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Rabies Virus Neutralizing Antibodies in Unvaccinated Rabies Occupational Risk Groups in Niger State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AG, JUJ, HMK and AAD designed the study, seek for ethical approval, data analysis and drafted the initial manuscript and final review. Authors AG, MSA, PAO, AAO, AZ and MDH designed and involved in sample collection, shipment across the Niger State to Zaria, Nigeria and some literature search. Author AG supported by other laboratorians in CDC, USA made necessary arrangements for the shipment of samples to CDC, Atlanta, USA, conducted the laboratory procedures (RFFIT), literature review and reviewing of the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the presence of rabies virus neutralizing antibodies (rVNA) as well the potency of the rVNA in rabies occupational risk humans in Niger State of Nigeria.

Study Design: Cross-sectional study.

Place and Duration: Research was conducted at the Department of Veterinary Public Health, Ahmadu Bello University, Zaria, Nigeria and Rabies Unit, Centers for Disease Control and Prevention, CDC, Atlanta, USA, between May, 2012 and March, 2013

Materials and Methods: A total of 185 human volunteers were recruited from rabies risk occupational groups who filled a structured questionnaire on their previous bite history and vaccination status, between May and July, 2012. A 2 ml each of blood from volunteers was collected and centrifuged at 3000 rpm for 10 minutes and sera separated into pre-labeled vacutainers. Standard Rapid fluorescent focus inhibition test (RFFIT) was used to detect the presence of rVNA in the sera. Further end point titration of the rVNA positive human sera was conducted to determine the potency.

Results: The results indicated that, detectable titre of rVNA was recorded in 16.4% (23 of 140) viable human sera screened. Although from the questionnaire survey, 21.7% (5 out of the 23 positives) responded to have been vaccinated over ten years prior. At least 3 of the respondents (1 dog butcher and 2 dog meat consumers) who responded not previously vaccinated had some neutralizing antibody titre range of 0.65 – 0.7 IU/ml which is above the minimum protective titre (0.5IU/ml) recommended by WHO. Similarly, 3 respondents (2 veterinarians and 1 animal health personnel) who responded to have been previously vaccinated (> 10 years earlier) yet had a high titre range of 0.5 – 5.4IU/ml. The highest specific rate for rVNA of 25% each was seen amongst the dog butchers and pet owners followed by hunters (20%) and dog meat consumers (14.8%). Up to 125 (67.6%) of the volunteers do consume dog meat with only 12 (9.6%) of them being dog butchers who source dogs for slaughter from households within and outside their territories.

Conclusion: Although the WHO minimum protective titre of rVNA is 0.5 IU/ml, the presence of relatively high titres amongst these risk groups in this report is an indication of a serious public health threat. This study recommends the vaccination of rabies high risk groups and further screening of rabies occupational risk and non risk groups in the study area and Nigeria at large.

Keywords: Rabies antibodies; occupational risk groups; RFFIT; Niger State; Nigeria.

1. INTRODUCTION

The genus *Lyssavirus* is a group of single-stranded, negative-sense RNA viruses that cause rabies. Rabies virus (RABV) is the prototype *lyssavirus*, and all subsequently discovered *lyssaviral* species are collectively known as the rabies-related *lyssaviruses* [1].

The genus *Lyssavirus* contains up to 14 genotypes [2] but the 12th member Shimoni bat virus and the 13th member Bokeloh bat lyssavirus were newly proposed in 2010 [3] and in 2011 [4] respectively. Additional *lyssavirus* (Ikoma virus) from an African Civet was discovered in Tanzania making a total of 14 members of the genus *lyssavirus* [2]. The first member classical rabies virus (RABV) genotype 1 has a worldwide distribution, while Lagos bat virus (LBV) genotype 2, Mokola virus (MOKV) genotype 3 and Duvenhage virus (DUVV) genotype 4 have distribution restricted to Africa [2]. All *Lyssavirus*

cause illness in humans indistinguishable from classical rabies RABV except *Lagos bat virus* [5].

