Rabies control in Belgium: from eradication in foxes to import of a contaminated dog

Rabiësbestrijding in België: van het uitroeien bij vossen tot de invoer van een besmette hond

S. Van Gucht, I. Le Roux

National Reference Laboratory of Rabies, Department Pasteur Institute, Scientific Institute of Public Health, Engelandstraat 642, B-1180 Brussels

svangucht@pasteur.be

ABSTRACT

Rabies is a lethal viral encephalitis of mammals. This article reviews important aspects of rabies control in Belgium. From the sixties to the nineties, the virus caused an epidemic in foxes in the south of Belgium. Thanks to successive vaccination campaigns starting from 1989, the disease was eradicated in foxes. Belgium was declared free by the World Organisation for Animal Health (OIE) in 2001. Different measures are in place to maintain this free status, including surveillance in wild carnivores and domestic animals and the European pet travel scheme. This scheme implies that pets can only be imported from risk regions after vaccination and confirmation of immunity by an antibody test. Data on the distribution of negative and positive results with this test in vaccinated animals are presented in this article. Further, a new case of rabies in a dog in Beersel, 3.5 months is described after import from Morocco. Finally, the unique role of bats in rabies epidemiology is elaborated.

SAMENVATTING

Rabiës is een dodelijke virale encefalitis bij zoogdieren. In dit artikel worden belangrijke aspecten van de rabiësbestrijding in België besproken. Het virus veroorzaakte een epidemie bij vossen in Wallonië van de jaren zestig tot negentig. De ziekte werd uitgeroeid bij de vos door opeenvolgende vaccinatiecampagnes vanaf 1989. België werd vrij verklaard door de Wereldgezondheidsorganisatie voor Dieren (OIE) in 2001. Om deze rabiësvrije status te behouden, wordt een minimale surveillance bij wilde carnivoren en huisdieren onderhouden en is het vervoer van honden, katten en fretten aan strikte Europese regels onderworpen. Deze regels houden in dat dieren enkel kunnen ingevoerd worden uit risicoregio's na vaccinatie en een bevestiging van de immuniteit met een antistoffentest. Er worden enkele cijfers weergegeven in verband met de verdeling van negatieve en positieve resultaten van deze test bij gevaccineerde dieren. Verder wordt een nieuw geval van rabiës bij een hond in Beersel beschreven, 3,5 maanden na invoer uit Marokko. Tenslotte wordt de unieke rol van vleermuizen in de epidemiologie van rabiës besproken.

INTRODUCTION

Rabies virus is a neurotropic lyssavirus (genus *Lyssavirus*) that belongs to one of the largest families (*Rhabdoviridae*) and orders (*Mononegavirales*) of viruses. The virion consists of a single-stranded negative-orientated RNA strain of 12000 bases. It has five structural proteins and is surrounded by an envelope in which a glycoprotein (G) is embedded. It has a typical bullet-like morphology with trimeric G spikes protruding from the surface. G is responsible for the induction of protective immunity and contains motifs that define virulence and pathogenicity.

Rabies virus causes encephalitis in men and animals (Hemachudha *et al.*, 2002). Dogs and cats are often intermediate hosts between men and wildlife. The virus is excreted in saliva and transmitted by bites, scratches or licks. Once introduced in a wound, it replicates locally in the muscle cells. After an incubation of a few days up to several years, the virus crawls up in the peripheral nerves and reaches the brain via retrograde axonal transport. This is followed by extensive replication in the cytoplasm of neurons, brain dysfunction, coma and death. During the incubation, the infection can not be diagnosed. The virus slumbers at low levels and there is no clear immune response. Seroconversion will only occur in the late stage of the disease, when the virus is already extensively replicating in the brain.

Once symptoms of the disease develop, rabies is almost always fatal. One patient from Wisconsin survived the disease in 2005 (Willoughby *et al.*, 2005). The applied treatment protocol, consisting of a combination of life support, antivirals and drug-

induced coma, was repeated in several other patients without success.

DISEASE SIGNS

In animals, early "prodromal" signs are characterized by changes in behavior and temperament (King and Turner, 1993). An affectionate, docile pet may become dominant and aggressive, or vice versa. Foxes will lose their fear of man and sheep often head-butt objects. Cats show an uneasy facial expression, frequently mew, repeatedly extend and retract their claws and may exhibit a restless dancing movement of the front legs. Prodromal signs last 2 to 5 days and progress to the dumb (paralytic) (75%) or the furious form (25%) of rabies, depending on what signs prevail. Paralysis and death occur in both forms about 4 to 8 days after the onset of symptoms. Dysfunction of cranial nerves leads to protrusion of the third eyelid, facial and lingual paralysis. Paralysis of pharyngeal and laryngeal muscles leads to hypersalivation and abnormal vocalization. Fever, anorexia and pica are also common. A consistent sign is ascending paralysis. Weakness and incoordination of the hind limbs progressively evolves to paraplegia. The clinical picture varies strongly and may include all, some or none of these signs. In contrast to humans, animals do not exhibit hydrophobia.

