

Original Research Article

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

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

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 2ml each of blood from volunteers was collected and centrifuged at 3000 rpm and sera separated in 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: 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. Similarly, 3 respondents (2 veterinarians and 1 animal health personal) who responded to have been previously vaccinated (> 10 years earlier) yet had 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%).

Conclusion: Although the WHO minimum protective titre of rVNA is 0.5 IU/ml, the presence of high titres amongst these risk groups in this report is a serious public health threat. This study recommends the vaccination of rabies high risk groups and further screening of rabies occupational 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. 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 WHO and 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 South-western Nigeria [9]. Furthermore rabies neutralizing antibodies have been reported in 28.7% of unvaccinated humans in South-western 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 on 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 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 depredation 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. MATERIAL 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). Figure 1 represent administrative map of Nigeria in Africa, with Niger State bounded with arrows.

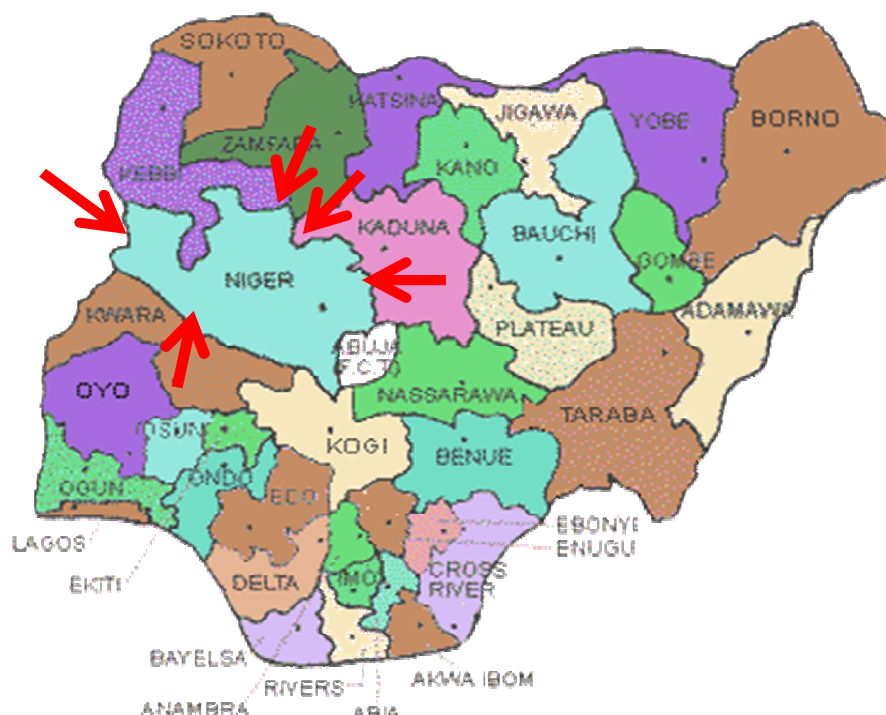


Figure 1: Administrative map of Nigeria showing 36 states and Abuja study area bounded with red arrows

2.2 Sample collection and laboratory 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 same period (May and July, 2012) in the study area. Veterinarians/Animal health personnels were recruited on visits to the clinics while, the other volunteers were recruited from amongst those volunteers seen at the five selected dog slaughter points in Bida, Minna, Kontagora and Suleja towns of Niger State. A consent form and a questionnaire 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-labeled 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* (1996)[6] on human sera in accordance with the CDC SOP for RFFIT [20]. Forty five of the sera samples were cytotoxic to the cell lines, only 140 sera were actually run for the RFFIT. End point titration was further conducted on the rVNA positive human sera. Potency of the rVNA was calculated based on Reed and Maunch method [21] as described in the CDC SOP using the expression below:

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

Statistical analysis: Data were analyzed using descriptive statistics and results presented in tables

3. RESULTS AND DISCUSSION

Results has 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 (tables 1). This suggests 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* (1987) had reported the presence of rabies virus antibodies in over 15.93% of unvaccinated dogs in south western part of Nigeria [9]. Similarly, Ogunkoya *et al* (1990) reported the presence of rabies antibodies in 30.7% and 28.6% of unvaccinated dogs and humans respectively in Nigeria [10]. This present finding has therefore augments the previous reports. The public health implication of this finding is whether or not these individuals or rVNA detected are 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 few hours, and may remain locally or have started movement on its pathway to CNS [22]. When it assumes activity it will move passively via the axons of the peripheral nervous systems at between 50 – 100 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 RFFIT protocol as employed [6]. Whether or not the disease is progressing in these individuals is not known, but 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 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 the 45 samples were those 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 from these groups was cytotoxic. It seems therefore, that these samples were not fully separated and possibly have some traces of red blood cells which might have interfered with the process and render the sera toxic to the cell line.

Table 1: Rabies antibodies detection on rabies occupational risk groups in Niger State, Nigeria

Volunteers	Total number collected	Cyto-toxic	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)

121 Key: * = they were previously vaccinated, ¹* = 1 previously vaccinated
122

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

135 However, in a report in India, some patients bitten by rabid foxes that were previously
136 vaccinated with PVCV and re-evaluated on days 870 and 1020 post initial vaccination had
137 titres < 0.5IU/ml. However, on a booster dose on day 1020 and re-evaluated (30 days later)
138 on day 1050, the patients have elicited a good anamnestic response, as indicated by the
139 increased values in antibody titres to 26.11IU [25]. This suggests that the PVCR vaccine
140 used by those vaccinated volunteers in our present study, may have remained viable for
141 such a long period of time, but need further evaluation.

142 It was also seen that even some unvaccinated volunteers had titres greater than the
143 minimum protective level 0.5IU/ml (table 2) recommended by the World Health Organization
144 [11]. This is another bewildering situation of our findings, but the question is are these
145 unvaccinated individuals yet with relatively high titres protected? To our understanding the
146 presence of high titres (0.6 – 0.7 IU/ml) may not suggest that the individuals are protected
147 and may suggest some two booster doses as recommended by WHO on previously
148 vaccinated individuals [26]. However, there were reports of some detectable titres of rVNA in
149 unvaccinated occupational rabies risk individuals. For example there was a report of the
150 presence of rVNA in an unvaccinated fox trapper with relatively high rVNA titre of up to
151 2.30IU/ml in Alaska [16]. Similarly, 11% (7 out of 63) of some villagers in Peru who were
152 believed to have had vampire bats depredation had rVNA with 3 of them having titres range
153 between 0.6 – 2.8 IU despite previously unvaccinated [17]. This indicated that the presence
154 of rVNA in occupational risk groups as in our present study is known to exist.

155
156 **Table 2:** Rabies antibody titre for all the RFFIT positive human volunteers in Niger
157 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 June2012	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.

4. CONCLUSION

This study concludes that there is serological evidence of rVNA in serum of 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. RECOMMENDATIONS

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

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 ethical committee and the Ahmadu Bello University, ethics committee (abuth/hrec/trg/36). 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 where applicable. all experiments have been examined and approved by the appropriate ethics committee"

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263 **APPENDIX**