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WARNING: REGULATIONS CAN DAMAGE YOUR HEALTH - THE CASE OF RABIES

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Introduction

More than one hundred years ago, Louis Pasteur (1822-1895) announced his first results on curative vaccination against rabies in a session of the Academy of Sciences in Paris, on Monday, October 26th, 1885. He declared that he had successfully vaccinated Joseph Meister with an attenuated vaccine and that the experiment had been repeated in another subject, jean-Baptiste Jupille. At the end of the treatment, Pasteur had subjected Joseph Meister to a genuine virulent challenge using a "fixed virus":

"...during the last days of the treatment, I inoculated Joseph Meister with the most virulent rabies virus, which originated from a dog and was reinforced by numerous passages from rabbit to rabbit, and which reproduces rabies in those animals (rabbit) after seven days of incubation and in dogs after eight to ten days. The vaccination trial was carried out according to previous experiments conducted on fifty dogs. When the immune status is reached, one can, without inconvenience, inoculate the most virulent virus, whatever the quantity. In every case, it was apparent to me that challenge has no other effect than to consolidate the refractory status against rabies. Joseph Meister, therefore, not only escaped from rabies due to biting, but also from rabies which I inoculated into him in order to control immunity conferred by the treatment, a rabies more virulent than the one originating from urban dog." (L Pasteur, *Comptes rendus de I 'Academie des Sciences* 1885, pp 765-774).

Pasteur's vaccine for the post-exposure treatment of humans against rabies was subsequently improved, step by step, to obtain the inactivated vaccines that are produced in cell culture today [1]. Apart from some experiments using an attenuated HEP FLURY strain of rabies virus in humans, only inactivated rabies virus vaccines have been licensed for human use.

The original experiments, in which vaccinated human beings were exposed to virulent rabies, were preceded by rigorous experiments in dogs. Preventive vaccines developed in animal trials largely contributed to dog rabies eradication in Western Europe at the beginning of this century. After the Second World War, rabies reappeared in several European countries linked to a wildlife rabies reservoir, namely the red fox (*Vulpes vulpes*).

Fox rabies is presently controlled in Western Europe by vaccination campaigns. Conventionally attenuated strains of rabies virus are still used by some countries for this purpose, notwithstanding their lack of safety and stability. A recombinant vaccinia rabies virus offers a better alternative, but its use is still forbidden in certain countries, mainly because of regulatory constraints.

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Rabies : a worldwide problem

Rabies is a fearful disease still prevailing in many countries in most parts of the world. It may be maintained in two, not necessarily inter-related, cycles: urban and sylvatic. Urban rabies, affecting stray and feral dogs and cats, is by far the most dangerous to man, accounting for an estimated 99 per cent of all recorded human cases and for 92 per cent of all human post-exposure treatments. Sylvatic rabies is characterized by the involvement of one or two main wild species in particular locations, and this pattern remains stable over many years. The wild animal species involved in maintaining the infection may vary according to geographical and ecological conditions. In North America, for instance, several wildlife species play a distinct role, such as the raccoon (*Procyon lotor*), the striped skunk (*Mephitis mephitis*), the red fox (*Vulpes vulpes*), the coyote (*Canis latrans*) and the Arctic fox (*Alopex lagopus*).

The present European terrestrial epizootic of rabies has spread some 1400 kilometers westward from Poland since 1939. Although it involves all susceptible species, both wild and domestic, the red fox is involved in more than 75 per cent of cases. In Western Europe, the fox seems to be the only species maintaining the present terrestrial. epizootic. Thus, if rabies were eliminated from the fox population, it would cease to be a problem in other wildlife or domestic species and, therefore, cease to be a problem for man.
The control of fox rabies is used as an example in the following review; nevertheless, many different epidemiological cycles exist in the world, either rural or sylvatic, involving many different animal species. Thus, the overall aim must be to develop control measures (e.g. through vaccination) that can be applied in as many different situations as possible.

Control of fox rabies

Prophylactic measures taken in the past, such as the destruction of foxes to reduce the fox population, did not prevent the spread of the epizootic. During recent years, most of the research on the control of fox rabies has concentrated on the development of methods of fox vaccination by the oral route, and this method has already been extensively used in all the contaminated countries belonging to the European Union. Research has focused on oral vaccination because it is the only means allowing the inmunization of a sufficient proportion (75%) of wild foxes through the distribution of vaccine baits. Therefore, the only applicable vaccines were either attenuated strains of rabies virus or live vectored vaccines.

Even so, as far as safety and stability are concerned, the use of attenuated rabies virus remains controversial because these virus strains are still pathogenic for laboratory and wild rodents [2], wildlife species, such as the chacma baboon (*Papio ursinus*) [3], or target species, such as the striped skunk [4]; moreover, these strains may still be pathogenic to man. Thus, humans exposed to SAD-derived attenuated strains of rabies must be treated with a conventional inactivated rabies vaccine. SAD-derived attenuated strains may also be inefficient in certain rabies vectors, such as the raccoon in North America [5]. Because of their residual pathogenicity, the use of attenuated strains of rabies virus for domestic animal vaccination in Western Epe has been discontinued.

