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ANTIGENIC ANALYSIS OF SOME NIGERIAN STREET RABIES VIRUS USING MONOCLONAL ANTIBODIES

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SUMMARY

The authors studied 12 street rabies virus isolates from 3 states of Nigeria using both the anti-nucleocapsid and anti-glycoprotein monoclonal antibodies and cross-protection tests. It was observed that all the viruses were rabies having divergent antigenic presentation. Also noticed was an antigenic shift when the viruses were passed through different hosts. Two antirabies vaccines used did not protect animals when challenged through the orthodox route and only traces of protection by one of the vaccines were observed when the route of challenge was intramuscular. In the virus isolation and adaptation the BHK 21 cell lining was found to be only 37% efficient while the Murine neuroblastoma cells were about 75% efficient.

KEY WORDS: Antigenic, Nigeria street rabis virus, monoclonal antibodies.

INTRODUCTION

The accepted view about rabies is that all of the rabies viruses in the lyssa virus serogroup of the rhabdovirus family were considered to be antigenically closely related regardless of their species and geographic origin. Previously, the diagnosis of rabies infection in human and animal was carried out with antisera obtained from animals immunized against rabies (WHO, 1975). This antiserum does not distinguish individual viruses belonging to the rabies group or diagnose infections caused by the rabies

related viruses, like Lagos, Mokola and Duvenhage, all of which belong to the lyssavirus genus (WHO, 1977) some of which were isolated in Nigeria and caused fatal infection in man (Fumilusi et al., 1972).

Wiktor and Koprowski (1978) found that the use of rabies specific hybridoma monoclonal antibodies permits the detection of different specificities expressed by viruses of the rabies group. After the analysis of several strains of fixed and street rabies virus, Wiktor et al (1980) showed evidence of antigenic differences. These antigenic differences were also noticed by Sureau *et al* (1983). These antigenic differences have been suspected to be responsible for occasional vaccine failure even when the vaccine can be assumed to be potent and the treatment prompt (Hattwick et al., 1972; CDC, 1976).

With the nascent knowledge of the antigenic discrepancies and the resultant effect on animal and human lives, it has been suggested (WHO, 1982) that a wide application of monoclonal antibodies to the antigenic analysis of rabies virus isolates may lead to a better understanding of the epidemiology of rabies.

MATERIALS AND METHODS

Sources of Infected Materials

Infected dog brains were collected from the Department of Veterinary Pathology of the Veterinary Faculty of Ahmadu Bello University, Zaria, Kaduna State. The National Veterinary Research Institute Laboratory at Vom, Plateau State and from the Veterinary Clinic at Akure in Ondo State all in Nigeria. The brain specimens were from rabies suspected dogs that were sent from different centres for confirmatory diagnosis. All cases involved human exposures. The brain specimens were kept frozen at 20°C (Table I). Few days to being transported to the United States of America, some few grammes of the brain were transferred into 50% glycerol in sterile phosphate buffered saline (PBS) pH 7.4 and others were left frozen. All specimens were transported in a cooler to the U.S.A.

Mice Inoculation:

The pieces of brain kept in the 50% glycerol buffer were washed seven times in PBS (pH 7.4). Then a 10% suspension by weight was made in PBS containing
5% Bovine serum and 1% antibiotics (penicillin-streptomycin).The suspension was centrifugated for 5-10 minutes at 15,000 rpm to remove the gross particles.

White weaning mice were used and 0.03 ml of the suspension was inoculated intra-cerebrally into each mouse as described (WHO, 1975). The brains of mice which died 5 to 28 days after inoculation were harvested, FA examined and positive brains were stored frozen at 70°C.

Inoculation of other animals

Fifteen Baby skunks (*Menphitis menphitis*) were dissented and allowed to recover for a week. The brain suspension of the original virus was injected (0.02-.03mls) periorbitally. One specimen (Owo) was also inoculated periorbitally into a dog. All these animals were closely monitored for signs and symptoms demonstrated. Harvests of FA positive brain were stored in
-70°C.

Propagation of Virus in cell Cultures

Brain tissues were homogenized in Eagle's minimum essential medium supplemented with 10% fetal calf serum (MEM-10) to form a 10% brain suspension, which was clarified by centrifugation (1600 rpm for 15 min).

