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Rabies and rabies-related viruses: a modern perspective on an ancient disease

F. Cliquet & E. Picard-Meyer

Laboratory of Research on Rabies and Wildlife Diseases, OIE (World Organisation for Animal Health) Reference Laboratory for Rabies, World Health Organization Collaborating Centre for Research and Management in Zoonoses Control, Community Reference Institute for Rabies Serology, Agence française de sécurité sanitaire des aliments – Nancy, Domaine de Pixérécourt, B.P. 9, 54220 Malzéville, France

Summary

Rabies is a worldwide zoonosis caused by a lyssavirus, with many host species acting as reservoirs for infection. The epidemiology of rabies has changed over recent years, as this disease has been brought under control or eliminated in many terrestrial animal species in Europe and North America.

A large number of *Lyssavirus* variants have now been characterised, and their distribution and animal hosts have become known. However, new lyssaviruses have been isolated from bats, prompting scientists to question the efficacy of the existing human and veterinary vaccines against these new strains. The epidemiology of bat rabies should be fully explored, so that the precise risks to the health of humans and domestic and wild carnivores may be determined and methods of preventing the disease among people who handle bats can be discovered. Rabies is still a significant public health problem, particularly in areas where canine rabies is still endemic, such as countries in Africa and Asia.

Keywords

Bat rabies – Bats – Epidemiology – Lyssavirus – Rabies – Terrestrial rabies.

Introduction

The emergence of pathogenic infectious diseases, associated with a range of underlying causal factors, represents a global threat to human and animal health. It has long been recognised that the infectious disease agents which cause several zoonotic diseases have the ability to cross the species barrier. Many of the new, emerging or re-emerging diseases in humans, at the end of the 20th Century and the beginning of the next, are known to be caused by pathogens which originated from animals or products of animal origin. However, this apparent increase in the incidence of disease may be the result of enhanced surveillance and awareness. When one considers the wide range of domestic and wild animal species involved and the pathogens concerned, which may be viruses, bacteria or parasites, then effective surveillance, prevention and control of zoonotic diseases poses a significant challenge to public health (67).

New viral zoonoses, such as Nipah virus, may emerge whenever environmental conditions are favourable. Nipah virus, which was first identified when a cross-reaction occurred with Hendra virus antisera, is the paramyxovirus responsible for porcine respiratory and neurological syndrome. This new virus, which remained undetected for some time, was responsible for 265 human cases of viral encephalitis in Malaysia, resulting in 105 deaths between September 1998 and May 1999. Most cases in humans (93%) were reported among those involved in pig-farming (67). The evidence currently suggests that fruit bats (*Pteropus* spp.) may be the natural hosts of, and provide the wildlife reservoir for, Nipah virus, Hendra virus and some other rabies-related viruses.

Ribonucleic acid (RNA) viruses, such as the lyssaviruses, are the fastest-evolving organisms due to the fact that they have a polymerase devoid of proof-reading mechanisms (12). The lack of a proof-reading mechanism creates a diverse viral population able to inhabit new conditions and escape defence mechanisms. This property makes RNA viruses among the most dangerous of pathogens. Understanding their evolution and the nature of the emergence of RNA viruses in human populations and domestic and wild animal species may help to control them.

Rabies is a viral zoonosis which causes encephalomyelitis. Rabies infection is caused by the viruses of the *Rhabdoviridae* family of the genus *Lyssavirus*, which has seven genotypes (Gt), and is maintained in reservoir mammals, mainly carnivores (dogs) and bats. Genotype 1 comprises the classical rabies virus (RV) strains which are found in almost every country in the world (Fig. 1). Genotypes 2 to 7 include the rabies-related viruses (RRV), more specifically, as follows:

– Lagos bat virus (LBV), (Gt2)

– Mokola (MOK) virus, (Gt3)

– Duvenhage virus (Gt4)

– European bat lyssavirus 1 (EBLV-1), (Gt5)

– European bat lyssavirus 2 (EBLV-2), (Gt6)

– Australian bat lyssavirus (ABLV), (Gt7) (3, 23, 24, 42) (Table I).

Badrane *et al.* (12) suggested that lyssaviruses may have originated from an insect rhabdovirus which bats contracted from insects some 7,080 to 11,631 years ago. It is thought that the RV switched hosts from bats to carnivores approximately 888 to 1,459 years ago.

The identification of RRV dates from the 1970s; however, the oldest recorded descriptions of the disease, which were found in Mesopotamia, are over 4,300 years old. The *Lyssavirus* genus (84) was created to group together all the viruses that produce a rabies encephalitis in inoculated mice and react with anti-nucleoprotein antibodies. Rabies-related viruses can be differentiated from RV by a decrease in the cross-reactivity in virus neutralisation tests with the antibody directed against the glycoprotein of the rabies virus, and in certain cases, by a failure of rabies virus vaccines to confer protection (reviewed in Messenger *et al.* [68]).

In 1978, Wiktor and Koprowski described the production of monoclonal antibodies to identify RV (94). Through the discovery of these monoclonal antibodies with specific reactivity, a great deal of knowledge was obtained about the characterisation and classification of RV and RRV. Moreover, as molecular biology tools were developed in the 1990s, it became possible to identify definitively the different viral species in the *Lyssavirus* genus, with the assistance of phylogenetic analysis of the nucleoprotein gene sequences (24, 42).

