Vaccines and vaccination against rabies for domestic and wild animals in Europe*

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Summary: Objectives of vaccination always place priority on the protection of human beings from rabies by vaccinating either the vector species (the fox in most countries), the relay species (domestic animals), or human beings themselves. Other objectives of vaccination are to reduce economic loss or to avoid creating a secondary cycle of rabies among domestic animals.

Vaccines containing live virus (Flury, ERA, Vnukovo) and those possessing residual virulence (Fermi type) are tending to be replaced by the safer vaccines prepared from inactivated virus, particularly those containing an adjuvant of immunity.

Testing of these vaccines is performed on the final product and its effect in the target species by serological titration. Testing of the final product is easier to standardise (in mice) in the case of vaccines containing inactivated virus. The international transport of animals requires a minimum antigenicity (1 International Unit per dose), calculated by comparison with a reference preparation of the European Pharmacopoeia.

Domestic animals are vaccinated with the aim of protecting either zones still free from the disease, or animals at greatest risk, or species most dangerous to man, and this is done by obligatory measures or incentive schemes.

Foxes are the subject of trials concerning oral vaccination in many European countries, with field trials in progress in the Federal Republic of Germany, Switzerland and USSR.

Results, as judged by the proportion of animals vaccinated, the rate of serological conversions and the incidence of rabies are very good in the case of domestic animals (primarily cattle and dogs), and encouraging in the case of foxes.

KEY WORDS: Cat - Cattle - Dog - Domestic animals - Fox - Horse - Quality control - Rabies virus - Vaccines - Vaccination - Wild animals.

INTRODUCTION

The purpose of this paper is to examine the current situation in those European countries where fox rabies occurs, with regard to the objectives of vaccination of domestic and wild animals, the vaccines commonly used, the testing to which they are submitted, their practical application, the results of such application, and general conclusions concerning the medical prophylaxis of rabies.

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OBJECTIVES

The primary objective of vaccination of animals, whether domestic or wild, is to protect the human population from rabies. This objective may be achieved at three levels, numbered 1, 2 and 3 in Figure 1. The effectiveness of vaccination and its cost vary according to the level chosen.

- **Level 1.** Vaccination of foxes is theoretically the most effective because it is an active measure which reduces the need for interventions 2 and 3, but has a relatively high cost, intermediate between that of levels 2 and 3.

- **Level 2.** Vaccination of domestic animals is highly effective, although a defensive measure, because it reduces the need for level 3, and is the least costly.

- **Level 3.** Vaccination of human beings before (but more often after) infection, is a purely defensive measure, and the most expensive of the three. It should be considered solely as a last resort, and it reflects a failure of veterinary measures.

Other objectives are of minor importance and they differ according to the species of animal, husbandry conditions, their numbers, etc. The aim may be to protect the animals in order to avert economic loss (in the case of livestock) or emotional loss (in the case of pets).

Another objective should be to avoid creating a secondary cycle of rabies through infected domestic animals, although this eventuality seems to be most unlikely in the light of our current knowledge of fox rabies (5, 8).

*Note that,* as far as European fox rabies is concerned, the aim of vaccinating domestic animals (level 2) is, of course, to protect the maximum number of animals at risk, because the efficacy of this measure is proportional to the number vaccinated. However, it is unnecessary to aim at the minimum of 80% vaccination ("rule of Charles Nicolle"), because the vaccination of domestic species cannot stop the cycle of rabies in foxes, the vector species, which alone are responsible for maintaining the disease.

VACCINES, THEIR PRODUCTION AND PROPERTIES

Among the numerous vaccines against rabies which have been developed since the time of Pasteur, only two types are in current use in Europe (1): vaccines prepared from "live" virus, either modified (Flury, ERA and Vnukovo) or partly inactivated (Fermi type), and vaccines prepared from inactivated virus.

