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EPIDEMIOLOGY OF RABIES: BIOLOGICAL AND SEROLOGICAL ASPECTS OF RABIES IN VAMPIRE BATS *DESMODUS ROTUNDUS* (E. GEOFFROY) CAPTURED IN VALE DO PARAÍBA, SOUTHEASTERN REGION OF BRAZIL

M.C.A.M. Souza[1], F. Bernardi, F.H. Ito

[1]Branch Laboratory at Pindamonhangaba, Biological Institute, Rua Soldado Roberto Marcondes, 324, CEP 12400-000, Pindamonhangaba, SP, Brazil.

ABSTRACT

Desmodus rotundus captured in Vale do Paraíba, SP were studied to detect the presence or not of rabies neutralizing antibodies and submitted to virus isolation from brain and pooled organs. Detection of viral antigen in brain and heart was performed through immunofluorescent (IF) technique. Among 138 specimens captured from June 1992 to April 1994, 7 (5.07%) brains and 4 (2.90%) pooled organs were positive for rabies through the mouse inoculation technique (MIT), and one brain positive through IF. With the mouse serum neutralization (SN) test, 9/133 (6.70%) of the sera presented measurable antibodies titers. Two bat isolates presented the mean viral titers of $10^{4.71}$ and $10^{4.57}$ $MICLD_{50}$/0.03 mL, respectively for the isolates 17/4C/94 and 17/1C/94, lower than that of CVS. SN test performed with a potent positive antirabies serum indicated the presence of serological difference; the use of a positive standard antirabies serum having 812 IU/mL presented the 50% neutralizing titers of $10^{2.50 \pm 0.39}$, $10^{4.44 \pm 0.41}$, $10^{2.28 \pm 0.55}$ and $10^{3.21 \pm 0.07}$ for 17/4C/94, CVS, M03/87 and 17/1C/94 isolates. Detection of rabies antibodies titers in bat sera should be used as an additional tool for epidemiological surveillance, identifying the areas of high risk of rabies.

KEY WORDS: Epidemiology, *Desmodus rotundus*, rabies, neutralizing antibody, viral titers.

RESUMO

EPIDEMIOLOGIA DA RAIVA: ASPECTOS BIOLÓGICOS E SOROLÓGICOS DO VÍRUS DA RAIVA OBTIDOS DE MORCEGOS HEMATÓFAGOS *DESMODUS ROTUNDUS* (E.GEOFFROY) CAPTURADOS NO VALE DO PARAÍBA, REGIÃO SUDESTE DO BRASIL. *Desmodus rotundus*, capturados no Vale do Paraíba, SP, foram estudados com o objetivo de isolar o vírus a partir do cérebro e "pool" de visceras em camundongos. A determinação do antígeno viral foi feita por imunofluorescência direta (IF), em impressões do cérebro e do coração. Do total de 138 morcegos capturados, entre Junho/92 a Abril/94, sete (5,07%) cérebros e quatro (2,90%) "pool" de órgãos resultaram positivos para a prova de isolamento e apenas um cérebro foi positivo à prova de IF. Com a técnica de soroneutralização (SN) em camundongos, nove (6,70%) apresentaram anticorpos. Duas amostras isoladas de morcegos apresentaram título viral médio em camundongos de $10^{4.71}$ e $10^{4.57}$ DL_{50}/ 0.03 mL para as amostras 17/4C/94 e 17/1C/94. A prova de SN realizada com um potente soro anti-rábico positivo, indicou diferenças sorológicas entre as amostras. O uso de soro anti-rábico positivo de referência padrão, contendo 812 UI/mL apresentou título neutralizante 50% de $10^{2.50 \pm 0.39}$, $10^{4.44 \pm 0.41}$, $10^{2.28 \pm 0.55}$ e $10^{3.21 \pm 0.07}$, para as amostras 17/4C/94, CVS, M03/87 e 17/1C/94. Título de anticorpos nos soros de morcegos poderia ser usado como um método adicional de vigilância epidemiológica da raiva, para identificar áreas de alto risco da doença.

