

Experimental infection of vampire bats *Desmodus rotundus* (E. Geoffroy) maintained in captivity by feeding defibrinated blood added with rabies virus

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Abstract

In vampire bats, food sharing behavior would contribute for the oral transmission of rabies virus among the roostmates. To test this hypothesis, 10 captive *Desmodus rotundus* bats were fed defibrinated swine blood containing mice brain suspension of PV-strain of rabies virus. Other 10 bats were fed blood mixed with a mice brain suspension of T-9/95 vampire-bat-field isolate of rabies virus. Another group of 10 bats was inoculated intramuscularly with a mice brain suspension of the T-9/95 isolate. Other 20 bats were maintained without treatment and fed defibrinated swine blood for 158 days. All animals found dead during the observation period or those sacrificed at the end of the experiment were necropsied and specimens such as the brain and non-nervous tissues were collected for rabies examination. Four bats inoculated intramuscularly developed clinical rabies, with signs lasting 1-2 days, and the survival periods ranged from 11-14 days. The initial rabies diagnosis was based on direct fluorescent antibody (dFA) and mouse inoculation test (MIT) performed only on brain specimens, and subsequently, brains and the non-nervous materials were further reexamined by means of dFA, MIT and heminested-polymerase chain reaction (ht-PCR) technique. The intake of the PV-strain caused rabies in 2 bats, with survival period of 25 and 32 days, while the three bats ingesting the T-9/95 isolate presented periods of 26-31 days. Although discrepant results were found among the diagnostic tests, viruses have disseminated to the central nervous system and other organs, as seen in bats inoculated intramuscularly.

Key words:
Rabies virus.
Oral inoculation.
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Chiroptera.

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Introduction

The first mention of vampire bats participating in the epidemiology of rabies in Southern Brazil was made at the beginning of the 1900's by Carini.¹ Several transmission experiments involving parenteral inoculation of rabies virus into vampire bats were made in early 1930's and their results definitively

proved that vampire bats can transmit the virus, especially to herbivores.^{2,3} The *Desmodus rotundus* vampire bats are the most important rabies virus transmitters in many Latin American and Caribbean countries and *D. rotundus* bats inoculated with rabies virus could eliminate the virus in saliva for four and half months, without presenting signs of disease.⁴

The rabies virus is primarily

transmitted to other bats by bites of a rabid vampire bat, through contaminated saliva^{2,3}, but other routes such as aerosol transmission in a heavily populated environment have also been reported⁵. In vampire bats, the blood regurgitation behavior to feed the young or the roostmate was described⁶, but according to Queiroz Lima⁷, the vampire bats do not acquire rabies virus by suckling the blood from the infected cows.

Rabies transmission involving the digestive tract in Humans is a queer mode of infection, although this is theoretically possible in the case of open abrasions in the gastrointestinal mucosa.^{8,9} On the other hand, rabies virus could be experimentally transmitted through oral route in laboratory rodents^{10,11,12} and according to Correa-Giron et al.¹³, the infection would be initiated through buccal and lingual mucosa, lung and intestines. In another experiment, 132 mice (*Mus musculus*) feeding bovine brain tissues taken from outbreaks transmitted by vampire bats, only three died of rabies infection and 22 presented seroconversion to rabies.¹⁴

Vampire bats were orally inoculated with 10LD_{50%} of a vampire bat-rabies virus variant - CASS-88, diluted in 1-2 ml of defibrinated sheep blood, and other bats were inoculated intramuscularly. After an average of 16 days of incubation period, both intramuscularly and orally inoculated animals showed clinical signs of altered reflexes, tremor and paralysis, but none showed aggressiveness and rabies virus was recovered from the brain, tongue and brown grease.¹⁵

More than 70 years after the early rabies transmission experiments in vampire bats; information on oral inoculation of rabies virus in vampire bats is scarce. The licking behavior and food sharing in vampire bats among the roostmates through regurgitation, reported by Wilkinson¹⁶, would play an important role in the dissemination of rabies virus within a colony, without involving the parenteral route.