VACCINES PREPARED FROM "LIVE" VIRUS

**Vaccines prepared from the "Flury" strain of modified virus.**

This strain was isolated in 1939 from the brain of Miss Flury, infected by a dog, and adapted to embryonated eggs. After 50 or 130 passages it was proposed for use as a live vaccine under the name "Flury Low or High Egg Passage" (LEP or HEP). This vaccine is currently used in Europe, the virus being propagated either in eggs or in cell culture. Certain fatal accidents (in cats and in immunodeficient animals) have led to a restriction or even a prohibition of its use (Flury LEP), but it is still used (e.g. in Italy and Switzerland) on account of the low cost of production and the good immunity which it confers when correctly manufactured and tested.
CYCLE OF WILDLIFE (FOX) RABIES IN EUROPE

Level 1: Oral vaccination of foxes (cost 5-30 Ecu* per fox)

Level 2: Vaccination of domestic animals (cost: 2-10 Ecu a head)

Other domestic species

Level 3: Vaccination of human beings (cost: 20-30 Ecu before exposure, 500-1000 afterwards)

* ECU = European Currency Unit = FF 6.85
= £S 0.57
= US.$ 0.73

FIG. 1
Levels and costs of vaccination against rabies in Europe (1985)
Vaccines prepared from the modified "ERA" and "Vnukovo" strains of virus.

The initial strain from which "ERA" and "Vnukovo" vaccines have been derived was isolated in Alabama and named "Street Alabama Dufferin" or SAD; it has been subjected to multiple serial passages in mice or in cells.

It is currently produced by culture in kidney cells from hamsters, dogs, cattle or pigs. Connaught Laboratories in Canada call it ERA (after Eva Gaynor, Rokitniki and Abelseth), while Russian laboratories call it "Vnukovo 32" after the name of a Russian airport and the temperature 32°C, which is the optimum temperature for replication of the selected clone.

Vaccines prepared from partly inactivated virus.

Such vaccines, called "Fermi type", are prepared from suspensions of fixed virus (derived from the Pasteur strain), partly inactivated by phenol. They still contain up to $10^2$ mouse i.e. LD$_{50}$.

General characteristics of vaccines prepared from "live" virus.

Innocuity of Flury, ERA and Vnukovo vaccines depends on the genetic stability of the strain, and also the conditions under which they are used (vaccine dose, and age, sex or immune status of the vaccinated animal). That of the Fermi type also depends on the degree of inactivation of the fixed virus.

Efficacy varies according to the nature, antigenic integrity and extent of replication of the strain within the animal (which depends on the titre of the inoculated virus). The immunity conferred may last for 2-3 years under good conditions.

This type of vaccine is usually lyophilised and not combined with other antigens, nor enriched with adjuvant. It is injected at the same dose for all species of animals.

VACCINES PREPARED FROM INACTIVATED VIRUS

There is a wider variety of such vaccines than the preceding type. Many more vaccine strains are employed, with various types of in vitro and in vivo replication of the virus. Since attenuation of the pathogenicity of a vaccine strain for the "target" species is no longer important, both strains and substrates may vary from one laboratory to another. However, it is still possible to distinguish two major groups of these vaccines:

Vaccines prepared from virus propagated in vivo are still produced because of the relative ease of virus production. This may be achieved by inoculating adult animals (not recommended), but young or newborn animals are mainly used, particularly young mice, which provide viral titres 10 to 100 times higher than those obtained in adult animals, and provide a viral antigen free from the sensitising "neuro-allergenic" factor.

Vaccines prepared from virus propagated in vitro differ according to the substrate used for replication and the strain of virus used:

— Cell systems: hamster kidney cells (of a continuous or a diploid line) are often used, but also chick-embryo fibroblasts, and kidney cells from dogs, pigs or other species.

— Virus strains: most are derived from the Pasteur strain isolated in 1882 from a cow which died after being bitten by a dog. This has been subjected to a wide variety
of passages in various species of animal or in cells, and has been renamed “Chal­lenge Virus Standard” (CVS), Pitman-Moore (PM), Pasteur virus of the 11th pas­sage (PV11), Kissling, etc. Some vaccines utilise the Flury strain (and its clone HEP 675) and the Vnukovo strain.

**General characteristics of vaccines prepared from inactivated virus.**

— **Innocuity** is practically total if the vaccine has been prepared correctly, and there is no risk of “vaccinal rabies” with this type of product. However, the degree of inactivation has to be verified by reliable and sensitive tests.

