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RABIES IN A SET OF EIGHT-WEEK OLD PUPPIES IN NIGERIA: THE NEED FOR REVIEW OF CURRENT DOG ANTIRABIES VACCINATION SCHEDULE

Nanven Abraham Maurice[1], Pam Dachung Luka[2], Magdalene Nanven Maurice[3*], Emmanuel Ochefije Ngbede[4], Ponfa Nden Zhakom[5], Philip Paul Mshelbwala[6], Ishaya Sini Tekki[4], Utibe-Abasi Hilary Udoh[7], Udoh Akpan Inyang[7], Nsika Joseph Ekanem[7], Okon John Ifut[7], Philip A. Okewole[1], David Shamaki[1], Lami Hanatu Lombin[8]

[1]Department of Diagnostic and Extension, National Veterinary Research Institute, Uyo Laboratory, Akwa Ibom State, Nigeria; [2]Biotechnology Division, National Veterinary Research Institute, Vom. Plateau State, Nigeria; [3]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Kaduna State, Nigeria; [4]Department of Pathology and Microbiology, College of Veterinary Medicine, University of Agriculture, Makurdi, Benue State. Nigeria; [5]Rabies Laboratory, National Veterinary Research Institute, Vom., Plateau State, Nigeria; [6]Department of Veterinary Medicine, Faculty of Veterinary Medicine, University of Abuja, Nigeria; [7]Department of Animal Science, Faculty of Agriculture, University of Uyo, Akwa Ibom State, Nigeria; [8]Faculty of Veterinary Medicine, University of Jos, Plateau State Nigeria.

***Corresponding Author's E-mail**: mnanven@hotmail.com

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Abstract

Background: Rabies is endemic in Nigeria with clinical cases reported mainly in dogs and occasionally in livestock from all the geo-ecological zones of the country. Detection of rabies virus antigen in puppies at the age of five to ten weeks and in apparently healthy dogs shedding the virus in their saliva have been reported in some parts of Nigeria.
Material and Method: This report describes a clinical rabies in a set of eight weeks old puppies confirmed by antigen detection using the direct fluorescent antibody test (DFAT), the direct rapid immunohistochemical test (DRIT), and RT-PCR.
Results: it was positive for all test used including the RT-PCR which amplified at 750 bp from the gel electrophoresis.
Conclusion: Occurrence of rabies in puppies of this age, within which they are acquired and owned by other unsuspecting members of the public, is of grave public health consequences. People that love puppies, especially children, who are fond of carrying and playing with them, are also faced with the risk of exposure to rabies. Consequently, review of the existing dog antirabies vaccination schedule at twelve weeks of age in Nigeria, is recommended to ensure effective immunization of this age group of dogs and for the overall safety of the vulnerable members of the public.

Keywords: Puppies, Rabies, Vaccination schedule, DFAT, DRIT, RT-PCR, Nigeria.

Introduction

Rabies is caused by a single-stranded, RNA virus that belongs to the genus *Lyssavirus* and family *Rhabdoviridae* (Walker *et al*., 2015). The highly neurotropic rabies virus causes a rapidly progressive encephalomyelitis or meningoencephalitis (Tekki *et al*., 2014). Rabies affects all mammals but primarily domestic and wild dogs; other hosts include foxes, jackals, skunks, mongooses, raccoons and bats (Nandi and Kumar, 2011; Noah *et al*., 1998). However, there are reports of rabies in livestock like cattle, sheep and goats in Nigeria (Ojo and Adeoye, 1967; Okoh, 1981; Bello et al., 2007; Promed, 2012; Tekki *et al*., 2014). RABV persists in Eastern Europe and border crossings of rabid foxes resulting in the reintroduction of rabies in Italy (2008), Macedonia (2011) and Greece (2012). In Western Europe, the indigenous rabies reserves are now limited to bats, for EBLV-1 and EBLV-2. Europe is facing with cases of imported rabies, mostly linked to the illegal introduction of animals from countries not free of rabies: 21 cases (dogs or cats) between 2001 and 2013 (Kirandjiski *et al.,* 2012; Tsiodras *et al.,* 2013). However some countries in the United Kingdom, New Zealand, Hawaii, Australia, Japan and Antarctica are free from the rabies virus through

successful eradication programs or historically rabies have not been reported in these region (Hoffmann *et al*., 2010). Rabies has persisted in Asia and Africa where the burden is highest globally. In Nigeria, exposure to rabies through bites from rabid dog accounts for 100% of the confirmed cases (Garba *et al*., 2010; Ameh *et al*., 2014; Odeh *et al*., 2014). The epidemiology of rabies has been described in Nigeria with dogs as the reservoir of the disease (Oduye and Aghomo, 1985; Garba *et al.*, 2010; Ameh *et al*., 2014; Odeh *et al.*, 2014).