Virus-containing supernatant (0.25 ml) was mixed with 1 ml of freshly trypsinized BHK-21 cells (1 x 10^6 cell/ml) in disposable plastic Falcon T25 tissue culture flasks and left standing thirty minutes and incubated at 37°C for 10 minutes.

Five ml of MEM-10 was then mixed with the cells and 0.3 ml of this cell suspension was placed in each of two wells of eight-chamber labtek slides or 10 lamdar in the wells of Terasaki histoplates.

The T25 flasks, the labtek slides or the Terasaki histoplates were incubated as parallel cultures at 37°C in a CO_2 incubator. The medium was changed after 24 hrs to remove brain debris and the cultures were incubated for additional 2-3 days. The slides were fixed in cold acetone (80% acetone when using the terasaki histoplates) and cell monolayers were stained with fluorescein-labelled monoclonal anti-body (MCA) 502-2 which reacts with rabies and rabies related viruses (Lagos bat, Mokola and Duvenhage) and with NCA 422-5 which reacts with only rabies-related viruses as described by Wiktor et al (1983).

The medium from the T25 flasks that show growing virus (FAT) was collected and stored at 70°C. Infected cells were trypsinized and new cultures initiated using a 1:4 split ratio as described above. An aliquot of cells was placed again in the labtek slides or Terasaki

histoplates for monitoring the extent of infection using FAT. This procedure was repeated until when more than 50% of the cells in the culture were infected, the culture was tested with panel of anti-nucleocapsid monoclonal antibody anti-NC-MCA and when all the cells in the culture were infected the culture was used in the virus neutralization assay after being titrated with the panel of MCA specific for rabies and rabies related GP antigen as described by (Wiktor et al., 1983, 1984).
The same procedure was followed when the neuroblastoma cell culture was used, but the Dulbeccos modified Eagles Medium with high glucose was used in place of MEM.

Characterization of virus Nucleocapsid (NC) antigen

Infected cells from cultures showing at least 50% positive cells were adjusted to a concentration of 2 x 10^6 cells/mol as described by Wiktor et al (1980) and distributed into 36 wells of Terasaki plate (Falcon 304) at 10 il/well. Plates were incubated at 37°C for 24 hrs washed once with PBS, and fixed for 30 minutes in 80% acetone in distilled water and dried. Ten il each of 36 monoclonal antibodies of the panel was placed in individual wells and incubated for 30 min. at 37°C. The plates were washed twice in PBS and dried. Five il of fluorescein-labelled anti mouse gamma globulin was added to each well and incubated for 10-20 minutes at 37°C. Plates were then drained. All plates were examined under ultraviolet light with microscope equipment for epi-illumination.

Characterization of virus glycoprotein (GP) antigen by virus neutralization:

Titration of virus

Five fold dilutions of the Isolates were prepared in Falkon Micro-Test II plates in a total volume of 100 il of MEM-10 BHK-21 cells (1 x 10^6 cells/ml), were mixed with the virus dilutions and 10 il of this mixture was transferred into wells of the Terasaki plates. Cultures were incubated for 4 days at 37°C in 5% CO_2, fixed in 80% acetone and 20% DW and stained with fluorescein-labelled anti NC antibody of rabbit origin. The longest dilution of virus showing FA-positive cells was considered as the end point of titration.

Neutralization with Antiglycoprotein Monoclonal Antibodies

The virus neutralizing activity of 44 monoclonal antibodies was measured against different virus isolates by determining the virus neutralization index as described by Wiktor et al (1983). This test was performed only if the infectivity titer of the virus to be tested was 10^3-10^4 infective units per 10 il. The test was carried out as described by Wiktor et al (1984). A reduction in virus-titer of more than 100 infective units in the presence of Monoclonal Antibodies was considered as positive virus neutralization.

Characterization of virus Nucleocapsid antigen by mouse brain impression smear.

A panel of monoclonal antibodies directed against the nucleocapsid antigens of rabies virus using an indirect immunofluorescence test on the brain smears of infected mice was carried out as previously described by Wiktor et al (1980); Sureau et al (1983).

