THE EVOLUTION OF RABIES EPIDEMIOLOGY IN WILDLIFE

by Jean Blancou, Director General, Office International des Épizooties
12 rue de Prony, 75017 Paris, France

INTRODUCTION

Nearly all the available historical texts on the origins of disease associated human rabies with canine rabies, and dogs have been regarded as the main vector of this zoonosis since ancient times.

Nevertheless, rabies has long been reported for many centuries in wild animals, and the latest advances in molecular epidemiology have proved that rabies virus has indeed existed in various species of animals for millennia [reviewed by Bourhy & Rotivel, 1995].

However, epidemiological information gathered since the discovery of the rabies virus by Louis Pasteur shows that this disease has undergone change during the 20th century.

The purpose of this account is to review and anticipate this evolution based on historical information and current scientific knowledge concerning rabies in terrestrial mammals and bats.

HISTORICAL INFORMATION

In his *History of animals* written in the 4th Century BC, Aristotle wrote that 'if the rabid dogs bites, all the animals bitten become rabid' [Théodoridès, 1986, p. 26].

The Indian *Susrutasamhita* of the 1st Century AD states that in dogs, jackals, hyenas and tigers the tail, jaw and shoulders droop, and the animals drink a lot [Théodoridès, 1986, p. 21]. Arab and Persian authors of the 11th and 15th Centuries (including Avicenne and Sidi Siouti) add the wolf, fox and beech marten to the list of rabid wild mammals [Théodoridès, 1986, p. 48 & 51]

This information indicates clearly that, since ancient times, rabies has affected dogs and wild carnivores simultaneously, but nothing is known of the original vector of the disease. Early authors never cite bats as animals affected, and it was the conquistadors of South America who first reported bats as possible vectors of the virus [Baer, 1991, p. 390].

The rabies virus can infect every warm-blooded animal. However, natural infection of birds seems to be exceptional, and is still unconfirmed. Only inoculation of virus into the brain can overcome the natural resistance of birds to infection. Therefore, birds will not be mentioned again in this account, which is confined to terrestrial mammals and bats.

RABIES IN TERRESTRIAL MAMMALS

It is difficult to describe the evolution of rabies in various wild mammals, because the available epidemiological information is disparate or incomplete.

Therefore, this account is presented in three steps, so as to distinguish what is known from what remains hypothetical: an examination of the facts, relevant scientific observations and conclusions.

The facts

Although cases of rabies in wildlife (sylvatic rabies) have been reported since ancient times, such reports do not state whether they were isolated cases or genuine epizootics, and it was not until the Middle Ages that more precise information became available.

This account is confined to authentic epizootics of sylvatic rabies, affecting entire populations of wild mammals. The terminology employed by the early authors (e.g., epidemic instead of epizootic) will be conserved, as will the geographical terms of the period.

Rabies in Wolf

Many authors draw attention to the ancestral fear of wolves (*Cans lupus*), which was largely attributable to the fact that rabid wolves, like rabid dogs, lose all fear of human beings and would attack them savagely. According to Barbier [Barbier, 1929, pp. 75-116] and Heusinger [Heusinger, 1853, pp. 655-661], who researched all the ancient chronicles, the principal episodes of wildlife rabies occurred during the following periods:

- *1271*: Epidemic of wolf rabies in Franconia;
- *1590*: epidemic at Belfort (one wolf bit 12 people, 9 of whom died of rabies);
- *1790*: epidemic in Silesia
- *1801*: epidemic in Ohio involving foxes.

Wolf rabies still survives in some Asian and Middle Eastern countries, usually as a spillover of canine rabies rather that a true cycle of wolf rabies. Pack die-offs have been observed from time to time in Canada and Alaska [Wandeler, personal communication]. As in the case of the jackal, nothing is really known about the wild or domestic carnivores was the primary host of the virus.

Rabies in Fox

In contrast to the wolf, rabid foxes seldom attack human beings, and consequently epizootic of fox rabies has not attracted as much attention as rabies in wolves and dogs [Blancou *et al*., 1991a]. Epizootic were reported in the following periods:

- *1578-1581*: major epidemic of rabies in **red foxes** (*Vulpes vulpes*) in Europe, controlled by killing foxes;
- *1771*: major epidemic in North America;
- *1776*: major epidemic in North Africa;
- *1803-1840*: prolonged epidemic in Switzerland and Germany;

- *1810*: epidemic in Ohio also affecting wolves;
- *1866-1872*: brief epidemic in Corinthia;
- *1921-1928*: major epidemic in the Dijon region.
- *From 1947 to the present day*: extensive epidemic in Europe, spreading at a rate of 40 km a year, from Czechoslovakia and Poland [Serokowa, 1968, p. 70] to France and Italy (Fig. 2). Viruses isolated during the latter epizootic are phylogenetically identical to those from ancestral foci of canine rabies in Europe and the Middle East [Kissi *et al.*, 1997, p. 529*]*. In 1985, the westward progression of this epidemic came to a natural halt, and one of the explanations proffered for this was that immunity developed in the fox population resulted from infection by an evolved strain passaging I blood [Blancou *et al.*, 1987, p. 4; 1988, p. S608; 1990, p. 543].

