PATIENT-SIDE RT-PCR FOR DIAGNOSIS OF RABIES – A CASE REPORT

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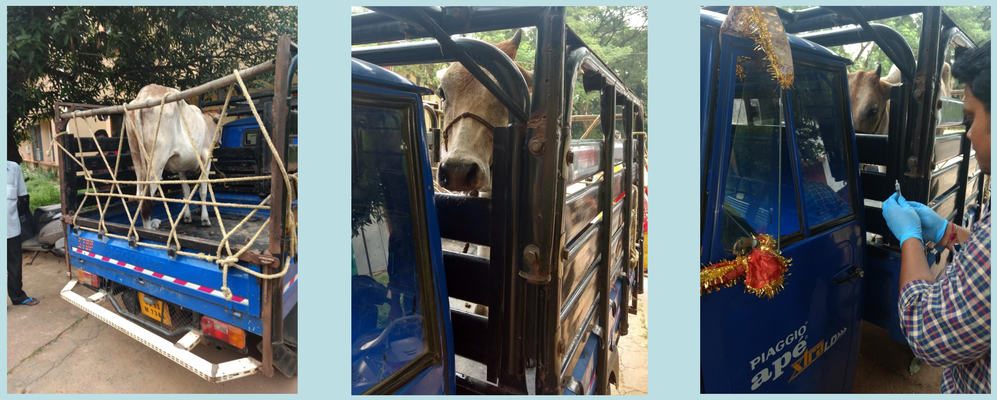
Rabies, one of the oldest viral diseases, affects all warm-blooded animals across the globe. Caused by bullet-shaped lyssa virus of Rhabdoviridae family, transmission occurs primarily through direct or indirect contact with the saliva from rabid animals. In India, about 15 million people are bitten by animals, mostly dogs, every year(Menezes, R. 2008). In developing countries like India, 90% of human deaths from rabies are caused by dog bites only. Clinical signs and the history of exposure to rabid animals continue to be the deciding factor for the clinician to initiate post-exposure prophylactic measures. This system of diagnosis has its own disadvantages. Hence, simple, rapid and accurate diagnosis is extremely essential for proper planning and follow up programmes in suspected cases. Present study deals with correlation of clinical signs with the results of a chip-based quick RT-PCR for confirmatory diagnosis of rabies.

A 2-year-old heifer from village Bhingarpur (Dist: Puri, Odisha) was presented in the Department of Epidemiology and Preventive Medicine, College of Veterinary Science and Animal Husbandry, OUAT on October 9, 2018 with the complaint of sudden anorexia with complete refusal to water, roughages & concentrates and aimless movement since two days. Moderate salivation with foam was noticed on close clinical examination (Fig 1a-1c). Interrogation failed to reveal any direct/indirect exposure to rabid animal. Animal was apparently healthy prior to this episode. However, history coupled with clinical signs aroused suspicion of rabies. At this crucial juncture, the usefulness of a newly developed animal-side test was tested.

The heifer succumbed 2 days after presentation with the typical signs suggestive of rabies.



(a) (b) (c)

Fig1(a-c) : Infected heifer loaded in a mini truck with collection of saliva.

With the history and clinical signs in backdrop, the saliva present either on the floor or adhering to the mouth was collected properly with the help of a sterile swab and disposable syringe. The sample was transferred to a vial containing 2.5 ml of lysis buffer supplied by M/S Molbio. The swab was touched to saliva for 8-10 times with each time dipped into the lysis buffer and pressed against the side wall of vial. The saliva sample thus collected was subjected to real time PCR using a small portable electric/battery operated equipment manufactured and supplied by M/S Molbio (Fig 2) where nucleic acid extraction and amplification were undertaken in two different units viz., Trueprep Auto and Truelab UNO Dx, respectively. The entire process from collection of bio-sample till availability of results was completed within an hour.