Symptoms in humans include persistent fever, paresthesia or hyperesthesia at the site of entry, paralysis, difficulty swallowing, phobic and inspiratory spasms, autonomic dysfunctions such as hypersalivation and spontaneous ejaculations, behavioral changes such as hyper(re)activity, aggression, hallucinations, aerophobia and hydrophobia (Hemachudha *et al.*, 2002). The furious form predominates. The mechanisms by which the virus causes these nervous abnormalities are not understood and are a matter of current research.

RABIES IN THE WORLD: A TRAVELLERS' DISEASE

Rabies is a worldwide health problem. An estimated 55 000 people die from rabies each year, and another 10 million people are treated after contact with suspected animals (www.who.int). According to the registers of the authors, about 1000-1500 Belgians are vaccinated each year prior to travel to endemic regions, including most parts of Asia, Africa or South-America. A map of infected regions in the world can be found on the WHO website. Preventive vaccination is recommended for persons that plan a long stay and/or will do risk activities (military missions, back pack travelling, developing aid, etc.) in an endemic region.

Unvaccinated people who are bitten by an affected animal, can still be protected if they receive immediate post exposure prophylaxis according to WHO guidelines. This includes passive immunization with virus-neutralizing immunoglobulins (day 0) and a regimen of 4 (day 0 (×2), 7 and 21) or 5 injections (day 0, 3, 7, 14 and 28) with an inactivated rabies vaccine. Passive immunization and vaccine-induced immunity are assumed to neutralize the virus when it is still in the wound, before it gains access to the central nervous system, where it is protected from the immune system. Vaccine-induced immunity will only kick in after a few days, leaving some time for the virus to enter the central nervous system. In case of severe exposure, especially in the face, administration of immunoglobulins is therefore crucial to prevent later manifestation of the disease. People with low risk contacts often receive vaccine only. Each year, about 50 to 150 Belgians receive urgent post exposure prophylaxis after a bite from a rabies-suspected or -confirmed animal. In contrast to Third World countries, the easy access and consequent application of preventive and post exposure prophylaxis in individuals at risk have limited the number of human cases in Belgium. The last indigenously acquired case occurred in 1922. One case of foreign acquired rabies was reported by the Brussels Brugmann Hospital in 1981. Nevertheless, vigilance remains necessary since, despite all efforts, several imprudent West-European travellers have died over the past years from the disease (www.who-rabies-bulletin.org).

Immunoglobulins are purified from sera of vaccinated humans, which makes them expensive and limits supply. In addition, vaccine companies more and more fail to meet the (inter)national demand for human-use rabies vaccines. The WHO recommends developing cheaper alternatives for classic immunoglobulin preparations. Efforts are done by different research groups and companies to develop cocktails of monoclonal antibodies as an alternative. No such product has been commercialized yet.

SURVEILLANCE AND CONTROL OF RABIES IN BELGIUM

In Europe, foxes are the main reservoir of classic rabies virus (genotype 1, Table 1). In contrast to the situation on the American continent European bats are not a reservoir of classic rabies. They are, however, hosts of two related genotypes, namely European bat lyssavirus-1 and -2 (EBLV-1 and -2).

Figure 1 shows the evolution of surveillance and positive cases in Belgium from 1966 to 2007. In the beginning of the twentieth century, Belgium was still afflicted by sporadic cases of urban rabies in domestic carnivores. Thanks to sanitary control of the dog population, urban rabies disappeared in 1930. Belgium was presumed free of classic rabies for several decades.

Since World War II, there had been a progressive spread of rabies in foxes from Eastern to Western Europe. The epidemic started near the Russian-Polish border in 1939 and moved westwards at a speed of 30-40 kilometres per year. The first positive fox in Belgium was diagnosed in 1966 in Manderfeld, Liège. The following decades, the disease spread in the region below the rivers Sambre and Meuse with thousands of

