ORIGINAL ARTICLE

Comparison of Immunochromatographic Test and Reverse Transcriptase Polymerase Chain Reaction for Detection of Rabies virus in Live as well as Post mortem animals

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Abstract

Rabies is an ancient global fatal disease of central nervous system (CNS) and most significant zoonotic and neglected viral disease that affects almost all kinds of mammals, including humans. The aim of the present study was to study the ante-mortem detection of rabies virus from saliva samples as well as post mortem detection of rabies virus from brain samples by Immunochromatographic test kit (ICT) and Reverse transcriptase polymerase chain reaction (RT-PCR) from different locations of Gujarat state. A total of 12 samples (6 brain samples and 6 saliva samples) were aseptically collected from rabies suspected live and dead animals (viz. dog, buffalo, cow and horse) for rabies virus detection. Results of this study revealed that all the six brain samples were found positive (100%) and three saliva samples out of six samples were found positive (50%) by both the tests, ICT and RT-PCR. Results obtained by ICT and RT-PCR for rabies diagnosis were almost similar implying that both the tests have yielded almost comparable results, and ICT can very well be adopted as a field level test for rabies diagnosis.

Key words: Rabies, Brain, Saliva, ICT, RT-PCR, Gujarat

Introduction

Rabies is historically one of the most significant zoonotic diseases, because of nearly 100% case fatality rate and ubiquitous global distribution (Blanton et al., 2008). Rabies is considered as a reemerging zoonosis in many parts of the world, particularly in countries of Asia, Africa and Latin America except Antarctica where the disease is enzootic despite the availability of proven prevention and control tools but more than 95% of human deaths occur in Asia and Africa (Sudarshan et al., 2007). Globally, Rabies is categorized as either urban (where dogs and cats are the major reservoir hosts), or sylvatic (major reservoirs are foxes, wolves, bats and other wildlife) rabies (WHO, 2013). It occurs mainly in the urban form in India, in which dogs play an important role as the reservoir and transmitter of the disease to humans and domestic animals (Sudarshan et al., 2007).

Rabies virus is the prototype member of the genus Lyssavirus of the family Rhabdoviridae under the order Mononegavirales (Wunner et al., 1995). It is a single stranded, negative-sense RNA virus, Lyssavirus (genotype 1)

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Some of the important aspect of this article is reused from the original thesis work of the Author

with a genome size of approximately 12 kb (Bourhy et al., 1993). Rabies viruses are bullet shaped measuring about 75 nm X 200 nm in size and can be roughly divided into a structural and a functional unit: the ribonucleocapsid core and the viral envelope (Badrane and Tordo, 2001; Rupprecht et al., 2002). Viral genome encodes total five structural genes which are separated by four non-coding intergenic sequences from 3' terminus to 5' terminus in the order of N-P-M-G-L, which encode respectively the nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and large subunit of transcriptase (L) (Wunner et al., 1998).

Conventionally, Rabies diagnosis generally revolves around direct visualization by electron microscopy, direct or indirect FAT (OIE recommended confirmatory test), virus cultivation in cell lines, mouse inoculation test (MIT), immunohistochemistry, enzyme immunoassay but these tests are only feasible after the death. Molecular technique such as the RT-PCR has been applied by various researchers to improve the sensitivity and specificity of ante- as well as post-mortem diagnosis of rabies (Kamolvarin et al., 1993). Recently, Researchers have developed a novel diagnostic test for rabies virus using immunochromatographic techniques which can achieve rapid and sensitive detection for rabies virus using MAb which recognize the N protein of rabies virus. In this study we have shown the uniqueness of rapid diagnostic test kit and evaluated its efficacy by comparing it with RT-PCR. The rapid diagnosis test is simple, time- and cost-saving. It can be used anywhere in the world and needs no special reagents or equipment (Nishizono et al., 2008).

Research endeavour on rabies in India has been very less in spite of the fact that India accounts for the maximum human incidences and death thereby reflecting the significance of animal rabies. The present study was aimed for the ante-mortem detection of rabies virus from saliva samples as well as post mortem detection of rabies virus of suspected rabies cases from brain samples by Immunochromatographic test kit (ICT) and RT-PCR from the samples collected from different locations of Gujarat state, India.

Materials and Methods

Collection of Samples

Brain samples (n=6) were collected aseptically from rabies suspected animals during post mortem examination at the Department of Veterinary Pathology, College of Veterinary Science & Animal Husbandry, AAU, Anand and were also received from field through various organizations (Figure1).

