

Canine Rabies with Short Incubation Period in an Unvaccinated Nigerian Indigenous Breed of Dogs: “Case Report”

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Abstract: Canine rabies remains endemic and a serious public health hazard in Nigeria. A case of suspected rabies in an unvaccinated Nigerian Indigenous Breed of dog bitten by a stray dog was reported to the Veterinary Teaching Hospital, University of Abuja. The incubation period was three days and the bitten dog died on day-ten from rabies-like signs. Pen side test, Direct Fluorescence Antibody Test and Reverse Transcription Polymerase Chain Reactions, confirmed rabies virus infection. The incubation period and the number of days post-exposure during which mortality occurred were shorter compared with general held belief of 14-90 days post-rabid animal bite, hence our patient was likely bitten around its head or infected with a virulent wild rabies virus strain. The short incubation period could be dangerous if not promptly reported, therefore, the need for public awareness on the importance of dog vaccination against rabies. The report of high level of unvaccinated dogs in satellite towns of Abuja agreed with this case report. This poor practice poses significant public health risk as such unvaccinated dogs serve as reservoirs for transmission of rabies. Thus, concerned authorities need to take prompt action to initiate and enforce mass rabies enlightenment campaigns and vaccination of dogs in Abuja Capital Territory.

Keywords: Rabies, Short incubation period, Unvaccinated dogs, Stray dogs.

1. INTRODUCTION

Rabies is an acute fatal zoonotic viral encephalomyelitis of all warm-blooded mammals, including humans. It is caused by members of the genus *Lyssavirus* in the family *Rhabdoviridae*, which are bullet-shaped, enveloped, non-segmented and have negative-sensed RNA genome, of approximately 12 kb. Despite being vaccine-preventable [1], the case fatality rate of rabies is almost 100% and it is estimated to cause 40,000-100,000 human deaths each year worldwide. Consequently, the disease is of great public health importance especially in the developing countries [1, 2]. Canine rabies remains endemic and a serious public health concern in Nigeria with dog bites as main mode of transmission to humans and other animals. It occurs in all parts of the country but grossly under reported [3, 4]. Rabies has high socio-economic burden on any country where it is endemic and is responsible for the loss of over 1.8 million

disability adjusted life years) every year [DALYs [5]]. Between January, 2006 to December, 2007 prevalence rate of 46.9% was reported of the total samples submitted to the National Reference Laboratory at National Veterinary Research Institute (NVRI), Vom, Plateau State, from different regions of the country [3]. The prevalence of rabies in Nigeria is between 15 to 20% and the true picture cannot be easily determined because of under reporting [6].

Rabies has been reported in states neighboring the Federal Capital Territory (FCT), Abuja and the movement of rabies infected dogs between the FCT and the neighbouring states is possible [6, 7]. Although there are scanty reports of Rabies in the FCT, Abuja, [4] reported that over 90% vaccination coverage of the dogs' population in the Abuja municipal, while large proportion of the dogs (47.4%) in FCT satellite towns were not vaccinated against rabies. Also, 95.6 % of the

exotic breeds of dogs were reported to be vaccinated, and only 22.9% of the Nigeria Indigenous Breed (NIB) of dogs were vaccinated in the FCT [4].

There can be more pragmatic and proactive systematic activities in eradicating rabies in the FCT than being reactionary. For instance, [8] reported that in October, 2019, FCT Veterinary Services, Department of Agriculture and Rural Development Secretariat (ARDS) conducted vaccination exercise for dogs and cats at Nyanya, following attack on a boy at Mambilla Barracks by a rabid dog. Such reactionary approach cannot be adequate to control rabies; there must be strategic planned programme of holistic campaign targeting all satellite towns and entire FCT to achieve coverage of 70% of vaccination of dog population in order to attain eradication of rabies by 2025, ahead of the global strategic plan to end human death due to dog mediated rabies by 2030 [9]. In this case report, we underscored the public health burden of rabies in FCT satellite communities due to dog bite and the need for sustained routine annual vaccination programmes, which is essential for adequate immunization of dogs and elimination of rabies.

2. CASE HISTORY

On Monday 31st August 2020, a client brought a complaint to the Veterinary Teaching Hospital (VTH), University of Abuja, that his dog was bitten by a stray dog. The incident occurred about seven days earlier in Gwagwalada area council, one of the satellite towns of the FCT, Abuja. The client explained further that about 3 days after contact with the stray dog, behavioural changes were observed in his dog; which became unnecessarily

aggressive and attempting to bite at any friendly approach. It also refused water and food. The dog is a male, NIB, of about eight (8) months old and had no history of vaccination against rabies.