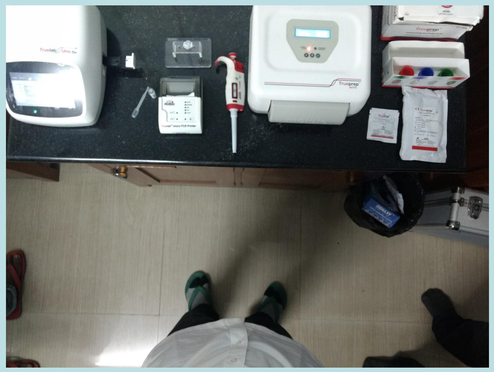
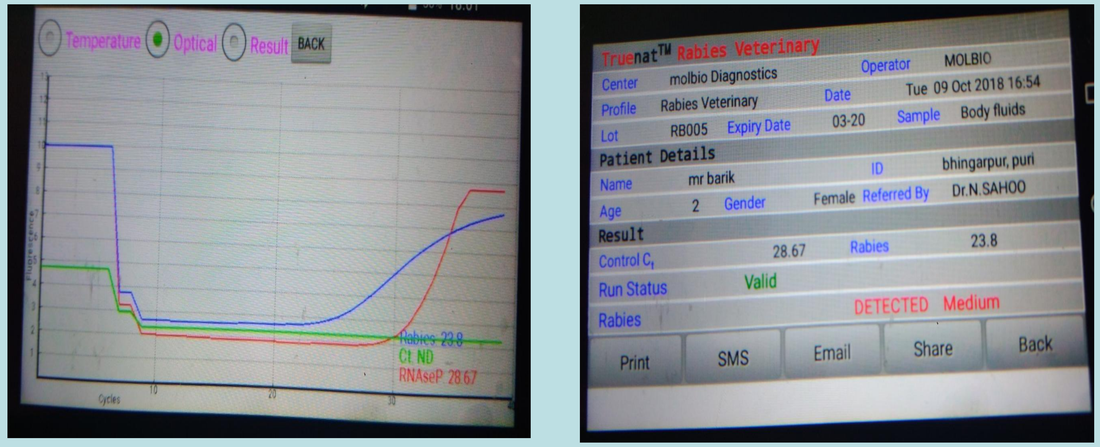


Fig 2 : M/S Molbio Trueprep Auto and Truelab UNO Dx

Saliva sample collected from the heifer found positive for rabies with a Ct value of 23.8 (Fig 3a & 3b) confirmed our clinical tentative diagnosis.



(a) (b)

Fig 3(a & b) : Results of real time PCR with Ct value

Rabies is manifested in two forms – furious and dumb. Furious rabies is characterized by aggression, aimless movement, excessive salivation, hypersensitivity to sounds, voiceless attempts to bellow/change in voice and complete anorexia whereas paralysis is the dominant feature of dumb form (Radostits *et al.,* 2009). But, the heifer under discussion had no typical signs suggestive of any one form of rabies at the time of presentation. It is a fact that many a times clinical signs alone during early phase of illness is unreliable and often confused with several other diseases having nervous manifestations viz., lead poisoning, lactation tetany, listeriosis, polioencephalomalacia, vitamin A deficiency, ketosis, hyperthermia, plant poisoning, louping ill, etc. The quick, simple and sensitive ante-mortem diagnostic technique developed by M/S Molbio showed positive results.

Laboratory diagnosis based on post-mortem examination of infected brain specimen by fluorescent antibody test (FAT) is considered as a gold-standard. However, it is time-consuming, requires experienced personnel and sophisticated laboratory facilities having biosecurity management system.

Extraction and amplification was initially carried using 1ml of lysis buffer mixed sample where low viral load and a higher Ct value(32.33) was detected. On increasing the quantity of sample to 1.5ml, moderate quantity of virus and a comparatively lower Ct value(23.8) was found . Though the test is simple and quick in ante-mortem rabies detection, the recommended procedure needs to be standardized with respect to the minimum quantity of saliva required to give positive results.

Quick real time PCR developed by M/S Molbio found to be a helpful and reliable diagnostic tool for field level diagnosis of rabies.

1. Radostits, O.M., Gay, C.C., Hinchcliff, K.W., Constable, P.D.(2009). Veterinary Medicine, A Textbook of the Disease of cattle, horses, sheep, pigs and goats. 10th Edition, 1384-1393.
2. Menezes, R.(2008). Rabies in India. CMAJ J, 178(5): 564–566