The aim of this work was to investigate the possibility of *D. rotundus* bats

become infected by ingesting the rabies virus added in the blood meal. During the maternal care period, a young bat or a roostmate being fed regurgitated blood by an infected mother eliminating the rabies virus into saliva, would acquire the virus from the food sharing behavior.

Material and Method

Bats: *Desmodus rotundus* vampire bats were captured using mist-nets, from the neighborhood of the city of Pilar do Sul, Southwestern São Paulo, a region known to be free of rabies in vampire bats, according to the veterinarians dealing with the control of vampire bats and belonging to the São Paulo State Livestock Defense Office (Escritório de Defesa Agropecuária-EDA), São Paulo State Secretary of Agriculture. Once transported to the city of São Paulo, 30 bats were divided randomly into three groups of 10 bats each, and were maintained in wire cages for observation for varied periods until use. Other 20 bats were maintained in a larger wire cage, without any treatment until the end of the observation. The capture and maintenance of bats in captivity to perform the experiment was authorized by the Brazilian Ministry for Environment and Brazilian Institute of the Environment (IBAMA), license No. 46/02, IBAMA protocol No. 020270035/02.61.

Mice: Swiss albino mice (*Mus musculus*), CH-3 Rockfeller lineage, weighing 12-14 g were provided by the animal facility of the Department of Preventive Veterinary Medicine and Animal Health, the animals were housed and handled with ethical principles and their use was authorized by the Bioethical Commission of the Faculty of Veterinary Medicine and Zootechny, University of São Paulo, Licence No. 106/2002.

Defibrinated swine blood: to feed the bats in the captivity, swine blood was collected from a slaughterhouse located at São José dos Campos - SP, and after defibrinating (stirring with a twig) and filtrating with gauze, 200 mL aliquots were

stored at -20°C until use. One aliquot of the pooled swine sera was submitted for mouse serum neutralization test (SN₅₀) to evaluate the rabies antibody titer. The thawed defibrinated blood was administered in Petri dishes with a volume of 200 mL for each 10 bats per day.

Rabies viruses: Viruses used were the PV fixed strain of rabies virus, provided by the ex-Centro Panamericano de Zoonoses - CEPANZO, Argentina, maintained in liquid nitrogen at -196°C, and passed four times intracerebrally in mice, and a field isolate T-9/95, isolated from a *D. rotundus* bat, stored at -196°C and also passed four times in mice. These viruses had been characterized previously through the complete N gene sequencing, by the research partners of the College of Bioresource Sciences of the Nihon University, Fujisawa, Kanagawa, Japan, respectively as a fixed rabies virus strain and a vampire bat-related rabies virus variant (VRRV), genotype I of classic rabies virus¹⁷.

Direct fluorescent antibody (dFA) test: Impression smears made from brain and non-nervous tissues of vampire bats were submitted to the immunofluorescent conjugate from Sanofi® at a working dilution of 1:120, according to the technique described by Dean et al.¹⁸.

Mice inoculation test (MIT): this technique was used according to the procedures described by Koprowski¹⁹.

Serum neutralization test (SN₅₀): the sera obtained from swine and vampire bats were submitted to mouse neutralization test, using the “constant amount of virus and varied dilutions of serum”, according to procedures described by Atanasiu²⁰. The standard equine rabies positive serum having 812IU/mL was provided by the Butantan Institute of the São Paulo State Secretary of Health. The rabies SN₅₀ neutralizing titers were calculated according to Reed and Muench²¹ and expressed in IU/mL²².

