CONFIRMATION OF RABIES IN BUFFALO FROM ORGANIZED FARM OF NORTH GUJARAT

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ABSTRACT

Rabies is an infectious, fatal viral disease caused by a neurotropic virus and transmissible to all mammals through bite by rabid animals viz. dog, mongoose etc. In the present study rabies was tentatively diagnoses on the basis of history and clinical manifestations from buffalo of an organized farm of north Gujarat. This article summarizes the typical characteristics of rabies in a buffalo including its confirmatory diagnosis using florescent antibody test (FAT) and histopathology, which were consider as gold standard test for rabies diagnosis. Finding was also confirmed using polymerase chain reaction. Based on the study it was hypothesized that it might have been transmitted from rabid dog or mongoose.

Keywords: rabies, fluorescent antibody test, buffalo

INTRODUCTION

Rabies is a viral zoonotic disease. It is one of the oldest, dreadful infectious diseases known to mankind since early civilization. Infection is mainly transmitted by bite of rabid animal (mainly dog bite). In general disease is propagated by bite from animal to animal and animal to man. Disease is characterized by signs of abnormal behavior, nervous disturbances, impairment of consciousness, ascending paralysis and death. It is caused by Rabies Virus (RV), of the genus *Lyssavirus* of the family *Rhabdoviridae* under the order *Mononegavirales* (Rupprecht *et al*., 1995). It has characteristic bullet shape, which help for easy distinguishing it from other viruses. Developing countries like India contributing a significantly in total loss caused by rabies. Beside direct losses, rabies in animal is also act as source of infection for human rabies. Thus it's important to diagnose and control rabies in animal, in order to control rabies in human. In our country, buffalo forms the backbone of India's dairy industry and is rightly considered as the India's milking machine (Balani, 1999). India, with 32 million tons is world's topmost buffalo milk producer accounting for 64% of the world's production. We have 16 internationally accepted buffalo breeds and have special importance in Indian economics. So confirmation and control of such dreaded and neglected disease may help in minimizing economic loss to the country. In present study, an attempt was made to diagnose rabies from a rabies suspected buffalo having no history of bite from rabid animal.

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MATERIALS AND METHODS

History and observations

A dairy veterinary doctor providing veterinary services to a well-organized and managed farm of village Mumanvas (Danta takuka and District Banaskantha) of North Gujarat having 80 adult buffaloes and many young animals. As a part of routine treatment he started treatment of one sick buffalo showing dullness and off-feed. He had tentatively diagnosed it as rabies on third day, when symptoms are getting prominent and are indicative of it. So, immediately he isolated that animal and informed our laboratory for confirmation of disease and guidance for treatment and control. On farm visit we observed that, farmer had isolated one buffalo of six year age; showing anxious look, frequent micturition, kinked tail, constipation, attempt to eat sand and passing wind from rectum with loosened sphincter along with various nervous symptoms indicative of beginning of paralysis. Animal was frequently rubbing his head against the ground and nearby inanimate objects. The skin over the forehead was peeled off and serum/blood was oozing out. On clinical examination of the animal, no lesions were seen in the mouth, tongue and feet, and body temperature is found around 104°F. Based on the history, clinical signs and conversation with owner and doctor, we tentatively diagnosed that buffalo might be suffered from rabies.

Sample collection

Blood and smears (Corneal impression and saliva) from live animal was collected with proper precautions. And on death, animal was transported to Veterinary College of Sardarkrushinagar Dantiwada Agricultural University; where post mortem was performed (Figure 1) and part of brain tissue was collected in 50% glycerol saline and 10% formalin for diagnosis and confirmation of etiology. Brain tissue impression smears were also prepared for FAT and Seller's staining.

Fluorescent antibody test (FAT)

Brain tissue impression smear was fixed with 80% chilled acetone (80/20 in PBS) and stained with rabies anti–nucleocapsid FITC conjugate (Catal # REF 800-092, FDI, Fujirebio Diagnostic, Inc, USA) following the method describe by Patel *et al*. (2015). Stained slide was observed after mounting with mounting fluid (50% v/v Glycerol) under fluorescent microscope (Motic).