The virus neutralization methods (rapid fluorescent focus inhibition test or RFFIT and fluorescent antibody virus neutralization or FAVN) are the current reference methods prescribed by World Health Organization (WHO) and Office International des Epizooties (OIE) for detection of rVNA [6,7,8].

In about three decades ago, serological evidence of rabies virus antibodies has been reported in 15.93% of unvaccinated dogs in Southwestern Nigeria [9]. Furthermore rabies neutralizing antibodies have been reported in 28.7% of unvaccinated humans in Southwestern Nigeria [10]. About 10 million people receive post-exposure treatments each year after being exposed to rabies-suspect animals [11]. However, millions of humans, particularly the occupational risk groups in the developing countries remain unvaccinated due to lack of

awareness of the risks involved, non-availability and the prohibitive cost of the rabies vaccines.

The increased sourcing and slaughter of dogs for human consumption within and outside Niger State of Nigeria has been reported [12]. Presence of rabies antigen in the brains of some slaughtered dogs in Nigeria has also been documented [13,14,15]. The public health risk of presence of rabies antigens in slaughtered dogs is enormous, particularly on the occupational risk groups. Additionally, in a survey on fox trappers in Alaska in 1994, 3 trappers had rVNA though two were previously vaccinated, but the 3rd member had never received pre – or – post exposure yet has rVNA titre of 2.30IU/ml [16]. In a more recent report in Peru 11% (7 out of 63) of some villagers screened had rVNA in their sera, they were believed to have had bats deprecations in their locality [17]. The serological evidence of rabies virus neutralizing antibodies (rVNA) in occupational risk humans in Niger State of Nigeria was investigated and hereby discussed.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Niger State, Nigeria, a state in the north central region of Nigeria. The state is located between Latitudes 8° 30' N and 11° 30' N and between Longitudes 3° 30' and 7°20'E, comprising of three senatorial zones (Bida, Kontagora and Minna) with 26 local government areas. The state has a total human population of over 3.9 million [18] with abundant livestock resources having 199,812 dog populations in the state [19]. The state falls within Guinea (southern) Savanna Belt of Nigeria, with about 90% of its population living in the rural areas practicing subsistence farming. Niger State is the largest state in the country in terms of land area, which is about 76,000sq Km (or nearly 9 per cent of Nigeria's total land area). Fig. 1 represents an administrative map of Nigeria in Africa, with Niger State bounded with arrows.

