# Antibody levels against rabies among occupationally exposed individuals in a Nigerian University

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**Summary**

The authors investigated the levels of anti-glycoprotein antibodies against rabies virus in the sera of occupationally exposed humans at the University of Ibadan, Nigeria. A quantitative indirect enzyme-linked immunosorbent assay (ELISA) was used to detect rabies virus anti-glycoprotein antibodies in sera from 20 zoological garden workers, 20 veterinarians and 30 clinical veterinary students at the University of Ibadan. The sera were obtained between September 2008 and February 2009. Of these 70 healthy individuals, 29 (41.4%) consisting of 15 zoological garden workers (75.0%), 13 veterinarians (65.0%) and 1 veterinary student (3.3%) were immune to rabies virus (antibody titre ≥0.5 equivalent units per ml), while 41 (58.6%) were not immune. The prevalence of rabies anti-glycoprotein antibody was higher within the older segment of the study population than among the younger veterinary students. Almost all those who had spent at least 10 years on the job had higher levels of rabies vaccination compliance and were immune. Our results indicated that there is low anti-rabies immunity among occupationally exposed individuals at the University of Ibadan. There is a need for a complete course of primary and booster vaccinations of professionals exposed to the rabies virus. The impact of these results on rabies control in Nigeria is discussed.

**Keywords**

Antibody, Exposure, Ibadan, Immunity, Neutralising antibody, Nigeria, Occupational hazard, Rabies, Vaccination.

## Livelli anticorpali contro il virus della rabbia in individui esposti a causa dell'attività svolta all'Università di Ibadan, Nigeria

**Riassunto**

*Gli autori hanno ricercato i livelli di anticorpi anti-G contro il virus della rabbia in campioni di siero di individui esposti a causa dell'attività svolta all'Università di Ibadan, Nigeria. Campioni di siero di 20 operatori del giardino zoologico, 20 veterinari e 30 studenti di Clinica Veterinaria sono stati sottoposti a dosaggio quantitativo indiretto con metodica ELISA. I campioni sono stati raccolti nel periodo settembre 2008-febbraio 2009. L'analisi dei sieri dei 70 operatori, tutti soggetti sani, ha permesso di stabilire come 29 individui (41,4%), comprendenti 15 operatori del giardino zoologico (75,0%), 13 veterinari (65,0%) e 1 studente di veterinaria (3,3%), fossero immuni al virus della rabbia (titolo anticorpale ≥0,5 eu/mL) a differenza dei restanti 41 soggetti (58,6%). La prevalenza dell'anticorpo antiglicoproteina della rabbia è risultata maggiore nella fascia di età più elevata della popolazione presa in esame rispetto agli studenti di veterinaria. La quasi totalità degli individui presenti nella struttura operativa da*

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*almeno 10 anni ha manifestato livelli più elevati di compliance al vaccino della rabbia risultando immune. Lo studio effettuato ha indicato una bassa immunità verso il virus della rabbia degli individui esposti il che sottolinea la necessità di un ciclo completo di prime vaccinazioni e richiamo per i professionisti esposti al virus. Si discute l'impatto dei risultati ottenuti sul controllo della rabbia in Nigeria.*

**Parole chiave**

Anticorpo, Anticorpo neutralizzante, Esposizione, Ibadan, Immunità, Nigeria, Rischio occupazionale, Rabbia, Vaccinazione.

## Introduction

Rabies is a highly fatal disease. It is mainly transmitted in developing countries through bites of rabid dogs (9, 16). Rabies is recognised as a neglected disease by the World Health Organization (WHO) and, perhaps more accurately, a disease of neglected communities (7). Emphasis of governments and non-governmental agencies in Africa is mainly focused on human immunodeficiency virus/ acquired immunodeficiency syndrome (HIV/ AIDS), poliomyelitis, tuberculosis and malaria, yet rabies constitutes a much greater health challenge and economic burden in Africa than imagined (7, 15). Approximately 24 000 people (mainly children) die of the disease each year in Africa (7, 15). In view of the extremely high fatality rate of human rabies, the prevention of rabies infection is of utmost importance (9, 16). Therefore, individual pre-exposure immunisation of people at continued risk of exposure to rabies and post-exposure treatment is recommended (6, 25). About eight million people receive costly post-exposure prophylaxis each year in Asia and Africa after having been bitten by rabid or suspected rabid animals (15, 21). Recommended protocols and booster injections rely on rabies virus antibody titres in the serum of individuals. In particular, boosters should be administered when the titre falls below 0.5 IU/ml (24, 25, 26). The quantification of anti-rabies antibodies is therefore of great interest and is frequently employed to determine the immune status following pre-exposure prophylaxis.