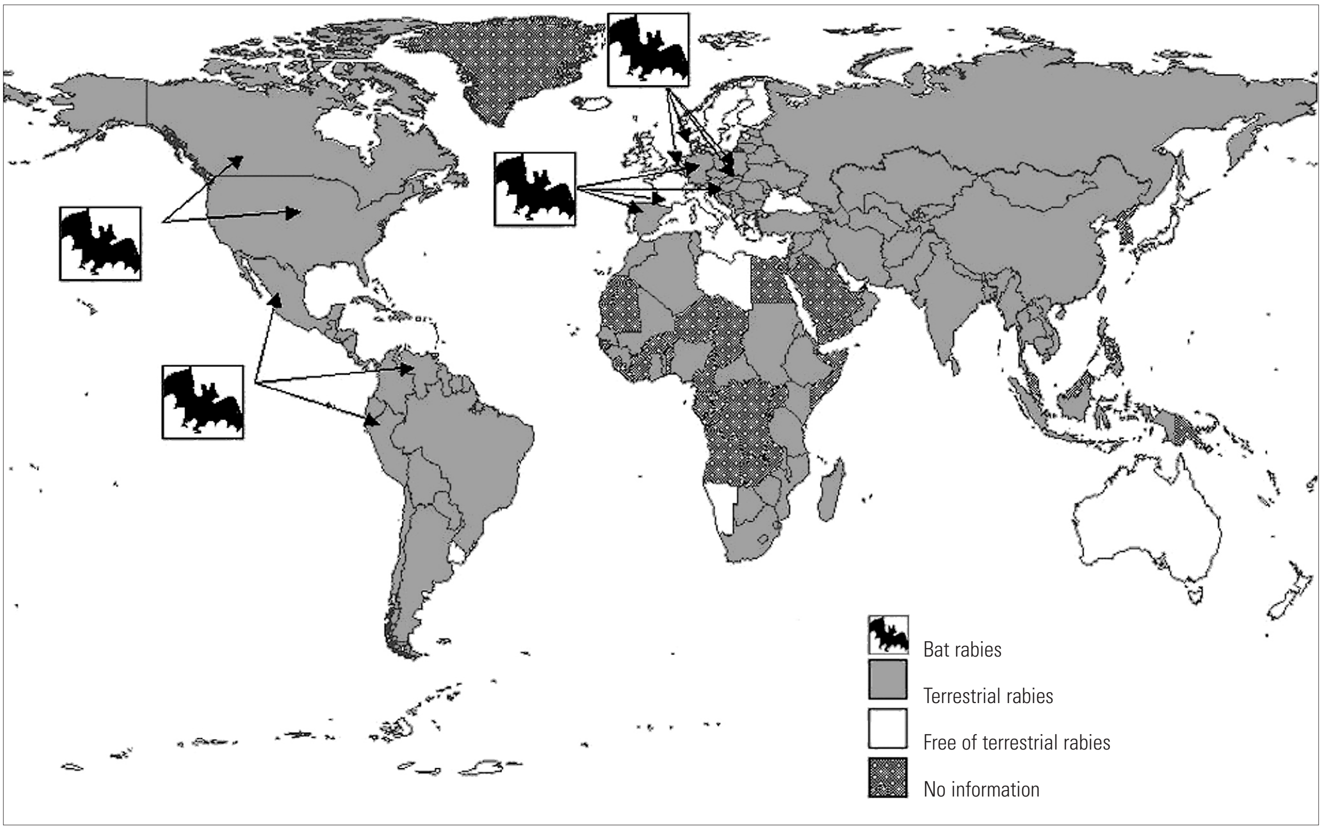

Fig. 1
Geographical distribution of rabies throughout the world in 1999
Source: World survey of rabies for the year 1999, World Health Organization, 1999 (96)

Table I
Viruses of the *Rhabdoviridae* family, *Lyssavirus* genus (3, 7, 20, 23, 24, 40, 42, 59, 90)

Genotype	Serotype/ phylogroup	Virus name	Distribution	Source species	Human deaths	Other known susceptible mammal hosts
1	1/I	Rabies virus	Widespread except in Australia, Britain, Iceland, Ireland, New Zealand, Scandinavia	Dogs, foxes, raccoons, bats in the Americas and others	70,000 per year	Wide range of mammals
2	2/II	Lagos bat virus	Central African Republic, Nigeria, Senegal, South Africa Senegal South Africa, Zimbabwe Ethiopia	Fruit bats: *Eidolon helvum, Micropterus pusillus Epomophorus wahlbergi* Insectivorous bats: *Nycteris gambiensis* Cats Dogs	Has not been detected in human beings	Dogs, cats
3	3/II	Mokola virus	Cameroon, Nigeria Ethiopia, South Africa, Zimbabwe Zimbabwe Central African Republic	Shrews Domestic cats Dogs Rodents	2 (Nigeria: 1969, 1971)	Shrews, rodents, dogs, cats
4	4/I	Duvenhage virus	South Africa Zimbabwe	Insectivorous bats: *Miniopterus schreibersii* *Nycteris thebaica*	1 (South Africa: 1971)	None detected
5	5/I	European bat lyssavirus 1a European bat lyssavirus 1b	Denmark, France, Germany, Hungary, Netherlands, Poland, Russian Federation France, Netherlands, Spain	Insectivorous bats (especially *Eptesicus serotinus*)	2 (Russia: 1985; the Ukraine: 1977)	Sheep (one case of bat rabies in a stone marten has also been reported)
6	6/I	European bat lyssavirus 2a European bat lyssavirus 2b	Germany, Netherlands, Ukraine, United Kingdom Switzerland	Insectivorous bats (especially *Myotis dasycneme* and *M. daubentonii*)	2 (Finland: 1985; Scotland: 2002)	None detected
7	1/I	Australian bat lyssavirus	Australia Philippines	Insectivorous bats (*Saccolaimus flaviventris*) Fruit bats (*Pteropus alecto, P. poliocephalus, P. scapulatus* and *P. conspicillatus)*	2 (Australia: 1997, 1998)	None detected
Proposed genotype	?/?	Aravan virus	Southern Kyrgyzstan	Insectivorous bats *(Myotis blythii*)	Not detected	None detected
Proposed genotype	?/?	Khujand virus	Northern Tajikistan	Insectivorous bats *(Myotis mystacinus*)	Not detected	None detected
Proposed genotype	?/?	Irkut virus	Eastern Siberia	Insectivorous bats (*Murina leucogaster*)	Not detected	None detected
Proposed genotype	?/?	West Caucasian bat virus	Caucasus	Insectivorous bats (*Miniopterus schreibersii*)	Not detected	None detected

Lyssaviruses of terrestrial (non-flying) mammals belong to Gt1, and have been isolated all over the world. Only a few countries are free of terrestrial rabies, as follows:

– New Zealand
– Australia
– Japan

– the United Kingdom
– Ireland
– Scandinavia
– Iceland.

Other countries, for instance, several European countries, have become free of terrestrial rabies through oral vaccination. Genotype 1 isolates are also found in the Americas in flying mammals (such as haematophagous and insectivorous bats).

This paper is intended to give a global view of human and animal rabies epidemiology in different parts of the world, describing the principal methods of control and prophylaxis and the difficulties associated with preventing this disease. Obviously, many deficiencies in surveillance, control and prevention are linked to inadequate financial resources due to a political lack of interest. However, whenever feasible, making some practical changes to the way in which anti-rabies campaigns are managed would probably be beneficial without increasing the global costs.

Rabies-related viruses in Australia

Until 1996, Australia and the Antarctic were the only two continents recognised as being free of RV and RRV. However, in June 1996, a *Lyssavirus* infection was diagnosed in a frugivorous bat belonging to the Megachiroptera family (*Pteropus alecto*) in Australia (41). The presence of RRV was then demonstrated in another species of insectivorous bat (*Taphozous flaviventris*) (48). The virus that was isolated from these bats, ABLV, which most closely resembles the Gt1 strains in terms of antigen and genotype, became Gt7 in the *Lyssavirus* genus.