Seller's staining and H and E staining

Brain tissue impression smear was immerse in the seller's staining solution (2 Part of Methylene blue and 1 Part Basic fuschin) for 1 to 5 seconds and rinse with tap water. After drying observed under microscope.

Formalin fixed brain tissue samples was thoroughly washed in running water; dehydrated in ascending grades of alcohol and acetone; cleared in benzene and embedded in paraffin tissue embedding media. The paraffin embedded tissues were sectioned at 5 µ thickness and stained by haematoxylin and eosin (H and E) method (Lillie, 1965).

Polymerase chain reaction

For detection of rabies antigen using PCR, forward primer 5'- ACAA AGCTTAATGGATGCCGACAAGATTGT-3' and reverse primer 5'- CCA GGATCCCATGAGTCACTCGAATATGTCTT-3' were used, which amplify 1353 bp fragment of the nucleoprotein gene (Gupta *et al*., 2005). PCR was carried out in 200 µl PCR tubes using Nexus gradient Master cycler (Eppendorf, Germany).

Figure 1. Photograph showing the opened cranial cavity for collection of brain sample.

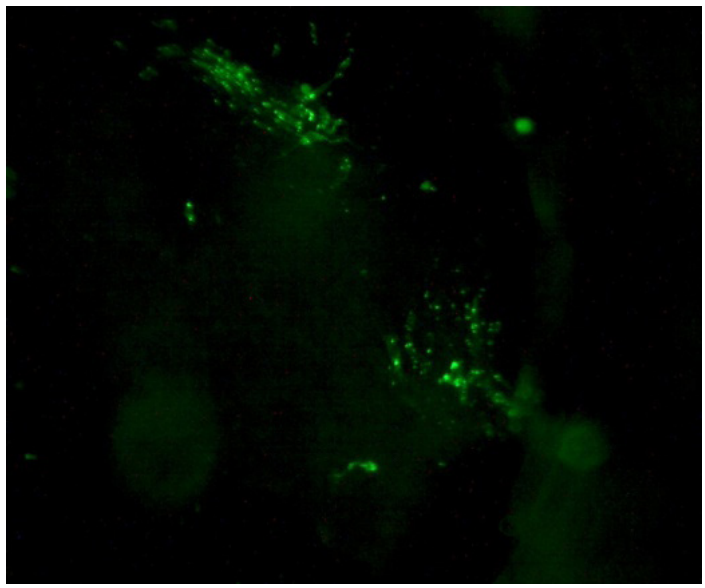

Figure 2. Florescent antibody test apple green coloured fluorescent indicate presence of rabies specific antigen in brain tissue by indirect FAT.

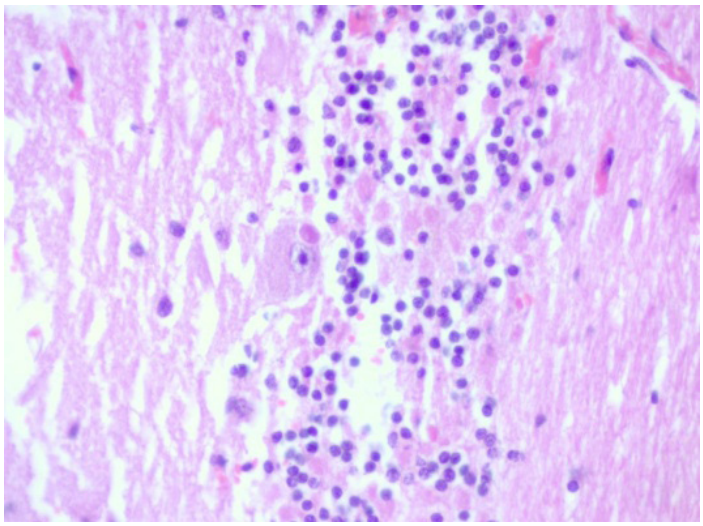

Figure 3. H and E staining arrow showing the intra cytoplasmic basophilic inclusion body (Negri Body) on staining the brain tissue section with H and E staining.