Specific immunity after a correct primary vaccination with a WHO-approved vaccine against rabies offers protection for at least two years (4, 19). Rabies researchers need to be laboratory tested every six months, employees in rabies diagnostic laboratories once a year and veterinarians every two years (6). This recommendation is popularly adhered to in the developed countries of Europe and Americas (8); however, in some developing countries of Africa, including Nigeria, it is largely unheeded (9, 10, 12, 16). The situation in Nigeria is one of inconsistencies in vaccination programmes and vaccine failures (17, 19, 22). There is a dearth of published reports on protection levels against rabies among occupationally exposed individuals in Nigeria.

The virus neutralisation tests (rapid fluorescent focus inhibition assay or RFFIT and fluorescent antibody virus neutralisation or FAVN) are the current reference methods (14, 20, 21, 26), prescribed by the WHO and the World Organisation for Animal Health (*Office International des Épizooties*: OIE). However, these methods are time-consuming and expensive. Furthermore, many of these techniques require very well-trained technicians and laboratory facilities to observe the precautions necessary for handling live rabies virus (20, 26). In view of these challenges, the Platelia Rabies II enzyme-linked immunosorbent assay (ELISA) was developed and evaluated in terms of sensitivity (98.6%), specificity (99.4%), accuracy and precision for virus neutralising antibody detection (13, 20). Platelia Rabies II ELISA was considered to be as sensitive and specific as the current standardised reference method. The method is simple, safe and rapid and can be considered a useful alternative to the neutralisation test (13). The Platelia Rabies II ELISA was used in this study in view of its relative advantages for limited laboratory facilities and technicians.

The objective of this study was to determine and quantify levels of anti-rabies glycoprotein antibodies in sera from veterinary students, veterinarians and zoo workers at the University of Ibadan in Nigeria, to measure humoral immune response to anti-rabies

 

vaccination. This paper presents the effect of vaccine dose, length of presence in occupation and funding behaviour of university administrations towards anti-rabies vaccination in Ibadan for those members of staff of the university who are occupationally exposed to rabies. The World Rabies Day 2008, tagged 'Working together to make rabies history', was a unique opportunity for a multidisciplinary team of investigators (physicians and veterinarians) at the university to assess the efficacy of the biennial pre-exposure rabies vaccination used at the university. The results of this investigation and those conducted on second-year clinical veterinary students of the university who are also occupationally exposed to rabies, are described and evaluated to show the levels of seroconversion and immunity against rabies among the different groups.

# Materials and methods

## Sampling method and specimen collection

Using stratified randomisation, 70 occupationally exposed members of staff and students of the University of Ibadan were selected for this study (Table I). This comprised 20 staff members of the Veterinary Teaching Hospital (VTH) (of a total of 92 members of staff of the hospital who worked in contact with animals which included 47 veterinary clinicians, 23 animal health technologists and 22 animal handlers; consultants were not included in this study); 20 zoological garden workers (out of a total of 30) and 30 second-year clinical veterinary students (out of a total of 62). Relevant information, including rabies immunisation records, was obtained through semi-structured questionnaires submitted to each person tested. With the aid of sterile needles and syringes, 2.5 ml of blood was collected through the cephalic vein into plain sample bottles (without anticoagulants) and allowed to clot. Sera were obtained by centrifuging at 3 000 rpm and these samples were temporarily stored at –4°C until the test was conducted.

## Detection of rabies anti-glycoprotein antibodies

A quantitative indirect ELISA (i-ELISA), using the Platelia™ Rabies II kit (Bio-Rad, Marnes-la-Coquette) (13, 21), was used to detect rabies virus anti-glycoprotein antibodies. The kit included a microplate that was pre-coated with rabies glycoprotein extracted from the inactivated and purified virus membrane. The optical density (OD) values for the test specimens were compared with the OD values of positive controls and antibody titres expressed as equivalent units per ml (eu/ml), were obtained from a standard OD antibody titre curve. All steps were conducted in accordance with the instructions of the manufacturer (13, 21) and results were read using an ELISA reader (IRE 96™, Saint Jean d'Illac) at a wavelength of 450-620 nm. Subjects were considered to be immune against rabies virus infection if they produced ELISA titres of ≥0.5 eu/ml (13, 21).