Pathogenicity of attenuated rabies virus strains can be abolished by mutating arginine residues at position 333 of the rabies virus glycoprotein. This has led to the development of a new attenuated vaccine strain, which is already in use in the field [6]. Another inconvenience of attenuated strains of rabies viruuros is their heat-sensitivity, which reduces their potential efficacy in field conditions.

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Thus, in order to improve both the safety and stability of the vaccines used for fox vaccination in the field a recombinant vaccinia virus has been developed that expresses the immunizing glycoprotein of rabies virus. This virus vaccine has been tested in the field for oral vaccination of foxes against rabies [7,8•].

Development of a vaccinia - rabies vector vaccine for oral vaccination of wildlife against rabies

The glycoprotein of rabies virus is the sole viral protein present on the external surface of the viral membrane. It is the only viral antigen capable of eliciting the production of rabies virus-neutralizing antibodies and has been shown to be capable of conferring immunity to rabies. Thus, the rabies virus glycoprotein is an ideal candidate for use in the construction of a subunit marked vaccine.

The rabies virus glycoprotein gene has been inserted into the thynlidine-kinase (TK) gene of vaccinia virus (VV), generating a selectable TK-virus [9,10] known as VVTGg RAB, which is safer than the parental strain [11]. VVTGg RAB has been tested for efficacy and safety in the main target species in Western Europe and North America: fox, raccoon and striped skunk. The duration of protection conferred by VVTGg RAB (a minimum of 18 months in adult animals) corresponds to the length required for fox vaccination in the field due to the high turnover of the fox population.

The preclusion of epizoological risks, such as the emergence of asymptomatic carriers of wild rabies virus, is also of major importance. This situation could occur in the field by vaccination of naturally infected animals during the incubation period. The influence of vaccination with VVTGg RAB, both on the onset of the disease, and on the delay before death in foxes previously infected with wild rabies virus, has been investigated [12]. The results show that 'early' and 'late' death phenomena occur as a consequence of interactions between oral vaccination with VVTGg RAB and rabies infection, but preclude the risk of the emergence of asymptomatic carriers of wild rabies virus after vaccination.

It is also preferable that a vaccine virus used for oral vaccination of wildlife should not be horizontally transmitted to unvaccinated animals. Accordingly, no transmission of immunizing amounts of VVTGg RAB was found to occur in adult or young foxes. Changes in tissue tropism were also not observed [13]. In areas of Europe earmarked for vaccine distribution, several non-target wild species were chosen for safety testing, both because of their opportunistic feeding behaviour, and because of their presence [14]; similar experiments were carried out on wild species from North America. In every case, the recombinant virus was always perfectly safe. More recent experiments have also shown that the recombinant virus, administered either by scarification or by the oral route, is also safe for squirrel monkeys (*Saimiri sciureus*) and for chimpanzees (*Pan troglodytes*) [15••]. Additional experiments were performed on several species (including cows) in contact with control animals to test for horizontal transmission of VVTGg RAB. Without exception, the results showed that no horizontal transmission took place.

The only remaining perceived risk to be investigated was the eventual recombination of the recombinant virus with a wild orthopox virus. For such an event to occur, both parental viruses must multiply during the same period of time in the same cells of the same animal. As no serological evidence for orthopoxvirus infection in the fox population has been found, however, this risk may be discarded in the main target species. Moreover, experimental inoculation of cowpox virus into foxes via the oral route results in viral multiplication only at a low level and for a short duration in the mouth cavity.

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Taking into account these epidemiological and experimental data, it is most unlikely that recombination between VVTGg RAB and another orthopox virus could occur in the vaccinated foxes. It is, therefore, preferable to choose a recombinant vin1s that has no counterpart in the wild (e.g. vaccinia virus) and that, besides a long history of use in uncontrolled conditions, has never been established in wildlife. Thus, a vector virus previously unencountered by wildlife, but with a wide host range, is, for safety reasons, better than another virus isolated from a target species (e.g. raccoonpox virus) that is still prevalent in the wild. The fact that vaccinia virus has been used for more than 150 years without any undesirable ecological impact, such as installation in wildlife, also argues strongly for its choice.

Deliberate release of the vaccinia-rabies recombinant virus for oral vaccination of foxes against rabies

On the basis of all the available experimental data concerning the safety of the VVTGg RAB for target and non-target species and its efficacy in foxes, limited field trials of fox vaccination with the recombinant virus were authorized, first by the Belgian [16] and then by the French public health authorities.

