Science World Journal Vol. 20(No 2) 2025 https://dx.doi.org/10.4314/swj.v20i2.22
www.scienceworldjournal.org
ISSN: 1597-6343 (Online), ISSN: 2756-391X (Print)
Published by Faculty of Science, Kaduna State University

Full Length Research Article

RABIES SAMPLE SUBMISSION TO DIAGNOSTIC CENTRES: A POTENTIAL BIAS IN DISEASE SURVEILLANCE DATA

*[1]Zhakom Ponfa Nden, [2]Egbe Nkechi Euchara, [3]Dibal Deborah, [2]Abdulsalam Mohammed, [1]Ishaya Sini Tekki

[1]National Veterinary Research Institute, PMB 01, Vom, Plateau State
[2]Department of Biotechnology, Nigerian Defence Academy. Kaduna State
[3]Department of Biological Science, Nigerian Defence Academy, Kaduna State

*Corresponding Author Email Address: ponfaz@gmail.com

ABSTRACT
Understanding of rabies epidemiology is required for efficient planning and implementation of prevention, control and elimination. This is achieved through accurate and reliable data collected through Surveillance. This study aimed to determine the distribution and pattern of rabies samples submission across the country at the National Veterinary Research Institute (NVRI) Vom, Plateau State and Nigeria, within five months (May –September 2020). A total of seventy-eight suspected rabid animal samples Canine (Dog, n=72; 92.3%), Chiroptera (Bat, n=2; 2.6%), Ovine (Sheep, n=1; 1.3%) and Caprine (Goat, n=3; 3.8%) were submitted for diagnosis at the Rabies Diagnosis and Research Laboratory NVRI Vom Plateau State, Nigeria. A total of 78 samples, 72% were brought from Plateau State, 10 % from Bauchi State, 5.3% from Kano State, 4.0% from Akwa Ibom State, 2.6% from Kaduna, while Bayelsa, Ebonyi and Gombe states had 1.3% respectively. The geographical distribution of the samples revealed a significant proportion (72%) was collected from Plateau State. This reflects a potential bias due to proximity of the Diagnostic Laboratory, which is located within the state. This uneven distribution may limit the generalization of the findings across Nigeria, highlighting the need for more comprehensive surveillance to reflect representative sample collection across different regions of the Country. In this study, the greater part (92.3%) of rabid suspect animal species was domestic dogs. These findings underscore the critical role of dogs in the epidemiology of rabies and the need for targeted interventions amongst canine populations. It is recommended that comprehensive national rabies surveillance be conducted, especially among domestic dogs in the country.

Keywords: Rabies, Rabies Virus, Sample Submission, Surveillance Data

INTRODUCTION

Rabies is one of the most dreaded and fatal zoonotic diseases of all mammals including man (Beigh *et al.,* 2014). It is caused by a neurotropic virus belonging to the genus *Lyssavirus* in the family *Rhabdoviridae* (Kuhn *et al*., 2021; Walker *et al*., 2022). Rabies is found all over the world except for regions with a strict quarantine system, rigorous eradication or natural barriers like mountains and rivers, plus regular vaccinations (Wyatt, 2007; Adedeji *et al.,* 2010). The highest number of cases is reported in Africa and Asia, particularly the Indian sub-continent, with mortality from the disease exceeding 30,000 and 23,000, respectively (Knobel *et al.,* 2015). In Nigeria, no fewer than 10,000 people are exposed to rabies annually (Okoh *et al.,* 2018). According to Otolorin *et al*. (2014), 78 human deaths due to rabies occurred in Nigeria from 1998 to 2014. In another study, Tekki *et al*. (2015) reported a prevalence of 66.1% laboratory-confirmed dog rabies involving human exposure in Nigeria from 2005 to 2014. In a review by Mshelbwala *et al*. (2021), a prevalence of rabies virus antigen detection between 3% and 28% has been reported, with more studies in the north, a region where the NVRI, Vom, saddled with the responsibility of rabies diagnosis, is located. Mshelbwala *et al*. (2021) reported south-south region having a prevalence of 6%, south-east between 5% and 9%, south-west between 2% and 11%, north-west between 4% and 50% %, north central, between 5% and 17% and the north-east between 2% and 44%.

