IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 15, Issue 4 Ser. I (April 2022), PP 08-11
www.iosrjournals.org

Chip based RT PCR test (TrueNat PCR) for peripheral level diagnosis of Rabies in animals: A Pilot Study

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Abstract
Accurate and timely diagnosis of animal rabies is the back bone of medical decision making for initiating post exposure prophylaxis (PEP) of rabies exposed victims. As part of global efforts to eliminate dog mediated human rabies by 2030, there is an urgent need to improve laboratory facilities in the peripheral level. The traditional reference methods of rabies diagnosis, fluorescent antibody test or PCR have several limitations for wide scale implementation in settings that lack economic and infrastructural resources. This documentation is the report of evaluation of a chip based Realtime PCR test as a peripheral level diagnostic tool for rabies. The study demonstrated a sensitivity, specificity and diagnostic accuracy of 92.3, 100, and 95.8 percent respectively when compared with Fluorescent Antibody Test, the gold standard diagnostic test for rabies. The study, conducted on twenty four brain tissue samples of animals suspected of died due to rabies, concluded that TrueNat PCR is a simple, easy to use, economical and reliable rapid test for rabies diagnosis especially in resource poor peripheral settings.

Key Words*: Fluorescent Antibody Test, RT PCR, Immuno chromatographic method, TrueNat PCR*

Date of Submission: 27-03-2022 Date of Acceptance: 07-04-2022

I. Introduction

Rabies is one of the most fatal zoonotic diseases which have tormented humans since antiquity and is still a significant public health problem in many parts of the globe. As per WHO estimates, India accounts for 36% of the global and 65% of the south East Asian human rabies death[1]. Laboratory based surveillance of rabies is severely constrained in resource poor developing countries. Lack of availability of laboratory facilities at field level, access to reference labs, biosafety issues in collection and transport of samples, sample spoilage during transport to distant referral laboratories and various practical issues pose challenges in improving rabies surveillance.

As part of global efforts to eliminate dog mediated human rabies by 2030, there is an urgent need to improve laboratory facilities in the peripheral level. Developing easy to use and affordable diagnostic tools is the need of the hour. Brain is the preferred sample for rabies confirmation and Fluorescent Antibody Test (FAT) is the gold standard method for confirmatory diagnosis of rabies in both animals and humans. But the method is expensive and requires trained, experienced and skilled laboratory personnel making it infeasible for large scale application. Molecular methods like conventional RT PCR or Realtime PCR are the other reference methods. However it is challenging to set up full-fledged molecular testing laboratory with large floor space, high end instruments, trained personnel and uninterrupted power in peripheral settings. Hence there is an urgent need to explore newer easy to use and affordable field tests without compromising accuracy of results. Immuno chromatographic based tests (rapid lateral flow device) have greatly improved peripheral level diagnosis of rabies[2]. But can be employed only as a screening test. Negative results demand confirmation by more sensitive tests like FAT or PCR.

In this study we have evaluated the diagnostic accuracy of a novel TrueNat assay, which is a chip-based Realtime PCR test. The instrument and accessories are portable, light-weight, battery operated and hence can be used in remote areas with limited infrastructure.

II. Materials And Methods

The study was conducted at the State Institute for Animal Diseases, Thiruvanathapuram, Kerala on animal brain samples received for diagnostic confirmation as part of routine disease surveillance activities. The study evaluated twenty four brain tissue samples from 16 dogs, 6 cats and 2 rabbits. TrueNat PCR results were compared with Fluorescent Antibody Test, the gold standard test for rabies diagnosis.

Test Methodology:

Direct Fluorescent Antibody Test on brain impression smears was carried according to *Meslin et al.*,1996[3] and results were interpreted as bright/dull/dim apple-green round to oval intracellular fluorescence accumulations or no fluorescence and were graded as per *Tepsumethanon et al.,* 1997[4].

TrueNat PCR: Test was conducted on brain issue as per manufacturer's protocol. TrueNat work station consists of a nucleic acid extraction device and a PCR reaction analyser (Fig 1) along with accessories like RNA cartridges and microchips. It is portable, battery operated and can run 6 hrs on single charge. The whole procedure from RNA extraction from samples to final reading of result takes about 90 minutes. The results are declared either as detected (high, medium, low or very low); not detected or as test invalid. Ct value is also given (Fig 2) and one can visualize the reaction real time.

Fig 1 : TrueNat Workstation

Data Analysis

The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy were calculated by 2 x 2 table method.

III. Results And Discussion

Among the 24 brain samples tested, 12 (50.0 %) and 13 (54.1%) samples were positive by TrueNat PCR and FAT respectively (Table 1). Taking the results of FAT as gold standard, TrueNat PCR test showed a sensitivity, specificity and diagnostic accuracy of 92.3, 100 and 95.8 percent respectively. One sample out of the twenty four (4.1%) tested showed a difference between the two tests The comparative study displayed a positive predictive value of 100% and negative predictive value of 91.7% for the test under evaluation, TrueNat PCR.

