

*Short communication*

## DETECTION OF RABIES VIRAL ANTIGEN IN CATTLE BY RAPID IMMUNOCHROMTOGRAPHIC DIAGNOSTIC TEST

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**ABSTRACT:** In recent years, improved quality, accuracy and speed for diagnosis of rabies has been adopted for rabies control strategies in developing countries. In field condition, rapid immunochromtographic diagnostic test (RIDT) is a true requirement for rapid epidemiological surveillance of rabies. In the present study, a total of ten numbers of rabies suspected cattle brain sample form different parts of West Bengal, India were examined through RIDT. The results revealed that one sample was found to be positive. The test was established as powerful screening tool for rabies with high sensitivity and specificity. Thus, RIDT can be employed as a reliable and quick approach for diagnosis and control of rabies under field condition.

**Key words:** RIDT, Rabies, Cattle brain.

Rabies, a zoonotic disease, is fatal encephalomyelitis infecting all warm blooded animals. It is caused by RNA virus under genus *Lyssa* virus and the family *Rhabdoviridae* (Pringle 1991). The virus travels to the central nervous system from site of infection resulting brain death of the rabid affected animals. Rabies is spread mainly through saliva when an infected animal scratches or bites another animal. Every year, 55,000 deaths were estimated due to rabies throughout the world (Lozano *et al.*, 2012, Knobel *et al.*, 2005). More than 95% of rabies death occurs in Africa and

Asia (Fact Sheet 2014).

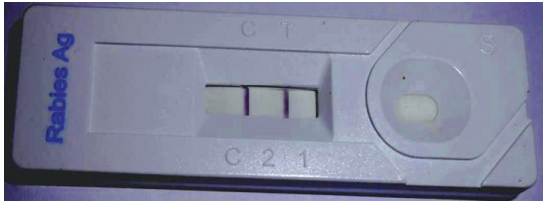
Rabies is 100% fatal and at the same time it is 100% preventive. Therefore, pre-exposure prophylaxis should be a significant step to combat with the disease. For that, convenient and rapid diagnostic approach on rabies is essential under field condition. At present, Fluorescent Antibody Test (FAT) is a gold standard test for detection of rabies virus and confirmatory diagnosis of rabies as per WHO and OIE recommendation (Dean *et al.*, 1996, Meslin *et al.*, 1996). But, the main drawbacks of FAT reside in the use of well structured

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**Fig. 1 The RIDT showing positive in test samples.**



**Fig. 2 The RIDT showing negative in test samples.**

laboratory with expensive instruments and well-trained technicians. Despite, FAT requires fresh brain sample for experiment, which would be difficult to get in distant specimens. Other laboratory diagnostic methods including mouse inoculation test, rabies tissue culture test have limiting uses because these tests are costly, time consuming and difficult to perform in field condition. Although the sensitive molecular diagnostic approaches like nested RT-PCR, hemi-nested RT-PCR (HnRT-PCR) has been evolved for detection of rabies in recent days; it requires sophisticated laboratories and highly skilled technicians (Heaton *et al.*, 1997, Picard-Meyer *et al.*, 2004).

To alleviate the difficulties in diagnostic approach under field condition, here we used commercially available Rabies antigen detection kit for diagnosis of rabies in cattle.

### **Procedure**

A total of ten tissue samples from cerebellum and hippocampus of suspected cattle were collected from Animal Health Centers of different districts under Animal Resources Development Department, West Bengal. The suspected animals were suffering from pyrexia, anorexia, sticky salivation, posterior paralysis followed by restlessness, attacking mood and death after 4 days.

The Anigen Rapid Rabies Ag test Kit was used for qualitative detection of rabies viral

antigen. This is a chromatographic immune assay. For each sample, 10% brain homogenate was prepared in PBS. Then the sample was collected using swab and mixed in the sample diluents tube provided in the test kit. Finally, sample was added into the sample hole of the test device using a disposable dropper. The purple color band moved across the result window in the centre of the test device indicating proper loading of the sample. Results were interpreted within 5-10 minutes.

### **Finding of the study**

Out of 10 cattle brain sample tested by RIDT, both the control band and test band were shown in one sample indicating the positive result with this assay (Fig. 1). While the single control band was observed in negative samples (Fig. 2). This result suggested that out of ten suspected cattle with the symptoms of rabies, one cattle was infected in rabies.

Rabies is a deadly disease of all warm blooded animals having significant impact on global zoonosis. In spite of availability of effective vaccines, rabies causes serious global threat by killing thousands of people every year. Therefore, rapid diagnosis of rabies is essential for initiating prompt measure in treatment and controlling rabies. Wild carnivores are natural reservoir of rabies virus infection which passes on domesticated animals and human through scratching and biting. In every year, roughly

36% of the world's rabies deaths occur in India predominantly by contact with rabid dogs (Bulletin of the WHO 2009). India has highest number of cases of rabies in world with 20,000 (36%) of an estimated global annual 55,000 rabies deaths (Bulletin of the WHO 2009).

