

**CONTROL OF RABIES IN HERBIVORES - TECHNICAL MANUAL**  
**Brazil - 2009**

(Translated version)

MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY SECRETARIAT OF ANIMAL  
AND PLANT HEALTH  
PORTARIA Nº 168 OF 27 SEPTEMBER 2005.

THE SECRETARY FOR ANIMAL AND PLANT HEALTH OF THE MINISTRY OF AGRICULTURE, LIVESTOCK AND FOOD SUPPLY, using the powers vested in him by Article 42, of Appendix I, Decree no. 5,351, enacted 21 January, 2005, pursuant to Normative Instruction 5, dated 1 March, 2002, and case file no. 21000.004608/2005-04, resolves to:

Article 1. Approve the TECHNICAL MANUAL FOR THE CONTROL OF RABIES IN HERBIVORES, 2005 edition, drafted by this Secretariat's Department of Animal Health, for use by government personnel in the activities of Brazil's National Program for the Control of Rabies in Herbivores throughout the territory, attached to the present Ordinance.

Article 2. Order the publication and ample dissemination of the Manual, which is to be placed on the Ministry of Agriculture, Livestock and Food Supply website.

Article 3 This Ordinance shall come into force on its publication date.

GABRIEL ALVES MACIEL

**Foreword**

Since its identification in Brazil's herds, rabies has resulted in serious losses to the national livestock patrimony, and requires a firm commitment by Brazilian society to seek its effective control. The issue is a complex one, and ongoing discussion is necessary in order to standardize control measures and enhance working conditions, as well as permanently train the personnel carrying them out. Steady progressive reduction in the occurrence of the disease will essentially depend on the quality and continuity of such actions.

For this reason, during the National Meeting of the Program to Control Rabies in Herbivores, in December 2004, a proposal for a Manual was put forward in order to provide technical support to the rabies control activities in herbivores throughout Brazil.

Since then, coordinated by PNCRH, the proposal initially put forward has received valuable contributions from professionals throughout Brazil, enabling this study to be consolidated.

Jorge Caetano Junior  
Director of DSA/SDA/MAPA

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AC	Competent Authority
ALGORITHMS	Rules or a set of rules that specify how to solve a problem.
PRIMO-VACCINATED ANIMALS	Animals vaccinated for the first time
POSITIVE CASE OF RABIES	Either a case proven by laboratory results, or all animals with clinical pictures compatible with rabies encephalitis
SUSPECTED CASE OF RABIES	any sick animal with a clinical picture suggestive of rabies encephalitis, with epidemiological history.
CGCD	General Coordination for Combating Diseases
COSALFA	South American Commission for Combating Foot and Mouth Disease
CRMV	Regional Veterinary Medical Council
DSA	Department of Animal Health
EEB	Brazilian abbreviation for BSE—Bovine Spongiform Encephalopathy
EFFECTIVE CONTROL	the situation achieved by the State, or part of the State (of Brazil), where the incidence of rabies in herbivore is virtually zero, without vaccination, and with population control of <i>Desmodus rotundus</i> and an active surveillance system.
OUTBREAK OF RABIES IN HERBIVORE IS PRIMARY OUTBREAK	Any farm where at least one positive case of rabies in domestic herbivores has been found and where epidemiological investigation confirms that the animal was infected in that place. A farm where the disease likely manifested first in a given sanitary episode.
GPS	Global Positioning System.
GTA	Animal Movement Permit (GTA - <i>Guia de Trânsito Animal</i> )
IBAMA	Brazilian Institute for the Environment and Renewable Natural Resources
INCIDENCE	Number of new cases of infected animals in a given population, during a given period.
MAPA	Ministry of Agriculture, Livestock and Food Supply
INDEPENDENT VETERINARIAN	A Veterinarian who is not part of the Official Inspection Service
OFFICIAL VETERINARIAN	Veterinarian of the Official Veterinary Service
PREDICTIVE MODEL	A model able to carry out some type of prediction.
MS	Ministry of Health

OIE	World Animal Health Organization
WHO	World Health Organization
OPAS (PAHO)	Pan-American Health Organization
PANAFTOSA	Pan-American FMD Center.
PECRH	State-Level Program for Controlling Rabies in Herbivores
PNCRH	National Program for Controlling Rabies in Herbivores
PREVALENCE	This means the total number of cases of the disease, in a specific period, represented by a proportion of the total number of animals in the population.
OWNER	Anyone who is a depositary possessor, or keeps domestic herbivores in his or her power in any capacity.
HERD	A collectivity of animals raised under common handling conditions, in a single raising establishment.
SDA	Secretariat of Animal and Plant Health
SEDESA	Animal and Plant Health Service of the Federal Agricultural Superintendent's Office
SFA	FEDERAL SUPERINTENDENCY OF AGRICULTURE
SIVCONT	Continental Surveillance System
SNC	Portuguese abbreviation for Central Nervous System—CNS
SVS	The Ministry of Health's Secretariat for Health Surveillance.
Official Animal Health Service	The Animal Health Service at federal, state or municipal level.
ULV	Portuguese abbreviation for Local Veterinary Unit—an office of the state animal health service, coordinated by an official veterinarian, and in charge of surveillance and veterinary response activities in one or more municipalities.

## 1. INTRODUCTION

Rabies is considered to be one of the most important zoonoses in public health, not only because of its drastic course and lethality, but also because of its heavy social and economic costs.

Bovine rabies is estimated to cause hundreds of millions of dollars' worth of damage in Latin America, through the death of thousands of head, as well as indirect expenses resulting from the vaccination of millions of cattle and countless post-exposure treatments (serovaccination) for people who have been in contact with suspect animals.

The main vector of rabies in herbivores is the common vampire bat of the species *Desmodus rotundus*. Because this species is abundant in livestock raising regions, several Latin American countries have developed programs to control it, because the vaccination of domestic animals prevents neither the occurrence of economic losses, nor the propagation of this viral infection among wild populations.

In 1966, through its Animal Health Division, the Ministry of Agriculture introduced a Plan to Combat Rabies in Herbivores, currently known as the PNCRH, now executed by the Department of Animal Health (DSA), of the Ministry of Agriculture, Livestock and Food Supply (MAPA).

PNCRH aims to control rabies in herbivores throughout Brazil, rather than enabling coexistence with the disease. It achieves this goal by strategically vaccinating susceptible species and by controlling the population of its main vector, *Desmodus rotundus*, along with other prophylactic and surveillance measures. The federal legislation approving Technical Norms for the Control of Rabies in Herbivores in Brazil is currently Ministerial Normative Instruction n° 5, dated 1 March 2002 (Appendix I).

Several states of Brazil have their own legislation giving specific measures to implement the program at a state level, supporting the federal norms.

These states carry out organized programs, with activities defined not only to control *Desmodus rotundus*, but also educational activities, laboratory diagnosis, the promotion of vaccination of domestic herbivores, registration of the bats' roosting sites and epidemiological surveillance. All of these organized actions need

to be extended to every state in Brazil.

This Manual covers the procedures and activities recommended by PNCRH in order to standardize veterinary care and sanitary surveillance, thus enabling prompt and high quality response to signs of the risk of recurrence of rabies in domestic herbivores throughout Brazil. Its main aim is to underpin the animal health services in their control of rabies in herbivores, enabling the choice of the best strategy to be employed in each situation encountered.

In light of surveillance in the ruminants in Brazil for all neurological syndromes, but particularly rabies, bovine spongiform encephalopathy and scrapie, Ministerial Normative Instruction nº 5, in article 2, made it mandatory for owners immediately to notify the Official Veterinary Service of the occurrence suspected occurrence of cases of rabies.

## 2. BRIEF BACKGROUND INFORMATION

Paralytic rabies of bovines was diagnosed for the first time in the state of Santa Catalina by Carini (1911), when Negri bodies were identified in the nerve tissues of the brains of bovines killed by an as-yet unknown disease. Settlers in the region believed that the disease was being caused by hematophagous bats, and Carini mentioned this in his article which was published in *Annales de L'Institut Pasteur de Paris*. At that time, researchers dubbed Carini's report as a mere tropical fantasy. Haupt and Rehaag, two German veterinarians hired by the Santa Catalina government, identified rabies virus in the brains of hematophagous bats in 1916. Their report was hotly contested because, as with Carini's, the world was reluctant to accept that bats could be "reservoirs" of rabies virus, especially since that time Louis Pasteur declared that "for it to be rabies, there would have to be the involvement of a rabid dog". In the Santa Catarina episodes there were no reports of the disease in dogs. Between 1925 and 1929 botulism was reported in bovines and ascending poliomyelitis in humans in the island of Trinidad, in the Caribbean. Two physicians, Hurst and Pawan, confirmed that the disease in bovines and humans was rabies, and that it was transmitted by vampire bats. The studies of Queiróz Lima (1934), Torres and Queiróz Lima (1935) and Hurst and Pawan (1931- 1932) finally led to acceptance of the idea that vampire bats could transmit rabies to animals and Man.

Today, thanks to advances in research and the implementation of methodologies enabling greater understanding of rabies in all its aspects, we have technological tools for the epidemiological surveillance of the disease. The introduction of molecular biology techniques and the use of monoclonal antibodies have enabled the main wild reservoirs of rabies virus to be discovered, and their association with geographical areas.

In 1996 PAHO began a project to study the molecular epidemiology of the rabies virus isolated in the Americas and in the Caribbean, including the use of a panel of monoclonal antibodies provided by the Centers for Disease Control and Prevention (CDC), Atlanta, USA. The study enables certain reservoirs to be associated with antigenic variants of the rabies virus, such as variant 3, which is associated with the hematophagous bat *Desmodus rotundus* (the main reservoir in Brazil); variants 1 or 2, related to rabies in populations of dogs; and also variant 4, related to the rabies virus maintained and transmitted by populations of the insectivorous bat *Tadarida brasiliensis* and other established populations.

## 3. INSTITUTIONAL RESPONSIBILITIES

Coordination, the drafting of norms, and oversight of the activities of the PNCRH are the responsibility of MAPA, as are the definition of strategies to prevent and control rabies, and the accreditation of laboratories for the diagnosis of rabies and other diseases with nerve symptomatology.

It also falls to MAPA to promote information system and surveillance actions, carry out audits on the state level animal health services, health education activities, capacity building for human resources with continual updating of techniques in all procedures (surveillance, prevention, diagnosis, prophylaxis and control), as well as to provide financial support by means of agreements for the activities in the control of rabies in herbivores performed by the State Secretariats of Agriculture.

In all the states of Brazil, as well as in the Federal District, control of rabies in herbivores is coordinated and supervised by the Federal Agricultural Superintendents' Offices (*Superintendências*—SFA), which have technical personnel trained to carry out animal health defense activities.

Every SFA has a SEDESA, where an official MAPA veterinarian is responsible for managing PNCRH in the state.

The State-level Animal Health Organizations are responsible for implementing the activities of the PNCRH, in its operationalization in the State, and above all the registry of farms, the registration and monitoring of the roosting sites of hematophagous bats, the implementation of surveillance in at-risk farms and areas, and response to outbreaks of the disease. Furthermore, there are health education activities, the organization and participation of organized society in municipal animal health committees, promotion and inspection of vaccination of herds, and the capacity-building for human resources and the regular supply of information to MAPA concerning activities to control rabies in herbivores in the state.

It falls to the accredited laboratories to process suspect samples sent in order to confirm the rabies diagnosis, and to submit negative samples to differential diagnosis. Whenever there are positive results, the central body of the State Animal Health Defense Service, and the State Secretariat of Health must be immediately notified. If the samples taken from herbivores have been sent in by independent veterinarians, other professionals, or by the owners, the animal health defense service must be notified of the suspected case so that an official veterinarian can be sent to visit the farm. If the samples have been sent from other states of the country, laboratories must inform the SEDESA of the state of origin of the suspected case and send results there, as well as to the State Secretariat of Health, in compliance with their respective jurisdictional competencies.

In order to boost interactive participation and make PNCRH more transparent and more credible, MAPA set up a Consultative Scientific Committee on Rabies, made up of a multi-institutional and multidisciplinary working group bringing together specialists from the fields of health, agriculture, the environment, from research institutions and other such institutions, in order to provide DSA with technical and scientific support. It is for this consultative committee to issue technical opinions and draft proposals to improve Brazil's system for the control of rabies in herbivores and put forward norms on surveillance and prophylaxis against rabies.

The states are encouraged to set up Consultative Scientific Committees to discuss and evaluate strategies to implement control and prophylaxis against rabies in herbivores in their territory.

#### 4. CURRENT SITUATION AS REGARDS RABIES IN HERBIVORES IN BRAZIL

Rabies in herbivores may be considered endemic in Brazil, although it varies according to the regions.

The main factors leading to the insidious and worrying spread of rabies in Brazil are:

- increased availability of food, represented by marked growth in the size of herds; chaotic land occupation, characterized by huge environmental changes such as deforestation, the building of highways and hydro-electric power plants (HEPPs), which change the environment in which the bats live, forcing them to look for new areas and other sources of food;
- the availability of artificial roosting sites in the shape of construction works like tunnels, water tanks, abandoned houses, culverts, decommissioned charcoal furnaces, and so on;
- deficient execution in some states of Brazil of the State Program to Control Rabies in Herbivores.