Heminested-PCR (ht-PCR) applied for brain and non-nervous tissues: the technique used was described by SOARES et al.²³, using the Primer sets

P510/P874 (P510: ATA GAG CAG ATT TTC GAG ACA GC; P784: CCT CAA AGT TCT TGT GGA AGA) and P510/P492 (P942:CCC ATA TAA CAT CCA ACA AAG TG). Specimens previously tested by FAT and MIT and stored at -20°C were submitted for total RNA extraction by using TRIzol LS method, according to manufacturer's instructions (Gibco BRL). Reverse transcription was performed with extracted product (7 µL) added to a final volume of 20 µL containing 1mM of each dNTP, 20 pmols of primer P510, 1 x RT buffer (Gibco BRL), 1 mM dTT, 200 units of M-MLV reverse transcriptase (Gibco BRL) and 0.01% DEPEC treated ultra pure water and the mixture was incubated at 42°C for 60 minutes. Primary amplification was made in 5 µL of the reverse transcribed-cDNA template in a final volume of 50 µL, containing 0.2 mM of each dNTP, 25 pmols of primer P510, 25 pmols of primer P942, 1.5 mM of MgCl₂, 1 x PCR buffer (Gibco, BRL), 1.25 units of Taq DNA polymerase (Gibco, BRL) and ultra pure water and amplification made on a MJ Research PTC-200 Thermal Cycler. The heminested amplification was performed in 5 µL of primary amplification template using the primers P510 and P874. The cycling conditions for the primary amplification were: initial heating at 94°C/3min, 35 cycles at 94°C/45 sec, 55°C/60 sec, 72°C/90 sec and a final extending step at 72°C/10 min. The thermal cycles for the nested assay were the same, with amplification phase of 25 cycles. Products of PCR were run in 2% agarose gel electrophoresis in standard TBE and stained with ethidium bromide 0.5 µg/mL and gels were examined under UV light and photographed.

Procedures:

After being kept for 23 days in captivity, the first group of 10 bats was fed defibrinated swine blood (total volume of 200mL) added with 20mL of a 20% (weight/volume) viral suspension (infective dose of 20x10^{7.23} MILD₅₀/220mL) prepared from the rabid mice brains inoculated with the PV strain of fixed rabies

virus, and they were observed daily for rabies signs up to 72 post-inoculation days and five surviving bats were submitted for blood collection through intracardiac puncture, and euthanized soon after using ethyl-ether. The sera of these bats were pooled and further submitted to SN₅₀ test. After 36 days in captivity, the second group of 10 bats was inoculated intramuscularly at the external thigh with a dose of 10^{4.16} MILD₅₀/0.03mL of a 20% (w/v) brain suspension prepared from mice inoculated with a T-9/95 field rabies virus isolate and observed daily for signs of rabies. Rabies-positive bats of this group were defined as the “standard positive bats”. The third group of 10 bats, after 78 days of captivity, received 200 mL of defibrinated blood added with 20 mL of a 20% (w/v) rabid mice brain suspension (infective dose of 20x10^{5.68}MILD₅₀/220mL) of T-9/95 field rabies virus. Twenty-eight days after feeding the viral suspension, two bats were bled through cardiac route and were submitted to euthanasia, and the pool of sera was further submitted for SN₅₀ test. All the bats found dead after the parenteral or oral administration of rabies virus were necropsied and the organs like the brain, lung, kidney, heart, liver, testicle, tongue, brown grease, submaxillary salivary gland and the spleen were collected to be examined by dFA, MIT, and ht-PCR technique. The 20 control bats were maintained without administration of virus and had been fed

with defibrinated blood through the entire period of observation of 158 days. At the end of the observation period, all surviving bats were exsanguinated and the pool of the sera was submitted for SN₅₀ test. After the sacrifice and necropsy, the brains of bats of this group were submitted for dFA and MIT.

Results

The vampire bats promptly accepted the defibrinated swine blood added either with the PV-strain or the T-9/95 field rabies virus mice brain suspensions, but individual daily blood intake was not recorded. The rabies SN₅₀ titer of pooled swine sera used for feeding the bats was found with <0.02 IU/mL, and the pooled serum samples of PV-treated bats which had been sacrificed at the 72nd post-inoculation day presented SN₅₀ titer <0.02 IU/mL, while the pooled sera of the T-9/95 orally treated group presented SN₅₀ titer of 0.2 IU/mL. The control bats sacrificed after being maintained for 158 days of observation did not present any signs of rabies and the pooled sera showed SN50 titer of <0.02 UI/mL.