Vaccination and challenge test

Thirty 3 weeks old white mice, all male obtained from Veterinary Diagnostic Laboratory of the College of Veterinary Medicine, Iowa State University Ames Iowa's colony were used. Two vaccines-human diploid cell culture inactivated vaccine, Wyeth Laboratories Inc. Marietta, PA 17547 control No. 372A1-10601 and duck embryo-dried killed virus, Lilly & Co. Indianspolis IN 46206 were used.

Forty - two mice were used for each of the vaccines. Each mouse was given 0.03 ml of vaccine intramuscularly. A booster was given 14 days after the first dose. The infectivity titers of the challenge viruses were determined by intra-cerebral inoculation of unvaccinated control mice.

The mice were challenged intra-cerebrally (i/c) and intramuscularly (i/m) with 0.03ml serial 10

fold dilutions of virus isolates (titre of 10^{-4}) 12,8c and 5c of viruses from Nigeria 30 days after vaccination. Mice were examined daily for 4 weeks for signs of rabies.

RESULTS

Eight mice were inoculated with each virus. Death was observed within 6-18 days. Isolates 8 and 12 elicited extremely short incubation periods. The Mice started to die by day 6 and they all died within 2 days. Isolates 9, 10 and 11 were slow in killing mice; deaths did not occur until 12 days post-inoculation. All deaths from each isolate occurred within an interval of 5 days following the first death (Table II).

All the juvenile striped skunks developed fatal infections. Isolate 8, and 12 killed the skunks within 14-18 days, isolate 6 killed the skunk in 21 days with all 3 skunks displaying a violent type of rabies. The skunks that received isolates 8 and 12 were extremely violent. The fourth isolate killed the skunk in 30 days and an experimentally infected dog in about 45 days, but neither the skunk nor the dog showed any furiousness. Of particular interest was the dog that was eating and drinking normally one day and the following morning was found dead. Rabies virus was detected by FA test on brain smears of the dead animals.

Five of the virus isolates were cultured in BHK-21 cells cultures (Table III). One of these was directly from the original brain tissue preserved in 50% glycerol, 3 were from first passage homogenized mouse brains and one was from homogenized juvenile skunk brains and one was cultured from both mouse and skunk passage harvest. Three isolates, 9, 10 and 11 respectively did not replicate in the BHK-21 cell cultures.

Seven of the isolates were cultured in murine neutrablastoma (NB) cell cultures, all from the original dog brain tissues kept in 50% glycerol, and each from at least 1 additional animal passage harvested. Virus isolates, 9, 10 and 11 which failed to grow in BHK-21 cells cultures grew very slowly in neutroblastoma cell cultures.

As shown in Table III, brain impression smears from mice infected with all isolates gave positive staining reactions with monoclonal antibody 502-2 and all but one, isolate 10 gave a negative reaction with monoclonal antibody 422-5, indicating that all isolate but 10 were true rabies viruses and not rabies-related viruses. Virus isolate 10 gave a positive staining reaction to all the monoclonal antibodies and a week positive to 422-5. Table V also shows the immunoreactivity of 36 monoclonal antibodies with 7 isolates of dog viruses passaged through mice, as also reported by Ezeokoli et al (1984); Flamand, et al (1980) and Sureau et al (1983). The original brain of dog 6 and 8 strain passage through a baby skunk, and isolate 12 passaged through a baby skunk and finally isolated by passaging it in BHK21 cells cultures. All the isolates reacted negatively to monoclonal antibody 422-5 and positively to monoclonal antibody 502-2, which further confirmed that all these isolates were rabies viruses. Unfortunately, isolate 10 was not successfully adapted to BHK-21 cells for monoclonal antibody studies. The result also confirmed that all tested viruses were street viruses and not CVS because they all stained positively to monoclones.