The historical series of notified cases of rabies in herbivores, by State and Year, can be consulted at ([www.agricultura.gov.br](http://www.agricultura.gov.br)).

#### 5. NOTIFICATION OF THE OCCURRENCE OF RABIES

The owner must immediately notify the Official Veterinary Service of suspected cases of rabies in herbivores and of the presence of animals with bite marks of hematophagous bats, or of their roosting sites. Failure to notify jeopardizes the health of herds in the region and may place humans themselves at risk of the disease. Rabies is compulsorily notifiable; therefore owners who fail to do so may face legal punishment.

Whenever the Official Veterinary Service is notified of the suspicion of Rabies in herbivores and of the attack on the herd by vampire bats, it must respond as rapidly as possible to the notification. When necessary, material must be collected for laboratory diagnosis as laid down in the Manual of Procedures for the Diagnosis of Central Nervous System Diseases in Bovines (on the MAPA website at [www.agricultura.gov.br](http://www.agricultura.gov.br)), and the control of the *Desmodus rotundus* population in the region, and guidance must be given about rabies vaccination in and around the outbreak site.

Most importantly, material suspected of rabies must be sent to the laboratories by:

- Official Service Veterinarians. The State Animal Health Service must always prioritize the involvement of official veterinarians;

- Independent veterinarians may also send the material for analysis. These professionals must be aware that whenever there is a suspected case of rabies, they must notify the competent sanitary authority in the region quickly. It is recommended that the State Animal Health Service send an official letter via the CRMV to all the state's private-sector veterinarians to inform them of the activities of execution of the program as well as of the addresses of the veterinary care units and official veterinarians responsible for the State Program to Control Rabies in Herbivores (PECRH).

- Other professionals or owners. Because Brazil is so vast and many farms are hard-to-reach, and furthermore because in many states the municipalities do not have either an official or a private veterinarian, samples are sometimes sent for diagnosis to laboratories by the owner or by other professionals. In this case the recommendation is sometimes to send the head of the suspect animal to the laboratory, or in the case of small wild animals, the entire body should be sent.

#### SAMPLES SENT TO THE LABORATORY MUST ALWAYS GO WITH THE SINGLE FORM FOR REQUESTING TESTS FOR NEUROLOGICAL SYNDROME (Appendix II)

All professionals directly involved in disease-control activities must receive preventive vaccination themselves, and their immunity must be proven by serology, as determined by the WHO (World Health Organisation).

Compliance with this demand will also be subject to a PNCRH audit.

#### 6. PROGRAM STRATEGY

The program strategy is fundamentally underpinned by:

- Epidemiological surveillance;
- Guidance for the vaccination of domestic herbivores;
- Control of hematophagous bats of the species *Desmodus rotundus*, whenever there is a risk of transmitting rabies to herbivores.

#### 7. CHARACTERIZING AT-RISK AREAS

From 1910 to 1940, bovine rabies was mainly confined to the coastal regions of Brazil, possibly as a result of land occupation processes. Deforestation of the Atlantic Rainforest to obtain access to more fertile land, the introduction of cattle raising and the building of highways, railways, dams, tunnels, reservoirs, the channeling of rivers and creeks - all these were factors that changed bats' habitats, especially those of vampire bats. Later on, outbreaks of bovine rabies occurred in the hinterlands of states, *pari passu* with major environmental changes brought about by such activities as livestock-raising and mining.

The epidemiology of bovine rabies involves natural factors, such as a favorable habitat for the bats, the presence of rabies virus in the forest cycle, and social factors that establish how Man performs economic activities in nature. The epidemiology of bovine rabies is therefore directly influenced by environmental factors triggered by humans; in order to understand the epidemiological model of bovine rabies, therefore, the organization of space must first be understood.

The key for characterizing at-risk area for rabies in herbivores is a function of how Man appropriates the geographical space.
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Understanding the socio-economic drivers behind the occurrence, maintenance and evolution of bovine rabies is crucially important, both in order to explain its epidemic behavior and to determine more effective measures to control it in endemic regions.

The epidemiological model proposed aims to identify and monitor the presence of rabies virus in the

*Desmodus rotundus* population.

The core of the problem is the ecology of *Desmodus rotundus*, and this is the basis of the proposed model.

Herbivores are accidental hosts for the rabies virus, because, despite being part of the epidemiological chain of rural rabies, they only contribute as sentinels for the existence of the viruses. Their part in the process is restricted to the death of the animal rather than any involvement in the transmission process to other species - except accidentally. This statement is based on the fact that rabies in herbivores has little or no likelihood of transmission to other animals; its main characteristic is paralytic, unlike the furious symptomatology which one finds in rabies among carnivores.

Occupation of the space will condition the ecological behavior of the transmitter; this behavior is driven by the availability of shelter and food.

Given how *Desmodus rotundus* interacts with the environment, the risk of rabies in herbivores may be explained by two major components:

RECEPTIVENESS is a set of variables that express the ecosystem's ability to shelter *Desmodus rotundus* populations. The drivers of receptiveness are the availability of food and shelter.

The main drivers of receptiveness are:

- Food-related:
  - density of herbivores/usable area;
  - herbivores/pasture area;
  
- Related to suitable roosting sites:
  - % of the area with limestone soil;
  - % of the area under permanent forests; steepness of the terrain;
  - number and location of permanent and temporary natural shelters;
  - number of artificial shelters and number of buildings with potential, used by the transmitter as a shelter;
  - altitude.

Receptiveness may be classified as high, medium, low or null, depending on the presence and intensity with which the above-mentioned factors are expressed, and the inter-relations among them.

VULNERABILITY is a set of factors related to the ability of a transmitter to enter an area and to viral circulation. These factors enable diffusion of the disease to new areas, and they facilitate the process.

The drivers of vulnerability are:

- the construction of HEPPs, deforestation, the building of highways and railways, the making of new pasture areas, the disruption of the food supply, flooding and other environmental changes;
- cases of rabies in herbivores or the presence of *Desmodus rotundus* in the municipality and/or in neighboring municipalities; cases of rabies in other chiroptera and mammals (variant 3).

Vulnerability may be high, medium, low or null, depending on the presence or inter-relationship of the cited factors.

The predictive risk model takes both receptiveness and vulnerability into consideration. The database should be fed every twelve months in order to predict in timely fashion the implementation of the proper control measures. The vulnerability factors observed should be given, both retrospectively (up to two years prior to the first report) and prospectively, when such information is available; for prospective data, up to the next updating of the database.

Information gathering for the database, whether through active or through passive surveillance, should be deemed to be a pillar of the program. As soon as problems are detected concerning a given region, such as the absence or inconsistency of information, it will be treated as a silent area.



The database to build the predictive model will use municipalities as the epidemiological units of interest.

The algorithm used by the risk model will take the association between receptiveness and vulnerability into consideration. At municipality level this association will provide a risk score, to be plotted onto a geotagged map of the topographical grid map of Brazil's municipalities, enabling areas of greater or lesser risk for rabies, and areas where the disease is already present, to be seen. This will enable specific localized control measures to be triggered, and the major benefit will be that the State Animal Health Service will be proactive thus optimizing time and resources.

Taking one or other control measure, or several together, will depend on the risk situations that the predictive model will show.

## 8. VACCINATING DOMESTIC HERBIVORES

Normative Instruction no. 5, dated 1 March 2002, sets forth that vaccination of herbivores requires an inactivated virus, at a dosage of 2ml per animal, regardless of age, given subcutaneously or intramuscularly.

Compulsory vaccination is recommended during an outbreak of the disease and should preferably be adopted in bovidae and equidae of three months of age or over. However, in animals below three months of age, guidance may be given on a case by case basis in accordance with the technical evaluation of a veterinarian.

Primovaccinated animals must be revaccinated 30 days after the first vaccination. It should be stressed that animals born after vaccination of the herd must be vaccinated when they reach the recommended age of three months.

States may bring in supplementary vaccination on the need for compulsory systematic vaccination in at-risk areas, based on the model shown in the preceding item.

## COMPULSORY VACCINATION SHOULD BE TEMPORARY AND SUSPENDED AS SOON AS THE STATE PROGRAMS REACH SATISFACTORY LEVELS OF RABIES CONTROL, ENSURING THE SANITARY CONDITION OF THE HERDS.

When a State decides to adopt compulsory systematic vaccination, the suggestion - in order to ensure compliance with it - is to introduce a requirement for proof of rabies vaccination whenever Animal Transit Permit GTAs are issued.

To prove vaccination, the owner of the animals must be required to present an invoice for the purchase of the vaccine giving the batch number, expiry date and laboratory where the vaccine was produced. The owner must also give the date of vaccination and the number of animals vaccinated by species.

For the purposes of revaccination, the duration of immunity provided by the vaccine will be deemed to be 12 months maximum.

## 9. CONTROL AND SALE OF RABIES VACCINES AND VAMPIRICIDAL PRODUCTS

### a) Rabies Vaccines:

In Brazil, all rabies vaccines are produced in cell culture and undergo quality control (innocuousness, sterility, effectiveness and potency) at MAPA's National Animal and Plant Laboratory in Campinas, SP. After approval the batch of vaccines may only be sold when given a holographic seal to guarantee quality. From production to application the rabies vaccine will be kept refrigerated within a 2°C to 8°C temperature range, without direct exposure to sunlight. In retailers, immunobiologicals must be kept in exclusive freezers with two thermometers for maximum and minimum temperatures.

This vaccine must never be frozen. Freezing changes the vaccine's components and disrupts its immunogenic power.

The expiry date printed on the vial must be strictly followed. State Animal Health Services will implement the system to control the sale of rabies vaccines for use on domestic herbivores, and retailers will be obliged to

notify purchase, sale and stock of vaccines, by batch and laboratory.

Retailers may only sell vaccines that are properly stored in isothermal boxes, containing ice, guaranteeing maintenance of the legally-required temperature.

b) Vampiricidal products:

State Animal Health Services will operate a system to control the sale of these bat poisons, and retailers will be obliged to notify their purchase, sale and stock, identifying the purchaser, farm and municipality, all of which information is easily found in the invoice.

The expiry date printed on the packaging must be strictly followed.

Official veterinary services must carry out periodical visits to livestock-farmers using these products in order to understand the true dimension of the problem, and identify the rate of attack by hematophagous bats on the herd on the farm and herds in the region, and to provide guidance to farmers as to the necessary precautions and handling of these products.

## 10. CONTROL OF VECTORS

Vampire bats are found from the north of Mexico to the north of Argentina and on some Caribbean islands, in regions where the average altitude is below 2,000m. There are only three hematophagous species worldwide: *Desmodus rotundus*, *Diphylla ecaudata* and *Diaemus youngi*, and which are found in Brazil. *Desmodus rotundus* is the main vector transmitting rabies to herbivores, because it is the most abundant species of hematophagous bat and herbivores are its major source of food. Herbivores may, rarely, become infected in attacks by dogs, cats and other rabid wild animals.

Herbivore rabies control teams must be completely familiar with the region where they work and with the potential transmitters occupying the area. Appendix III describes the equipment necessary to effect capture. The method chosen to control vectors will depend on the animal species involved, the topography and possible legal restrictions (environmental protection areas, indigenous peoples' reserves and so on). The method used to control hematophagous bats is based upon the use of anticoagulant substances, particularly warfarin.

The control methods must be selective and executed correctly so as to target *Desmodus rotundus* specifically without causing harm or disruption to other species that play an important role in the ecological balance.

Selective methods may be direct or indirect:

- In the direct selective method the hematophagous bat must be captured and the vampiricide applied topically to its back. Ingested by a bat that has contact with it, the active ingredient causes internal hemorrhage, killing it (Figure 1). To implement this method, the hematophagous bat must preferably be captured near its source of food (capture near the livestock pen). *Desmodus rotundus* individuals may be captured directly in their roosting place, when artificial, and close by natural roosting sites like caves and deep sinkholes known as "furnas". When authorized by Brazil's Environment Authority (IBAMA), in exceptional circumstances, bats may be captured inside their natural roosting-sites. Correct execution of the direct selective method may only be conducted by properly trained and equipped personnel of the official services, who must return to the farm to verify whether measures have been effective.

STATE-LEVEL ANIMAL HEALTH SERVICE PERSONNEL, WHENEVER REQUESTED OFFICIALLY AND IN EXCEPTIONAL CIRCUMSTANCES, MAY ASSIST PUBLIC HEALTH AUTHORITIES IN THE CONTROL OF OF HEMATOPHAGOUS BATS THAT ARE ATTACKING HUMANS.

