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Prevalence of antibody against rabies among confined, free-roaming and stray dogs in a transit city of Nigeria

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Summary

The prevalence of anti-glycoprotein antibodies against rabies virus is studied in the sera of confined, free-roaming and stray dogs in Ilorin, the capital city of Kwara State, Nigeria. A quantitative indirect enzyme-linked immunosorbent assay (i-ELISA) was used to detect rabies virus anti-glycoprotein antibodies in sera from 116 confined, 61 free-roaming, and 13 stray dogs. The sera were collected between June and December 2008 from apparently healthy dogs. Of these 190 dogs, 81 (42.6%), consisting of 57 confined (49.1%), 23 free-roaming (37.7%) and 1 stray (7.7%), had antibody titres that exceeded the positive threshold of 0.5 equivalent units (eu)/ml against rabies, while 109 (57.4%) presented titres that were below the threshold. Prevalence of rabies anti-glycoprotein antibody was higher in the confined dogs compared to free-roaming and stray dogs. Our results indicated low anti-rabies sero-prevalence (42.6%) in the dog population of Ilorin, a transit city that lies between northern and southern Nigeria. This is the first community-based prevalence report on the anti-rabies serological profile of dogs in Nigeria. The need for primary and booster mass vaccination of dogs and the impact of these findings on rabies control in Nigeria are discussed.

Keywords

Antibody, Anti-glycoprotein antibody, Dog, ELISA, Enzyme-linked immunosorbent assay, Indirect ELISA, Nigeria, Rabies, Virus.

Prevalenza dell'anticorpo antirabbia in cani confinati, liberi e randagi in una città di transito in Nigeria

Riassunto

Gli autori hanno indagato la prevalenza di anticorpi anti-glicoproteina al virus della rabbia nel siero di cani confinati, liberi e vagabondi a Ilorin, capitale dello stato del Kwara (Nigeria). I campioni di siero sono stati raccolti nel periodo giugno – dicembre 2008 da cani apparentemente sani. Gli anticorpi anti-glicoproteina del virus della rabbia sono stati rilevati nel siero di 116 cani confinati, 61 liberi e 13 randagi mediante dosaggio con immuno-assorbente legato all'enzima quantitativo indiretto (i-ELISA). Di questi 190 esemplari testati, 81 animali (42,6%), 57 cani confinati (49,1%), 23 liberi (37,7%) e un randagio (7,7%), hanno presentato titoli anticorpali superiori alla soglia di positività di 0,5 eu/ml per la rabbia, i restanti 109 cani (57,4%) hanno mostrato titoli al di sotto della soglia. La prevalenza dell'anticorpo anti-glicoproteina della rabbia è risultata superiore nei cani confinati rispetto a quelli liberi e randagi. Questi risultati hanno indicato una bassa

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sieroprevalenza antirabbia (42,6%) nella popolazione canina di Ilorin, città di transito situata nella zona centrale della Nigeria. Questo è il primo rapporto di prevalenza del profilo sierologico antirabbia riferito a una comunità di cani in Nigeria. Il presente contributo discute della necessità di sottoporre la popolazione canina a vaccinazione primaria e richiamo di massa e, del relativo impatto di queste azioni sul controllo della rabbia nel Paese.

Parole chiave
Anticorpo, Anticorpo anti-glicoproteina, Cane, ELISA, i-ELISA, Nigeria, Rabbia, Test con immuno-assorbente, Virus.

Introduction

Rabies is a fatal disease that is mainly transmitted, at least in developing countries, by the bites of rabid dogs (17). The infection is known to account for the death of approximately 55 000 people across the world each year (30). Although emphasis of governments and non-governmental agencies in Africa is mainly focused on human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS), poliomyelitis, tuberculosis and malaria, rabies constitutes a much bigger health challenge and is a greater economic burden in Africa than envisaged, with about 24 000 people (mainly children) dying of the disease each year and about eight million people a year receiving costly post-exposure prophylaxis (PEP) following bite injuries from rabid animals (16). Furthermore, while a significant reduction in human rabies associated with domestic dog rabies has been achieved in Mexico, South America and the Caribbean over the last two decades, dog-associated human rabies has increased in parts of sub-Saharan Africa in the same period (30).