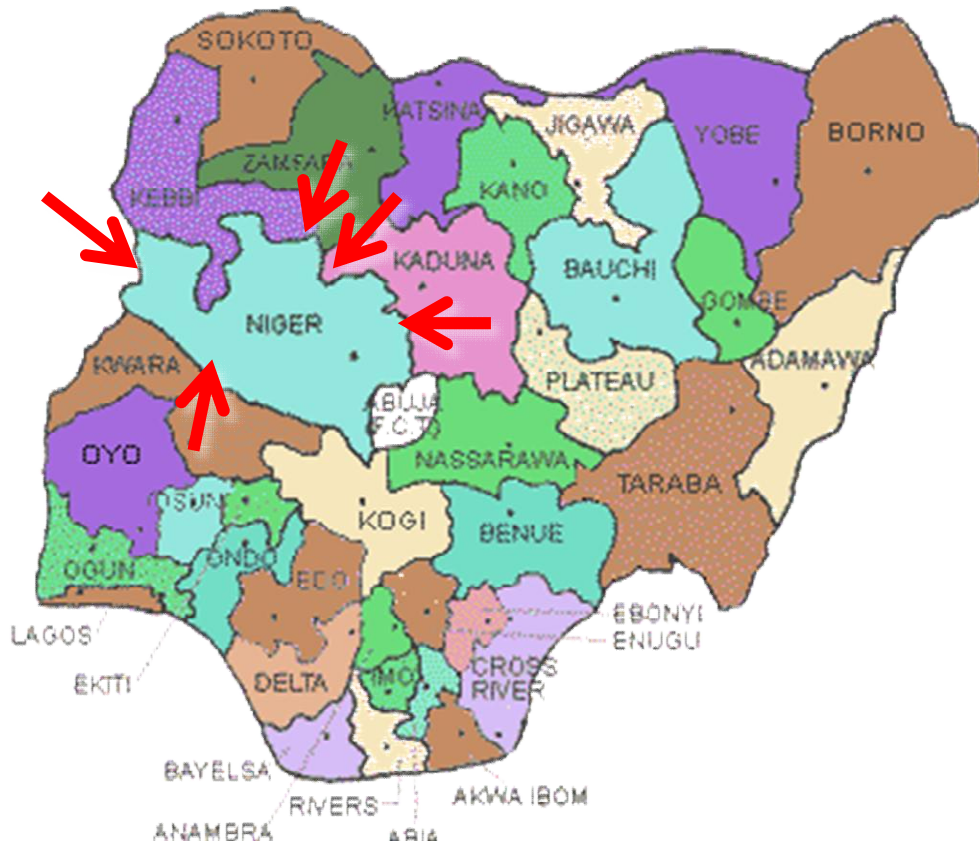


Fig. 1. Administrative map of Nigeria showing 36 states and Abuja study area bounded with red arrows

2.2 Sample Collection and Laboratory Procedure

The research was conducted following ethical approval as obtained. Using aseptic procedure, a 2mls of blood samples each were collected from 185 human rabies occupational risks volunteers (dog butchers, dog meat consumers, pet owners, hunters with dogs and Veterinarians/Animal health personnels) during the period (May and July, 2012) in the study area. Veterinarians/Animal health personnels (numbered 30) were recruited on visits to the Veterinary clinics. While, the other volunteers (numbered 155) were recruited from amongst those volunteers (dog butchers, dog meat consumers, pet owners and hunters with dogs) seen at the five selected dog slaughter points in Bida, Minna, Kontagora and Suleja towns of Niger State, Nigeria. The slaughter points are areas where bars exist in which dog meat and alcohol/beer are sold in the study area. A consent form and a questionnaire (containing the demographic information, history of dog bite, anti rabies vaccination, dog slaughter and consumption, sourcing of dogs for slaughter etc.) were filled by each volunteer earlier before the blood collection. The 2ml blood samples were allowed to clot on the spot, taken to the laboratory on ice pack, then centrifuged at 3000rpm for 10 minutes for serum separation and transferred to pre-labelled vacutainers and stored refrigerated. A total of 185 human sera with the questionnaires were shipped on dry ice to Rabies Program, Centers for Disease Control and Prevention (CDC), Atlanta, USA. A standard RFFIT test was conducted as described by Smith et al. [6] on the human sera in accordance with the CDC SOP for RFFIT [20]. Forty five of the serum samples were cytotoxic to the cell lines, only 140 sera were actually screened for the RFFIT. End point titration was further conducted on the rVNA positive human sera. The potency of the rVNA was calculated based on Reed and Maunch method [21] as described in the CDC SOP using the expression below:

$$\text{Number of IU/ml} = \left[\frac{\text{End-point titer of the test serum}}{\text{End-point titer of the reference}} \right] \times 2 \text{IU/ml in the reference serum}$$

2.3 Statistical Analysis

Data were analyzed using descriptive statistics and results presented in tables.