3. DIAGNOSIS

3.1. Physical Examination

The dog was aggressive and refused to take water. It later laid on lateral recumbence, displaying spasm on all the limbs and died on 2nd September 2020, about ten days after contact and exposure to the stray dog. The decapitated. The head of the dog was decapitated wrapped in a water-resistant polythene bag and transported with ice packs in a cooler to the Central Diagnostic Laboratory, NVRI, Vom Plateau State. Post-mortem of the head was conducted to collect brain sample for confirmatory diagnosis.

3.2. Sample Submission

The decapitated dog’s head was wrapped in a water-resistant polythene bag and transported on ice packs in a cooler to Rabies Laboratory, NVRI, Vom Plateau State, for confirmatory diagnosis.

3.3. Laboratory Diagnosis

Pen Side Test (One step Antigen Rapid Test Kit)

Brain tissue sample was aseptically collected during post-mortem examination of the decapitated dog head. The brain tissue sample was subjected to lateral flow rabies virus antigen detection using one step antigen rapid rabies lateral flow test kit (BioNote, Incooperation, Korea-<http://www.bionote.co.kr>), and the result was interpreted according to manufacturer’s instructions (Figure 1).

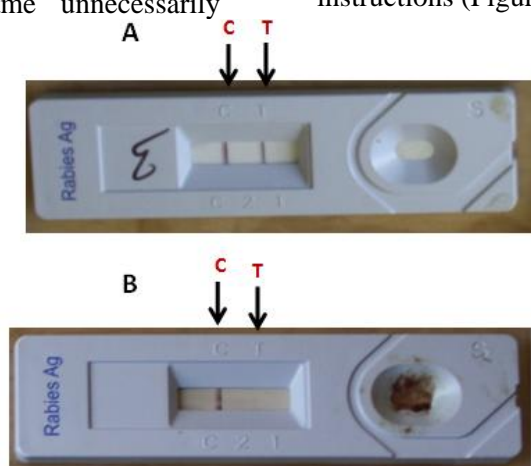
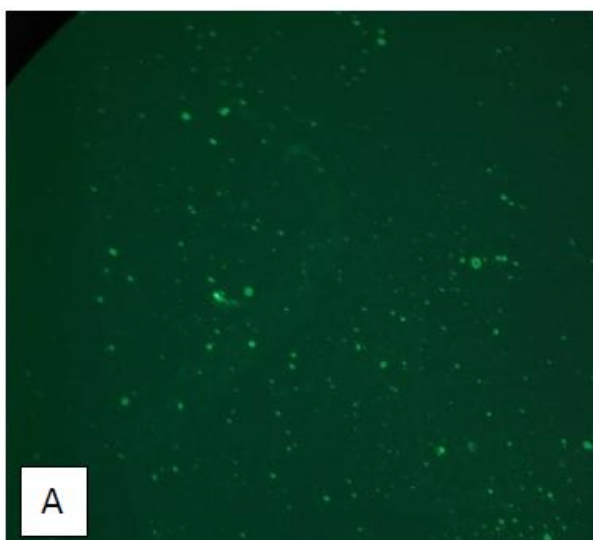


Figure1. (A) One step rapid rabies antigen test result depicting presence of rabies virus antigen in brain tissue sample of rabies infected dog (C-control-black arrow; T-test sample-white arrow - positive); (B) test result

depicting absence of rabies virus antigen in brain tissue of negative sample (uninfected brain sample) (C-Control - black arrow; T-Test sample - negative - white arrow).

Direct Fluorescent Antibody Test (DFAT)

Confirmatory diagnosis was carried out on the brain tissue sample by DFAT as described by [10], using FITC Anti-rabies Lot. 800-092-500-01T (Fujiribio Diagnostic AB Sweden) according to manufacturer’s instructions. The stained smears were then observed for apple green fluorescence under the fluorescent Microscope (Zeiss, Axiovision at 20x magnification) for the characteristic apple-green fluorescence by *Lyssavirus* antigen.



The stained suspected brain sample smears showed apple green fluorescence appearance under the fluorescent Microscope (Zeiss, Axiovision at 20x magnification). The appearance of apple green fluorescence indicates the presence of *Lyssavirus* antigen (Figure 2A). The uninfected brain sample smear is the negative control sample showing no apple green fluorescence (Figure 2B) indicating absence of *Lyssavirus* antigen in the negative control brain smear.