Rabies infection in humans is the most dreaded aspect of this zoonosis because it is ultimately fatal if left untreated (8, 10, 11). Cases of atypical rabies have attracted greater attention to the disease in Nigeria. For example, an apparently healthy family dog with good home care and an adequate record of vaccination with Flury strain low egg passage (LEP) rabies vaccine was reported to have bitten a 12-year-old girl. The death of the dog four days after biting the girl did not qualify it as a rabies suspect (19). The death of the girl six months later forced a change in the autopsy protocol at the Ahmadu Bello University Teaching Hospital in Zaria, Nigeria. Although several studies have been conducted on clinical rabies in Nigeria (3, 6, 14, 21, 27), some rabies cases do present difficulties for dog owners and clinicians when they present as atypical cases without specific rabies signs or symptoms (19).

Rabies can be prevented by vaccination. In humans, post-exposure treatment includes three aspects, as follows: immediate washing of the wound, the rapid administration of high quality rabies immunoglobulins for severe cases, and a complete course of rabies immunisation with modern cell culture vaccines (29, 30). Vaccine manufacturers usually recommend 5 doses of the vaccine to be administered on days 0, 3, 7, 14 and 28 (29). Pre-exposure vaccination of dogs in Nigeria usually involves a primary dose of the LEP Flury strain vaccine at 3 or 4 months of age, with a booster dose every year to sustain threshold immunity against rabies (5, 8, 15, 20). There is therefore a need to inform dog owners as well as policy makers of these important schedules to ensure effective vaccination programmes.

Although numerous studies have been conducted on rabies in Nigeria, comprehensive information on the dog population and on rabies anti-glycoprotein antibody levels (herd immunity) in Ilorin is not available in the literature. This limits informed public health decision-making on rabies control for the city and for Nigeria as a whole because of the uniqueness of Ilorin as a transit city between the north and south of Nigeria. Most of the reports on population immunity of dogs in Nigeria are based on records of dogs that were taken to private- and government-owned veterinary hospitals and clinics (5, 20). The present study is a community-based investigation of anti-rabies immunity among confined, free-roaming and stray dogs in Ilorin, a transit city where people and animals often rest and refresh en-route to both parts of the country.

Materials and methods

Study area

Ilorin lies between latitude 8°25′N to 8°32′N and longitude 4°30′E to 4°41′E, close to the confluence of the Rivers Niger and Benue, the two rivers that demarcate the northern and southern regions of Nigeria (22, 23). With a population of 0.85 million (18), Ilorin has a small industrial activity base, and the inhabitants are predominantly civil servants or small business operators. The city is 50.2 km² in area and is situated approximately 400 km from the Federal Capital Territory. Its strategic location as the gateway between the southern and northern areas of the country makes it easily accessible to all parts of the country by air, road and rail transport. By road, Ilorin offers reliable road transport services to cities in the north and the south of Nigeria (22, 23). Every day, the city receives travellers by road from the northern to southern areas of Nigeria or vice-versa, where most refresh en route (Fig. 1).

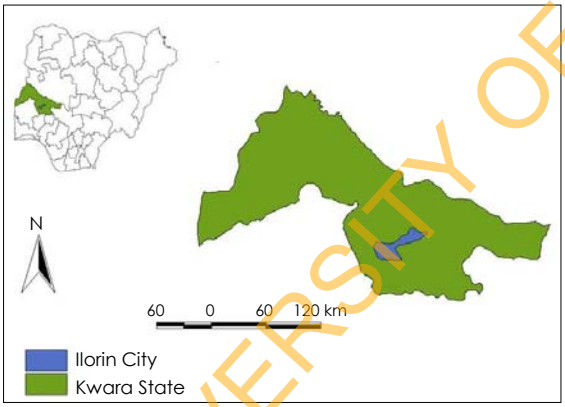

Figure 1
Study area located in a central transit city of Nigeria

Sampling method and specimen collection

Using stratified randomisation, 190 healthy dogs of both sexes (106 males and 84 females) were selected for this study across districts, streets and households in the city. Their points of confinement or capture were geo-referenced using a global positioning system (GPS) (details of the method were reported by Olugasa *et al.*) (25). The sample comprised 116 confined, 61 free-roaming and 13 stray dogs (Table I). Samples were taken from three major land use areas of the city (22, 23), namely: residential areas (including high-income government residential areas [GRA], medium-income residential areas and low-income traditional settlements), the non-residential areas (including government offices, commercial, industrial and educational areas) and the transit areas (made up of vehicle terminals for cars, buses and lorries coming from northern to southern Nigeria and vice-versa).