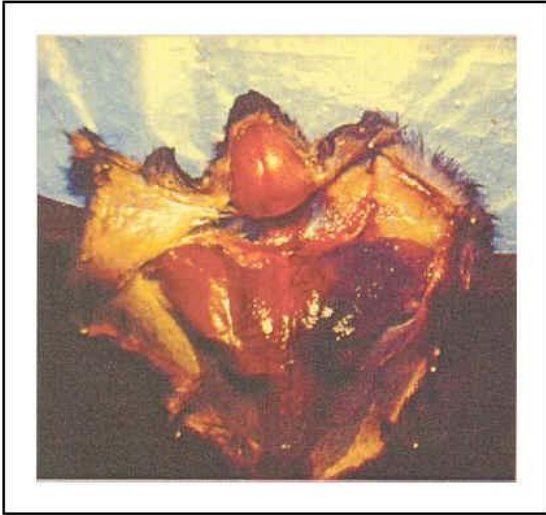


Figure 1: Hemorrhage caused by warfarin in *Desmodus rotundus* (photo Silvia B. Silva)

- In the indirect selective method there is no need to capture the vampire bats. This method consists of topical application of two grams of vampiricide paste around the recent bite wounds caused by the bats. Other vampiricide products may also be used and are particularly useful in livestock. In these control systems only aggressive hematophagous bats are eliminated because they tend to feed to return to the same wound on consecutive days. Topical use of the paste may be repeated while the animal is being targeted by the bats (Figures 2, 3 and 4). This may be done by the owner of the animal victim, guided by the veterinarian, preferably in early evening, and the animal should remain where it had been bitten the previous night. This practice should be encouraged by the State-level animal health personnel.



Figures 2 and 3: Application of vampiricide paste to animal wounds.  
(Photographs: Rogério S. Piccinini)



Figure 4: *Desmodus rotundus* feeding on wound treated with vampiricide paste (photo Rogério S. Piccinini)

Sanitary handling of herds, the habit of monitoring animals for the presence of vampire bat bite wounds. Owing to the importance of this topic, a specific chapter was included on the biology and control of the *Desmodus rotundus* population (Chapter III).

#### 11. REGISTRATION AND MONITORING OF ROOSTING SITES

If control of rabies in herbivores is to be effective, the State Animal Health Service must maintain a routine of registering *Desmodus rotundus* roosting sites / shelters (Appendix V), monitored at least once-annually, within the regional characteristics of each State.

The shelters must be geotagged using GPS. In Chapter IV there is a summary of this tool, which is of fundamental importance for the population control of bats.

When possible, specimens of *Desmodus rotundus* must be collected from the shelter site and sent to the laboratory. If these bats are suspected of carrying rabies, they must be captured and sent to the laboratory for diagnosis.

If rabies is transmitted to herbivores by other wild mammals, the Official Animal Health Service must carry out an epidemiological survey including identification of the virus involved in order to investigate the origin and extent of the outbreak. After the survey has been completed, it must be sent to the IBAMA Regional superintendents office, requesting support for controlling rabies in wild mammal species (identifying which ones), and a copy must be sent to the National management of PNCRH in Brasilia.

#### 12. OTHER EPIDEMIOLOGICAL SURVEILLANCE MEASURES

For the epidemiological surveillance of rabies, there is an information system including mandatory notification of cases with continuous updates.

State-level Coordination Offices for the Program to Control Rabies in Herbivores must maintain up-to-date diagnoses of the epidemiological situation, assessing distribution and drivers of propagation, in order to enable immediate adoption of control / prophylaxis measures for rabies.

The criteria for defining priority in response to notifications, such as the number of animals suspected of being affected by the disease, the number of animals attacked by *Desmodus rotundus* and the average number of attacks against a single animal, must all be evaluated.

There must be continuous exchange of information on cases of rabies within the frontier and the control measures/strategies adopted between the states of Brazil. To facilitate operationalization, an inter-frontier strip of approximately 12km must be adopted.

On international borders, the National Coordination of the PNCRH must be notified of cases of rabies in

herbivores in municipalities along the border, so as to set off the process of communication to neighboring countries. The epidemiological surveillance strategy and the working plan adopted must be revised annually or whenever necessary.

### 13. OPERATIONS IN OUTBREAKS

The implementation of sanitary measures in an outbreak area is the responsibility of the Official Animal Health Service, the executor of the State Program for Control of Rabies in Herbivores.

In a suspected case of rabies or any other nerve syndrome, the official veterinarian must fill out the Initial Disease-Investigation Form (Form-In).

After notification of laboratory confirmation of the diagnosis of rabies, a team will travel to the infected animal's origin farm and carry out epidemiological investigation. This visit must take place within 24 hours of notification.

In accordance with the epidemiological investigation and the information of location and chronological reporting of the notifications, vaccination activities and control of bats will be carried out on the basis of either of the two models presented below (Figures 5 and 6).

The concentric-circle model is more efficient in cases where, in a given region, the outbreaks occur in dispersion without a logical pattern, and where the direction in which further cases will travel cannot be predicted. The second model, linear blocking, is to be used when the outbreaks follow a specific linear path. In this case, spread of the disease may follow the line of a river, a mountain range, the shores of a reservoir, or along a highway or railway. Whatever the circumstance, the blocking actions against the progression of the virus must be carried out from the periphery inwards towards the center. That is because the infected bat may transmit the virus to other colonies up to twelve kilometers away from the initial outbreak.

Attention must be paid to rabies in animals brought onto the farm and transferred during the disease's incubation period. In this case, after epidemiological investigation, the farm of origin may be considered the primary outbreak.

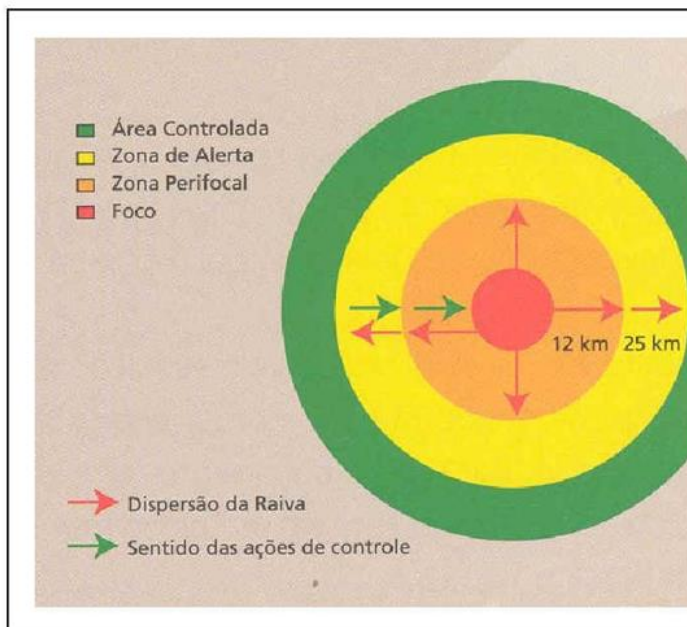


Figure 5: Concentric Circle Model for Response to Rabies Outbreaks (Adapted from Piccinini, R.S, - 1985)

At the discretion of the official veterinarian, focal and perifocal vaccination may be adopted so as to cover all herbivores on farms within a 12 (twelve) kilometer radius, in accordance with local topography.

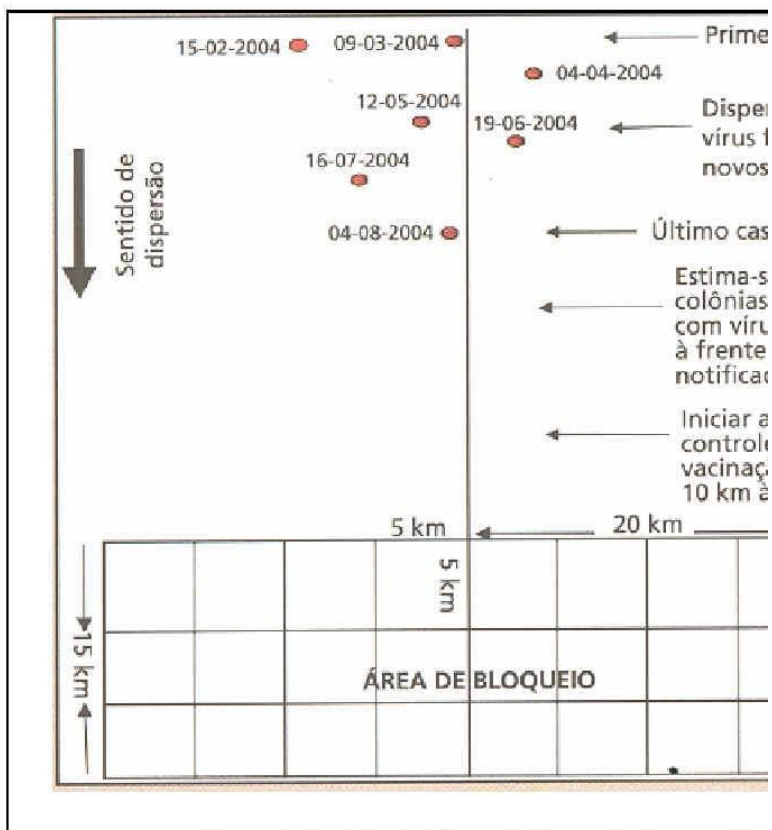


Figure 6: Linear Blockade Model to the Progression of Rabies (Adapted from Piccinini, R.S. 1985)

The control of vectors must be intensified in the outbreak area, in a 12-km radius, respecting the local topography.

If other animals die in this area, it will be the responsibility of the official veterinarian to necropsy them and take samples to be sent for laboratory tests for rabies and other diseases compatible with the clinical signs, such as the other encephalitis diseases caused by poisoning, Aujeszky's disease, cerebral babesiosis, listeriosis, equine encephalitis, and so on.

After the effective sanitary measures recommended to control the outbreak have been taken, new cases of rabies are not expected to occur within a time line equivalent to twice the average incubation period for the disease, which is 45 days. However, if this does occur, the strategy in the outbreak area should be reassessed.

A rabies outbreak should be declared closed 90 days after the last death to occur on the farm, and the final Supplementary Disease-Investigation Form (Form-Com) should be filled out.

Permanent measures to be taken in those regions should be conducted in accordance with the areas' risk classification.

The Disease-Investigation Forms (Form-In and Form-Com) filled out for surveillance of neurological syndromes do not necessarily have to be sent directly to DSA in Brasília, unless the official veterinarian's presumptive diagnosis is of a disease considered exotic or liable to the adoption of emergency actions.

#### 14. SAMPLE TAKING AND LABORATORY TESTS

Laboratory diagnosis is essential for defining an outbreak because it will only be actually deemed a rabies outbreak when there are one or more cases of the disease confirmed by laboratory tests.

The taking of samples from animals suspected of having contracted rabies must be performed by a veterinarian or a professional trained by a veterinarian, and who has received suitable training as well as being properly immunized. However, sampling and sending material suspected of rabies must always be the exclusive responsibility of a veterinarian (official or independent).

Samples of the Central Nervous System must be taken from the animal suspected of infection with rabies. For ruminants this is the encephalon (cortex, cerebellum and brain stem) in accordance with the Manual of Procedures for the Diagnosis of Diseases of the Central Nervous System in Bovines. For equines, this is the encephalon and spinal cord. Material must be sampled for laboratory diagnosis from all animals that died with symptoms compatible with encephalitis diseases.

Bats captured and sent for investigation of the presence of rabies virus must whenever possible have at least 1ml of their blood taken so that 0.2-0.5ml of blood serum can later be sent to the laboratory along with the specimen to be tested. If serum samples cannot be sent, the bats must be anesthetized using ether and sacrificed according to the recommended bioethical principles. The entire animal must be sent, either frozen or chilled, for laboratory testing.

The sample collected must be packed in a vial with a cap or a hermetically sealed double plastic bag, identified and placed into an isothermal box containing recyclable ice to keep the temperature between 2°C and 4°C. The sample for differential histological testing for other encephalitis diseases must be put in a capped vial or a specific plastic bag and fixed in 10% formol. If there is a long delay between sampling and dispatch to the laboratory for rabies diagnosis, it is recommended that the sample be frozen after the parts for differential diagnosis have been separated from it.

**NEVER FREEZE SAMPLES FOR BOVINE SPONGIFORM ENCEPHALITIS (BSE) DIAGNOSIS.**

**THE LABORATORY MUST BE WARNED IN ADVANCE OF THE DISPATCH AND OF THE TIME OF ARRIVAL OF THE SAMPLE, AND SAMPLES SHOULD NOT BE SENT NEAR TO OR DURING WEEKENDS WITHOUT PRIOR COMMUNICATION.**

The sample should preferably be sent ad/or delivered to the laboratory within 24 hours of extraction, in a perfectly sealed isothermal box, bearing the symbol of biological hazard (Figure 7) and a label

with the wording: **URGENT, PERISHABLE BIOLOGICAL MATERIAL.** The Unique Form Requesting Neurological Syndrome Tests (Appendix II) should be attached to the lid of the isothermal box, with case information inside a plastic bag.

