Ecology and control of infectious disease in vampire bats
November,2 - 2016 - Peru

Photo: Marilene Almeida

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Indirect immunization of *Desmodus rotundus* bat in captivity, with anti-rabies V-RG vaccine.

MOTIVATION

- the number of human rabies cases transmitted by bats;
- the number of bat rabies cases;
- the number of rabies cases in herbivorous transmitted by hematophagous bats;

- the necessity of assessing other control rabies strategies in epizootic and enzootic situation;
- to offer an alternative to the vampiricide.

Preservation of the *Desmodus rotundus*

What is the ecological role of haematophagous bats?

Feces "guano" can be used as fertiliser (Nowak, 1991).

"Guano" is the base of food chain in the caves (detritivora chain), which permit the survive of one high number of parasitic species. (Aguiar & Taddei, 1996).

Numerous invertebrates live in and over the semi-liquid feces (Winsatt, 1970).

Properties of the VBSP-Vampire Bat Salivary Plasminogen activator - antithrombotic therapy (Gardell et al, 1990; Mellott et al., 1992, Witt et. Al, 1992).

The anticoagulant properties of the saliva was shown to increase blood flow in stroke patients.
(January 10, 2003, J. the American Heart Association).

Due their habit of hygiene and behaviour

Clustered in their niche

Maintaining close contact with all individuals in the colony

These bats use their tongues and feet to clean their bodies.

Photo: Marilene Almeida

Greenhall (1965)
Linhart *et al.* (1972)

These observations suggested that any substance could be applied on the back of one bat, would be ingest by the vampire bat and the substance could be spread among the colony


Photo: Marilene Almeida

Why not one vaccine?

HYPOTHESIS

THE ADMINISTRATION OF V-RG VACCINE THROUGH VASELINE PASTE APPLIED IN THE BACK OF ONE BAT CAN INDUCE IMMUNITY IN IT AND IS ABLE TO INDIRECTLY PROTECT OTHER BATS FROM THE SAME COLONY


Photo: Marilene Almeida

CAPTIVITY


Photo: Marilene Almeida

The cages are made in policarbonate, with two independent systems, one air inlet and one air outlet.

There is a pre-filter unit (retention of 97% for particles of 3 to 7 um) to increase the life of the main filters, an inner and outer HEPA filters.

The circulation of air was made directly in the interior of cages. The system was incorporated considering the possibility of aerosol formation and secondary infections. Circulation rate: fifty/hour.

The bats were fed with defibrinated swine blood, 20 to 30 mL a day per animal.

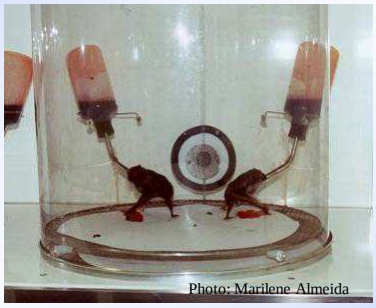

Photo: Marilene Almeida

MONITORING

weight of animals

Consumption of blood

Photo: Marilene Almeida

Photo: Marilene Almeida

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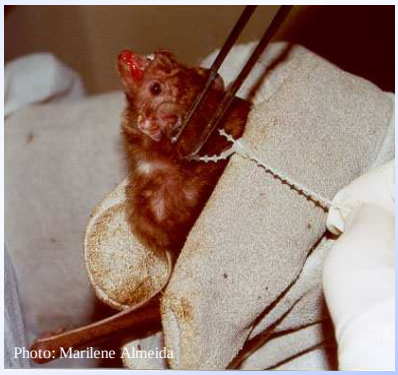
Natural and artificial shelters

Adaptation period: 30 to 45 days

Separation by sex and shelter



Letters are used as a form of identification of one individual in a group, facilitating the capture of specific individual for technical proceedings.

The cages showed a good adaptation for the bats: The adequate consumption of defibrinated blood, the increase or maintenance of weight and the low mortality of the bats (19.8%), demonstrate this fact.
The cages design proved to be adequate and secure to bats observation.