With the aid of sterile needles and syringes, 2.5 ml of blood was collected from each dog, 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 were temporarily stored at –4°C until tests were conducted.

Detection of rabies anti-glycoprotein antibodies

A quantitative, indirect enzyme-linked immunosorbent assay (i-ELISA), the Platelia™ Rabies II kit (Bio-Rad, Marnes-la-Coquette) (12) was used for the detection of 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 (12) and results were read using an ELISA Reader (IRE 96™, Saint Jean d'Illac) at wavelength of 450-620 nm. Subjects were considered to have threshold level of antibody against rabies virus infection if they produced ELISA titres of ≥0.5 eu/ml (29).

Survey conducted among dog owners

A follow-up questionnaire was provided to the owners of donor dogs who completed forms that were analysed using Microsoft Excel®. An individual questionnaire was conducted within two months of the field work so as to

Table I
Sample size and data collection among dogs in a central transit city of Nigeria (June-December 2008)

Location and capture area	Confined	Free roaming	Stray*	Total
Transit area (vehicle terminals)	10	10	5	25
Residential area				
High income	46	9	0	55
Medium income	32	20	3	55
Low income	4	13	3	20
Non-residential area	24	9	2	35
Total	116	61	13	190

* location of stray dogs as at the time of capture for blood collection

assess anti-rabies vaccination history and to collect data on type of ownership, husbandry management, breed, usage (i.e. purpose for keeping the dog), age, place of vaccination (if ever) and rabies history in the community (species involved, breed, age and location). Those interviewed were asked to produce vaccination certificates as proof of vaccination. In cases where a vaccination certificate was not available, the names of dog owners and of the dogs were recorded for verification of vaccination status from the immunisation register at the veterinary clinic indicated. Reasons for failure to present dogs for vaccination were also requested. Student's t-test was used to evaluate statistical significance of some of the findings.

Results

The Flury strain anti-rabies LEP vaccine is produced locally in Nigeria by the National Veterinary Research Institute in Vom, Plateau State. Kwara State Government vaccinated 1 500 dogs in 2007 and 500 dogs in 2008 in a state-wide, free anti-rabies immunisation campaign that included Ilorin City, using this vaccine. Some 148 (77.9%), 23 (12.1%) and 19 (10%) of the 190 dogs sampled were reported as vaccinated at least once, not vaccinated and unknown vaccination history, respectively. Of the 148 vaccinated, 97 (65.5%) were inoculated at private veterinary clinics, 35 (23.6%) at government mass vaccination campaigns and 16 (10.8%) at both private veterinary clinics and the government mass vaccination campaigns (Fig. 2). Mean antibody titre among seropositive dogs varied from 0.84 ± 0.4 eu/ml among free-roaming dogs in the transit areas to 3.2 ± 1.2 among confined dogs in high-income residential areas and 3.51 ± 1.0 eu/ml among free-roaming dogs in the non-residential areas (Table II). Figure 3 shows the relationship between vaccination status and threshold antibody titre in dogs sampled in the study area.

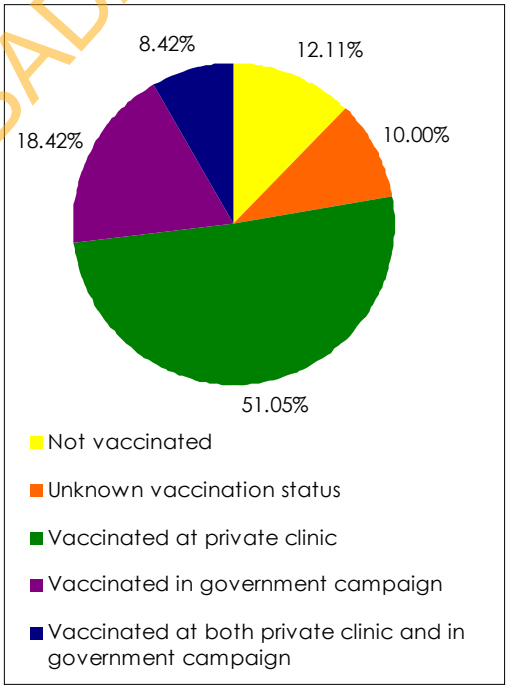

Figure 2
Vaccination status of dogs in a central transit city of Nigeria

Of the 116 confined dogs, 57 (49.1%) had rabies anti-glycoprotein antibody levels that were equal to or greater than 0.5 eu/ml in their serum; this was the case for 23 out of 61 (37.7%) free-roaming and 1 out of 13 (7.7%) stray dogs (Table II). Seroprevalence of 69.6%