Rabipur™ (Chiron Behring Vaccine Ltd, India), a purified chicken-embryo cell rabies vaccine, was used for pre-exposure vaccination of occupationally exposed staff of the University of Ibadan. One dose (1 ml of ≥2.5 IU/ml vaccine potency) was administered intramuscularly every two years at the University

Table I
Some demographic characteristics of the three study groups

| Group | No. | Male | Female | Duration of occupational exposure (years) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 1-5 | 6-10 | 11-15 | 16-20 | 21-25 | 26-30 |
| Veterinary students | 30 | 20 | 10 | 30 | 0 | 0 | 0 | 0 | 0 |
| Veterinarians | 20 | 16 | 4 | 9 | 4 | 5 | 0 | 1 | 1 |
| Zoo workers | 20 | 14 | 6 | 5 | 6 | 5 | 1 | 1 | 2 |
| Total | 70 | 50 | 20 | 44 | 10 | 10 | 1 | 2 | 3 |

Health Service, Jaja Clinic, in Ibadan. The most recent was in November 2008.

## Donors' questionnaire survey

A follow-up questionnaire was provided to the donors and completed forms were analysed using Microsoft Excel®. An individual questionnaire was conducted within two months of the vaccination day so as to assess the vaccination history and to collect data on the length of service at the zoo and VTH (i.e. job specification and age) and animal bite history (i.e. species, breed and age) characteristics and, for unvaccinated persons, the reasons for failure to present for vaccination. Interviewees were asked to produce vaccination certificates as proof of vaccination. In cases where a vaccination certificate was not available, the names of the individuals were recorded for certification of vaccination status from the vaccination register at the University Health Centre.

# Results

Thirteen out of 20 (65.9%) veterinary staff, 15 out of 20 (75.0%) zoo workers, and 1 out of 30 (3.3%) veterinary students had rabies antibody levels that exceeded 0.5 eu/ml (range 0.5 to 4.1 eu/ml) (Table II). As shown in Table III, only 14.3% of the veterinary staff and zoo workers who had spent 1 to 5 years on the job were immune to rabies, while individuals who had spent 6-10 years and 11-15 years on the job all had antibody levels that exceeded the 0.5 eu/ml threshold. In comparison to this,

Table II
Rabies antibody titres expressed as equivalent units per ml among occupationally exposed humans tested

| Group | Male | | | Female | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Titre >0.5 eu/ml | Mean ±SD | No. tested | Titre >0.5 eu/ml | Mean ±SD | No. tested | Titre >0.5 eu/ml | Mean ±SD | Size |
| Veterinary students | 0 | 0±0.0 | 19 | 1 | 0.9±0.0 | 11 | 1 | 0.90±0.0 | 30 |
| VTH workers | 10 | 3.91±0.21 | 15 | 3 | 4.07±0.06 | 5 | 13 | 3.95±0.19 | 20 |
| Zoo workers | 13 | 2.83±1.18 | 18 | 2 | 2.60±2.12 | 2 | 15 | 2.80± 1.23 | 20 |
| Total | 23 | 3.30±1.10 | 52 | 6 | 3.48±1.33 | 18 | 29 | 3.25±1.14 | 70 |

SD standard deviation
VTH Veterinary Teaching Hospital, Ibadan

Table III
Rabies antibody levels in relation to number of years in occupation

| Years of occupational exposure | Total | Male | | | Female | | | Antibody titre mean ±SD |
|---|---|---|---|---|---|---|---|---|
| | | Titre >0.5 eu/ml | Antibody titre mean ±SD | Size | Titre >0.5 eu/ml | Antibody titre mean ±SD | Size | |
| 1-5 | 44 | 3 | 2.35±1.91 | 31 | 1 | 3.8±0.0 | 13 | 2 | 1.0±0.14 |
| 6-10 | 10 | 10 | 3.11±1.45 | 8 | 8 | 3.01±1.40 | 2 | 2 | 2.45(2.19) |
| 11-15 | 9 | 9 | 3.33±0.82 | 8 | 8 | 3.25±0.83 | 1 | 1 | 4.0(0) |
| 16-20 | 1 | 1 | 3.0±0.0 | 1 | 1 | 3.0±0.0 | 0 | 0 | 0(0) |
| 21-25 | 2 | 2 | 4.05±0.08 | 2 | 2 | 4.05±0.07 | 0 | 0 | 0(0) |
| 26-30 | 4 | 4 | 4.05±0.09 | 2 | 2 | 4.0±0.14 | 2 | 2 | 4.1±0 |
| Total | 70 | 29 | 3.25±1.15 | 52 | 23 | 2.88±1.27 | 18 | 6 | 3.03±1.58 |

eu equivalent unit
SD standard deviation

antibodies above this threshold were found only in 1 out of 30 (3.3%) veterinary students tested. Overall, antibody levels increased from 1-5 years to 10-30 years on the job. This pattern is seen in Figure 1. Thus, the prevalence of antibodies against rabies was lowest in veterinary students and veterinarians and zoo workers who were new on the job, increased with the number of vaccinations on the job, and remained almost consistently at the same high level for those who had been in the same occupation for over 10 years.