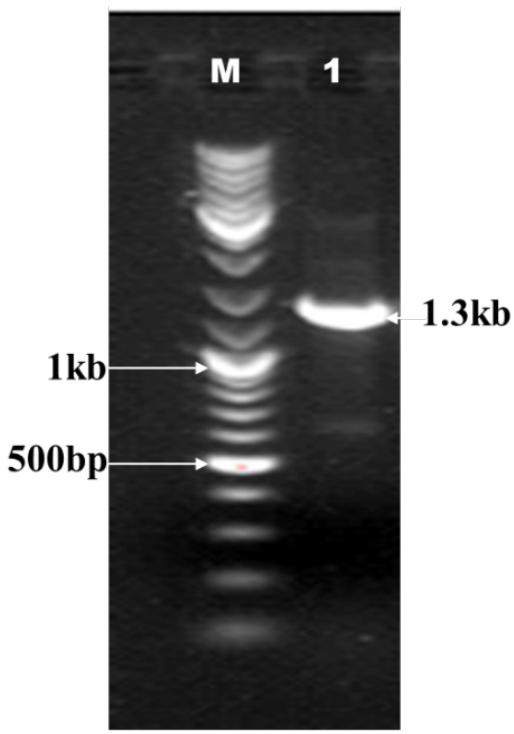

Figure 4. Polymerase chain reaction (PCR) agarose gel electrophoresis showing the amplification of N gene product of 1.3 KB size.

Each 25 µl of the PCR mixture comprised of 1.5 µl of DNA, 12.5 µl of 2 × PCR master mix, 1.0 µl of each forward and reverse primer and 9.0 µl nuclease free water. The PCR conditions includes initial denaturation at 95°C for 5 minutes; followed by 30 cycles of 95°C for 30 seconds (denaturation), 55°C for 60 seconds (annealing) and 72°C for 90 seconds (extension); with a final extension step of 72°C for 4 minutes. 5 µl of amplified PCR product was mixed with 1 µl of 6X gel loading dye and subjected to electrophoresis in 1.5% agarose gelalong with 1 kb plus DNA ladder. The images were captured and documented using gel documentation system (Bio Rad., U.S.A).

RESULT AND DISCUSSION

We had observed rubbing of forehead on ground and with other inanimate objects like wooden pegs, wall of mangers etc. which leads to peeling of skin and oozing of serum/blood; which is one of the important and prominent sign in rabid buffalo, similar observation was also documented by other researchers for buffalo and cattle. In order to rule out the possibility of other closely related diseases, blood sample was examined for presence of haemoprotozoan parasites. But on examination no such parasites was observed. Blood sample is also subjected for differential blood cell count, in which neutrophilia was observed. It has no direct relation with this disease but this might be due to secondary infection to self-instinct trauma. Surprisingly, corneal impression and saliva smear does not showed any specific fluorescence on FAT. This might be due to improper preparation and transportation of smear or virus may not present in sufficient quantity at the time of smear preparation. But FAT of brain tissue had showed rabies specific apple green colour fluorescent (Figure 2), which had confirm rabies in this buffalo. This findings is also supported by demonstration of Negri bodies by Seller's staining of brain tissue sample and H and E staining of formalin fix brain tissue section (Figure 3). In PCR also specific amplicon of 1.3 KB size was successfully amplified, which further confirm the present finding (Figure 4). As FAT is internationally consider as gold standard test for diagnosis of rabies, we conclude that present case is of rabies. Thus, farmer is suggested to go for prophylactic vaccination of all closed contact animals using vaccine from reputed company on day 0, 3, 7, 14, 30. Since then herd is under observation of local veterinary officer, but no case of rabies has been reported from that farm. In order to find out the source of infection, once again we contacted and asked questions to farmer and other management staff of the farm. They do not recall any incidence of dog or mongoose bite in the herd. But it was observed and confirmed from their staff that the animals were kept loose in a large open enclosure having incomplete wire fencing and nearby area have presence of stray dogs and mongoose. Based on these observation we had concluded that rabies virus might be transmitted to this buffalo from rabid dog or mongoose and farmer is not aware of it.

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