Table II
Anti-rabies seroprevalence in dogs in relation to land use areas of Ilorin City, Nigeria

Location and capture area	Confined				Free roaming				Stray				Total
	No.	Titre ≥0.5 eu/ml	%	AB titre (eu/ml) (mean ± SD)	No.	Titre ≥0.5 eu/ml	%	AB titre (eu/ml) (mean ± SD)	No.	Titre ≥0.5 eu/ml	%		
Residential area													
High income	46	32	69.6	3.2 ± 1.2	9	4	44.4	2.21 ± 1.6	0	0	0		55
Medium income	32	10	31.3	2.7 ± 1.5	20	7	35.0	2.45 ± 1.5	3	0	0		55
Low income	4	1	25	1.4 ± 0.0	13	4	30.8	1.18 ± 0.8	3	0	0		20
Transit area (vehicle terminals)	10	0	0	0.0 ± 0.0	10	2	20	0.84 ± 0.4	5	0	0		25
Non-residential area	24	14	58.3	2.9 ± 1.2	9	6	66.7	3.51 ± 1.0	2	1 (1.13)	50		35
Total	116	57	49.1	2.95± 1.2	61	23	37.7	2.32 ± 1.5	13	1	7.7		190

AB antibody
eu equivalent unit
SD standard deviation

(32/46) was recorded among confined dogs living within the high-income residential areas (Table II), while a seroprevalence of 58.3% (14/24) was recorded among confined dogs in non-residential areas. A single stray dog captured in the non-residential area was seropositive (1.13 eu/ml) for rabies antibody (Table II). Free-roaming dogs in medium-income residential areas revealed 35% (7/20) prevalence. All confined dogs in the transit area were seronegative.

Overall seroprevalence of 42.6% (81/190) was recorded among sampled dogs (Table III). Dogs aged between 3 and 8.9 years were more frequently seropositive than dogs below 1 year of age (Table III). There was a significant difference between mean antibody titre of seropositive females compared to seropositive male dogs (p<0.05). Male dogs had higher seroprevalence rates of 45.3% (48/106) compared to females which were 39.3% (33/84).

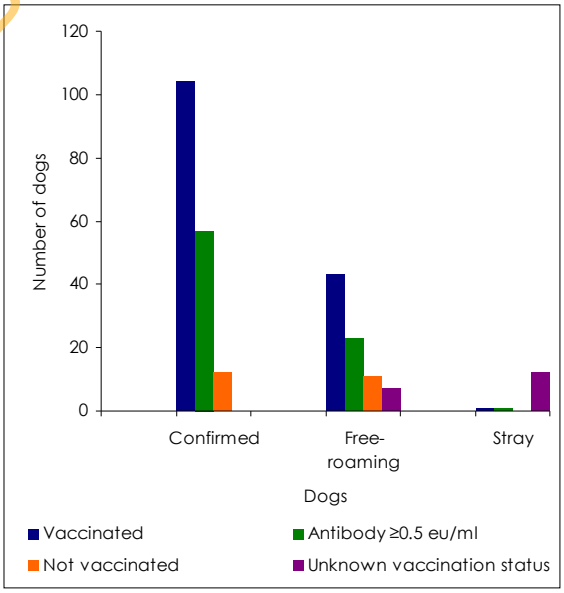

Figure 3
Vaccination and anti-rabies seropositive status in dogs in a central transit city of Nigeria

Table III
Rabies antibody levels in dogs in a central transit city of Nigeria by age group and sex

