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Detection of rabies virus antigen in brain tissue of dogs slaughtered for human consumption in Taraba State, Nigeria

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Abstract

Rabies as an ancient zoonosis constitutes a threat to public health by causing over 59,000 annual human mortalities worldwide. The aim of this study was to detect rabies virus in brain tissue of dogs slaughtered for human consumption in Taraba State, Nigeria. A total of 150 dogs comprising 136 adults and 14 puppies consisting of 82 males and 68 females was sampled from slaughter points in five Local Government Areas. Brain samples were collected from each dog in labeled sterile sample bottles and screened for rabies virus antigen using direct fluorescent antibody test (DFAT). Results showed that 3 out of the 150 (2%) brain samples screened were positive for rabies virus; out of which 2 were from Unguwan Kasa (14.3%) and 1 was from Quarter Five (7.1%). This therefore suggests the presence of rabies virus in dogs slaughtered for human consumption in Taraba State, Nigeria and their role as reservoirs of the virus. Therefore, there is need for awareness education on safe handling of dog meat to minimize the risk for butchers/meat handlers.

Keywords: Brain samples, dogs, Prevalence, Rabies, Taraba State

Introduction

Rabies is a viral encephalomyelitis transmitted to humans following exposure to infected mammals, mainly dogs, through bites, scratches or licks on damaged skin or mucous membranes (Léchenne *et al*., 2016). However, transmission of rabies through solid organ transplantation has been reported (Zhang *et al*., 2018). The disease is caused by a neurotropic virus of the genus *Lyssavirus* of the family *Rhabdoviridae*, and is transmissible to all mammals including humans (Shankar, 2009). Following infection, the virus migrates along neural pathways into the central nervous system (brain). From the brain, it travels centrifugally to the peripheral and autonomic nervous systems, eventually migrating to the glands and organs (Zhou *et al*., 2016). It is widespread in African domestic dogs and certain wild canine populations (Bingham, 2005). This disease represents a global public health concern, causing an

estimate of 59,000 human deaths per year, mainly in low income countries (Leung *et al*., 2007; Hampson *et al*., 2015). The limited control measures in many countries and lack of governmental concern make the disease a neglected tropical disease (Knobel *et al*., 2005; Hampson *et al*., 2015). The burden of rabies is influenced by age-related and socioeconomic factors: rabies is most commonly reported in children below 15 years of age and in poor and low-income people that have no access to treatment facilities (Sudarshan *et al*., 2007). Control of rabies in the animal reservoirs (domestic dogs) is the only means to disrupt the transmission cycle of the disease and to eliminate both dog and human rabies cases in the world (Cleaveland *et al*., 2006; Zinsstag *et al*., 2009).

It has been reported that apparently healthy dogs harbour and excrete the virus in Nigeria (Baba, 2006; Aliyu *et al.*, 2010). These healthy carrier dogs are sometimes slaughtered and consumed by humans, thus creating a potential risk of transmission of the virus (Baba, 2006). There are also reports of previously vaccinated dogs coming down with rabies in some parts of Nigeria (Okoh, 2000). Dog bite cases by apparently healthy dogs and other dogs in Taraba State of Nigeria have become common (TSSHMR, 2014). The consumption of dog meat by humans is a common practice in Taraba State and many parts of Nigeria, and the public health implication of this practice is yet to be fully assessed (Ameh *et al*., 2014). Therefore, the aim of this study was to determine the prevalence of rabies virus in apparently healthy dogs slaughtered for meat in Taraba State, Nigeria.

Materials and Methods

Study area

The study was conducted in some selected dog markets/slaughter points in Taraba state, North-Eastern Nigeria. The state lies between latitudes 6°25'N and 9°30'N and between longitudes 9°30'E and 11°45'E (Taraba State Ministry of Land and Survey, 2005). It has 16 Local Government Areas with land mass of 55,920.00 sq. km, population of 2,300,736 people and population density of 54 per sq. km (Taraba State Ministry of Land and Survey, 2005).

Sample size determination

The sample size for the study was calculated using the formula of Mahajan (1997).