3. RESULTS AND DISCUSSION

The results have revealed that about 16.4% of rabies occupational risk humans had some detectable titre of rabies virus neutralizing antibodies (rVNA) in their blood with higher specific rates seen in the butchers and pet owners (Table 1). This suggests a prior exposure of these individuals to rabies viral antigen either at once or repeatedly in smaller doses due to their occupation or hobby. The detection of rabies antibodies in unvaccinated dogs and humans has long been reported by some workers in Nigeria. Aghomo et al. [9] had reported the presence of rabies virus antibodies in over 15.93% of unvaccinated dogs in the southwestern part of Nigeria. Similarly, Ogunkoya et al. [10] reported the presence of rabies antibodies in 30.7% and 28.6% of unvaccinated dogs and humans respectively in Nigeria. This present finding has therefore augmented the previous reports. The public health implication of this finding is whether or not these individuals or rVNAs detected is protective or it is just a mere presence of the antibodies. This study has no specific answers to this question, but our prior knowledge of the rabies virus and pathogenesis of the disease; at any point of entry of the virus, it assumes an eclipse state where the virus will remain dormant for a few hours, and may remain locally or have started moving on its pathway to CNS [22]. When it assumes activity, it will move passively via the axons of the peripheral nervous systems at between 0.5 – 1 mm/hr [23,24]. By that time body's immune response to the presence of the virus would have been present and could be detected with the RFFIT protocol as employed [6]. Whether or not the disease is progressing in these individuals is not known. However, it is pertinent to note that the principal immunological correlate of protection produced by vaccination or natural infection is neutralizing antibody [25]. Hence, the presence of rVNA is a strong pointer of previous exposure to rabies virus, particularly in the unvaccinated individuals/volunteers.

It has been shown that 25% each of dog butchers and pet owners, then 20% hunters and 14.8% dog meat consumers tested had detectable rabies virus neutralizing antibodies (rVNA) (Table 1). This suggests that dog butchers and pet owners have greater tendencies of being infected with rabies antigen in their occupations if the dogs they process or keep as pet have the rabies antigen. Furthermore, it was noticed that about 45

(24.35%) of samples processed were cytotoxic to the cell line used (Table 1). What is responsible for this finding is not known, but it can be seen that about 44 out of the 45 samples were those samples collected from other occupational groups at the dog slaughter points. It appears that the environment is not as controlled as those samples obtained from veterinarians/animal health personnels in the clinics which were immediately clotted and centrifuged hence only one sample of the latter group was cytotoxic. It seems, therefore, that these samples from the former groups were not fully separated and possibly have some traces of red blood cells which might have interfered with the process and rendered the sera toxic to the cell line.

However, based on the principle of immunology, T-helper cells actually contribute to the development of immunity, whereas cytotoxic T cells do not appear to play a role in protection and may actually be detrimental to the host [25]. This could be another possible reason why cytotoxicity was observed in these 45 samples in this study.

It was, however, observed that few of these positives (5 out of 23 positives) in the present study, from the questionnaire results had a history of anti-rabies vaccination over 10 years earlier but with relatively high titres presently seen (Table 2). This may suggest that the presence of rVNA in these individuals may be due to previous vaccinations received. Even then WHO recommends annual revaccination/immunization of individuals at risk, so the absence of annual re-immunization of these individuals for about 10 years, yet with a relatively high titre value range (0.5 – 5.4IU/ml) (Table 2). It leaves us with yet another epidemiological niche in rabies immunization. The vaccine type used in the last 10 years on these individuals was Purified vero cell vaccine, PVCV (Verorab^R). Does the potency of the vaccine type used has such long duration of protection is not known. Could these individuals, possibly, been exposed to the rabies antigen in their day to day activity of recent, which triggered this response, is also not known.

However, in a report in India, some patients bitten by rabid foxes that were previously vaccinated with PVCV and re-evaluated on days 870 and 1020 post initial vaccination had titres < 0.5IU/ml. However, on a booster dose on day 1020 and re-evaluated (30 days later) on day 1050, the patients have elicited a good anamnestic response, as indicated by the

increased value in antibody titres to 26.11IU [26]. This suggests that the PVC rabies vaccine used by those vaccinated volunteers in our present study, may have remained viable for such a long period of time, but need further evaluation.