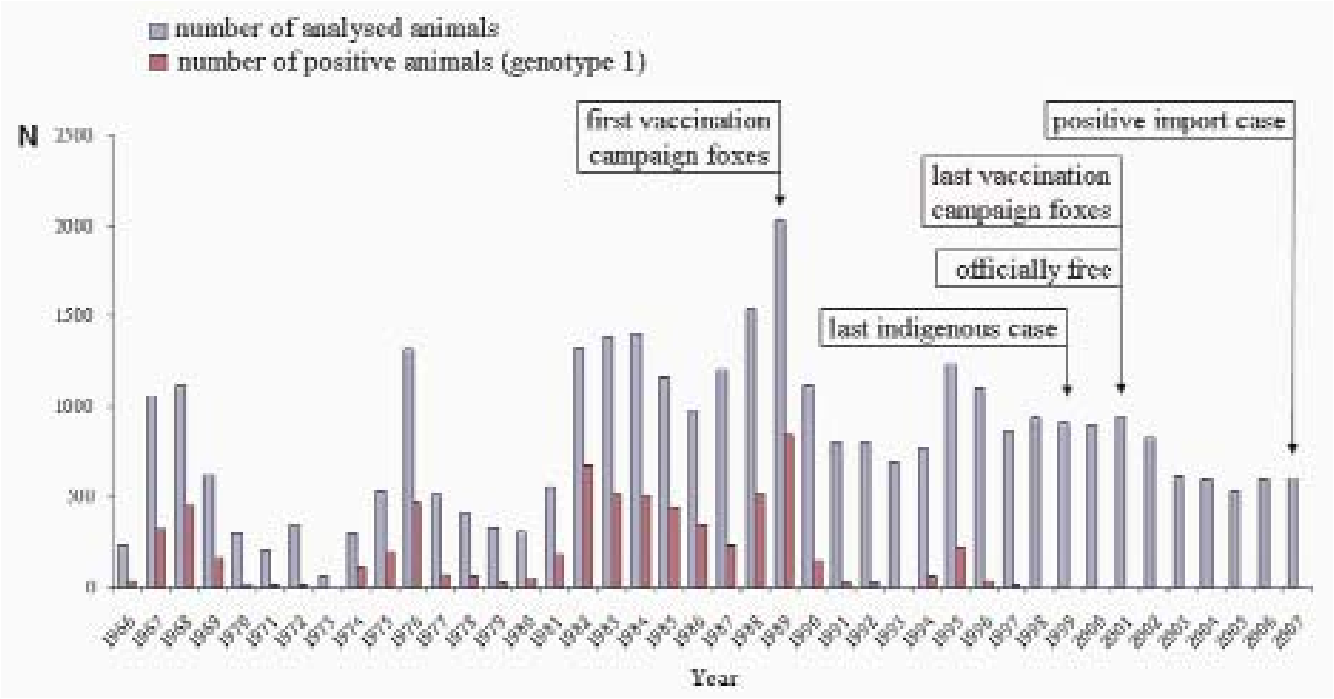

Figure 1. Surveillance of rabies in Belgium.

positive cases in wildlife and domestic animals. The epidemic reached its maximum incidence in the eighties. The contaminated area reached from Russia to France, and from the Netherlands to Italy. In Belgium, a record of 841 positive cases was diagnosed in 1989 and some positive animals were now also detected north of the rivers Sambre and Meuse. Considering the increasing risk for human health, the restrictions for international pet travel (e.g. the 6-month quarantine measure of Great Britain) and the augmenting cost of preventive and curative vaccinations in humans, the European Community, together with national authorities, decided to eradicate the disease by setting up large scale vaccination campaigns in foxes. Initially, two vaccines were used: the attenuated rabies virus SAD B19 and a recombinant vaccinia virus (a pox virus) that expresses the rabies virus surface protein G (Raboral V-RG®). The latter appeared to be more stable in the environment and became the vaccine of choice in Belgium. Vaccine baits were dispersed in the environment in autumn and spring. They were made of a fishmeal shell with a sachet, containing the vaccine suspension, tucked inside. Distribution was done by helicopter or plane in rural areas or on foot in urban areas. Vaccination campaigns were organized from 1989 until 2001. More information on the design and follow-up of these campaigns is given by Brochier *et al.* (1994). These expensive campaigns led to eradication of classic rabies in Belgium by the end of the nineties. The last case was diagnosed in a bovine from Bastogne in 1999.

Belgium was declared officially free of rabies in 2001 according to the World Organisation for Animal Health (OIE) guidelines (Terrestrial Animal Health Code 2007). This free status means that: (1) no case of indigenously acquired rabies has been confirmed in man or animal since the past 2 years and (2) no imported case in carnivores has been confirmed outside a quarantine station for the past 6 months. Bats infected with EBLV-1 or -2 are not taken into account. In October 2007, the free status was withdrawn for 6 months due to import of a contaminated dog (see further).

The Rabies Laboratory of the Scientific Institute of Public Health (IPH) is the only laboratory in Belgium responsible for surveillance and diagnosis of rabies in wild and domestic animals, and occasionally humans. Basic surveillance is an international obligation to guarantee the official rabies-free status. In Belgium, about 600 animals are examined yearly for rabies. These include ruminants suspect of mad-cow disease, dogs and cats with rabies-like symptoms (often after bite incidents), deer and wild carnivores (foxes, badgers, martens) that presented nervous disease or were found dead.

National legislation, which dates back to 1967, obliges to vaccinate dogs beneath Sambre and Meuse and dogs in camping sites. In the future, legislation will likely be changed to abolish the systemic vaccination of dogs in the south of Belgium and only impose vaccination in certain risk groups of carnivores (e.g. hunting dogs). All wildlife and most domestic carnivores will thus become fully susceptible to the virus. It is therefore crucial to maintain an early detection system to swiftly contain localized cases and prevent spread. It is a unique achievement to become free of an infectious disease, but to remain free afterwards requires a continuous commitment and investment by authorities, despite sometimes fading public interest.