Since 1996, other indigenous Australian species of frugivorous and insectivorous bats have been shown to be reservoirs for ABLV (39, 48). The distribution for ABLV corresponds to the geographic dispersion of these flying mammals in mainland Australia (44, 91).

The ABLV isolates are separated into two clades: frugivorous bat isolates and insectivorous bat isolates. The presence of two ABLV variants in Australia suggests an exchange or 'cross-over' of the virus from one species of bat to another at a specific time in the past (43). The differences observed between the protein sequences of these two variants reflect the virus adapting to a new host.

Since 1996, two cases of human infection due to ABLV have been reported in Australia. The first case was diagnosed in November 1996, in a thirty-nine-year-old woman, five weeks after she had probably been bitten by an insectivorous bat, *Saccolaimus flaviventris*. The patient was an animal handler and she died twenty days after the onset of the illness (46). The second case was reported in another woman, aged twenty-seven, in 1998. This was more than two years (n = 27 months) after she had been bitten on the finger by a frugivorous bat (from the *Pteropus* family). Neither of these women had been vaccinated against rabies (2, 46).

Following these human cases, investigations were conducted on three cats and three puppies inoculated with virulent ABLV to study the pathogenicity of the virus on domestic carnivores. The preliminary results (62) showed that the animals survived, but the test period was limited to only three months after inoculation.

Shortly after the first human case, preventive anti-rabies vaccination was strongly recommended for people who are occupationally or recreationally exposed to bats and advice was given on what to do when scratched or bitten by bats (4).

Rabies in Asia

In Asia, the main transmission route for rabies is a rabid dog bite: between 94% and 98% of rabies deaths are due to canine bites (37).

Table II demonstrates that the number of human rabies cases in Asia is by far the highest in the world. Rabies is particularly rife in over-populated countries (Bangladesh, India and Pakistan).

Over the last few years, there has been a decrease in mortality rates in countries where efficient disease control measures have been implemented, e.g. Thailand and Vietnam (52). In other countries, such as the People's Republic of China and the Philippines, the number of cases has increased over recent years. There are several possible reasons for this increase, as follows:
– in particular, an increase in rabies surveillance
– an increase in dog populations
– inadequate post-exposure treatment
– the lack of effective rabies vaccines (100).

Most people (usually children) who die of rabies have not been treated or have not received adequate treatment because of the high cost of vaccines and rabies immunoglobulins. Furthermore, both rabies immunoglobulins and vaccines are not always available.

Moreover, although the World Health Organization (WHO) recommends using only cell-culture vaccines, some Asian countries (particularly Bangladesh and Pakistan) still manufacture cheap vaccines produced from infected brains. Apart from being less efficient, these

Table II
Epidemiological data of rabies in animals (domestic and wild) and humans, reported to the World Health Organization (WHO) between 1990 and 1999

Continent	Year	No. of bat cases	Total no. of animal cases	No. of human cases	No. of people treated for exposure to domestic animals	No. of people treated for exposure to wild animals	Total no. of people treated
Africa	1990	1	3,883	282	65,117	1,460	66,577
	1991	0	4,018	335	68,752	405	69,157
	1992	1	4,743	494	47,154	2,223	49,377
	1993	0	4,733	507	48,159	258	48,417
	1994	0	4,139	141	14,051	126	14,177
	1995	0	4,361	206	39,091	1,351	40,442
	1996	0	2,857	238	46,124	1,761	47,885
	1997	0	2,344	200	21,757	605	22,362
	1998	2	4,365	204	409,379	4,071	413,450
	1999	0	3,507	147	15,380	1,060	16,440
North America and Canada	1990	686	6,655	1	92	ND	92
	1991	755	8,649	3	ND	ND	ND
	1992	699	11,021	1	ND	ND	ND
	1993	808	11,515	3	ND	ND	ND
	1994	670	9,087	6	ND	ND	ND
	1995	806	8,356	4	ND	ND	ND
	1996	781	7,421	4	ND	ND	ND
	1997	958	8,646	4	ND	ND	ND
	1998	1,076	8,325	1	ND	ND	ND
	1999	1,031	7,505	0	ND	ND	ND
Latin America	1990	16	15,513	167	77,780	1,026	78,806
	1991	28	15,570	145	45,556	470	46,026
	1992	18	14,934	219	62,748	527	63,275
	1993	31	9,599	196	29,345	329	29,674
	1994	ND	8,504	144	40,661	691	41,352
	1995	38	7,731	149	34,017	571	34,588
	1996	77	11,126	179	74,338	4,962	79,300
	1997	3	7,840	110	3,446	244	3,690
	1998	5	6,286	86	284,219	14,980	299,199
	1999	17	9,345	75	74,838	4,135	78,973
Asia	1990	0	8,942	821	49,580	1,239	50,819
	1991	1	7,610	802	209,699	3,397	213,096
	1992	0	10,149	33,880	669,504	1,870	671,374
	1993	ND	11,818	30,500	225,484	5,085	230,569
	1994	0	14,111	33,801	181,437	2,510	183,947
	1995	ND	26,087	35,191	23,791	1,933	25,724
	1996	0	4,700	32,772	39,702	20,544	60,246
	1997	0	9,695	33,008	674,322	2,766	677,088
	1998	0	7,258	33,075	883,395	7,894	891,289
	1999	0	6,583	1,613	758,382	1,062	759,444
Europe	1990	39	17,181	21	176,260	6,344	182,604
	1991	15	14,446	41	116,418	6,219	122,637
	1992	14	12,751	20	33,593	3,746	37,339
	1993	18	9,178	17	35,795	6,952	42,747
	1994	6	8,908	18	156,097	65,557	221,654
	1995	6	9,618	33	49,036	8,881	57,917
	1996	6	8,059	16	39,702	20,544	60,246
	1997	29	5,098	13	46,534	4,208	50,742
	1998	24	6,108	7	36,801	3,651	40,452
	1999	40	7,201	31	33,355	8,034	41,389
Oceania	1990	0	618	492	7,096	604	7,700
	1991	ND	604	0	2,865	494	3,359
	1992	ND	1,420	317	11,654	318	11,972
	1993	ND	0	0	ND	ND	ND
	1994	ND	0	0	0	0	0
	1995	ND	0	0	ND	ND	ND
	1996	ND	0	0	ND	ND	ND
	1997	ND	0	0	0	0	0
	1998	0	0	0	7	2	9
	1999	0	0	0	6	4	10

Source: compilation of world surveys of rabies, RABNET, WHO (96)
Depending on both countries and years, data are inconsistently reported to WHO, excluding a detailed and comparative analysis of these data
ND: no data

cheaper vaccines need heavier vaccine protocols and can present residual pathogenicity. These vaccines, when they are available, are usually offered to the poor who need treatment.