EXPERIMENTAL INFECTION

Lethal dose

Challenge

EXPERIMENTAL INFECTION

10 D.rotundus - 100MICLD50

10 D.rotundus - 1,000MICLD50

10 D.rotundus - 10,000MICLD50

10 D.rotundus – 100,000MICDL50

13 *D.rotundus* – 1.000,000MICLD50

Control Group (PBS)

Intramuscular route, pectoral muscle (0,1mL)

Observation period: 90 days

Rabies virus variant: brldr2918, isolated from *Desmodus rotundus* naturally infected.

Rabies Diagnostic: FAT Fluorescent antibody
Mouse InoculationTest
RT-PCR

Sequencing at Center for Diseases Control and Prevention - CDC Atlanta

Serology: RFFIT

4 samples from each animal
(one before, three after the rabies virus inoculation)
0.5IU/ml like cut-off

Photo: Marilene Almeida

Brain and salivary glands: FAT - MIT
RT-PCR

Results

Rabies experimental infection of Desmodus rotundus bat

MICLD50	mortality (%)	bat	incubation period (days)	symptoms	morbidity (hours)
100	0	--	--	--	--
1,000	20	A2	19	-	?
		B2	41		?
10,000	20	H7	29	+	24
		Y7	32		
100,000	60	Q12	5	+	48
		X12	5		
		Z12	5		
		K12	9		24
		R12	9		
		F12	14		18
1.000,000	69.2	X3	6	+	24
		S3	5		
		F3	5		
		V3	5		
		Y3	5		
		P4	5		
		S4	5		
		W4	5		
		C4	7		
control	0	--	--	-	--

Experimental infection of *Desmodus rotundus* rabies virus according diagnostic test

FA, MIT / RT-PCR		FA MIT / RT-PCR	
brain		salivar glands	
Infected	+	4	6
control	-	-	-
survivor	-	-	-

CLINICAL ASPECTS

All animals that developed rabies showed signs of paralytic rabies: weakness, muscular tremors, muscular spasms, impossibility to stand on their feet and thumbs, lack of coordination of posterior limbs, irritability to light, wind and sounds, paralysis and prostration.

None clinical manifestation associated with furious rabies was observed in the sick animals.

CLINICAL ASPECTS

Video 3

Photos: Marilene Almeida

CLINICAL ASPECTS

Photos: Marilene Almeida

EXPERIMENTAL INFECTION
Serology - - Results

Serum samples were seronegative to VNA before the experiment.

97.2% of the bats showed increase in the level of antibodies on day 30.

VNA titres above 0.5IU/mL were found in 52.7% of bats on day 30, 43.7% on day 60 and 34.5% on day 90 after inoculation.

EXPERIMENTAL INFECTION
Serology - Results

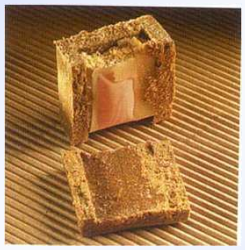
It was observed resistance to experimental infection in animals with low titres of antibodies (<0,5IU/mL) in all groups, independently of the dose inoculated.

Animals that received the highest inoculums developed the highest antibodies titres. (p=0.0002).

EXPERIMENTAL INFECTION
Conclusions

Bats were able to survive a large dose of rabies virus and produced antibody titers that would be considered "protective" in humans

IMUNOGENICITY OF VACCINE AND ORAL ROUTE



Vaccine: Raboral V-RG - Merial

Recombinant: A life-modified Vaccinia Virus (Copenhagen strain) express the rabies glycoprotein (ERA strain).

Potency: 10^8 e 10^9.

A sachet containing rabies vaccine is inserted into the baits and sealed in with wax.

Red fox

(*Vulpes vulpes*)

Raccoon

(*Procyon lotor*)

... oral vaccination is not a panacea, and should be viewed as an important adjunct to traditional prevention and control techniques in human and veterinary medicine. Local outbreak suppression of rabies among free-ranging wildlife is documented, and regional elimination of particular virus variants among specific, targeted carnivore hosts is demonstrable. Rupprecht et al., Dev Biol 2004;119:173-84.

dose	animals
0.25ml	7
0.75ml	8
1.25ml	7
2.0ml	6
3.5ml	10

Photo: Marilene Almeida

3.5ml group, vaccine concentred (0.7ml) by dialysis in saturate solution

Challenge: twenty six days after vaccination by intramuscular route with the lethal dose established in the experimental infection.