THE EUROPEAN PET TRAVEL SCHEME: VACCINATION AND SEROLOGY TO PREVENT IMPORT OF CONTAMINATED PETS

The EU has restricted pet travel to strict regulations to prevent introduction of rabies in free countries (EU Regulation 998/2003). These apply to non-commercial movement of dogs, cats and ferrets and depend on the rabies epidemiology in the country of origin and destination. Pets can enter Belgium if they are vaccinated with an authorized rabies vaccine. Moreover, if the animal comes from a country where rabies is not controlled, a blood sample has to be taken 1 to 12 months after vaccination and tested for protective antibodies to confirm the efficacy of the vaccination. This is done by a virus-neutralizing assay on cells. Two variants of this assay are approved by the OIE and EU, namely "Fluorescent Antibody Virus Neutralisation" (FAVN) and "Rapid Fluorescent Focus Inhibition Test" (RFFIT). The latter is used in the IPH Rabies Laboratory. Yearly, the IPH Rabies Laboratory performs 5.000 to 6.000 RFFITs (95% animal and 5% human samples). If well-protected (titer of ≥ 0.50 IU/ml), the animal can enter Belgium not earlier than

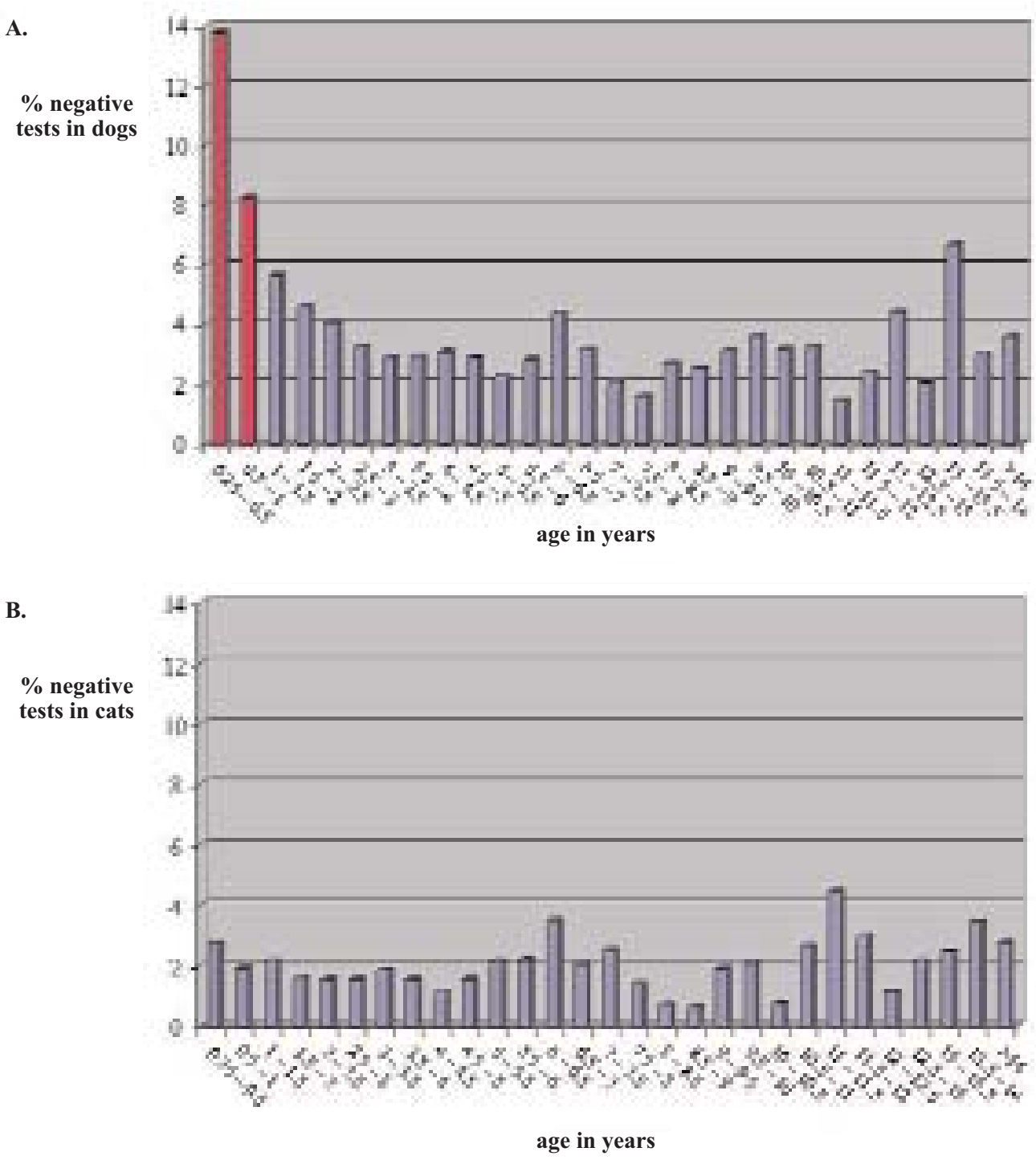

Figure 2. Proportion of negative RFFIT results upon vaccination in dogs (graph A) and cats (graph B) according to age.