PALAVRAS-CHAVE: Epidemiologia, *Desmodus rotundus* raiva, anticorpos neutralizantes, títulos virais.

INTRODUCTION

Since antiquity, vampire bats have been shrouded in mystery and malignant atmosphere and they have been raising interest, curiosity and fear in human beings. These facts have contributed to an utter but unfounded awe, making bats one of the most unwanted animals (MENDEZ, 1972).

CARINI (1911) described paralytic rabies in cattle in Santa Catarina state, southern Brazil, and was the first to associate vampire bats as the reservoir of the disease; HURST & PAWAN (1931); TORRES & QUEIRÓS LIMA (1935, 1936) have established the responsibility of hematophagous bats in transmitting rabies in herbivores, especially in Latin American countries. Rabies transmitted by non-hematophagous bats was first reported in the United States in 1953, in a *Lasiurus intermedius* specimen, sent to the laboratory after biting a child (SCATTERDAY, 1954). Many reports of rabies related to insectivorous bats were made in the world. In the United States, 647 cases were reported in 1992 (BRASS, 1994), only the States of Hawaii and Alaska among the American territories are considered free of rabies in bats (NATIONAL ASSOCIATION OF STATE PUBLIC HEALTH VETERINARIANS, 1995). In Europe, rabies positive cases in bats increased from 15 in 1985, to 142 in 1987 (WHITBY et al., 1996).

In Brazil, a report from UIEDA et al. (1995) describes four rabies cases in insectivorous bats in different cities of São Paulo State during the period of 1988 to 1991, two of them downtown São Paulo. In the same year, MARTORELLI et al. (1995) reported a rabies positive diagnose in a specimen of *Myotis nigricans* in the region of Ribeirão Pires, southeast Brazil.

MORENO & BAER (1980) reported that the question of vampire bats acting as sound carriers of rabies virus must be reviewed, because in their experiments no healthy bats have excreted virus through saliva, and no sick animals had recovered from the disease. Similarly DELPIETRO et al. (1985), did not find the existence of sound carrier bats or chronic shedders of the virus, like those described by TORRES & QUEIRÓS LIMA (1935, 1936) and PAWAN (1936).

The early rabies outbreaks in São Paulo state were firstly noticed in Ubatuba in 1935 and in Itu, (CARNEIRO, 1936) and the animal health authorities were worried about the possibility of the disease reaching the "Holstein" cattle raised in Vale do Paraíba, not affected at that time (PACE, 1943).

The Vale do Paraíba, according to the report of Divisão Regional Agrícola / Coordenadoria de Assistência Técnica Integral (DIRA / CATI), 1982, is located in the Southeastern region of Brazil. At the right riverside it is the range called Serra do Mar, at the left riverside, the Serra da Mantiqueira, and the landscape is varied as it is the climate. Lands are used for varied crops and livestock production. Losses due to cattle deaths in consequence of rabies in Vale do Paraíba were referred by SUGAY & NILSSON (1966). NILSSON et al. (1968) reported that the region was endemic to rabies and cattle owners desperately vaccinated their animals repeatedly. The situation of Vale do Paraíba nowadays is still endemic to rabies (SOUZA et al., 1994) and annual estimated losses due to rabies are still very high. Many owners and field practionners have questioned the efficacy of rabies vaccine being used, suggesting the existence of immunologically distinct rabies virus variants.

The methodology of monoclonal antibodies or the sequencing of aminoacids or the use of reverse PCR technique are not usual in our country. TIGNOR et al., apud KING & CRICK (1988) stated that serologically different rabies virus strains presented low intensity of fluorescence even with the use of the conjugate at a lower dilution. Antigenically different rabies virus strains should not be adequately neutralized with the presently available rabies vaccines (TORDO, 1991). This phenomenon has to be studied, because claims are that the currently available vaccines were not controlling efficaciously the disease.