In Asia, dog populations can be divided into three groups, as follows:
– dogs with owners
– community dogs
– stray dogs.

Data from Thailand for the year 2000 demonstrate that 53% of human cases of rabies were caused by dogs with owners. However, 47% were caused by stray dogs (77). It is much more difficult to implement control measures (particularly the parenteral antirabies vaccination) for strays. Most countries have a reasonably regular and organised system for slaughtering stray dogs, but this system is often not very well accepted by the population for both cultural and religious reasons. Moreover, these slaughtering campaigns, which are usually conducted on a small scale, are not sufficient to eliminate all the stray dogs in one region.

Since 1994, the World Society for the Protection of Animals, in close collaboration with the animal protection organisations of the countries concerned, has been implementing control measures for stray dog populations; the situation varies from country to country and the measures are adapted accordingly (99).

In India, for example, between 1993 and 1998, 97,000 stray dogs (from a total dog population of an estimated 25 million) were captured and then released after being neutered and vaccinated against rabies. According to analysis, if these programmes of birth control are regularly conducted, they should lead to the stabilisation of stray dog populations within five to seven years (61).

Where the actual control of canine rabies is concerned, only a few countries (Indonesia, Malaysia, the People's Republic of China, the Philippines, Sri Lanka, Thailand and Vietnam) have a satisfactory system of disease surveillance and control. Despite distinct improvements during the last few years, epidemiosurveillance campaigns are often established only partially or 'piecemeal', and only in those regions with trained personnel and adequate existing facilities, such as a laboratory.

These control programmes are based on regular mass parenteral vaccination campaigns, either throughout the country or in certain territories. The aim is to vaccinate 75% to 80% of all dogs against rabies. Certain countries, such as Malaysia, have opted for strategies that aim to develop a 'belt' of immunity along their frontiers or to protect certain densely populated urban areas (such as Hanoi in Vietnam) (97). Depending on the country, vaccine coverage is estimated at between 10% and 94% (37). Other countries, e.g. Sri Lanka, have successfully initiated control programmes which combine parenteral vaccination, oral vaccination (73) and sterilisation of dogs which have owners. (In Sri Lanka, the annual turnover of the dog population is 25%.)

In 1997, a rabid dog introduced rabies onto an island in Indonesia (Flores). Soon after, the entire island was infected. As there was no veterinary infrastructure, rabies control was based principally on reducing the canine population. In fact, 44% of the original population was eliminated. This involved considerable participation from the community and local government (16).

It is uncertain how the rabies cycle is maintained in Asia. Wild animal species such as the mongoose, wolves, jackals (*Canis mesomelas*) or bats have been suggested as reservoirs for the virus, but there are no data to support such hypotheses. A recent study reported the presence of antibodies that seroneutralise the ABLV isolates in frugivorous and insectivorous bats in the Philippines (7), despite the fact that these animals had no *Lyssavirus* infection in their brains. These data, which need to be confirmed on a larger scale, suggest that there is an ABLV-related lyssavirus in the Philippines, and perhaps also in Southeast Asia, which is transmitted by certain bat species. However, no epidemiological sequencing supported by molecular data is available to demonstrate the existence of a possible rabies cycle in wild animals. The only cycle which has been observed is the one maintained by the canine population.

Until recently, very few studies of rabies isolates had been conducted in Asia, but over the last few years four new isolates have been described in insectivorous bats (Table I). The Aravan virus was isolated in *Myotis blythii* in Kyrgyzstan (6) in 1991 and the Khujand virus in *M. mystacinus* in Tajikistan in June 2001 (58). The Irkut virus, which is phylogenetically similar to Gt4 and Gt5, was detected in a *Murina leucogaster* in 2002 in eastern Siberia and the West Caucasian bat virus was isolated in a *Miniopterus schreibersii* in the Caucasus (20). West Caucasian bat virus is phylogenetically similar to the cluster of Gt2 and Gt3 and is the most distinct lyssavirus detected so far (20).

Rabies in Africa

Epidemiology of rabies viruses in terrestrial host species

In Africa, the dog is the principal vector and reservoir of rabies. Dogs represent more than 75% of rabid animals in most African countries. Most of the reported human infections occur in children under ten years of age.

Although sporadic rabies cases in wild mammals have been documented throughout the African continent, the disease has not been extensively studied in these countries. This is understandable, because applying control measures and educating the general public is difficult when there are so many other public health priorities, e.g. acquired immune deficiency syndrome (AIDS), malaria, and tuberculosis.

Canine rabies has existed in Algeria, Morocco, Tunisia and Egypt for centuries and in each of these countries, more than 100 cases a year are reported in dogs (28).

In Tunisia, a national rabies control programme was implemented in 1982. Mass campaigns of parenteral dog vaccination were conducted every two years at first, then, with the rapid dog population turnover, annually, with quality vaccines produced locally. The number of rabies cases decreased accordingly (81). In 1999, 178 cases of rabies were recorded in animals and there was one case in humans.

In Morocco, in 2002, there were 446 cases of rabies in animals and 23 cases of human mortality. These human cases occurred mainly in rural and suburban areas and were transmitted by dogs. It is important to note that 86% of the people who died of rabies between 1995 and 2001 in Morocco had not received any anti-rabies vaccine treatment (36).

Canine rabies is present in all of the countries situated between the Sahara desert and the Equator. The role of jackals and hyenas (*Crocuta crocuta*) is occasionally important in some areas, but as spillover from dogs (i.e. additional to canine rabies) rather than as an independent disease cycle (19).

The area between Sudan and Guinea presents the same epizootic characteristics in the south as those that exist in the sub-Saharan zone to the north, but with the addition of accidental infection of wild species (monkeys, bats, rodents, etc.). Nigeria occupies a special place, having been the source of numerous RRV isolates from shrews (*Sorex* spp.) and bats infected by Lagos virus and Mokola virus (MOKV).

In Tanzania, it has been demonstrated that the number of cases of human rabies is clearly underestimated, with a recent study by Cleaveland *et al.* suggesting that there are actually 4.9 human deaths per 100,000 inhabitants, 100 times more than the number officially recorded (29). In the Cleaveland study, rabies mortality was estimated according to ground data on the following:

– the incidence of wounds from animal bites
– the accuracy of the rabies diagnosis
– the number of bite wounds received
– the treatment post-exposure (28).

The number of human cases is high, due principally to:

– the high cost of post-exposure treatment
– the rapid growth of human and dog populations
– the increasing mobility of rural populations
– the decline in the infrastructure and resources available for disease control (27).