3 months after the date of blood sampling. The United Kingdom, Ireland and Malta require a waiting period of 6 months. This waiting period reduces the risk that an incubating animal can enter the country of destination. Indeed, vaccination protects an animal against future infection, but does not prevent the disease in an already incubating animal (Hanlon *et al.*, 2002). Sweden and Norway apply different rules and require that the blood sample is taken at least 4 to maximum 12 months after vaccination. If positive, the animal can enter immediately. Some countries have additional requirements concerning treatment against ticks and *echinococci*, but this is beside the scope of this article. A comprehensive review of pet travel regulations in Europe is presented in Annex I.

It is important that veterinarians inform their clients in advance on the possibility of an insufficient antibody test result, despite prior vaccination. In case of a negative test, the entire procedure of vaccination, blood testing and waiting period has to be repeated. Not rarely, this creates an awkward situation for the veterinarian, who may be presumed incompetent by the owner, and for the owner, who is forced to postpone or annul the voyage.

We have examined the RFFIT results of 28412 canine and 7757 feline blood samples submitted to the IPH Rabies Laboratory between 2000 and 2005. All samples were taken 1 to 12 months after vaccination, specifically for the rabies antibody test. We calculated the proportion of positive and negative test results according to species, age and time point of sampling after vaccination. Odds ratios (OR) for a negative test result were calculated using GraphPad Instat®.

6.35% of canine and 2.10% of feline blood samples tested negative (<0.50 IU/ml). Clearly, dogs respond less well to rabies vaccination than cats (OR = 3.16, P <0.0001). In dogs, the percentage of negative samples is strikingly higher below the age of one year. This is illustrated in Figure 2. 14% of dogs between the age of 3 to 6 months (OR = 4.34, P <0.0001) and 8% of dogs between the age of 6 to 12 months (OR = 2.44, P <0.0001) tested negative. Above the age of 1 year (OR = 1, reference category), the percentage of negative samples varied around 3% and did not seem to increase with high age. Probably this is because young dogs have been vaccinated only once, whereas older animals have often received one or more additional vaccinations earlier in life. Unfortunately, we can not prove this statement, since we usually do not dispose of the entire vaccination history: only the last vaccination has to be reported.

The ideal time period of sampling is 1 to 2 months after vaccination (4.42% negatives). Intervals of 3 months or more are associated with a significant higher probability of a negative test result (8.81% negatives, OR = 2.54, P <0.0001). Indeed, most problems of negative test results occur for owners that want to travel to Sweden or Norway, since blood can only be taken as late as 4 months post vaccination.

For naïve dogs, we advise to vaccinate twice prior to blood sampling to guarantee maximal success of the serological test. We also recommend to use monovalent vaccines. In dogs, the probability of a negative test result is significantly higher with multivalent vaccines (rabies + *Leptospira*, 16.43% negatives, OR = 3.15, P <0.0001), compared to monovalent ones (rabies only, 5.77% negatives). Rarely, we encounter cases that receive multiple vaccinations and fail to seroconvert. These non-responders are usually dogs and their frequency is estimated to be 1/1000.

THE DANGER OF REINTRODUCING RABIES

Rabies virus has proven to be very successful to maintain itself and continuously re-emerge in the animal kingdom. The earliest reports of rabid dogs date from 2300 BC in Mesopotamia. A success factor is its quiescent incubation which can take an unpredictable long time (up to 6 years in humans) (Smith *et al.*, 1991). This allows the virus to persist long time in a population and spread large distances. Also, the existence of animal reservoirs, such as bats, from which the virus makes occasional jumps to other species, contributes to the success. Phylogenetic studies support the hypothesis that rabies viruses of carnivores are derived from bat strains, which adapted to a new carnivore host and caused temporary or long lasting infection chains (Tordo *et al.*, 2006). Reintroduction of rabies can occur in three ways:

Import of an animal incubating the virus: a Belgian example

In August 2007, a family from Beersel adopted an abandoned puppy of a few weeks old during their visit to Morocco. They found the animal near a camping ground and imported it to Belgium by airplane, after a local veterinarian had declared the animal "rabies-free". The animal was not vaccinated against rabies, nor tested for antibodies, and unfortunately passed international borders without control. Three-and-a-half months after arrival, the animal developed subtle mood changes, became exceptionally friendly and less fearsome. The general condition of the dog worsened and 3 days later, on Octobre 19th 2007, the animal presented fever and changes in behavior, such as excessive husky barking, biting objects and aggression. The owner submitted his dog to a veterinary clinic, where it was quarantined, observed for 12 hours and euthanatized. The cadaver was sent to our facilities for autopsy and testing. We diagnosed rabies by direct immunofluorescence staining of viral antigens in the hippocampus on Octobre 22th 2007. Two days later, this was confirmed by virus isolation in neuroblastoma cells. Immediately after the first test, the authorities were warned, including the "Federal Public Service of Health, Food Chain Safety and Environment", "Flemish Agency for Care and Health" (preventive medicine in humans) and the "Federal Agency for the Safety of the Food Chain" (animal disease control).

