www.ajbrui.net

Comparison of the Fluorescent Antibody Test and Direct Microscopic Examination for Rabies Diagnosis at the National Veterinary Research Institute, Vom, Nigeria.

Ehizibolo, D.O[*1]; Nwosuh C.I[1]; Ehizibolo, E.E[2]; Kia, G.S.N[3]

[1]*National Veterinary Research Institute, Vom, Nigeria.*
[2]*Department of Community Health and Primary Health Care, Lagos State University College of Medicine, Nigeria.*
[3]*Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.*

ABSTRACT: One hundred and eighty-nine (189) dog brain samples submitted to the National Veterinary Research Institute, Vom, Nigeria for rabies diagnosis were assayed by the Direct Microscopic Examination (DME) for Negri bodies, the Fluorescent Antibody Test (FAT) for rabies antigens, and the Mouse Inoculation Test (MIT). The MIT was used as a confirmatory test. There were 12(12.5%) false negative and 6(6.7%) false positive results with DME, while there was 2(1.7%) false negative and 2(1.7%) false positive with FAT. The FAT was more sensitive (98.26%) than the DME (87.5%), and also more specific, 97.29% and 93.55% respectively. The FAT predicted positive and negative results more accurately than the DME. Positive predictive value of FAT was 98.26% compared with 93.33% of DME, and the negative predictive value of FAT was 97.29% compared with DME's 87.88%. The pre-test probability of rabies in Vom was 60.8%. This finding shows that FAT is a sensitive and reliable rabies diagnostic test than DME and its continued use is recommended in Nigeria whenever is feasible. The DME is still useful as a screening test, and the MIT must be used to confirm both FAT and DME

Keywords: *Fluorescent antibody test, mouse inoculation, direct microscopic examination, rabies diagnosis, Nigeria.*

INTRODUCTION

The predominant rabies virus reservoir hosts are bats and carnivores. Among these, rabid dogs represent a substantial public health problem particularly in developing countries (Rupprecht *et al.*, 2002).
Rabies is the only major disease in which laboratory diagnosis of the disease in an animal directly affects human treatment (Messenger *et al.*, 2003). Prompt and reliable diagnosis of rabies is essential to patient post-exposure prophylaxis and to guide epidemiologic surveillance. The National Veterinary Research Institute (NVRI) Vom is presently the major diagnostic Laboratory for rabies in Nigeria with the capacity to carry out the Direct Microscopic Examination (DME), Fluorescent Antibody Test (FAT) and the Mouse Inoculation Technique (MIT).

The Direct Microscopic Examination (Seller's staining for Negri bodies) is the standard screening test for rabies in NVRI because it is easy, cheaper, faster and economical to perform. However, it has the disadvantage of low sensitivity, ranging from 75% to 85% (Velleca and Forrester 1981, Fekadu and Smith 1989, Miranda and Robles 1991) and may fluctuate based on the species of animal involved and the time of the animal's death. The presence of cytoplasmic inclusion bodies produced by other pathogens (canine distemper virus and canine hepatitis virus) and artifacts in the animal's brain sometimes gives a number of false-positive results (Velleca and Forrester 1981, Fekadu and Smith, 1989; Boolert, 2005). Animals sacrificed prematurely may present a false negative result (Tierkel, 1973). The specificity and accuracy of rabies diagnosis was improved when the direct fluorescent antibody technique (FAT) was introduced (Goldwasser and Kissling, 1958). Among the laboratory tests recommended by the World Health Organization (WHO), the FAT is the accepted

Manuscript received: June 2008; Revision Accepted November 2008

*Address for correspondence: kingdavid_e@yahoo.com

standard for rabies diagnosis (Rupprecht *et al*, 2002), with a sensitivity closely approximating that of the mouse inoculation test (MIT) and Tissue Culture infection Technique (TCIT) (Bourhy, *et al.*, 1989). However, it is less effective with decomposed tissues compared to the polymerase chain reaction (PCR) and the need for an ultraviolet (UV) microscope makes the test very expensive to perform. The mouse Inoculation Test (MIT) is a virus isolation method usually for confirmation with sensitivity of almost 100%. However, Velleca and Forrester (1981) reported that in rare cases, false positives may occur when the virus is no longer viable, and it takes a period of 21 days before the result may be available which may be too late to be valuable in treatment decisions for humans.
This study is therefore designed to compare the sensitivity and specificity of the FAT and DME with MIT in the diagnosis of rabies at NVRI, Vom, Nigeria in order to establish and adopt a more reliable practical rabies diagnostic technique in the country.