Observation period: 90 days after challenge

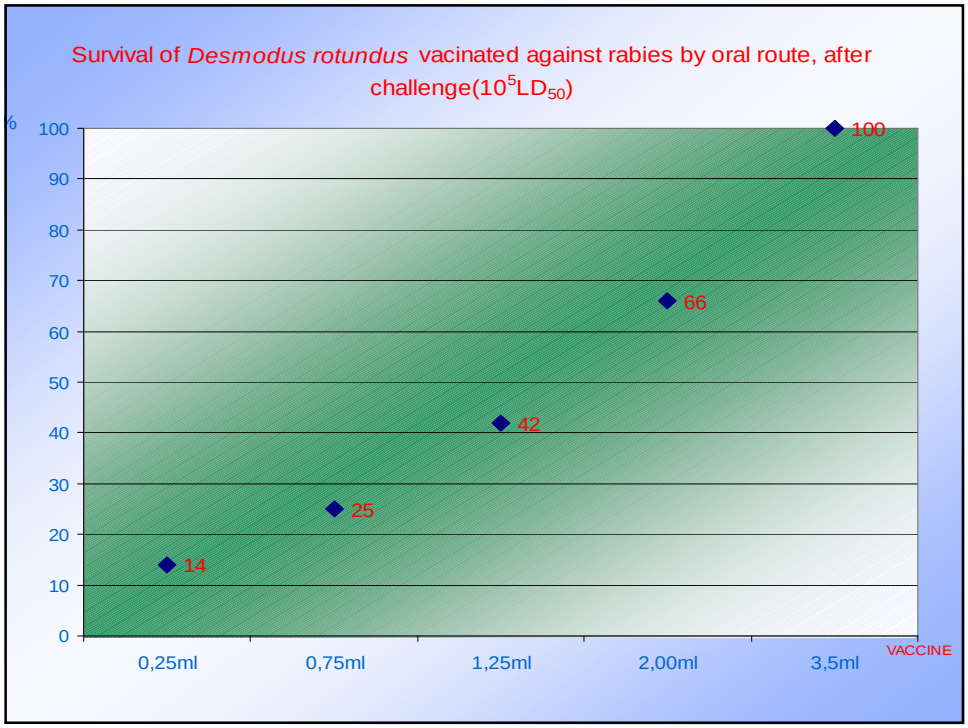
Serology: RFFIT - before

24 days after vaccination

20 days after challenge

day of sacrifice

Diagnosis: FAT Brain



Oral route - Serology

The majority (94.7%) showed increase of titers 20 days after vaccination, but VNA titers above 0.5IU/ml were found only in 26.3% of bats.

After the challenge, 92.1% of the animals showed titers above 0.5IU/ml and 80% of the survivors showed titers above 0.5IU/ml on day 90 (0.6 to 3.2IU/ml).

Animals with low titres of antibodies resist to challenge after vaccination.

Oral route - Conclusion

V-RG vaccine by oral route showed immunogenic to *Desmodus rotundus* and the survive after challenge showed dependent dose.

The survival rate increased when the administered dose increased.

PASTE+COLOR+VACCINE


Photo: Marilene Almeida


Photo: Marilene Almeida

8 groups - 1 or 2 animals of each group

The vaccine was concentrated by dialysis in super saturate solution of sacarose and it was homogenated in paste and color.

8 groups - 1 or 2 animals of each group

A to **E** - volume of 9 vaccines concentrated in 1.2 to 1.5ml

F to **H** - volume of 18 vaccines concentrated in 2.0ml

This volume was homogenated in 1.5grams to 3.0grams of paste and color.

Challenge:

Twenty-six days after vaccination by intramuscular route with the lethal dose established in the experimental infection

Observation period: 90 days after challenge

Serology: RFFIT - before

24 days after vaccination

25 days after challenge

day of sacrifice

Diagnosis: FAT brain

SURVIVAL AFTER VACCINATION AND CHALLENGE

Dose Vaccine	Number of bats	Incubation Period (days)	Morbidity Period (hours)	SURVIVAL %
Nine (A)	7	6 to 15	18 to 24	42,8
Nine (B)	7	7 to 11	18 to 24	71,4
Nine (C)	7	5 to 9	24 to 48	57,1
Nine (D)	7	5	24 to 48	57,1
Nine (E)	7	5 to 6	24	71,4
Eighteen (F)	8	5	48	62,5
Eighteen (G)	9	5 to 10	24 to 48	55,5
Eighteen (H)	10	5 to 11	18 to 24	60

Paste route - Serology

The majority (71.7%) showed increase of titers 24 days after vaccination, but VNA titers above 0.5IU/ml were found only in 20% of bats.