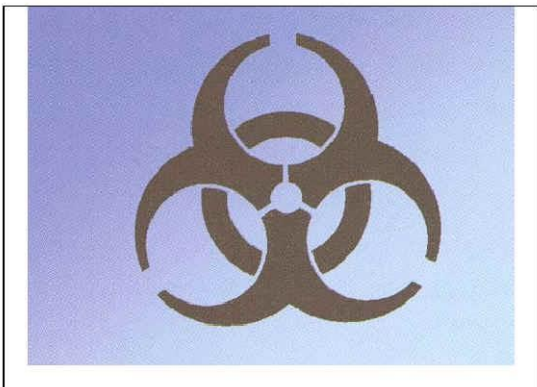


Figure 7: Bio Hazard symbol

Samples from animals suspected of having rabies sent for laboratory testing will be processed by direct immunofluorescence and biological testing (inoculation in mice or in cells). Histopathology or immunohistopathology will provide differential diagnosis.

## 15. INFORMATION SYSTEM

Trade relations in live animals and in animal products between several countries have been significantly affected by health. The need to enhance the credibility of official veterinary services is increasingly obvious in this scenario. Therefore one point that deserves special attention is the transparency of National

Epidemiological Surveillance and Information Systems. The monthly rabies form must be sent to the DSA epidemiology division by the 15th of each following month.

PANAFTOSA, supported by several countries including Brazil, recently made changes in the Continental Information System, setting up a computerized system known as SivCont which enables web access to any unit informing the database from any country. SivCont underpins the effectiveness of National Surveillance Systems in three ways:

- sensitivity: related to the capacity to detect episodes of a given disease (passive + active mechanisms to detect events);
- specificity: the ability to rule out other sanitary events detected which might present clinical signs compatible with the disease for which the surveillance is being carried out;
- timeliness: how fast the information leads to action.

Within this new concept, the core of weekly communications is no longer the quadrant of the country where the disease has been suspected of occurring, and is now the notification of events based upon observation of syndromes compatible with the diseases that are the target of surveillance. Furthermore, the unit of observation is now Notification, and the quadrant is reinforced as the epidemiological unit of geotagging, and the information per Epidemiological Week is replaced by the concept of Week of Acquisition of Information: each country must inform the Continental System at least once a week, so that it is possible to classify the event by Epidemiological Week starting from the Date of Notification or of Probable Commencement, which enables better temporal evaluation of the events that have occurred.

SivCont is configured for three groups of syndromes:

- Vesicular Syndromes: the target diseases for this group are foot-and-mouth disease and vesicular stomatitis;
- Swine Hemorrhagic Syndromes: the target diseases for this group are Classical Swine Fever and African Swine Fever;
- Nerve or Neurological Syndromes: the target diseases for this group are rabies, BSE and equine encephalitis.

SivCont thus provides essential data for the characterization of areas at risk for rabies, such as the number of outbreaks and their respective geographic coordinates.

## 16. HEALTH EDUCATION AND DISSEMINATION OF PREVENTIVE ACTIONS

The major goal of animal health education is to promote animal, human and environmental health by raising awareness and by the resulting commitment of all links in the production chain and society at large.

To achieve this goal for rabies, the techniques, resources and means of communication - as well as specific educational activities to involve cattle farmers effectively in the immediate notification of each and every suspected case of rabies as well as of the notification of animals attacked by hematophagous bats and of awareness of the existence of bat refuges - must be used. They must also be advised to use vampiricide paste on animals that have been bitten.

In seeking solutions effectively to control rabies in herbivores, the organization of a range of social representations in the community, such as farmers' associations, rural unions, cooperatives, rural societies, government and non-governmental organizations, municipal or intermunicipal animal health councils, integrated with a state-level council, all make for a favorable situation for liaison and execution of pre-established disease-control measures. Municipal Health Councils and Rural Development Councils should also take part.

The educational activities of professionals involved in the program should encourage farmers to change their behavior, so that they:

- a) inform the Animal Health Service nearest to their farm of any suspicion of Rabies or of the attacks by hematophagous bats on animals on their farms or in the region;
- b) vaccinate their herds whenever necessary;



- c) apply vampiricide paste around recent lesions, in their cattle, provoked by hematophagous bats;
- d) notify official veterinarians of the deaths of animals.

## 17. TRAINING OF PERSONNEL INVOLVED IN THE PROGRAM

The Central Coordination of Federal and State-level Animal Health Services must enable continuous education programs to be held so that professionals, technicians and auxiliaries who are in charge of controlling rabies in herbivores in their respective areas can receive specialized on-going training in epidemiology, biostatistics, the planning and administration of health campaigns, laboratory diagnosis, the ecology of bats, the control of hematophagous bats and in the methodology of health education.

## CHAPTER II - REVISION OF RABIES 1.

### 1. INTRODUCTION

Rabies is an acute disease of the CNS - Central Nervous System that can affect all mammals including humans. It is characterized by a fatal encephalomyelitis caused by a Lyssavirus.

The World Animal Health Organization (OIE), in its Terrestrial Animal Health Code, lists rabies in the category of diseases common to several species.

### 2. ETIOLOGY

The order Mononegavirales groups those viruses made up of single-stranded RNA (ssRNA), that are unsegmented and with negative-polarity. The following families are included: Filoviridae, Paramyxoviridae, Bornaviridae and Rhabdoviridae.

Rhabdoviridae are sub-divided into two plant virus groups, one fish virus and three mammalian virus groups, the latter being the genera:

- Vesiculovirus, linked to vesicular disease in animals;
- Ephemerovirus, linked to ephemeral fever in bovines;
- Lyssavirus, linked to fatal encephalomyelitis in mammals.

Currently, viruses of the Lyssavirus genus are included in seven genotypes, in accordance with a resolution of the International Committee on the Taxonomy of Viruses (ICTV), and an eighth genotype has been put forward.

Meeting in Niagara Falls, EUA, in 1994, rabies experts put forward a change to "genotypes" to replace "serotypes", the term used up until that time to designate different members of the Lyssavirus genus.

The Rabies virus has a characteristic morphology: it is bullet-shaped, with an average diameter of 75nm and a length ranging from 100nm to 300nm, depending on the sample. The virion comprises a coating made up of a double phospholipid membrane from which glycoprotein spiculae of approximately 9nm emerge. The coating covers the coiled nucleocapsid made up of negative, non-segmented, single-strand RNA.

Rabies virus is usually transmitted by direct contact; it is vulnerable to chemicals (ether, chloroform, mineral salts, strong alkali and acids), to physical agents (heat, ultraviolet light) and to environmental conditions, such as dessication, luminosity and excessive temperatures. For the chemical disinfection of surgical instruments, clothing or the setting in which the necropsy of a rabid animal will take place, several agents are indicated: 2% hypochloride, 10% formol, 1-2% glutaraldehyde, 2% sulfuric acid, 5% phenol and hydrochloric acid, 1% creolin, and others. For disinfecting rooms, 0.25 - 0.9% formalin solution, and 1%-2% sodium bicarbonate rapidly and efficiently inactivate the viruses. Loss of infectiousness occurs in two minutes at 80°C, and in sunlight in 14 days at 30°C.

Even in adverse environmental conditions rabies virus can maintain infectiousness of relatively long periods, and is naturally inactivated by autolysis. Putrefaction destroys the virus slowly, over approximately 14 days.

### 3. CHARACTERIZATION OF VARIANTS ISOLATED IN BRAZIL

Antigen typification with monoclonal antibodies (Mabs), developed in 1978 by Victor & Koprowski, and nucleotide sequence analysis more recently, have been used throughout the world to identify the viral variants associated with rabies outbreaks. The data, in conjunction with data obtained by epidemiological surveillance, can effectively help identify the animal reservoir involved.

Six pre-established antigen profiles have been identified in Brazil since 1996 using an indirect immunofluorescence test and a panel of monoclonal antibodies against the viral nucleoprotein, produced by the Centers for Disease Control and Prevention (CDC), Atlanta, USA, and pre-established by OPAS to study samples isolated in the Americas:

- variant 2 dog, also isolated in humans and wild terrestrial animals;
- variant 3 *Desmodus rotundus*, also isolated in other bat species, pets, domestic animals, wild animals and humans;
- variant 4 *Tadarida brasiliensis*, also isolated in other non-hematophagous species and pets;
- a variant similar to variant 5 and also linked to the isolation of hematophagous bats in other countries, isolated in non-hematophagous bats and in pets;
- variant 6 *Lasiurus cinereus*, isolated in insectivorous bats and a profile that shows positive reactions to all the Mabs used, observed in samples of non-hematophagous bats, dogs and humans.

In addition to these variants, a further six antigen profiles not compatible with those pre-established in the panel have been observed, linked to insectivorous bats and affecting other animals, in addition to a profile linked to humans and small primates such as marmosets (*Callithrix jacchus*) in the north-east of Brazil. These different profiles, in later genetic studies, were sometimes linked to reservoir species, as is the case of the variant isolated in north-eastern marmosets or the variant associated with insectivorous bat *Histiotus velatus*.

The particular nucleotide exchanges detected in the different isolations of rabies virus in the field enabled identification of viral variants associated with different endemic cycles or coming from different domestic and sylvatic reservoirs. However, phylogenetic studies of these variants are not very important if one does not have epidemiological surveillance data corresponding to each case in order to identify the circumstances in which the outbreak was triggered, as well as the animal species involved and those aspects that enabled perpetuation of the virus in nature.

### 4. TRANSMITTERS

In countries where canine rabies is controlled and there are no hematophagous bats, the major transmitters are terrestrial sylvatic animals such as foxes (*Vulpes vulpes*), coyotes (*Canis latrans*), wolves (*Canis lupus*), Arctic foxes (*Alopex lagopus*), raccoon-dogs (*Nyctereutes procyonoides*), common raccoons (*Procyon lotor*), and skunks (*Mephitis mephitis*), to name a few.

On the other hand, where the disease is not controlled, such as most countries on the continent of Africa, Asia and Latin America, the virus is maintained by several species of domestic and wild animals.

In Brazil, the major animal vector for rabies in humans remains the dog, although bats are increasingly implicated, and may in fact be the main species responsible for maintaining the virus in the wild. Positive identification of rabies virus has been described in several wild animals of the Brazilian fauna, such as the "hoary zorro" (*Dusicyon vetulus*), skunks (*Conepatus sp.*), raccoons (*Procyon cancrivorus*), marmosets (*Callithrix jachus*), Savannah foxes (*Cerdocyon thous*), and both hematophagous and non-hematophagous bats.

### 5. PATHOGENESIS

Pathogenesis describes the pathway taken by viruses, from their point of inoculation (Gateway of entry) up until their elimination or shedding:

a) Gateway of entry:

Inoculation of rabies virus particles in a susceptible animal's organism occurs through skin lesions usually

caused by the bite of an infected animal which is shedding viruses in its saliva. It is also possible that infection is caused by wounds or other discontinuities of the skin, when in contact with the saliva and organs of infected animals. The likelihood of blood, milk, urine or feces containing a sufficient quantity of viruses to trigger rabies is remote.

There have been reports of the transmission of rabies orally. The exact mechanism involving oral transmission has not yet been clarified, although one of the ways of immunizing wild animals currently adopted by some countries is by means of baits (for ingestion) containing attenuated virus vaccines. Incidents suggestive of oral or nasal infection have been reported concerning human rabies transmitted by aerosols in laboratories and in caves densely populated by bats. In humans, transplantation of corneas and other infected organs has been linked to the development of rabies in the recipient patients.

#### b) Incubation Period:

The variability of the incubation period depends on such factors as invasive capacity, pathogenicity, viral load of the initial inoculum, point of inoculation (the closer to the CNS, the shorter will be the incubation period), age, the animal's immunocompetency, and other factors.

In humans the average incubation period is from 20 to 60 days, although there have been reports of exceptionally long periods. In turn, determination of the natural rabies incubation period in animals is hard to prove, because it is difficult to register the exact moment when the virus is inoculated. However, experimental infection studies carried out with different animals, and using viral samples from different origins, have shown variations, with extremely long or extremely short periods.

In dogs, the average incubation period is from 3 to 8 weeks, with extremes ranging from 10 days to 6 months. In skunks (*Mephitis mephitis*) periods ranging from 105 to 177 days have been observed, 20 to 165 days in bovines experimentally submitted to biting by infected *Desmodus rotundus*, 60 to 75 days in bovines kept in the field and 25 to 611 days in bovines experimentally inoculated intramuscularly. In experiments involving intramuscular inoculation in Rinds and bovines with samples of rabies virus obtained from *Dusicyon vetulus*, from north-eastern Brazil, the incubation period ranged from 17 to 18 days. In asses, inoculation with the same sample produced a period ranging from 92 to 99 days and in equines from 179 to 190 days.

The World Animal Health Organization's Terrestrial Animal Health Code reports that the incubation period for rabies is 6 months.

#### c) Spread:

Morgagni in 1769 postulated that rabies virus migrates "via nervo". After a variable incubation period, followed by viral replication in the connective tissue and muscular tissue surrounding the inoculation site, infection spreads rapidly to the CNS. In certain circumstances particles may directly penetrate the peripheral nerves, without prior replication in non-nervous tissue.

Amputation experiments on animals proved the transmission of infection via peripheral nerves. Viral replication involves several steps: adsorption, penetration, denuding, transcription, translation, genome replication, maturation and sprouting.