Materials and Methods

On July 16, 2015, a client presented a six-year bitch which just whelped for the sixth time with the set of three puppies to the National Veterinary Research Institute (NVRI) Outstation, Uyo, Akwa Ibom State, Nigeria for routines check-up. Two of the puppies were female while one was a male. The owner also had a four years old male mongrel in the house.

On September 3, 2015, the dog owner reported to the NVRI Laboratory that one of the female puppies went out of the house-hold gate early in the morning and was bitten on the upper and lower jaws by a stray dog suspected to be rabid. The bite-wound was simply dressed and 0.3ml of antibiotics (Penstrep) was administered by parenteral route to the puppy per day for five days to facilitate wound healing by controlling secondary bacterial infections. A couple of weeks later, the owner reported abnormal behaviours such as aggression, hyper salivation in the puppy. It was also reported to have bitten other puppies in the kennel before it died on September 21, 2015 at 5:15 pm, three weeks after it had been bitten by the rabid suspect stray dog. Subsequently, the remaining two puppies also died at eight weeks old. The case was tentatively diagnosed as Rabies. The heads of the two puppies that died later were severed and shipped on ice to the Rabies laboratory, NVRI, Vom, Plateau State, for confirmation, the animal's brain was collected by opening the skull as described (Barrat and Blanco, 1998).

Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. An Institutional Ethical Clearance - NVRI AUCC REF No: AEC/02/23/15 was obtained from the Animal Use and Care Committee of National Veterinary Research Institute, Vom, Nigeria, to carry out the study.

Laboratory Diagnosis

Direct Fluorescent Anti-body Test (DFAT): DFAT was carried out as described by CDC (2006).

Direct Rapid Immuno-histochemical Test (DRIT): DRIT was performed on formalin fixed impression smears of the brain specimens as described by CDC (2011).

RNA Extraction and RT-PCR

Sample preparation: Approximately one gram of brain tissue from each sample was weighed and homogenized using a pestle and mortar. After homogenizing, 10% tissue suspension was made in PBS, pH 7.2 and clarified in a refrigerated centrifuge at 10,000 rpm for 5 minutes. The supernatant was decanted into a sterile tube and kept at 4°C for RNA extraction. RNA extraction: Total RNA was extracted using QIAamp Viral RNA Mini kit following the manufacturer's guidelines. Extracted RNA was kept briefly at 4°C pending cDNA synthesis. cDNA synthesis and conventional RT-PCR: Reverse transcription and subsequent amplification were performed on all samples, using a previously described protocol (Markotter *et al*., 2006) with primers 001lys and 550B. RT-PCR was performed on samples using the following protocol. One microliter of the primary amplified PCR product was added to a final volume of 50µl containing 5.0µl 10× PCR buffer (New England Biolab®, USA), 4.0µl dNTP mixture (10 mM) (Promega), 10 pmol forward primer 001lys and reverse primer 550B, and 0.5µl Taq polymerase (2 U/µl; New England Biolab®, USA). Amplification was performed on a GeneAmp PCR System 9700 (Applied Biosystems, Germany). After denaturation at 94°C for 1 min, reactions were cycled 40 times at 94°C for 30s, 37°C for 30s, and 72°C for 90s, with final extension at 72°C for 10min. Products were visualized on 1% agarose gels stained with ethidium bromide.

Results and Discussion

Apple-green fluorescing rabies viral particles were present in the brain sample smears processed by FAT (Figure 1). Similarly, DRIT result revealed red inclusions within bluish cell bodies as shown in Figure 2 below, indicating that the sample was positive for rabies. The presence of the virus was also confirmed by RT-PCR (Figure 3).