The 44 anti-glycoprotein monoclonal antibodies listed in Table V were evaluated for neutralizing activity against the 7 isolates. In group IA, the only isolates of the viruses which were neutralized by monoclonal antibody 509-6 were the two baby skunk passage harvests of isolates 8 and 12. In group IIB, no reactivity was noticed. In group IIC, mouse passage and baby skunk passage harvests to isolates 9 were both neutralized but neither mouse passage nor baby skunk passage harvests of isolate 12 were neutralized by monoclonal antibody 1107-1. Mouse passage harvest of isolate 9 was not neutralized by monoclonal antibody 613-2 but the baby skunk passage harvest was. In group IIIA, only the baby skunk passage harvest of isolate 8 reacted with monoclonal antibody 248-8, whereas 194-2. In group V, monoclonal antibodies showed differential affinities for the 7 isolates tested. Monoclonal antibody 523-1 reacted with mouse passage harvests of isolate

279-82 and 277-82 and with baby skunk passage harvests of isolates 8 and 12, but did not react with the rest. Monoclonal antibody 193-2 only reacted with baby skunk passage harvest of isolate 8. Monoclonal antibody 1108-1 did not react with mouse passage harvest of isolate 5, but did react with the rest.

Mice immunized were not protected when challenged intra-cerebrally by both vaccines. The human diploid tissue culture vaccine showed some evidence of protection when the challenge was made intramuscularly fig. 4.

TABLE I: Sources of 12 Rabies virus passage in cell cultures

Lab. No.	Local No.	Material	Source	Comments
N 1	Dog 1	Original dog brain	Kaduna State	The two boys showed signs
N 2	Dog 2	Original dog brain	Kaduna	of encephalitis and posterior paralysis before death.
N 3	Dog 3 -332-81	Original dog brain	Oyo State	Human bite involved, died in quarantine
N 4	Dog 4 -444-80	Original dog brain	Ondo State	Human bite occurred, killed and brought to Vet Clinic
N 5	279-82	Original dog & mouse	Kaduna State	Human bite occurred, killed and brought to Vet. Clinic
N 6	259-83	Original dog and first mouse brain passage	Kaduna State	Human bite occurred
N 7	277-82	Original dog and first mouse brain passage	Kaduna State	Human bite occurred
N 8	Adeyanju-83	Original dog and first mouse brain passage	Kaduna State	Human bite occurred. Many human beings exposed.
N 9	Owo	Original dog and first mouse brain passage	Ondo State	Several human bites occurred
N 10	188	Second mouse Brain passage	Vom, Plateau	Human bite occurred
N 11	185	Second mouse Brain passage	Vom, Plateau	Human bite occurred
N 12	Vilmot-83	Original dog and first mouse brain passage	Zaria, Kaduna	Puppy of 2 months. Bit the owner, owners houseboy and the bitch. Showed very furious type rabies

TABLE II: Immnoreactivity of hybridoma antibodies with nucleocapsid antigens against Nigerian strains of rabies viruses.

	Nigerian strains of rabies virus						
Monoclonal antibody group and number	**279-82 mouse passage**	**259-82**	**277-82 mouse passage**	**Adeyanju mouse passage**	**Adeyanju baby skunk passage**	**Wilmoth mouse passage**	**Wilmoth baby skunk passage**
1 502-2	+	+		+	+	+	+
2 103-7	+	+	+	+	+	+	+
3 206-1	+	+	+	+	+	+	+
4 209-1	+	+	+	+	+	+	+
5 229-0	+	+	+	+	+	+	+
6 590-2	+	+	+	+	+	+	+
7 515-3	+	+	+	+	+	+	+
8 104-4	+	+	+	+	+	+	+
9 111-2	+	+	+	+	+	+	+
10 111-14	+	+	+	+	+	+	+
11 239-10	+	+	+	+	+	+	+
12 289-1	+	+	+	+	+	+	+
13 377-7	+	+	+	+	+	+	+
14 102-27	..+	+.+	&	+.+	+	+..	+
15 222-9	+	+	+	+	+	+	+
16 237-3	+	+	+	+	+	+	+
17 120-2	+	+	±	+	+	+	+
18 364-11	+	+	+	+	+	+	+
19 714-3	+	+	+	+	+	+	+
20 422-5	&	&	&	&	&	&	&
21 816-1	+	+	+	+	+	+	+
22 817-5	+	+	+	+	+	+	+
23 818-5	+	+	+	+	+	+	+
24 822-7	+	+	+	+	+	+	+
25 701-9	+	+	+	+	+	+	+
26 703-8	+	+	+	+	+	+	+
27 715-3	+	+	+	+	+	+	+
28 721-2	+	+	+	+	+	+	+
29 802-1	+	+	+	+	+	+	+
30 802-2	+	+	+	+	+	+	+
31 803-6	+++	..+	..+	&	+	+++	+
32 804-9	+	+	+	+	+	+	+
33 805-3	+	+	+	+	+	+	+
34 806-1	+	+	+	+	+	+	+
35 807-5	+	+	+	+	+	+	+
36 808-2	+	+	+	+	+	+	+

