

DIAGNOSIS OF CANINE RABIES BY THE DIRECT FLUORESCENT ANTIBODY TECHNIQUE IN PLATEAU STATE, NIGERIA

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SUMMARY

One hundred and eighty-nine (189) dog brain specimens submitted to the National Veterinary Research Institute (NVRI), Vom, for rabies diagnosis were tested by the direct fluorescent antibody test (DFA). One hundred and fifteen (60.8%) specimens tested positive. The incidence rates of rabies in female dogs tested (63.6%) was not significantly higher ($p > 0.05$) than the males (58.4%). The age specific incidences for dogs less than and above 6 months old were 55.9% and 61.9% respectively. The difference was however not statistically significant ($p > 0.05$). The results of this study showed a higher incidence of rabies in the months of June to July, and October to December. This current finding is suggestive of a public health risk of the area studied and calls for surveillance, public enlightenment, adequate vaccination coverage and control of stray and free-roaming dogs. The pre-exposure immunization of people at high risk, and collaboration of all stakeholders to ensure effective control of the disease is also advocated.

KEY WORDS: Rabies, Diagnosis, Direct fluorescent antibody test, Dog, Nigeria

INTRODUCTION

Rabies is fatal encephalitis caused by "Lyssavirus genotype-1" of the Family Rhabdoviridae, a neurotropic RNA virus commonly transmitted through the bite of an infected animal (Arai *et al.*, 2003). The virus is usually introduced at the site of inoculation to the central nervous system (CNS) via peripheral nerves. Rabies has traditionally been associated with dogs more than any other animal, and in parts of the world where domestic animal control and vaccination programs are limited; dogs remain the most important reservoir of the disease. The disease is one of the most feared zoonosis in the world,

WHO indicated that there are at least 60,000 human deaths and 10 million postexposure

treatments each year due to dog bite (Sonika *et al.*, 2007). Information on rabies deaths is sparse in Nigeria and West Africa as a whole due to misdiagnoses and underreporting. However, it is estimated that there are about 10,000 human exposures per year in Nigeria (Nawathe, 1980). The disease is endemic in Nigeria (Umoh and Belino, 1979) and has continued to plague its communities. Human rabies was first reported in the country in 1912 (Boulger and Hardy, 1960) while the first laboratory diagnosis of canine rabies was made in 1925 at the Yaba rabies laboratory (Nawathe, 1980). Since then, there have been several reported cases and outbreaks of rabies in Nigeria (Boulger and Hardy, 1960; Anon, 1957; Adeyanju and Addo, 1977; Onunkwo *et al.*, 1980). As observed by Adeyemi *et al.* (2005) there is still inadequate rabies vaccination coverage in the country, thereby increasing public health risk. The microscopic examination (seller's staining) of the

brain (Ammon's horn) is the common screening test for rabies in Nigeria, owing to its ease and affordability. However, due to its low sensitivity and tendency to give false-positive result, a more accurate and standard test, the fluorescent antibody test is recommended worldwide for rabies diagnosis (Rupprecht *et al.*, 2002) by the World Health organization (WHO). In Nigeria, only a few rabies laboratories have the capacity to carry out this standard technique. This study aims at determining the status of canine rabies by the direct fluorescent antibody technique.

MATERIALS AND METHODS

Specimen

One hundred and eighty nine fresh dog head samples from different parts of Plateau State were submitted to the rabies diagnostic laboratory of the National Veterinary Research Institute (NVRI) Vom from January to December, 2006, for rabies testing. All the samples were from suspected dogs involving human exposures. Over 95% of the dogs were stray and free-roaming. The brain (Ammon's horn) of each sample was immediately removed by dissecting the dog's head with a hack-saw on a clamp and kept frozen at -20 °C until used.

Direct fluorescent antibody (DFA) techniques

The DFA was based on the techniques described by Meslin *et al.* (1996). Impression smear preparations of the hippocampus (Ammon's horn) were placed in a coplin jar containing acetone and fixed at -4 °C for 30 minutes. The slides were air-dried and stained with fluorescein-labelled monoclonal anti-rabies immunoglobulin (CHEMICON International, CA). These were then incubated at 37 °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 400X magnification. Bright/dull/dim apple-green or yellow-green, round to oval or dust/sand-like particles were observed as shown in plate 1. A negative result shows a blank dark background (Plate 2). Positive and negative controls were run together with the test specimens. The DFA was usually backed up with the mouse inoculation technique (MIT) as described by Meslin *et al.* (1996), using 3 to 5-day-old suckling mice.