Saliva samples (n=6) were collected from live animals which were suspected for rabies at the Teaching Veterinary Clinical Complex (TVCC), College of Veterinary Science and Animal Husbandry, A.A.U., Anand (Figure 2). The details of samples are given in table 1. Brain homogenate (10%) was prepared in sterile phosphate buffer saline. Saliva suspension was prepared by dipping saliva swabs collected from live suspected animals in 4 ml of phosphate buffer saline. Brain homogenates and saliva suspensions were stored at -40° C in deep freeze for further use.

Immunochromatographic Test

Immunochromatographic test (ICT) was used for the qualitative detection of rabies virus antigen from saliva and brain homogenates. The ICT was performed using Anigen Rapid Rabies Ag Test Kit (BioNote Inc., Korea). If enough Rabies virus antigen is present in a sample, a purple test line will be visible in the result window. To carry out the test, swab was dipped in prepared brain homogenate or saliva suspension and then inserted into the specimen tube containing 1 ml of assay diluent. Four drops of the sample were added into the sample hole using the disposable dropper provided in the kit. Result of the test was interpreted within 10 minutes.

RNA extraction and RT-PCR

The RNA was extracted from brain homogenates and saliva suspensions using QIAamp Viral RNA mini kit, 50 reactions (Catlog No. 52904, QIAGEN, Valencia, CA) as per the manufacturer's instructions. RT-PCR was performed

using Qiagen one-step RT-PCR Kit (Qiagen, Germany, cat no. 210210). The primer set used for one step RT-PCR was JW12(F) ATGTAACACCTCTACAATG and JW6(R) CAATTAGCACACATTTTGTG targeting 605bp size amplicon of the nucleoprotein gene of rabies virus (Arvindhbabu et al., 2014). RT-PCR was carried out in a final reaction volume of 25 µl using 200 µl capacity thin walled PCR tubes comprising of 5 µl Qiagen one-step RT-PCR Buffer (5×), 1 µl of dNTP mix (10mMol), 1.5 µl of each primer (10 pmole), 1 µl of Qiagen one-step RT-PCR enzyme mix, 5 µl of RNA template (30ng/ul) and 10 µl of RNAse free water. The RT-PCR reactions were performed in thermocycler (Biorad, USA) with following cycles; reverse transcription 50°C for 30 min, initial denaturation of 95°C for 15 min followed by 35 cycles of denaturation, annealing and extension at 94°C for 30s, 50°C for 30s and 72°C for 60s, respectively, and the final extension was carried out at 72°C for 10 min.

Electrophoresis

RT-PCR amplified product was confirmed by Electrophoresis. To confirm PCR amplification, 5 µl product from each PCR tube was mixed with 1 µl of 6X gel loading buffer and placed in the well and electrophoresed along with 100bp DNA molecular weight marker (GeneRuler, MBI Fermentas) on 2% agarose gel containing ethidium bromide at the rate of 1 µl / 20 ml gel, at constant 100 V for 45 min in 0.5X TBE buffer. The amplified product was visualized as a single compact band of expected size under UV light and documented by gel documentation system (SynGene, Gene Genius Bio Imaging System).

Results

Immunochromatographic test

All the brain samples were found positive (100%) by Immunochromatographic test by showing two purple lines, one in control and second in test. In saliva samples, three saliva samples out of six samples were found positive (50%)by Immunochromatographic test. The three negative saliva samples showed only one line in control on Immunochromatographic test kit(**Table 1, Figure 3**).

RT-PCR

In RT-PCR, all the six brain samples were positive (100%) whereas out of six saliva samples, three samples were found positive (50%) by amplifying the expected 605bp size amplicon on gel documentation system. Negative samples had not showed any band (**Table 1, Figure 4**).

Discussion

In present study, Immunochromatographic test revealed 100% positivity in case of brain samples and 50% positivity in case of saliva samples. Reta et al. (2013) found 82 positive samples out of 115 brain samples from rabid animals by immunochromatographic test, while 85 samples were found positive by d-FAT. Servat et al. (2011) revealed 100% positivity of rabies from brain material of Europian mammals. Markotter et al. (2009) detected 100% positivity of African lyssaviruses from brain materials. Nishizonoet al. (2008) also found 95.5% positivity using brain samples from rabid dogs. Kang et al. (2007) with total 110 samples including saliva and brains and found 52 positive samples by immunochromatographic test, while 57 samples were found positive with more sensitive and specific techniques such as d-FAT and RT-PCR. Studies on usefulness of immunochromatographic test have also been carried out by Kasempimolporn et al. (2011), Ahmed et al. (2012) and Savaliya et al. (2015).