In South Africa, Angola, Botswana, Namibia, Zambia and Zimbabwe, canine rabies represents an important risk for humans. The situation is complicated by independent rabies cycles in wild mammals and by the presence of RRV (Gt 2, 3 and 4) in wild cats (72). Jackal populations support the epidemic of canine RV generally associated with the domestic dog. The same RV found in canines has also been discovered in the bat-eared fox (*Otocyon megalotis*). Several variants of this strain also exist. One is the canine biotype closely related to the European strains of RV, which is maintained by members of the Canidae family, the bat-eared fox, jackal species and the domestic dog.

The incidence of canine rabies is increasing in sub-Saharan Africa, and few successful dog vaccination programmes have been implemented in the last twenty years (29). Some attempts at oral vaccination have been conducted in jackals (17), free-ranging African wild dogs (*Lycaon pictus*) (55) and domestic dogs (11).

Rabies-related viruses in Africa

Genotypes 2, 3 and 4 have been isolated in Africa (Table I). The isolation of MOKV has been reported sporadically and rather infrequently from only a small number of African countries. This is the only lyssavirus that has never been isolated in a bat. However, surprisingly, it has been isolated in a number of other different hosts, such as shrews, cats, dogs and rodents.

To date, RRV of genotypes 2, 3 and 4 has never been isolated outside of Africa, with the exception of one case of rabies in the frugivorous bat (*Rousettus aegyptiacus*), reported in France in May 1999. This bat had been imported from Africa to France through Belgium, and molecular data demonstrated that the virus was an LBV (30).

Rabies in America

North America and Canada

In the 1950s, canine rabies was endemic in the United States of America (USA), but carefully planned control measures and parenteral vaccination have brought it under control. In the USA and Canada today, rabies reservoirs are to be found among wildlife. Different epidemiological cycles exist in the following species (the figure in parentheses indicates a percentage of the total number of rabies cases):

– raccoons (*Procyon lotor*) (40%)

– skunks (*Mephitis mephitis*) (30%)

– bats (approximately 17%)

– red and grey foxes (*Vulpes vulpes* and *Alopex lagopus*) (approximately 6%). These species are infected with several RV variants (Gt1).

Usually, the geographical distribution of the virus strains which belong to a particular species is delimited. The virus variants circulating in the raccoon are located along the Atlantic coast as far as the Appalachians, those circulating in the skunk are found in California and in the North Central and South Central states, and those circulating in the grey fox (*Urocyon cinereoargenteus*) are found in Alaska, Arizona and Texas. Finally, on the border between Texas and Mexico, coyotes (*Canis latrans*) and unvaccinated dogs maintain a rabies cycle with a canine virus. On the other hand, the cycle maintained by insectivorous bats is distributed throughout the continent of the USA.

The domestic species most often infected (representing about 7% of the total number of cases) are cats, dogs and bovines. One study has shown that cats and dogs are usually infected by the most common wild reservoir species at the infection site (64).

Since the 1950s, at least 39 human cases of rabies linked to infection by a rabid bat have been recorded in the USA, and in most cases it was unclear how the victims were infected because most had no memory of being scratched or bitten. The species most often involved are the eastern pipistrelle (*Pipistrellus subflavus*) and the silver-haired bat (*Lasionycteris noctivagans*) (57).

Spillover infection has been observed from other wild animals into domestic carnivores. In the same way, the viruses present in bats are sporadically found in cats and dogs (64). Since 1990, these viruses have been implicated in 92% of indigenous human cases of rabies (57).

As in Europe, the first rabies control measures applied in the USA consisted of attempts to reduce the reservoir populations, but these were unsuccessful (85). Oral vaccination has proved to be effective in significantly reducing the number of rabies cases in red and grey foxes, as well as in raccoons and coyotes, but oral vaccination is much more difficult in the USA than in Europe, where the number of reservoir animals is limited and the surface areas to be covered by vaccination are much smaller. Oral vaccination of raccoons in each state of the eastern seaboard seems to be the best way of avoiding the spread of the virus within the USA, but this method is very expensive (92).

There is no effective means of controlling rabies in such inaccessible vector species as the insectivorous bat. Control measures include public education campaigns (1), preventive measures for certain occupational groups (in particular, vaccination for rabies researchers) and post-exposure treatment for people who are infected.

Latin America

Most of the recorded human cases of rabies are caused by domestic animals. Some biomolecular studies also suggest the presence of several wild reservoirs for the disease (in particular, the mongoose) (86).

In Latin America there are two epidemiological cycles, both with Gt1. The first cycle is terrestrial and is maintained by dogs; canine rabies being enzootic in most Latin American countries, including the Caribbean islands. The second is aerial and is carried by bats. Several species of haematophagous bats, the main one being *Desmodus rotundus* (vampire bat) (93), represent the principal transmission vector of RV to domestic carnivores and livestock, causing considerable economic losses (65). In historical terms, the presence of RV in Latin America was suggested for the first time in insectivorous bats in Brazil in approximately 1910, and confirmed in the insectivorous and frugivorous bat in Trinidad in 1931. In Latin America, as in the USA, several variants of RV exist (86).

Sporadic cases are recorded in humans. The risk of transmission from vampire bats to people is estimated at 0.96 cases for every 100 inhabitants in Amazonia in Brazil (100). Insectivorous bats can also infect humans (38). These species represent an important reservoir for rabies, particularly in urban environments (34). Haematophagous bats, on the other hand, are found in rural environments, close to livestock breeding areas.

Rabies control measures are being targeted at terrestrial and aerial reservoirs, as well as at preventing human infection. Since the beginning of the 1980s, an important canine rabies control programme has been implemented in Latin America (15), with the technical co-operation of the Pan American Health Organization. This programme is based on campaigns for the mass parenteral vaccination of dogs. The results of these mass vaccination campaigns, which are remarkably well organised in some countries (for example, nearly 10 million dogs were vaccinated in one week in Mexico in 1999 [14]), are very encouraging and have led to a significant reduction in the number of animal and human rabies cases (Table II).

For vampire bats, the main disease prevention measure consists of reducing or eliminating certain populations of *Desmodus desmodus*. These bats are very sensitive to the effects of anti-coagulants, which can be injected either into bovines, on which they feed, or into captured bats (25).