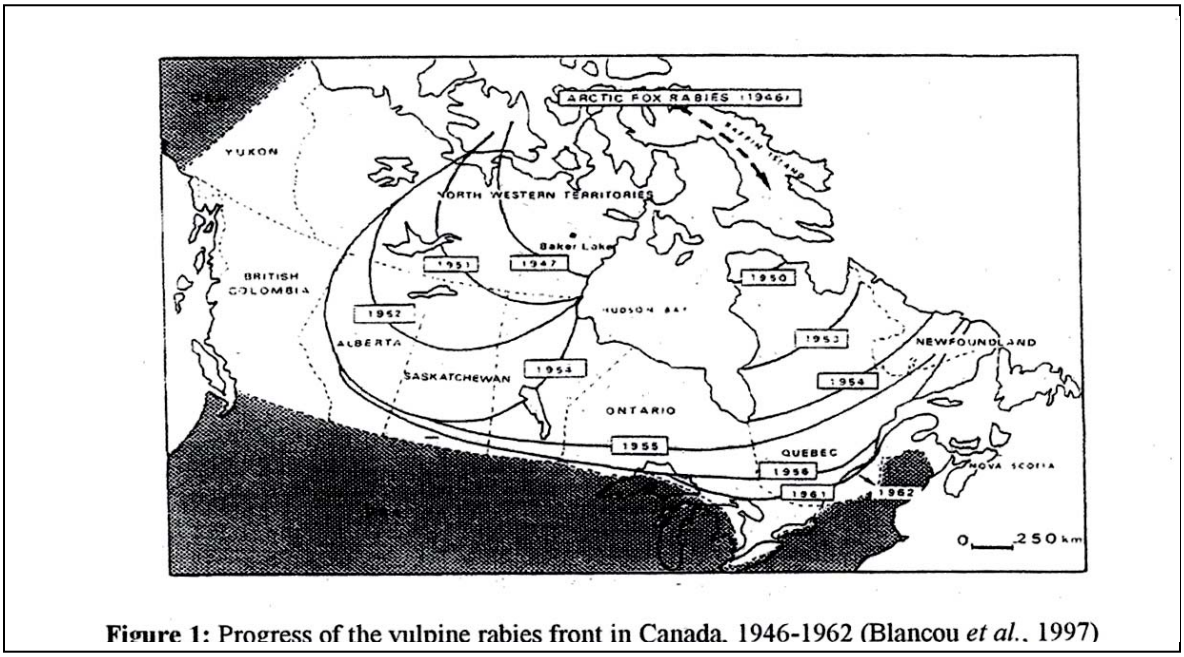

Figure 1: Progress of the vulpine rabies front in Canada, 1946-1962 (Blancou *et al.*, 1997)

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Rabies in **artic foxes** (*Alopex lagopus*) seems to have existed since 1859 (the date at which the term 'crazy foxes' was first recorded) and has spread since 1945 to various territories within and south of the Artic Circle: Alaska, Greenland, Russian Federation (where the disease is known as 'polar madness') and Canada [Crandell, 1991, p. 291].

Rabies is currently retreating from Europe, due to oral vaccination of foxes, although the disease persists Central and Eastern Europe.

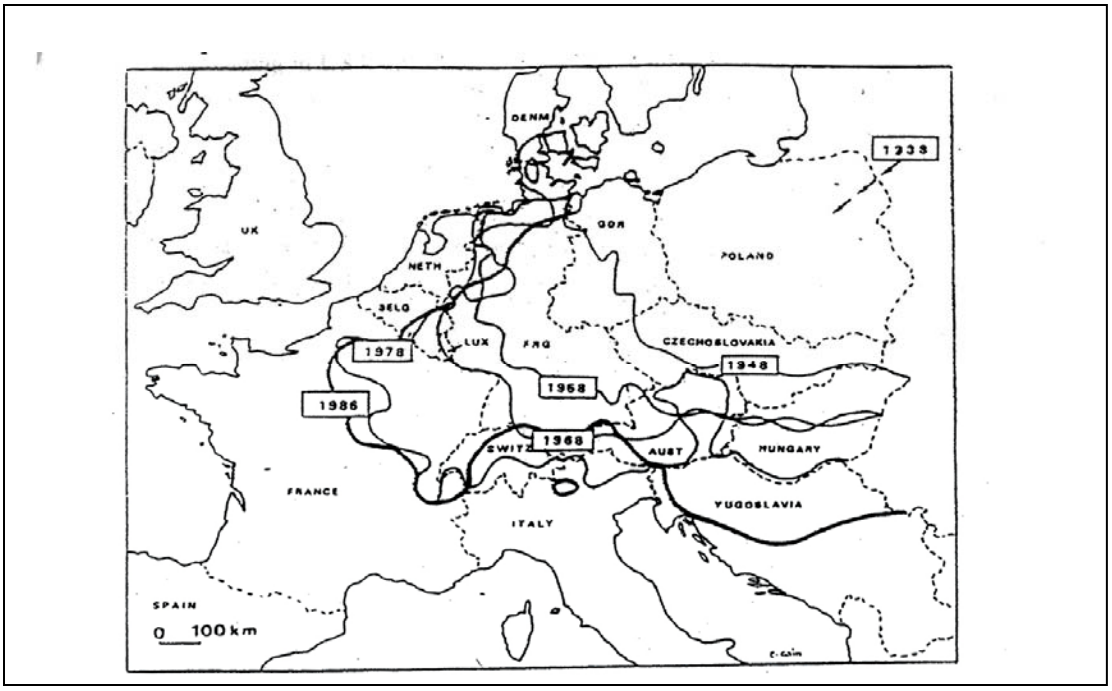

Figure 2. Successive limits of the front of vulpine rabies in Eastern Europe, 1938-1986 (Blancou *et al*., 1987)

Rabies in Skunk

The striped skunk (*Mephitis mephitis*) can contact rabies through contact with other wild carnivores, particularly foxes.

- *1870*: severe epidemic of rabies in the United States of America (USA);
- *1874*: this became so serious I Kansas and Colorado (41 human deaths) that Harvey proposed to call it *Rabies mephitica* {Steele & Fernandez, 1991, p. 9]
- *1950*: a new epidemic commenced I the USA and Canada,
- *1980s*: the peak was reached [Charlton *et al*., 1991 p. 308].

According to many authors, skunk rabies resulted from adaptation of vulpine virus to this species [Wandeler, 1991a, p. 127; Tordo *et al*., 1993, p. 322]. This seems plausible, because foxes and skunks are equally susceptible to a virus isolated from skunk, which are no more resistant to vulpine virus than foxes. The situation may therefore be different according to the region: spillover from fox rabies in Ontario and Quebec, or an independent cycle in California, the American Midwest ant, the Canadian prairie provinces [Charlton *et al*., 1991, p. 318; Wandeler *et al.,* 1994 p. 316].

Rabies in Raccoon

Reported in raccoons (*Procyon lotor*) for the first time in the USA in 1936, this form of rabies appears to have spread from dogs.