It was also seen that even some unvaccinated volunteers had titres greater than the minimum protective level 0.5IU/ml (Table 2) recommended by the World Health Organization [11]. This is another bewildering situation of our findings, but the question is are these unvaccinated individuals yet with relatively high titres protected? To our understanding the presence of relatively high titres (0.6 – 0.7 IU/ml) in unvaccinated volunteers may not suggest that the individuals are protected. We advocated for some two booster doses one week apart as recommended by the WHO on previously vaccinated individuals [27]. It should, however, be noted that there were reports of some detectable titres of rVNA from unvaccinated occupational rabies risk individuals reported elsewhere. For example, there was a report on the presence of rVNA in an unvaccinated fox trapper with a relatively high rVNA titre of up to 2.30IU/ml in Alaska [16]. Similarly, 11% (7 out of 63) of some villagers in Peru who were believed to have had vampire bats depredation had rVNA with 3 of them having titres range between 0.6 – 2.8 IU despite they were previously unvaccinated [17]. This indicated that the presence of rVNA in occupational risk groups as in our present study is known to exist.

About 73.9% (17 out of 23) volunteers who showed detectable rVNA in the present study had titres below the minimum protective level (Table 2). It has been emphasized that natural rabies virus infections only rarely induce a protective immune response in humans [28,29]. This may probably explain why most of the rVNA detected from the unvaccinated volunteers were below the WHO recommended minimum protective level (0.5 IU/ml) (Table 2).

It was noted and observed from the questionnaire data that amongst the 155 volunteers (dog butchers, dog meat consumers, hunters and pet owners) whose blood were collected at the dog slaughter points, a total of 125 of them do consume dog meat. It appears that the consumption of dog meat cuts across all or many occupational groups in the study area. Of the one hundred and twenty five respondents that consume dog meat, 51 (40.8%) were civil servants, while 12 (9.6%) engaged in the actual

dog butchering, sourcing their dogs for slaughter from households within and outside their territories (Table 3). This outcome may suggest that the eating of dog is widely accepted in the study area especially amongst the civil servants. Based on the fact that all the samples (except the 30 from veterinarians/animal health personnels) were collected at the dog slaughter and consumption points and 125 volunteers do eat dog meat. It appears that exposure to the virus and subsequent response to detection of rVNA in some volunteers may be due to gradual and continuous exposure to the rabies virus in

the possibly rabies antigen contaminated environment or utensils where dogs are slaughtered and processed.

Furthermore, it was evident that two of the volunteers were bitten by a dog and were vaccinated between 19 and 21 years earlier (Table 3), yet, none of these individuals developed rVNA either due to vaccination or infection. Probably longer duration of vaccination and type of vaccine used may have been responsible for the non detection of rVNA in these individuals.

$$\text{Number of IU/ml} = \frac{\text{End-point titer of the test serum}}{\text{End-point titer of the reference}} \times 2 \text{ IU / ml in the reference serum}$$

Table 1. Rabies antibody detection of rabies occupational risk groups in Niger State, Nigeria

Volunteers	Total number collected	Number cytotoxic	Negative	Positive (%)
Butchers	12	4	6	2 (25)
Dog meat consumers	113	32	69	12 (14.8)
Pet owners	24	8	12	¹ *4 (25)
Hunters	6	0	5	1 (20)
Veterinarians	6	0	4	*2 (8.7)
Anim. Hlth. Personnels	25	1	21	*2 (13.8)
Total	185	45	117	23 (16.4)

Key: * = they were previously vaccinated, ¹* = 1 previously vaccinated

Table 2. Rabies antibody titre for all the RFFIT positive human volunteers in Niger State, Nigeria