$N = Z^2pq / d^2$

Where N = Sample

Z = the appropriate value from the desired confidence (1.96)

p = expected prevalence

q = 1 - Prevalence

d = Allowable error

Using relative prevalence of 7.98 % from previous study by Ameh *et al*. (2014)

$N = 1.96^2 \times 0.0798 \times (1-0.0798) / 0.05^2$

N = 112.8

In order to increase the chances of detecting the antigen, 150 dog brain samples were collected.

Dog sampling

Convenience sampling method was used to select 5 Local Government Areas (Jalingo, Ardo Kola, Gassol Bali and Takum) for this study based on the availability of existing dog markets/slaughter points. The criteria used for the selection of the dog markets/dog slaughter slabs (points) included in the study were the metropolitan and/or heterogenous nature of the towns taken for the study, the recognized dog markets with the availability of a good number of slaughtered dogs of at least 6 per market day being brought from all over the state, the understanding and consent of slaughter points owners, owners of the dogs, and cooperation and participation of the butchers.

Simple random sampling technique was employed to select slaughtered dogs intended for human consumption from the aforementioned sampling units over a period of three consecutive months (July to October, 2014).

Specimen collection

Prior to slaughter, the age, sex, breed and vaccination history were obtained and recorded. A total of 150 dog brain tissue samples were collected as described by Barrat and Blancou (1988) from dog slaughter points in the five Local Government Areas of Taraba State, Nigeria. The brainstem was obtained by inserting a straw through the occipital foramen, placed in pre-labelled sample bottles and stored at -20°C in a small Hisense® freezer and later transferred to -80°C freezers in the National Veterinary Research Institute (NVRI) Vom, Plateau State Nigeria.

Rabies virus antigen detection

The brain samples were subjected to the direct fluorescent antibody test (DFAT) for the presence of RABV antigens as described by Dean *et al*. (1996) at the National Veterinary Research Institute (NVRI) Vom, Plateau State, Nigeria. This was performed using monoclonal fluorescein isothiocyanate-labeled anti-rabies virus antibodies (FITC) (Fujirebio Diagnostics Inc., Malvern, Pennyslvania PA USA) and polyclonal antibody–conjugate (Bio-Rad, Australia).

The results were recorded based on the specific aggregates of rabies virus antigen detection (as apple green fluorescence) using fluorescence microscope.

Results

From the 150 local dogs sampled, 136 were adults while 14 were puppies; 82 were males while 68 were females. Using direct fluorescent antibody test (DFAT), 3 (from adult male dogs) out of the 150 (2%) brain samples collected and screened showed an apple green fluorescence (Figures 1a and 1b) for the two conjugates, hence were positive for rabies virus. Out of the 3 positive samples, 2 were from Unguwan Kasa (14.3%) and 1 was from Quarter Five (7.1%) (Table 1).

Discussion

A total of 150 dog brain samples from apparently healthy local dogs were collected and examined by DFAT from 11 dog markets/slaughter points in the 3 Senatorial districts of Taraba State, Nigeria. The 2% dog brain samples positive for rabies antigen by DFAT is lower than 7.98% by Ameh *et al*. (2014) in Wukari Metropolis, Taraba State; 31 % by Ajayi *et al*. (2006) in Maiduguri Borno State; 43 % by Sabo (2009) in Plateau State; 44% by Aliyu *et al*. (2010) in Yola Adamawa State; 7.0% by Otolorin *et al*. (2014) in Aba, Abia State. The lower prevalence reported in this study could be due to the lower number of dogs sampled and the short time duration of sampling (3 months). Also, since only the brainstem was collected, the detection of the antigen could possibly be decreased hence, the lower prevalence. Dogs are

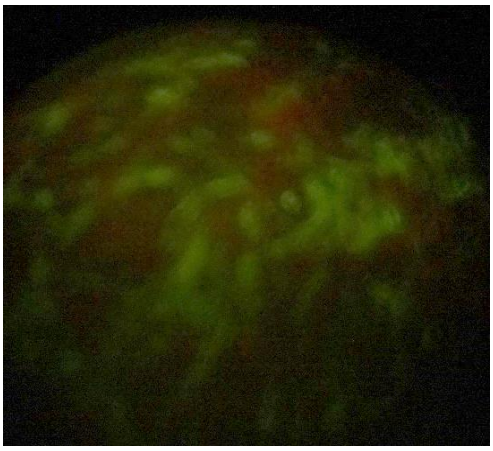

Figure 1a: DFAT negative test sample showing no apple green fluorescence indicating rabies negative sample

