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Detection of Rabies Antigen in the Brain Tissues of Apparently Healthy Dogs Slaughtered in Ogoja - Cross River State, Nigeria.

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SUMMARY

Rabies is a serious public health hazard and recently outbreaks of the disease have been reported in three local government areas in Cross River State. Detection of rabies antigen in the brain tissues of apparently healthy dogs indicates the presence of rabies virus and this is a significant factor in the transmission and spread of the disease. A cross sectional study was set up to investigate the presence of rabies antigen in the brain tissues of dogs slaughtered for human consumption in Ogoja. The results showed that out of 177 brain samples tested by Fluorescent Antibody Technique, 6(3.39%) were positive for rabies antigen. The rate of infection was higher in females 5(6.33%) than in males 1(1.02%). All the positive samples were from adult dogs. Observations during sampling revealed that processors of dog meat were not wearing protective clothings and had no pre or post exposure prophylaxis against rabies. These findings imply that those involved in handling and processing of dog meat are greatly at risk of exposure to rabies either from bite by these dogs or from infected nervous tissues or saliva that may accidentally contaminate open wounds or bruises and even intact mucous membrane (oral and ocular) during dog meat processing. Therefore public health intervention is required to create awareness about the dangers of the disease.

Key words: Rabies antigen, slaughtered dogs, Ogoja, Nigeria.

INTRODUCTION

Rabies is caused by a virus of the family *Rhabdoviridae* and genus *Lyssavirus*, now called lyssa virus 1 (RABV gen 1). It manifests in three classical stages, prodromal stage, excitatory stage and paralytic or silent stage (Idachaba *et al.,* 2009). Rabies virus causes acute encephalitis in all warm blooded hosts including humans and the outcome is almost always fatal (Nadin-Davies, 2000; WHO, 2010). Although all species of mammals are susceptible to rabies virus infection, only a few species are important as reservoirs for the disease (Garba *et al.,* 2009). The domestic dog is the established reservoir for rabies virus (RABV) in Nigeria and the virus is maintained in this host (Dzikwi *et al.*, 2010; WHO, 2010). It is the principal reservoir and transmitter host for rabies to animal and humans (Oboegbulem, 1994; Osinubi *et al.,* 2009). Although rabies is a vaccine-preventable disease, it still poses a significant public health problem in many countries in Asia and Africa where 95% of human deaths occur even though safe and effective vaccines for both human and veterinary use exist (WHO, 2010).

Developing countries including Nigeria are considered risk areas for rabies exposure and the vaccination status of the suspected animal alone should not be used or considered whether to initiate post-exposure prophylaxis or not (CDC, 1983; WHO, 2008; 2010) as human deaths have resulted even from bites of dogs

reported to have been adequately immunized against rabies. The presence of rabies antigen in the saliva and brain tissues of apparently healthy dogs slaughtered for human consumption in some parts of Nigeria and the obvious consequences of bites from these healthy dogs resulting in human deaths have been reported (Ezeokoli and Umoh, 1987; Baba, 2006; Garba, 2007; Garba *et al.*, 2008 and Aliyu *et al.*, 2010). Dogs are being slaughtered for human consumption in some parts of Ogoja, thus there is a risk of exposure to rabies. Several reports or studies (Ajayi *et al.*, 2006; Garba *et al.*, 2008; Sabo *et al.*, 2008; Akombo, 2009;) have shown prevalence of rabies antigens in the brain tissues of apparently healthy dogs slaughtered for human consumption in some parts of the country. Also, Asuquo *et al.* 2005; Adeyemi *et al.* 2005; Idachaba *et al.* 2009 and Owai, 2009 have reported the prevalence for rabies in Okoyong community in Cross River State, Ibadan, Plateau and Calabar respectively, thus confirming the presence of the disease and its health risk to the general public.