Key

- = Negative staining
± = Variable staining
+ = Positive staining

TABLE III: Laboratory propagation studies on 12 dog brain specimens brought from Nigeria. Methods of preservation, passage through laboratory animal hosts and isolation in BHK-21 and neuroblastoma cell cultures. BHK-21 cells used here were the fibroblastic type supplied by Dr. Wiktor, Wistar Institute and the neuroblastoma cells used were supplied by Dr. G. Baer, CDC, Atlanta, Georgia.

Lab.	Labora-tory Identify	Frozen brain (A)	Brain in glycerol (B)	Mouse Passage (C)	Juvenile skunk passage (D)	Iowa dog passag e (E)	BHK-21 Isolatio n	Passages before 100% Infectivit y	Neuroblastom a cell isolation	Passages to reach 100% infectivit y
N 1	Dog 1	+		X	X	X	X	*	*	O
N 2	Dog 2	+		X	X	X	X	*	*	O
N 3	Dog 3/322-81	+		X	X	X	X	*	*	O
N 4	Dog 4/144-80	+		X	X	X	X	*	*	O
N 5	279-82 (82020)		+	+	X	X	5C	3	5B 5C	-2
N 6	259-82		+	+	+	X	6D	5	6B	3
N 7	27782		+	+	X	X	7C	3	7B 7C	2
M 8	Adeyanju		+	+	+	X	8C	3	8B	2
N 9	Owo	+	+	+	+	+	X	0	9B 9C 9D 9E	4-6 4 3 4
N10	188 Vom		+	+	X	X	X	0	10B 10C	4 2
N11	185 Vom		+	+	X	X	X	0	*	*
N12	Wilmot		+	+	+	X	12C	3	12B 12C 12D	3 1 2

Key
* - Not attempted
x - Not employed

TABLE IV: Summary of the Reactivity of the tested strains of viruses against a Panel of 40 Anti-glycoprotein monoclonal antibodies

Number of monoclonal antibodies that neutralized strains	Percent	Number of monoclonal antibodies that did not neutralize strains	Percent
22	55	18	45
25	62.5	15	37.5
27	67.5	13	32.5
23	57.5	17	42.5
19	47.5	21	52.5
28	70	12	30.0
27	67.5	13	32.5

Nigerian strains of rabies virus

Monoclonal Antibody roup and number	277-82 mouse passage	Wilmoth mouse passage	Adeyanju mouse passage	Owo	279-82 mouse passage	188 Vom	259-82 mouse passage
#1							
102-27	-	-	+	+	-	+	-
103-7	+	+	+	+	+	+	+
104-4	+	+	+	+	+	+	+
111-2	+	+	+	+	+	+	+
111-14	+	+	+	+	+	+	+
120.2	+	+	+	+	+	+	+
206	+	+	+	+	+	+	+
209-3	+	-	+	+	+	+	+
#2							
222-9	+	+	+	+	+	+	+
222-10	+	+	+	+	+	+	+
229-1	+	-	+	+	+	+	+
237-3	+	+	+	+	+	+	+
239-10	+	+	+	+	+	+	+
364-11	-	-	-	-	-	+	+
377-7	+	+	+	+	+	+	+
389-2	-	-	-	+	+	+	+
							-
390-1	+	+	+	+	+	+	+
422-5	-	-	-	-	-	+	-
502-2	+	+	+	+	+	+	+
515-3	+	+	+	+	+	+	+
590-3	+	+	+	+	+	+	+
anti mouse	-	-	-	-	-	-	-
direct FA	+	+	+	+	+	+	+

