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Advances in Brain Specimen Collection for Rabies Diagnosis

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A B S T R A C T

An attempt was made to collect brain samples without opening the skull to diagnose rabies. Brain samples were collected using AI sheath through the Occipital Foramen Route (OFR) and Retro Orbital Route (ROR) and subjected to fluorescent antibody test (FAT) and staining for Negribodies. Results by these methods showed equal sensitive on comparison with the classical method. This study suggests that the OFR and ROR were suitable to collect brain specimens in field where a laboratory structure with biosafety facility is not available.

Introduction

Rabies remains massive threat and zoonotic diseases of mankind. The classical method to collect the brain samples from rabid suspected animals is done by opening the skull and time-consuming operation (Zerai Woldehiwet, 2005 and Shankar, 2009). Furthermore, brain sample meant for polymerase chain reaction (PCR) require typical methods that preclude contamination from other specimens (WHO, TRS 982; Kadam *et al.,* 2011).

To overcome these limitation, attempt for occipital foramen route and retro-orbital route have been made to collect brain samples without opening the skull which will expedite.

The sample collection for clinical laboratory diagnosis of rabies was done as follows.

Materials and Methods

Plastic and rubber articles

Disposable plastic wares like scintillation vials, artificial insemination sheath (AI-Sheath), plastic pipette (2ml, 5ml), drinking straw and rubber gloves were used in this study.

Standard solutions

Phosphate Buffered saline (PBS-pH - 7.2), PBS with tween 20 and 50% glycerol saline and Williams modified Van Gieson's stain were used.

Conjugate

Lyophilised, adsorbed anti- rabies nucleocapsid antibody conjugate with fluorescein isothiocyenate (Biorad) was used.

Sample collection

Brain samples were collected through the occipital foramen route (Barrat, 1996), retro-orbital route (Hirose *et al.,* 1991) and open method (Lepine and Atanasiu, 1996) as described by the authors in parenthesis respectively.

Impression smear preparation

Impression smear were made from all the samples and subjected to methanol and cold acetone fixing and tested for Negribodies (Basheer *et al.,* 1997) and fluorescent antibody test (Dean *et al.,* 1996 and Bonny *et. al.,* 2015) respectively.

Results and Discussion

Out of 54 samples collected by both OFR & ROR from 40 canine, 10 bovine and 4 caprine species, 39 were positive for rabies and 15 were negative by two methods (FAT and staining) but FAT detected more number of rabies cases. Both occipital foramen and Retro-orbital routes of brain sampling were found to be equally sensitive compared with the classical method of sample collection. Of 54 brain samples collected by the classical method, 38 were positive for rabies which included 24 canine, 9 bovine and 5 caprine. The sensitivity of Negribodies by classical method is 94.87% and specificity 93.33% (Table 1).

The brain sample collected by the occipital foramen route, 26 animals showed Negribodies in the impression smears which included 17 canine, 4 bovine and 4 caprine. The sensitivity of negribodies by OFR is 41.02% and specificity is 33.33% but the sensitivity of ROR route is 38.46% (Table 1).

In the present study, it was found that the AI sheath was suitable for collecting the brain specimens rather than using drinking straw and disposable pipette in which the former was not so strong enough to pass through the brain and the latter was found to be too hard to cut by scissors, and hence unsuitable for preservation of samples. But, when AI sheath was used, it could be easily cut by using ordinary scissors and cut portion could be preserved in formalin after collection.

Table.1 Sensitivity and specificity of negribodies compared with fluorescent antibody technique

Species	Classical Method		OFR Method		ROR Method	
	NB	FAT	NB	FAT	NB	FAT
Canine	25	25	17	25	17	25
Bovine	9	9	5	9	4	9
Caprine	5	5	4	5	3	5
Total	39	39	26	39	24	39
Sensitivity	94.87%		41.02%		38.46%	
Specificity	93.33%		33.33%		40.00%	

NB - Negribodies
FAT - Fluorescent Antibody Technique
OFR - Occipital Foramen route
ROR - Retro-orbital route

Plates.1

(a) Occipital foramen route

(b) Retro orbital route

Both OFR & ROR were found to be suitable for brain specimen collection and also they are equally sensitive and specific when compared with that of classical method (Table 1).

This was in accordance with the Barrat (1993) Bingham *et al.*, (2002) and Iamamoto *et al.*, (2011) findings, who stated that both techniques were equally sensitive and could be used in field conditions when a laboratory structure was not available for sampling. Field veterinarians could also adopt these techniques, as it is very simple, rapid and safe.

It is concluded that it is possible to use ROR and OFR for collection of brain sampling comparable to the classical method of brain sampling and for further epidemiological surveillance of rabies. The ROR appears to be particularly rapid and safe for the collection samples in the field.

This type of collection reduces the cross-contamination, easy to transport brain samples from field to lab and minimizes the chances of accidental exposure to the virus.

The rapid collection procedures described are also suitable for total RNA extraction in rabies diagnosis by the polymerase chain reaction (Silva *et. al.*, 2013) because only disposable materials are used like AI sheath and drinking straws are used.

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References

Barrat J. 1996. Simple technique for the collection and shipment of brain specimens for rabies diagnosis. In: Laboratory Techniques in rabies. 4th Edn. WHO, Geneva. 425-432

Basheer M.A., Ramakrishna J and Manickam R. 1997. Clincal observations in rabid calves and sero conversion studies of different post-infection immuization schedules with anti-rabies vaccines. *Veterinary Archiv*, 67(3): 87-92.

Bingham J and Maria van der M. 2002. Distribution of rabies antigen in infected brain material: determining the reliability of different regions of the brain for the rabies fluorescent antibody test. J. Virol. Methods. 101(1-2): 85-94.

Bonny Mayes and Charles E. Rupprecht. 2015. Current Laboratory Techniques in Rabies Diagnosis, Research and Prevention, Vol 2: 83-92

Dean D.J., Abelseth M.K and Atanasiu P. 1996. The fluorescent antibody test. In: Laboratory techniques for rabies. 4th

Edn. WHO, Geneva. Pp. 88-95.

Hirose J. A.M, Bourhy, H and Sureau P. 1991. Retro-orbital route for brain specimen collection for rabies diagnosis. *Veterinary Record*. 129: 291-292.

Iamamoto K., Quadros J and Queiroz L. H. (2011). Use of Aspiration Method for Collecting Brain Samples for Rabies Diagnosis in Small Wild Animals. Zoonoses and Public Health. 58: 28–31.

Kadam S. S., Sherikar A. A and Pingale V. S. 2011. Comparative Analysis of Routine Laboratory Diagnostic Tests for Rabies, *Indian Journal of Virology*, 22(2): 142

Lepine, P and Atanasiu, P. 1996. Histopathological diagnosis of rabies. Laboratory Techniques in rabies. 5th Chapter, 4th Edition, WHO, Geneva: 66-79.

Shankar B.P. 2009. Advances in Diagnosis of Rabies. Veterinary World, 2(2): 74-78.

Silva S. R, Katz I. S. and Mori E. 2013. Biotechnology advances: a perspective on the diagnosis and research of Rabies Virus. Biologicals, 41(4): 217–223.

World Health Organization, "WHO Expert Consultation on Rabies. Second report," World Health Organization Technical Report Series 982, 2013. Pp. 25-27.

Zerai Woldehiwet. 2005. Clinical laboratory advances in the detection of rabies virus. Clinica Chimica Acta, 351: 49 – 63.

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