In Plateau State, several incidences of rabies have been documented with high prevalence between 36 to 60.17% (Kujul *et al*., 2012; Karshima *et al*., 2013). However, with the paucity of data in Plateau State and Nigeria at large, a true representation of the scenario of rabies may not be ascertained (Duwong *et al*., (2024). Dogs are the principal hosts and transmitters of rabies and are adjudged as the cause of human rabies deaths in 99% of all cases globally (Hampson *et al.*, 2015). Canine rabies is endemic in Nigeria and grossly underestimated. Exposure to rabies through bite from rabid dogs accounts for almost 100% of the confirmed cases (Garba *et al*., 2010 and Konzing *et al*., 2019). In Africa, the data situation regarding rabies remains deplorable across the continent. Obstacles to more epidemiological insight are found on all levels of surveillance, and the resulting lack of information hinders effective intervention and drives neglect (Nel, 2013 and Lechenne *et al*., 2017). Dog-related data are even less available, because dog owners rarely utilize the Veterinary Services, or even most Veterinary Service Centres lack appropriate facilities to handle animal rabies diagnosis (Lechenne *et al*., 2017). Rabies is of a significant public health concern globally, and accurate surveillance data is essential for its control and elimination (Ashley et al., 2013). While Laboratory-based data from sample submission of suspected rabid animals has proven to be an effective rabies detection, prevention and control strategy, rabies has been detected in apparently healthy dogs in Nigeria (Konzing *et al.,* 2015 and Kia *et al.,* 2018). However, biases in sample submission to Veterinary Diagnostic Centers can impact the reliability of surveillance data

The study was aimed to determine the distribution and pattern of rabies samples submission across Nigeria at NVRI Vom, Plateau State. This report will also help in understanding the implications of sample submission on disease surveillance data.

MATERIALS AND METHODS

The study was carried out at Rabies Laboratory, National Veterinary Research Institute Vom, Plateau State, Nigeria. A total of 78 animal brain tissue samples were received over a period of 5 months (May to September 2020) from 9 states of Nigeria (Figure

1). Factors such as sample source, geographic distribution, animal species and diagnostic test (direct Fluorescent Antibody Test) results were examined.

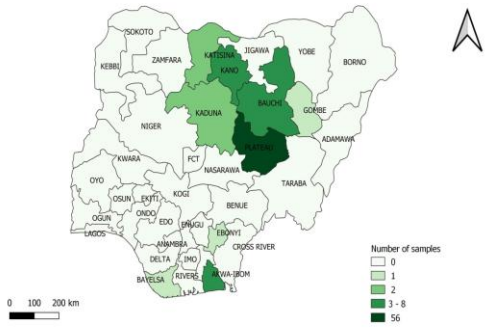

Figure 1: Map of Nigeria showing sources of Suspected Rabies Animals by State

Fluorescent Antibody Test (FAT)

Fluorescent Antibody Test was carried out according to the procedure described by the OIE (2004) and WHO (1996). Three portions (about 1g) of the cerebellum, hippocampus and the medulla were cut and homogenized. An impression smear was made on the pre-labelled glass slides by turning the slide over the sample homogenate. The smear was air dried at room temperature and fixed in chilled acetone for 30 minutes at -20°C. The slide was air dried at room temperature and transferred to a humid chamber and stained with 150 µL of a working dilution (1:1000) of Fluorescent Isothiocyanate conjugate (FITC Anti-Rabies Monoclonal Globulin, Sweden). The slide in the humid chamber was incubated at 37°C to stain for 30 minutes and thereafter washed briefly in phosphate buffered saline (PBS) pH8.5, and blotted. A drop of 50% buffered glycerol was added to the smear, and a cover slip applied. Both negative and positive controls samples (obtained from brain tissue of mice inoculated with known positive and negative rabies samples) were run alongside the test sample. All slides, including controls were examined under fluorescence microscope (Zeiss AXIO, Germany) using x20 and x40 magnification. The presence of shining apple green oval or ellipsoid fluorescing particles indicates the presence of rabies antigen (positive result), while the lack of fluorescence shows the absence of rabies virus.