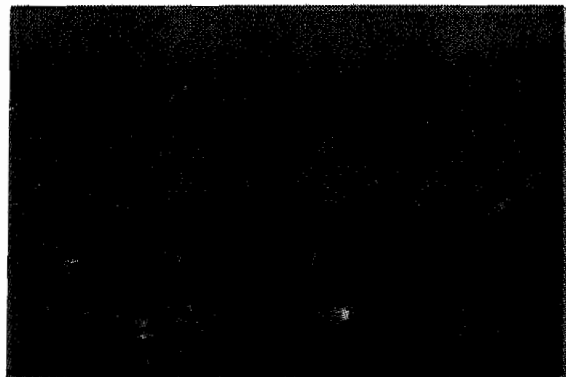


Plate 1: Positive DFA result

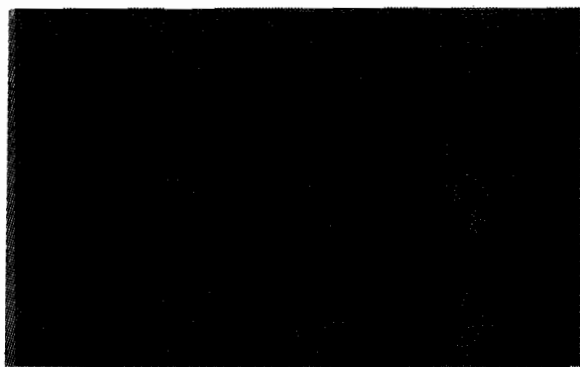


Plate 2: Negative DFA result

RESULTS

One hundred and eighty-nine (189) dog brain specimens were tested for rabies antigen by the Direct Fluorescent Antibody (DFA) techniques. They comprised 101 (53.4%) males and 88 (46.6%) females. Thirty-four (18%) were less than 6 months old while 115 (82%) were above 6 months of age. One hundred and fifteen (60.8%) of the specimens studied were positive for rabies antigen.

Table I shows the incidence of canine rabies detected by the fluorescent antibody techniques in Plateau State, in 2006 based on sex. There were higher rates in female dogs, (63.6%) than the males, (58.4%). The difference was however not statistically significant ($P > 0.05$). The rate of canine rabies in the different age groups is shown in Table II. Nineteen (55.9%) of the positive cases were less than 6 months old while 96 (61.9%) were older than six months. There was also no significant differences ($P > 0.05$). Three puppies of 8-week-old tested positive in the group below 6

months. The monthly incidence and laboratory diagnosis of canine rabies in Vom are presented in Table III and Figure 1 respectively.

TABLE I: Incidence of canine rabies detected by DFA at NVRI Vom, Plateau State in 2006 based on sex

Sex	No of Samples Tested	No. Positive (%)
Male	101	59 (58.4%)
Female	88	56 (63.6%)
Total	189	115 (60.8%)

(df=1, $X^2 = 0.34$, $P > 0.05$)

TABLE II: Incidence of canine rabies detected by DFA at NVRI Vom, Plateau State in 2006 based on age

Age	No of Samples Tested	No Positive (%)
< 6 months	34	19 (55.9%)
6 months - 1 year	29	18 (62.1%)
> 1 year	126	78 (61.9%)
Total	189	115 (60.8%)

(df=2, $X^2 = 0.43$, $P > 0.05$)

TABLE III: Monthly incidence of canine rabies detected at NVRI laboratory Vom, Nigeria in 2006

Month	No of Samples Tested	Incidence (%)
Jan	20	10 (50)
Feb	14	9 (64.3)
March	13	7 (53.8)
April	14	5 (35.7)
May	23	11 (47.8)
June	18	13 (72.2)
July	10	7 (70)
August	22	13 (59)
September	13	6 (46.2)
October	13	10 (76.9)
November	15	12 (80)
December	14	12 (85.7)
Total	189	115 (60.8)

