International Scholars Journals

International Journal of Veterinary Medicine and Animal Health ISSN 2756-3871 Vol. 15 (3), pp. 001-013, March, 2024. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Seroprevalence of rabies virus antibodies in dogs within FCT, Abuja

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Received January 30, 2024; February 24, 2024 and Published April 15, 2024

Abstract

Rabies is a deadly zoonotic viral disease of warm blooded vertebrates globally. This study was carried out to determine the seroprevalence of rabies virus antibodies in dogs using indirect ELISA. One hundred and eighty four (184) sera samples assayed from three Area councils (Gwagwalada, Kwali, and Kuje) randomly by balloting without replacement, adopting a cross sectional study design showed that 150 (81.5%) were positive and 34 (18.5%) were negative for rabies antibodies out of which 129 (83.7%) were previously vaccinated while 21 (70%) were not vaccinated. The distribution of breed, sex, age, location and vaccination history/status in relation to antibody titre showed more male dogs had more antibodies 82 (44.7%) among German Shepherd breed 57 (31.0%), within the age range 1-5yrs 92 (50%) especially in Gwagwalada 83 (45.1%) among vaccinated dogs 129 (70%) which was significant for vaccination status (P value < 0.05). This study suggests high level of vaccination awareness among dog owners within the study area with a small proportion of potential unvaccinated dog population at risk of rabies infection which can affect the increasing population following the incidence of dog bite. In conclusion, this study indicates evidence of rabies antibodies in both vaccinated and unvaccinated dogs. The study provided valuable information for public health authorities and policymakers involved in rabies control programs. Sustained public awareness campaign should be encouraged.

Keywords: Seroprevalence, Rabies Virus, Dog, ELISA, FCT, Nigeria.

1. INTRODUCTION

Rabies is a fatal viral disease that affects all warm blooded vertebrates (Zulu *et al*., 2009). It is one of the most important and widespread zoonotic diseases and a global dilemma (Blancou, 1988). The virus causing the disease belongs to genotype 1 of the genus *Lyssa* virus in the family *Rhabdoviridae* (Blancou, 1988). The rabies virus genome consists of a single stranded, non-segmented, negative sense RNA of approximately 12kb (Tordo *et al*., 1986). The *Lyssa* virus genus, within the Rhabdoviridae family, is subdivided into seven genotypes based on RNA sequencing (Bourhy *et al*., 1993): These include Classical rabies virus (RABV, genotype 1 found worldwide), Lagos bat virus (genotype 2 found in Africa), Mokola virus (genotype 3 most common in Africa), Duvenhage virus (genotype 4 most common in Africa), European bat lyssavirus 1 (EBLV-1, genotype 5 most common in Europe), European bat *lyssa*virus 2 (EBLV-2, genotype 6 most common in Europe), and Australian bat*lyssa*virus (ABLV, genotype 7 most common in Australia) (Gould *et al*, 1998), Also four presume viruses

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(Aravan, Khujand, Irkut and West Caucasian Bat Virus) were isolated. Shimoni bat virus as well as Bokelohbat*lyssa*virus were isolated in 2009) (Freuling *et al.*, 2011) and the Ikoma lyssavrus (Marston *et al*., 2012) were discovered in 2011 and 2012 respectively. *Lyssavirions* are helical symmetric and have a cross-sectional area of around 75 nm and a length of about 180 nm. (Drew ,2004).

A mechanism known as pinocytosis is triggered when the trimeric spikes on the membrane surface of the virus connect with a particular cellular receptor, presumably the acetylcholine receptor. Through the creation of an endosome as a result of this mechanism, the virus can enter the host cell (CDC, 2009). The virus then utilizes the endosome's need for an acidic environment, adheres to its membrane, and releases its five proteins and single strand RNA into the cytoplasm (CDC, 2009).

The virus starts to reproduce once it enters a muscle or nerve cell. Using cytoplasmic nucleotides, the L protein starts the transcription of five mRNA strands and a positive RNA strand from the original negative RNA strand. These mRNA strands are then translated by ribosomes in the cytoplasm into the corresponding proteins (P, L, N, G, and M). Post-translational modifications may be necessary for some proteins (CDC, 2009). As an illustration, the G protein moves through the rough endoplasmic reticulum, where it experiences further folding, and is then delivered to the Golgi apparatus, where it is glycosylated (CDC, 2009).