Search for rabies antibodies in bats sera could be used as an alternative tool for epidemiological surveillance, in addition to virus isolation from brain material. In fact, TRIMARCHI & DEBBIE (1977); PRICE & ERERARD (1977); DELPIETRO et al. (1972) reported the presence of rabies antibodies in detectable levels. In Northeastern Brazil, in Bahia state, vampire bats sera from Amargosa, Barra da Estiva, Elísio Medrado, Irajuba and Iramaia regions revealed varied antirabies serum neutralizing titers, mostly between < 4 and ≥ 32 (SILVA et al., 1974).

The aim of this work is a study of bat rabies in Vale do Paraíba by capturing hematophagous bats from

Table 1 - *Desmodus rotundus* vampire bats captured in different localities of Vale do Paraíba, SP, during the period of July/92 to April/94. São Paulo, 1997.

Number of lot	Place of capture	Date of capture	Number of bats sent to the laboratory (sex)
1	São Luís do Paraitinga	30/6/92	07(3males; 4females)
2	São Luís do Paraitinga	20/10/92	09(4males; 5females)
3	Guaratinguetá	23/11/92	05(3males; 2females)
4	Pindamonhangaba	5/3/93	07(4males; 3females)
5	Pindamonhangaba	8/3/93	03(03males)
6	Lavrinhas	14/4/93	07(4males;3females)
7	Lagoinha	20/4/93	03(2males;1female)
8	Jambeiro	26/7/93	04(4females)
9	Pindamonhangaba	27/7/93	08(8females)
10	Roseira	3/8/93	02(2males)
11	São Bento do Sapucaí	14/9/93	11(8males; 3females)
12	Santo Antonio do Pinhal	15/10/93	06(1male; 5females)
13	Guaratinguetá	26/10/93	05(5males)
14	Cunha	09/11/93	07(2males; 5females)
15	Natividade da Serra	11/11/93	06(2males; 4females)
16	Natividade da Serra	25/1/94	11(5males; 6females)
17	Guaratinguetá	3/2/94	18(11males; 7females)*
18	São Bento do Sapucaí	2/3/94	09(5males; 4females)
19	Guaratinguetá	15/3/94	03(3males)
20	Guaratinguetá**	9/4/94	07(5males; 2females)
TOTAL			138(76males; 62females)

* 5 bats remained without blood collection, died during transportation

** return to the same gallery of lot 17.

the outbreak region and assessing zoonotic potential and mainly to evaluate the importance of serological results in these animals, when used as a tool for epidemiological surveillance, combined with the use of virus isolation. To assess if there is or not any difference in serologic and biologic behaviour among the rabies virus, the isolates were then compared with the fixed rabies virus strains.

MATERIAL AND METHODS

Bats: *Desmodus rotundus* was captured from different areas of Vale do Paraíba, São Paulo state, during different periods of the year (Table 1). The capture of bats was performed by the specialized staff of Serviço de Defesa Animal / Secretaría da Agricultura e Abastecimento (SDA / SAA) using mist nets set up at cave entrances, and pipes and galleries under roads. In the case of pipes and the galleries under the roads, the two open extremities were closed with mist nets and two operators entered with nets, making noises in order to force them out to be trapped. The classification and the identification were according to VIZOTTO & TADDEI (1973) and VIEIRA (1942) and only vampire bats were used in this experiment. To the half of the captured vampire bats was applied the topical treatment with anticoagulant and freed immediately after; the other half was kept alive in cages and was transported to the Laboratório Regional do Instituto Biológico (LR/IB) at Pindamonhangaba, SP.

Mice: albino mice weighing 11 to 15g and approximate age of 21 days were provided by the Department of Preventive Veterinary Medicine and Animal Health-FMVZ, USP.

Hamsters: Four pregnant golden female Syrian hamsters, weighing close to 120g were used for the

assessment of the viscerotropism of the isolates. After a week from the birth, the offsprings were sacrificed for the search of the rabies virus. The hamsters were inoculated through intra-pad route, using dose of 0.05 mL and observed daily for clinical signs of rabies.