Between 1948 and 1952, numerous cases (spillover of canine rabies?) occurred in New Your State, while at the beginning of the 1950s a true epidemic commenced in Florida.
It lasted for 12 years, and then spread (through displacement of rabid raccoons) to the Mid-Atlantic States, where a peak was recorded in 1983 while 1,608 cases [Torrence *et al.*, 1991].

The epidemic is still spreading in the USA northwards 9The Canadian border) and eastwards (the disease has already reached Ohio).

Rabies in Jackal

Records since the 19th Century shows that African jackals (*Canis aureus, C. mesomelas* and *C adustus*) act as rabies vectors. However, whether dogs or jackals were the first reservoir of the disease in this region is unknown [Smith *et al*., 1993, p. 311].

Rabies of Mongoose

Two epidemics of different types of rabies have been reported in mongooses.

One, occurring in southern Africa among yellow mongooses (*Cynictis penicillata* ssp.) for many decades, currently accounts for 70% of cases of wildlife rabies in South Africa [Chapparo & Esterhuysen, 1993, p. 373].

The other epidemic affects Indian mongooses (*Herpestes auropunctatus*) in the West Indies, where mongooses were imported during the 1860s (to control rats in sugar-cane plantations). On Grenada, rabies antibody was present in 18%-43% of mongooses captured in 1985 [see Chaparro & Esterhuysen, 1993, p. 374]. At present, the disease persists in an endemic state in certain islands, notably Puerto Rico [Krebs *et al.,* 1996].

Most authors believe that there is a genuine cycle of sylvatic rabies among African mongooses, while that in the Caribbean seems to be a spillover of canine rabies in which the virus is poorly adapted to its new host. This is demonstrated by a large number of animals, which possess the antibodies [King *et al*., 1993, p. 299; Nel *et al*., 1003, p. 305]. However, because mongoose rabies in the Caribbean survived for long periods of time, it is suspected that the induction of immunity is an adaptative trait of the virus in this particular case, helping to buffer mortality which could lead to virus extinction in the reduced host population [Wandeler, personal communication].

Rabies in Coyote

An epidemic of rabies among coyotes (*Canis latrans*) was reported in Canada in *1940,* but it disappeared spontaneously within a few years, leaving instead an epidemic of vulpine rabies [Tinline, 1988, p. 301].

In the early *1990s,* another epidemic of coyote rabies commenced in Texas, undoubtedly as a result of the establishment of a cycle of canine (Mexican) rabies virus within the species. A northwards spread of this epidemic in the USA is currently being controlled successfully by oral vaccination [Meehan, 1995].

Rabies in Racoon-dog

Instances of infection of raccoon-dogs (*Nyctereutes procyonoides*) with rabies from foxes were reported during the 1980s. A specific epidemic cycle developed in Eastern Europe, where the animal was introduced from Asia for the manufacture of fur hats [Cherkasskiy, 1988].

Rabies is also endemic among indigenous raccoon-dogs in the Republic of China and Korea.

Rabies in other wild carnivores

Accidental or regular cases of rabies have been reported in some 30 species of carnivores of the Canidae, Felidae, Mustelidae and Viverridae, families. These cases do not appear to have been the origin of genuine epidemic cycle in these species. However, in certain cases, particularly in jackals and bat-eared foxes (*Otocyon megalotis*), the large number of outbreaks gives rise to fear of the onset of specific epidemics [Thomson & Meredith, 1993]. Numerous hypotheses have been advanced to explain this general absence of a specific cycle, most of which point to ethological factors (social organization), ecological factors or behavioural factors (the 'passive' attitude of rabid animals) [Blancou & Pastoret, 1993, p. 31; King *et al*., 1993, Smith *et al*., 1993].

Rabies in herbivores

In areas where rabies is endemic, herbivores are regularly victims of bites from rabid dogs. Such sporadic cases can assume the proportions of genuine epizootics, as happened on two occasions in Europe, and also in Africa.

In the United Kingdom, fallow deer (*Dama dama*), kept within enclosures, were victims of rabies on two occasions: 269 of 1,00 died in Richmond Park (Surrey) in 1886 and 1887, while in 1889, 500 of 600 died at Ickworth in Suffolk. The cycle arose as a result of bites [Barbier, 1929, p. 74].
In Namibia, kudu (*Tragelaphus strepsiceros*) were affected in 1977, and about 50,000 died from rabies over an eight-year period. The epidemic seems to have been caused by contamination through drinking water or eating prickly shrubs [Schneider, 1985, p. 520].

Rabies in rodents

Although some rodents (rats, squirrels, beavers, woodchucks…) may become infected with rabies, there is actually very little naturally occurring rodent rabies, and the real risk following rodents bites remains very low [Winkler, p. 405].

Scientific observations

The causes and mechanisms of various waves of sylvatic rabies, and their possible links to canine rabies, have been the subject of much research and numerous hypotheses since the discovery of the rabies virus. Many authors now believe that these successive waves originated from a progressive adaptation of the rabies virus, originating from a primary vector or reservoir, to different species, followed by serial passage in these species. These passages served to gradually enhance the pathogenicity of the virus for its host; this was demonstrated initially by a shortening of the incubation period of the disease, an impairment of the immune response, and the increased excretion of virus in saliva. However, the course of these serial passages can eventually have a counter productive result, through adaptation of the virus to its host and spontaneous cessation of the epidemic (see below).

These different explanations or hypotheses are based on experimental observations given below:

Artificial adaptation of the rabies virus to a species which is not the natural host

The earliest experiments demonstration the possibility of adapting rabies virus to a species, which is not the natural host, were conducted by Louis Pasteur in 1883. To produce a vaccine

against canine rabies, Pasteur attempted to adapt canine virus by serial inoculation into the brain of monkeys. This completely changed the patogenicity of the strain for monkeys (which became very susceptible to the new virus) and for dogs (which became very resistant), and he concluded that the application of these findings would result I a vaccine against rabies. Pasteur extended the technique of serial passage to rabbits, which enabled him to obtain a 'fixed' virus of potential value as a vaccine for human rabies. Marie also applied this technique to chickens in 1902, and later by Koprowski in 1948, which produced Flury vaccines of low egg passage (LEP) and high egg passage (HEP). The same procedure of successive passages in laboratory animals (and then in cultured cells) was used to produce the modified Street Alabama Dufferin (SAD) strain, from which most of the vaccines now used for immunizing wild animals by the oral route have been derived [reviewed by Wandeler, 1991b]. The procedure is now used to examine the effect of serial passages on the structure of 'quasi species' of the rabies virus, and for genetic fixation of the mutations obtained [Kissi *et al*., 1997].
This forced adaptations of the virus consists of enhancing the pressure of selection of mutants most virulent for a given species, following serial passages in that species.