The information on the incubation period, clinical period, and survival period found in bats fed defibrinated blood added with the PV fixed strain of rabies virus is presented in table 1.

In bats inoculated intramuscularly with the T-9/95 field isolate of rabies virus, four

Table 1 - Incubation period, clinical period, and post inoculation survival period in *D. rotundus* vampire bats fed defibrinated swine blood containing 20x10^{7.23}MILD₅₀/220mL of a fixed strain of PV rabies virus and rabies confirmed on brain tissues by direct fluorescent antibody test (dFA) and mouse inoculation test (MIT) - São Paulo – 2003

Bat's #	Incubation Periods (in days)	Clinical period (in days)	Post inoculation survival period (in days)	Rabies confirmation on brain tissues DFA/MIT
1♂	24	1	25 ⁽¹⁾	+/+
2♂	...	--	72 ⁽¹⁾	-/-
3♀	31	1	32 ⁽¹⁾	+/+
4♀	...	--	53 ⁽¹⁾	-/-
5♀	...	--	55 ⁽¹⁾	-/-
6♀	...	--	57 ⁽¹⁾	-/-
7♀	...	--	72 ⁽²⁾	-/-
8♂	...	--	72 ⁽²⁾	-/-
9♂	...	--	72 ⁽²⁾	-/-
10♂	...	--	72 ⁽²⁾	-/-

⁽¹⁾Found dead without rabies signs; ⁽²⁾Bleeding day and euthanasia; - - Absence of signs or symptoms; ... = Not determined; + = Positive; - = Negative; ♂=Male; ♀=Female, # = number; +/- =positive for both tests; -/-= negative for both tests

were found with wing paralysis, salivation, urinary incontinence, altered reflexes, followed by coma and death. The clinical period was observed for only 1 or 2 days. The incubation period, clinical period, and post inoculation survival period are presented in table 2.

Similarly, the information on the incubation period, clinical period, and survival period found in bats fed defibrinated blood added with a mice brain suspension of T-9/95 field rabies virus isolate is presented in table 3.

The presence of rabies antigen, virus or viral RNA in the brain and other non-nervous organs in bats administered the PV virus were confirmed in two individuals. In one bat, the dFA, MIT and ht-PCR techniques identified the presence in lung, and

in the other, only dFA registered positive result with the lung material, while liver reacted positively to ht-PCR, as presented in table 4.

The results of dFA, MIT and ht-PCR examinations of specimens collected from the intramuscularly inoculated bats are indicated in table 5.

Brains of two bats receiving T-9/95 isolate through alimentation were found positive by dFA and MIT, and did not react positively to ht-PCR. On the other hand, brain material of another bat did not react to dFA, MIT and ht-PCR techniques. Only liver were found positive by the three techniques. Additionally, ht-PCR reacted positively at least once or twice with lung, liver, kidney, salivary gland, and brown grease specimens, as seen in table 6.

Table 2 -Incubation period, clinical period, and post inoculation survival period in *D. rotundus* vampire bats inoculated intramuscularly with 0.03mL of mice brain suspension of the T-9/95 vampire-bat rabies virus isolate with an infective dose of $10^{4.16}$ MILD₅₀/0.03mL and rabies confirmed on brain tissues by direct fluorescent antibody test (dFA) and mouse inoculation test (MIT) - São Paulo - 2003

Bat's #	Incubation period (in days)	Clinical period (in days)	Post inoculation survival period (in days)	Rabies confirmation on brain tissues dFA/MIT
1♂	10	1	11	+/+
2♀	11	2	13	+/+
3♂	11	2	13	+/+
4♂	12	2	14	+/+
5♂	...	--	23 ⁽¹⁾	-/-
6♂	...	--	35 ⁽¹⁾	-/-
7♀	...	--	2 ⁽¹⁾	-/-
8♂	...	--	2 ⁽¹⁾	-/-
9♂	...	--	3 ⁽¹⁾	-/-
10♂	...	--	3 ⁽¹⁾	-/-