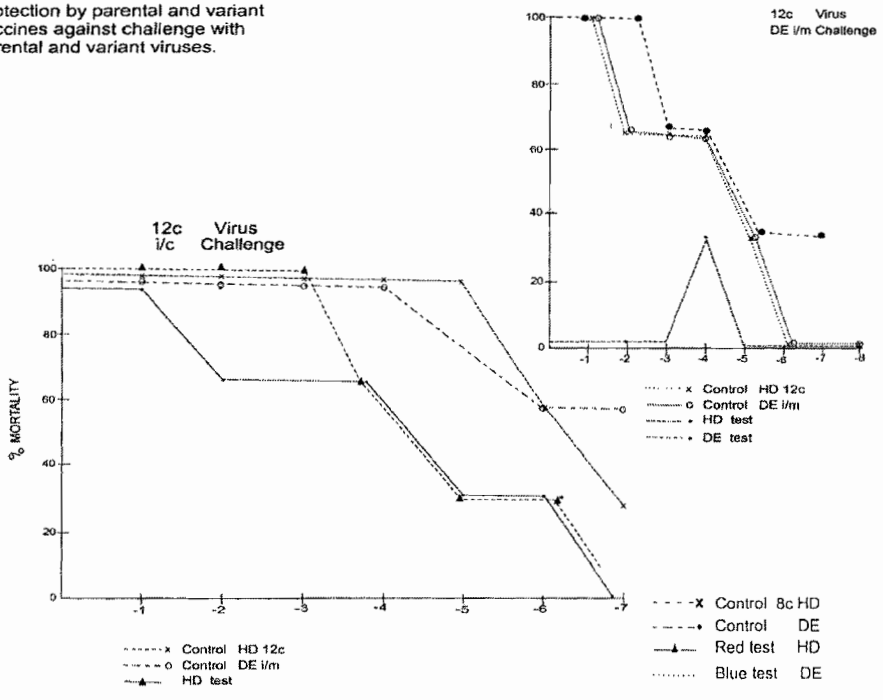
Key+ = variable or weak staining; ± = positive staining; - = negative staining

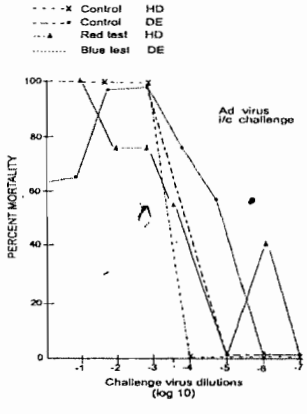
TABLE V: Immunoreactivity of hybridoma antibodies with glycopr otein antigens against Nigerian strains of rabies virus.