Sera: Bats sera were collected through cardiac punction, using 1.0 mL B-D disposable syringe, 26 G ½ needle. The blood samples were allowed to clot, and sera were then separated and centrifuged at 1,500 rpm and put into small vials, properly identified, and stored at - 20ºC until use.

Antirabies sera: it was used a standard reference antirabies equine serum, potency of 812 IU/mL, and the other potent non-reference rabies positive serum was also prepared in horse, potency in IU/mL not determined.

Virus: CVS ("Challenge Virus Standard") - fixed strain of rabies virus, from CEPANZO, strain 31/2 was used. The strain, maintained at - 196ºC was reactivated twice in mice through i.c. inoculation before its use as source of antigen for SN and FA tests.

Pasteur Virus - the strain was maintained at -196ºC and inoculated twice in mice for its reactivation and used for the SN test.

M 03/87, a canine rabies virus, was isolated in 1987 from Mogi Mirim-SP municipality. After its identification through FA and SN tests, the isolate was then maintained at -196º C and reactivated twice in mice through i.c. route before its use.

Bat rabies virus isolates from brain specimens or pooled visceral organs were successively maintained in mice, and aliquots of brains corresponding to each passage were stored at -20º C and used when needed.

Rabies FA conjugate: Conjugate was prepared from serum of hamsters hyperimmunized with a commercially available rabies vaccine (Pfizer), constituted of a classic PV strain of rabies virus, and conjugated to fluorescein isothiocyanate (Sigma). The working dilution of the conjugate was 1:60.

Diluent: The diluent used to prepare brain suspension and to dilute serum and virus was a solution of sterilized distilled water, with 2% normal equine serum, previously inactivated at 56ºC for 30 minutes, free from rabies antibodies and containing 1,000 IU/mL of penicillin and 1.25 mg/mL of streptomycin.

Virus isolation: the techinque used was i.c. inoculation into young-adult mice using a dose of 0.03 mL (WEBSTER & DOWSON, 1935) in groups of ten mice each, with daily observation for a period of 30 days.

FA test: the technique followed was of the GOLDWASSER & KISSLING (1958).

SN test: the test was run according to ATANASIU (1967) using constant virus LD_{50} and serial dilution of sera. To calculate the virus titer, Log_{10} transformation was used and the method was according to REED & MÜENCH (1938), also using the formula of PIZZI (1950) to estimate the standard error (SE) of LD_{50}. The confidence interval (CI) was constructed using a = 0.05 and Z = 1.96 using the following formula: CI of $LD_{50} = LD_{50} \pm (1.96)$ (SE LD_{50}).

Procedures:

The hematophagous bats were submitted to cardiac punction; then the brains were collected and four impression smears were made on slides for each specimen, and after being dried at room temperature, the slides were immersed in cold acetone at -20ºC for 2-4hs. After acetone treatment, slides were kept in a wooden box and stored at -20ºC until use.

From each brain, 1:5 (W/V) suspension was prepared and after centrifuging at 1,500 rpm, 0.03 mL of this suspension was inoculated via i.c. in each group of ten mice, which were observed for 30 days, searching for signs of rabies. Animals found dead, paralyzed or in agonizing state had their brains collected for confirmation of rabies through FA test. Similarly, visceral organs collected from bats, including heart, lungs, liver and brown fat were pooled in a suspension which was inoculated into mice via i.c. for virus isolation.

Bats sera, after thawing, were inactivated at 56ºC for 30 minutes and an aliquot of 0.2 mL was transferred into a tube and added to the same volume of CVS suspension, containing approximately 100 LD_{50}/ 0.03mL of CVS strain. This serum-virus mixture was incubated for 90 minutes at 37ºC; after this the mixture was maintained cooled in ice bath and then inoculated into five mice per dilution. The mice were observed for 21 days, searching for rabies signs. All sera reacting to 1:2 dilution were submitted again to SN test, using a two-fold dilution until the final dilution of 1:32. To calculate the SN titers, the method of REED & MÜENCH (1938) was used. To calculate the LD_{50} endpoints of rabies virus isolates, groups of ten mice each were used for each dilution, based on the method of REED & MÜENCH (1938) and PIZZI (1950).