Where there are enough proteins, the viral polymerase will start to create new negative strands of RNA using the positive strand RNA as a template. These negative strands will next combine with the N, P, L, and M proteins before moving to the cell's inner membrane, where a G protein has inserted itself. The G protein then coils around the N-P-L-M complex of proteins, including parts of the host cell membrane into the process. This creates the virus particle's new outer envelope. The virus then emerges from the cell as buds.

The viral polymerase starts the synthesis of new negative RNA strands from the positive RNA strand template when there are enough proteins present (Gluska *et al*., 2014). These negative strands form complexes by joining with the proteins N, P, L, and M. These complexes then go to the host cell's inner membrane (Gluska *et al*., 2014). The N-P-L-M protein complex is wrapped around by the membrane-embedded G protein, which also absorbs parts of the host cell membrane to create the viral particle's outer envelope (Gluska *et al*., 2014). Then the virus separates from the host cell (Gluska *et al*., 2014). Upon entry, the virus is neurotropic and follows the neural pathways into the central nervous system (Gluska *et al*.,2014). Initially, the virus infects muscle cells near the site of infection, where it can replicate undetected by the host's immune system (Gluska *et al*.,2014). Once the virus has multiplied sufficiently, it starts to attach to the neuromuscular junction's acetylcholine receptors (p75NR) (Gluska *et al*., 2014). The virus then moves retrogradely through the nerve cell axon as a result of an interaction between its P protein and the protein dynein, which is found in the cytoplasm of nerve cells. Once within the cell, the virus leaves quickly towards the central nervous system (CNS), where it replicates in motor neurons before making its way to the brain (Cotran *et al*., 2015). Following infection of the brain, the virus moves centrifugally to the peripheral and autonomic nerve systems before eventually moving to the salivary glands, where it is prepared to infect the following host (Baer, 1991). In dogs, the virus is acquired through bites from infected dogs

In humans, the incubation period—the time between infection and the onset of symptoms—generally lasts 1-3 months (Giensa *et al*., 2015). Depending on the location, size, and severity of the infected wound as well as the amount of virus delivered, incubation periods as short as four days and as long as six years have been observed (Giensa *et al*., 2015). Initial rabies symptoms and indicators, like a fever and a headache, are frequently vague. People may experience a variety of signs and symptoms, including partial paralysis, anxiety, insomnia, disorientation, agitation, strange behavior, paranoia, panic, and hallucinations as the rabies infection progresses and causes inflammation in the brain and/or meninges (Giensen *et al*., 2015). According to Giensen *et al*., (2015), these symptoms could get worse and result in delirium and coma. Hydrophobia could also develop in the infected person (Giensen *et al*., 2015). According to Gienssen *et al*., (2015), death often happens 2 to 10 days following the onset of the first symptoms. Even with the provision of appropriate and urgent care, survival is essentially unknown once symptoms have appeared (Giensen *et al*., 2015; Rupprecht *et al*., 2006). Jeanna Giese, however, made medical history in 2004 as the first Milwaukee protocol patient to survive rabies without getting effective post-exposure prophylaxis (Jordan, 2008).

Rabies was previously known as hydrophobia, or "fear of water" (Smallman-raynor *et al*., 2004). According to Smallman-Raynor *et al*., (2004), hydrophobia describes a group of symptoms that often appear in the latter stages of a viral infection. When offered liquids to drink, the affected individual panics and has trouble swallowing. They are also unable to quench their thirst. Any mammal that has the virus may show symptoms of hydrophobia (Smallman-raynor *et al*., 2004). It is simpler to spread the virus through biting since the virus has a tendency to grow and assimilate in the salivary glands of the infected animal (Smallman-raynor *et al*., 2004). Infected people who produce too much saliva may experience severe laryngeal and throat spasms that make it difficult to

swallow (Smallman-raynor *et al*., 2004). The virus's capacity to spread by biting would be greatly diminished if the infected person could swallow saliva and drink (Smallman-raynor *et al*., 2004).

