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A 30-year Retrospective Laboratory Surveillance of Wildlife Rabies in Nigeria

Ishaya Sini Tekki[1], Julius Olaniyi Aiyedun[2], Emmanuel Sunday Hambolu[1], Ladi Chabiri[1], Joseph Alex Davou[1], Oladapo Oyedeji Oludairo[2*], Henry Olanrewaju Jegede[3], Chika Nwosuh[1], Olatunde Babatunde Akanbi[4], Oluwafemi Babatunde Daodu[5] and Reuben Ocholi[1]

(1)National Veterinary Research Institute, Vom, Nigeria.
(2)Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ilorin.
(3)Veterinary Teaching Hospital, University of Ilorin.
(4)Department of Veterinary Pathology, University of Ilorin.
(5)Department of Veterinary Microbiology, University of Ilorin.
*Corresponding author: oludairo@hotmail.com *Received: 24/8/2021 Accepted: 29/6/2022*

ABSTRACT

Rabies is a neglected, fatal zoonotic disease that poses great diagnostic challenge in developing countries. It remains a serious public health hazard in many developing countries where dog bite is the main mode of transmission to man. Accurate laboratory diagnosis of rabies is important in the confirmation of the disease in man and animals. This study was designed to evaluate the existence of rabies in wild animals in Nigeria between 1990 and 2019. Annual records of rabies which were confirmed through laboratory diagnosis at the National Reference Laboratory for rabies in Nigeria, The National Veterinary Research Institute (NVRI), Vom, Plateau State, were retrieved, analysed and presented using descriptive statistics. A total of Eighty- four (84) wildlife specimens tested for rabies during the period under review, 17 (20.34%) were positive for rabies while 67 (79.76%) were negative. Squirrels (8%) and monkeys (5%) had the highest occurrence of the disease. Thus presenting baseline information on the occurrence of rabies in wildlife in Nigeria.

Keywords: Surveillance, Nigeria, Rabies, Wildlife.

INTRODUCTION

Rabies is a highly fatal zoonosis caused by eleven viral species belonging to the genus *Lyssavirus* and family *Rhabdoviridae* (Rupprecht et al., 2002). It causes encephalomyelitis in infected man and animal including most wildlife (Rupprecht et al., 2002). The occurrence of the disease in Nigeria is as early as the history of human existence in the country, virtually all ethnic groups have vernacular names for canine rabies (Adedeji et al., 2010). Urban rabies is well known in Nigeria but little is known about sylvatic/wildlife rabies (Rupprecht et al, 2002). A rabid wildlife may approach towns and attack domestic animals and humans (Miller et al, 2013). Rabies fatality could be up to 100%. An approximately 50,000 to 100,000 humans and countless animal both wild and domestic die annually due to rabies (WHO, 2020). This virus is transmitted mainly through bite or scratch of an infected animal (infectious saliva) (WHO, 2020). The natural host of ten of these viral species are bats while dog are the major transmitter to human (Fisher, 2018). Epidemiologically, rabies has two cycles which are the urban and wildlife/sylvatic. Urban rabies have been almost eliminated in developed nations leaving only wildlife cycle of rabies (OIE, 2009; WHO, 2018; Khalafalla and Ali,

2021; Suluku et al., 2021). The wildlife cycle usually reverts to urban cycle due to frequent contact between rabid wildlife and stray domestic/pet animals (Ogunkoya et al., 2003). There is no effective treatment for rabies once the symptoms develop (CDC, 2019). Lyssa viruses is widely distributed across the globe except Antarctica (WHO, 2020). Rabies is endemic and widespread in Nigeria with insufficient public health resources, poor surveillance (Aiyedun and Olugasa, 2012). Mammals are susceptible to rabies but only few species serves as reservoirs. Rabies transmission risk and its link with wildlife have been documented in Nigeria (Aiyedun et al., 2017).