MATERIALS AND METHOD

Specimens: A total of 189 samples of fresh rabies suspected dog heads were submitted to the rabies laboratory of the NVRI, Vom, for testing between January and December 2006. All the cases involved human exposure. The brain of each dog head sample was carefully removed and kept frozen at -20^{o}C until tested. The samples were tested for rabies by the DME, FAT and the MIT.

Direct microscopic examination: A fresh touch impression smear of the hippocampus (Ammons horn) of each sample was made on a clean glass slide. DME was performed according to the technique described by Tierkel (1973). Each smear was stained with 1% solution of basic fuchsin and methylene blue in absolute alcohol (seller's stain) for 1-3 seconds and rinsed in running water. The slides were viewed under X100 (oil immersion) objective. Negri bodies were detected on positive slides as cherry red or magenta staining depending on its density with sharply defined spherical, elongated or oval bodies with dark blue medium to large granules within the matrix of the body.

Fluorescent antibody test: FAT was carried out according to Meslin *et al.*, (1996). Impression smear preparations of the hippocampus were placed in a coplin jar containing acetone and fixed at -4^{0}C for 30 minutes. The slides were air-dried and stained with fluorescein-labelled monoclonal anti-rabies immunoglobulin (CHEMICON International). These were then incubated at 37 ^{0}C for 30 minutes in a humid chamber and further washed with Phosphate Buffered Saline (PBS) in 3 successive washes for 5-10 minutes. The slides were rinsed with distilled water; air-dried and mounting buffered glycerol applied, then visualized under an immunofluorescent microscope (Zeiss) at X400 magnification. Bright/dull/dim apple-green or yellow-green, round to oval intracellular accumulations were observed. Positive and negative controls were run together with the test specimens

Mouse inoculation test: The intra-cerebral mouse inoculation test (MIT) was conducted according to Meslin *et al.*, (1996). The hippocampus was macerated in a mortar, diluted to 10% in PBS (PH 7.2) containing penicillin
50 IU – Streptomycin 2mg/ml and centrifuged (1500 rpm for 5 minutes). At least 5 suckling mice (3 days old) were inoculated intracerebrally, each with 0.03ml of the supernatant and observed daily for 21 days for any signs of rabies (trembling, in-coordination, paralysis, humping, seizures, prostration). PBS, PH 7.2 was used for inoculation of the negative control.

Analysis of Results: Using the MIT for comparison, the sensitivity, specificity, predictive values of positive and negative results of the FAT and DME were evaluated. The pre-test probability of rabies in Vom was also determined.

RESULTS

The results of the MIT compared with that of the DME are shown in Table 1. There were 6 false positive results with the DME and 12 false negatives compared with MIT. The MIT results were also compared with that of FAT (Table 2). Out of the 12 false negatives with DME, only 2 showed negative for FAT.

Table 1: Comparison between results obtained by DME and MIT on 189 dog brain received at NVRI for rabies diagnosis

	MIT RESULTS		
DME Results	+	-	**Total**
+	84	6	90
-	12	87	99
Total	96	93	189

+ Positive
- Negative

Table 2: Comparison between results obtained by FAT and MIT on 189 dog brain received at NVRI for rabies diagnosis

	MIT RESULTS		
FAT Results	+	-	**Total**
+	113	2	115
-	2	72	74
Total	115	74	189

Two of the 6 false positive results with DME were also positive with FAT although weakly. The overall performance of both DME and FAT compared with the MIT, and the determination of the pre-test probability is summarized in Table 3. In all, the FAT was more sensitive and specific than DME and can also predict positive and negative results more accurately than DME. The pre-test probability was shown to be 60.8%.

Table 3: Comparison between results obtained by DME and FAT with the MIT based on sensitivity, specificity, predictive value of positive results (PV+), predictive value of negative results (PV -) and the pre-test probability of the 189 dog brain samples

	DME	**FAT**
Sensitivity %	87.5	98.26
Specificity %	93.55	97.29
PV + %	93.33	98.26
PV - %	87.88	97.29
Pre-Test probability %	60.8	60.8

DISCUSSION

The need to establish a more reliable and accurate test for rabies diagnosis in our laboratories for proper decisions regarding the treatment of potentially exposed individuals cannot be over emphasized. This can be a cost effective approach to the management of dog bite victims considering the high cost of rabies post-exposure treatment (Robles and Miranda, 1992)

Our observation that most of the dog heads submitted to the laboratory was presented several hours and even overnight after the dogs were killed or died without any form of preservation may have influenced the false positive result demonstrated by the DME and FAT compared to the MIT which was negative for such samples. The same observation was made (Robles and Miranda, 1992) in a study of 337 brain samples using MIT and FAT, in which one FAT-positive sample gave negative MIT result.

