



A CASE STUDY OF RABIES IN A SIX MONTH OLD CALF IN ZARIA, NIGERIA

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ABSTRACT

Rabies though endemic in Nigeria, it is under reported this has contributed to claim by World Health organization (WHO) that there is no rabies in Nigeria, yet humans and animals are dying of rabies. On 26th March, 2013 a herdsman brought a complain to Ambulatory unit of The Ahmadu Bello University Veterinary Teaching Hospital, Zaria, Kaduna State, Nigeria of his six month old calf which was suspected to have been bitten by a dog three months ago showing nervous signs and anorexia. The calf showed the following signs during field observation, bellowing, hyper salivation, straining. Congested ocular mucous membrane and engorged jugular vein were also observed. Calf died while on transit to the Hospital, brain tissue was collected for confirmatory diagnosis, it was highly congested showing sign of encephalitis. The brain was found positive when it was tested with Rapid immunochromatographic test using 10% homogenate of the brain stem. A portion of the brain was sent to National Veterinary Research Laboratory Vom, Plateau state, Nigeria, for Confirmatory Diagnosis using Fluorescent antibody test (FAT) which also gave positive result for rabies. However the saliva was negative using Rapid immunochromatographic test. The implication of the above finding conclude that cattle rabies is in abundance and can be transmitted to other animals, though cattle are dead end host, but exposure to humans, via aerosol, contact with saliva during slaughtering is possible.

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This case study, recommend the use of rapid immunochromatographic test in areas distant to laboratories where FAT is conducted for quick screening and intervention before samples are sent to for confirmatory diagnosis, vaccination of livestock to prevent exposure and economic losses and education of herdsmen in order to make them capable of recognizing the signs and symptoms of rabies whenever it occurs for proper eradication and a successful control program.

1 Introduction

Rabies is a viral disease of mammals which is transmitted by the bite of a rabid animal. Rabies virus (RABV) infects the central nervous system, causing encephalopathy and ultimately death (George et al., 1996; Timoney et al., 1988). The virus is a single stranded RNA virus belonging to the genus *Lyssavirus* of the family *Rhabdoviridae* (Timoney et al., 1988; Wunner et al., 1995). The incubation period varies inversely with the proximity of bite to the central nervous system, but has been reported to vary from 21-80 days in dogs to upto 209 days in horses and cattle (Bizimenyera, 1998; Shankar et al., 2012). The clinical disease in cattle manifests as change in natural behaviour, excessive salivation, excitability mania, which ends in motor paralysis and death. In man and cattle mortality is 100% (Bizimenyera, 1998; Beigh et al., 2013). Rabies was first documented in Nigeria in humans in 1912 and in dogs in 1925 (Boulger & Hardy, 1960) and since then many workers (Umoh & Belino, 1979; Ekele & Okoh, 1984; Oboegbulem, 1994; Ogunkoya, 1997; Garba et al, 2005; Garba et al, 2008) have continued to report cases of rabies, suggesting that the disease is endemic in Nigeria, with more reports (WHO, 2010) showing that prevalence of the disease is increasing. Poor record system and absence of diagnostic laboratory in most States has further worsened the situation in the country (Ogunkoya et al., 2012).

Recently, eight people died in Cross River State, the cause was traced to dog bite, but before reporting to the Veterinary authorities in the affected state, the animals were killed and probably consumed (OIE, 2012) this in turn preclude confirmatory diagnosis as no sample could be taken, due to the tradition of dog consumption in the affected state (Ogunkoya et al., 2012). Rabies is endemic in Nigeria, the extent of which is yet to be ascertained. The principles of rabies control and eradication has been tested to be effective in other countries, but in Nigeria the prerequisite for this eradication are grossly inadequate (Ogunkoya, 2013) and still rabies is a problem. This case study sort to establish the role of rapid immunochromatographic test, in rabies diagnosis under field condition.

2 Case report

On the 26th, march 2013, a herdsman at Ugwan Zubairu brought a complain to the Ambulatory Unit of the Ahmadu Bello University, Zaria of his six Month old Calf which was anorexic and showing nervous manifestations. History revealed it was suspected to have been bitten by a dog three months

back, during field observation, the following signs were seen bellowing, hyper salivation, straining. Congested ocular mucous membrane, prominent engorged jugular vein, sunken eye ball were all observed. on an attempt to transport it to the hospital it died, its brain was extracted and subjected to rapid immunochromatographic test and thereafter transported in ice pack to National Veterinary Research Institute, Vom, Nigeria for confirmatory diagnosis.