Sensitisation accidents (delayed type hypersensitivity and anaphylaxis) are also rare when the injections of vaccine are few and well spaced.

— **Efficacy** (level and duration of immunity conferred) depends above all on the antigenic make-up of the strain, conditions of replication of the virus, the inactivating agent and the final concentration of antigen, which should meet the national and/or international requirements (see below).

The duration of immunity conferred should extend to 2-3 years under good con­ditions, as with live vaccines. The vaccine may be lyophilised (remaining stable for 18 months) or issued in liquid form (stable for at least 12 months). Adjuvants of immunity, such as aluminium hydroxide, are often added to these vaccines, particu­larly those prepared from cell cultures, in order to enhance the level and duration of the humoral immune response in the target species.

It is becoming more common to incorporate other antigens, such as leptospiro­sis, distemper and viral hepatitis antigens in vaccines for dogs, panleukopenia anti­gen in vaccines for cats, and foot and mouth disease antigen in vaccines for cattle.

**NOTE**

Major progress has been achieved in recent years in the case of vaccines produced in Europe.

- **General technical advances** in cell culture (large-capacity fermentors, “micro­carriers”, automation, etc.) have improved the quantity and quality of the yields, making it possible to produce a vaccine containing 3-5 IU per dose.

- **Concentration and partial purification** of the viral antigen (by ultrafiltration or zonal ultracentrifugation) have led to lower concentrations of the useless non-viral protein nitrogen, and higher concentrations of specific viral antigen, which can be assayed with high precision.

However, up to now no industrially produced vaccine has been able to utilise iso­lated viral glycoprotein, despite the high degree of purification obtained, and the same applies to chemically defined antigenic “sub-units”. By contrast, the opportu­nities offered by “immunosomes” (preformed liposomes representing the original antigenic structure) are very promising. The possibility of utilising genetic manipula­tion to produce this glycoprotein, and programming it for other micro-organisms (e.g. vaccinia virus) is one of the paths of European research which may have funda­mental consequences with important applications in the future (13).

- **Knowledge of antigenic determinants of the virus** has been promoted by the recent development of monoclonal antibodies, which are capable of recognising strains by their nucleocapsid marker, providing each strain with an identity card which will
be extremely valuable for laboratories producing rabies vaccines (9). In Europe, it seems that the vaccines at present in use (most of which are derived from the Pasteur strain of virus) provide excellent protection against the field virus involved in the current enzootic of fox rabies.

- **Addition of an adjuvant of immunity** to vaccines for use in herbivores, and also in carnivores, constitutes a major advance in Europe during the past few years. Thanks to adjuvant vaccines it has been possible to recommend (or require) the abandonment of vaccines prepared from live virus (or virus possessing residual virulence) in most European countries, to the advantage of modern, perfectly safe vaccines.

### THE TESTING OF VACCINES

#### TESTING THE FINAL PRODUCT

The method of testing depends on whether the vaccine is an inactivated one or not (6).

**Vaccines prepared from inactivated virus** are submitted to two tests:

*Innocuity* is verified by intracerebral injection into mice and by intramuscular injection into at least two animals of the species in which the vaccine will be used.

*Efficacy* has for some time been evaluated by the Habel test, which consists of challenging vaccinated and unvaccinated mice with increasing doses of virus in order to measure the difference in the 50% lethal dose between one group and the other; this difference should be at least 1:1000. This test is being abandoned because of its imprecision for vaccines of average efficacy, and because of difficulties in standardisation on the European scale.

The Habel test has been generally replaced by that of the National Institutes of Health (NIH), in which mice given two intraperitoneal injections of different dilutions of the vaccine under test are challenged with a uniform dose of virus. The efficacy of a vaccine is measured by the final dilution which will still protect 50% of mice, and it has the advantage of being expressed in international units (IU) because of the use of an international reference preparation or one of its subsets. This is why the European Pharmacopoeia has adopted the same principle, though with a single injection of vaccine (see below).

