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Full Length Research Paper

Risk of typical rabies in dog meat-eating human population, in Enugu, Nigeria

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This study was designed to ascertain if the rabies virus antigen found among apparently healthy dogs were viable and to which genotype they belong. One hundred and fifty two dog heads were randomly collected from markets and restaurants where dogs are slaughtered and consumed as delicacy in Enugu State, Nigeria. The brain samples from the heads were examined for the presence of rabies virus antigen by direct fluorescent antibody technique, while positive ones were isolated by the mice inoculation test. Viral genotypes of the positive samples were determined by reverse transcriptase polymerase chain reaction and sequencing. Out of the one hundred and fifty two dog brain samples examined, six (4%) were positive by direct fluorescent antibody technique and mice inoculation test. All the isolates belong to genotype 1 rabies virus. The result of this study showed that some of the apparently healthy dogs slaughtered for meat in Enugu state have classical rabies virus and possibly shed the virus in their saliva without showing obvious clinical signs. This has a serious public health implication and thus calls for a re-evaluation of rabies endemicity in Nigeria.

Key words: Rabies virus, Slaughtered dogs, DFAT, MIT, RT-PCR, Genotyping.

INTRODUCTION

Rabies disease is caused by rabies virus of the genus lyssa virus which occurs worldwide and in a variety of animals including man. It belongs to the order Mononegavirales and family Rhabdoviridae (viruses with distinct "bullet" shape) (CDC, 2008). The virus is neurotropic, single-stranded organism which causes fatal encephalitis with high mortality rate in a wide variety of mammalian species (Nadin-Davis, 2002). Besides poliomyelitis and pox, rabies is one of the longest known infectious diseases in human history (Krauss *et al*., 2003). In Nigeria, rabies is endemic and one of the most significant but neglected zoonotic diseases. Despite the development of safer and more effective vaccine in recent time, rabies remains an important zoonotic and re-emerging disease worldwide. It is estimated that more than 55,000 people die of rabies annually and over 95% of these deaths occur in Asia and Africa (WHO, 2005). Dogs are susceptible to rabies and they are the most likely animal host that spread infection to humans (Nadin-Davis, 2002). While dogs are the major animal reservoirs in the developing nations, wild animals maintain the cycle of infection in developed countries with consequent emergence of new strains of virus (Ruppretch *et al*, 2006). A model had earlier been proposed in which rabies virus survives by adaption to the preferred host (dog), in such a way that a mutually non-detrimental ecological balance or "climax" was established (Chambers and Scott 1969). In this "Canid climax" it was suggested that the dog suffered in-apparent infection or transient illness and recovered animals became "healthy carriers". Occasional infection of the "climax" hosts may result in overt disease and death and spillover into the less adapted wildlife or human host may result in epidemics. Evidence to support this model is the existence of "*oloufato*" in West Africa a non-fatal disease of dogs which had been identified as rabies (Chambers and Scott, 1969). In general, many reported cases in Africa and elsewhere show that clinical rabid dogs may

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sometimes not die but recover and continue to live as carriers (Arko *et al*., 1973, Fekadu, 1975; Bell, 1975). This suggests that virus carrying and virus excreting "healthy" dogs are assumed to exist. The implication of healthy carriers, in-apparent infection and recovery from clinical rabies is that they compound human exposure to unrecognized carriers (Ajayi *et al.*, 2006; Nadin-Davis, 2008). In Nigeria, there had been reports of the presence of rabies antibodies in non-vaccinated dogs and evidence of rabies antigen has also been reported in apparently healthy dogs, although these authors did not demonstrate that the antigens seen were viable (Fekadu, 1975; Ogunkayo *et al*., 1984; Garba *et al*., 2010). Dogs live in close proximity to human and sometimes they are slaughtered for consumption. These slaughtered dogs may be in-apparent carriers of rabies virus, and thus serve as potential risk in infecting humans (Wertheim *et al*., 2009). Prior to this study no isolates of rabies virus have been obtained from the brain of these apparently healthy dogs hence this study to determine the risk associated with dogs slaughtered for consumption.

MATERIALS AND METHODS

Markets were conveniently selected based on history of dog-meat consumption, and not more than ten heads of dog were sampled from each market. None of the slaughtered dogs had a history of rabies vaccination or any clinical sign indicative of rabies as at the time of sale or slaughter. One hundred and fifty two (152) heads of apparently healthy slaughtered dogs were bought from fifteen markets where dogs are slaughtered for human consumption. Various sections of the brain and salivary glands were harvested as previously described (Atanasiu, 1975). Direct fluorescent antibody test (DFAT) was performed according to the method described by Dean *et al*., (1996) using monoclonal fluorescein isothiocyanate-labeled anti-rabies antibodies (Fujirebio Diagnostics Inc., Malvern, PA, USA). Mouse inoculation tests (MIT) were performed on all DFAT positive and doubtful samples as previously described by Koprowski, (1967).

Total RNA was extracted from all the positive sample using Trizol reagents according to the manufacturer's instruction (Life Technologies, Carlsbad, California, USA). The N-gene was amplified by reverse transcription–polymerase chain reaction (RT-PCR) in two overlapping amplicons, as described by Trimarchi and Smith (2002). Sequence analyses and phylogenetic reconstructions were performed using Bio Edit (Hall 1999) and MEGA 6 software platforms (Tamura *et al*., 2013). Phylogenetic analysis was conducted by comparing the partial rabies sequences with other rabies isolates selected from the Genbank representing rabies virus isolates that circulate in Africa and other rabies-related viruses.