It is important to genetically identify rabies species found in wildlife since antigenic and genetic studies have revealed that rabies virus strains circulating in specific host species undergo genetic adaptation and evolve into distinct biotypes that differ in antigenicity and pathogenicity (Nadin-Davis and Fehlner-Gardiner, 2019**)**. Most studies in Nigeria involved antigen detection by FAT (the gold standard technique for rabies diagnosis) and antibody detection by ELISA while genetic identification tools like PCR were employed by some researchers (Aghomo et al., 1990). FAT is regarded as the main diagnostic tool while PCR is recommended as confirmatory diagnostic tool (Fooks et al., 2012).

Rabies has been isolated in various wildlife in Africa like mustelids, viverrids and canids in Southern Africa (Nel et al., 2005). African wild dog in South Africa (Hofmeyr et al., 2000). African wild dog in Kenya (Kat et al., 1995), African civet (Enurah et al., 1988) in addition to mongooses and jackals in Nigeria (Atuman et al., 2014a).

This study investigated Laboratory diagnostic surveillance of the occurrence of wildlife rabies in the last 30 years (1990-2019) by the National Veterinary Research Institute, been the National rabies reference laboratory in Nigeria. This will give the number of confirmed cases of rabies in wildlife in the country.

MATERIALS AND METHODS

Rabies Diagnostic Methods Employed at the National Veterinary Research Institute Wild Animal brain samples submitted for confirmatory diagnosis of rabies at the National Reference Laboratory for rabies (National Veterinary Research Institute (NVRI), (Vom), Nigeria, between 1990 and 2019 were tested by histopathological technique (Lépine, 1966), the direct microscopic examination (DME) using the Seller's stain technique (Tierkel and Atanasiu, 1996) and the direct Fluorescent Antibody Test (DFAT) (Dean et al., 1996). Samples obtained from animals that had bitten humans and tested negative by either of the above mentioned techniques were subjected to the Mouse Inoculation Test (MIT) (Meslin et al., 1996) for confirmation.

Data Collection

All the data on animal brain samples that tested positive for rabies using all diagnostic tests employed from 1990 to 2019 were retrieved from the archival records of rabies laboratory in NVRI, Vom, Nigeria for surveillance purposes to arrive at the number of animal/wildlife samples submitted and tested for rabies including the number of samples from wildlife that tested positive using the diagnostic test employed at the centre.

Data Analyses

Using Microsoft excel version 2013, data collected were compiled, processed and analysed using descriptive statistics, and results presented in tables and graphs.

RESULTS

Histologic Examination of Brain Tissues

Evidences of rabies encephalomyelitis and meningitis in rabies suspect brain tissues were: Mononuclear infiltration and Perivascular cuffing with lymphocytes or polymorphonuclear cells. Others were

Babes nodules consisting of glial cells and presence of Negri bodies (inclusion bodies) seen in the cytoplasm of neuronal cells especially purkinje cells (Figures 1a and 1b).

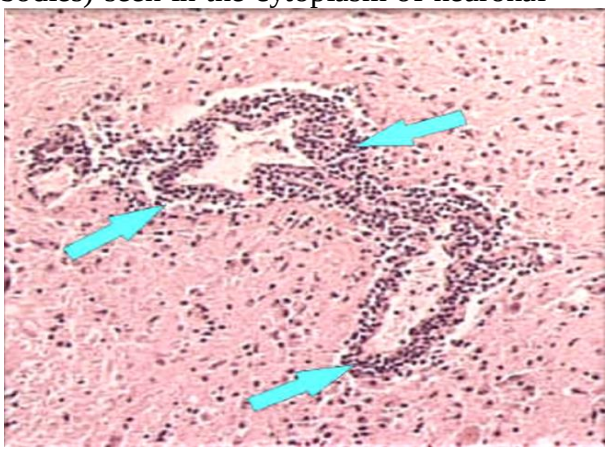

Figure (1a): Perivascular Cuffing around a blood vessel of H&E stained histological section of brain tissue (arrows) (CDC, 2011).