The question of whether the same phenomenon can occur in the natural selection of mutants better adapted to a species should be asked. Experiments on mice [Blancou *et al*., 1996] and the facts presented below provide the initial proof. It is quite obvious that rabies virus has colonized different host species and that this access to new principal host species can only be explained by assuming that a virus mutant happened, by chance, to be adapted to the new host [Wandeler, 1993, p. 348 and personal communications].

Natural adaptation of the rabies virus: biotypes

Rabies viruses obtained from the core of rabies epidemics among different species have been examined by cross-inoculation experiments. Research conducted by various workers has shown that viruses of different origin always belong to serotype 1[a], but vary considerably in pathogenicity for animals to which they are inoculated.

The first experiments of this type were performed in the early 1960s when skunks, raccoons, opossums and foxes were inoculated with vulpine virus. Foxes were between 100 and 40,000 times more susceptible to this virus that other species. The vulpine virus was present more often in their saliva than in the saliva of other inoculated species [smith & Baer, 1988, p. 272; Wandeler, 1991a, p. 127]. More extensive series of experiments were undertaken in foxes in Canada in 1970, then in Europe in raccoon-dogs, foxes and raccoons [reviewed by Blancou & Aubert, 1997], and in Africa in mongooses and jackals [Chaparro & Esterhuysen, 1993] – see Table I

[a] Both 'true' and 'related' rabies viruses belong to the genus *Lyssavirus* of the family *Rhabdoviridae.* They are currently classified into four serotypes ad six genotypes. Classification by serotype (from 1 to 4) is based on the protective power of polyclonal antibodies produced in laboratory animals immunized with various strains, or on an in vitro reaction with selected monoclonal antibodies *[*Wiktor *et al*., 1980]. Classification into *genotypes* (1 to 6) is based on a comparison of amino acid (or nucleotide) sequences corresponding to the entire region that codes for the gene of viral nucleoprotein [Bourhy *et al.,* 1993]. Serotypes 1 to 4 correspond to genotypes 1 to 4. For each serotype or genotype there is a prototype virus, and these comprise: rabies virus proper (= serotype 1/genotype I), Lagos bat virus (= serotype 2/genotype 2), Mokola virus (=serotype 3/genotype 3) and Duvenhage virus (= serotype 4/genotype 4). 'European bat lyssavirus' (EBL1) is the prototype of genotype 5, and EBL2 is that of genotype 6. Genotypes 2 to 6 are not regarded as 'true' rabies viruses, they are 'viruses related to rabies virus'. Other viruses have not been classified definitively, notably those isolated in 1995 from bats in Australia.

Table I:
Natural resistance of some carnivores to inoculation with virus obtained from different species

Species of carnivore tested	Origin of challenge virus (a)_	Resistance of carnivore (b)	Ref.
Canis familiaris	*C. familiaris*	±	(1)
	Vulpes vulpes	+++	(1)
Cynictis penicillata	*C. pennicillata*	+	(2)
	C. familiaris	+++	(2)
Nyctereutes procyonoides	*N. procynoides*	+	(3)
	V. vulpes	+++	(3)
Procyon lotor	*V. Vulpes*	++	(1)
Mephitis mephitis	*M. mephitis*	±	(4)
	V. vulpes	+	(4)
Mustela putorius furo	*V. vulpes*	++	(1)
Vulpes vulpes	*V. vulpes*	±	(1)
	C. familiaris	+++	(1)
	N. procyonoides	++	(1, 3)
	Eptesicus focus	++++	(1, 5)
	E. serotinus	+++	(6)

(a) isolates obtained from the usual vector species and which has not passaged in another species

(b) ± = very weak resistance
+ to ++++ = increasing resistance (by a factor of 10 to 10^6) measured in intracerebral lethal doses 50 for mice (LD_{50}/ic/mouse)

-(1) Black & Lawson, 1970; Artois *et al*., 1989; Blancou & Aubert (1997)
-(2) Chapparo & Esterhuysen, 1993
-(3) Blancou & Aubert, 1991b; Botvinkin, 1984
-(4) Charlton *et al*., 1991; Sykes 1966
-(5) Blancou *et al*., 1986; Rupprecht *et al.*, 1991
-(6) Soria Baltazar *et al*., 1988.

These experiments all reached the same conclusion, namely the virus isolated during authentic epidemics of sylvatic rabies originating from the animal species, which acts as the main vector has a particularly high pathogenicity for that species, which means that rabies in different species of animals can be placed in separate compartments [b] [Smith & Baer, 1988, p. 271]. This compartmentalization can be quantified by determining the dose of virus lethal for 50% of the animals of a given species, inoculated intramuscularly. This dose [c] will be 100 to 100,000 greater than that determined in other species, which are not acting as vectors in the epidemic. In 1986, such observations led to a proposal to distinguish different biotypes of rabies virus by applying all the characteristics observed after animal inoculation namely; pathogenicity, excretion of virus in saliva, immune response, etc. [Blancou, 1986, p. 135 & 1988 p. S607].

[b] This separation into compartments (or compartmentalisaton) is accentuated by another phenomenon: a virus which is particularly pathogenic for one species is not necessarily pathogenic for others. Therefore, the vector of such virus is not only usually harmless for other species, but these species can become vaccinated either by bites [Blancou, 1985, p. 459], or by cannibalism with ingestion of infective tissue [Wandeler, 1991a, p. 130]. This explains the presence of natural antibodies in certain animals that have been in contact with a virus of low pathogenicity for them [Chantal & Blancou, 1985, p. 289].