RESULTS

Six (4%) samples were positive by both the DFAT and mouse inoculation tests. Salivary glands of the corresponding six positive brain samples were also positive by MIT while others were negative. Results of the genetic analysis shows that all the isolates belong to rabies Genotype 1 with 95-100% nucleotide similarity with previous rabies isolates from Nigeria and none showed homology with any rabies related viruses (RRV). All the isolates grouped together except KJ921999 which had a very close identity with EU888724 – a previous isolate from the northern Nigeria (Figure 1).

DISCUSSION AND CONCLUSION

The result of this study has for the first time demonstrated viable rabies virus in apparently healthy dogs that were slaughtered for human consumption in Nigeria. This result is in agreement with the report of previous study which suggested that dogs may suffer in apparent infection or transient illness in "canid climax" model particularly in Africa, and recovered animals may become "healthy carriers" capable of transmitting the disease to other animals and possibly humans (Chalmer and Scott, 1969).

Our finding suggests possible carrier state of rabies among dog population in Enugu state of Nigeria including those sold for human consumption. This finding is consistent with the results of previous workers (Kureishi *et al.,* 1992; Wallerstein, 1999; Wertheim *et al.,* 2009) who reported an association between slaughtering and processing of dogs and human rabies. That isolate KJ921999 is identical with isolate EU888724 (an isolate from Northern Nigeria) is not surprising as most of the dogs slaughtered in the south are purchased from the northern part of the country. In Nigeria, rabies antibodies have been demonstrated in some unvaccinated and apparently healthy dogs, with a potential consequence that these animals could excrete rabies virus in their saliva for a long period without showing any symptoms of the disease (Aghomo *et al*., 1986; Baba, 2006; Garba *et al*, 2010).

Although death is the ultimate end when dogs are usually infected (Baer, 1991), our finding has shown that, that position may no longer be valid as these dogs 'live' with the virus without any obvious clinical signs. Ogunkoya *et al* (2003) in an earlier report arrived at similar conclusion. It will appear that dogs without clearly observable clinical outcome pose serious health risk to other animals and humans and as such, it is advisable to implement immediate treatment to individuals who suffer from dog-bite irrespective of the status of the dog as all cases of bites in endemic countries should be seen as potential rabies case unless proven otherwise (Chhbara *et al*., 2007).

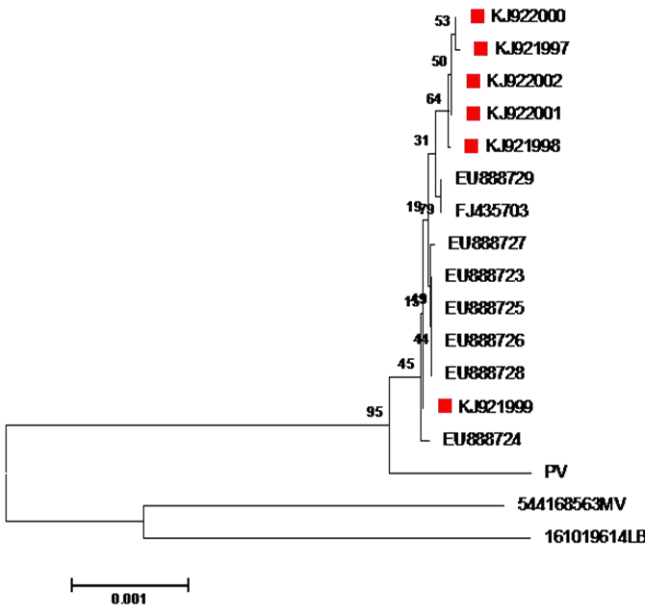

Figure 1. A neighbor-joining phylogenetic tree based on partial N gene sequences (400 bp) of the rabies using the K2P model and 1000 bootstrap replicate values. The genetic distance is shown by the horizontal branch lengths and vertical branches are noninformative and were used for clarity purposes only. 544168563MV is Mokola virus while 161019614LB is Lagos bat virus. The tree was rooted with Pasteur virus (PV).GenBank accession numbers of isolates in this study KJ921997; KJ921998; KJ921999; KJ922000; KJ922001; KJ922002).

Since MIT results revealed that the rabies virus isolates were virulent and caused fatalities in mice, it may be possible that clinical rabies would have developed in these dogs if they were not slaughtered. Based on the RT-PCR and gene typing, classical rabies virus and not rabies related viruses were confirmed. Okoh (1989) has previously stated that no rabies related virus have been isolated from dog brains examined for lyssavirus in Nigeria.

It will be necessary to consider further investigations since rabies isolates proven to have 100% morbidity and mortality in this study were recovered in apparently normal dogs without any clinical sign. The implication of healthy carriers, in-apparent infection and recovery from clinical rabies is that they compound human exposure to unrecognized carriers (Ajayi *et al*., 2006; Nadin-Davis, 2008).

We therefore conclude that carrier states of rabies in dogs do exist in Nigeria and this situation is grave for the country in view of the weak human disease surveillance and overtaxed healthcare systems (Fasina *et al.,* 2009). The excretion of virus in the saliva of such apparently health dogs may play a significant role in perpetuating the virus in nature and in the transmission of the disease. It is therefore obvious that these dogs shed rabies virus in the environment without apparent clinical signs, posing health risk problems especially to the marketers, butchers, processors and consumers. The public health risk of rabies in Nigeria is real and effort should be made by all stakeholders to address this.

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