## Discussion and conclusions

This study revealed that second year clinical veterinary students displayed significant neglect for rabies pre-exposure vaccination at the University of Ibadan. Approximately 50% (30/62) of students in the class donated their sera for the study and were tested. Only 1 (3.3%) was immune. The student was immune following post-exposure prophylaxis in a case of a suspected rabid dog bite. In a country where canine rabies is endemic, this situation indicates the high risk of exposure to rabies among veterinary students. Rabies in humans and animals has a long history in Nigeria and has been widely reported in the literature. Typical clinical cases (19, 21) and confirmed atypical signs of the disease, with a fatal outcome in humans and animals (17) have been reported. The virus was isolated from apparently healthy unvaccinated dogs (3), vaccinated dogs (19) and from an eight-week old puppy (1). All clinical veterinary students attend to dogs and cats routinely at the VTH in Ibadan and occasionally do home calls arranged privately.

All tested students responded to the questionnaires and were unanimous in their claims to understanding the risks involved in handling dogs when unprotected. All the students placed very high importance on pre-exposure rabies vaccination. Only 46.7% (14/30) admitted that the cost was affordable and 53.3% (16/30) would pay for it if the university authority would not. A dose of the vaccine costs two thousand five hundred naira (N2 500 which is equal to about US$22.7 at N110 = US$1) on average, but the WHO recommends a complete course of primary vaccination that requires three injections to be given on days 0, 7 and either day 21 or 28 (24, 25), costing N7 500 (approximately US$68.1) in a country with a low per-capita income. This socio-economic factor offers a clue to the behaviour of the students towards anti-rabies pre-exposure vaccination. About a decade ago (until 1998), the cost of anti-rabies vaccination for veterinary students and occupationally exposed staff was borne by the university administration. That practice has since changed,

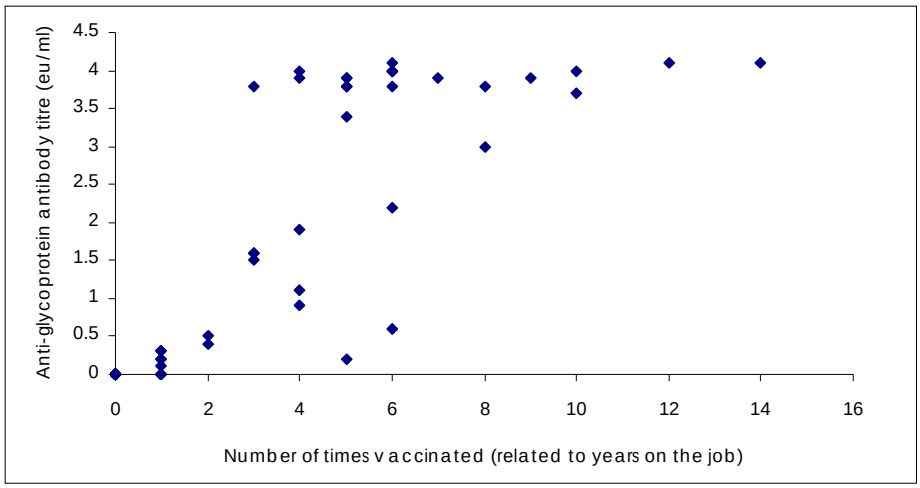


Figure 1
Antibody titre and frequency of vaccination among occupationally exposed staff (40) and students (30) at the University of Ibadan

but veterinary students still consider that the university administration should bear the cost for them.

The university continues to support staff anti-rabies pre-exposure vaccination. This may probably explain why the length of presence on the job has had a positive impact on the level of immunity. The level of rabies antibody in workers 1 to 5 years on the job at the zoological garden and at the VTH was significantly lower than among workers that had been 10 years and above on the job. In the same way, the majority of VTH and zoo workers believed that the employers should pay for their immunisation. This finding indicates that the level of rabies pre-exposure vaccination compliance among VTH and zoo workers may be influenced by changes in funding behaviour of the university authority. The cost of immunisation was considered expensive by most respondents, especially the lower income workers, including animal handlers at the VTH. If the university authority withdraws funding, most workers would not receive pre-exposure rabies immunisation. Thus, post-exposure vaccination would be the last resort for those who can afford it. Even then, most patients do not receive the recommended full course of post-exposure vaccine injections (12). In addition, post-exposure prophylaxis requires rabies hyperimmune globulin which is scarce (7, 11).