Rabies in Europe

In Europe, the main reservoir and vector of rabies, since the late 1930s, has been the red fox (*Vulpes vulpes*), followed by the raccoon dog (*Nyctereutes procyonoides*) in Central and Baltic Europe. In Estonia, the raccoon dog is the primary infected species, accounting for more than 44% of the rabies cases in 2003 (5). Once the virus is introduced into a rabies-free area, the resulting outbreak considerably reduces the local fox population. However, the population rapidly recovers, due to its high reproductive potential. With the exception of islands (i.e. Britain, Ireland) and large peninsulas (Norway, Sweden), most European countries have become infected (88). At the end of the 1970s, the farthest limits of the rabies wave to the north, south and west were the Netherlands, Italy and France, respectively. Turkey is the only European country where the domestic dog is the principal vector of rabies. However, a molecular analysis of different isolates (50) suggests that there has been recent spillover from the domestic dog to the fox, which could imply the development of an endemic cycle in wildlife.

Disease control measures, consisting principally of extensive fox culling, did not prevent the spread of the virus (9). The only control measure that has proved to be both lasting and efficient is oral vaccination. Vaccine baits, containing a capsule or plastic sachet filled with an anti-rabies liquid vaccine, are scattered throughout fox habitats. Switzerland was the first country to adopt such programmes, in 1978. To date, seven European countries are reported to be free of rabies as a result of oral vaccination programmes:

– Finland and the Netherlands since 1991
– Italy since 1997
– Switzerland since 1998
– France since 2000
– Belgium and Luxembourg since 2001.

In 1989, the European Commission (EC) began to subsidise rabies control programmes in wildlife (half of the total cost), on condition that co-ordinated strategies be established at country borders. As a result, countries in Central Europe began to progressively establish vaccination programmes, with a consequent and very significant decrease in the incidence of terrestrial rabies in these countries (Table III).

Today, the EC also provides support to the 'new countries' of the EC (the ten countries that entered in May 2004) in terms of animal disease control programmes. The most recent of these programmes were established in Hungary, Lithuania and Estonia.

Table II records the epidemiological data on rabies for all of Europe. Registered human cases of the disease are due, in most cases, to the following causes:

– canine rabies contracted in a non-European country
– infection by a rabid indigenous and imported wild or domestic carnivore (mainly in Russia and the Ukraine)
– infection by a rabid bat (four human cases have been reported since 1977).

Since the first case of rabies in bats was recorded in Europe in 1954, Gt5 (EBLV-1a) and Gt6 (EBLV-2b) have been detected throughout the continent. The EBLV-1 strain seems to be more prevalent, infecting approximately 95% of all bats testing positive for the presence of the virus between 1977 and 2000 (40).

Bats infected with EBLV-1 and EBLV-2 have been reported throughout Europe, from Russia to Spain, particularly in coastal regions (25). Isolates of EBLV-2 have been recorded in Central Europe, Switzerland, Eastern Europe and the Ukraine (25, 51), whereas EBLV-1 strains are distributed principally throughout Western European countries. The EBLV-1a strain has been found in Germany, the Ukraine, Russia, Poland, the Netherlands and Denmark, while EBLV-1b has been reported from France, Spain and the Netherlands (3, 25, 82).

It is interesting to note that only two countries are known to be infected by both EBLV-1a and EBLV-1b (the Netherlands and France). The EBLV-2 variant has never been isolated in France. However, this strain could arrive naturally from Eastern or Northern Europe.

Table III
Incidence of rabies in native terrestrial animals in European countries which conduct oral vaccination programmes

First year of vaccination programme	Country	Number of native terrestrial animal cases 1989	1998	2003
1978	Switzerland	60	0	0
1983	Germany	6,823	104	24
1984	Italy	55	0	0
1986	Austria	1,890	3	1
1986	Belgium	842	1	0
1986	France	4,213	2	0
1986	Luxembourg	139	0	0
1988	Finland	6	0	0
1988	Netherlands	23	0	0
1988	Slovenia	761	14	8
1989	Czech Republic	1,463	85	0
1992	Hungary	1,061	554	172
1992	Slovakia	250	414	326
1993	Poland	1,891	1,332	382

Source: Rabies Bulletin Europe (5)

As stated above, since 1977, four people have died of rabies after being infected by bats. None of these people had been immunised against the disease; nor did they receive any treatment after exposure. Apart from these four fatal human cases, EBLVs adapted to insectivorous bats have not been reported as crossing the species barrier. However, this might reflect under-reporting caused by the difficulties associated with cryptic cases of rabies in sylvatic species. Some spillover of EBLV-1 from bats to terrestrial mammals has been documented in Europe. In Denmark, in 1998 and 2002, two sheep were infected with EBLV-1a and in Germany in 2001, a stone marten (*Martes foina*) became infected with EBLV-1 (69, 78).

Discussion

Rabies is a disease for which all the necessary remedies exist, in contrast to the situation with many other diseases. That is, it is possible to prevent, control and treat rabies; the present human and veterinary vaccines produced by cell culture are safe and effective and industrially-produced antirabies immunoglobulins exist, although they are not as freely available as one would wish. Yet, in spite of this, WHO records more than 40,000 human deaths from rabies each year. These deaths are nearly always associated with infection by a Gt1 virus from a dog. Children aged between five and fifteen are the most affected and about 7.5 million people receive post-exposure treatment each year. The number of cases in humans and animals is still believed to be underestimated. Moreover, as epidemiological surveillance is absent, irregular or insufficient (28) in several developing countries of Asia and Africa, the levels of under-reporting are difficult to estimate.

Treatment and control: problems and recommendations

One of the principal difficulties in controlling rabies is the lack of funds dedicated to this particular disease, perhaps because rabies has made no significant political or economic impact thus far. There are still too few precise data on the actual number of cases and the amount of money that could be saved by successfully controlling the disease (32).

Recent studies have developed a tool for analysing the impact of rabies (32): the disability-adjusted life year, which is already used for other diseases. This method enables researchers to compare the effects of several different diseases on public health and economic losses. When this tool was used in Tanzania (28), it demonstrated the importance of rabies, when compared to other diseases which had previously been considered as being of higher priority. This type of analysis could be put to good use in several Asian and African countries, and could also help in persuading countries to recognise rabies as a notifiable disease. For the last three years, the WHO has been encouraging discussion on a plan of action which would ensure, as follows:

– better control of rabies in Asia

– better monitoring of the disease

– identification of all the interested parties ('stakeholders') and information required to promote political support for such action (97).

Most people who die of rabies have either never been treated or have received some treatment, but not necessarily in the time required or in accordance with the WHO protocol. However, the WHO post-exposure protocol, widely used in developed countries, may be difficult to apply in developing countries because it is both costly and complicated. Owing to the expense and difficulty of obtaining human immunoglobulins, some developed countries use horse immunoglobulins. Moreover, in many developing countries there is a shortage of rabies vaccines. This is frequently accompanied by a lack of medical infrastructure and medical personnel are often insufficiently trained. There is also a lack of good communication and public education to ensure that rabies information is readily available and that people know what to do when bitten by a rabid animal. The simple low-cost step of cleaning the wound thoroughly with a large amount of soap and water is not always taken, although it is essential in decreasing the viral load at the point of infection and thus increasing the chances of survival (54). This fact needs to be more widely known, particularly when children are bitten.