The acetylcholine receptor (AchR) has been suggested as an important element for the penetration of viral particles into the axons of neuromotor junctions, where through glycoprotein it specifically binds to the receptor, impinging upon the peripheral nerves, progressing centripetally towards the CNS, following the retrograde axoplasmic flow, traveling 100-400mm per day.

During the incubation period, and before CNS involvement, the presence of the virus can no longer be shown by conventional diagnostic methods, and some researchers have called this period viral eclipse.

The particles reach the neuronal cells of the brain stem, hippocampus, thalamus, spinal cord and cerebellum. The lesions of rabies polyencephalomyelitis are characterised by perivascular infiltration by mononuclear cells, focal and regional gliosis and neuronophagy. Degeneration of the neuron, surrounded by macrophages, and occasionally by other inflammatory cells, creates a nucleus of neuronophagy called Babe's nodules. Vacuolization eventually produces a spongiform lesion in rabies. Demyelination also occurs. Groups of viral proteins make up corpuscles of intracytoplasmic inclusions known as Negri bodies, particularly found in the cytoplasm of neurons and Purkinje cells in the cerebellum.

Production of interferon (IFN) has been shown by several rabies virus inoculation experiments, although

the elicitation of high titers of IFN in the brain did not inhibit viral replication in mice.

#### d) Elimination of the Virus:

Reaching the CNS after intense replication, the viruses proceed centrifugally towards the peripheral and autonomous nervous system is, affecting such organs as the lung, the heart, the kidneys, the bladder, the uterus, the testicles, the hair follicles, and above all the saliva glands, where they are shed in the saliva.

In natural infection, stimulation of B lymphocytes to produce antibodies occurs late on, after the onset of symptoms. The action of these antibodies is to block extracellular viruses, before reaching muscle cell receptors, inhibiting spread at the site of inoculation and progress towards the CNS.

Functional changes in the neurons are moderated by B and T-cell mediated immunity or by other non-specific non-immune mechanisms. The intense proliferation of inclusion bodies within neurons leads to functional changes in the nerve cells, and involvement of the limbic system, giving rise to behavioral changes.

Viral particles may be identified in the saliva days before the onset of clinical signs.

## 6. CLINICAL ASPECTS OF RABIES

Clinical signs in herbivores:

After the incubation period has passed, several different signs of the disease may arise, paralysis being the most common; however, the furious form may occur, leading the animal to attack other animals or human beings.

In the case of bat-transmitted rabies, marked differences between clinical manifestations in bovines, equines, assinines, mules and other economically important domestic animals such as caprines, ovines and pigs have not been observed. The initial sign is that the animal isolates itself, holding apart from the herd, presents a certain apathy and loss of appetite, may hang its head and seem indifferent to its surroundings. Other signs follow, such as increased sensitivity and pruritus in the bite site, constant lowing, tenesmus, hyperexcitability, increased libido, abundant viscous salivation and difficulty swallowing (appearing to suggest that the animal is choking).

As the disease evolves, the animal presents uncoordinated head movements, muscle tremors and tooth grinding, midriasis and absence of pupillary reflex, motor dyscoordination, staggering gait and involuntary muscle contractions. Once lying down, the animal can no longer rise, and peddling movements occur, respiratory difficulty, opisthotonus, asphyxia and finally death, which usually occurs from 3 to 6 days after the onset of signs, although in some cases it may stretch out for up to 10 days.

Once the clinical signs of rabies begin, there is nothing more to do except isolate the animal and wait for its death, or sacrifice it in the agonic phase. Because signs in bovines and equines may be mistaken for other diseases of encephalitis, the differential laboratory diagnosis is crucial.

The meat of animals suspected of having rabies should never be used for consumption. Viral particles have been found at detectable levels in the heart, lung, kidney, liver, testicle, saliva glands, skeletal muscle, and brown fat of a range of domestic and wild animals.

Handling the carcass of a rabid animal is highly dangerous, especially for butcher's shop professionals, cooks, or meat processing industry employees. Extreme caution must be taking when dealing with suspect animals, because there is a risk when untrained people handle the head or brain, or if they place their hands in the mouths of the animals in an attempt to relieve their choking. When this happens to anyone, they must immediately go to a primary health clinic for attention.

For readers' benefit, the symptoms in the human, occurring in three stages, are given below:

The first stage, known as prodromal, lasts for roughly 2-10 days and is characterised by headache, fever, nausea, fatigue and anorexia.

The second stage is where sensory excitation, or a phase known as the "acute neurological period", occurs, lasting for 2 to 7 days. Bizarre behavior patterns occur, such as extreme aggression, anxiety, insomnia, increased libido, tingling, priapism, hypersalivation, aerophobia, photophobia, reactions to sounds, muscle contractions, convulsions, hydrophobia, and a tendency to bite and chew.

The third stage is characterised by coma and paralysis, lasting from several hours to several days, marked by a state of mental confusion, hallucinations, cardiorespiratory stoppages, and paralysis of the neck or the region around the inoculation site. Falling into a coma, the patient may die within a very few days. In cases of human rabies linked to transmission by bats, the major symptomatology of the disease observed is paralysis. Humans presenting symptoms similar to those described above should ALWAYS be taken to the nearest primary health care unit, and the health authorities must immediately be notified.

## 7. PERIOD OF TRANSMISSIBILITY

In cats and dogs, excretion of the virus through the saliva may be detected from 2 to 4 days before the appearance of clinical signs, and this may persist throughout the course of the disease, which is inevitably fatal. The death of the animal usually occurs from 5 to 7 days after the onset of signs. For this reason, cats and dogs suspected of rabies must be kept under observation for 10 days after the date of aggression. For wild animals, few studies have been carried out about the transmission period; it is known that it varies from species to species. There is a report of the rabies virus being shed in the saliva for a period of up to 202 days in the case of a *Desmodus rotundus*, without apparent signs of the disease.

The period during which herbivores can transmit the disease is not known exactly. Although some species of herbivore do not have suitable dentition to cause deep wounds, there are reports of rabies transmitted to humans by herbivores. It is therefore recommended that anyone place their hands into the mouth of any animal species with nerve symptoms without the use of suitable protective equipment.

The OIE's Terrestrial Animal Health Code states that the period of infectiousness in rabies in domestic carnivores begins 15 days before the appearance of the first clinical signs and ends with the death of the animal.

## 8. PROPHYLAXIS

Prophylaxis consists mainly of the immunization of susceptible animals.

In the case of herbivores, the guidelines already laid down in this manual and in Normative Instruction no. 5, recommend population control of *Desmodus rotundus*, along with other prophylactic actions for rabies. In the case of cats and dogs, follow the norms laid down by the Ministry of Health.

## 9. TREATMENT

There is no treatment and the disease is invariably fatal after the onset of clinical signs.

Only in the case of human beings are rabies vaccines indicated for post-exposure treatment. Use may also be made of the application of homologous or heterologous anti-rabies serum (HRIG). Passive immunity conferred by anti-rabies immunoglobulin lasts at most for only 21 days.

## 10. Clinical

### DIAGNOSIS:

Clinical observation only allows rabies to be suspected, since the signs of the disease are not characteristic and may vary from animal to animal or among individuals of the same species. A diagnosis of rabies should not be concluded without clinical and epidemiological observations, because there are several other diseases and genetic, nutritional and toxicological disorders whose clinical signs are compatible with rabies, as may be observed in Appendix VI.

### Laboratory diagnosis:

To date there is no conclusive laboratory diagnostic test before the death of the sick animal that can express absolute results. However, there are internationally standardized laboratory procedures for post-mortem samples obtained in animals or humans suspected of having contracted rabies. The laboratory techniques are preferably applied to tissues removed from the CNS. Fragments of the hippocampus, brain stem, thalamus, cortex, cerebellum and medulla oblongata are traditionally the materials of choice.

### Diagnostic techniques:

The laboratory diagnosis may be performed using two types of routine procedure:

a) Immunochemical identification of the viral antigen: a.1) Direct immunofluorescence test:

The most widely used test for diagnosing rabies is direct immunofluorescence (DIF), recommended by the World Health Organization (WHO) and by the World Animal Health Organization (OIE). This test may be used directly on a print of tissue on a microscope slide or to confirm the presence of rabies virus antigen in cell culture. The DIF gives reliable results in a few hours, when performed with fresh samples, in 95-99% of cases. For direct diagnosis, prepared prints of the hippocampus, cerebellum and medulla oblongata are stained with a specific conjugate marked with a fluorescent substance (antirabies antibodies + fluoresceine isothiocyanate). In the DIF specific aggregates of the nucleocapsid are identified by observed fluorescence. DIF may be used in samples preserved in glycerin after repeated washes.

b) Viral isolation:

This test detects the infectiousness of the sample by inoculating a suspension of tissues extracted from the suspect sample in biological systems, enabling the agent to be isolated. It is used concomitantly with DIF has recommended by the World Health Organization (WHO, 1996).

b.1) Inoculation in mice:

A group of mice aged from 3 to 4 weeks or neonates at 2 to 5 days of age are inoculated intracerebrally. The young adult mice are observed for 30 days and each dead mouse is examined using DIF. To accelerate the results of inoculation of neonatal mice, one mouse at a time is recommended to be sacrificed at days 5, 7, 9 and 11

post inoculation, and this is followed by DIF. In vivo isolation in mice is a costly test and whenever possible should be replaced by isolation in cell culture.

b.2) Test in cell culture:

The cell lineage recommended for this type of test is murine neuroblastoma (NA-C1300). Viral replication is revealed by DIF. The results of the test is obtained 18 hours post-inoculation. Incubation is generally continued for 48 hours, and in some laboratories for up to 4 days. This test is as sensitive as the inoculation in mice test. If a laboratory has a cell culture unit, that test should replace inoculation in mice, thus avoiding the use of animals, as well as being less costly and more rapid.

Other identification tests not routinely adopted are described on the MAPA website ([www.agricultura.gov.br](http://www.agricultura.gov.br)).

## CHAPTER III - BIOLOGY AND CONTROL OF *DESMODUS ROTUNDUS*

### 1. CHARACTERISTICS

The most widely studied species of hematophagous bat is *Desmodus rotundus*, owing to its economic and social significance. *Desmodus rotundus* is harmful to the raising of herbivores because apart from attacks on the animals, it can transmit rabies, when infected by the rabies virus. This shows the need for a focus on this bat in rabies control.

*Desmodus Rotundus* is highly versatile in its use of shelters, which may be natural such as caves and hollow trees, or artificial, such as abandoned houses, bridges, culverts, charcoal furnaces, and so on. There are different types of shelter: daytime or permanent, where they roost most of the time; nocturnal, where they roost as long as is necessary for digester and after feeding, then going back to their permanent roosting site. Maternity shelters hold females, their young, and the dominant males. The roosting sites are dark, cool, and highly humid, and these conditions are most frequently found in natural caves lit by the sun only in the morning. Roosting sites for single males hold young individuals who have not yet reached sexual maturity when they will form their harems.

Most groupings of *Desmodus rotundus* comprise from 20 to 200 individuals. They have a complex social

structure based on the formation of harems, where a dominant male defends a group of females (approximately 12) and their young. Colonies of over 50 individuals usually contain several groups of 10 to 20 females with young. Young males, from 12 to 18 months of age, are expelled from the group by the dominant male.

Single males who have been expelled from colonies may travel for up to 100km, although their range of action is less than 15km. They make up smaller clusters, near to the harem, biding their time to dispute the place of the dominant male.

The behavior of grooming (licking) other individuals of the species is found mainly among females, guaranteeing the integrity of the group and the sharing of food. The licking action stimulates regurgitation of the food by a satiated female, enabling another female that has not fed to eat. Females who do not collaborate in sharing food are expelled from the group.

The species has a low level of reproduction because the gestation period lasts 7 months, and giving birth to only one young per year favors population control.

Hematophagous species (*Desmodus rotundus*, *Diphylla ecaudata* and *Diaemus youngi*) are exclusively found in the neotropical region and usually occur from Mexico to Argentina.

Morphologically, *Desmodus rotundus* is a medium-sized chiropteran, with a wingspan of 37cm and weighing approximately 29g. Its ears are short and pointed at the tip, its eyes are large, although smaller than those of other hematophagous species (*Diphylla ecaudata* and *Diaemus youngi*), its lower lip has a V-shaped medial groove (Figure 1). The thumb is long, with three calluses or pads, one small, round one at the base, one long, broad one in the mid-portion, and a small one at the thumb tip. The interfemoral membrane is poorly developed, measuring roughly 19mm in its median region, with sparse short hair scattered about its dorsal surface. Calcaneus is reduced in size, looking like a small wart. The body is covered by a short dense brown hairs, those on the pack being darker than those on the belly. Varying by a region of Brazil and/or by the age of the animal, this coloring may tend towards golden or gray.



Figure 1: *Desmodus rotundus* (photo: Clayton Gitti)

## 2. RABIES IN BATS

How the disease behaves in the bat is virtually unknown. The most important factor to consider is that the species may harbor rabies virus in its saliva glands before the clinical manifestations of the disease, for longer periods than those observed in other species. The disease has been reported to take the following forms in bats: typical furious rabies, with paralysis and death; furious rabies and death without paralysis; and typical paralytic rabies and death.