Table 2 - Results of rabies virus isolation and FA test in brains and pooled organs of vampire bats (*Desmodus rotundus*) captured in Vale do Paraíba, SP. São Paulo, 1997.

Lot number	number of bats	FA test		Isolation	
		brain	heart	brain	pooled organs
1	7	-	-	-	-
2	9	-	-	-	-
3	5	-	-	-	-
4	7	-	-	-	-
5	3	-	-	-	-
6	7	-	-	-	-
7	3	-	-	-	-
8	4	-	-	-	1*
9	8	-	-	-	-
10	2	-	-	-	-
11	11	-	-	-	-
12	6	-	-	-	-
13	5	-	-	-	-
14	7	-	-	-	-
15	6	-	-	-	-
16	11	-	-	-	-
17	18	1**	-	7	4
18	9	-	-	-	-
19	3	-	-	-	-
20	7	-	-	-	-
TOTAL	138	1	0	7	5

- negative for rabies

* negative for rabies according to FA test, although being positive for mice inoculation

** positive for rabies with fine particles and small intracytoplasmic inclusion bodies.

The bats isolates were maintained in mice through i.c. route and the biological characteristics were compared with CVS and PV strains and with M03/87 canine isolate. One of the bats isolates was inoculated into hamsters via intra-pad in order to assess the viral dissemination into non-nervous tissues. After the death, brain, heart, liver, lung, uterus and kidneys were collected from the mothers; pooled suspensions were made from visceral organs and brains of offsprings and inoculated into mice for virus isolation. From these materials, impression smears for FA examination were made.

RESULTS

The results of the isolation of rabies virus from brain tissue or pooled organs of vampire bats, captured in Vale do Paraíba, and the corresponding results of FA test are summarized in Table 2.

As illustrated in Table 2, lot number 8 corresponded to capture made at Jambeiro; the suspension prepared from pooled organs, identified as 08/1P/94, was positive for inoculation test, with 8 mice dead, with mean incubation period of three days and without showing the characteristic signs of rabies and negative results for the FA test. This material was subsequently inoculated twice in mice via i.c., however, the results of the second and the third passages were negative for FA test.

Material shipped from Guaratinguetá, lot 17, was positive for rabies in 7/18 brain suspensions submitted to mice inoculation. The presence of rabies virus was confirmed in four pooled suspensions. The incubation period of these isolates ranged from 8 and 21 days. Virus titration was not performed in the first isolation. By means of FA test, rabies was confirmed in the brain of only one bat. Nevertheless, the FA test confirmed the diagnosis in the brain of all mice inoculated with brain and pooled organs, as presented in Table 3.

Table 3 - Results of rabies virus isolation and FA test in specimens taken from vampire bats (*Desmodus rotundus*) captured in Guaratinguetá, Vale do Paraíba, SP. São Paulo, 1997.

	Virus Isolation		FA test	
Results	brain	pooled organ	brain	heart
Positive	1,3,4,5,6,8,16*	1,4,8,16	1	-

* numbers correspond to bats of the lot 17

- none detected

Bats numbered 1,4,5,8 and 16 died during transportation to the laboratory, blood not collected for SN test.

Detectable levels of antibody were found in few bats through the SN test, as illustrated in Table 4. Most of the examined sera was found with SN titers < 2. Among the sera with noticeable titers, 2 sera of lot 16 were detected with SN titers, one showed titer of 4 and the other, 11; lot 17 presented 3 sera with titers: one with 4, and two with titers >16; lot 20 presented 3 sera reacting at a titer of 4 and one, > 32, however, its endpoint was not determined.

