DETECTION OF RABIES ANTIGEN IN BRAINS OF SUSPECTED RABID DOGS USING SELLER'S STAINING TECHNIQUE AND ENZYME IMMUNOASSAY

NIMZING LOHYA*[1] and ZWANDOR NANBOL[2]

[1]Department of Medical Microbiology
University of Jos – Nigeria.

[2]National Veterinary Research Institute,
Vom-Nigeria.

*To whom correspondence should be addressed
P.M .B. 2084, Jos-Nigeria.

ABSTRACT

Objective:

To detect the presence of rabies antigen in brains of suspected rabid dogs.

Materials and Methods:

Ninety six (96) brain specimens from suspected rabid dogs were examined for the presence of rabies antigen using Seller's staining technique and enzyme immunoassay.

Results

The two techniques were both effective for the detection of rabies antigen, however seller's staining method detected more positive cases 42(43.8%) than enzyme immunoassay 27(28.2%) ($P<0.05$). This may be attributed to the many pitfalls of the seller's staining technique as putrid brain materials can lead to false-positive results.

Conclusion

In view of these findings, enzyme immunoassay if carefully carried out may be more useful for the detection of rabies antigen in surveillance studies since it is more rapid and convenient for the assay of large number of specimens. However, fewer specimens may still require the older staining method where available facilities are limited.

Key Words: Rabies antigen, Seller's Staining/Enzyme-immunoassay, Vom-Nigeria.

INTRODUCTION

Rabies a viral zoonosis remains a dreadful, gruesome human communicable disease that kills about 50,000 people per year, mostly in Asia, Africa, South America and Central Europe[10]. Rabies still persists as a major health problem in this age of high technology[3,5] with high endemicity in many parts of the world with the exception of a few countries and areas that have been historically free of the disease[1]. Rabies mortality ranks tenth among all infectious diseases worldwide[2].

It is a viral encephalitis, agonizing in its manifestations and an infectious disease involving the central nervous system to which all mammals are susceptible. The dog is incriminated as the animal vector responsible for transmission of rabies to man and other dogs[6]. The vast majority (95-98%) of the 60,000 annual death cases worldwide occur in canine (dog)

rabies endemic regions with large stray dog population. Dogs are responsible for nearly 80% of animal bite wounds[9].

Diagnosis commonly utilize fluorescent antibody techniques, microscopic examination of brain for negri bodies, isolation of the virus from tissue specimen and confirmation by neutralization tests. Polymerase Chain Reaction (PCR) methods for the detection of rabies have also been developed[4,7]. Fluorescent antibody technique has been reported as more sensitive and faster than virus isolation in mice. However where such a technique equipment is not available, an enzyme immunoassay is a valuable alternative which is as specific as indirect immuno-fluorescent technique[5].

MATERIALS AND METHODS

Dog brain (hippocampus; Ammon's horn) specimens.

Ninety-six (96) brain specimens were obtained from suspected rabid dogs brought to the National Veterinary Clinic at Vom Plateau State. Some of the brain specimens were from dogs killed after human bites. Others were from suspected rabid dogs. The suspected dogs were quarantined for some 10-14 days in the Veterinary Clinic to observe for the clinical signs of rabies. After death the head is removed and sent to the virus diagnostic laboratory for analysis. The brain specimens were obtained by cutting up the cranium to get the hippocampus or Ammon' horn.

Seller's Staining Method

Each dog brain sample obtained was squashed and an impression smear made on a glass grease-free slide. Subsequently staining was done by the use of seller's stain and washing carried out using buffered saline pH 7.0. Each slide was then dried and examined under a light microscope for negri bodies.

Enzyme Immunoassay Method

Collection and Dilution of Anti-rabies Serum from Dogs.

20ml of blood were collected from each of two dogs vaccinated against rabies. The sera separated were pooled together to obtain the anti-rabies serum. This was diluted in 4 using carbonated bicarbonate buffer pH 9.6 before being used for the sensitization of micro plates for the enzyme immunoassay procedure.

Treatment of the Dog Brain Specimens.

About 1g of each dog brain specimen was ground in a mortar and homogenization done in four volumes of tissue culture media. Centrifugation was carried out at 500rpm for 30 minutes. To each supernatant, penicillin/streptomycin of 100µl/ml and 100g/ml were added. Each sample was then tested by the enzyme immunoassay. Rapid Rabies Enzyme Immuno Diagnosis (RREID) kits were obtained from Diagnostic Pasteur Marnes la Coquette, France. The test was carried out as specified by the manufacturers using the method of Perrin et al,[8]. In brief, the microplates were sensitized by adding 50µl per well of the diluted anti-rabies serum and incubated at 30°C for 2 hours. Wells were washed four times with the washing solution and further incubated at 37°C for 1 hour with the horse radish peroxidase conjugate. The washing procedure was repeated and further incubation was done in the dark at room temperature with 0-phenylenediamine – 2NHCl substrate. The resulting reaction was stopped by adding 1ml of 1NHCl. The amount of viral antigen present was determined spectrophotometrically at a wavelength of 492nm.

RESULTS

Out of the 96 brain specimens analysed, 42(43.8%) were positive by the seller's staining technique while 27(28.15%) were positive by the enzyme immunoassay. Fifteen (15.7%) specimens were positive by both techniques (Table 1). The two techniques were both efficient for the detection of rabies antigen. However a significant difference was observed in the positive cases between the two techniques ($P<0.005$).

Table 1: Comparison of Seller's Staining technique and Enzyme-immunoassay for the detection of rabies antigen in brains of suspected rabid dogs.

No. of specimens screened for rabies Antigen	No. Positive (%)		
	Seller's staining Method	Enzyme immunoassay	Both techniques
96	42(43.8%)	27(28.1%)	15(15.7%)

DISCUSSION

The results of this study show that both techniques were effective in the detection of rabies antigen. However, the Seller's staining technique detected more positive cases than enzyme immunoassay. This is similar to other reports particularly between mouse inoculation Seller's[8]. This may be attributed to the many pitfalls of the seller's staining method. The high number of positive cases may therefore be attributed to improper staining and examination of smears thereby mistaken artifacts and red cells from bloody brain materials in degenerating specimens for negri bodies, since these can take up the Seller's stain giving rise to false positive results. The enzyme immunoassay is obviously sensitive and useful quantitatively. Its specificity is comparable to immuno fluorescent technique for the diagnosis of rabies [8].

The enzyme immunoassay is more rapid and useful for the detection of the rabies antigen in the brains of infected dogs. It is convenient when large number of specimens are being assayed. A wider application of this technique is required in this laboratory to replace the old staining technique. A rapid and sensitive method such as the enzyme immunoassay will be needed when surveillance studies requiring large number of specimens are being considered.

The dog is the main terrestrial vector of rabies and the principal cause of human transmission. Continuous studies using enzyme immunoassay to survey the incidence and seasonal trends of rabies in domestic animals particularly the dogs are needed. This will help in proper design of efficient rabies control programmer within geographic areas since appropriate knowledge of the local epidemiological cycles are required.

ACKNOWLEDGEMENT

We thank the Director N.V.R.I. Vom for permitting us use their facilities. We also thank Mr. Moses Makinde who helped in providing the dog brain materials used in this study and also late Mr. A.N.C. Okeke who participated to make this work a success.

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