S/no.	Sample ID.	Group	Sample date	Titre	IU/ml	Location of volunteer
1.	001H	DB	26 May 2012	5	0.05	Bida
2.	007H	DC	26 May 2012	7	0.07	Bida
3.	019H	DC	26 May 2012	11	0.11	Bida
4.	020H	DC	26 May 2012	11	0.11	Bida
5.	031H	DC	27 May 2012	65	0.65	Minna
6.	032H	DB	27 May 2012	65	0.65	Minna
7.	040H	PO	28 April 2012	8	0.08	Minna
8.	045H	DC	28 April 2012	13	0.13	Minna
9.	048H	DC	24 May 2012	42	0.42	Bida
10.	049H	PO	24 May 2012	11	0.11	Bida
11.	073H	DC	24 May 2012	29	0.29	Bida
12.	076H	DC	24 May 2012	11	0.11	Bida
13.	092H	DC	31 May 2012	13	0.13	Minna
14.	096H	PO**	1 June 2012	7	0.07	Minna
15.	108H	DC	1 June 2012	11	0.11	Bida
16.	111H	HT	2 June 2012	13	0.13	Bida
17.	114H	DC	2 June 2012	7	0.07	Bida
18.	127H	PO	5 June 2012	13	0.13	Minna
19.	139H	DC	22 June 2012	70	0.7	Bida
20.	167H	AH**	20 July 2012	50	0.5	Minna
21.	177H	VET*	20 July 2012	50	0.5	Minna
22.	181H	AH*	20 July 2012	17	0.17	Minna
23.	182H	VET*	20 July 2012	540	5.4	Suleja

Key: DB = Dog butcher, DC = Dog consumer, PO = Pet owner, HT = hunter, AH = Animal health personnel, VET = Veterinarian, * = Vaccinated, ** = Bitten & Vaccinated, H = Human sera. Reference serum titre used was 200

Table 3. Summary of demographic information about dog meat consumers in Niger State, Nigeria

Occupation	Number of dog meat consumers (%)	Vaccination against rabies	Source of dog for slaughter
Civil Servants	51(40.8)	^{φφ} Never	*Only consume
Business	24(19.2)	^φ Never	*Only consume
Students	22(17.6)	Never	*Only consume
Farmers	7(5.6)	Never	*Only consume
Private practitioners	6(4.8)	Never	*Only consume
Hunters	3(2.4)	Never	*Only consume
Dog butchers	12 (9.6)	Never	**Households
Total	125(100)	Never	

Key: ^{φφ} = one respondents bitten by dog and vaccinated 21 years earlier, the other bitten but not vaccinated 8 years earlier,

^φ = one respondent bitten by dog and vaccinated 19 years earlier

* = do not slaughter dogs, ** = slaughter and consume dog meat

On a general consideration, the rabies viruses spread from peripheral sites of entry to the central nervous system (CNS) tissues via axonal transport, thereby bypassing the specialized features of the neurovasculature, the blood-brain barrier (BBB) [30]. Once the virus reaches CNS tissues three alternative outcomes are likely to occur as hypothesized [30]: (1) the BBB remains intact and the infection is lethal due to the absence of an antiviral CNS immune response (2) immune effectors cross the BBB and mediate a CNS antiviral immune response with extensive immunopathology that contributes to the disease, or (3) immune effectors cross the BBB and clear the virus from the CNS without significant pathological consequences. It is well known that in humans naturally infected with rabies virus the latter outcome is exceedingly rare [30]. In our present study, whether the virus is in the CNS or not, whether the immune effectors (the T- helper cells, rabies specific B-cells) have crossed the BBB or not are not known. One fact is that peripheral immune response was confirmed by the present presence of rVNAs as a response to natural infection due to occupational exposure by unvaccinated volunteers.

4. CONCLUSION

This study concludes that there is serological evidence of rVNA in the serum of vaccinated and unvaccinated rabies occupational risk groups in Nigeria. Rabies occupational risk groups are at risk of contracting rabies without overt symptoms of rabies, but serological response with some reasonable titres that may or may not be protective.

5. RECOMMENDATION

This study recommends further survey/screening of rabies occupational risk groups for rVNA as well as vaccination of rabies occupational risk groups and public awareness campaigns in the state and Nigeria at large.

CONSENT

A participant information leaflet was given and each volunteer filled and signed a consent form as contained in the protocol before sample collection.

ETHICAL APPROVAL

Ethical approval has been given both from the state ethics committee and the Ahmadu Bello University, ethics committee No. ABUTH/HREC/TRD/36. Rabies antibody positive volunteers were vaccinated free. Similarly, we hereby declare that "principles of laboratory animal care" (NIH publication no. 85-23, revised 1985) were followed, as well as specific national laws were followed. All experiments have been examined and approved by the appropriate ethics committee".

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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