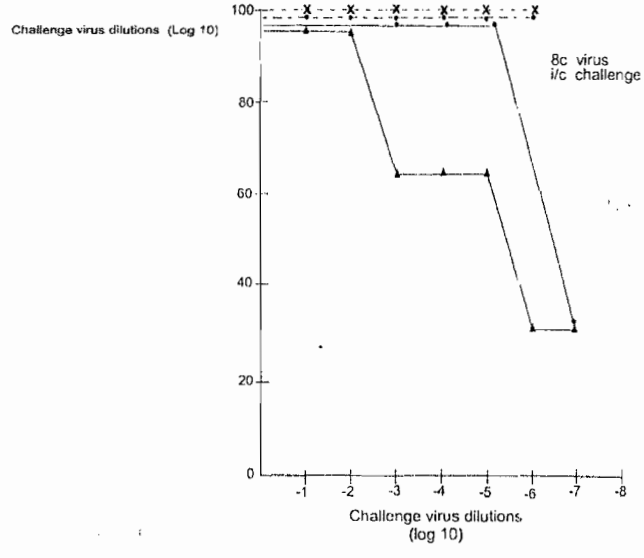
			Nigerian strains of rabies virus						
Monoclonal antibody group and number			**279-82 mouse passage**	**259-82**	**277-82 mouse passage**	**Adeyanju mouse passage**	**Adeyanju baby skunk passage**	**Wilmoth mouse passage**	**Wilmoth baby skunk passage**
9A	1	509-6	O	O	O	o	X	o	x
11A	2	231-22	X	X	X	x	X	x	x
	3	220-8	O	O	O	o	X	o	x
B	4	162-3	X	X	X	x	X	x	x
	5	1116-1	X	X	X	x	X	x	x
	6	1119-24	X	X	X	x	X	x	x
	7	1121-2	x	X	X	x	X	x	x
	8	101-1	x	X	X	x	X	x	x
	9	1111-1	x	X	X	x	X	x	x
C	10	1112-1	x	X	X	x	X	x	x
	11	1105-3	x	X	X	x	X	x	x
	12	1107-1	x	X	X	x	X	x	x
	13	240-3	o	O	O	o	O	o	o
	14	613-2	x	X	X	x	X	x	x
	15	719-3	o	O	O	o	O	o	o
	16	1117-8	o	X	X	o	O	o	o
	17	226-11	o	O	O	o	O	o	o
IIIA	18	194-2	x	X	X	x	X	x	x
	19	248-8	o	O	O	o	X	o	o
	20	1113-1	o	O	O	o	O	o	o
B	21	507-1	o	O	X	o	X	o	o
	22	120-6	o	X	X	x	X	o	x
	23	120-6	o	X	X	x	X	o	x
	24	1103-4	x	X	X	x	X	x	x
	26	1122-3	x	X	X	x	X	x	x
	27	127-5	o	O	O	o	O	o	o
IV	28	110-3	x	X	X	x	X	x	x
V	29	1118-6	x	X	X	x	X	x	x
	30	1120-10	x	X	X	x	X	x	x
	31	1109-3	x	X	X	x	X	x	x
	32	1114-2	x	X	X	x	X	x	x
	34	176-2	x	X	X	x	X	x	X
	35	193-2	x	X	X	x	X	x	X
	36	904-4	o	O	O	o	O	o	O
	38	523-1	x	O	X	o	X	o	X
	39	1108-1	o	X	X	x	X	x	X
	41	504-1	o	O	O	o	O	o	O
	42	508-9	o	O	O	o	O	o	O
	43	419-2	o	O	O	o	O	o	O
	44	422-2	o	O	O	o	O	o	O

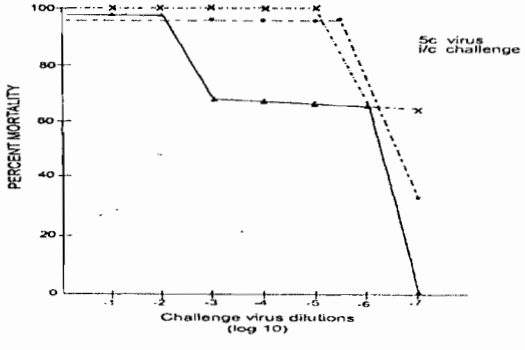
o = no neutralization, i.e., positive staining: x = neutralization took place, i.e., negative staining.

Protection by parental and variant vaccines against challenge with parental and variant viruses.









DISCUSSION

The isolation and propagation of street rabies viruses using cell cultures have been reported (Smith et al., 1978; Umoh et al., (1983). The finding in this study also confirms that the murine neuroblastoma (NB) cells were superior to the BHK-21 cells in isolation of street rabies viruses. The results showed that 17 isolates were successfully obtained through NB cells, including 6 of 8 normal dog brain samples kept in 50% glycerol (100%) from the 7 mouse brain passaged isolates, 3 (100%) from the 3 skunk brain passaged isolates and of Iowa infected dog brain sample. With BHK-21 recoveries were made from 1 (12.5%) of 8 original dog brain samples in glycerol, 5 (62.5%) of 8 mouse brain passaged isolates, 2 (66.6%) of 3 skunk brain passaged isolates. In general, the BHK-21 cell line was 38.8% as efficient in isolation of rabies street viruses as was the murine NB cell line. The recovery efficiency of the BHK-21 cell line was only high in mouse brain and skunk brain passaged isolates (Table 3) indicating that for the BHK-21 to be successfully employed in street rabies virus isolation, the virus titre of the original material must be high. The murine NB, on the other hand, was very efficient in street rabies virus isolations, yielding about 75% efficiency in isolation from original materials. With this cell line, high yields of virus were achieved after only 2-4 serial passages of infected dog brains, and from mouse and skunk brain 1-2 passages. Apart from this, rabies antigens could be detected by FA as early as 24 hours after infection of the cell cultures. This potential of NB cell cultures would make it useful for rapid rabies diagnosis and could perhaps make the use of this particular cell line superior to the mouse inoculation test in rabies diagnosis.