Furious rabies, which affects 80% of those with rabies (Smallman-raynor *et al*., 2004), is frequently linked to hydrophobia. The paralytic type of rabies, which affects the remaining 20% of cases and is characterized by muscle weakness, loss of sensation, and paralysis but does not typically result in dread of water, can induce these symptoms(Smallman-raynor *et al*., 2004).

Domestic dogs are a common host species for the disease agent, which is maintained and transmitted by a range of different host species (Ngoepe *et al*., 2009). Due to their intimate contact with humans (McKenzie *et al*., 1993), domestic dogs (Canis familiaris) play a crucial role in the transmission of rabies, with 85–95% of human rabies cases being attributed to dog bites (Tang *et al*., 2005). According to the World Health Organization, ten million people worldwide Wild animals (such as bats, raccoons, and foxes) serve as the virus' maintenance host in the wildlife (sylvatic) cycle of rabies (WHO 2018).

MATERIALS AND METHODS

Study Area

The study was carried out in the Federal Capital Territory, commonly known as FCT, or loosely as FCT-Abujawhich is a federal territory in central Nigeria. Abuja, the capital city of Nigeria, is located in this territory. FCT was formed in 1976 from parts of the states of Nasarawa, Niger and Kogiwithin the Middle Belt region of the country. The territory is located just north of the confluence of the Niger River and Benue River. It is bordered by the states of Niger to the West and North, Kaduna to the northeast, Nasarawa to the east and south and Kogi to the southwest. Lying between latitude 8.25 and 9.20 north of the equator and longitude 6.45 and 7.39 east of Greenwich Meridian. The Federal Capital Territory has a landmass of approximately 7,315 km^2, and it is situated within the Savannah region with moderate climatic condition .The territory is currently made up of six Local Government Area Councils, namely;Abaji, Abuja Municipal, Gwagwalada, Kuje, Bwari and Kwali(FCT State, 2018)

Study Design

The study adopted cross sectional study. Blood samples were collected randomly from Gwagwalada Area Council, Kuje Area Council, Kwali Area Council of FCT Abuja, after balloting without replacement. In the various sampling sites, blood samples were collected from dogs brought to Veterinary clinics, house to house call based on owners consent. Demographic and zoographic information including vaccination history, sex, breed, age and location of dogs were obtained at the point of sample collection from the dog owners. Associations between the demographic variables, vaccination status and rabies antibody titer of each dog were assessed. Retrospective Analysis was also carried out. using hospital records on dog bite cases in 3 locations namely: University of Abuja Teaching Hospital Gwagwalada, Kwali General Hospital Kwali, Kuje General Hospital Kuje which spanned from 2010 to 2019 (10yrs). Data collected and analyzed included demographic information of affected dog bite victims.

Laboratory Analysis

Samples collected and processed were subjected to indirect Enzyme linked immuno-absorbent assay (ELISA) technique as previously conducted by Ohor *et al.,*(2007) in accordance with manufacturer's recommendation using qualitative indirect ELISA Kits from (Elabscience Biotechnology USA). All reagents were brought to room temperature before use. The wash buffer 30x were diluted with distilled water to yield 500ml of 1x buffer. All reagents standard solutions were prepared as instructed in the Kits manual. The kit is based on reverse phase enzyme imune assay technique. The micro titer plate was precoated with a target monoclonal antibody. Positive/negative controls or samples were added to the wells and incubated. Antibodies in the sample bound to the antigen on the plate. Unbound antibody was washed away during a wash step. A Horseradish Peroxidase (HRP) conjugated detection antibody was then added and incubated. Unbound avidin-HRP was washed away during a washing step. TMB substrate was then added and color developed. The reaction was stopped by addition of acidic stop solution and color changed into yellow that was measured at 450 nm. The OD of sample was then compared to the OD of the positive and negative controls in order to determine the presence of rabies virus antibody. The detailed procedure was conducted as outlined below: The Sample and control were numbered, and a record of control wells and sample kept 2 wells for negative and 2 wells for positive control were set. 100ul of positive and negative control were added to positive and negative control well. 100ul of serum were added to other sample well covered with plate sealer, mixed thoroughly and incubated at 37^0c for 30mins. The liquid in each well was removed, and then 300ul of wash buffer was added to each well and washed. This procedure was repeated for 5 times, 30secs interval/time. The plate was inverted and tapped against thick clean absorbent paper. Then 100ul of HRP conjugate was added to each well and covered with plate sealer, incubated at 37^0c for 30mins in shading light**.** The

