Field and Laboratory Detection of Rabies Antigens in Saliva and Brains of Dogs in Nigeria: An Approach Using Rapid Immunochromatographic Test

Audu SW1*, Adawa DAY2, Mshebwala PP3, Simon YA2 and Yakubu B4

1Department of Veterinary Medicine, Ahmadu Bello University, Zaria
2Veterinary Teaching Hospital, Ahmadu Bello University, Zaria
3Department of Veterinary Medicine, University of Abuja, FCT Abuja
4Molecular and Biotechnology division, National Veterinary Research Institute, Vom

*Corresponding Author: Audu SW, Department of Veterinary Medicine, Ahmadu Bello University, Zaria, Tel: +2348065535565, E-mail: Solomonaudu.ss@gmail.com


Abstract

A rapid diagnosis associated with bite will quickly explain whether a bite constitute an exposure or not; brains and saliva of rabid animals has been documented to contain rabies antigens and serves as medium for the transmission of the infection to susceptible host and 98% of rabies in human is caused by bite from rabid dogs. In Kaduna State, Nigeria there are several active dog markets and slaughter areas where dogs are slaughtered for meat, there are also Veterinary Clinics where destroyed, euthanized and suspected rabid animals are send for examination or quarantined. Study was carried out in five slaughter areas and a clinic within 3 Local Government Areas in Kaduna State to evaluate the efficacy of field and laboratory detection of rabies antigens in saliva and brains of apparently healthy dogs slaughtered for consumption and suspected rabies cases that were brought to the Clinic dead or alive dogs using rapid immunochromatographic diagnostic tool for detection of rabies antigen in the saliva and brains sample of rabies suspect, Saliva and brains of 50 apparently healthy dogs slaughtered for consumption were screened for rabies antigens and eight (16%) were found to be positive for rabies antigens using Rapid Immunochromatographic test for rabies antigens and Direct Flourescent Antibody Test (DFAT). In the clinic saliva of ten dogs involved in bite and showing signs consistent with rabies were tested for rabies antigen using Rapid Immunochromatographic Test following either natural death or euthanasia and were positive. The brain of same dogs was tested for rabies antigen using RICT. All extracted brains were tested for rabies antigens using the goal standard test, DFAT and all those that were positive for Rapid Immunochromatographic test for rabies antigen in the field and laboratory were also positive for DFAT. Considering the distance between diagnostic and reference centres from rural communities, we strongly recommend the use of this diagnostic test. It is handy, cost effective and can reduce cost of management of dog bite victims.

Keywords: Brain; DFAT; Saliva; Rabies Antigen; RICT

Introduction

Rabies is an acute, contagious and highly fatal disease of all warm blooded animals caused by a Rhabdo virus that is single stranded, negative sense RNA and belongs to lyssa genus [1,2]. The virus has tropism for peripheral nerve tissues and the central nervous system of both humans and animals [3-6]. The disease is endemic in Nigeria and the actual level of burden of the disease in the country has not been achieved due under reporting [7, 8]. The saliva of rabid dogs has been documented to contain high concentration of rabies viruses and serves as a medium for transmission of the infection [9-11]. Although, non-bite exposures can occur through licks or splash of infected saliva into wound, 98% of human deaths have been documented to have been caused by almost and always bite of a rabid dog [12]. The virus causes a non suppurative viral encephalomyelitis with characteristic clinical forms of rabies such as dumb, furious and asymptomatic (inapparent) forms [13-15]. Once clinical sign sets in, death is the end result. However, some recoveries have been reported both in Man and Animals world-wide [3,16].

The procedure for the direct Fluorescent Antibody Test (DFAT) was first described by Goldwasser and Kissling...
The immuno chromatographic lateral flow strip test is a one-step test that facilitates low-cost, rapid identification of various analytes including viruses [22]. Briefly, suspected material is subjected to a test device similar to a lateral flow device. Conjugated detector antibodies attached to two different zones on a membrane indicate the presence of viral antigen. Preliminary validation studies with a limited number of samples showed that the rapid immuno chromatographic test (RICT) might have great potential as a useful method for rabies diagnosis without the need for laboratory equipment and electricity [22].