In cooperation with the rabies and epidemiology

units of IPH, the latter agencies conducted a survey to identify people, dogs and cats that had been in contact with the infected animal during the contagious period. We defined this period as 15 days before the appearance of the first symptoms until euthanasia. Indeed, experimental data show that virus excretion in the saliva is possible during this period (Fekadu, 1988). Forty people received post-exposure treatment, because of close contact with the animal. Nine of them were bitten or scratched. The second dog of the family, which lived in close contact with the infected dog, was euthanatized. Another twenty-one animals were identified as possible or confirmed contacts, including 16 dogs and 3 cats. Many of these contacts occurred in a nearby park, where the infected dog was walked and played without a leash on a daily basis during the contagious period. Placards were hung in the park to inform people and identify contacts. Contact animals were vaccinated and monitored on a regular basis by veterinary inspectors for 6 months. Incubation of the disease can take several years in humans, but usually does not exceed 6 months in carnivores. Importantly, rabies can not be diagnosed during the incubation period.

The affected town and all the neighbouring towns were designated as a region with increased biosecurity measures for the following 6 months. People were recommended to vaccinate their dogs and cats, to report animal bites to their physician or veterinarian, and to keep cats inside. Dogs had to be kept on a leash. Dead-found or diseased foxes had to be submitted for rabies diagnosis. No secondary case was detected. Belgium will regain its rabies-free status by the end of April 2008, 6 months after initial diagnosis.

This is a reminder to include rabies in the differential diagnosis of pets with nervous disease signs, especially after a history of import from a country outside Western or Northern Europe. Most cases in the past years in Western Europe were imports from Northern Africa (e.g. 5 cases in France in 2001-2004), but attention should also go to dogs imported from Eastern Europe. Many new EU member states and the Russian Federation still have fox rabies on their territory. Import from these countries only requires vaccination, and no serological testing or waiting period. Abolishment of border controls within the EU facilitates smuggle of unvaccinated pets.

Host jump from bat reservoirs

Bats are hosts of different types of lyssaviruses, depending on species and geography (Table 1). In Europe, certain bat species are hosts of genotype 5 and/or 6 (Fooks *et al.*, 2003). These European bat lyssaviruses can cause typical rabies in other animals or men. Fortunately, transmission to other species has proven to be rare, but vigilance to the epidemiological and genetic evolution of these viruses is recommended. In Europe, 5 persons died from EBLV after being bitten by a bat, sometimes after an unprovoked attack (Fooks *et al.*, 2003). On the American continent, bats are hosts of genotype 1 and represent the main source of infection for humans. The importance of bats in rabies epidemiology is illustrated by two recent incidents. In the Netherlands, December 2007, a thirty-four-year-old woman died from rabies after being scratched by a bat in Kenia (source: Eurosurveillance). In France, November 2007, a house cat was diagnosed with rabies, after developing typical signs and biting its owner (source: AFSSA). The cat had never left the country and rarely went outside the house. The animal had contracted the disease from bats which lived in the attic of the owners' house.

Table 1. Rabies genotypes.

Genotype	Name	Reservoir	Distribution
1	Classic rabies virus	Carnivores Bats	Worldwide
2	Lagos bat virus	Bats	Africa
3	Mokola virus	?	Africa
4	Duvenhage virus	Bats	Africa
5	European bat lyssavirus-1 (EBLV-1)	Bats	Europe (seropositive bats in Belgium)
6	European bat lyssavirus-2 (EBLV-2)	Bats	Europe
7	Australian bat lyssavirus	Bats	Australia

In Belgium, a variable number of people, ranging from 0 to 12, are treated each year after being bitten by a bat. Usually, they were bitten by a bat that was captured and presented by their pet dog or cat. In most cases, the bat is no longer available for analysis and rabies prophylaxis is administered as a measure of precaution.

To study local epidemiology, the IPH Rabies Laboratory has set up a network of passive surveillance. Agreements were made with (amateur) chiropterologists from Wallonia (Plecotus) and Flanders (Vleermuizenwerkgroep Natuurpunt VZW),

Figure 3. Recovery of a weakened bat (*Pipistrellus nathusii*) by an amateur chiropterologist from Antwerp. Photograph taken by W. Willems, Vleermuizenwerkgroep Natuurpunt VZW. Printed with permission.

to submit dead-found bats for rabies analysis. In Flanders, bats can be deposited in a network of 30 freezers, known as the "Marternetwerk", which is maintained by the "Research Institute for Nature and Forest" (www.inbo.be). By the end of 2007, we examined 108 bats for rabies antigens and/or RNA in the brain. All tested negative. This approach remains biased since we can not control the sampling. Most cadavers submitted for analysis belong to *Pipistrellus spp.* (Figure 3), which are very common in Belgium, but probably least likely to be infected according to foreign studies (Van der Poel *et al.*, 2005).

A preliminary study was undertaken to look for antibodies in living bats (active surveillance) in the Ardennes region (Audrey *et al.*, 2007). One-hundred-thirty-two bats were caught at night with nets. Blood was collected from the wing vein. From 58 bats, the recovered volume was sufficient to pursue antibody titration. Serological assays for EBLV-1 and -2 were performed at the Pasteur Institute of Paris. Antibodies against EBLV-1 were found in 9 bats (*Myotis myotis*, *Myotis natterreri* and *Plecotus spp.*). No antibodies against EBLV-2 were found. Seropositive bats appeared in good health, indicating that EBLV-1 circulates in Belgian bats without causing lethal disease. Possibly, they undergo transient infection or become subclinical carriers. More research on epidemiology and virulence characteristics of bat lyssaviruses is warranted.