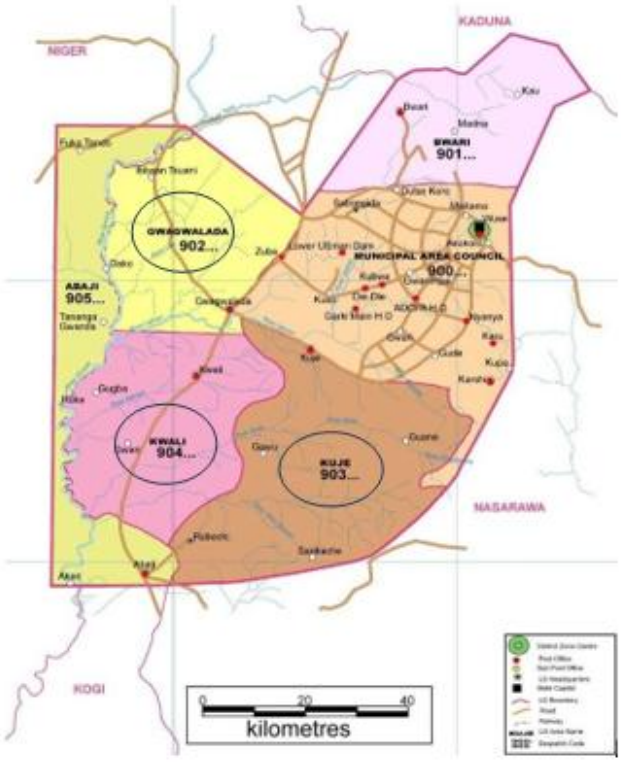

Figure 1: Map of FCT Abuja showing the Six Area Council above (Google map).

liquid in each well was washed, following same process of washing. While 50ul of substrate Reagent A and 50ul of substrate Reagent B was added into each well covered with plate sealer and mixed thoroughly, incubated at 37^{O}c for 15mins. Finally, 50ul of stop solution was added into each well and mixed thoroughly. The absorbance value (A-value) of each well was measured by using a micro plate reader with 450nm in wavelength.

RESULTS

Out of the 184 samples subjected to Indirect ELISA test showed that 150 (81.5%) were positive while 34 (18.5%) were negative for RBV IgG antibodies. According to the breed distribution of RBV IgG which indicated that German Shepherd 57 (31.0%) positive and 15 (8.1%) negative. Caucasian breed were 32 (17.4%) positive and 8 (4.3%) negative. Boerboel 20 (10.9%) positive and 3 (1.6%) negative. Bullmastiff 1 (0.5%) negative and 0 positive. Cane corso 5 (2.7%) positive, 1 (0.5%) negative. Rotweiller 11 (6.0%) positive 3 (1.6%) negative. Lhasa Apso 3(1.6%) positive. 1(0.5%) negative. Belgian Malinoise 6 (3.3%) positive and 0 negative. American Eskimo 4 (2.2%) positive and 1(0.5%) negative. Indigenous breed were 12 (6.5%) positive and 1(0.5%) negative. Rabies antibodies distribution according to sex showed that 82 (44.7%) male dogs were positive and 18 (9.8%) females were negative. Rabies antibodies distribution according to vaccination status was 129 (70%) vaccinated dogs positive and 25 (13.6%) negative. Non-vaccinated were 21 (11.4%) positive and 9 (4.9%) negative. Rabies virus distribution according to age showed dogs within the age range of 3-6 months were 23 (12.5%) positive and 10 (5.4%). Dogs within 6-12months were 20 (10.9%) positive and 8 (4.3%) negative. Dogs within 1 -5 years were 92 (50%) positive and 16 (8.7%) negative. Dogs above 5 years were 15 (8.2%) positive as shown. Rabies virus distribution according to location showed 83 (45.1%) dogs from Gwagwalada Area council positive and 20(10.9%) negative. 46 (25.0%) dogs from Kuje were positive and 12 (6.5%) negative. 21 (11.4%) dogs from Kwali were positive, 2 (1.1%) negative.