The clinical signs vary in sick animals, but special attention must be paid to individuals not presenting habitual behavior, such as flying more feeding during daytime, or fallen on the ground.

## 3. METHODS TO CONTROL *DESMODUS ROTUNDUS*

The techniques already described in this manual must be carried out correctly and selectively, so as only to affect bats of the species *Desmodus rotundus*, and not harm or disturb other species of bats such as insectivorous, frugivorous, pollenivorous, carnivorous and piscivorous bats, because the latter are an essential factor in the ecological balance.

Owing to the biology, habitat, area of action, feeding patterns, habits, social organization and specific

behaviors of *Desmodus rotundus*, techniques have been developed to control its populations, by using anticoagulants.

*Desmodus rotundus* population control techniques are:

a) The Direct Selective Method

The technique needs perfectly trained and aware teams to carry out the activity within the standards of biosecurity, given that there is a certain degree of risk in the activities which require special precautions. This technique may be carried out near natural and artificial roosting-sites (caves, sinkholes, tunnels, hollow trees and so on ) or near sources of food (corrals, sties, poultry houses and so on). The technique consists of capturing *Desmodus rotundus* in mist nets, and applying the anticoagulant to their backs before releasing them (Figures 2.a and 2.b). When they return to their roosting sites, these individuals will interact with other members of the colony, passing the product to them.

To harmonize procedures, it should be considered that for each bat (*Desmodus rotundus*) properly treated with anticoagulant paste, 20 other bats of the same species will die. The product causes hemorrhage that is fatal within 4 to 10 days.

Hematophagous bats avoid flying during the brightest periods of the night, particularly full moon. Therefore, every month there will be two weeks that are more favorable for capturing the hematophagous bats: the week prior to and the week after the new moon.

The nets used to capture birds are the most suitable for controlling hematophagous bats. They are manufactured in a range of sizes, measurements and colors. Black ATX Nets are generally the most suitable. They come in several sizes, from 6 to 12 meters long, 2 - 3 meters high, and with 3 or 4 horizontal struts. Nets smaller in area may be used specifically outside the roosting sites, taking into consideration the size of the entrance to the shelter. We recommend the use of light metal tubes, rigid PVC tubes, bamboo stalks or wooden posts, according to availability, as the support structure.

They should be laid out in a single plane in such a fashion that the guide wires are aligned in parallel one on top of the other, in a horizontal plane. They must be kept closed until just before dark, in order to avoid capturing birds and insects.

When opening them, the handles must be spaced out at regular distances, leaving the lower handle some 5 to 10cm off the ground. The space between the horizontal struts or guide wires running through the net must form pouches into which the bats will fall and entangle themselves as they struggle. A common mistake made by inexperienced people is to fully stretch out the net. If it is set up in this way, it will not form the bag-like pouches, and thus the bats will not be captured because they will bounce off the net rather than entangle themselves in it (slingshot effect).

All personnel and equipment must be fully prepared at the moment that the nets are opened. Each individual must be aware of their role and work in harmony, avoiding excess anxiety and getting in each other's way.

The team must wait silently near the net so as to enable shining a light on it when it is suspected that it contains bats. If it does, the bat must be removed immediately. The sooner it is removed, the less its level of stress, and the less chance it will have to tangle and damage the net.

Having identified which side the bat has been captured, remove it first by the feet, disentangling them. Now that its feet are free, begin to disentangle arms and head. The bat, having been freed, must be placed in a proper cage where it will be kept until the completion of the capturing work, therefore separate cages should be used for males and females, or individual cloth bags.

After the bats have been captured and the nets folded, two grams of the anticoagulant must be applied to the back of each *Desmodus rotundus*, after which they will be released. Males and suspect bats should preferably be sent for laboratory tests. Information on the number and gender of hematophagous and other bats captured that night should be reported on a specific form (Appendix IV).

If the person in charge sees fit, further captures can be carried out. However, colonies are normally reduced satisfactorily by a single capture.

a.1) Capture near cattle pens

For this modality of capture, the team should arrive on the farm in the afternoon, by 16:00, when it will have time to make the around the pen in daylight. If they are set up before the cattle enter, keep the nets closed at the top of the poles, avoiding capture of daytime birds, and enabling the cattle to pass under them.



The animals should ideally all be kept separate before the next set up.

Bear in mind that bats, like other species, are part of the food chain and thus also fear predators. They therefore avoid coming out in the brightest nights (waxing moon and full moon), when they are more vulnerable. The ideal lunar phases for capture are thus those periods where the moon is waning and the new moon, when it gets dark early, encouraging the bats to come out in search of food. These phases of the moon also favor capture activities ending early, around 22:00.

If the cattle usually spend the night in pasture, it will be necessary to ask the owner to place them in the nearest pen or paddock for at least three consecutive nights, so that the bats can identify their new location. For capture around pens, you should choose a pen or corral that will adequately hold all the cattle while enabling complete surrounding by nets, bearing in mind the natural barriers. The nets are normally set up at a distance of one to two meters from the corral to enable space for inspection and avoid the nets tangling on sticks or wires.

Bats from more than one colony may be caught in this type of capture, which enables the control of the populations of different roosting sites within the same region.

Teams taking part in the capture (source of food or shelter) must inspect the nets, removing the trapped bats, at least every 20 to 30 minutes.

Those *Desmodus rotundus* trapped must be kept in cages until the end of capturing activities; separate cages may be used for males and females.

Non-hematophagous species must be kept in separate cages from the hematophagous bats and released at the end of the capture activity, thus avoiding their being entangled in the nets again.

The time of completion of the capture activities depends on the season of the year, the hour the moon rises, and the number of hematophagous bats captured.

At the end of the capture activity and after the nets have been put away (the nets must be properly cleaned) two grams of anticoagulant paste must be spread on the back of each *Desmodus rotundus* captured, and then they must be released.

In order to maintain effective surveillance, 10% of the captured *Desmodus rotundus* should be sent to a laboratory, preferably those bats with suspected rabies.

#### a.2) Capture outside the roosting site

This modality of capture is carried out at night and is influenced also by the phases of the moon. The nets are set up outside the entrance to the shelter, taking care first to clear away sticks and twigs from the location, to avoid them tangling in the nets and damaging them. A sheet of plastic or tarpaulin one meter wide may also be placed on the ground under the entire extension of the net. The nets must be set up leaving 5 to 10cm clearance from the ground at the lowest level, because these bats tend to fly only centimeters off the ground, above all when returning from feeding, when they are heavy.

#### a.3) Capture inside the roosting site

As seen previously, both artificial and natural roosting sites exist. Capture within caves should only be carried out in exceptional cases, when there is insufficient response to other actions taken until then, and after authorization from the competent authority.

This modality can be carried out both during the day and during the night, and is not influenced by the phases of the moon; but it requires special precautions on entering the caves and working inside them, above all behavior that does not harm the environment or its components.

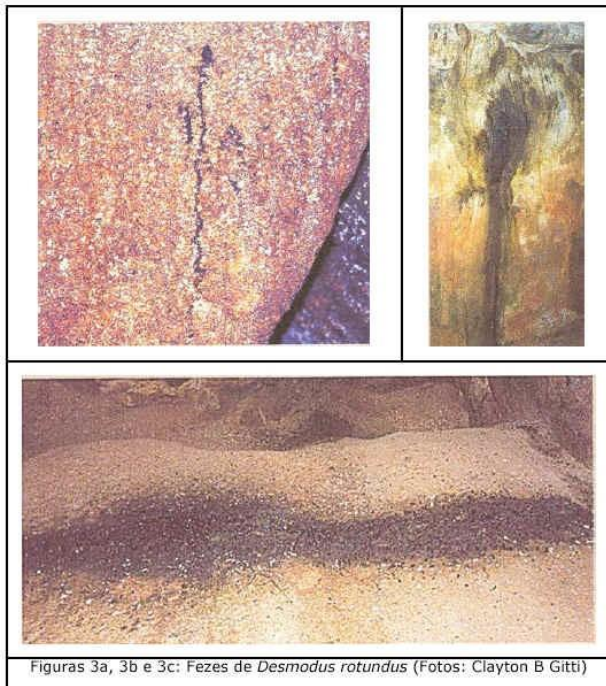
The use of half-face masks with activated coal filters, goggles, long-sleeved overalls, calf-length rubber boots and rawhide gloves is recommended for working in caves.

Fuel driven lighting methods may not be used inside shelters. Battery-powered torches or flashlights are recommended.

Once inside the shelter the presence of *Desmodus rotundus* must be confirmed; this is done by observing feces on the ground, walls or other structures of the shelter. These feces are black and take the form of small droplets (when only a few individuals roost in the site) or may be spatter stains and pooling of feces (when there are many). It should also be determined if this is an active roosting site, in other words, if hematophagous bats are using it at that moment; or an inactive site, if they have been

present but no longer are.

In active sites the feces are very dark, shiny, and viscous, somewhat like drops of burnt oil. In inactive sites, these feces will be dry and opaque. The ammoniac odor of digestive blood is characteristic of these sites.



Depending on the internal dimensions of the roosting site, either a capture net or a butterfly net may be used. Fishing nets may also be used to delimit the capturing space.

After the capture process has ended, two grams of the anticoagulant must be applied to the back of each hematophagous bat, after which they will be released. Males should preferably be sent for laboratory tests. Information on the hematophagous and other bats captured that night should be reported on a specific form (Appendix IV).

The need for a further capture is determined by inspection of the shelter, observing the presence of bats or fresh feces, as well as the large number of attacks on livestock.

This should be done so as to keep the stress on the bat colony to a minimum.

#### b) Indirect Selective Method

b.1) Topical use on the biting site Two grams of paste are applied in this method around the bite marks of cattle that have been attacked. This system of control eliminates only those hematophagous bats that are attacking domestic animals, and should be carried out by the farmer, under the guidance of a veterinarian. It has been observed and confirmed that *Desmodus rotundus* tends to return to the same wound to feed on consecutive days; therefore this method applies while the animal is still being assaulted. The paste should be applied in the late afternoon, and the animal should stay in the same spot where it was the previous night.

Owners must be instructed to include the habit of monitoring their animals for lesions caused by hematophagous bats in their sanitary handling of their herds.

This method should always be in courage because it is extremely efficient.

b.2) Use of gel on the back of the assaulted animal This method also aims to eliminate only those hematophagous bats that are the aggressors.

It consists of using the vampiricide gel on the back of the animal being bitten, because most vampire bats begin their approach to the animal on the animal's back.

This technique should be used mainly for pasture-raised animals, and the farmers themselves are responsible for carrying it out.

#### 4. ASSESSING THE EFFICACY OF THE CONTROLS CARRIED OUT

Satisfactory *Desmodus rotundus* population control in a given region should be assessed by the reduction of the rate of biting of domestic herbivores, and also by the number of bats found dead in the roosting sites. The occurrence of new cases the following year may be explained by repopulation of roosting sites by individuals from other infected colonies.

IF CASES OF PEOPLE ATTACKED BY BATS ARE REPORTED, OR OF PEOPLE WHO HAVE HAD CONTACT WITH ANIMALS SUSPECTED OF HAVING RABIES, THESE PEOPLE SHOULD BE SENT TO THE NEAREST COMMUNITY HEALTH POST AND THE COMPETENT AUTHORITIES SHOULD BE NOTIFIED OFFICIALLY.

### CHAPTER IV - GLOBAL SATELLITE POSITIONING, AN ESSENTIAL TOOL IN PROMOTING ANIMAL HEALTH.

#### 1. INTRODUCTION

GPS (Global Positioning System) is a methodology developed by the American Government's Department of Defense for accurate positioning on the surface of the globe. The system was initially conceived for military use, and a systematic signal error was built in so that it could not be used by hostile forces. However, this restriction was abolished and the GPS system is widely used today in a whole range of human activities in all countries, for locating points, routes, and for navigation. The issue of the built-in error in the satellites, albeit on a small scale, remains, to avoid use in warfare.

A GPS system basically consists of two main components: a "constellation" of orbiting satellites, and the receiver device.

The technology is based on the constellation of satellites in orbit above the Earth. At any point on the surface of the globe, and at any moment, the satellite will be moving in their orbit, providing total coverage of the Earth's surface. By locating and communicating with 3 or more satellites, a GPS receiver can locate itself on the surface of the Earth. There is no doubt as to the overwhelming advantage of using this technology as against printed maps or compasses.

The accuracy of the coordinates provided by GPS may vary, depending on the number of satellites located by the receiver, on the intensity of the signal captured, of the number of satellites at the moment of reading, and the topography of the terrain and on atmospheric conditions.

The GPS device consists of three separate parts: an antenna, the receiver and a position storage device. After

connection is made with satellites, the device calculates the location of the unit and saves it in a file. The data may be saved as points, lines or polygons, and the information may be transferred to a computer or to a palmtop.

GPS devices can also measure distances, average speed and present speeds, altitudes (some present altimetry graphs), temperature and atmospheric pressure.