[c] The 50% lethal dose is expressed as 'LD_{50}/ic/mouse', in other words lethal doses for 50% of mice inoculated intracerebrally.

Studies on the evolution of these characteristics have provided an explanation of the arrest of the spread of the European vulpine rabies epidemic during the 1980s [Blancou *et al*., 1987; Aubert *et al*., 1991]. Nevertheless, determination of the profiles of these different biotypes is laborious compared with monoclonal antibody assay and genetic typing.

Monoclonal antibodies: a wide diversity of antigenic variants

Parallel to the animal experiments described above, application of the monoclonal antibody technique to the *in vitro* characterization of different rabies viruses has made an important contribution to epidemiological studies since the early 1990s [Wiktor *et al*., 1980]. It has become possible to demonstrate that viruses isolated from one species of animal conserve their original profile, even after passages in another species [Rupprecht *et al*., 1991, p. 85; Net *et al*., 1997, pp. 80 and 81].

The analytical power of monoclonal antibodies is sufficient to identify the broad geographical regions in which variants originate, and to determine the species affected by these variants or by various vaccine strains. However, the technique has certain limitations, particularly in its inability to predict the pathogenicity of a variant for a given animal species, for which animal experiments are essential [Wandeler, 1991a, p. 129].

Genetic typing of rabies viruses: variability, evolution and genotypes

Genetic typing is conducted by analyzing the amino acids of different viral proteins, or the nucleic acids, which code for these amino acids. This analysis is usually performed on viral nucleoprotein, but it can also be applied to glycoprotein, and non-coding regions (pseudogenes) of virus which change more rapidly [Bourhy *et al*., 1995]. The results have shown that the evolution of the rabies virus is associated with a genetic derivative, which forms from the accumulation of mutations during replication of virus in different hosts. Determination of these variations makes it possible to distinguish different genotypes of virus (associated with a given species), which remain stable during the early passages in another species [Bourhy et al., 1993; Nel et al., 1997, p. 82]. It also facilitates the construction of phylogenetic trees, which show the position of a virus in relation to other viruses, and the relative distribution of the genetic derivation of various strains, whether wild or vaccinal. This derivation evolves very slowly, and it seems certain that different animal species have become victims of serial passages of virus for some time. Nevertheless, it is still impossible to determine the original vector or reservoir with certainty.

Conclusions

Whether performed *in vivo* or *in vitro,* studies of the rabies virus in wild terrestrial mammals have given rise to some valuable conclusions concerning epidemiology.

- The rabies virus possesses *considerable genetic plasticity,* as shown by the number of antigenic variants and phylogenetic lines. However, the virus does not have the extreme variability [d] off certain other viruses, and this relative stability makes it possible to produce vaccines that are still effective, using strains isolated by Pasteur a century ago.

[d] The potential variability of the rabies virus is, in fact, limited by the constraints of adaptation to the host, which imposes a survival strategy [Amengual *et al*., 1997; Wandeler *et al*., 1994, p. 317].

- *Genetic evolution of the virus is slow*, but there is no reason why this should not continue over a long period, giving rise to new epidemic cycles among new species of wild mammals, which have previously acted as occasional hosts only.

- Whatever the phylogenetic line to which it belongs, a rabies virus *must become adapted to a given mammal* if it is to develop into a new biotype and create a durable epidemic cycle. Many distinct cycles can develop in this way in different parts of the world, based on the same model, by a phenomenon of convergence of adaptations.

- Repeated infection of species other that that which harbours a given biotype may result in the emergence of a new biotype after serial passage in that species. But in some cases, *the emergence of a new biotype may be precluded or delayed* by the presence of antibodies formed during non-fatal infection with the biotype responsible for the main epidemic cycle. This biotype will then act as a true live viral vaccine, resulting in an endemic rather than epidemic rabies status in other species.

- The species barrier, resulting from the existence of biotypes is capable of *reducing the risk of importing rabies* into a country, either because there is no species susceptible to the imported virus, or because the existing susceptible species have little chance of coming into contact with the vector of that biotype.

RABIES IN BATS

Rabies in bats (of which there are no fewer than 950 species) is best studied separately form rabies in other wild species. The disease usually evolves in an independent manner, even though bats can infect other mammals and, in particular, human beings.

The facts

Rabies in haematophagous bats

The first report of rabies related to a bat bite seems to have been made in 1511. During the fourth voyage of Christopher Columbus, Anghiera mentioned that bats as big as turtle doves flew around the Spaniards, and full of fury, bit them so cruelly that they became engaged [Théodoridès, 1986, p. 77]. In 1526, De Oviedo also reported numerous deaths among Spanish soldiers, victims of bat bites in Yucatan (Mexico). Deaths of livestock due to these bites were reported from Guatemala (16th Century), Ecuador (18th Century) and the Caribbean (20th Century). On Trinidad, the cause of deaths among cattle and human beings bitten by haematophagous bats (vampires) was definitely attributed to rabies: 2,000 cattle and 56 people died between 1931 and 1936 [Baer, 1991 p. 390].

At present, rabies in vampire bats is a problem in nearly every country of Latin America. Losses of cattle as a result of these bites is considerable, and figures have been estimated at 200,000 head in Brazil, 90,000 in Mexico and 334,000 in the rest of the continent [Smith & Baer, 1988, p. 292].

Rabies in other species of bats

Rabies in non-haematophagous bats (fruit-eating, insectivorous, etc.) was not recognized for a long time, and human mortality from the bites of these species must have been underestimated before the 20th Century.

In the Americas, the first cases of rabies among fruit-eating bats were reported in Brazil in 1921, then in Port-of-Spain (Trinidad) in 1931. The first full investigation was not made until 1953 in the USA. On 23 June of that year, a young boy in Florida was attacked and bitten by an insectivorous bat (*Lasiurus intermediu*). The father of the victim, who had worked in Mexico, believed the bat to be rabid, and this was confirmed in the laboratory. Soon afterwards, a search was made for rabies virus in bats in various States of the USA and, in the seven years that followed, the virus had been isolated in 30 States from bats of the genera *Lasiurus, Eptesicus, Myotis, Tadarida* an others. Since then, many hundreds of reports are received each year in the USA (787 in 1995), accounting for about 10% of wildlife rabies [Krebs *et al*., 1996]. It appears that in these species, the rabies virus can be transmitted by aerosol, particularly in caves where thousands of individuals cohabit. In Canada, the first case of rabies in an insectivorous bat was reported in 1957; the disease was confined mainly to *Eptesicus fuscus.* In Latin America, rabies has been recorded in over 50 species of nonblood-lapping bats, including some fish-eating bats. Strains of virus isolated from these species (particularly *Tadarida brasiliensis*) differed from those obtained from vampire bats (*Desmodus rotundus*) [Baer & Smith, 1991, p. 353].