3 Materials and methods

3.1 Saliva test Using rapid immunochromatographic diagnostic test

On reaching the hospital, sterile swap stick was used to take sample from the saliva dropping from the mouth of the dead calf, it was then inserted into the provided assay buffer tube and stirred to ensure a good sample extraction. The rapid immunochromatographic test cassette was then removed from the foil pouch and place horizontally. Using a sterile dropper, four drops of the extracted sample was then dripped into the sample hole in the cassette and result was interpreted within five to ten minutes, according to manufacturers instruction (BioNote, Inc).

Brain was extracted at the rabies post mortem room using the method described by Kaplan and Koprowski (1980). It was then placed in a polythene bag and sent to National Veterinary Research Institute, Vom Plateau state, Nigeria, in ice pack for confirmatory diagnosis following rapid immunochromatographic test.

3.2 Rapid Immunochromatographic Diagnostic Test(RIDT)

The RIDT is based on the principles of immunochromatography using a gold labeled monoclonal antibody (MAb). Purified anti- Nucleoprotein Mabs are immobilized in a test zone of the nitrocellulose membrane, while purified goat anti-mouse IgG are immobilized in the control zone of the membrane to capture unbound MAb. The sample, once added in the sample pad, migrates through the gold MAB pad, the test zone and the control zone respectively.

The RIDT was performed as described by the manufacturer (BioNote, Inc). A 10% (w/v) brain homogenate was prepared in culture medium, collected with a swab, and then dipped in the buffer supplied in the kit. After mixing for 10 seconds, four

drops of the suspension were added with a dropper to the sample hole of the test strip. Results were interpreted between 5 and 10 min after the beginning of the migration.

The test is considered as positive when two bands are revealed (one in the test zone, one in the control zone), while the test is considered as negative if only one band is observed.

3.3. Fluorescent antibody test (FAT)

Rabies direct fluorescent antibody assay (Monoclonal antibody-conjugate) reagents from Fujirebio Diagnostic Inc Malvern, P.A 19355 was used and the working (reagent) dilution after titration was achieved at 1:40 in accordance with the manufacturers recommendations and as described by Flamand et al. (1980).

A small fraction of the brain sample was smeared using wire loop on one part of a slide and then was air dried and fixed in cold acetone for one hour at -20°C . The slides were air dried and then the rabies conjugate was applied at 1:40 and incubated for 30 minutes at 37°C in a humid chamber after which excess conjugate was removed from the slides by rinsing it with 7.4 pH PBS solution about 3-5 minutes and was allowed to air dry. The cover slips were mounted with buffered Glycerol Mounting medium and the slides were examined using a fluorescence microscope within 2 hours after staining. When brilliant apple-green fluorescence colored or greenish yellow objects is exhibited against a black background then the test slide is positive

4. Results and Discussion

Fluorescent antibody test which is the gold standard test for rabies and rapid immunodiagnostic test were both positive, however saliva sample was negative with Rapid immunodiagnostic test.

Plate 1 and 2 Shows the results of rapid immunochromatographic test and fluorescent antibody test (FAT), the presence of two bands within the result window in plate 1 indicate positive result for rabies, while in plate 2 brilliant apple green particle is an indication of positive result. Nomadic herdsmen in Nigeria are in habit of keeping dogs for security, although they love doing that, routine vaccination is not taking into consideration and dogs stand a high risk of exposure as they mixed with stray dogs around. In this case, human exposure through aerosol and contact is possible as calves are in habit of licking ,herdsmen handle their animals when sick and sell adult sick once for meat.

Rabies infection occurring through the handling and skinning of rabid animals has been reported (Tarig et al., 1991) as such exposure through handling is possible in this case. Vaccination of the dog could not be ascertained and the dog was not seen at the time of visit. In this case, the saliva was negative with immunochromatographic test, this finding differ from that of (Delpierio et al., 2001) in his work, sterile swab was rubbed at different parts of the oral cavity, which led to the isolation of rabies virus from 1.6% of saliva sample, in this case, the negative result could be attributed to the fact that the swab was taken from the saliva found dropping from the oral cavity, the calf was dead at the time of sample collection, its likely the viral titre was low.