Materials and Methods

Convenient random sampling as described by Mike (2011) was employed [26]. The most accessible units of the population were used. Live dogs bought or brought for slaughter were sampled in slaughter areas designated in a selected dog markets within 3 selected local Government areas of Kaduna State (Kaduna South, Chikun and Sabon Gari). Cases of dog attacking and biting school children, Biting the owner and family members, biting animals in the community, restless and attacking moving objects and humans and dog with hanging jaw with stringy saliva, unable to eat and use its hind limbs Dog bite cases with suspected exposure and history of mental disorientation were selected for the study.

A rapid diagnosis associated with bite will quickly explain whether a bite constitute an exposure or not [11,23]. The brain or saliva of suspected biting dog is used for the detection of the rabies antigen [24]. In African and Asian countries, at least 10% of brain samples received at the laboratories for rabies diagnosis are decomposed because of lack of storage facilities, proximity of the incident area to the laboratories, poor transportation, inadequate prompt diagnostic tests that are field based, all these factors lead to late and misdiagnosis. Diagnosis is fundamental in the surveillance and control of rabies. In Nigeria, dFAT is often performed on dogs at postmortem where dogs involved in bite cases are destroyed or euthenised to confirm if they are rabid or not. This avoidable practice often cause losses to dog owners and breeders [8,12,14,25]. Therefore, the absence of a rapid confirmatory test can result in the inappropriate management of animal bite, unnecessary administration of post exposure prophylaxis (PEP) which will cause an economic burden to the owner. It can also cause delay in rabies PEP which can cause human death. In Kaduna state, there are several active dog markets where dogs are slaughtered on a daily bases without ante-mortem and/or post-mortem examination, this pose as a potential danger to butchers and those involved in dog trading. Distance of diagnostic centres from most rural clinics serves as a major challenge to prompt diagnosis and management, in Kaduna state the only diagnostic centres are located in Mando and Zaria. A rapid diagnosis can initiate a rapid response. The study was carried to identify whether rapid immunochromatographic diagnostic test is suitable for use both in field and laboratory for diagnosis of rabies using the saliva and brain of dogs.

Materials and Methods

Study Method

Convenient random sampling as described by Mike (2011) was employed [26]. The most accessible units of the population were used. Live dogs bought or brought for slaughter were sampled in slaughter areas designated in a selected dog markets within 3 selected local Government areas of Kaduna State (Kaduna South, Chikun and Sabon Gari). Cases of dog attacking and biting school children, Biting the owner and family members, biting animals in the community, restless and attacking moving objects and humans and dog with hanging jaw with stringy saliva, unable to eat and use its hind limbs Dog bite cases with suspected exposure and history of mental disorientation were selected for the study.

Study Area

The study was conducted in Kaduna State which falls within the Guinea Savannah zone and covers an area of about 48,473.2 square kilometers and situated at longitude 06°10’ and 09°00’ East of Greenwich Meridian and Latitude 09° 10’ and 11°30’ North of the Equator. Dog markets/ slaughter slaps located in Basawa Army Barracks Mammy market, Ungwan Tabo Samaru and Jushi in Northern Senatorial district and Kamanzou, Trikania, Television Garage, in Central senatorial district based on availability.

Sampling Method

The study employed convenient random sampling technique as described by Mike (2001) [26]. The most accessible
Rapid Immunochromatographic Test kit Cat.No: RG18-0, Lot.No: 1801DD003 (Rabies Ag Test kit, Bionote) for the detection of rabies antigen in the brain and saliva of dogs was employed. Saliva from dogs presented for slaughter were sampled and tested as described by the manufacturer. Briefly, a sterile swab stick was inserted into the mouth of the dog before slaughtering. The wet swab was then inserted into an assay buffer tube and stirred to ensure a good sample extraction. The immunochromatographic test cassette was then removed from the foil pouch and placed horizontally. Using a sterile dropper, three drops of the extracted sample was then dripped into the sample hole in the cassette and result was interpreted within five to ten minutes.