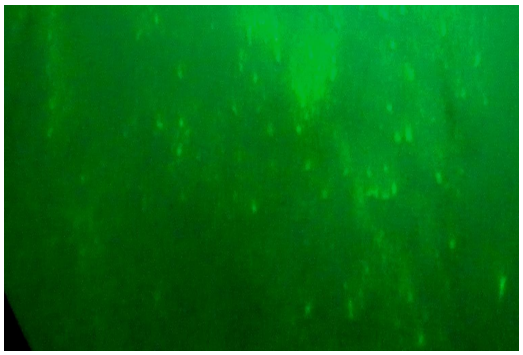

Figure 1. DFAT positive sample

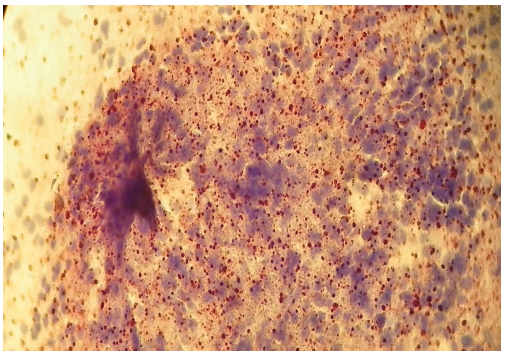

Figure 2. DRIT positive sample

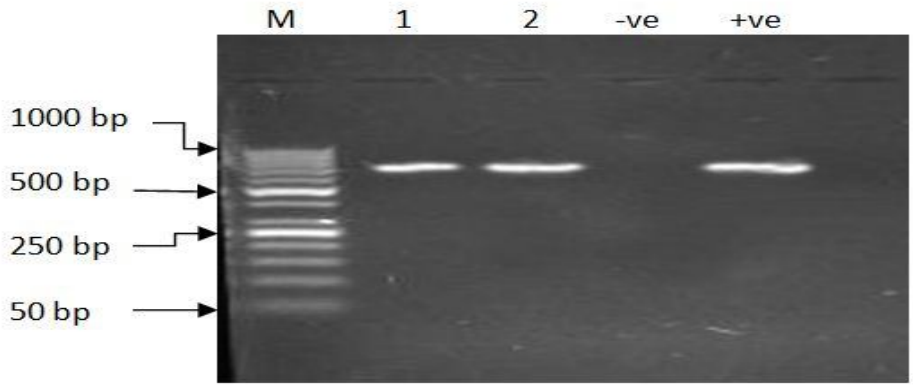

Figure 3: M- Molecular marker, Lane 1 and 2 samples, -Ve- Negative control, +Ve- positive control. The samples were amplified at 750bp from the gel electrophoresis.

Rabies is endemic in Nigeria and this has been attributed to dog-to-dog, dog-to-livestock and dog-to-human transmission (Bello *et al*., 2007; Aworh *et al*., 2011; Abubakar *et al*., 2012; Hambolu *et al*., 2013 Tekki *et al*., 2014). Generally, effective control of the disease can best be achieved by achieving a minimum of 70% vaccination coverage of dogs (Coleman and Dye, 1996). This high coverage is especially important to maintain the population level immunity and to break transmission of rabies in situations where stray dogs predominate (Hambolu *et al.,* 2014). In Nigeria, all dogs aged three months and older are vaccinated against rabies (Umoh *et al*., 1988), with the assumption that younger ones are protected by maternal derived antibody (MDAs). Occurrence of rabies in puppies of less than three months of age in this report is therefore of a serious public health concern. Although the general public is at risk of infection, the strong attachment of most owners and other lovers of dogs, especially children, to puppies or dogs generally, increase the risk of human infection with the causative virus. This therefore calls for review of dog vaccination schedule for effective control of rabies in Nigeria. In previous studies, rabies has been reported in a ten weeks old puppy in Uyo, Akwa Ibom State (Offiong *et al*., 2013) and in eight weeks, six weeks and five weeks old puppies in parts of Nigeria (Adeyanju and Addo, 1977; Onunkwo *et al*., 1980; Kujul *et al*., 2010). Outside Nigeria, rabies has been reported in a 10 weeks old male border collie-cross puppy in Saskatoon in Western College of Veterinary Medicine Teaching Hospital (Jennifer *et al*., 2007). It is obvious therefore that puppies infected with rabies virus are veritable sources of infection to man and other animals as they would eventually be disposed of by the dog breeder and acquired by new owners.