The Belgian authorizations were preceded by safety assessment (i.e. risk versus benefit) of the use of recombinant vaccinia-rabies virus for *f9x* vaccination against rabies. It was concluded that there was considerable risk of exposure to rabies infection in the target area and that this risk could be reduced through the use of a vaccine (i.e. Wl'Gg RAB) more efficacious (in terms of immunogenicity and stability) than the vaccines already in use. As far as safety was concerned, clear and identified risks were associated with the use of conventionally attenuated rabies virus strains, such as the SAD B19 strain. It was possible to abolish the risk associated with vaccination by substituting the attenuated virus either with recombinant vaccinia-rabies virus or with rabies virus strains in which arginine at position 333 of the glycoprotein had been modified.

With the safety of the VVTGg RAB confirmed by these small trials, the Belgian authorities agreed to an enlarged open field trial. The vaccine was subsequently shown to be very stable, even following natural freezing and thawing cycles. The VVTGg RAB vaccine retained its capacity to immunize for at least one month in field conditions, a period that corresponds to the longest delay of uptake that baits may undergo in the field. Following this enlarged trial, three fox-vaccination campaigns using VVTGg RAB were carried out in Belgium in November 1989, April 1990 and October 1990 in order to check for efficacy in an area of 2200 km2 [17].

Towards elimination of rabies?

The above trials, in which the VVTGg RAB was deliberately released over a 2200 km2 area of Southern Belgium, were intended to test the feasibility of rabies elimination over a large area. On this occasion, the economics of the vaccine-bait dispersal program were also investigated. The average yearly cost of rabies infection in Belgium (in the period 1980-1989), including post-exposure treatments of humans, animal diagnosis, compensation to farmers for the culling of infected live- stock, and the culling of wild foxes, was estimated to be 400000 ECUs (10000 km)^2, or 88000 ECUs per annum for the area under study. (These figures include neither the cost of vaccination of domestic animals nor the salaries of civil servants.) In comparison, the overall expenditure during the three campaigns of vaccine-bait distribution in Belgium was estimated

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to be 118000 ECUs. In addition, as vaccination following elimination can, in principle, be interrupted or subsequently limited to the borders of vaccinated zone, long-term maintenance of a rabies-free area by peripheral vaccination with VVTGg RAB is economically justifiable.

The use of VVTGg RAB has now been extended to all contaminated areas in Belgium and the grand duchy of Luxembourg as well as to large areas of France. The vaccine is presently being tested in the United States. As far as Belgium is concerned, rabies is nearly reaching the stage of elimination. Rabies elimination in Belgium has already had beneficial effects besides improvement in animal health. First, the decrease in number of humans requiring post-exposure treatments correlates with the decrease in rabies incidence in animals (mainly cattle). Second, the diminution of the incidence of rabies in wildlife has had a beneficial effect on the survival of threatened wild species, such as the Eurasian badger (*Metes metes*), in the contaminated area. Estimation of the badgers' population in the treated area shows a gradual increase in number. In fact, Belgium is slowly recovering badger numbers similar to those before 1966, which was when rabies was reintroduced from Germany. Finally, elimination of rabies will help to authorize free movement of pets within the European Union.

Conclusions

The story of fox vaccination against rabies using the recombinant vaccinia-rabies virus shows, above all, that the choice of a recombinant product can greatly improve the safety of a vaccination procedure. As demonstrated previously, the recombinant virus is much safer than older products, such as the conventionally attenuated strains of rabies virus. Nevertheless, some European countries (e.g. Germany) refuse to use the recombinant vaccine for regulatory (or political) reasons. Moreover, recent press campaigns in Germany have argued against the use of this vaccine, claiming unjustly that it caused a human death. The cause of this human death is well documented [18). It did not result from infection with the recombinant vaccinia-rabies virus; rather, it resulted from an infection with a wild cowpox-like virus transmitted by a cat. The patient (an 18-year-old man) had not been previously vaccinated against smallpox and was intensively immunosuppressed by medication to cure another condition. We consider that the press campaign has been completely irrational. Since Germany does not use the recombinant vaccinia-rabies virus, the question arises where did the infection come from?

It should be remembered that the use of cowpox virus to prevent smallpox at the beginning of 19th century provoked the same irrational reactions. In spite of this, its use led to the eradication of the disease worldwide [19]. Certain countries still stonewall the introduction of recombinant vaccines through regulatory obstacles, even if efficacy and safety are well documented in other countries on a large scale with clear success and without any detrimental effect [20]. The choice of vaccinating, not vaccinating, or merely waiting is also not neutral; meanwhile, people and animals are still dying from rabies. To conclude, one should remember this citation from Edward Jenner 0749-1823):

"The skepticism that appeared, even among the most enlightened of medical men, when my sentiments on the important subject of the cowpox were first promulgated, was highly laudable. To have admitted the truth of a doctrine, at once so novel and so unlike anything that had ever appeared in the annals of medicine, without the test of the most rigid scrutiny would have bordered on temerity."

Regulations, therefore, play a beneficial role when they do not hinder the scrutiny and application of scientific evidence.

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ABBREVIATIONS : TK-thymidine kinase; W-vaccinia virus.

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