Age group (years)	Male				Female				Total
	No.	Titre ≥0.5 eu/ml	%	Antibody titre (eu/ml) (mean ± SD)	No.	Titre ≥0.5 eu/ml	%	Antibody titre (eu/ml) (mean ± SD)	
<1	9	2	22.2	0.95±0.5	0	0	0	0	7
1-2.9	34	13	41.2	2.74±1.3	36	11	30.6	2.72±1.5	70
3-5.9	54	25	46.3	2.6±1.4	37	18	48.6	3.06±1.2	91
6-8.9	8	7	87.5	2.77±1.3	8	4	50	3.9±1.6	16
≥9	1	1	100	0.5±0.0	3	0	0	0	4
Total	106	48	45.3	2.55±1.4	84	33	39.3	3.05±1.2	190

eu equivalent unit
SD standard deviation

Discussion and conclusions

This study provides the rabies antibody profile of dogs in a central transit city of Nigeria using an ELISA technique which was considered to be a suitable alternative to the neutralisation test (12), and revealed a seroprevalence rate of 42.6% in a community-based assessment. No earlier studies of this nature were available for this city. Nevertheless, two reports indicated 37.5% and 71.4% anti-rabies seroprevalence in dogs, respectively, in another nearby city of Ibadan in the south-western area of Nigeria (5, 20). These reports on Ibadan were not based on community populations, but rather on hospital records of dogs that were taken to government-owned veterinary hospitals which may not truly represent the prevalence of rabies antibody in dogs in that city. The present findings on Ilorin City indicate a more accurate report on anti-rabies seroprevalence in dog population in a central transit city between the northern and southern areas of Nigeria.

The low prevalence of antibody against rabies observed in this study may indicate a combination of factors, including lack of stable rabies vaccination programmes for the majority of dogs in the city and vaccination failure in some of the cases, which is not uncommon in Nigeria (5, 15). Expensive exotic dogs vaccinated at a private veterinary clinic with booster in this city did not attain the threshold antibody level. This may be attributable to vaccination failure. In addition, the 2007 and 2008 free anti-rabies vaccination in dogs performed by the Kwara State Veterinary Services was aimed at increasing antibody levels and prevalence in dogs in the city against rabies. However, it did not attain the World Health Organization prescribed 70%-80% epizootiological baseline of herd immunity in the community. The combination of private and government vaccination efforts against the disease has not resulted in adequate seroprevalence in the dog population. This low seroprevalence favours large-scale epizootic or focal outbreaks of rabies, with an increased risk for humans (5).

High antibody levels obtained in some of the confined and free-roaming dogs may be attributed to potent booster vaccination received in some instances. In general, dogs in the age range of 3-8.9 years were seropositive and most frequently found with high antibody titre in the community. This is most likely due to the fact that they might have received more vaccinations than the animals below 1 and 1-2.9 years of age. Dogs of less than one year of age were mostly below the threshold of anti-rabies antibody. This finding is similar to a report on occupationally exposed humans in a Nigerian University (26). Low levels of antibody associated with young individuals on the job were associated with non-compliance with booster dose regime recommended by vaccine manufacturers (26).

The observation of low vaccination compliance among humans and dogs in Ibadan and Ilorin (25, 26) and in this study may well be

representative of a rabies pre-exposure immunisation situation in other cities and villages of Nigeria. A communal risk pattern associated with clinical human rabies in this transit city indicated high exposure among children who had to commute to schools from their homes in low-income residential areas. Bites from infected dogs were more common in non-residential areas, especially around a beef market, where waste foods, including condemned meat and other abattoir waste and wastewater were available (1, 2, 4, 9, 24, 25) to sustain free-roaming and stray dogs. Constant flux of people and animals in this transit city implies an outbreak of rabies in this city can be easily spread to other parts of Nigeria. A more effective and efficient strategy for anti-rabies vaccination (8, 13, 15, 17) that would attain 70% to 80% of dog population coverage with potent vaccine and booster doses is crucial. The dog population in Ilorin has recently been estimated at 1 258 (7).

Subsequent monitoring and evaluation of dog vaccination exercises were critically lacking, yet essential to achieving effective and efficient population seroconversion. Limited state resources, especially in a highly populated country like Nigeria, requires the efficient use of what is available. There is therefore an increasing need for animal health authorities to utilise a combination of sero-surveillance and modern geographic information systems to focus pre-exposure anti-rabies vaccination of dogs in Nigerian communities. Potent booster vaccination in dogs needs to become a compulsory part of a regular programme for dog vaccination in Nigeria. It is critical that this approach is promoted in Nigeria if the country is to achieve rabies control as reported by Thailand and some other Asian countries (11, 28).

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