Public education is not just a priority for developing countries, for example, France still has much progress to make in informing the general public about bat lyssaviruses, without causing panic, which might lead to the irrational and unnecessary destruction of bat colonies. Information on the risks of rabies and rabies regulations should also be given to the increasing number of people travelling in rabies-endemic areas. In some cases, such travellers have illegally, but often unwittingly, brought a dog back into a rabies-free zone (26). In 1999, a frugivorous bat (*Rousettus aegyptiacus*) was imported from Africa through Belgium to France. The bat carried a Gt2 isolate and, as a result, nearly 130 people who came in contact with the bat had to receive post-exposure treatment. Laws on animal imports should be more strictly enforced and customs controls should be more rigorous.

Today, several possibilities are being explored to try to improve access to treatment for infected humans. Monoclonal antibody 'cocktails' (mixtures) could be developed to compensate for the lack of readily available immunoglobulins (79). Systematic vaccination could become part of the Expanded Programmes on Immunisation (60). Finally, local manufacture of immunoglobulins and vaccines in countries at risk would enable these countries to reduce costs. At present, the WHO is compiling the results of all experiments concerning post-exposure treatment by the intradermal route (66). New, more efficient biological products which are cheaper and easier to produce are necessary to allow the local transfer of technology among countries in Asia and Africa.

The ultimate control measure for rabies is to eliminate the virus from its reservoir populations. However, better co-ordination between the public health sector, the veterinary sector and local government departments would go a long way towards making control programmes more effective. The WHO has established guidelines which explain in detail how to organise local rabies control programmes (98).

Problems encountered in the control of canine rabies stem notably from lack of the following:

– financial resources
– effective surveillance
– adequate veterinary health infrastructure
– readily available information
– public awareness
– ecological studies on local dog populations.

Ideally, there should be a national reference laboratory for rabies diagnosis in every country. Dogs usually receive rabies vaccinations when they are 3 months old, but very often they become infected before they reach this age. Most of the infected dogs are destroyed, but some may be kept and quarantined because of the religious beliefs of the owner. The authors have already demonstrated that vaccination before the age of three months is effective, even in an animal with maternal antibodies (31). Puppies should be vaccinated along with adult dogs during any mass parenteral vaccination campaign. This would help to broaden vaccine coverage and reduce the incidence of rabies in children.

The destruction of all stray and feral dogs, which is usually very unpopular with the general public, does not, in the long term, constitute a realistic method of disease control. In some cases, selective destruction may be an effective accompanying measure. Research is now in progress to try to develop a contraceptive vaccine, which would limit dog reproduction in a reversible manner. The ideal would doubtless be to have a rabies immunocontraceptive vaccine (21). Oral anti-rabies vaccination, which has already proved effective in dogs, should be used more often during parenteral vaccination campaigns to try to reach inaccessible dogs. In some countries, better management of household waste would certainly lead to a reduction in the density of dog populations, as their reproduction capacity is linked to sources of available food. Similarly, better management of slaughterhouse waste would provide better tools for the control of both rabies and *echinococcosis*/hydatidosis.

Countries should also be encouraged to develop the local production of cell-culture vaccines. Deoxyribonucleic acid (DNA) vaccines, which have proved to be efficient under experimental conditions, should soon be validated in the field.

As far as rabies control in wild terrestrial animals is concerned, the only efficient and cost-effective technique thus far (10) has been oral vaccination. In Europe and North America, oral vaccination has led to the elimination of the virus in several species of carnivore.

In Europe, there is a growing awareness of the importance of rabies control among new and 'candidate' EC countries, and since 3 July 2004, thanks to effective rabies control measures and the disease-free status of several European countries, legislation with regard to animal health requirements for the transport of companion animals has been harmonised throughout the European Union.

Further studies required

Bats have been found to be carriers of six genotypes of rabies (only Gt3 has not been isolated), but in Europe, for reasons still unknown, the Gt1 isolate commonly detected in insectivorous bats from North America has not been isolated so far. Further studies are needed to increase scientific knowledge about bat ecology and viral transmission between bats. This is particularly important, because as previously mentioned, four new genotypes have recently been isolated in insectivorous bats in Asia and Eastern Europe.

No failure in post-exposure vaccinal treatment has been recorded in France or in the rest of Europe. This suggests that the RV vaccines produced with Gt1 induce antibodies that should be capable of cross-neutralising and cross-protecting against at least some of the other genotypes. To evaluate the transmission risk of these viruses and of the new isolates, protection experiments should be undertaken in mice, using human and veterinary vaccines; according to these results, new pharmaceutical molecules or new chimeric vaccines could be developed to enhance the vaccinal protection against all lyssaviruses. Previous studies have been conducted with the most severely unnatural challenges (injection by the intracranial route).

As for carnivores, the rabies virus remains largely confined to bat populations, although some spillover from bats into terrestrial mammals and humans has been documented in Europe. Transmission of RV from one terrestrial mammal to another remains the most important source of infection for terrestrial mammals, when compared to terrestrial animals infected by bats. Nevertheless, experimental laboratory studies must be conducted to determine the viral pathogenicity of EBLV and ABLV for wild and domestic terrestrial mammals. The so-called emergence of EBLVs in Europe raises the question of vaccination for certain occupational groups, in particular medical and veterinary professionals, as well as bat handlers.

Diagnostic tools

Three principal methods of diagnosis have been standardised and recommended by the WHO, as follows:

– the immunofluorescence test (FAT)

– cell inoculation tests (i.e. the rabies tissue culture inoculation test or RTCIT)

– the mouse inoculation test (8, 35, 56).

However, although these techniques are standardised and routinely used in every laboratory (FAT and RTCIT being the most common), they vary in efficacy, specificity and reliability. The sensitivity of each method depends upon the biological quality of the specimen being diagnosed. Moreover, the sensitivity of most of these methods is high with rabies isolates (Gt1), but may be reduced with RRV isolates (74).

Thus, for the last twenty years, rabies diagnosis laboratories have been developing tools to enable virus typing, either with the help of monoclonal antibodies or by sequencing the amplified products of reverse transcription polymerase chain reaction (RT-PCR).

As a result of the development of molecular biology tools, other techniques for diagnosis have evolved, such as the following:

– RT-PCR (33, 47, 53, 70, 76, 80)

– RT-PCR followed by restriction fragment length polymorphism

– PCR-enzyme-linked immunosorbent assay (ELISA) (18, 22, 89)

– hybridisation *in situ* (69).