The bats isolates were maintained in mice for several passages, in comparison with CVS and M03/87 canine origin isolate. The bats isolates 17/3C/94, 17/5C/94, 17/6C/94, 17/8C/94 and 17/16C/94 were not titrated from the third passage in mice, since the isolates titers were relatively low, as illustrated in table 5. As the passages progressed in mice through i.c. route, the incubation period of the bats isolates has decreased; at the 6th passage, 17/1C/94 and 17/4C/94 were similar to that of CVS fixed strain.

The results of intrapad inoculation into hamsters using the isolate 17/1C/94, were the following: from two pregnant rabid hamsters, rabies antigens were positively detected in brain, heart, liver, lung, kidney and uterus by means of FA test; the offsprings were all negative. Similarly, virus isolation was positive in brain, heart, liver, lung, kidney and uterus and negative to the offsprings. Other two hamsters, inoculated with the same isolate, did not show any signs of rabies, and after being observed for 120 days, the mothers and offsprings were sacrificed and results of FA test and virus isolation were all negative.

The results of neutralization test of PV and CVS strain, and of 17/1C/94 and 17/4C/94 isolates against a potent rabies positive serum are summarized in Table 6.

The results of virus neutralization test using a rabies positive standard serum having 812 IU/mL, are presented in Table 7

DISCUSSION

From a total of 138 hematophagous bats captured in Vale do Paraíba, all of them were *Desmodus rotundus*, indicating high density of this species in the region, confirming the description made by SUGAY & NILSSON (1966); NILSSON et al.(1968) and SOUZA et al. (1994).

Although reports of rabies in cattle are very frequent, the searches and the finds of colonies of

Table 4 - Levels of rabies serum neutralizing antibodies in sera of vampire bats (*Desmodus rotundus*) captured in Vale do Paraíba, SP. São Paulo, 1997.

Lots(number of reactant sera/number of lots'sera)	SN antibodies titers
1(7/7), 2(9/9), 3(5/5), 4(7/7), 5(3/3), 6(7/7), 7(3/3) 8(4/4), 9(8/8), 10(2/2), 11(11/11), 12(6/6), 13(5/5), 14(7/7), 15(6/6), 16(9/11), 17(10/13), 18(4/9), 19(3/3), 20(3/7)	<2
16(1/11), 17(1/13), 20(3/7)	2 |— 4
...	4 |— 8
16(1/11)	8 |— 16
17(2/18)	16 |— 32
20(1/7)	>32

Table 5 - Evolution of viral titers (LD_{50} / 0.03mL) in mice through i.c. inoculation: CVS strain, canine isolate and bat (*Desmodus rotundus*) isolates from Vale do Paraíba-SP. São Paulo, 1997.

	VIRAL STRAIN AND ISOLATES viral titers in (LD_{50} ± 1.96 SE)* log_{10}			
number of passages via I.C.	CVS	M03/87	17/1C/94	17/4C/94
2nd	6.70 ± 0.28[a]	3.93 ± 0.30[b]	4.06 ± 0.43	4.60 ± 0.57
3rd	5.84 ± 0.29	3.93 ± 0.28	4.20 ± 0.53	3.44 ± 0.32
4th	6.04 ± 0.25	nd	5.06 ± 0.22	5.52 ± 0.27
5th	6.13 ± 0.23	nd	4.97 ± 0.25	4.77 ± 0.29
6th	5.28 ± 0.29	nd	5.52 ± 0.27	4.52 ± 0.27

* a = 0.05 and Z = 1.96, using the method of PIZZI (1950) and titers calculation based on REED & MÜENCH (1938).

[a], [b] = passages considered after reactivation in mice.

nd = not done

SE = standard error

vampire bats infected with rabies virus are almost casual. In this study, 76 male and 62 female bats were included, with 7 (5.07%) cases of positive isolation. This percentage does not constitute any epidemiological significance, because there was a compromise with SDA/SAA authorities, in treating half of the captured bats with anticoagulant and only the remaining were destinated to this research. Many times, the returning to the caves visited previously, was not successful because no bats were found. In these visitings, many carcasses were found, probably due to anticoagulant treatment. The choice of the visiting places was motivated by the convenience, as determined by its proximity to highways, easy access etc.