Conventional diagnostic approach of rabies includes histological identification of Negri bodies by direct microscopy, demonstration of viral antigen by FAT, virus isolation, mouse inoculation test, different viral neutralization assays etc. These tests have limitations with any of the characteristics like low sensitivity, sophisticated, time consuming or expensive. Moreover, in FAT minor variation of test procedure may produce dramatically change in the sensitivity of the results (Rudd *et al.*, 2005). Nested and hemi-nested PCR has been used to diagnose rabies, but it needs several hours with costly equipment to run.

In such scenario, rapid immunodiagnostic test (RIDT) has been developed as a useful method of rabies diagnosis without the need laboratory equipments. This immunochromatographic lateral flow strip test is a one-step test for rapid identification of rabies viral antigen. A simple and rapid immunochromatographic test kit for rabies diagnosis has been developed using polyclonal and monoclonal antibody that recognize epitope II and III of rabies virus nucleoprotein. The RIDT kit has potential to test saliva and brain tissue. In the present study, the RIDT kit has been proven to be useful for rapid diagnosis of rabies without using any equipment. Here, a total of 10 cattle brain samples have been screened, out of which one sample revealed the presence of rabies viral antigen in cerebellum specifically in hippocampus region. The efficacy of the rapid

immunodiagnostic kit was tested by several researches. In earlier study, the sensitivity, specificity and accuracy of the kit was reported as 91.66%, 100% and 94.11% respectively (Kang *et al.*, 2007).

In another study, the research group compared RIDT with FAT and found that, the saliva strip test was 94.4% specific and 93% sensitive. Thus, our result reflected the high sensitivity and specificity of the test with confirmatory diagnosis of rabies for the suspected cattle. The RIDT can be performed by using saliva of suspected animals. While comparing with nPCR, the saliva strip test was shown to have 98.7% specificity (Kasempimolporn *et al.*, 2011). So, RIDT is rapid, effective and trustworthy test for both antimortem and postmortem diagnosis of rabies under field condition. Likewise, our results suggest that the efficacy of RIDT is unquestionable for detection of rabies under field condition.

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#### **REFERENCES**

- Chatterjee P (2009) India's ongoing war against rabies. Bulletin of the World Health Organization. 87(12): 885 – 964.
- Dean DJ, Abelseth MK, Atanasiu P (1996) The fluorescent antibody test. In: Meslin FX, Kaplan MM, Koprowski H (Eds.), Laboratory Techniques in Rabies. World Health Organization, Geneva, Switzerland. 88–95.

Fact Sheet World Health Organization (2013)  
Retrieved 28 February, 2014.

Heaton PR, Johnstone P, Mcelhinney LM, Cowley R, O'sullivan E, Whitby JE (1997) Heminested PCR assay for detection of six genotypes of rabies and rabies-related viruses. *J Clin Microbiol* 135: 2762-2766.

Kang B, Oh JS, Lee CS, Park BK, Park YN *et al.* (2007) Evaluation of a rapid immunodiagnostic test kit for rabies virus. *J Virol Methods* 145: 30–36.

Kasempimolporn S, Saengseesom W, Huadsakul S, Boonchang S, Sitprija V (2011) Evaluation of a rapid immunochromatographic test strip for detection of *Rabies virus* in dog saliva samples. *J Vet Diagnos Invest* 23: 1197–1201.

Knobel DL, Cleaveland S, Coleman PG, Fe'vre EM, Meltzer MI (2005) Reevaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 83: 360-368.

Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K *et al.* (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. *Lancet*. 380: 2095-2128.

Meslin FX, Kaplan MM, Koprowski H (Eds.) (1996) *Laboratory Techniques in Rabies*. World Health Organization, Geneva, Switzerland.

Picard-Meyer E, Bruyère V, Barrat J, Tissot E, Barrat MJ, Cliquet F (2004) Development of a heminested RT-PCR method for the specific determination of European Bat Lyssavirus. Comparison with other rabies diagnostic methods. *Vaccine* 22: 1921-1929.

Pringle CR (1991) The order mononegavirales. *Arch Virol* 117: 137-140.

Rudd RJ, Smith JS, Yager PA, Orciari LA, Trimarchi CV (2005) A need for standardized rabies-virus diagnostic procedures: effect of cover-glass mountant on the reliability of antigen detection by the fluorescent antibody test. *Virus Res* 111: 83–88.

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