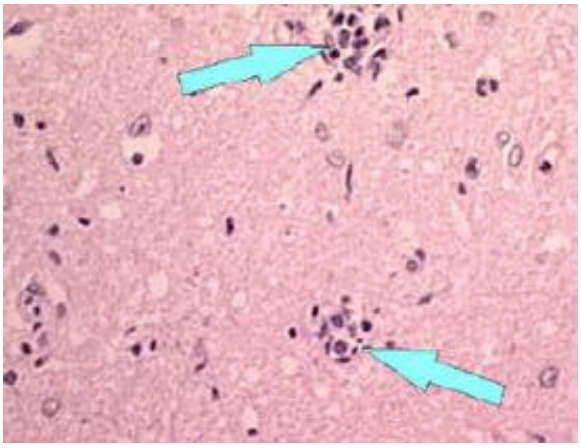

Figure (1b): Babe's nodules of glial cells of H&E stained histological section of brain tissue (arrows) (CDC, 2011).

Direct Microscopic Examination by Sellers Staining

Negri bodies corresponding to aggregates of viral proteins, which are specific for rabies virus infection were detected in positive test and positive control specimens (Figures 2a and 2b).

Figure (2a): Negri body in Sellers stained infected brain tissue (arrow) (CDC, 2011).

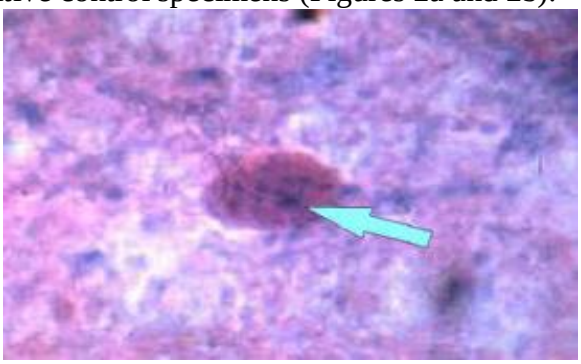

Figure (2b): Enlargement of a Negri body in Sellers stained brain tissue (arrow) (CDC, 2011).

Mouse inoculation test

Inoculated mice remained apparently healthy till varied signs of rabies manifested which are thriftiness, lethargy, paralysis (Figure 3a) and death (Figure 3b).

Figure (3a): Inoculated mice display signs of rabies infection (staggering) and self-isolation (arrows) post inoculation (PI) with infected brain sample

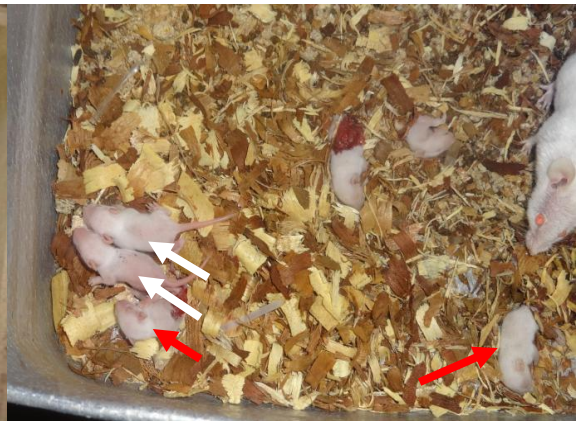

Figure (3b): Inoculated mice display paralysis (white arrows) and death (red arrows) PI with rabies infected brain sample

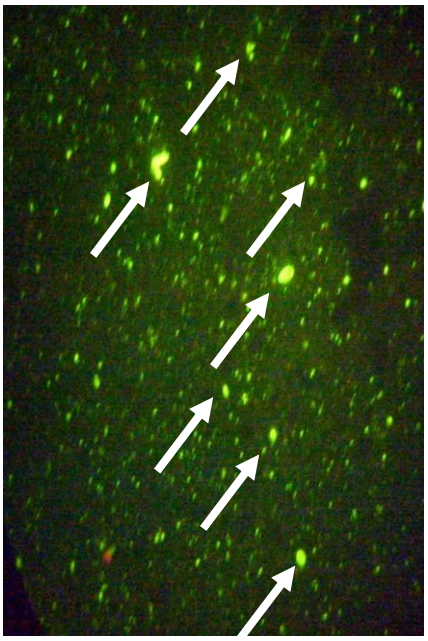

Figure (4a): Rabies FICT stained positive control specimen showing immunofluorescence