Concerning mice inoculation and FA test, 7 brain specimens and 5 pools of visceral organs have been found with positive results, although the pooled suspension of lot 8 was not considered as rabies. In this respect, SUGAY & NILSSON (1966) reported the difficulty in isolating virus from pooled visceral organs, this fact could possibly be attributed to the dilution factor of the virus. In the search of virus from bats, mouse inoculation should always be made, as well as FA test. Had we used only the FA test, we might have detected only one positve specimen and missed 6 positive results.

Among the seven positive isolates, five were of bats that had died during shippment to the laboratory. It is important to notice that during the capture with mist nets, it was almost impossible to distinguish which bats were rabid or not. Probably the five bats dead could be already sick or in their incubation period. In Bolivia, STOURAITIS & SALVATIERRA (1978) reported the isolation of rabies virus from *Desmodus rotundus* apparently in

Table 6 - Results of neutralization test using PV and CVS strain, and the isolates 17/1C/94 and 17/4C/94, from vampire bats (*Desmodus rotundus*) captured in Vale do Paraíba, SP. São Paulo, 1997.

		Virus strain and isolates							
Serum dilution	Number of	PV		CVS		17/1C/94		17/4C/94	
log_{10}	inoculated animals	D	A	D	A	D	A	D	A
- 3.9031	10	0	10	0	10	6	4	4	6
- 4.2141	10	0	10	0	10	10	0	7	3
- 4.5052	10	0	10	0	10	10	0	9	1
- 4.8062	10	4	6	2	8	10	0	9	1
- 5.1072	10	10	0	10	0	10	0	9	1

D= dead; A= alive

Table 7 - Results of neutralization test using a rabies positive standard reference serum against the CVS strain, M03/87 of canine origin, and the isolates 17/1C/94 and 17/4C/94, from vampire bats (*Desmodus rotundus*) captured in Vale do Paraíba, SP. São Paulo, 1997.

Serum* dilution $\log_{10}$	Number of inoculated animals	Virus strain and isolates							
		17/4C/94		CVS		M03/87		17/1C/94	
		D	A	D	A	D	A	D	A
- 1	10	nd	nd	nd	nd	0	10	nd	nd
- 2	10	0	10	0	10	1	9	0	10
- 3	10	10	0	0	10	7	3	0	10
- 4	10	10	0	0	10	9	1	5	5
- 5	10	10	0	9	1	10	0	9	1

D= dead; A= alive; nd= not done

* rabies standard reference serum with 812 IU/mL.

good health, as did VILA-RAMIREZ & ALVAREZ (1963) in Mexico.

The early studies in vampire bats had indicated that they could act as healthy carriers, thus transmiting the disease for long periods without demonstrating signs of the illness or even without dying of rabies (HURST & PAWAN, 1931; QUEIROZ LIMA, 1934). MORENO & BAER (1980), however, stated that vampire bats could not experimentaly be proved as healthy carriers of rabies virus; all bats which presented the signs of rabies died invariably. For the authors, after almost fifty years ago since the reports of QUEIROZ LIMA (1934) and HURST & PAWAN (1931), surprisingly very scarce information is available concerning inoculation experiment of rabies virus in hematophagous bats, suggesting that similar experiments could be made in the future using different isolates. DELPIETRO & NADER (1988) presented discouraging opinion about the existence of healthy bat carrier or chronic shedder of rabies virus. In our study, bats found positive could be already ill, or could be the healthy carriers or could be in its incubation period.

The dissemination of rabies virus to visceral organs is sometimes interpreted as an uncommon finding for the rabies virus, as described for Mokola serotype, however, many rabies isolates have been reported as highly viscerotropic (VILLA-RAMIREZ & ALVAREZ, 1963).

The viscerotropic capacity of the bat isolate was assessed in 4 pregnant hamsters; rabies virus could be detected in half of the inoculated animals. The offsprings born from those hamsters, however, were all negative; STEECE & CALISHER (1989) reported the transmission of rabies virus from a mother bat to its fetuses.

