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A CASE OF RABIES IN A STRAY DOG AT NSUKKA, SOUTHEASTERN NIGERIA

Lynda O. Obodoechi[1], Wilfred S. Ezema[2] and Chukwunyere O. Nwosu[3].

[1]Department of Veterinary Public Health and Preventive Medicine, [2]Department of Veterinary Pathology and Microbiology, [3]Department of Veterinary Parasitology and Entomology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State, Nigeria.

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ABSTRACT

As part of study to expand the epidemiology of rabies and to safeguard public health, brain (hippocampus, brain stem, cerebellum and cerebrum) and salivary glands of a stray dog reported to have bitten a pedestrian without provocation in Nsukka, South-East Nigeria, were examined for rabies virus. Smears of the specimens were stained and examined by direct florescent antibody technique at the Department of Veterinary Pathology and Microbiology, University of Nigeria Nsukka. The test was repeated at the National Veterinary Research Institute, Vom, Nigeria. In both laboratories, the smears fluoresced in presence of known rabies anti-serum to give apple green color which indicated presence of rabies antigen. Genotyping of the antigens by reverse transcriptase- polymerase chain reaction showed that the virus involved belonged to Rabies type 1. The results of this study suggest the continued existence of rabies in stray dogs in Nsukka area of Southeastern Nigeria.

***Keywords*:** Rabies, Stray dog, Nsukka, Nigeria.

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INTRODUCTION

Rabies virus is transmitted when an infected animal bites another animal or humans or when an open wound is contaminated with saliva of infected animals or other sources of the virus [1,2]. With the exception of Antarctica, the disease is endemic on all continents [1]. Its highest case incidence occurs in Asia and Africa where it is a threat to over 3 billion people [1]

Rabies is spread when an infected animal scratches or bites another animal or human [1]. Saliva from an infected animal can also transmit rabies if the saliva comes into contact with the mucous membranes of the mouth, nose, or eyes [1]. More than 99% of rabies cases in countries where dogs commonly have the disease are caused by dog bites [3]. The time period between contracting the infection and the manifestation of symptoms is generally one to three months. However, this time period can vary from less than one week to more than one year [1] .The time is dependent on the distance the virus travels to reach the central nervous system [4].

Correspondence: E-mail: onyinye.obodoechi@unn.edu.ng; **Tel:** +2348037989457
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Rabies causes about 26,000 to 55000 deaths worldwide every year [1, 5]. More than 95% of these deaths occur in Asia and Africa [1]. Death from rabies almost always occurs 2 to 10 days after first symptoms. Survival is rare once symptoms have presented, even with the administration of proper and intensive care [6]. Only a few people have survived a rabies infection after showing symptoms and this was with extensive treatment known as the Milwaukee protocol [7]

One of the first grand successes of the Pastuer's germ theory of disease was the development of rabies vaccine which was the first successful scientifically developed vaccine for prevention of human disease [8, 9]. There have been development of safer and more successful vaccine treatments, better and more effective vaccines and yet rabies remains the most important zoonotic and re-emerging disease worldwide. In Nigeria, the first recorded case of human rabies was in 1912, and in 1925 canine rabies was diagnosed at Yaba rabies laboratory [8]. Every year in Nigeria, about 10000 people are exposed to rabies [10]. A concomitant rise in rabies in both dog and human population in Nigeria portends danger [11].

Dog bite poses a major public health threat both in developed and developing nations. In addition to the severe physical trauma, and potentially permanent disfiguring wounds sustained from dog attack, dog bite victims are often burned with emotional and psychological trauma [12]. Bites expose victims to many potential zoonoses, particularly rabies [13] which is feared because of the extremely high fatality rate of almost 100%.

CASE HISTORY

A stray dog was reported to have bitten a woman at Obukpa Layout, Nsukka, Enugu State in Southeastern Nigeria. The bite was unprovoked. The dog was apprehended, killed and buried by the residents. On the report of the case the next day (after 24 hours of burial) and the suspicion that the animal might have been rabid, the remains of the dog was exhumed for further examination and laboratory diagnosis.