#### 2. USE OF GPS IN ANIMAL HEALTH

The use of GPS devices may help in the process of disease notification, as well as in triggering suitable sanitary measures. Animal health services must therefore be trained to obtain geographical location information properly, using these devices. It is a simple process to obtain this information, but some details are essential for the information obtained to be reliable.

Some steps must be followed so that the device works perfectly and the geographical coordinates are obtained correctly.

When the GPS is turned on, the device will seek satellite signals. Two circles will appear on the screen: an outer circle which indicates the satellites in orbit on the line of the horizon, and an inner circle indicating satellites in orbit at 45° to the horizon (Figure 1). For a perfect reading, the GPS must not be behind any obstacles, such as roofs, butterfly nets, trees, because the device will be unable to track the satellite signals.

Signal intensity is shown by bars on the same screen. Triangulating with at least 3 satellites, the device records coordinates automatically.

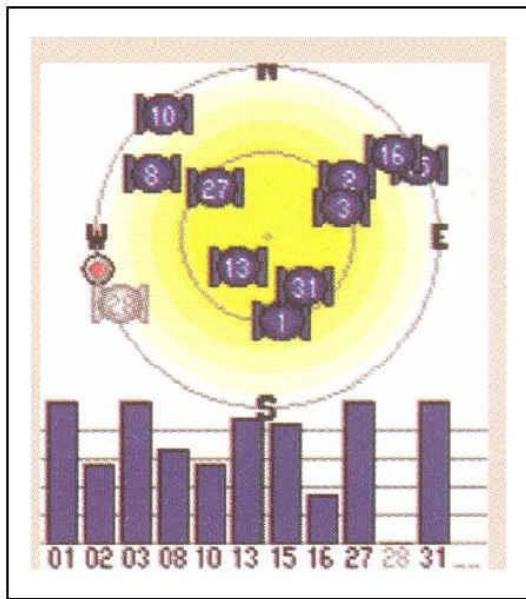


Figure 1. Satellite signal acquisition page.

However, for the geographical coordinate to be noted correctly, two main points are important: The geographical datum and the system of coordinates.

A geographical datum is a mathematical model approximating to the real shape of the Earth (which is irregular) and allowing coordinates to be calculated more accurately.

The system of coordinates means the formats in which the geographical coordinates are noted. There are two main coordinate systems, the geodesic (based on latitude and longitude) and UTM, in which the coordinates are

noted in distances of meters or kilometers.

The datum must be changed on the first use of the device, or whenever the batteries are changed (Figure 2).

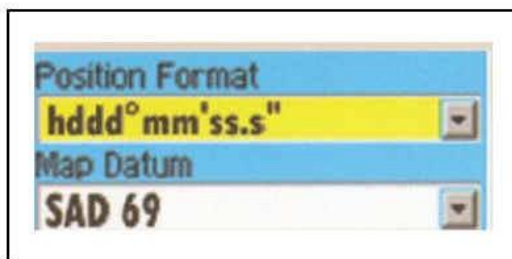


Figure 2. Configuration of the geographical datum and coordinate system.

Every region of the world has a specific geographical datum. For Brazil, the geographical datum to be used must be the South American 69 (SAD69). Failing to change the geographical datum to the corresponding region will mean obtaining coordinates that do not match the real location of the point.

The coordinate system should preferentially be the geodesic system, noting latitude and longitude as degrees and tenths, hundreds and thousands of a degree (hddd°mmss.s), because then the coordinates can be inserted directly into Geographical Information Systems (GIS) without any need for transformations to be carried out *a posteriori*.

This is a very important factor in the disease notification process, because the more important information systems such as PANAFTOSA's SivCont, an information system covering vesicular, nerve and swine hemorrhagic diseases, and the Animal Health Information System (*Sistema de Informações Zoossanitárias - SIZ*) of the Ministry of Agriculture, Livestock and Food Supply, require geographical

coordinates for outbreaks to be keyed in in the format described above.

As soon as the device is set up (with the geographical datum and system of coordinates), the geographical coordinates can be noted. They can be stored on the device itself and downloaded to a personal computer, plugged directly into SIG programs, or even jotted down on paper or in a computerized database.

To obtain a point's coordinate, press and hold Enter. The coordinates will appear on a screen called *puçá grafic*, as shown in Figure 3.

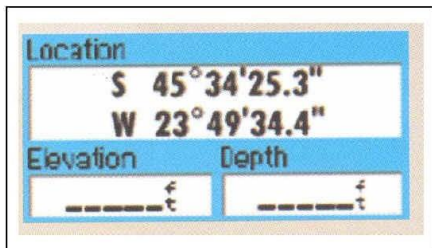


Figure 3. Example of the geographical coordinates screen.

If noting down the coordinates on paper or in a database, it is essential to avoid input errors. For example, on the band of longitude in which the state of São Paulo is located, and input error of one degree of latitude would mean a mismatch of approximately 110km between the point of attainment and the real coordinate.

### 3. BASIC NOTIONS OF CARTOGRAPHY

To use a GPS device correctly, certain basic notions of cartography are necessary, and given below:

Pythagoras (528 BC) triggered a great revolution in the concept of the shape of the Earth, by proposing that the planet was spherical. Since then, the concept has changed and we now know that the Earth is not as regular as we once thought.

The model put forward by Gauss (1828) speaks of an irregular surface owing to the action of gravity and the centrifugal force acting on the oceans. However, that model would cause great difficulty for finding a point on the surface. As a compromise, an ellipsoid geometrical model has been adopted, which is the shape of an ellipse flattened at the poles (Figure 4).

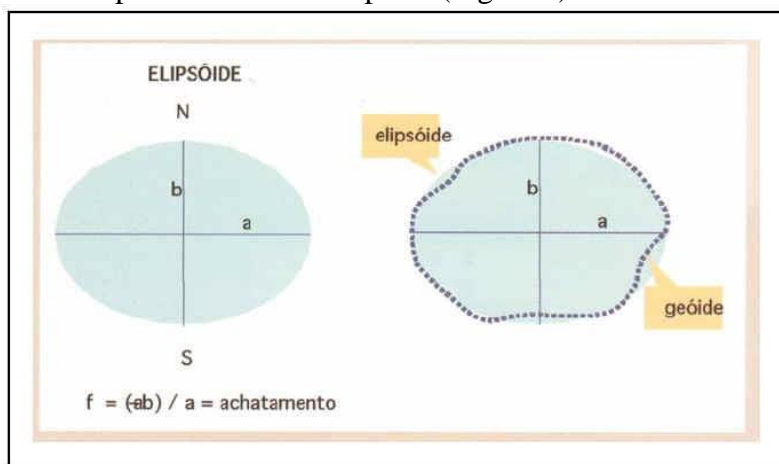


Figure 4. Representations of an ellipsoid and a geoid.

Locally, the shape of the ellipsoid and its position relative to the geoid define the so-called geodesic system (geodesic datum). Brazil has adopted the South American Geodesic System (SAD 69), which possesses the following parameters:

Reference ellipsoid UGGI 67  
Larger Semi-axis (a):  
6,378,160m

Flattening (f): 1/298.25 Origin of coordinates (planimetric datum) Station  
: Chuá vertex (MG)  
Coordinates: 19°4541.6527S 48°0604.0639W  
Geodesic azimuth for the Uberaba vertex: 271°3004.05

These parameters do not need to be input in a GPS device, is enough to key in the datum. The GPS system uses the datum called World Geodesic System 1984 (WGS 84). It is important to set up the GPS to the datum matching the region where the survey is being carried out. In Brazil, the datum to be used is the South American Geodesic System (SAD 69).

#### 4. CARTOGRAPHIC PROJECTIONS

Cartographic projections are the representation of a curved surface on a flat surface. There are many challenges involved, because extensions or contractions of the curved surface will always be needed in order to accommodate it to the plane. GIS systems carry out these adjustments automatically and in accordance with the given parameters. On GPS devices the cartographic projections need not be keyed in.

#### 5. SYSTEMS OF COORDINATES

These are needed for locating points by coordinates on a surface whether flat or curved. In the case of an ellipsoid, meridians and parallels are used. On a flat surface, Cartesian coordinates (x and y) I used. The meridians cut the Earth into two hemispheres from pole to pole. The meridian of origin is the Greenwich Meridian (0°). The parallels are circles that cross the meridians perpendicularly. The largest circle is that of the Equator (0°). The others shrink in size as they move away from the equator until they become the poles (90°) (Figure 5).

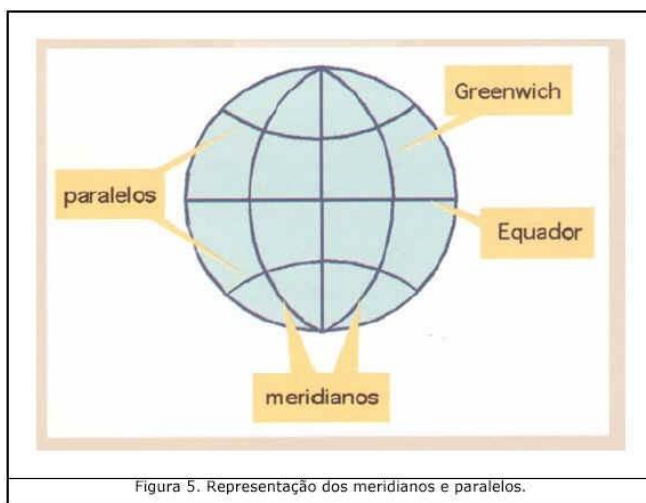


Figura 5. Representação dos meridianos e paralelos.

To locate a given point on the surface of the Earth, the coordinates are determined in terms of latitude and longitude (Figure 6).

Latitude is an arc above the meridian which passes through the point of interest, counting from the Equator up until that point. Its variation goes from 0° to 90°N (+90°) to the north, and from 0° to 90°S (-90°) to the south.

Longitude is the arc above the equator which goes from Greenwich to the Meridian passing through the point of interest. To the west of Greenwich, longitude ranges from 0° to 180°W (-180°), up until the International Date Line. To the east of Greenwich, longitude ranges from 0° to 180°E (+180°), up until the International Date Line.

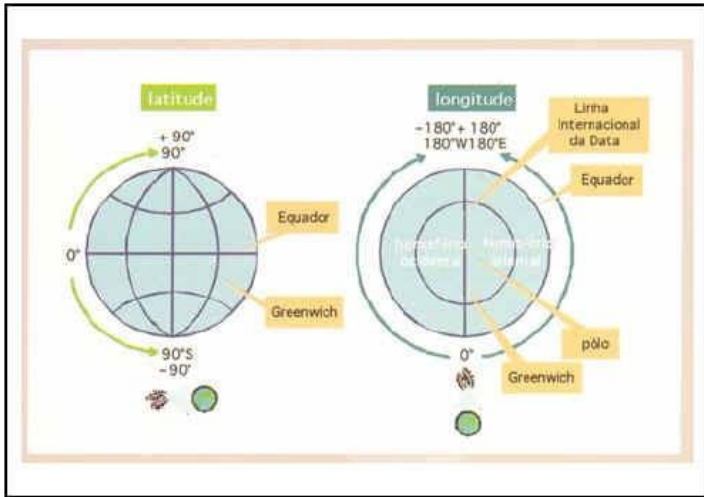


Figure 6. Schematic of the system of coordinates based on latitude and longitude.

Brazil's National Program for Controlling Rabies in Herbivores (PNCRH) demands that coordinates be noted using latitude and a longitude (geodesic system), in the format hddd°mmss.s", in other words, up to three figures for degree, two for minutes, two for seconds, and one for tenths of seconds. Attention should be paid to the coordinate sign (positive for the hemispheres North and East, and negative for hemispheres South and West, where most of Brazil lies).

## 6. UTM SYSTEM

PNCRH does not demand use of the UTM coordinate system, but it is important to understand the system, because many states still use it. Coordinates obtained in the system must be converted into the geodesic system.

First and foremost it is a military coordinate system. It is based on dividing the world into 60 Time zones each of 6° longitude. The numbering for the time zones begins with zone 1 (180°W to 174°W) and continues eastwards.

Each zone has horizontal bands of 8° latitude, called 'zones' stretching from 80°S to 84°N. Each zone receives a letter (from south to north) from C to X (I and O do not exist, to avoid confusion with 1 and 0). Letter X has 12° latitude.

In the polar region, the UTM system cannot apply, and the Universal Polar Stereographic System (UPS) must be used.

The UTM system is based on a greater system coinciding with the central meridian of the time zone and with the Equator. Each time zone is elongated by 30 at its extremities, over adjacent zones.

Coordinates from the UTM grades are expressed in distances of meters from the East (easting) and from the North (northing).

Eastings:

These measurements are referenced to the Central Meridian.