RESULTS

Geographic distribution of Sample submission;

The distribution of samples by state (Table 1) reveals that 72% of the samples collected came from Plateau State, 10 % from Bauchi State, 5.3% Kano State, 4.0% Akwa Ibom State, 2.6% each from Kaduna, and Katsina, while Bayelsa, Ebonyi and Gombe states had 1.3% respectively

Analysis of Suspected Rabid Animals Sample submission by Species:

Table 2 shows the analysis of the suspected rabid animals. The 78 samples used for this study indicates that, Canine (Dog, n=72; 92.3%), Chiroptera (Bat, n=2; 2.6%), Ovine (Sheep, n=1; 1.3%) and Caprine (Goat, n=3; 3.8%) were collected during the study period.

Detection of rabies virus (RABV) antigen in fresh animal brain tissues

The result of rabies antigen detection in fresh animal brain tissues (Table 3) shows that, out of the 78 fresh brain specimens examined by FAT, 51 (65.4%) were positive for rabies, while 27 (34.6%) were negative for rabies.

Table 1: Sources of Suspected Rabid Animals by State

STATE	NO. OF SAMPLES	PERCENTAGE (%)
Akwa Ibom	3	3.8
Bauchi State	8	10.2
Bayelsa State	1	1.3
Ebonyi state	1	1.3
Gombe State	1	1.3
Kaduna state	2	2.6
Kano State	4	5.1
Katsina State	2	2.6
Plateau State	56	71.8
TOTAL	**78**	**100**

Table 2: Suspected Rabies Animals used for the Analysis

SAMPLE	TOTAL	PERCENTAGE (%)
Canine (Dog)	72	92.3
Chiroptera (Bat)	2	2.6
Ovine (Sheep)	1	1.3
Caprine (Goat)	3	3.8
TOTAL	**78**	**100**

Table 3: Detection of RABV antigen in fresh animal brain tissues

S/N	Specie	Positive	Negative
1	Canine (Dog)	48	24
2	Chiroptera (Bat)	0	2
3	Ovine (Sheep)	0	1
4	Caprine (Goat)	3	0
	TOTAL	**51**	**27**

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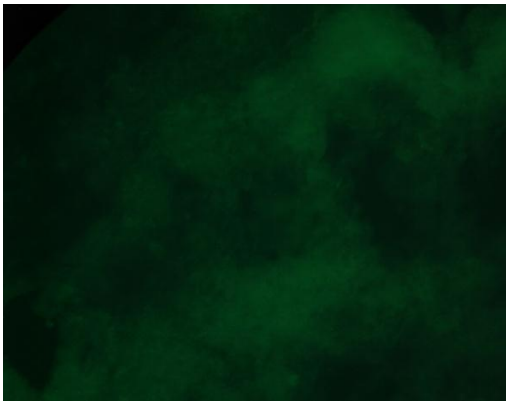

Figure 2A: Negative Slide showing the absence of apple green fluorescent particles

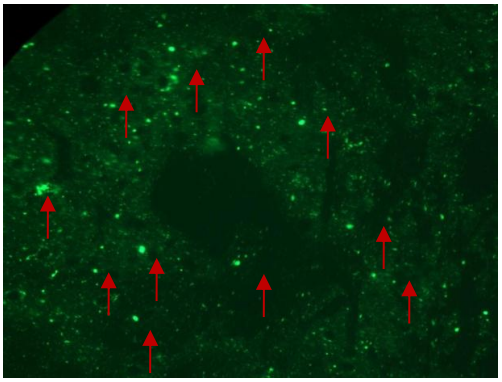

Figure 2B: Positive Slide showing the presence of apple green fluorescent particles (Arrows)