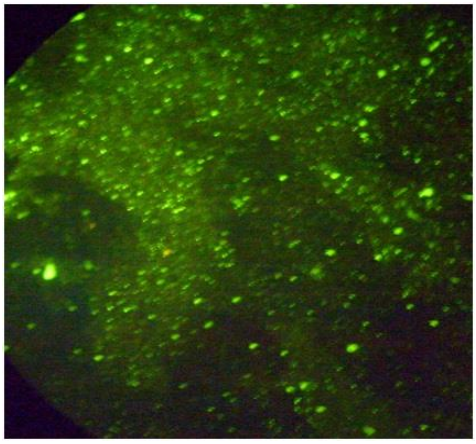

Figure 1b: DFAT positive test sample showing apple green fluorescence indicating rabies positive sample

Table 1: Distribution of dogs sampled for rabies virus in Taraba State

Location	No. sampled	Age (adult/ puppy)	Sex (male/ female)	Breed (local/ exotic)	DFAT positive	DFAT rate (%)
Unguwan Kasa	14	12/2	6/8	14/0	2	14.3
Gidan Yazawa	14	13/1	5/9	14/0	0	0.0
Mile Six	14	14/0	8/6	14/0	0	0.0
ATC Bye-pass	14	12/2	9/5	14/0	0	0.0
ATC Kofai Matai	13	12/1	6/8	13/0	0	0.0
Opp Tsokwa Lodge	13	13/0	9/4	13/0	0	0.0
Quarter Five	14	12/2	7/7	14/0	1	7.1
Ada Barracks	14	11/3	8/6	14/0	0	0.0
Unguwan Mummuye	13	11/2	7/6	13/0	0	0.0
Unguwan Dorawa	13	13/0	9/4	13/0	0	0.0
ATC Kasuwan Bera	14	13/1	8/5	13/0	0	0.0
Total	150	136/14	82/68	150/0	3	2.0

The rabies positive brain samples were from adult male dogs

bred from Plateau and Kaduna States and sold out to buyers from different part of the country including Taraba State of Nigeria. The detection of rabies virus antigen in these dogs in Taraba State was not uncommon as they were probably never vaccinated. Also, these dogs were brought from different part of the state and country with no vaccination records. Apparently healthy dogs are dogs that present no clinical characteristics of rabies such as lethargy, lack of appetite, agitation and anxiety on physical examination. However, they have been demonstrated to be reservoirs of rabies virus in China as reported by Lu *et al*. (2006) and Zhang *et al*. (2008). These dogs could bite humans and other dogs thus posing a great danger to the populace as their vaccination status cannot be ascertained. Human rabies deaths due to transmission of rabies through handling and skinning of infected carcasses and subsequent consumption of raw meat had been documented in Asia (Tariq *et al*., 1991; Hu *et al*., 2009). This indicated that dogs slaughtered for human consumption could be a source of infection to dog handlers and butchers as most of the individuals involved in dog meat processing do not utilize personal protective equipment. Also, the method of slaughter predisposes these dog meat processors to bites from dogs during handling.

In conclusion, rabies virus antigen was detected in slaughtered apparently healthy local dogs in Taraba State, Nigeria. To the best of our knowledge, this finding confirms the presence of rabies virus in apparently healthy dogs slaughtered for meat consumption in Taraba State, Nigeria. Further studies on the isolation, characterization and virulence of this virus should be carried out in this area. Therefore, implementation of measures to enhance prompt and adequate screening and vaccination of dogs should be intensified whether for human consumption or not. There is also need for awareness education on the risk and danger of rabies to dog handlers, butchers and consumers of dog meat.

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Conflicts of Interest

The authors declare no conflict of interest.

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