In Europe, bat rabies was first reported from Hamburg (Germany) in 1954, but subsequent investigations did not meet with the same success as in the USA, for only 14 new cases were identified up to 1985 [Nieuwenhuis, 1992]. The death of a human being in that year gave fresh impetus to the investigations, and between 1985 and 1991 rabies was diagnosed in over 400 bats in Europe. The common serotine bat (*Eptesicus serotinus*) seemed to be affected most often, with bats of other genera (including *Myotis* and *Pipistrellus*) being occasional victims of the disease. At present, bat rabies persists in Europe, but the annual number of reported cases has fallen since 1991 [Amengual *et al*., 11997, p. 2319].

In Africa, research was stimulated by the findings in other parts of the world, and in 1956 a rabies-like virus was isolated from a fruit-eating bat in Nigeria. This virus was subsequently classified as serotype 2, genotype 2 (prototype Lagos bat virus). In 1970, a new strain of virus was isolated in South Africa and named after a man who died from a bite by an unidentified insectivorous bat. This strain was classified as serotype 4, genotype 4, prototype Duvenhage. Since then, few isolates of rabies virus have been obtained from African bats, regardless of the species; their number does not exceed at few dozen [Perry, 1997].

In Asia there has been very few reports of bat rabies, despite systematic epidemiological surveys, particularly in the Philippines ad Malaysia. In India, one case was reported in 1980 in a flying fox (*Pteropus* sp.) [Fraser *et al*., 1996]

Rabies was considered to be absent from *Oceania,* including Australia, New Zealand and New Caledonia, until 1996. In that year, a *lyssavirus,* provisionally called 'Australian bas lyssavirus' was identified in two black flying foxes (*Pteropus alecto*) in New South Wales (Australia) [Fraser *et al*., 1996]. Since then, other *lyssaviruses* have been obtained from two other species of *Pteropus,* and from an insectivorous microchiropteran [Gleeson, 1997]. These viruses are still being examined, but it has been proposed to classify them in a new genotype, genotype 7 [Hooper *et al.,* 1997; Westbury, 1997].

Scientific observations

The most important question, from an epidemiological point of view, is whether there is a link, or might be a link, between rabies in terrestrial mammals and that in bats. By taking the four observations noted above in connection with mammalian rabies, it is possible to formulate the first steps towards answering this question.

Artificial adaptation of a bat virus to a terrestrial mammal

In most cases, viruses of serotype 1/genotype 1 isolated from bats have had little or no pathogenicity for other inoculated mammals, and this has precluded attempts to adapt the virus by serial passages.

The first attempts were made in 1966, following the occurrence of two cases of humans infected with rabies after indirect contact with bats of the species *Tadarida brasiliensis.* Many terrestrial mammals were inoculated (coyotes, opossums, raccoons and foxes), but most of them resisted infection, including a direct bite from rabid bats [Baer & Smith, 1991, p. 361].

In 1975, other attempts in Mexico infection with a vampire bat strain passaged once in cattle succeeded, but no further passages were undertaken. In 1986, experiments were repeated with a strain-isolated form an insectivorous big brown bat (*E. fuscus*), which proved to be pathogenic for cats, but no further passages were performed [see Soria Baltazar *et al.,* 1988, p. 620].

Another virus isolated from the same species was inoculated into the brain of laboratory animals, bats and skunks. The inoculated animals died from rabies, and there was practically no change in the antigenic composition of the virus after three to five passages [Rupprecht *et al.,* 1991, p. 85]. By contrast, the same virus, inoculated by the same route into red foxes, then recovered from a fox which had died, was devoid of pathogenicity for other foxes, whatever the inoculation route [Blancou *et al.,* 1986]. During a small outbreak of rabies in red foxes (*Vulpes vulpe*) on Prince Edward Island (Canada), virus isolated from three animals demonstrated the characteristics of a bat virus (serotype 1/genotype 1). The salivary glands of one of these three foxes contained the virus (in small amounts), which could have resulted in an intraspecific cycle of infection, but in fact such a cycle did not become established [Daoust *et al.,* 1996, p. 405].

In 1987 Bode reported that cats could be infected with the 'Stade' strain (European bat isolate, 1970, Germany) but that further passages in the same species were quite difficult [Schneider & Cox, 1994, p. 212].

More recent experiments have been performed on foxes with a virus of genotype EBL1 isolated from an insectivorous serotine bat (*E. serotinus*), which died of rabies in Denmark. This virus was of relatively low pathogenicity for foxes, and could not be recovered from the salivary glands of dead foxes [Soria Baltazar, 1988]. It is therefore extremely unlikely that a natural cycle of rabies could be established in foxes by means of a bat bite [e].

One wonders if contact with bat virus was the origin of the natural antibodies found in dogs in Ethiopia in 1956 [Chantal & Blancou, 1985], because such viruses had recently been isolated from dogs and cats in that country [Mebatson *et al.,* 1993].

[e] It is interesting to note that the same virus proved to be of very low pathogenicity for sheep when inoculated intramuscularly [Soria Balatazar, 1988], and was not at all pathogenic for dogs when inoculated intramuscularly [Fekadu, 1993, pp. 95-98].

Difficulty of natural adaptation of a bat virus to terrestrial mammals

No natural cycle of sylvatic rabies so far described among terrestrial mammals seems to have any relationship to a cycle, which occurs among bats. Of course, cases of rabies in terrestrial mammals (and particularly human cases) have been seen in many different regions of the world, and terrestrial mammals (wild or domestic [f]) could have become infected by the bat viruses, but this is a rare event. Experiments conducted in the USA have led to the conclusion that 'rabies in bats exists largely independent of rabies in terrestrial animals and does not contribute to enzootic maintenance of terrestrial rabies' [Baer & Smith, 1991, p. 361].