Demonstration of rabies virus by standard laboratory techniques viz.: DFAT (Figure 1), DRIT (Figure 2) and Amplification (750bp) of a region of the N gene using oligo primers 001lys and 550B as described by Markotter *et al*., (2006) (Figure 3) in the brain samples of the puppies in the present study; coupled with the previous reports above, pose a serious challenge to Veterinarians and other stakeholders in Nigeria in their efforts in achievement of the elimination of death due to dog rabies by 2030 (global Alliance for the Control of Rabies, 2015).

One wonders what the dynamics of the epidemiology of the virus is in domestic dogs in Nigeria currently, considering the occurrences of rabies in puppies at far below the recommended age of vaccination and the reported detection of rabies virus or some Lyssaviruses in apparently healthy dogs in parts of Nigeria (Aliyu *et al*., 2010; Mshelbwala *et al.*, 2013; Hambolu *et al*., 2013; Tekki *et al*., 2014).

Most of the puppies affected by rabies in Nigeria so far have been from mongrel bitches which are usually either not vaccinated or improperly vaccinated against rabies (because of the poor knowledge and attitude on the importance of vaccination and who is qualified to administer vaccine. This is in addition to the fact that majority of the owners of Mongrel are poor in the society). In the present cases however, the bitch had been vaccinated against rabies before having the puppies in question. Although the bitch was not screened for rabies virus neutralizing antibody, there was an indication that the bitch was either not immunized even though vaccinated, hence the puppies had no maternal derived antibody (MDAs), or the level of the MDAs was too low to protect them. Vaccine failure had occurred in 2.5 of every million doses in Nigeria as cited by Kujul *et al.* (2010) who reported the occurrence of rabies in puppies of a bitch that had previously been vaccinated against rabies. The commonest cause of non-seroconversion and protection of a vaccinated individual against rabies is vaccine failure, which is a reoccurring phenomenon in Africa and Asia. This is reported to have been caused by reduced antigenicity of the vaccines (Webster, 1939), largely due to poor handling (Oladokun *et al.*, 2010) and improper administration of the vaccines (Tierkel, *et al*., 1953). Possible changes in the strains of rabies vaccine viruses during stages of propagation in different animal hosts had been adjudged to reduce their usefulness as vaccines (Wiktor, *et al*., 1969). Therefore, to ensure maintenance of vaccine potency for effective immunization of dogs, there is need to carry out proper monitoring of handling and administration of rabies vaccines as loss of viability resulting from improper handling in the field has been reported in Nigeria (Oladokun *et al*., 2010).

If the dam of the affected puppies was protected and had passed adequate MDAs to the puppies, it then means that the puppies succumbed because the MDAs had waned at the time they were exposed. It is also possible that the vaccine was no longer potent at the time of administration resulting in vaccine failure and thus no MDAs to confer passive immunity were transferred to the puppies increasing their susceptibility. This finding corroborates the reports from the field that show increase in the incidence of dog rabies in Uyo, Akwa Ibom State which, according to Offiong *et al*. (2013) is exacerbated by the high rate of handling and consumption of dog meat as a special delicacy among the natives of the State. Most of the trade dogs slaughtered and consumed in Uyo are unvaccinated stray dogs captured from various places in northern part of the country. The dogs are usually transported together under stressful conditions to major holding centres in Uyo, warranting fighting and transmission of pathogens including rabies virus from infected to susceptible ones. From the local holding centres, the dogs are distributed amongst various slaughter spots without screening for rabies and fitness for slaughter (Ehimiyein *et al.*, 2014). This will certainly increase the risk of human exposure to rabies in the state if necessary measures are not taken to by stakeholders to enforce compulsory vaccination of dogs against rabies.

Conclusion

Although rabies is endemic in Nigeria, detection of the virus in puppies before the recommended age of vaccination suggests that there is a change in the trend of the epidemiology of the disease. Evidences of rabies in puppies that are younger than the official vaccination age in Nigeria (3 months) give credence to the need for change of the policy on dog vaccination against rabies in order to protect this age group of dogs and the general public from infection with rabies.

This therefore calls for review of dog vaccination schedule for effective control of rabies in Nigeria. Annual rabies mass vaccination campaign in addition to improved public health enlightenment campaigns on the devastating effect of rabies and the importance vaccination in the prevention and control of rabies are recommended.

Conflict of Interest: The authors declare that they have no conflict of interest.

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