All brain samples were tested using the rapid immunochromatographic test as described by the manufacturer. Briefly, a 10% suspension of brain sample was made in the buffer tube. The immunochromatographic test cassette was then removed from the foil pouch and placed horizontally on the table. Using a sterile dropper, three drops of the 10% sample was then dripped into the sample hole in the cassette and result was interpreted within five to ten minutes. All the brain samples tested using rapid immunochromatographic test were also subjected to Fluorescent antibody test (FAT) as described by Dean et al (1996) [21]. Briefly, DFAT was performed in the Viral Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, ABU, Zaria., Impression smear of the brain sample was prepared on a clean glass slide, air dried and fixed in cold acetone for 1 hour at – 20°C. The acetone fixed immersion smear of the sample was then stained with Fluorescien-labelled anti-rabies immunoglobulin (FITC anti-rabies monoclonal globulin, Fujirebio Diagnostic, Inc. (FDI), USA). The slides were incubated for 30 minutes at 37°C in a humid chamber and then washed with phosphate buffered saline (pH 8.5) three successive times over a period of 10 minutes. Slides were air-dried after rinsing with distilled water. The slides were viewed at X 40 using a Fluorescent microscope (ZEISS, Primo star Ileid, Carl Zeiss microimaging, Gottingen, Germany; series no. 3133000412). Results of the test were interpreted positive if a bright apple-green fluorescence of particles was observed under the microscope and negative when no specific apple-green fluorescence was observed on the slide under the fluorescent microscope.

Using Rapid Immunochromatographic test, 8 out of 50 dogs sampled were positive for rabies antigen that means shedding the virus in their saliva (16% prevalence). The brains of these same dogs were subjected to RICT and the same result with that of the saliva was obtained. These brain samples were again subjected to DFA test and were positive for rabies antigens same as in RICT with varying degree of positivity from ++ to +++ (Figure 1).

Clinical samples were tested using RICT for rabies antigen and all those that were positive were also tested using Fluorescence Antibody Test were also positive with varying degree of positivity between +++ and ++++ (Figure 2).

Figure 1: Rapid immunochromatographic test result of saliva of a dog showing positive

unit of the population was used. Here, live dogs bought or brought for slaughter were sampled in the different slaughter areas. Saliva and brains of dogs were sampled for the study.

Saliva

Brain

Laboratory diagnosis

Results
Figure 2: Comparative positive test result of brain of apparently healthy dog slaughtered for human consumption

Figure 3: Shows a DFAT positive brain slide of a clinical rabies case with apple green appearance

Discussion

Poor diagnosis and reporting system of rabies cases either as an exposure or death despite being an endemic area may be as a result of poor surveillance and reporting system, inadequate, cheap, prompt, rapid and accurate laboratory and field-based diagnostic rabies tests. The findings of this study revealed that rabies is endemic in Kaduna state and is found in both dogs that shed the virus and yet look apparently healthy and those that were destroyed or brought for clinical examination as a result of mental derangement or sudden change in behaviour. These apparently healthy dogs are often slaughtered for human consumption in different active dog markets located in different parts of the state, this study agrees with findings reported by [8,25,28,29], that rabies is endemic in some dog markets in Nigeria. Rabies antigens was confirmed from saliva and fresh dog brain samples collected from apparently healthy dogs in slaughter areas and clinical cases presented to clinic. All dogs that were saliva positive for RICT were also brain positive for rabies antigen using RICT and DFAT. The result shows a strong agreement between the RICT and the goal standard test. The study also revealed that RICT used under field condition, was also found to yield the same result as when
used in the laboratory. Although the DFA test is the gold standard test frequently used for rabies diagnosis, the RICT therefore has proven to be equally efficacious and reliable as DFAT in Field and Laboratory with the added advantage of being cost effective and prompt, can be interpreted within 10 minutes which will save time and serve as an effective tool for rabies diagnosis in developing and underdeveloped countries, where there is limited or no surveillance on rabies [30,32].

In Conclusion, the study reports a 16% prevalence of rabies among apparently healthy dogs slaughtered for consumption and recommends the rapid immunochromatographic test as an important and useful diagnostic method for the rapid detection of rabies virus under field and laboratory conditions. It’s cost effective, does not require special training and equipment and can be performed under room temperature and test result can be preserved for a long time under room temperature [33].

References
26. Mike B (2011) Convenience sampling: SAGE research methods online