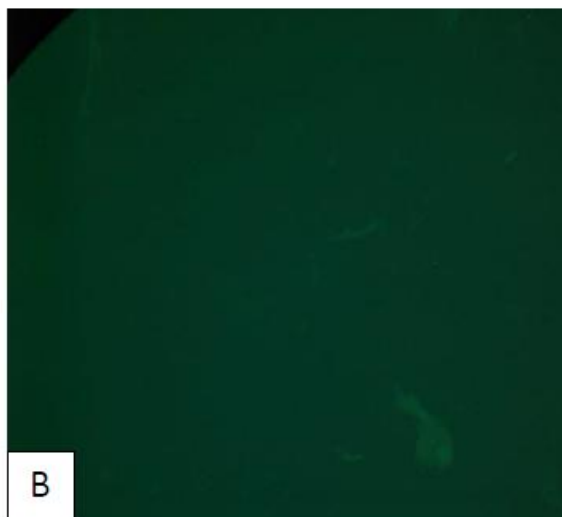


Figure 2.(A) Smear of infected dog brain samples stained with rabies FITC anti-rabies showing apple green fluorescence. (B) Smear of negative brain control sample stained with rabies FITC anti-rabies showing no apple green fluorescence

Reverse Transcription Polymerase Chain Reaction (RT-PCR) Test

A combination of oligonucleotide primers (001lys forward, 550B reversed and 541lys reversed) (Table 1), were used for the detection of rabies virus N gene as described by [11]. Briefly, total RNA was extracted using Bioneer extraction kits (Bioneer, Inc., 1000 Atlantic Avenue Alameda, CA 94501 USA), according to manufacturer’s instruction. The cDNA was synthesized using primer 001Lys and famenters kits (Bioneer, Inc, 1000 Atlantic Avenue Alameda, CA 94501 USA), following manufacturer’s instruction. Amplification of the targeted regions was done adopting the following protocols; 50 µl reaction mixture containing 5 µl of the cDNA, 0.25 µl (1.25 units) of Takara Taq DNA polymerase (Takara Biotechnology, Japan), 3 µl of 25 mM MgCl₂,

4 µl of 10 mM dNTP mixture, 8 µl each of 10 pmol of forward and reversed primers (001Lys and 550B; 541 for N gene, 5 µl of 10X Taq polymerase reaction buffer and made up to 50 µl with 24.75µl of nuclease free water. The amplification was carried out with an ABI 9700 thermocycler with an initial denaturation at 94 °C for 5 minutes, followed by 40 cycles of [94°C for 30 s, 45°C for 30 s, 72°C for 1m] and a final extension at 72°C for 5 minutes [11].

The suspected brain sample after amplicon extraction, RT-PCR amplification showed band size of 600 bp (Figure 3). Rabies virus antigens were detected in the smear of brain sample with all the three (3) assays used in the laboratory diagnosis (Table 2).

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Table1. Details of Rabies Virus N-Genes Primers used in the study

S/No.	NAME	SEQUENCE (5' to 3')	APPLICATION	POSITION ON GENOME
1	001Lys	ACGCTTAACGAMAAA	cDNA Synthesis, PCR	1-15
2	550B	GTRCTCCARTTAGCRCACAT	PCR, hnPCR	647-666
3	541lys	CACMGSNAAAYTAYAARACNAA	hnPCR	541-561

Table2. Test method employed in the laboratory diagnosis of rabies in the case under consideration and the results

S/No.	TEST METHOD	RESULT	REMARK
1	Rapid Pen side test	Positive	Positive
2	DFAT	Positive	Positive
3	RT PCR	Positive	Positive