Table 1: Distribution of Rabies antibodies in dogs according to breed.

Variable (Breed of Dog)	Result Positive(%)	Negative(%)	Total(%)
American Eskimo	4	1	5
Belgian Malinois	6	0	6
Boerboel	20	3	23
Bullmastiff	0	1	1
Cane Corso	5	1	6
Caucassian	32	8	40
German Shepherd	57	15	72
Indigenous	12	1	13
Lhasa Apso	3	1	4
Rotweiller	11	3	14
Total	**150**	**34**	**184**

Table 2: Distribution of rabies antibodies in dogs according to vaccination status in FCT.

	Sera Samples		
Vaccination status	**Positive**	**Negative**	**Total**
Vaccinated	129	25	154
Not Vaccinated	21	9	30
Total	**150**	**34**	**184**

DISCUSSION

Rabies antibody detection in the study area

In this study 154 (83.7%) of the dogs sampled in this study were vaccinated against rabies based on history obtained at the point of sample collection, which shows that many dog owners were aware of the dangers of rabies and the value placed on their highly priced exotic dogs. However, this study could not establish whether the rabies antibodies detected were due to vaccination or infection because the assay used was qualitative and not quantitative. Nevertheless, all the dogs vaccinated against rabies virus that had rabies antibodies titre value ≥ 0.5 were considered as protective titres due to vaccination while those dogs that were not vaccinated against rabies virus that had titres ≥ 0.5 were considered as antibodies due to infection.

Based on the above deduction vaccinated dogs 129 (70%) sampled had antibodies titre>0.5 which could be as a result immune response to vaccination and were considered protective antibodies due to vaccination. Furthermore, vaccination status of dogs was found to be significantly associated with antibody response, this implies that vaccination stimulates antibody response thereby raising the titre and conferring immunity. This was in agreement with other studies in India (Jayakumar *et al.,* 1989), Tunisia (Bahloul *et al.,* 2004), Namibia (Hikufe 2016) and in Ilorin, Nigeria (Aiyedun 2013). This implies that vaccinated dogs are (more likely to have protective rabies antibody titre than unvaccinated dogs and vaccinated dogs. The association between vaccination and protective antibody titre is understandable being that vaccination with rabies antigen elicits antibody response and production of immunoglobulin G proteins, which is in consensus with studies in Ibadan, Abuja, Bolivia and Germany(Subanya *et al.,* 2007) (Olayemi *et al.,*2016) (Jakel *et al.,*2004). This was in contrast to a study in Sri Lanka (Pimburage *et al.,* 2017) and India (Savaliya et al., 2015) where majority of vaccinated dogs sampled did not achieve protective titre. The result of the protective antibodies as a result of vaccination in the vaccinated dogs showed the level of control of rabies in the area under study as well as viability and potency of vaccines.

This study further revealed that 21 (11.4%) of unvaccinated dogs were seropositive for rabies antibodies but had no history of vaccination which suggest an ongoing infection due to presence of rabies virus in apparently healthy dogs (Fekadu et al., 1982). Furthermore, it could be due to previous exposure with a less virulent strain of the rabies virus a situation that may result in the distortion of accurate information of the disease in the Nigeria (Eze et al., 2020). This finding is

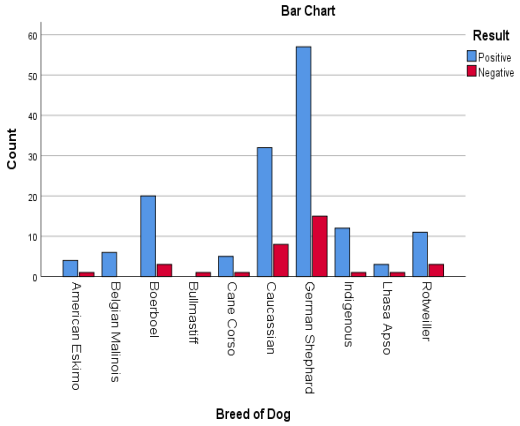

Figure 2. Distribution of rabies virus antibodies according to breed of dogs in FCT, Abuja.