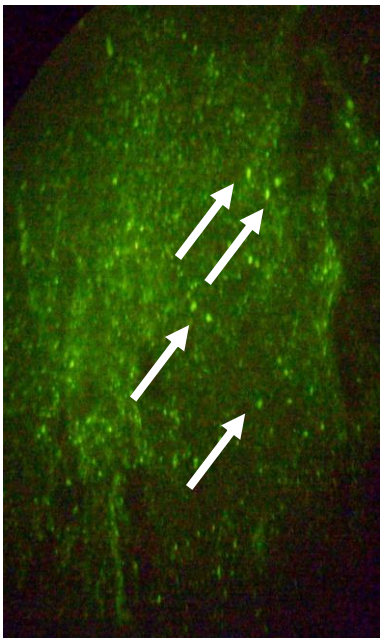

Figure (4b): Rabies FICT stained test specimen showing immunofluorescence (arrows)

Figure (4c): Rabies FICT stained negative control specimen showing no immunofluorescence

Results of DFAT. Antigen-antibody complexes formed between rabies virus antigens in fixed and stained tissue smears of the positive control and positive test sample/antibodies to the virus contained in the FICT conjugate appeared as apple-green fluorescence under fluorescent microscope. Positive Control virus (Figure 4a) had higher concentrations of stained complexes fluorescing in the smear compared to the test samples (Figure 4b). In both positive samples, the complexes appeared in various shapes, sizes, concentrations and degrees of clarity on the smears, but were fewer in concentration in the test samples smears. Since there were no virus particle to form complexes with the. Negative control, there was no fluorescence (Figure 4c).

The distribution of wildlife samples tested for confirmatory diagnosis of rabies using histopathology, Sellers staining, DFAT and/or MIT techniques were presented in Table 1. Out of the animal samples tested within the period under review, eighty-four samples (0.047%) were from wildlife. A minimum of one (1) wildlife sample was submitted and tested in 13 (1992, 1999, 2001, 2009-2016, 2018 and 2019) of the 30 years studied. In the remaining 17 years of the study period (1990, 1991, 1993-1998, 2000, 2002-2008 and 2017), no wildlife sample was submitted for rabies diagnosis. The highest annual wildlife sample submission/testing was 59 in 2013 followed by four (4) in 2016 (Table 1) while the rest ranged from zero to three (3) (Table 1). Records showed a total of 16 wildlife that tested positive for rabies over the period under review (Table 1, Figure 5). These consisted of two lions (one each in 2010 and 2012), four monkeys (one in 1992, one 2009 and two in 2019), one hyena (in 2011), one Chimpanzee (in 1999), one fox (in 2018) and 7 squirrels (all in 2013) (Table 1). The highest number of wildlife tested was squirrel (35), followed by rat (24) while the least were hyena (1), chimpanzee (1) and Porcupine (1) (Table 1, Figure 5). The animal species with the highest number of positive outcome was squirrel (7) followed by monkey (4) and lion (2) (Table 1, Figure 5). Other wild animals that tested positive were hyena (1), Chimpanzee (1), fox (1), totalling 16, while the rest had negative outcome (Table 1 and Figure 5). Out of the total wildlife samples tested during this period, 19.076% (16/84) were positive for rabies. Table 1 depicts the results of wildlife rabies during the thirteen years in which wildlife samples were received and tested for rabies. Figure 5 shows the categories of wildlife species tested for rabies and the numbers positive. Ten various species of wildlife was submitted and tested over the period of the three decades. These included lion, monkey, hyena, jackal, chimpanzee, fox, bat, squirrel, rat and porcupine (Table 1, Figure 5). Of these numbers, six (60.00%), including lion, monkey, hyena, chimpanzee, fox and squirrel tested positive for rabies while the remaining four (40.00%) tested negative (Figure 5). Out of the samples of ten wildlife species that were submitted for diagnosis, all (100%) specimens from hyena and chimpanzee were positive, followed by lion (66.66%). Squirrel, though had the overall highest number of samples submitted and the overall highest numbers that tested positive has the lowest percentage positivity (20%) (Table 1).