TIGNOR et al., apud KING & CRICK (1988) stated that antigenically different rabies viruses reacted with less brightness through FA test, even when conjugate was used at lower working dilution. Changing the working dilution of the conjugate, it was very difficult to confirm this fact in our experiment. The polyclonal conjugate is not suitable for this kind of analysis due to several variables, like the smears thickness, conjugate quality, and others.

Some authors reported that the incubation periods of bat isolates through i.c. inoculation into mice were extremely short (BAER et al.,1980). In this experiment, the incubation periods varied from 8-21 days in first isolation; none of the mice inoculated with these isolates needed to be observed for more than 30 days. By continuing the passages in mice, at the 6th passage, the incubation period was found to be similar to that of CVS strain.

Among the isolates, 17/1C/94 was found with the highest viral titer of $10^{4.60 \pm 0.57}$ LD_{50} /0.03mL and the only one to be detected by the FA test, and the 17/1C/94 was another isolate found with its LD_{50} titer close to the first one; both isolates were compared serologically with fixed rabies virus strains and differences in their reactivity to a potent rabies positive serum were then detected (TABLE 6). In fact, with PV and CVS strain, the 50% endpoint neutralization index was found between the serum dilution -4.8062 and -5.1072, while with 17/1C/94 was < -3.9031 and between -3.9031 and -4.2141, for 17/4C/94 isolate.

The CVS strain, the bat isolates and the canine isolate, when compared by means of neutralization

technique using standard rabies serum (812 IU/mL) until the dilution of -5.0 showed a similar reactivity pattern; the neutralization was more effective with CVS strain and then followed by 17/1C/94, M03/94 and 17/4C/94. Slight differences in their serological reactions using the reference serum could be due to their distinct virus titer, or due to previous adaptation to mouse brain. Isolate M03/87, although been stored at -196ºC, and been maintained twice in mice after its reactivation, presented the reactivity pattern closer to that of CVS strain.

In an experiment using potent antirabies positive serum, OLIVEIRA (1993) could not detect any immunologic difference among the 30 rabies virus isolates collected from different regions of the country. In this study, slight differences in serological reactivity pattern were detected. The neutralization of this potent antirabies serum occurred by using a minimum viral dose of 50,230 or a maximum of 1,099,000 LD_{50}/ 0.03 mL for PV strain; and for CVS strain, a minimum of 526,000 and a maximum of 1,989,850 LD_{50}/0.03 mL. Using the same interpretation, for isolate 17/4C/ 94, the minimum dose of virus used in the serum-virus mixture was 2,290 and the maximum of 6,180 LD_{50}/0.03 mL; and for the 17/1C/94, minimum of 7,960 and maximum of 29,580 LD_{50}/0.03 mL. Corresponding to these different viral doses used in the reaction system, the SN_{50} endpoint titers could be the minimum of 77,620 and the maximum of 169,820 for PV strain, while for CVS, it ranged from 64,560 and 102,320, and for 17/1C/94 the SN_{50} endpoint titer was < 8,000 and in a range of 7,760 and 13,490 for the isolate 17/4C/94. Thus, it was demonstrated that bat isolates although presenting lower viral titers than to that of fixed strains, 50% neutralization occurred at a higher concentration of the serum. In fact, recent isolates have been found with lower titers, it could be related to the matter of viral adaptation in mice, or due to an excessive consumption of rabies antibodies due to the presence of defective virus in fresh isolates. In field conditions, a partially immunized herd would present higher risk in acquiring the disease than adequately immunized animals. Rabies antibodies in serum would be maintained at high level in order to confer effective protection against virus having similar characteristics to those studied in this work. Additionally, as reported by TRIMARCHI & DEBBIE (1977); PRICE & ERERARD (1977); SILVA et al., 1974; DELPIETRO et al. (1972) the search of rabies antibodies in bats should be used as a complementar tool of epidemiological surveillance to indicate the viral activity in bat populations.

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