The IPH Rabies Laboratory is currently setting up in-house assays to quantify EBLV-1 and EBLV-2 antibodies. These assays will be used for prevalence studies in bats and validation of anti-EBLV immunity in vaccinated humans and cats. Indeed, current vaccines contain genotype 1 antigens and offer partial, but probably sufficient, protection against divergent genotypes of bats. Brookes *et al.* (2005) found protection against EBLV-1 and -2 in sera of 48 of 50 vaccinated humans. However, people with relatively low antibody titers (0.50 to 5.00 IU/ml) risk being insufficiently protected against bat strains (Brookes *et al.*, 2006)

Spread in foxes from Eastern to Western Europe

Figure 4 shows the distribution of rabies cases in Europe in 2006-2007, including the positive import case in Belgium. Most cases occur in Eastern Europe and involve foxes infected with genotype 1. Thanks to expensive and elaborate vaccination campaigns, rabies in foxes has been eradicated in Western Europe. The last residual focus was in Germany, about 300 km from the Belgian borders. In 2006, 3 foxes were still diagnosed positive in this region (www.who-rabies-bulletin.org). Thanks to vaccination campaigns, no new cases have been detected since and Germany was declared rabies-free in the course of 2008.

Rabies is still highly prevalent in foxes and raccoon dogs in the new Eastern European member states of the EU. It is important that Eastern Europe controls the local situation by surveillance and vaccination

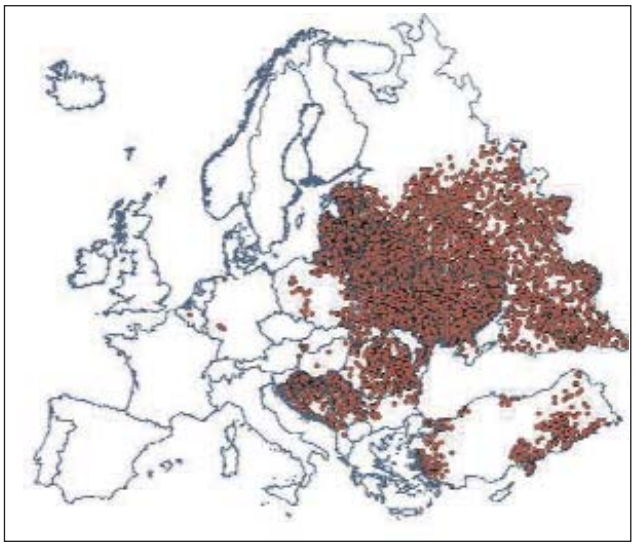

Figure 4. Positive rabies cases in Europe during 2006-2007. Adapted from the WHO Rabies Bulletin Europe, WHO CC for Rabies Research and Surveillance, Institute of Epidemiology, Friedrich-Loeffler-Institute, Wusterhausen/Germany, available at www.who-rabies-bulletin.org. Printed with permission.

programs. In previously freed areas, the rabies status in foxes should continue to be monitored, at least at a minimum level, to provide quick response in case of reintroduction.

In 2006 and 2007, respectively 94 and 141 foxes were analyzed by the National Reference Laboratory of Rabies in Belgium. Although this is less than the proposed 8 foxes/100 km^2/year by the WHO, it provides an indication of the current sanitary status in foxes. Fox cadavers submitted to the laboratory are also examined for *Echinococcus multilocularis* and *Trichinella spiralis*, two zoonotic parasites, in cooperation with the "Research Institute for Nature and Forest" and the "Institute of Tropical Medicine".

CONCLUSION

The rabies case in Beersel reminds us to remain vigilant to diseases which are presumed eradicated in a region. It is important to emphasize the role of veterinarians in the control of this public health disease. It was primarily thanks to the quick and accurate suspicion of rabies made by veterinarians from a clinic in Brussels, that a local catastrophe was prevented. If left undiagnosed, the incident would most likely have cost several human lives, with possible spread of rabies in a metropolitan region with a high density of dogs, cats and foxes. Diagnosis is not obvious considering that most Belgian veterinarians, especially in the Brussels and Flemish region, have never encountered rabies in practice.

ACKNOWLEDGEMENTS

The authors want to thank Dr. F. Klein and Dr. N. Praet for their pioneer work on rabies epidemiology in

REGULATION (EC) NR. 998/2003 (MODIFICATION OF DIRECTIVE 95/65/EEG): NON-COMMERCIAL MOVEMENT OF DOGS, CATS AND FERRETS

Pets should be identified (chip or until 2011: tattoo) and accompanied by a European passport or model veterinary certificate (vaccination status, valid period of vaccination, rabies antibody titer)

Comming from:

- **EU**
- **Associated countries: Andorra, Iceland, Liechtenstein, Monaco, Norway, San Marino, Switzerland, Vatican City State**
- **Favourable countries (see: Annex II part C of regulation 998/2003)**