Table 1: Results of laboratory diagnosis of rabies in wildlife by Direct Microscopic Examination, Direct Fluorescent Antibody Test and the Mouse Inoculation Test at the National Reference laboratory in Nigeria between 1990 and 2019:

Year	Lion		Monkey		Hyena		Jackal		Chimpanzee		Fox		Bat		Squirrel		Rat		Porcupine		Total animal samples tested	Total wildlife tested	Number of wildlife that tested positive
	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve			
1990	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**142**	**-**	**-**
1991	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**141**	**-**	**-**
1992	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**116**	**1**	**1**
1993	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**100**	**-**	**-**
1994	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**163**	**-**	**-**
1995	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**181**	**-**	**-**
1996	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**141**	**-**	**-**
1997	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**104**	**-**	**-**
1998	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**69**	**-**	**-**
1999	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	**76**	**1**	**1**
2000	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**79**	**-**	**-**
2001	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**174**	**1**	**-**
2002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**186**	**-**	**-**
2003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**217**	**-**	**-**
2004	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**214**	**-**	**-**
2005	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**182**	**-**	**-**
2006	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**195**	**-**	**-**
2007	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**131**	**-**	**-**
2008	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**242**	**-**	**-**
2009	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**427**	**1**	**1**

2010	1	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	1	-	-	**218**	**3**	**1**
2011	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**217**	**1**	**1**
2012	1	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**225**	**2**	**1**
2013	-	-	-	-	-	-	-	1	-	-	-	-	-	-	7	28	-	23	-	-	**187**	**59**	**7**
2014	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**220**	**3**	**-**
2015	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**197**	**3**	**-**
2016	-	-	-	-	-	-	-	1	-	-	-	-	-	3	-	-	-	-	-	-	**175**	**4**	**-**
2017	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	**182**	**-**	
2018	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	1	**173**	**2**	**1**
2019	-	-	2	-	-	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	**207**	**3**	**2**
TOTAL	**2**	**1**	**4**	**7**	**1**	**-**	**-**	**2**	**1**	**-**	**1**	**1**	**-**	**4**	**7**	**28**	**-**	**24**	**-**	**1**	**178, 108**	**84**	**14**