For the two last-named tests, the minimum antigenicity required by the WHO is 0.3 IU per dose. For the international movement of animals, the minimum requirement is 1 IU per dose (WHO-OIE).

**Vaccines prepared from “live” virus (Flury, ERA, Vnuko) are also submitted to two specific tests:**

*Innocuity* is verified by intramuscular injection into at least 20 guinea pigs and 2 animals of the most receptive species in which the vaccine will be used.

*Efficacy* is verified by determining the titre of the viral vaccine, and by intramuscular injection into at least 10 guinea pigs, 70% of which should survive challenge infection with virulent virus, while 80% of unvaccinated controls die.

**Vaccines possessing residual virulence** are submitted to a simple titration of virulence (which must not exceed $10^2$ intracerebral mouse LD$_{50}$) and, if necessary, tests for efficacy in laboratory animals and the target species.
TESTING IN ANIMALS OF THE TARGET SPECIES

Postvaccinal immunity may be measured by direct and indirect methods.

**Direct testing** consists of comparing the resistance to experimental or natural infection of vaccinated animals with that of unvaccinated animals. *Experimental infection* is the sole technique which guarantees the strength and the persistence of the immunity conferred by a rabies vaccine. Testing by *naturally acquired infection* is based on a detailed investigation of the number of cases of rabies in an enzootic area among vaccinated and unvaccinated animals. This may provide conclusive statistical evidence.

**Indirect testing** has the advantages of being simpler and cheaper, and therefore applicable on a larger scale. It may be done by testing serum from vaccinated animals (humoral immunity), or by tests for cell-mediated immunity.

— **Humoral immunity** is tested by titrating the immunoglobulin antibodies in serum, either by their neutralising property or by various other properties. The neutralising property of serum is tested by inoculating mice (the serum neutralisation test on mice, the current reference method) or by inoculating cultures of sensitive cells (plaque reduction test, or rapid extinction of fluorescent foci). In most cases these properties are well correlated with the resistance of vaccinated animals to virulent challenge. They may be expressed in international units if simultaneous titration with WHO reference serum is performed. The other properties may be evaluated by appropriate techniques, such as complement fixation, passive haemagglutination, counter immuno-electrophoresis, enzyme immuno-assay, etc.

— **Cell-mediated immunity** testing ought to supplement the assessment of humoral immunity, but the results achieved so far have proved unsatisfactory because of poor correlation with resistance to challenge.

**NOTE**

Considerable progress has been achieved recently by research workers in Europe concerning the testing of vaccines prepared from inactivated virus.

— **New tests for efficacy** include the measurement of antibodies in vaccinated mice (correlated with their resistance to challenge), the antibody binding test, the enzyme-linked immunosorbent assay (ELISA) and the simple radial immunodiffusion test, all of which have the advantage of being done *in vitro*. Their results agree with those of testing in animals, at least in the case of cell-culture vaccines.

The test of the European Pharmacopoeia employs just one injection of different dilutions of vaccine instead of two, and seems to give better results, because it is more capable of discriminating between vaccines of average antigenicity, something which cannot be done by the NIH test. The international reference preparation enables the titre of inactivated vaccines to be expressed in international units, but only small amounts of the preparation were formerly available (which led to the creation of numerous subsets of the standard), until adequate supplies of the “European Reference Preparation” were made available.

— Moreover, a *simplified test* employing only one dilution of vaccine provides a qualitative result, which can determine whether a vaccine should be submitted to the full testing procedure. This screening test is simpler and less expensive (2).

— Finally, a *peripheral test* is under collaborative investigation by European and
American laboratories. This involves intramuscular challenge of mice with a wild strain of virus (8).

THE USE OF VACCINES

In domestic animals.

Once the problem of the choice of a vaccine and the preparation of sufficient quantity has been resolved, the next question is its most logical application (animal species, number of animals) and its most efficacious use (ratio of quality to cost) in a given European country. At this stage national legislation plays a fundamental role, for the realisation of the objectives outlined at the beginning of this report depends on the preparation of legislation and its acceptance and implementation by the users. European legislation has arrived at two different principles: compulsory vaccination and/or recommended vaccination.