Plate 3 Shows the calf bellowing and straining, this was also seen in the finding of (Ismail and Nuri, 2009), it is also in agreement with the findings of (Barnard, 1979). In his observation of rabies in cattle for a period 10 years he documented 92% salivation, 69% bellowing, 47% aggression and 12% straining, all the above were seen in the case except aggression. In Plate 4 foamy saliva and congested ocular mucous membrane is seen, is similar to the findings of (Revesz, 1988; Imren and Sahla, 1994 and Hudson et al., 1996).



Plate 1 Result of Rapid immunochromatographic test. The presence of two bands within the result window indicating positive result.

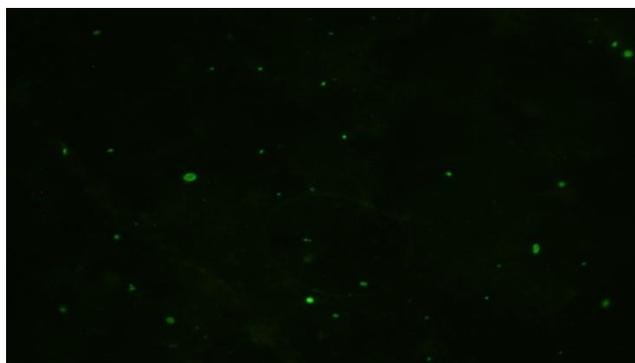


Plate 2 Fluorescent antibody test (FAT). Brilliant apple green particle indicating positive result for rabies.



Plate 3 Calf straining.



Plate 4 Calf with foamy saliva and congested ocular mucous membrane.



Plate 5 sunken eyes, prominent ribs as evidence of dehydration, engorged jugular vein as a result of straining.

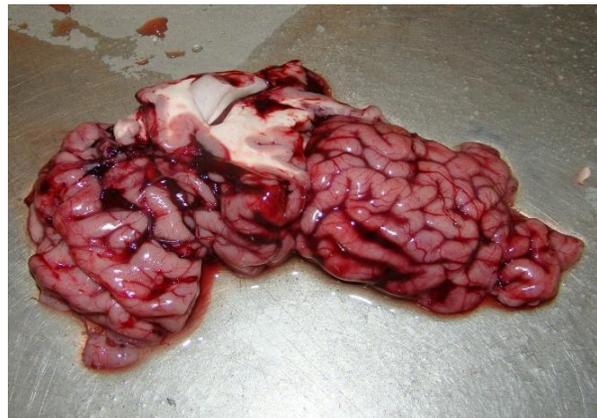


Plate 6 Harvested brain showing evidence of congestion and encephalitis.

100% of the cattle had excessive salivation and change in behavior, 70% bellowing. From the history giving, anorexia was observed by the herdsman, (Strinunthapanth, 1985) also reported anorexia and hyper salivation in rabid cattle and buffalo. In Plate 5 the ribs are seen prominent and eyes sunken, which are evidences of dehydration seen in rabies as the calf cannot suckle nor feed, this is similar to the findings of (Bizimenyera,1998) in his work he attributed that to the inability of the calf to suckle. Plate 6 is showing a congested brain with evidence of encephalitis which is the likely cause of nervous manifestation, in the work of (Ismail and Nuri, 2009) aggression was also seen.

Rapid immunochromatographic test have been used for rabies diagnosis and evaluated with different tests (Alexandre et al, 2012) under laboratory condition, this is the first time, it is been used to diagnose rabies in calf, under field condition in Nigeria, seeing from the result it is in agreement with the gold

standard test for rabies with the classical signs seen. Stray dogs are the sole propagating animal for rabies in Nigeria, conversely in Europe, the two main animal species involved in rabies transmission are the red fox and the raccoon dog (Alexandre et al., 2012) In rabies free countries, where a surveillance system has to be maintained (Cliquet et al., 2010), rabies cases are limited to illegally imported pets (Barrat, 2006) and to bats species (Fooks, 2003), however in Nigeria, reported cases are sporadic due to lack of knowledge by the herdsmen on rabies; this makes the formulation of effective control program difficult. There is therefore need to educate nomadic herdsmen to be able to recognize rabies when it occur so as to minimize public health hazard. Control of rabies in domestic dogs and vaccination of livestock like cattle, sheep and goat is necessary to prevent possible exposure and economic losses. This case study, recommend the use of rapid immunochromatographic test in areas distant to laboratories

where FAT can be conducted for quick intervention before samples are sent to for confirmatory diagnosis.

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