All these techniques have the advantage of being rapid, sensitive and specific, and provide information about types and epidemiology. Since the 1990s, considerable progress has been made in understanding the modes of circulation and distribution of rabies viruses among the different animal populations and geographical areas.

Recently, even faster and more sensitive techniques have been developed, such as the TaqMan test, to identify the RNA of the RV (49, 63). However, although they are both sensitive and rapid, all molecular biology techniques to date increase the risk of contaminating samples within the laboratory (and thus giving false positive results) (75). Consequently, the WHO Expert Committee on Rabies meeting in 1992 did not recommend the use of PCR in routine diagnosis (95). Hence, harmonisation of the classical methods of diagnosis with the new techniques would appear necessary. All those countries affected by RV, in addition to countries which are currently free of rabies, but potentially infected by RRV, should perhaps be advised to do the following:

– standardise diagnosis techniques

– validate all protocol modifications

– participate in interlaboratory comparison trials of serological tests (83) and rabies diagnosis (13)

– produce reference reagents to study newly isolated viruses

– confirm all positive diagnoses, especially in countries previously reported free of rabies, by sending the original brain to independent reference laboratories as soon as possible for typing.

As a complement to these reference techniques, RT-PCR methods followed by the sequencing of amplified products have become indispensable molecular biology tools for confirming positive diagnoses in research laboratories and for typing RV.

New techniques such as RT-PCR, PCR-ELISA and PCR-TaqMan could be used on the micro samples of saliva and blood collected from living bats, with minimum disturbance to the animal.

Anti-rabies antibodies can be induced by contact with the virus. The presence of viral RNA in the saliva indicates infection. This gives rise to an important question of post-exposure treatment when the RT-PCR response is positive in the saliva of animals (particularly the bat) and negative by RTCIT. Thus, should those who have handled bats, and have come into contact with bats in apparently good health, receive post-exposure treatment?

Conclusion

Rabies is continually evolving throughout the world. Significant advances have been made since the 1980s in the multi-disciplinary sectors of immunology, vaccinology, molecular virology and epidemiology, thus allowing a far greater understanding of RV circulation.

Continuing molecular epidemiological and surveillance studies are necessary to trace spillover transmission from reservoir species to non-reservoir animals and humans, and also to monitor the emergence of specific rabies strains into new species and geographic areas, which, to a large extent, is often prompted by human activities (i.e. the movement of wildlife and importation of animals).

Phylogenetic analysis strongly supports the theory that lyssaviruses evolved in bats long before the emergence of rabies in carnivores, which was very probably caused by regular host-switching from bats. Such host switching is also observed in wildlife; for example, the emergence of a *Myotis* bat variant strain in Arizona in 2002, which created an epizootic among skunks (87).

New causative agents of rabies continue to emerge, with the recent description of four novel lyssaviruses. The West Caucasian bat virus is the most divergent of known lyssaviruses, and, according to a report given at the 2003 International Conference on Rabies in the Americas, neither pre-exposure vaccination nor conventional post-exposure treatment provide any significant protection against it (45).

Bat rabies epidemiology should be more comprehensively explored so that precise risks to the health of humans and domestic carnivores can be identified and effective disease prevention measures can be applied to those who handle bats. Such further investigations should be conducted in close collaboration between bat biologists and laboratory scientists.

In Europe, the objective for the future is to eliminate terrestrial rabies from the continent. This will involve:

– co-ordinating all efforts to maintain the disease-free status of those countries which are presently free of rabies

– providing technical assistance and recommendations for European countries which are not already involved in rabies-prevention programmes, particularly those from:

a) eastern Europe (the Ukraine, European Russia)

b) northern Europe (the Baltic countries)

c) southern Europe (Romania, Bulgaria).

A further and more ambitious goal is to co-ordinate all efforts from all sectors to increase rabies surveillance programmes in Africa and Asia and thus ultimately to decrease the incidence of rabies on these continents.

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■

Le virus de la rage et les virus apparentés : un regard nouveau sur une ancienne maladie

F. Cliquet & E. Picard-Meyer

Résumé

La rage est une zoonose présente mondialement, provoquée par un lyssavirus, dont le réservoir infectieux est constitué par une multitude d'espèces hôtes. Au cours de ces dernières années, l'évolution épidémiologique de la rage s'est inscrite dans un contexte de maîtrise ou d'élimination de la pathologie chez de nombreuses espèces animales terrestres, tant en Europe qu'en Amérique du Nord.

Alors qu'un grand nombre de variants du lyssavirus ont été caractérisés et que leur répartition géographique et leurs animaux hôtes ont été déterminés, l'isolement de nouveaux lyssavirus chez les chauves-souris a amené les scientifiques à s'interroger sur l'efficacité des vaccins humains et vétérinaires employés actuellement contre la rage. La rage des chauves-souris doit faire l'objet d'une étude épidémiologique approfondie en vue d'apprécier son risque réel pour la santé de l'homme et des carnivores domestiques et sauvages, et trouver éventuellement des moyens pour prévenir son apparition dans les populations en contact avec les chauves-souris. La rage demeure un grave problème de santé publique, notamment dans les zones où elle conserve un caractère endémique, par exemple dans les pays d'Afrique et d'Asie.

Mots-clés
Chauve-souris – Épidémiologie – Lyssavirus – Rage – Rage des chauves-souris – Rage terrestre.

■

El virus de la rabia y virus afines: consideraciones modernas sobre una enfermedad antigua

F. Cliquet & E. Picard-Meyer

Resumen
La rabia es una zoonosis de alcance mundial causada por un lyssavirus capaz de alojarse en numerosas especies hospedadoras que ejercen de reservorio de la infección. La epidemiología de la rabia ha cambiado en los últimos años, a medida que en Europa y Norteamérica se controlaba o eliminaba la enfermedad en muchas especies de animales terrestres.
Se han caracterizado un gran número de variantes de lyssavirus, cuya distribución y especies hospedadoras han quedado descritas. No obstante, el aislamiento de nuevos lyssavirus en murciélagos ha llevado a los científicos a cuestionar la eficacia de las actuales vacunas antirrábicas humanas y veterinarias. Conviene investigar a fondo la epidemiología de la rabia del murciélago para determinar con exactitud los riesgos que presenta para la salud de humanos y carnívoros domésticos y salvajes, y encontrar el modo de prevenir la enfermedad en las poblaciones que tienen contacto con murciélagos. La rabia sigue constituyendo un importante problema de salud pública, sobre todo en zonas donde la rabia canina es todavía endémica, como en varios países africanos y asiáticos.

Palabras clave
Epidemiología – Lyssavirus – Murciélago – Rabia – Rabia del murciélago – Rabia terrestre.

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