In either case (but particularly the latter case) the proportion of animals vaccinated often depends, in the last resort, on the goodwill of the owners. Such goodwill varies according to the seriousness of the local epidemiological situation, the extent of incentives (or disincentives), the economic effectiveness of vaccination, the quality of public information, etc. Effective legislation should incorporate the following provisions, in descending order of priority:

- **protect disease-free zones** from infection from infected zones (by vaccinating animals in transit),
- **protect the species most dangerous to man** (dogs, cats and cattle),
- **protect animals at high risk** or of considerable economic value (cattle, horses, valuable breeding stock of all species).

The following incentives (or disincentives) are used with variable effect, according to the country:

- **taxation or fines** for not declaring the disease or not vaccinating animals,
- **destruction of unvaccinated animals** which have been exposed to infection,
- **restriction of movement** of unvaccinated animals.

Under the best conditions, these regulatory measures should provide a vaccination rate approaching 80% of the animal population.

However, it is often the animals most at risk which receive the least attention (e.g. guard dogs, farm cats, livestock under extensive husbandry) and which consequently pay the heaviest tribute to rabies. Insurance against rabies (in the case of livestock) might compete with vaccination, although it tends to become uneconomic when the incidence of rabies increases.

In wild animals.

Vaccination of wild animals against rabies by the oral route is currently applied solely to the European red fox. Experience gained during the past twelve years in France, Switzerland, the FRG and later in Belgium and Italy has shown that only live vaccines are effective when administered by means of bait, providing they have a minimum titre of $10^7$ infective units per ml. Innocuity of these vaccines depends on the strain of virus, the strains most frequently used being standard ERA (= SAD),
or SAD selected in the presence of monoclonal antiglycoprotein antibodies (= "SAD B19") (3, 4).

Although potentially pathogenic for certain non-target species (such as rodents), they are incapable of producing "laboratory rabies" transmissible by serial inoculations. Field trials were therefore authorised in 1978 in Switzerland (12), and in 1982 in the FRG (10) and the USSR (7). The results are being followed closely by the WHO and the European Commission with a view to extending their use to other European countries.

As with domestic animals, the aim of these vaccinations is to reduce the incidence of rabies before it reaches man and, if possible, to suppress the source of the virus in wild fauna.

RESULTS

DOMESTIC ANIMALS

The results of vaccination, regardless of the animal species involved, may be assessed by certain complementary criteria:

- **Proportion of animals vaccinated** (the ratio of the number vaccinated to the total population).
- **Rate and degree of serological conversion** among the animal population (= number of serological conversions in a representative sample of the population).
- **Incidence of rabies** among the population studied compared with that of a control population selected on an *ad hoc* basis.

*Note that* the criterion of failure of the rabies front to advance, or failure of the disease to extend to animals living in the region under investigation cannot be applied to the domesticated species. It is known that they cannot excrete the vulpine virus at a rate or in amounts capable of creating secondary foci of rabies, whether they have been vaccinated or not. The results generally obtained for each species of animal, when the national legislation stipulates vaccination, are as follows.

Cattle.

*The proportion of animals vaccinated* depends on the involvement of the owners (epidemic pressure, financial aid from the State, husbandry conditions, etc.), but it can quite easily approach 100% under satisfactory conditions in certain European countries.

*The rate of serological conversions* may also be close to 100% of vaccinated cattle providing they received a good vaccine, particularly after the first annual booster dose. Not until after this booster dose can one be certain that all the animals are protected, for most vaccination failures occur after the primary vaccination (at which the animal may be too young, or still possesses maternal antibody). The use of certain inactivated vaccines containing aluminium hydroxide guarantees an antibody titre higher than 1 IU per ml of serum, and 100% protection during the three years which follow the first booster dose.

*The incidence of rabies* in vaccinated zones compared with unvaccinated zones can be seen in the Table I, which applies to France* (11).