Detection of rabies antigen indicates presence of the virus which is a significant factor in transmission of rabies from slaughtered dogs to the processors and/or consumers. Other lyssa viruses which are of a zoonotic threat to humans are Mokola virus (MKV), Lagos Bat Virus (LBV), Duvenhage Virus (DHV), European lyssa viruses 1 & 2, Australian Bat genotypes (Nottidge *et al.*, 2007). There is a dearth of information on the epidemiology and public health significance of canine rabies in Ogoja despite the fact that dogs are being commonly slaughtered. The detection of rabies antigen in slaughtered dogs will give information on the possible role of these dogs in the epidemiology and spread of rabies in the area.

The information on canine rabies status will serve as an epidemiological tool and a basis for successful control and prevention or intervention strategy towards the disease. According to Widdowson *et al.*, (2002), controlling rabies in urban dog populations is seen as a more cost effective, long term approach to prevent human rabies than reliance on post-exposure human treatment.

MATERIALS and METHODS

Study area: The study was carried out in Ogoja located in the Northern senatorial district of Cross River State. It is about 40 kilometres away from Vandeikya in Benue state. Nigerian local breed of dogs are slaughtered mainly in the urban town and some villages in Ogoja.

Study design: This cross sectional study was conducted in Ogoja between February and May, 2012 to detect rabies antigen in the brain of slaughtered dogs.

Brain sample collection: A total of 177 brain stem samples from slaughtered dogs were collected from various slaughter points (relaxation places called 'Joints' where dogs are slaughtered and processed for consumption) within the study area. Out of the total number of dogs tested 98(55.4%) were males, [94(95.9%) > 24 months and 4(4.1%) 1-24 months old], while 79(44.6%) were females, [76(96.2%) > 24 months and 3(3.8%) 1-24 months old]. Ageing of the dogs was carried out by examining the canine teeth both on the lower and upper jaw. Information on the source and sex of the dogs presented for slaughtering were obtained and the health status of the dogs was ascertained. Brain samples were collected by opening the skull through the occipital foramen in the direction of an eye according to World Health Organisation recommendation (Barrat, 1996). Each slaughter point was visited twice daily (morning and afternoon) and brain samples were collected based on availability. The brain samples obtained were stored in a pre-labelled EDTA free bottle containing phosphate buffered 50% glycerol solution. Additionally, some specimens were frozen at -20^{0}C. All the samples were then transported to the Viral Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria for rabies antigen detection

by fluorescent antibody test as described by Dean *et al.*, (1996).

Fluorescent antibody test (FAT) procedure: Rabies direct fluorescent antibody assay (DFA) monoclonal antibody conjugate reagents (Fujirebio Diagnostic Inc. Malvern, P.A 19355, USA) was used and the working reagents dilution prepared according to the manufacturer's recommendation and as described by Flamand *et al.*, (1980). A small fraction of the brain sample was smeared on one part of a slide and then air dried and fixed on cold acetone for one hour at -20°C. The slide was then air-dried for 3 minutes and the rabies conjugate was applied and incubated for 30 minutes at 37°C in a humid chamber after which excess conjugate was removed from the slides by rinsing in three changes of PBS (PH 7.4) for about 5 minutes. The slides were air-dried and examined using a fluorescence microscope within 2 hours after staining with fluorescein isothiocyanate labelled antibody. The presence of brilliant apple green fluorescence or greenish-yellow particles against a dark background was regarded as positive result for rabies while absence of specific apple green fluorescence was regarded as negative for rabies.

Public health intervention: Public health education was instituted by preparing and distributing pamphlets and flyers to high risk groups in the study area. These materials contained information on rabies and its danger to public health, prevention and control. Interaction with processors and consumers of dog meat to encourage pre or post exposure prophylaxis and use of personal protective clothing during operations were also carried out.

Data analysis: The rate of rabies antigen detection was calculated as a proportion of the total number of dogs examined.

RESULTS

Out of the 177 samples tested for rabies antigen by direct fluorescent antibody technique, 98(55.4%) were males, [adults 94(95.9%), young 4(4.1%)] while 79(44.6%) were females, [adults 76(96.2%), young 3(3.8%)]. The results showed that a total of 6(3.38%) were positive for rabies antigen. The rate of infection was higher in females 5(2.82%) than in males 1(0.56%) [Table 1]. All the positive samples were from dogs above two years.