It is presumed (Bourhy *et al*, 1989) that delay in testing unpreserved samples may result in false negative MIT as a result of virus inactivation. Valleca and Forrester, (1989) and Koprowski (1996) also speculated that inhibitory substances such as rabies inhibiting substance, antibody or interferon may play a role. In this study, the 2 samples that tested negative by both FAT and DME were positive by the MIT. This is in agreement with similar findings by Robles and Miranda (1992), and shows that viral isolation is the most sensitive test in situations where the amount of antigen is too little to be detected by other tests. The MIT being the reference test in our study was assumed to have a specificity and sensitivity of 100%. However, it has been shown that the quality of the specimen submitted may interfere with the accuracy of the result. This study showed that the FAT is a highly sensitive and reliable test (98.26%) and slightly more specific (97.29%) than the DME (93.55%). The slight difference in sensitivity of FAT compared to the MIT suggests that all FAT-negative samples should be confirmed by MIT. The predictive values in this study demonstrate the fact that FAT can detect both positive and negative samples more accurately than DME. We also presume that the low predictive value of negative test of DME (87.88%) may have been influenced by the presence of non- specific inclusion bodies in some samples leading to a false positive result.

Our conclusion confirmed previous findings that FAT is a truly reliable diagnostic test and could well be the standard for rabies diagnosis as accepted and practiced world wide. But it should be supported by virus isolation (MIT) routinely for confirmation. The results furth er showed that DME was fairly sensitive (87.5%) and specific. In Nigeria, where many rabies laboratories cannot afford the high cost of FAT reagents and the UV microscope, the DME can still be used but must be confirmed by MIT. However, effort should be made to incorporate FAT. This test (DME) can serve as a routine diagnostic test and especially in cases where the circumstances of dog bite is suggestive and always using the MIT to confirm all negative test results. The pre-test probability (60.8%) showed that vaccination coverage may have been ineffective to prevent the occurrence of the disease. A further study to validate the role of sample quality in rabies diagnosis and to compare the above tests with the PCR in the area studied is being embarked upon.

Acknowledgement

We appreciate the support and motivation of the Executive Director, Dr (Mrs) L.H. Lombin, the Director Diagnostic and Extension, Dr A.A. Makinde and the technical

assistance of the staff of rabies diagnostic laboratory, National Veterinary Research Institute, Vom, Nigeria.

REFERENCES

Boolert, L. (2005). Laboratory techniques for rabies diagnosis in animals at Queen Saovabha Memorial Institute. J Med Assoc Thai 88 (4) 550-553

Bourhy, H, Rollin, P.E, Vincent, J and Sureau, P (1989). Comparative field evaluation of the fluorescent antibody test, virus isolation from tissue culture and enzyme immunodiagnosis for rabies. J Clin Microbiol. 27(3): 519-523

Fekadu, M and Smith, J.S (1989). Laboratory diagnosis of rabies. In: Winkler, W.G (ed). Rabies concepts for medical professionals Merieux Inst., Florida. P 38

Goldwasser, R.A and Kissling, R.E (1958). Fluorescent antibody staining of street and fixed rabies virus antigen. Proc Soc Exp Biol Med 98: 219-223

Koprowski, H (1996). The mouse inoculation test. In: Meslin, F.X, Kaplan, M.M and Koprowski, H, editors. Laboratory techniques in rabies 3rd edition WHO, Geneva. pp 80-87

Meslin, F.X., Kaplan, M.M. and Koprowski, H. (1996). Laboratory diagnosis of rabies. Geneva, WHO, pp 88-95

Messenger, S.L., Smith, J.S., Orciari, L.A., Yager, P.A,and Rupprecht, C.E (2003). Emerging patterns of rabies deaths and increased viral infectivity. Emerging Infect Dis 9: 151-154

Miranda, N.L.J. and Robles, C.G (1991). A comparative evaluation of a new immunozymatic test (RREID) with currently used diagnostic tests (DME and FAT) for dog rabies. Southeast Asian J. Trop Med Pub Hlth 22: 46-50

Robles, C.G. and Miranda, N.L.J. (1992). Comparative evaluation of the rabies fluorescent antibody test and direct microscopic examination at the research institute of tropical medicine. Phil J Microbiol Infect Dis 21(2): 69-72

Rupprecht, C.E., Hanlon, C.A. and Hemachudha, T. (2002). Rabies re-examined. Lancet Infect Dis 2: 327-343

Tierkel, E.S. (1973). Rapid microscopic examination for Negri bodies and preparation of specimens for biological test. In: Kaplan, M.M and Koprowski, H. (eds). Laboratory techniques in rabies. 3rd edition. WHO, Geneva. pp 41-50

Velleca, M.W. and Forrester, F.T. (1981). Laboratory methods for detecting rabies. CDC, Georgia