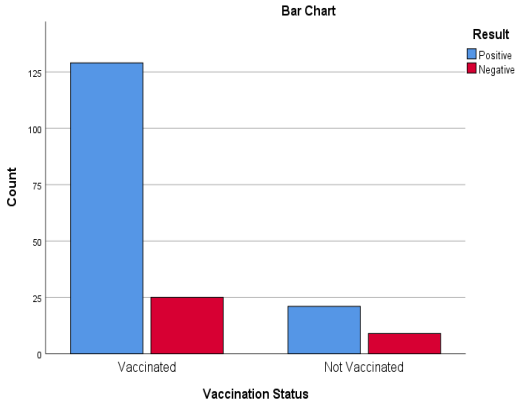

Figure 4. Distribution of rabies virus antibodies according to vaccination history of dogs in FCT Abuja.

similar to work carried out by Konzing et al., (2021) in Jos Metropolis where rabies antibodies were detected in 11 unvaccinated dogs. This agrees with Wosu (1990) which reported a prevalence of rabies antibodies in non-vaccinated dogs to be 17.5%. It was suggested that the antibodies were probably due to non-virulent prototype rabies or rabies related virus strain which is similar to the result of study carried out by cleaveland et al., (1999). The results of this study demonstrated that a proportion of unvaccinated dogs from the area under study had detectable levels of rabies antibodies. However no sufficient information seropositive dogs were reported to have died of suspect rabies. Other findings with regards to antibodies in unvaccinated dogs suggest that seropositive dogs were unlikely to be incubating rabies, but that seropositivity was consistent with aborted infection (Baradel *et al.,* 1988). Although this outcome differs from that classically described for rabies, much of our understanding about the pathogenesis of rabies is derived from experimental, not natural, infections. In natural

populations, the potential variation in infection routes, infective dose and virus characteristics may lead to a broader spectrum of outcomes than occurs in most experimental situations.

This also showed 25 (14%) dogs that had no protective rabies virus antibodies even though they were vaccinated, this could be due to wrong information provided while collecting the data or information regarding the outcome in dogs that merely seroconvert and do not reach protective levels. Kennedy *et al.,* (2007) discussed that the dog's total immunity does not reduce but only shifts from a more dominant IgM to a more IgG-based immunity. This means that absence of protective levels of rabies virus neutralising antibody titres in vaccinated dogs does not necessarily indicate that they are susceptible to rabies infection if challenged. Additionally, the role of cellular immunity and antibodies other than neutralising antibodies that contribute towards immunity from rabies requires further investigation to understand the protection that is observed sometimes in animals that do not show neutralizing antibodies without previous vaccination (Cleaveland *et al.,* 1999). Another reason could be vaccine failure either due to substandard use of vaccine in veterinary clinics or during mass vaccination campaign, a substandard vaccine has the potential to jeopardise the entire campaign as previously experienced in other mass dog vaccination efforts (Eng *et al.,*1994). Higher proportion of protective levels of antibody tires has been reported in other studies demonstrating the possibility of better seroconversion and maintenance of adequate levels of antibodies (Doddet *et al, .*2020) .

The study also showed that most of the dogs sampled for rabies antibodies detection were German shepherd and had highest rabies antibody titre in number. This is consistent with a previous study (Aiyedun, 2013). This may be due to location of the study (a semi-urban settlement where people keep mostly exotic breeds of dogs for security reasons especially German shepherd breed that is known for aggression and intelligence.

The study further revealed that 9 (5.0%) dogs were not vaccinated and were negative. This group constitutes a population of dogs at risk of exposure to rabies transmission and endemicity in the study area with potential public health significance especially in Gwagwalada where more of the seronegative dogs were reported.

CONCLUSION

This Study reveals evidence of rabies antibodies in both vaccinated (81%) and unvaccinated (11%) dog within FCT, Abuja suggesting high vaccination efforts among German Shepherd dogs. Hence awareness on the public health significance/threats of rabies and need for routine annual vaccinations is thus advocated.

Conflict of Interest

The writer declare no conflict of interest.

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