⁽¹⁾Found dead without signs of rabies; -- = Absence of signs or symptoms; ... = Not determined; += Positive; - = Negative; ♂=Male; ♀=Female; # = number; +/+ =positive for both tests; -/- =negative for both tests

Table 3 -Incubation period, clinical period, and post inoculation survival period in *D. rotundus* vampire bats fed with defibrinated blood containing $20 \times 10^{5.68}$ MILD₅₀/220mL of T-9/95 vampire-bat rabies virus isolate and rabies confirmed on brain tissues by direct fluorescent antibody test (dFA) and mouse inoculation tests (MIT) - São Paulo - 2003

Bat's #	Incubation period (in days)	Clinical period (in days)	Post inoculation survival period (in days)	Rabies confirmation on brain tissues dFA/MIT
1♀	...	--	46 ⁽¹⁾	-/-
2♀	26	2	28	+/+
3♂	...	--	82 ⁽¹⁾	-/-
4♀	...	--	48 ⁽¹⁾	-/-
5♀	...	--	52 ⁽¹⁾	-/-
6♀	26	1	27	+/+
7♂	...	--	56 ⁽¹⁾	-/-
8♀	29	2	31	+/+
9♀	...	--	28 ⁽²⁾	-/-
10♂	...	--	28 ⁽²⁾	-/-

⁽¹⁾Found dead without signs of rabies; -- = Absence of signs or symptoms; ... = Not determined; ⁽²⁾Bleeding day and euthanasia; += Positive; - = Negative; ♂=Male; ♀=Female, # = number; +/+ = positive for both tests; -/- = negative for both tests

Table 4 - Detection and isolation of rabies virus from organs of *D. rotundus* vampire bats orally infected by ingesting swine defibrinated blood containing the fixed PV virus, according to dFA, MIT and ht-PCR examinations - São Paulo – 2003

Bat's # organs\	Brain			Lung			Heart			Liver			Spleen			Kidney			Tongue			Salivary gland			Brown grease			Testicle		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1♂	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2♂	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3♂	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

♂ = Male; Tests: 1 = dFA; 2 = MIT; 3 = ht-PCR; + = Positive; - = Negative, # = number
 Bat # 2♂ used as the negative control

Table 5 - Detection of rabies virus, antigen or RNA in organs of *D. rotundus* vampire bats infected by intramuscular inoculation of 20% mice brain suspension of the of T-9/95 rabies virus isolate, according to dFA, MIT and ht-PCR examinations - São Paulo – 2003

Bat's # organs\	Brain			Lung			Heart			Liver			Spleen			Kidney			Tongue			Salivary gland			Brown grease			Testicle		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1♂	+	+	+	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	+
2♀	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	+	-	-	-	-	-	nt	nt	nt	nt
3♂	+	+	+	-	-	+	-	-	-	-	-	+	-	-	N	-	-	+	-	-	+	-	-	+	-	-	nt	-	-	nt
4♂	+	+	+	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+	-	-	-
7♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

♂ = Male; ♀ = Female; Tests: 1 = dFA; 2 = MIT; 3 = ht-PCR; Bat # 7♀ used as the negative control; nt= Not tested; + = Positive; - = Negative, # = number

Table 6 - Detection of rabies virus, antigen or RNA in organs of *D. rotundus* vampire bats orally infected by ingesting swine defibrinated blood containing the T-9/95 field isolate of rabies virus, according to dFA, MIT and ht-PCR examinations - São Paulo – 2003

Bat's # Organs	Brain			Lung			Heart			Liver			Spleen			Kidney			Tongue			Salivary gland			Brown grease			Testicle		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1♀	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2♀	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
6♂	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8♂	+	+	+	-	-	+	-	-	-	-	-	+	-	-	N	-	-	+	-	-	-	-	-	+	-	-	+	nt	nt	nt