TABLE 1. Distribution of positive samples by sex and age of dogs slaughtered in Ogoja, Cross River State Nigeria.

Gender	Age*		No. tested (%)	No. positive Sex specific rates (%)
	1-24mths (%)	>24mths (%)		
Male	0/4	1/94	98(55.4)	1 (0.56)
Female	0/3	5/76	79(44.6)	5 (2.82)
Total (%)	0/7	6/170	177(100)	6 (3.38)

Age* indicates number positive/ number tested

Distribution of brain tissue samples by source showed that 4(2.2%) were sourced from Ogoja, 14(7.9%) from Okuku, 6(3.3%) from Obudu all in Cross River State while 146(82.4%) were from Benue State and 7(3.9%) from Adamawa State. The highest number of positive samples 4(2.26%) were from Benue State while the remaining 2(1.12%) were from Ogoja and its environs (Table 2).

After distributing pamphlets and flyers to respondents, and educating them on the dangers of rabies and the need for taking pre and post exposure prophylaxis, washing dog bite wounds with detergent and water, wearing protective clothings during processing of dog meat and other risky behaviours, the response was very encouraging as participants expressed interest to change their attitude and practices towards rabies.

Table 2: Distribution of brain tissue samples by source of dogs slaughtered in Ogoja, Cross River State Nigeria.

	Source	No. examined (%)	No. positive (%)
1)	Cross River State		
	Ogoja	4 (2.2)	1(0.56)
	Okuku	14 (7.9)	1(0.56)
	Obudu	6 (3.3)	0 (0)
2)	Benue State	146 (82.4)	4 (2.26)
3)	Adamawa State	7 (3.9)	0 (0)
	Total	**177(100)**	**6 (3.38)**

DISCUSSION

The presence of rabies antigen in brain tissues of apparently healthy dogs slaughtered for human consumption is a significant factor in the epidemiology of the disease. The public health implication of this finding is that individuals involved in handling and processing of dog meat are at risk of exposure to rabies either from bites by these dogs or from infected nervous tissues or saliva that may contaminate open wounds or bruises during processing of dog meat. The presence of rabies antigen in apparently healthy dogs slaughtered for human consumption is in agreement with previous studies from several locations in Northern Nigeria (Sabo *et al.*, 2008; Garba *et al.,* 2008; Akombo, 2009; Aliyu *et al.,* 2010). In this study, it was shown that there were more female dogs positive for rabies antigen than male dogs. Adult dogs were mostly affected with rabies antigen than young dogs probably because more adult dogs were presented for slaughtering during the period of study. This result also agree with the findings of Baba (2006) and Aliyu *et al.*, (2010) which showed that more adult dogs tested positive for rabies antigen compared to puppies.

Majority of the positive samples 4(2.26%) were sourced from Benue State which is a neighbouring State to the study area. In an earlier study carried out by Akombo (2009) in Makurdi, Benue State, a prevalence of 15.8% was recorded in brain tissues of dogs slaughtered for human consumption. The implication of this finding is that rabies could possibly be imported to Ogoja through dog trade from the neighbouring state. The public health education materials distributed to high risk group (processors of dog meat) were effective mainly in informing the participants on how to deal with an animal suspected to be rabid, the earliest age for pets to receive anti-rabies vaccination (ARV) and repeat innoculation, and clinical signs of suspected rabid dogs. This was demonstrated in the participants' willingness to change their attitude and practices positively towards rabies. They were also used in creating awareness about adequate wound washing which should be the first line of action after animal bites before seeking medical attention.

The demonstration of rabies antigen in slaughtered dogs is an indication of a potential source of rabies infection and constitutes health hazards to dog handlers and consumers particularly in rural areas where dog meat processing is on the increase. Therefore public health campaign should be intensified especially in the rural areas where access to health facilities is limited.

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