* Reproduced with the kind permission of P. Précausta and J.-P. Soulebot (Rhône-Mérieux IFFA).
TABLE I
Comparison of the incidence of rabies between a vaccinated zone and an unvaccinated zone

<table>
<thead>
<tr>
<th>Status of the animals</th>
<th>Region A</th>
<th>Region B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. of cattle</td>
<td>No. of rabies cases</td>
</tr>
<tr>
<td>Unvaccinated</td>
<td>6 900</td>
<td>30 (4.3%)</td>
</tr>
<tr>
<td>After primary vaccination*</td>
<td>2 300</td>
<td>None</td>
</tr>
<tr>
<td>After at least one booster dose*</td>
<td>No data</td>
<td>30 377</td>
</tr>
</tbody>
</table>

* against rabies

Dogs.

The proportion of animals vaccinated depends particularly on the motivation and awareness of the owners, and unfortunately it is often better in urban areas (low risk areas) than in the rural population (high risk areas). Only exceptionally does the figure exceed 80 % of the population.

The rate of serological conversions among vaccinated dogs may be close to 95 % after the primary vaccination and 100 % after the first annual booster dose, which alone guarantees solid immunity. As in cattle inoculated with the same type of vaccine, a mean titre above 1 IU/ml can be maintained for 3 years after the first booster dose, and the animals can withstand any challenge infection (with vulpine virus) which is capable of killing 70 % of unvaccinated dogs.

The incidence of rabies in different zones cannot be compared, in practice, because the actual proportion of the animals at risk (whether vaccinated or not) is unknown. However, an excellent illustration is provided by the efficacy of a booster dose after exposure to infection. According to an investigation published in France (8), 3,479 out of 3,500 dogs were protected in this way.

Cats.

Values for the three criteria applied above to cattle and dogs are unknown in the case of cats, because of the difficulty of estimating their population (which is rarely declared or identified), and the difficulty of obtaining blood samples from this species, and of evaluating the individual risk of rabies.

The only valid data comes from laboratory experiments, which show that cats in good condition can be immunised as readily as dogs, while it is difficult to immu-
nise cats in a poor state of health (except if adjuvant vaccines are used). Experiments also show that the cat is very resistant (though less resistant than dogs) to vulpine virus, and that cats with fatal rabies, despite vaccination, do not usually re-excrete the infecting virus.

Other species.

There has been insufficient investigation of other species (mainly sheep and horses) to evaluate the results of vaccination. However, it would be appropriate to vaccinate flocks of sheep at high risk (since infection in this species often affects a large number within a flock), and also valuable horses out at grass.

WILD ANIMALS

In three countries which have carried out field trials (Switzerland, the USSR and the Federal Republic of Germany), the criteria for evaluating the results of oral vaccination of foxes against rabies are the same as those used in domestic animals.

The proportion of animals vaccinated can be estimated by labelling the bait (containing the vaccine) with tetracycline, which has shown that under good conditions around 80% of foxes within a zone where the medicated bait is distributed at 15-20 baits per km² are immunised.

The rate of serological conversions is rather less than that mentioned above, and may reach 60% among the animals in the type of vaccination zone specified above.

Comparison of the incidence of rabies in vaccinated and unvaccinated zones is difficult because of seasonal or periodic fluctuations in the epizootic among foxes. Within a suitable period (as in Switzerland in 1978-1981) there is no doubt that the incidence of the disease has been reduced or even suppressed in a zone correctly and regularly vaccinated twice a year.

GENERAL CONCLUSIONS

The medical prophylaxis of rabies is applied in a very variable manner in Europe, both concerning the quality of vaccines and the proportion of the different species of animals vaccinated.

Among domestic animals, every European country practises the preventive vaccination of certain species of animals, sometimes regularly, and sometimes to protect its disease-free territory.

The results of this vaccination have had no demonstrable influence in averting the creation of secondary foci of rabies, but it has proved valuable in:

— reducing the incidence of rabies in each species vaccinated, and
— reducing, in consequence, the number of human beings treated after exposure to infection.

Among wild species, only three countries (the USSR, the Federal Republic of Germany and Switzerland) have attempted to vaccinate foxes in the field, with initial results which are encouraging (no accidents, reduced incidence and reduced extension of rabies).

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REFERENCES
(See p. 246)