Key: +ve; Positive, -ve; Negative, -; Nil

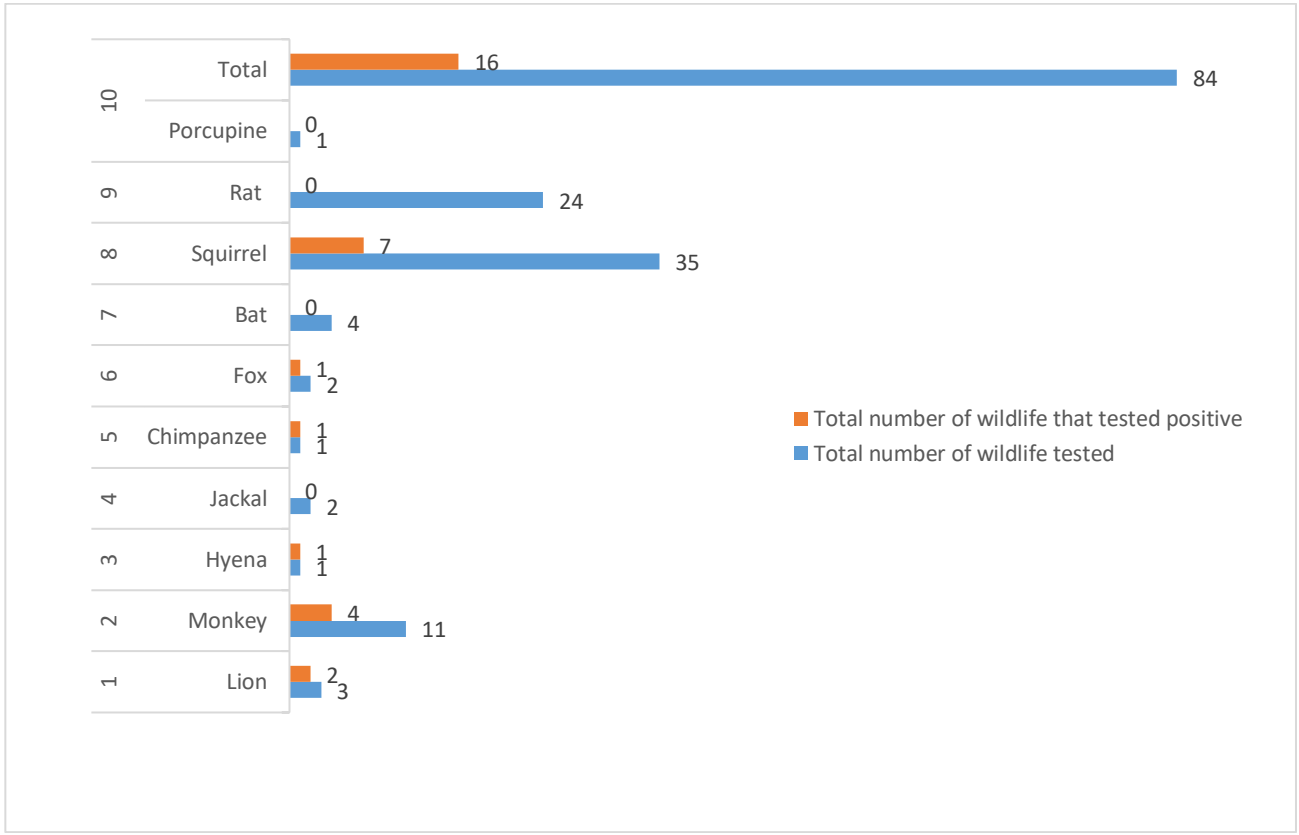

Figure (5): Distribution of wildlife tested, numbers tested and those positive for rabies in the study area.

DISCUSSION

Records of animal rabies laboratory diagnosis in Nigeria in the last three decades indicated that prior to the time modern methods such as direct fluorescent antibody test and RT-PCR became available in the country, rabies diagnosis was done using histopathological examination of fixed brain tissues. This was followed by Sellers staining technique on smear of fresh brain tissues, coupled with the clinical case history. Histopathology indications that a specimen was positive for rabies included the following: Mononuclear infiltration, Perivascular cuffing of lymphocytes or polymorphonuclear cells and Lymphocytic foci (CDC, 2011). However, because this technique is nonspecific in comparison to the newer methods. The newer methods are considered to be more specific and diagnostic for rabies (CDC, 2011).

The finding of the number of wildlife positive for rabies in this study does not show that wildlife may play major roles in rabies epidemiology in Nigeria. This may be due to the fact that limited number of wildlife were presented for confirmatory rabies diagnosis within the period under review and were those considered for the research. The result of this study is also in agreement with thereport that domestic animals, especially dogs may be responsible for the transmission of rabies to humans and other animals in 90% of reported cases in Nigeria (Oboegbulem, 1994). Whereas WHO (2020) classified domestic dog as major reservoir of rabies virus in Africa and Asia, Oboegbulem (1994) categorized wildlife such as rodents and hyenas as minor sources of rabies infection. Available records shows that only two cases of wildlife rabies (Kasali, 1977; Enurah et al., 1988) were reported in the last four decades in Nigeria. Incidentally, both cases occurred in Civet cats. To complement this assertion, Garba et al. (2008) reported that over 96% of animals rabies in Nigeria occurs in domestic dog between 1999 and 2008. The low incidence of wildlife rabies in the present study is however, in contrast with the assertion of MacDonald, 1993 and Blanton et al., 2009 that the incidence of urban and sylvatic rabies is on the increase in Africa. It is also in agreement with the report of Rupprecht et al. (2002), which stated that more frequent cases of rabies were diagnosed in wildlife than in domestic animals in the United States of America (USA) within the same period of time. In Nigeria on the other hand, the last effort made to control rabies through national mass vaccination of dogs against rabies was in 1982 during which over 70% of the country's dog population was vaccinated in line with WHO recommendation. (Mshelbwala et al., 2021).