The rarity of primary outbreaks of rabies originating from infection from bats, together with the scanty (or absent) re-excretion of virus in the saliva of infected animals, make it most unlikely that a bat virus will become adapted to a terrestrial mammal. On the other hand, intraspecific compartmentalization of rabies, particularly in migratory species of bats, does exist among American insectivorous bats a phenomenon also seen in terrestrial mammals [Baer & Smith, 1991, p. 350]. The same phenomenon seems to occur in European insectivorous bats, as the virus is recovered mostly from *E. serotinus* and mouse-eared bats (*Myotis* spp.), and seldom form other cohabitant species (see below).

Antigenic variability of bat viruses

Commencing in the 1980s, the use of monoclonal antibodies has provided more information of the relationships between different cycles of animal rabies, particularly by confirming that the viruses isolated from cycles established among terrestrial animals differ from those isolated from bats, whether blood-lapping of not [Baer, 1991, p. 395]. Insectivorous bats have been studied in greater detail, and here it seems that rabies is maintained in independent intraspecific cycles of different antigenic variants. Such variants may differ considerably, particularly among non-migratory species [Baer & Smith, 1991, pp. 350-351].

Genetic typing of bat viruses: geographical diversity and close adaptation to a host species

According to current genetic studies, rabies viruses from bats can be grouped into different genotypes (clusters) found in separate geographical areas. In the America, all viruses isolated so far belong to serotype 1/genotype 1 (prototype: 'true' rabies virus). Five groups of viruses from insectivorous bats have been identified, each infecting a different genus (*Eptesicus, lasiurus* and *Tadarida*); these viruses are closer to carnivore viruses than to those isolated from European bats. In Africa, the few strains isolated belong to serotype 4/genotype 4 (prototype Duvenhage virus), or to serotype 2/genotype 2 (prototype Lagos bat virus). In Europe, EBL1 virus (genotype 5) has been isolated most often from *E. serotinus*, while EBL2 (genotype 6) has been recovered from pond bats (*Myotis dasycneme*) and Daubenton's bat (*M. daubentoonnnii*) [Bourhy *et al.*, 1992]. Genotypes of viruses isolated in Oceania in 1996 have not yet been determined.

More recent studies (using the sequences of nucleotides coding for the terminal portion of viral nucleoprotein) have been conducted on forty-seven European and two African isolates of virus [Amengual *et al.*, 1997]. These studies have refined the classification of European viruses by subdividing group EBL1 into two subgroups (or lines), EBL1a and EBL1b.

[f] The terrestrial mammals most often infected with bat rabies are domestic cattle bitten by vampire bats. However, to date, no virus cycle has been established in these animals, or in wild herbivores of the Americas.

These two lines evolved separately, and an analysis of their geographical origin [g] suggests that line EBL1a evolved from east to Western Europe, while EBL1b evolved from south to north; both lines were present in the Netherlands. These may be two variants adapted to the same species of bat, EBL1b having been carried by animals migrating from North Africa via Spain [Amengual *et al.,* 1997]. Virus EBL1b may have had an African virus (Duvenhage virus) as its ancestor, while EBL1a had an Asian ancestor.

Whatever their origin, the weak intrinsic heterogeneity (i.e., genetic stability) of viruses EBL1 and EBL2 indicate that they are closely adapted to their hosts [Amengual *et al.*, 1997]. Thus the compartmental pattern of rabies in *E. serotinus*, and the existence of corresponding biotypes [Artois, 1993, p. 66] would be a convergence phenomenon similar to that observed in terrestrial mammals: each bat species harbours its own biotype, as is the case for foxes, dogs, mongooses, etc.

Conclusions

Many of the conclusions about viruses from terrestrial mammals also apply to bat viruses.

- The *genetic plasticity* of bat viruses seems to be just as pronounced as in terrestrial mammals in the case of viruses belonging to serotype1/genotype 1 (isolated in the Americas). It seems less pronounced for viruses of genotypes 4,5 and 6 (isolated in Africa and Europe), but it could be due to the small number of isolates examined.

- The *genetic evolution* of bat viruses is relatively important for viruses of genotype 1/serotype 1, as well as for genotypes 5 and 6, but overall it seems to be slower that in other rabies viruses from terrestrial mammals.

- *Adaptation of viruses* to different populations of bats has led to the establishment of stable cycles, particularly for genotypes 1 and 5. These viruses seem to have considerable difficulty in adapting to other species of mammals, which are consequently quite resistant to them.

- *Emergence of a rabies cycle in terrestrial mammals* by adaptation of a bat virus therefore seems to be improbable; bearing in mind the findings stated previously [h]. Two mechanisms can preclude or delay the creation of such cycles: resistance to infection, and development of natural antibodies following failure of infection. Such failure amounts to a natural vaccination by a live modified virus. Populations of terrestrial mammals immunized in this way can safely cohabit with bats.

- *The risks of importing* a virus of bat rabies into a rabies-free country are evidently considerable, bearing in mind the mobility of bats and the extent of their migrations. Import and sale of exotic fruit-eating bats in Europe (as companion animals), therefore, constitutes a risk that cannot be disregarded, because rabies virus has been isolated recently from *Rousettus aegyptiacus* (imported from Africa) in Denmark and in the Netherlands [Moutou, 1997, p. 8]. This virus is unlikely to spread to terrestrial mammals within the country [i]

[g] Geographical origin is estimated by evolution of the amino acid sequence of nucleoproteins by virtue of the distance separating two isolates. This evolution from a known geographical point (in this case the Straits of Gibraltar) may be explained by a 'bottleneck transmission mechanism' [Amengual *et al*., 1997, p. 2326; Clarke *et al*., 1993].

[h] The best example is no doubt the island of Trinidad, where rabies has been endemic among bats for more than 50 years, without a cycle being established in wild or domestic terrestrial mammals [Bear, 1997].