Entry in UK, Ireland, Malta, Australia	Entry in Sweden, Norway	Entry in the rest of EU[3]
Animal >3 months: 1. Vaccination 2. Serum sample: at least 1 month later (at least 10 days if booster) 3. If ≥0.5: 6 months delay [1,2] also: echinococcus and tick treatment	Animal >3 months: 1. Vaccination 2. Serum sample: >120 and <365 days 3. If ≥0.5: immediate departure	Animal >3 months: 1. Vaccination Animal <3 months: Accompanied by mother or declaration no contact with wild animals

Comming (or returning) from:

- **Unfavourable country**

Entry in the rest of EU:	Entry in UK, Malta, Ireland or Sweden:
Animal >3 months : 1. Vaccination 2. Serum sample: at least 1 month and maximum 1 year later (at least 10 days if booster) 3. If ≥0.5: 3 months delay[4]	Quarantine

Booster within valid period of vaccination (recommendations of the company: 1-3 years): no secondary antibody test or delay necessary

(1) Delay: starting from the date of serum sampling
(2) If <0.5: revaccinate and retest within same time frame and respect same delay
(3) Vaccination is still mandatory within Belgium when travelling south of the rivers Sambre and Meuse, but this might be abolished in the nearby future.
(4) No delay if animal returns to EU and was vaccinated and serologically tested in EU prior to departure from EU (advice: test animal before leaving EU)

Annex 1. Overview of rules for pet travel in Europe.

local bats and our technicians A. Francart, M.L. Blondiau and F. Mosselmans for all the work and commitment they dedicated to the laboratory. Special appreciation goes to Dr. R. Van Hoof and Dr. J. Colyn, medical doctors at the authors' institute, who treated several bitten people and assisted the Flemish Agency for Care and Health with the medical management of human contacts. The authors also wish to thank the authorities, the Flemish Agency for Care and Health and the Federal Agency for the Safety of the Food Chain for the efficacious control and management of the Beersel case subsequent to our laboratory confirmation and declaration. Koen Van Den Berghe (INBO), Alex Lefèvre and Wout Willems (Vleermuizenwerkgroep Natuurpunt VZW), Plecotus and Jacques Fairon (KBIN) are highly appreciated for their help with the collection of bats and willingly sharing their expertise on bat biology.

REFERENCES

Audrey L., Klein F., Bourhy H. Preliminary results of an active survey of bat rabies in Belgium. *The OIE/WHO/EU International Conference on 'Towards the Elimination of Rabies in Eurasia*. Paris, France, 27 - 30 May 2007, p. 90.

Brochier B., Boulanger D., Costy F., Pastoret P.P. (1994). Towards rabies elimination in Belgium by fox vaccination using a vaccinia-rabies glycoprotein recombinant virus. *Vaccine 12*, 1368-1371.

Brookes S.M., Healy D.M., Fooks A.R. (2006). Ability of rabies vaccine strains to elicit cross-neutralising antibodies. *Developments in Biologicals (Basel) 125*, 185-193.

Brookes S.M., Parsons G., Johnson N., McElhinney L.M., Fooks A.R. (2005). Rabies human diploid cell vaccine elicits cross-neutralising and cross-protecting immune responses against European and Australian bat lyssaviruses.*Vaccine 23*, 4101-4109.

Fekadu M. (1988). Pathogenesis of rabies virus infection in dogs. *Review Infectious Diseases 10*, 678-683.

Fooks A.R., Brookes S.M., Johnson N., McElhinney L.M., Hutson A.M. (2003). European bat lyssaviruses: an emerging zoonosis. *Epidemiology Infection 131*, 1029-1039.

Hanlon C.A., Niezgoda M., Rupprecht C.E. (2002). Postexposure prophylaxis for prevention of rabies in dogs. *American Journal of Veterinary Research 63*, 1096-1100.

Hemachudha T., Laothamatas J., Rupprecht C.E. (2002). Human rabies: a disease of complex neuropathogenetic mechanisms and diagnostic challenges. *Lancet Neurology 1*, 101-109.

King A.A., Turner G.S. (1993). Rabies: a review. *Journal of Comparative Pathology 108*, 1-39.

Smith J.S., Fishbein D.B., Rupprecht C.E., Clark K. (1991). Unexplained rabies in three immigrants in the United States: a virologic investigation. *New England Journal of Medicine 324*, 205-211.

Tordo N., Bahloul C., Jacob Y., Jallet C., Perrin P., Badrane H. (2006). Rabies: epidemiological tendencies and control tools. *Development Biology (Basel) 125*, 3-13.

Van der Poel W.H., Van der Heide R., Verstraten E.R., Takumi K., Lina P.H., Kramps J.A. (2005). European bat lyssaviruses, The Netherlands. *Emerging Infectious Diseases 11*, 1854-1859.

Willoughby R.E. Jr., Tieves K.S., Hoffman G.M., Ghanayem N.S., Amlie-Lefond C.M., Schwabe M.J., Chusid M.J., Rupprecht C.E. (2005). Survival after treatment of rabies with induction of coma. *New England Journal of Medicine 352*, 2508-2514.