The value of the Central Meridian is 500,000m, an arbitrary value, sometimes called false easting. The minimum and maximum values are, respectively:

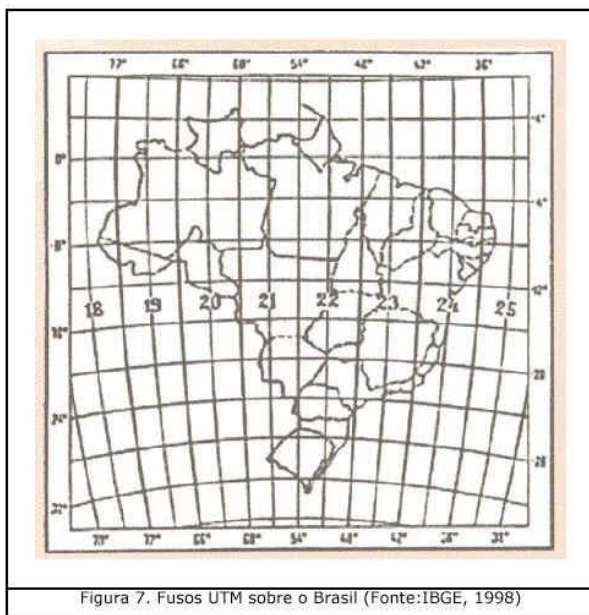
160,000m and 834,000m at the Equator;  
465,000m and 515,000m at latitude 84°N.

No value is ever equal to zero, because the 6° longitude zone and never exceeds 674,000m. Northings:

These measurements are referenced to the Equator. To the north of the Equator they receive increasing scores, the Equator itself receiving the value 0m N. to the south, they receive decreasing scores, and the Equator receives the value 10.000.000m N, to avoid negative values.

A problem that is also present in the UTM system is the deformation of the scale on a flat representation, because the time zone has a curved shape. Taking the scale factor of the Central Meridian as 1, the scale factor at the extremities of the time zone is approximately 1.0015. Adopting a scale factor equal to 0.9996 at the Central Meridian, convert the tangent cylinder into secant, which makes it possible to ensure a more favorable pattern of deformation throughout the time zone.

Brazil stretches over 8 UT zones as shown in Figure 7.



## 7. SUGGESTED SITES FOR FURTHER

RESEARCH <http://www.ibge.gov.br>

Projections:

<http://mac.usgs.gov>

UTM:

<http://www.maptools>.

with Conversions:

<http://www.cellspark.com>

## BIBLIOGRAPHY USED

Baer, G.M. The Natural History of Rabies, Second Edition. CRC Press, Boca Raton, Florida, USA, 1991, 620 pp.

BARROS, J. S.; FREITAS, C. E. A. A.; SOUSA, F. S. Raiva em animais silvestres no Estado do Ceará particularmente na raposa (*Dusicyon vetulus*). Zoonoses revista internacional, v. I, n.1, p. 9-13, 1989.  
Bourhy, H.; Kissi, B.; Tordo, N. Molecular diversity of the Lyssavirus Genus. Virology, v.194, p. 70-81, 1993.

Bourhy, H.; Rollin, P.E.; Vincent, J.; Sureau, P. Comparative field evaluation of the fluorescent antibody test, virus isolation from tissue culture, and enzymes immunodiagnosis for rapid laboratory diagnosis of rabies. J. Clin. Microbiol., v. 27, p. 519-523, 1989.



Burer, S. P. Distribuição de morcegos hematófagos *Desmodus rotundus*, *Diphylla ecaudata* e *Diaemus youngi* e a ocorrência da Raiva dos Animais Herbívoros no Estado do Paraná. Resumo de trabalhos. XXII Congresso Brasileiro de Medicina Veterinária, 1992, Curitiba.

Burer, S. P. Programa de Profilaxia e Controle da Raiva dos Herbívoros: Interfaces e Parcerias. In: II Curso de Atualização em Raiva dos Herbívoros. Secretaria de Estado da Agricultura e do Abastecimento do Paraná, Curitiba, p. 114-122, 1996.

Carini, A. Sur une grande epizootie de rage. *Ann. Inst. Pasteur* v. 25, p. 843-846, 1911.

(CDC) Centers for Disease Control and Prevention Update: Investigation of rabies infections in organ donor and transplant recipients Alabama, Arkansas, Oklahoma, and Texas. *MMWR Morb Mortal Wkly Rep.* 16; v. 53, n. 27, p. 615- 616, 2004.

CHARLTON, K.M.; CASEY, G.A.; BOUCHER, D.W.; WIKTOR, T.J. Antigenic variants of rabies virus. *Length Immunol. Microbiol. Infect. Dis.*, v. 5, n. 1-3, p. 113-115, 1982.

Delpietro, H.; Diaz, A. M.; Fuenzalida, E.; Bell, J. F. Determinación de la tasa de ataque de rabia en murciélagos. *Bol. Of. San. Pan.* v. 63, p. 222-230, 1972.

DIAS, R.A. Noções de cartografia geoprocessamento e sistemas de informações geográficas. Publicações da Universidade de São Paulo, LEB, p.1, fev., 2003.

FAVORETTO, S.R.; DE MATTOS, C.C.; MORAIS, N.B.; ARAÚJO, F.A.A.; DE MATTOS, C.A. Rabies in Marmosets (*Callithrix jacchus*) from the State of Ceará, Brazil. *Emerg. Infect. Dis.*, v. 7, n. 6, p. 1062-1065, 2001.

FAVORETTO, S.R.; CARRIERI, M.L.; CUNHA, E.M.S.; AGUIAR, E.A.C.; SILVA, L.H.Q.; SODRÉ, M.; SOUZA, M.C.A.; KOTAIT, I. Antigenic typing of Brazilian rabies virus samples isolated from animals and humans, 1989-2000. *Rev. Inst. Med. Trop. São Paulo*, v. 44, n. 2, p. 91-95, 2002.

Flores Crespo, R.; Burns, R.J.; Fernández, S.S. Evaluación de una técnica para combatir los vampiros en sus refugios. *Bol. Of. San. Pan.* v. 65, p. 427-432, 1974.

Flores Crespo, R.; Said Fernández, S.; De Anda López, D.; Ibarra Ververde, F.; Amaya, R. M. Intramuscular inoculation of cattle with warfarin: A new technique for control of vampire bats. *Bull. Pan. Am. Health Org.* v. 13, p.147-161, 1979.

Greenhall, A.M. Use of mist nets and strychnine for vampire control in Trinidad. *J.Mamm.* v. 44, p. 396-399, 1963.

HEINEMANN, M.B.; FERNANDES-MATIOLI, F.M.; CORTEZ, A.; SOARES, R.M.; SAKAMOTO, S.M.; BERNARDI, F.; ITO, F.H.; MADEIRA, A.M.; RICHTZENAHIN, L.J. Genealogical analyses of rabies virus strains from Brazil based on N gene alleles. *Epidemiol. Infect.*, v. 128, n. 3, p. 530-411, 2002.

Hooper, P.T.; Lunt, R.A.; Gould, A.R.; Samaratunga, H.; Hyatt, A.D.; Gleeson, L.J.; Rodwell, B.J.; Rupprecht, C.E.; Smith, J.S.; Murray, P.K. A new lyssavirus the first endemic rabies-related virus recognised in Australia. *Bull. Actual no. Pasteur*, v. 95, p. 209-218, 1997.

ITO, M.; ITOU, T.; SAKAI, T.; SANTOS, M.F.C.; ARAI, Y.T.; TAKASAKI, T.; KURANE, I.; ITO, F.H. Detection of rabies virus RNA isolated from several species of animals in Brazil by RT-PCR. *Journal of Veterinary Medical Science*, v. 63, n. 12, p. 1309-1313, 2001.

ITO, M.; ITOU, T.; SHOJI, Y.; SAKAI, T.; ITO, F.H.; ARAI, Y.T.; TAKASAKI, T.; KURANE, I. Discrimination between dog-related and vampire bat-related rabies viruses in Brazil by strain-specific reverse transcriptase-polymerase chain reaction and restriction fragment length polymorphism analysis. *Journal of Clinical Virology*, v. 26, p. 317-330, 2003.

Linhart, S.B.; Flores Crespo, R.; Mitchell, G.C. Control de murciélagos vampiros por medio de un anticoagulante. *Bol. Of. San. Pan.*, v. 73, p. 100-109, 1972.

Lord, R.D. Control of vampire bats. Ed. Greenhall, A.M. ; Schmidt, U. CRC Press, Boca Raton, Florida pp. 215-226, 1988.

Lord, R.D. Seasonality of bovine rabies and seasonal reproduction by vampire bats. *J. Wildl. Disease*, v. 28, p. 292-294, 1992.

Lord, R.D.; Fuenzalida, E.; Delpietro, H.; Larghi, P.; Diaz, A. M.; Lazaro, L. Observation on the epizootiology of vampire bat rabies. *Bull. Pan. Am. Health Org.*, v. 9, p. 189-195, 1975.

Meslin, F-X.; Kaplan, M. M.; KoprowIski, H. Laboratory techniques in rabies WHO, 4 ed. Geneva, Switzerland., 476 p., 1996.

Pawan, J.L. Rabies in the Vampire Bat of Trinidad with Special Reference to the Clinical Course and the Latency of Infection. *Ann. Trop. Med. Parasitol*, 30, p.401-422, 1936.

Pozzetti, PS; Latorre, M. do R.D.de O.; Ito, F.H. Estudo epidemiológico da Raiva em Animais Herbívoros no Estado de São Paulo nos anos de 1996 a 1999. Tese (doutorado em epidemiologia experimental e aplicada as zoonoses) Universidade de São Paulo FMVZ-VPS, São Paulo 2001.

ROEHE, P.M.; PANTOJA, L.D.; SHAEFER, R.; NARDI, N.B.; KING, A.A. Analysis of Brazilian Rabies isolates with monoclonal antibodies to lyssavirus antigens. *Revista de Microbiologia*, v. 28, p. 288-292, 1997.

ROMIJN, P.C., VAN DER HEIDE, R.; CATTANEO, C.A.; SILVA, R.D.E.C., VAN DER POEL, W.H. Study of lyssaviruses of bat origin as a source of rabies for other animal species in the State of Rio De Janeiro, Brazil. *American Journal Trop.Med.Hyg.*, v. 69, n. 1, p. 81-86, 2003.

RUPPRECHT, C.E.; HANLON, C.A.; HEMACHUDHA, T. Reviews-Rabies reexamined. *The Lancet Inf. Dis.*, v. 2, p. 327-343, 2002.

SILVA, R. A.; BRECKENFELD, S. G. B. Ocorrência da raiva em lobo-guará (*Chrysocyon brachyurus*, Illiger, 1815). Instituto de Pesquisa e Experimentação Agropecuária do Centro-Sul. EPE, Ministério da Agricultura. Boletim Técnico n° 70, Separata da Pesquisa Agropecuária Brasileira, v. 03, 1968.

Silva, J.A.; Moreira, E. C.; Haddad, J.P.A. et al. Uso da terra como determinante da distribuição da raiva bovina em Minas Gerais, Brasil. *Arq. Bras. Med. Veterinarian Zootec.*, v. 53, n 3, 2001.

Silva, J.A.; Moreira E. C.; Haddad, J.P.A. et al. Distribuição temporal e especial da raiva bovina em Minas Gerais, 1976 a 1997. *Arg. Bras. Med. Veterinarian Zootec.*, v. 53, n 3, 2000.

SOARES, R.M.; BERNARDI, F.; SAKAMOTO, S.M.; HEINEMANN, M.B.; CORTEZ, A.; ALVES, L.M.; MEYER, A.D.; ITO, F.H.; RICHTZENHAIN, L.J. A heminested polymerase chain reaction for the detection of Brazilian rabies isolates from vampire bats and herbivores. *Mem. Inst. Oswaldo Cruz*, Rio de Janeiro, v. 97, n.1, p. 109-111, 2002.

TORRES, S.; QUEIROZ LIMA, E. A raiva e sua transmissão por morcegos hematófagos infectados

naturalmente. Revista do Departamento Nacional de Produção Animal. Publicação oficial do ministério da Agricultura, Ano II, p. 1-55, 1935.

WARRELL, M.J.; WARRELL, D.A.M. Rabies and other lyssavirus diseases. *Lancet*, v. 363, p. 959-69, 2004.

WIKTOR, T.J.; KOPROWISKY, H. Monoclonal Antibodies Against Rabies Virus Produced by Somatic Cells Hybridization. Detection of Antigen Variants. *Process Nat. Acad. Sci. (USA)*, v. 75, p. 3938-3942, 1978.

WIKTOR, T.J.; KOPROWISKY, H. Use of monoclonal antibodies in diagnosis of rabies virus infection and differentiation of rabies and rabies-related viruses. *J. Virological Methods*, v. 1, p. 33-46, 1980.

World Health Organization Expert Committee on Rabies. Eighth Report World Health Organization Technical Report Series n. 824, 84 pp, 1992.

WHO WORLD SURVEY OF RABIES, 32 For the year, 1996 Diseases surveillance and control. WHO/EMC/ZDI/98.4, 1996.

WHO WORLD SURVEY OF RABIES, 35 For the year, 1999 Diseases surveillance and control. WHO/EMC/ZDI/98.4, 1999.

Wunner, W.H. Rabies Virus. In Jacteson, A. C.; Wunner, W.H. *Immunology. Eds Rabies Academic Press, London*, p. 23-76, 2002.

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