[i] A country where only bat rabies caused by virus of typed EBL1 or EBL2 occurs is still regarded as being free from rabies by the Office International des Epizooties [OIE, 1997].

GENERAL CONCLUSIONS

Four main points emerge from this overview of the evolution of rabies in wildlife:

Current situation of sylvatic rabies

At present the main cycles of rabies in wild terrestrial mammals seem to be confined to North America (Canada and the USA), Europe and southern Africa, and the principle cycle of bat rabies is located in the Americas. Some of these cycles have been established relatively recently (20^{th} Century), but historical evidence suggests that others may have originated, developed and then disappeared during past centuries.

It is possible that certain cycles of sylvatic rabies are more important than is thought, and inconspicuous cycles may occur among terrestrial mammals or bats in central Africa, Asia, and elsewhere. It is a curious fact that sylvatic rabies has been identified, reported and studied mainly in developed countries, whereas elsewhere, canine rabies dominates the picture. In developing countries, sylvatic rabies never becomes a national problem, because few people (rabies 'sentinels'0 become infected from wildlife, and the number of cases in animals is probably underestimated as a consequence. Such under estimation is all the greater because many rabid wild animals do not attack human beings directly, and human infection usually arises through the intermediary of dogs.

It is very difficult to obtain a clear picture of sylvatic rabies because the various cycles are in constant evolution. In some cases, an epizootic wave of wildlife rabies may come to a sudden stop. In other cases, the virus becomes adapted to new species by successive passages, and finally a virus might act as a live vaccine for other species, stopping the spread of rabies among them. Therefore, one has to anticipate the appearance and detection of new epidemic cycles of sylvatic rabies among terrestrial mammals or bats, but it is highly improbable that such cycles will last long, and they will not affect many species simultaneously.

It may be stated that the transfer of rabies from an infected to a rabies-free country has no chance of real success unless a host corresponding to the biotype occurs in the rabies-free country and that this host comes into direct contact with the vector of the biotype [Forman, 1993, p. 82].

Independence of rabies cycles in bats and other mammals

All the historical and epidemiological information available, together with the results of various experiments performed *in vivo* and *in vitro*, point to the complete independence of cycles of rabies in wild terrestrial mammals and bats, even though bats may sometimes infect wild mammals. However, when two cycles coexist, genetic studies cannot prove which preceded the other [j].

This situation is different than the rabies cycles in terrestrial mammals, which can intermingle and be replaced by another, as viruses evolve.

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[j] It is still difficult to know in which order rabies appeared in terrestrial mammals and bats, because viruses isolated from bats may be phylogenetically distinct. Whereas viruses isolated from American fruit-eating bats (serotype1/genotype1) are genetically close to those from dogs (but it is not known which species passed the disease to the other and when this occurred). There seems to be no link with European bat viruses, which belong to quite different genotypes.

Importance of the host/virus equilibrium

All that has been written above applies purely to the natural evolution of rabies in wildlife. This is governed by pathogenicity of the virus, length of the incubation period, duration of illness, amount of virus re-excreted in saliva, duration of this re-excretion, behaviour of rabid subjects, etc. [Carey & McLean, 1983; Wandeler, 1991a].

The evolution of a pathogen is almost strongly influenced by the populations of its potential hosts. Such populations vary (in size and geographical distribution) and are subject to environmental changes, particularly climatic changes, whether in ancient times (ice bridge between Asia and America) or at present (global warming). It is incontestable that human actions are decisive in most cases. They may favour the spread of rabies: the most striking examples are those of over development of vampire bat populations following the introduction of cattle from Europe into the Americas, or the mongoose populations imported into the Caribbean, or raccoon-dogs imported into Europe. Humans may also intervene directly by effectively reducing rabies epidemics in foxes, raccoon-dogs or coyotes by distributing baits containing rabies vaccine [reviewed by Wandeler, 11991b].

Study of the rabies viruses *in vivo* and *in vitro*

The range of pathogenicity of different isolates of the rabies virus, determined by animal inoculation, is considerable (up to $10^{6.5}$). This has suggested to various authors that biotypes might exist within might exist within different serotypes or genotypes [Blancou, 1988; Bourhy *et al.*, 1992; Chaparro & Esterhuysen, 1993]. A subdivision into biotypes seems to be reasonable due to the fact that pathogenicity is based on the amino acid composition of the virus and because it is known that it depends on a single substitution at arginine 333 of the viral glycoprotein [Flamand *et al.*, 1989, p. 75]. Thus the identification, by an *in vivo* test, of a change in a single amino acid could have considerable discriminatory power. In certain cases, this would refine the present classification based on serological or genetic analyses, which generally concerns an assembly of antigenic patterns, amino acids or nucleotides involved in only a small part of the genome.

The limit of the discriminatory power *in vitro* analyses may be illustrated by the fact that a panel of the usual monoclonal antibodies is incapable of distinguishing between a fully virulent wild virus and the same virus rendered nonpathogenic after passages in cells [Blancou *et al.*, 1983]. Similarly, an African canine virus and a European vulpine virus, of very different cross-pathogenicity, could be distinguished only by exhaustive genetic analysis [Tordo *et al.*, 1993, p. 319], and the viruses of American bats fall into the same genotype 1 as those of terrestrial mammals, even though they are of little or no pathogenicity for the latter. Nevertheless, this failure could simply mean that the monoclonal antibody with the proper epitope specificity is lacking [Wandeler, personal communication].

On the other hand, the discriminatory power of *in vivo* techniques has also its limits, when a virus undergoes adaptation to its host: the pathogenicity of the virus no longer obeys the dose/effect law, which makes characterization of its biotype very difficult (e.g., foxes/skunks in Canada

Therefore, at present, recourse to cumbersome *in vivo* techniques is only justified for epidemiological research and preferably when a genetically well-characterised virus is used [Bourhy & Rotivel, 1995, p. 32; Kissi *et al.*, 11997]. Such research could refine the

classification of genotypes, and could provide a better explanation of the evolution of certain epidemics of sylvatic rabies in time.

Above all, *in vivo* inoculation remains the only currently available method of estimating, in a practical and reliable way, the risks of transferring a given biotype from an infected country to a country free from rabies virus.

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