♂ = Male; ♀ = Female; Tests: 1 = dFA; 2 = MIT; 3 = ht-PCR; Bat # 1 used as the negative control; nt= Not tested; + = Positive; - = Negative; # = number

Discussion

The *D. rotundus* bats accepted the defibrinated swine blood, but they are very sensitive animals in captivity. In the experimental groups, many bats died without showing any signs of rabies after contact with the rabies virus, however, rabies were not confirmed in these animals. Among the 20 bats of untreated group, half died during the observation period, and rabies was not confirmed in these animals by means of dFA, MIT and ht-PCR techniques. The pooled sera of the control bats, sacrificed at the 158th observation day, was found with SN₅₀ titer <0.02 IU/mL. This fact corroborates the information that they were captured from a region known to be free of rabies in bats. The sera obtained from the swine blood used for the

alimentation of bats showed rabies SN₅₀ antibodies level <0.02 IU/mL, attesting that the slaughtered hogs were not vaccinated against rabies.

Vampire bats inoculated intramuscularly with rabies virus did not show any detectable levels of rabies antibody after one year of observation²⁴. In our experiment, 72 days after feeding of PV fixed virus, the pooled SN₅₀ antibody titer of bats was <0.02 IU/mL. However, 28 days after the virus intake, bats ingesting the T-9/95 virus isolate were found with SN₅₀ titer of 0.2 UI/mL and this level of antibody could be interpreted as an evidence of the humoral response against the rabies virus.

The PV strain used in this experiment revealed 100.00% identity of complete N nucleotides with the PV-Brasil (AF357308) available in GenBank. In this experiment, this

strain administered orally caused in bats a mortality rate of 20%, although the same PV strain, reported by Alves et al.²⁵ presented high mortality rate through intracerebral and intramuscular inoculation in mice and hamsters, but the oral administration in hamsters did not show any overt signs of the disease. The dilution effect and the total volume of blood-virus mixture ingested by the bats may have contributed to these results in bats, but in the experiment of rodents fed rabid brains, reported by Delpietro et al.¹⁴, the infectivity by the oral route was very low since only three rodents out of 132 died of rabies infection.

Sétien et al.¹⁵ inoculated *D. rotundus* bats through intramuscular route using a field isolate from *D. rotundus* bat origin, the variant named "CASS-88" of rabies virus, at an infective dose of $10^{6.0}$ ICLD₅₀/0.03mL. The authors found a mortality rate of 89%, with minimum incubation period of 7 days and a maximum of 30 days. In our experiment, none of the four intramuscularly inoculated animals receiving a dose of $10^{4.16}$ ICLD₅₀/0.03mL that were found positive for rabies showed any aggressive behavior and the clinical period lasted 1-2 days.

Laboratory animals were forced to ingest brains of mice inoculated with CVS fixed rabies virus, or they were inoculated by using an esophagic tube to introduce the inoculum¹³, with the infective dose similar to that used in this experiment, but the virus concentration needed to infect bats through oral route showed to be higher, when compared to intramuscular inoculation. In this experiment, the PV fixed virus showing a titer of $20 \times 10^{7.23}$ ICLD₅₀/220mL of defibrinated blood and freely ingested by bats, only two died of rabies with clinical signs.

Viscerotropism of rabies virus in naturally infected vampire bats has been reported^{26,27} and in this experiment, both PV fixed strain and the T-9/95 field isolate of rabies virus administered orally were found disseminated to nervous and non-nervous tissues. The PV fixed strain was detected in the brain, lung and liver fragments, showing

that the fixed strain is less invasive than the T-9/95 field isolate. The presence of the isolate T-9/95 was recognized in the brain, lung, liver, kidney, salivary gland and brown grease, especially with the ht-PCR technique.