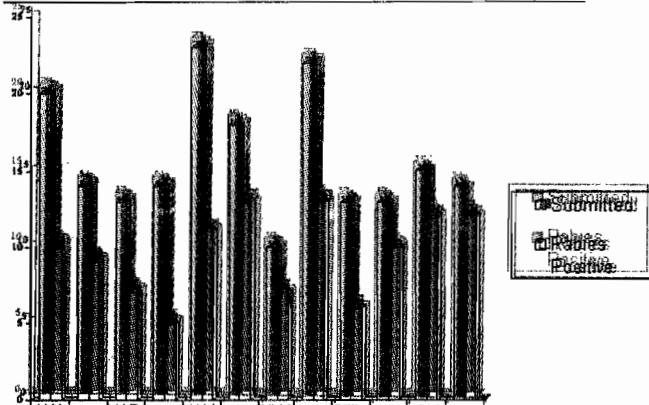


Fig 1: Monthly laboratory diagnosis of canine rabies by DFA in Vom in 2006

DISCUSSION

Based on the samples of 189 dog brain submitted for diagnosis at NVRI in 2006, 115 (60.8%) were positive for canine rabies antigen by the DFA. This finding is higher than previous reports in the same study area (Ogbe *et al.*, 2002; Garba *et al.*, 2005).

Female dogs had higher incidence (63.6%) than the males (55.4%). This was however, not statistically significant. In this study, dogs below 6 months of age had lower incidence (55.8%) than those above 6 months old (61.9%). This may be as a result of the protective maternal antibodies and the proper handling and/or confinement of puppies that limits their interaction with other animals and humans. Three puppies, 8-week-old tested positive corroborating the findings of Adeyanju and Addo (1977) and Onunkwo *et al.* (1980) that rabies can occur in dogs three months old or younger. Thus, we therefore support the need for reappraisal of the recommended 3 months vaccination age (Sikes, 1968; Bisseru, 1972) for dogs.

In our investigation, we observed a higher incidence of canine rabies in the months of June, July and October to December, corresponding with dog breeding seasons in the State, although more samples were received in the months of January, May and August, indicating higher human exposure. Similar cluster of January/March/November from the same study area (Garba *et al.*, 2005), April/September in Zaria, Kaduna State (Ezeokoli and Umoh, 1987) and September/October in Accra, Ghana (Belcher *et al.*, 1976) have been observed. During the breeding periods, the male dogs usually roam around for their female counterparts, intense dog fights are common and may result in biting of other animals or people around. A nursing bitch is also likely to be protective over her pups and may bite at the slightest provocation. Therefore, there is need to exercise caution when handling dogs or when within the vicinity of excited dogs to prevent bites.

The high incidence of canine rabies (60.8%) reported in this study supports the claim by Adeyemi *et al.* (2005) that current vaccination coverage may be inadequate to prevent the regular

occurrence of canine rabies in Nigeria, coupled with other problems like vaccine breaks mostly attributed to incorrect vaccine application, failure of some dogs to respond to even repeated doses of vaccine and previous exposure to street virus (Kappus, 1976), and could possibly be due to antigenic variations among several field viruses as suggested by Wiktor (1983). However, Okoh (1990) had reported that the LEP (Flury) vaccine used in Nigeria appears to meet local requirements in view of antigenic variations encountered. We also observed that over 98% of the samples submitted to the laboratory for rabies diagnosis in the year under review were from dogs. This reflects the role of domestic dog in the epidemiology of rabies in Plateau State.

The current findings are of epidemiological and public health significance and call for surveillance, public enlightenment, the enforcement leash laws to minimize the incidence of rabies. In Nigeria, canine rabies is a main threat to public health; there are significant numbers of strays, many owned dogs are not vaccinated due to the prohibitive cost and dog bites are very common. Therefore, we advocate the need for pre-exposure immunization of people at high risk like the Veterinarians, Hospital staff attending to rabies patients, Veterinary clinical students, and Laboratory personnel handling infected material, dog wardens, and dog catchers' to protect them in the event of exposure to this deadly disease. The ultimate control measure for rabies is to eliminate the virus from its reservoir hosts through adequate vaccination programmes and the control of stray dogs (Cliquet and Picard-Meyer, 2004). This is rather difficult because funds are not usually committed to this particular disease in Nigeria.

CONCLUSION

There is a need for better collaboration between the public health sector, the veterinary sector and the local government departments in the various states in order to make control programmes very effective.

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