The majority of the other attempts at mass vaccinations were at the State level and 99% reported vaccination rates of 15–66%, well below the WHO recommendation, except the programme in Niger State that reported 70% vaccination of dog population (Mshelbwala et al., 2021).

Data gaps and inadequate funding were some of the identified obstacles to implementing a national control program in Nigeria (UAR, 2020).

Thereafter, only pockets of campaigns has been embarked upon by interest groups such as the Nigerian Veterinary Medical Association, the Nigerian Veterinary Medical Students during the world rabies day, and some local and state governments in response to outbreak.

Despite the fact that rabies is endemic in Nigeria and wildlife rabies has been reported as far back as 1950s, 1970s and 1980s (Boulger and Porterfield, 1958; Kasali, 1977; Enurah et al., 1988 and Macdonald, 1993), it was observed that wildlife samples were submitted for test only in 13 years of the three decades studied in retrospect. However, submission of majority of the samples within the last

one decade (2009-2019) coincided with the involvement or participation of the veterinarians and veterinary students in public awareness creation on rabies since 2009 in Nigeria. Not-withstanding, lack of routine surveillance and under reporting may be responsible for the recorded non submission of samples from wildlife in 17 of the 30 years. Although Wu et al. (2009) stated that squirrels, chipmunks, rats, mice, hamsters, guinea pigs, gerbils, rabbits and hares are not usually infected with rabies, seven of the 35 squirrels tested in this study were positive for the viral antigen. This might have resulted from spread of urban rabies, through apparently healthy hunting dogs to wildlife as asserted by Ogunkoya et al. (2003), who added thatsuch spread typically occurs through infected hunting dogs. Dzikwi et al. (2010) also stated that the main host that maintains and transmits rabies to other animals including wildlife in Nigeria is the dog. In a reverse order, the domestic dog may be infected when exposed to infected wildlife (Ogunkoya et al., 2003).

Sixteen (16) of the 84 (19.05%) wildlife species tested turned out to be positive for rabies. The result is in agreement with Blanclanton et al., 2009, who submitted that frequently, one to three species of wildlife is responsible for rabies incidence in any ecosystem and that in most parts of the world, wildlife rabies is reported every month. Although rat was the second highest number of animal species tested, none was positive.

Since the first report of Rabies in 1912 in Nigeria (Bougler and Hardly, 1960), the disease has been endemic in the country. This is evidenced in the report of Kehinde et al. (2009) that submitted that rabies is well established in Nigeria and control measures are still very inadequate. It is important to genetically identify Lyssa virus species found in wildlife since antigenic and genetic studies have revealed that rabies virus strains circulating in specific host species undergo genetic adaptation and evolve into distinct biotypes that differ in antigenicity and pathogenicity (Tuffereau et al., 1989; Wunner et al., 1988).

Fluorescent antibody test (FAT) (which is regarded as the gold standard for rabies diagnosis in Nigeria) and serology are the diagnostic methods employed in most rabies studies in Nigeria (Aghomo et al, 1990). The advent of genetic identification tools like PCR, necessitate their use as important diagnostic tool (Fooks et al., 2012). A further benefit of RT-PCR has been for the detection and classification of novel members of the Lyssa virus genus, when the viral genomes are sequenced and analysed (Fooks et al., 2012).

CONCLUSION

This study highlighted the challenges of laboratory investigations and surveillance of rabies in wild animal species with the aim of effectively identifying and diagnosing the disease in Nigeria. It presented baseline information on the occurrence of rabies in wildlife in Nigeria for proper design of rabies control programs. The result of the study will ensure strategic measures are adopted for effective control and prevention of rabies in wildlife. Periodic Surveillance of wildlife rabies is essential for effective control and prevention of rabies in Nigeria. There is need for proper funding and provision of adequate diagnostic tools for rabies in Nigeria while the level of political commitment to rabies control should also be improved. There should be heightened public campaigns and vaccination programme which are expected to lead to significant reduction in incidence of urban rabies in Nigeria.

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