As shown in Table II and III, all the isolates completely tested were rabies viruses; strain 9, 10 and 11 was not conclusively tested because of their failure to grow in BHK-21 cell cultures. The definite pattern of reactivity observed indicated that all strains were antigenically uniform. This confirms the observations of Wiktor et al., (1983) that rabies strains of viruses originating from the same geographical area showed identical or nearly identical patterns of reactivity.

The principal diagnostic procedures used in rabies laboratories in Nigeria are the immunofluorescent test, examination for Negri bodies, and to a lesser extent, mouse inoculation.

Some previous studies on the epidemiology of rabies in Kaduna State (Ezeokoli et al., 1984), showed that rabies diagnostic tests which produced very dull fluorescence and those that did not yield fluorescence were discarded without further testing whether or not they were positive by mouse inoculation (Ogunkoya, 1986). Consideration was not given to possible infection of other lyssa-viruses. This diagnostic procedure may be responsible for selection of only antigenically related viruses in the country that influenced this result.

Table IV shows the immunoreactivity of monoclonal antibodies against glycoproteins and Table V shows the degree of reactivity of each of the virus isolate tested. Except for strain 12, all strains reacted with more than 50% of the panel of 40 monoclones which means that all but this one tested virus strain had common antigenic sites recognizable by the antiglycoprotein monoclonal antibodies.

A WHO report (WHO, 1982) on the application of monoclonal antibodies in the characterization and differentiation of laboratory and field strains of rabies virus stated that analysis of rabies variants and the grouping of variants that although initially neutralized by one or more of panel of monoclonal antibodies had lost this reactively be identified on the basis of three functionally independent antigen sites. In the study it was evident that an average of about 15 of the anti-glycoprotein monoclonal antibodies would not neutralize the tested strains of virus; i.e. all these strains comprised at least three functionally independent antigen sites.

In an attempt to characterize the antigenic determinants of glycoproteins that correlated with pathogenicity of rabies virus, it was found that all fixed strains of rabies virus that were

pathogenic for adult mice were neutralized by monoclonal antibodies 248-8 (Bemhard et al., 1983). In this study, all the viruses tested were neutralized by monoclonal antibody N194-2 but only one, the harvest of isolate 8 passaged through baby skunk, was neutralized by monoclonal antibody 248-8. All of the viruses tested in this study were pathogenic to baby and adult mice. This agrees with the suggestion that other determinants may be involved in the pathogenicity of street rabies virus.

It was considered important that passages of the rabies virus strains through mice and baby skunks, respectively, yielded harvest reacting differently in the monoclonal antibody studies (Table IV and V). The strain 12 passaged through mice was neutralized by 19 (47.5%) of the anti-glycoprotein monoclonal antibodies in the panel, whereas the same isolate 12 passaged through baby skunk was neutralized by 27 (67.5%) of the panel.

The isolate 8 passaged through mice was neutralized by 23 (57.5%) of the panel of monoclonal antibodies, and the same strain 8 passaged through baby skunk was neutralized by 28 (70%) of the panel. In addition, this virus harvest was the only virus which was neutralized by monoclonal antibody 248-8.

Vaccine break in dogs have been reported in Nigeria (Anon, 1972; Okoh, 1982; Ogunkoya, 1986). In 1983, an American who was bitten by his own dog that had previously been vaccinated in Nigeria died of rabies (CDC, 1976). Failure of vaccines to protect exposed individual human beings has been attributed either to low potency of the vaccine or to delays in beginning treatment. Occasionally however, failures have occurred even when vaccines could be assumed to have been potent and the treatment proper (Fumilusi et al.,1972; CDC, 1976). As such antigenic differences between strains of rabies virus used for the vaccine preparation and strains of viruses present in various localities have been suggested as possible cause of vaccine breaks and failure (Wiktor et al., 1980, Sureau et al., 1983). Failures of commonly used vaccines to protect Nigerian virus further strengthen Wiktor's suggestion, that vaccines made for use in different parts of the world should be prepared from prevailing viruses (Wiktor et al., 1983).

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