Although all the people who had worked in the zoological gardens or at the VTH for over 10 years were immune and had relatively high antibody titres that ranged between 3.3 eu/ml and 4.1 eu/ml, these values were low compared to results of other countries, including Slovenia, Thailand, United Kingdom and the United States of America (5, 6, 13, 20, 21, 23). While there was an indication of poor responders among two of the donors, the practice of incomplete primary vaccination, boosted on the second year, which does not comply with the recommendations of the WHO and those of the manufacturer on vaccine administration (especially during the first immunisation of a person), may be a major reason for the low levels obtained in this study. Thus, the biennial anti-rabies immunisation of VTH and zoological garden staff 'induced seroconversion to levels considered protective', only after a minimum of three years which implies two injections for most subjects (Fig. 1). It is assumed that most individuals remained unprotected during these three years. There is a need to revise this practice and use the dosage recommended by the WHO and a course of pre-exposure treatment.

Correct anti-rabies vaccination provides protective levels of immunity in nearly 100% of individuals following primary vaccination (11). This is guaranteed under strict compliance with the WHO recommendation of three injections of the vaccine administered on days 0, 7 and 21 or 28 of primary vaccination for prophylactic immunisation, and provided individuals are not immuno-compromised (4, 6, 9, 10). The authors observed from the survey responses that prophylactic primary vaccination of occupationally exposed staff of the University of Ibadan (especially from 2000 until today) included one injection of the vaccine that was administered on day 0, but no injection on days 7, 21 or 28 (thus one injection instead of three suggested by WHO and the manufacturer). We assume year 0 to be the year of primary vaccination. The second injection (first booster) was administered in year 2 after the primary vaccination. The third injection (second booster) was given in year 4 after the primary vaccination. A booster was administered once every two years. Therefore, the third and fourth injections were given in years 6 and 8 after the primary vaccination, respectively. The most recent was given in November 2008. In reality, incomplete vaccination has been practised. This accounts for the pattern of the immune curve shown in Figure 1. This situation may be linked to the financial burden created on the university. It was assumed that vaccinated individuals would personally complete the course of prophylaxis. However this seldom happened.

Active advocacy is now needed among students and staff to further educate them on the inherent risk and false impressions that may be held in regard to their immune status. This may enable behavioural change towards

 

self-protection against rabies. There is need to encourage occupationally-exposed workers of the university to supplement the cost of vaccination. Since veterinary students are highly exposed during training, it is necessary to determine a policy, either to include the cost of anti-rabies vaccination in their school fees, probably at a subsidised rate, or request proof of immunisation in their annual registration exercise. This is very important because the complete post-exposure treatment of rabies requires an injection around the bite site of rabies hyperimmune globulin that is scarce in many developing countries. Adequate pre-exposure immunisation makes the use of rabies hyperimmune globulin unnecessary when there is need to initiate rabies post-exposure treatment (23, 24).

The results of this study on the anti-rabies immune status of elite populations in Nigeria confirm an earlier report on post-exposure anti-rabies prophylaxis compliance in a cross-section of socio-economic classes of people in Nigeria (12). There often is incomplete immunisation during pre- and post-exposure treatments. While there are reports that indicate high vaccination coverage of dogs in Ibadan, up to 70% of the population, these reports were not based on community populations, but rather on hospital records of dogs that visited government-owned veterinary hospitals (2, 18). There may therefore be high risk of exposure to rabies virus for VTH workers and students. This may corroborate the fact that more effort is needed to control rabies in Nigeria and some other African countries (7).

In conclusion, the benefit of complying with rabies pre-exposure immunisation appears to be poorly appreciated by veterinary students and many young veterinarians. There is therefore a need to organise a periodic scientific and educational forum during which the dangers inherent in this carefree attitude would be highlighted. Occupationally exposed staff, especially in developing nations, should always remember that rabies is potentially fatal and the cost of achieving and maintaining adequate protection against the disease is worth enduring. Platelia Rabies II ELISA shows prospects for revealing the rabies risk status of people in neglected communities, fulfilling the goal of World Rabies Day now in its third year in 2009 (9).

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