All the six brain samples and three saliva samples were found positive by RT-PCR. Similar study was performed by Arvindhbabu et al. (2014), which resulted in detection of N gene of rabies virus in 43 out of 53 brain samples with a specific 605bp band size using the same primers as used in present study. McElhinney et al. (2014) detected rabies viral RNA from decomposed samples by RT-PCR on days 70, 48 and 48 at 4˚C, 25˚C and 35˚C, respectively and suggested that when decomposed samples are likely to be submitted, RT-PCR can be used to accompany

OIE-prescribed rabies diagnostic tests. Sharma et al. (2014b) found 20 positive samples by RT-PCR from 34 skin biopsies samples of clinically suspected animals (11 buffaloes, 8 cows, 13 dogs, 1 cat and 1 horse). Similarly, Bansal et al. (2012) detected nine positive samples out of 20 skin biopsy samples by conventional RT-PCR. Number of other workers viz. Heaton et al. (1999), Ito et al. (2001), Dantas Junior et al. (2004), Biswal et al. (2007), Nishizono et al. (2008), Arvindhbabu et al. (2012), Dandale et al. (2013) have also endorsed the usefulness of RT-PCR for rabies diagnosis. On comparison of both these tests, it is revealed that results obtained by ICT and RT-PCR for rabies diagnosis were almost similar. This implies that both the tests have yielded almost comparable results. ICT gives rapid results compared to RT-PCR hence ICT can very well be adopted as a field level test due to its rapid diagnosis, its easy procedure and less demanding in terms of facilities required.

Conclusions

The present study revealed that the Immunochromatographic test and RT-PCR yielded comparable results for detection of rabies virus from brain and saliva samples with an additional advantage of using them in live animals. Immunochromatographic test can very well be adopted as field level test for prompt diagnosis of rabies. It implies that modern tools like RT-PCR can be useful in detecting rabies virus in a sensitive way and has more relevance with regard to confirmatory diagnosis in live animals using saliva samples.

Table 1: Details of samples and results obtained by Immunochromatographic test and RT-PCR

Sr. No.	Animal	Type of sample	Location	Immunochromato-graphic test	RT-PCR
1	Buffalo	Brain	Mehsana	+	+
2	Buffalo	Brain	Mehsana	+	+
3	Buffalo	Brain	Mehsana	+	+
4	Buffalo	Brain	Mehsana	+	+
5	Cow	Brain	Surat	+	+
6	Buffalo	Brain	Rajkot	+	+
7	Dog	Saliva	Ambali	+	+
8	Dog	Saliva	Anand	+	+
9	Buffalo calf	Saliva	Anand	-	-
10	Dog	Saliva	Ode,Anand	+	+
11	Dog	Saliva	Borsad,Anand	-	-
12	Horse	Saliva	Tarapur,Anand	-	-

Figure 1: Collection of brain sample from rabies suspected dog during post mortem