Abnormal progression of rabies virus were described in the abortive, latent, persistent, inapparent, tolerant, recrudescing, or even slow virus infections, but the mechanisms involved are not known²⁸. The liver found positive by the dFA, MIT, and ht-PCR technique in a bat that ingested the T-9/95 isolate could be interpreted as an abnormal result or a technical error might have occurred when brain material was examined by dFA and MIT techniques and due to the small amount of available brain material, reexamination was not feasible. Specimens of brain, lung, liver, kidney, salivary gland and brown grease of one bat of the same group were found positive by the ht-PCR technique. In this experiment, non-nervous specimens reacted positively to ht-PCR technique, indicating the high sensitivity of the test. Or the PCR technique is indicating the presence of viral RNA circulating in the organisms of those inoculated bats, and the liver and lung would be the organs involved in the mechanism of the virus clearance. The use of the PCR and other amplification techniques for rabies diagnosis is not currently recommended for routine post-mortem diagnosis, otherwise the molecular techniques can be applied for epidemiological surveys in laboratories having strict quality control procedures, experience and expertise²⁹.

Although presenting some discrepant results among the diagnostic tests used in this experiment, the viruses administered orally to vampire bats could infect the nervous tissues and disseminate to other organs, similarly to that described in naturally acquired rabies in vampire bats^{26,27}. Rabies infection through oral administration of virus and its further dissemination to nervous and non nervous tissues determining the condition of a carrier, being completely asymptomatic,

was not observed in this experiment.

Considering the food sharing behavior in *D. rotundus* bats, the regurgitation of blood from an infected mother and then feeding the offspring or other roostmates, the saliva containing the rabies virus would

contaminate the regurgitated blood and the oral transmission of rabies virus might occur in nature, and we conclude that this type of transmission would contribute to the maintenance of the virus in the population of vampire bats.

Infecção experimental de morcegos hematófagos *Desmodus rotundus* (E. Geoffroy) mantidos em cativeiro e alimentados com sangue desfibrinado adicionado de vírus da raiva

Resumo

Em morcegos hematófagos, o hábito de compartilhar alimento poderia contribuir na transmissão oral do vírus da raiva. Para verificar esta hipótese, 10 morcegos *Desmodus rotundus* em cativeiro foram alimentados com sangue suíno desfibrinado, contendo suspensão de cérebros de camundongos infectados com vírus rábico PV. Outros 10 camundongos receberam sangue contendo suspensão cerebral de camundongos infectados com vírus de morcego hematófago (T-9/95). Um grupo de 10 camundongos foi inoculado intramuscularmente com suspensão de vírus T-9/95. Outros 20 morcegos foram mantidos sem tratamento e alimentados com sangue desfibrinado por 158 dias. Todos os animais encontrados mortos durante o período de observação ou sacrificados no final do experimento foram necropsiados e os cérebros e órgãos não-nervosos foram colhidos para a confirmação da raiva. Quatro morcegos inoculados intramuscularmente apresentaram raiva clínica, com sinais persistindo por 1-2 dias e os períodos de sobrevivência variaram de 11-14 dias. O diagnóstico da raiva inicialmente foi realizado somente com os fragmentos do cérebro, submetendo-os às provas de imunofluorescência direta (IFD) e inoculação em camundongos (IC). Subseqüentemente, os cérebros e os órgãos não-nervosos foram reexaminados com as técnicas de IFD, IC e *hemimested-polymerase chain reaction* (ht-PCR). A ingestão do vírus PV causou raiva em dois morcegos, com período de sobrevivência de 25 e 32 dias, enquanto que os três morcegos que ingeriram o isolado T-9/95 apresentaram períodos de 26-31 dias. Embora encontrando resultados discrepantes entre as técnicas diagnósticas utilizadas, os vírus ingeridos pelos morcegos foram detectados no sistema nervoso central e outros órgãos não-nervosos, como nos morcegos inoculados intramuscularmente.

Palavras-chave:
Vírus da raiva.
Inoculação oral.
Sangue.
Chiroptera.

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