LABORATORY DIAGNOSIS

Brain removal

The head was taken to the post mortem Room, Department of Veterinary Pathology and Microbiology University of Nigeria, Nsukka. Samples (hippocampus, brain stem, cerebellum and cerebrum) of the dog were harvested as described by Aliyu *et al* [14]. Essentially, the head was held firmly in a vice fitted on the operation table with the rostral end facing downward, and the dorsal surface of the head facing the operator. A midline incision was made on the dorsal surface of the head using scapel and blade. The skull was then exposed by dissecting away the skin, aponeurosis and temporal muscles and reflecting them laterally. The skull was sawed using hacksaw by making two latero-medial cuts through the occipital bone, then the temporal bones and finally joining these two lateral cuts at midpoint just above the eyes. The calvarium was lifted off by the aid of a strong thumb forceps while the meninges and the optic nerves were cut with the help of a rat tooth forceps and a pointed scissors. The two parts of the head were then untied from the vice and turned upside down. Using a scapel blade, the brain was then transferred onto a polythene bag and placed on the table to remove the hippocampus, cerebrum, the cerebellum and the brain stem. These samples were stored at -20^{o}C until processed.

Laboratory Investigations

The samples were processed at the Department of Veterinary Pathology and Microbiology, University of Nigeria, Nsukka and tested by FAT. Part of each sample was taken to National Veterinary Research Institute, (NVRI) Vom to repeat the test for confirmation of the result.

Direct Fluorescent Antibody Technique (DFA):

Glass slides were properly degreased and labeled accordingly. A known positive **sample normally challenge virus strain** (positive control) and a normal 3-week-old mice brain (negative control) were

used as controls. Both antigens were sourced from the NVRI, Vom, Nigeria) Thin impression smears of the negative control, positive control and test brain samples were made on the centre of each of the properly labeled glass slides on which smears of the suspected brain samples were made.

The smears were air-dried at room temperature and fixed in cold acetone contained in coplin jar for thirty minutes at -20^{o}C. The slides were air-dried for thirty minutes at room temperature and the smears encircled with wax pencil. 150 µl of the diluted aliquot of the FITC anti-rabies monoclonal globulin conjugate made by Fujirebio Diagnostics Inc. Malvern, PA, USA that detects the four African lyssaviruses (Rabies virus, Lagos bat virus, Mokola virus and Duvenhage virus) was applied to cover the smears within the encircled areas of the slides in the moist chamber and incubated at 37^{o}C for 30 minutes. Afterwards, the slides were properly washed in phosphate buffered saline (pH 8.5) for 30 minutes to remove the unbound DFA reagent in the smears. The slides were allowed to air-dry at room temperature. Two drops of 50% buffered glycerol mounting medium was added and cover slips were mounted and the slides were viewed under florescent microscope. The test impression and the positive and negative control impressions stained with FDI FITC anti- rabies monoclonal globulin were examined with a fluorescence microscope. A test impression was scored as positive when brilliant apple-green fluorescence was observed. The test was taken as valid if brilliant apple-green fluorescence was observed in the positive control, but there was no apple- green fluorescence in the negative control. For the test impressions, when there was no apple-green fluorescence, the sample was read as negative for rabies antigen. When there was apple-green fluorescence, the sample was read as positive for the antigen.

Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Seventeen street rabies viruses isolated in mice and one DFA positive ample were subjected to RT-PCR. The single tube method using multi enzymatic liquefaction of tissue (MELT ^{TM}) Total nucleic Acid isolation system was used in the isolation, extraction and purification of the RNA according to manufacturer's instructions (Applied Biosystems). Challenge Virus Standard (CVS)-positive RNA was used as the positive control in all the semi nested RT-PCR. The positive RNA was obtained, following the intra-cerebral inoculation of mice with CVS. The mouse brains were confirmed as DFA positive prior to RNA extraction as earlier described. While extracting RNA from a panel of isolates, negative mouse brain was also extracted at the same time in order to provide a negative control. The negative mouse brains originated from normal uninfected mice. The RNA was quantified using the Nanodrop machine (following the manufacturer's instructions). The RNA was diluted in HPLC purified water ensuring that it was not weaker than 1ng/µl. This was divided into aliquots of 5ul and stored at -80 ^{o}C until used. A hemi nested reverse transcriptase PCR (hnRT-PCR) assay that uses a cocktail of primers capable of detecting the six established genotypes of rabies and rabies-related viruses was conducted according to the method of Heaton *et al* [15] and Nadin –Davis [16]. These genotypes included classical rabies represented by "p" Pasteur virus, Lagos bat virus "L", Mokola virus "M", Duvenhage virus "D" and European lyssaviruses 1 and 2 "E". The primers were obtained from Inqaba Biotec Industries, South Africa. The primer designs were based on previous work of Heaton *et al* [15]. These primers were designed to recognize regions with high degree of homology between the nucleoprotein encoding genes. The expected amplicon size was 405bp and this was when sequence could be used to differentiate genotypes. Positive PCR results were observed in the form of bright band corresponding to 405 bp of a 100 bp marker.

RESULTS

The brain smears were found to show presence of fluorescing rabies viral antigens. Thus, the dog was positive for rabies disease before it was killed. The PCR and gene typing suggests that the rabies virus was classical rabies virus.

DISCUSSION

The reference method for diagnosing rabies is the florescent antibody test (FAT, an immunohistochemistry procedure) which is recommended by the WHO [17]. The FAT relies on the

ability of a detector molecule (usually fluurosescein isothiocynate) coupled with a rabies-specific antibody, forming a conjugate, to bind to and allow the visualization of rabies antigen using florescent microscope. The results of this study confirm that the stray dog has rabies virus at the time it was killed and that suffering from rabies may have been the reason for the unprovoked bite in this case. This appears to be the first reported case of rabies in a stray dog in the study area since there are no previous records of rabies in dogs generally in the study area. However, Wosu and Anyanwu [18] had previously detected rabies antibodies in vaccinated dogs in parts of Eastern Nigeria including the study area.

Confirmation of rabies in the stray dog calls for concern. This is because the dog was exhumed following the consent of the Veterinarians as killing and burying of any stray dog that bites humans has been the culture of the people in the area. Reports from the people showed that stray dogs often bite humans in the community and such dogs are usually killed in the belief that death of the dogs will prevent death of their victims. Often times, these bites are ignored and unreported. This issue of under reporting poses a threat to efforts to control and eradicate rabies in Nsukka area of Enugu State and Nigeria in general, as there will be neither relevant information nor samples for a proper diagnosis. This gives credence to the report of Fagbami *et al* [19] that reliable data on rabies and rabies–related lyssaviruses are scarce in many parts of the world, especially in the developing countries of Africa like Nigeria, because rabies is erroneously perceived as an animal and not human disease and is therefore, often under reported.

This case of dog bite was in the rural area where poverty, ignorance, and poor dog ownership culture abound. Dog bites in this area, are most times, treated with herbal medications and only presented to the hospital when obvious clinical signs start manifesting as has been reported to be the case in South Africa. [20]. The rate of human dog bites caused by stray dogs is responsible for suggesting that control of stray dogs is vital to success of efforts to control rabies in both animal and human populations in developing countries [21].

RT-PCR assays have proved to be sensitive and specific test for routine diagnostic purposes [22]. The PCR result confirmed that the virus detected in the brain specimen was classical rabies virus and not a rabies- related virus. This agrees with earlier reports [22, 23] that no rabies related virus has been isolated from dog brains examined for *Lyssavirus* in Nigeria.

Rabies in dogs is a serious public health problem in Enugu State especially in Nsukka because of under reporting, herbal medication and their belief which could have caused deaths of many dog bite victims.
One way to prevent rabies in humans is to focus on controlling it in animal reservoirs hence, we recommend regular interventions targeted at controlling stray dogs by establishing and maintaining high vaccination coverage in the dog population through public enlightenment /awareness campaigns. We also, recommend a joint sensitization of physicians and veterinarians about the need for detailed investigation of cases of dog bite in humans. Good dog ownership including confinement of dogs should be enforced on dog owners by relevant authorities to minimize incidences of dog bite.

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