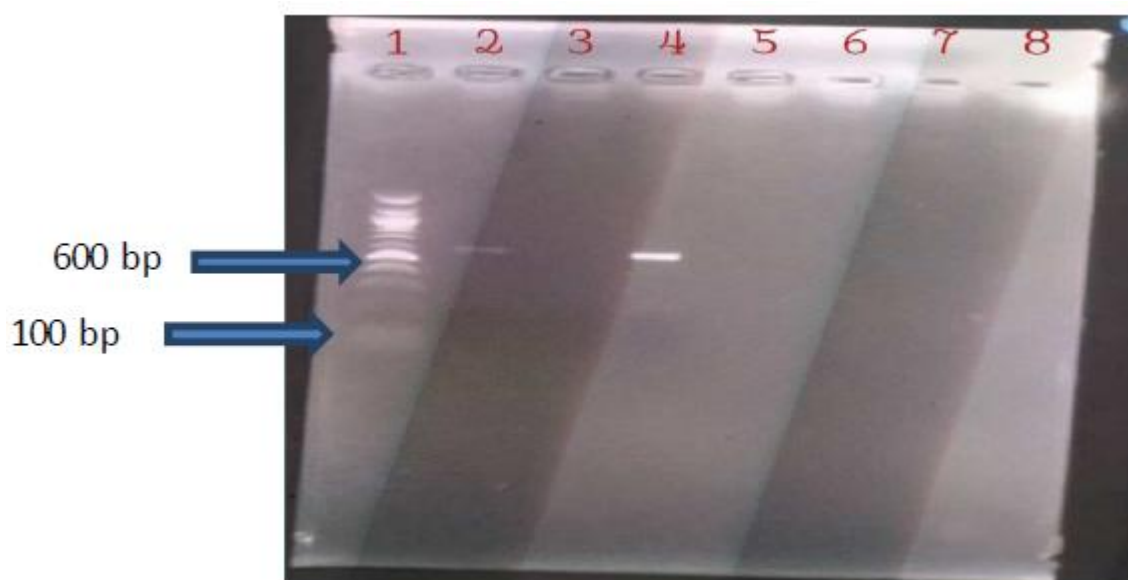


Figure3. Agarose gel image of one step RT-PCR showing Analysis of the fragment formation in One-step RT-PCR for the rabies virus sample. The rabies sample showed band size of 600 bp. Lane 1: is the Molecular marker of 100bp, Lane 2 is the positive controls, Lane 3 is the negative control and lane 4 is the strongly positive rabies sample. Lane 5, 6, 7 and 8 are empty wells.

4. DISCUSSION

Dog bite is of great public health concern because it is the main mode of transmission of rabies to humans and animals in Nigeria [4]. Resulting from deplorable financial situation and rise in insecurity in the last one decade in Nigeria, increase in dog population, particularly in the urban setting as both sources of income and for the security purposes have been witnessed. To worsen the situation, majority of these dogs are not vaccinated against rabies, and they freely roam the streets, increasing the risk of getting infected with rabies virus and transmitting same to other susceptible dogs. This way, continuous circle of transmission and spread of the wild virulent rabies virus strains amongst unvaccinated dogs is maintained.

Therefore, for Nigeria to achieve zero human death due to dog rabies, by 2030, stringent measures need to be taken in providing public awareness on responsible dog ownership and routine vaccination of 70% to 80% of dogs against rabies by all stakeholders. Authorities concerned should initiate vaccination programmes for dogs and enforce their route annual vaccination to mitigate rabies problems as this would break the circle of continuous transmission and spread of the virus.

The incubation period of three days and mortality on day ten in this 8 months old dog appeared shorter than the widely held view that the incubation period of the disease following a rabid animal bite is generally 14-90 days and that most (75%) of cases fall between 20 and 90 days [1]. However, there

have been reported development of symptom within days ([12]. Rare cases of several years of incubation period, usually associated with non-bite exposures have also been reported [13, 14]. Factors that influence the incubation period of rabies include the proximity of the exposure to the central nervous system, magnitude of viral load inoculated into the bite site as well as the nearness of the exposure to innervated tissues. Thus, the incubation is shorter for bites in the head and neck area compared to those in peripheral regions, especially the extremities [1]. In view of this, our patient in this case, was likely bitten around the head, which allowed the virus to access and replicated in the central nervous system within few days. Where clinical diagnosis is relied upon for lack of laboratory facilities, short incubation period could constitute danger, as rabies may be rolled out, considering the short incubation period vis-a-vis the 14-90 days usually reported. This will consequently amount to ‘false negative’ result (based on clinical judgment) and eventually lead to failure to administer post-exposure prophylaxis to exposed persons. Consequently, there is the dire need for public awareness on occurrence of rabies in dogs with short incubation period.

The length of time mortality occurred in this study agreed with [3] who reported eleventh day mortality in the nine months old vaccinated dog after quarantine. This report has further exposed the dangers and the public health burden of unvaccinated NIB of dogs. [4] reported that most (77.1%) of the Nigerian indigenous breeds of dogs in the satellite communities of FCT, Abuja are unvaccinated. In Nigeria, there has been control and prevention law/regulation for vaccination of dogs against rabies but unfortunately the laws are obsolete and are not being enforced. The result of this study suggests that NIB of dogs may serve as reservoir for rabies virus if not vaccinated. Hence, there is need for veterinary authorities through public education and purposeful animal vaccination to initiate, enforce and maintain dog vaccination campaigns geared towards breaking the circle of dog-to-dog transmission of rabies virus. This will invariably minimize dog-to-human transmission of rabies, especially in rabies endemic regions of the world.

5. CONCLUSION AND RECOMMENDATION

This case report shows that not all dog owners in Gwagwalada satellite town of FCT, Abuja vaccinate their dogs against rabies. This practice constitutes dangerous public health risk of contracting infection from bite or scratch of infected animals, especially domestic dogs. Thus, we strongly recommend that relevant government authorities, NGOs and other stakeholders need to institute prompt action for massive rabies enlightenment campaigns and vaccinations of dogs against rabies in the satellites communities in FCT, Abuja, in order to mitigate the spread of rabies.

AUTHORS' CONTRIBUTIONS

EOE reported the case to the VTH, University of Abuja. SIE, PUU and JAO carried out the preliminary observation, physical examination and submitted the laboratory samples to NVRI; ANE, IST and BBD carried out the laboratory tests; ANE, JAO, PUU and SIE developed the manuscript. All authors listed read, reviewed and approved the manuscript for publication in a reputable journal.

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