After the challenge, 79.3% of the animals showed titers above 0.5IU/ml and 57.1% of the survivors showed titers above 0.5IU/ml on day 90.

Animals with low titres of antibodies resist to challenge after vaccination.

Serology

	mouth/dialysis	paste/dialysis
after vaccination	94.7%	71.7%
0.5IU/ml	26.3%	20%
after challenge	92.1%	79.3%
sacrifice	80%	57.1%
SURVIVAL	100%	71.4%

Conclusions

- V-RG vaccine by mix showed immunogenic to *D. rotundus* and the survive after challenge showed dependent contact (with the animal that received the mix and if this contact was intense).
- The vaccine induced immunity in it and was able to protect other bats from the same group.
- None injuries / lesion associated to vaccinia virus was observed in the dorsal muscle in the days after the application of paste with V-RG vaccine.

ALL ANIMALS SHOWED SIGNS OF CONTACT WITH THE MIX (VACCINE/PASTE/COLOR)

This contact can't be determined numerically, just estimated through survival after challenge.

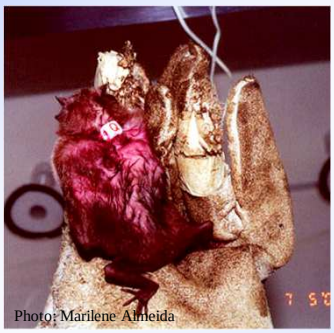


Photo: Marilene Almeida

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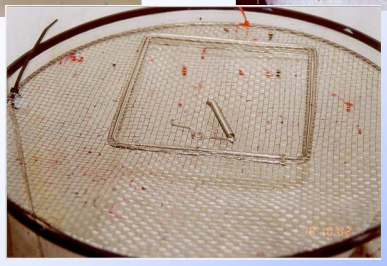
Part of the paste applied in the back of the bat was lost. This loss can't be quantify.



Photos: Marilene Almeida

Conclusions

One much more concentrated vaccine and one neutral vehicle with the adequate consistency should be tested

VACCINE PASTE ROUTE

ULTRAFILTRATION

Other method to concentrate the vaccine in order to obtain one mix (paste + vaccine + color) with the adequate consistency.


Photo: Marilene Almeida

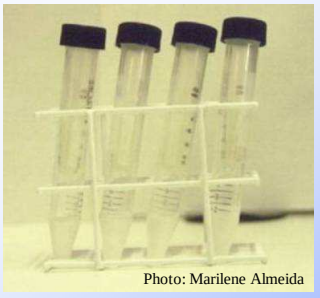


Photo: Marilene Almeida

Ultrafiltration by cellulose membrane with porous of 50.000 Daltons

VACCINE BY MIX ULTRAFILTRATION

Three groups of seven bats each were tested.


Photo: Marilene Almeida


Photo: Marilene Almeida

SURVIVAL AFTER VACCINATION BY PASTE AND CHALLENGE

Ultrafiltration

Survival proportion

71.4% (Groups 9 and 12)

100% (Group 10)

(9 sachets of vaccine – 0.8ml final volume – 7 bats)

Dyalisis

Survival proportion - 71.4% (Groups B and E)

(9 sachets – 1.2/1.5ml final volume - 7 bats)

VACCINE BY MIX ULTRAFILTRATION

Conclusion

The method to vaccine concentration by ultrafiltration improved the prior results increasing the number of animals protected to rabies challenge

Serology		
	paste/dialysis %	paste/ultrafiltration %
after vaccination	71.7	84.2
0.5IU/ml	20	63.1
after challenge	79.3	93.3
sacrifice	57.1	100

The method to vaccine concentration by ultrafiltration improved the prior results in the serology