence of rabies antibodies; these portend public health risk. There is therefore a critical need for more effective and efficient strategy for rabies vaccination that would attain 70% to 80% of dog population coverage with potent vaccine. There is a need for enforcement of compulsory pre-exposure vaccination compliance and annual booster vaccinations using properly stored immunogenic vaccines against rabies in dogs as part of responsible dog ownership. Animal health authorities should conduct regular rabies sero-profiling of dogs, both those presented and vaccinated at veterinary hospitals and the general dog population in Abeokuta, Ogun State. These interventions should be given urgent attention in order to achieve the goal of zero rabies prevalence by the year 2030.

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