DISCUSSION

The role of Rabies surveillance data in the prevention, control and elimination of rabies cannot be over emphasized. The disease accounts for about 75,000 human deaths each year in canine rabies-endemic regions of the world (Wyatt, 2007). In Nigeria, more than 10,000 people are exposed to rabies annually (Okoh *et al.,* 2018). The geographical distribution of samples revealed a significant proportion (72%) was collected from Plateau State, with the northern region recording more submissions compared to the southern part. This reflects the ease of sample submission due to proximity to the diagnostic centre and by extension, impacts on the availability of data for decision making. From different articles published on rabies virus in Nigeria, researchers reported the prevalence of rabies virus in different states with the Northern parts of the Country recording higher percentage of rabies virus. Mshelbwala *et al*. (2021), reported higher prevalence rate of rabies across the northern region of Nigeria; north-west between 4 and 50%, north central, between 5 and 17% and the north-east between 2 and 44%, compared to the lower prevalence rate recorded in the southern region; the south-south 6%, south-east between 5 and 9%, south-west between 2 and 11%.

Reporting the high prevalent rate of rabies virus in the northern part of Nigeria may not be the true representation of rabies prevalent distribution in Nigeria as a result of the following factors: bias in sample submission for rabies diagnosis to NVRI Vom as Plateau State submitted highest number of samples due its proximity to the reference laboratory (NVRI) Vom, thereby making it easier and faster for samples to be submitted. Collection, preservation and transportation of sample may be hectic for some states that are far from Plateau state and this explained the reason behind high prevalent rate in some northern states in Nigeria. No sample was received from any state in the Western region of Nigeria during the period covered for this study. Only one each from two states of the South- south. The fact that rabies samples did not come from these regions does not mean that they are free from endemicity of rabies. It may simply show that there is a big gap in rabies surveillance across the states in Nigeria. Reporting and accepting the prevalent rate of rabies in the Northern region of Nigeria compared to other regions may not be taken as a fact unless there is a more comprehensive survey of rabies virus across the states and regions, without any bias in sample collection. The study also shows that the uneven distribution may limit the generalisation of findings across Nigeria, highlighting the need for more comprehensive surveillance and sample collection across different regions of the Country.

The result of rabies antigen detection in the 78 animal brain specimens examined by FAT showed that 51 (65.4%) were positive for rabies, while 27 (34.6%) were negative for rabies. This implies that rabies is still endemic in Nigeria. Duwong *et al*. (2024) also reported the endemicity of rabies in Nigeria with a prevalence of 60.17%.

In this study, the majority (92.3%) of rabid suspected animal species were dogs. Hampson *et al.* (2015) reported that dogs are responsible for the vast majority of human rabies cases globally, while Tekki *et al.* (2016) noted a significant prevalence of laboratory-confirmed dog rabies cases in Nigeria. These findings underscore the critical role of dogs in the epidemiology of rabies and the need for targeted interventions amongst canine populations.

Conclusion

This study emphasises the need for a holistic approach to having a more robust rabies surveillance data and not relying only on sample submission. Addressing this bias can improve the accuracy and reliability of surveillance data, thereby informing more effective public health strategies for rabies prevention, control and elimination. It is recommended that comprehensive national rabies surveillance be conducted in the country so as to cover all locations, and a dire need to awaken all rabies stakeholders in all states of the federation to the submission of samples for diagnosis.

Acknowledgement

The authors wish to thank the management of National Veterinary Research Institute (NVRI), Vom and the Department of Biological Sciences, Nigeria Defence Academy for their immense support. We also wish to appreciate Dr Leviticus Konzing, Dr Hambolu Emmanuel, Mr. Livinus Clement, Mr Joseph Alex and Mr Haruna Rabo for the technical support they provided during the course of this work.

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