Table 1 : Rabies positivity on FAT and TrueNat PCR

Number of samples	FAT	TrueNat PCR
Positive :	13	12
Negative :	11	12
TOTAL (n)	24	24
Test positivity (%)	**54.1**	**50.0**

Table 2 : Cross analysis of results of two tests

		FAT		
		Positive	Negative	Total
TrueNat PCR	Positive	12 a	0 b	12
	Negative	1 c	11 d	12
	Total	13	11	24 (n)

Table 3 : Grading of viral antigen load

	FAT 4+ degree fluorescence (Positive)	FAT: 3+ degree fluorescence (Positive)	FAT: 2+ degree fluorescence (Positive)	FAT 1+ degree fluorescence (Positive)	FAT: No fluorescence (Negative)
Number of samples	4	4	2	3	11
	TrueNat Detected high (Positive)	TrueNat Detected medium (Positive)	TrueNat Detected low (Positive)	TrueNat Detected very low (Positive)	TrueNat Not Detected (Negative)
Number of samples	6	3	1	2	12

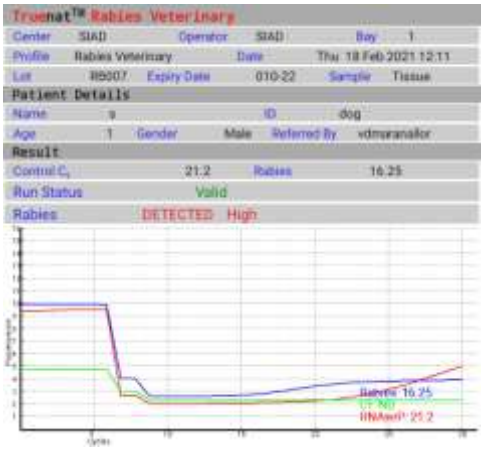

Fig 2 : TrueNat PCR result

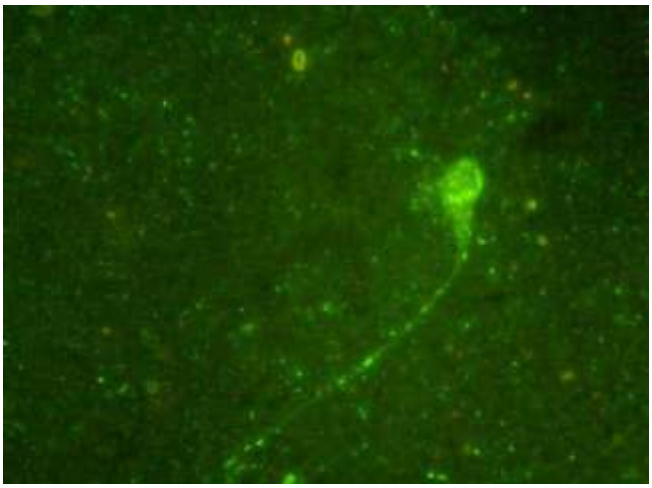

Fig 3 : FAT 3+ degree fluorescence

Rabies is a classic "One Health" challenge transmitted only through animal vector. Accurate and timely diagnosis of animal rabies is the back bone of medical decision making for initiating post exposure prophylaxis (PEP) of rabies exposed victims. TrueNat PCR is a battery operated, portable device, a proprietary product of M/s Molbio Diagnostics Private Limited, Goa, India. The test works on pre- loaded, ready to use and disposable micro PCR chips. Extraction procedures are automated and simple requires only minimal training. The device has an automated reporting system and is GPRS/Bluetooth enabled for real time data transfer. It is semi quantitative. This assay has been evaluated and endorsed for human pathogens like Mycobacterium[5] and SARS CoV-2 [6, 7] and the test is commercially available for detection of more than 25 pathogens. Recently it has been approved for drug resistant tuberculosis[8, 9]

As the concept looked promising for improving rabies diagnosis at field level or in peripheral clinical laboratories, the present study was designed to evaluate it for rabies virus detection as compared to gold standard test, FAT. Till date there have been no field trials evaluating its scope for rabies diagnosis in animals or humans.

The result of this study looks promising as the test has demonstrated a sensitivity and specificity of 92.3% and 100% respectively. Test is rapid and provides results in 90 minutes as compared to 10-12 hour required for conventional RT PCR or Immunofluorescence test. Operational cost is comparable with RT PCR or FAT though not cheaper. More over the sample collected in lysis media which immediately lyses infective virus making it non-infectious reduces the biosafety concerns in sample preparation. Method is semi quantitative providing results along with Ct values thus giving information on the antigen load (Table 3) and to decide whether to go for a second confirmation especially when applied on clinical specimens. Contamination chances are less when compared to conventional RT PCR as micro-chips, cartridges and extraction reagents are individually packed for each specimen. One limitation of this method is that it cannot handle large number of samples simultaneously. However, Rabies classically appears in nature as incidences rarely as outbreaks and so practically situation demanding testing large number of samples at a time is remote.

We admit that the number of samples being evaluated in this study is small and limited to brain specimens only. The challenges of collecting brain in field conditions can overcome to certain extend if the method proves its efficiency in non-invasive samples. Further studies on a larger sample set and on different clinical samples like saliva, skin or other body fluids like tears are recommended to elucidate more on its application as a point of care test in humans and pen side rabies diagnostic method in animals.

IV. Conclusion

It can be concluded that TrueNat PCR is a simple, easy to use, economical and reliable rapid test for rabies diagnosis especially in resource poor peripheral level clinical laboratories and will prove to be a game-changer in the concerted efforts of improving laboratory based surveillance in the journey towards "Zero rabies by 2030" campaign.

Acknowledgement

We acknowledge Director of Animal Husbandry, Kerala for the facilities provided under R&D head and M/s Molbio Diagnostics Private Limited, Goa, India for developing the test on request.

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Swapna Susan Abraham, et. al. "Chip based RT PCR test (TrueNat PCR) for peripheral level diagnosis of rabies in animals" *IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 15(04), 2022, pp. 08-11.