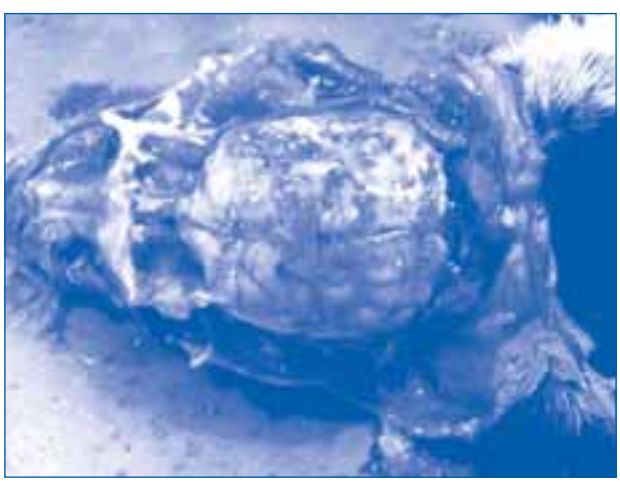


Figure 2: Collection of saliva sample from rabies suspected dog

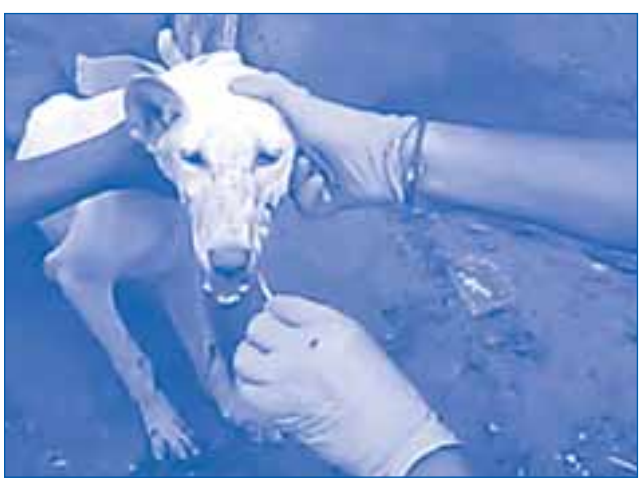


Figure 3: Immunochromatographic test for rabies diagnosis

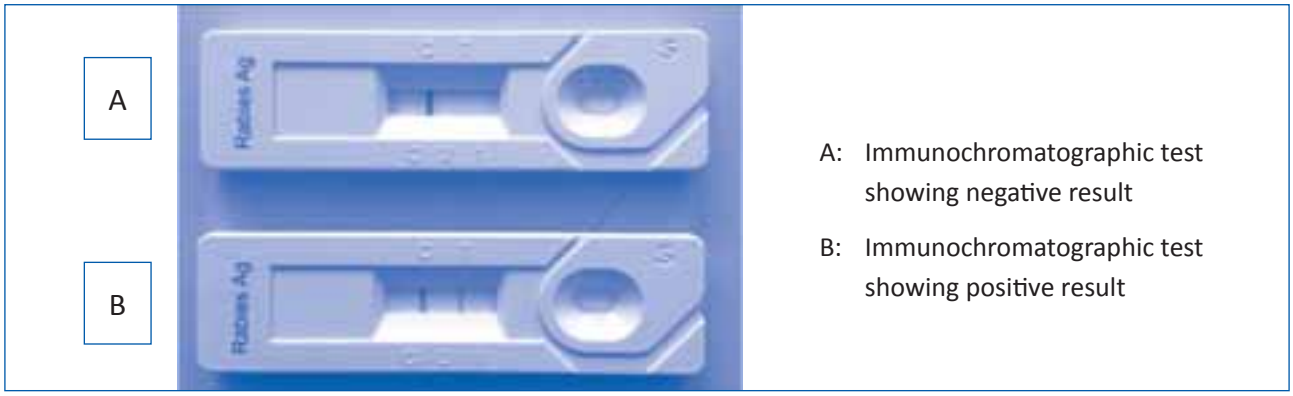
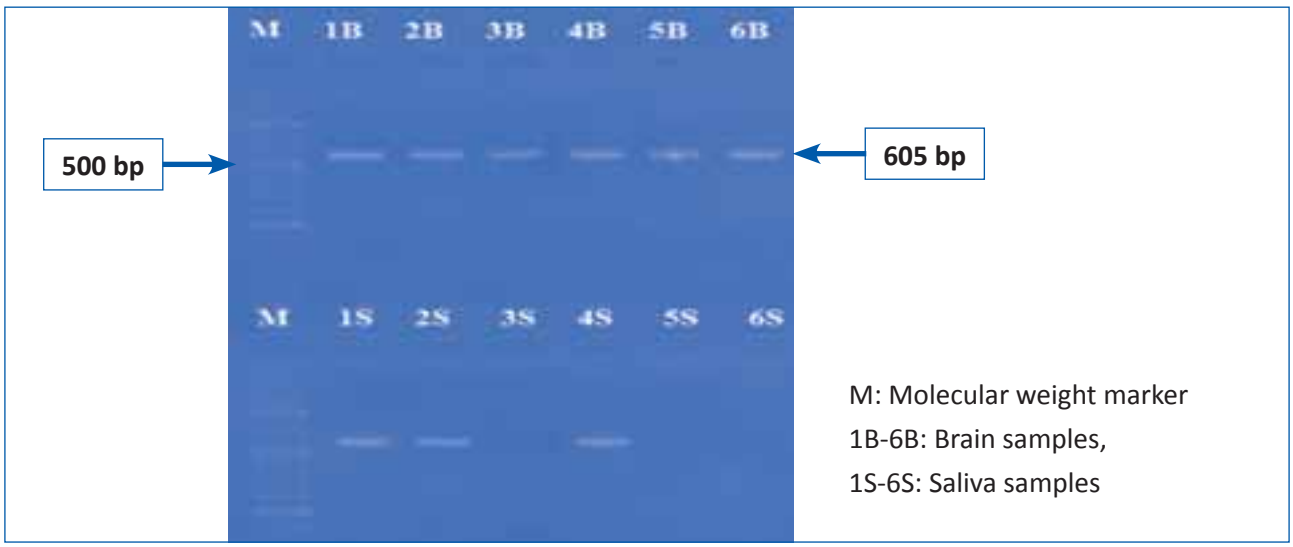


Figure 4: RT-PCR results using brain and saliva samples for rabies diagnosis



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