Rev. sci. tech. Off. int. Epiz., 1988, 7 (4), 1005-1013.

Vaccinating wild animals against rabies

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Summary: The authors review the current situation regarding vaccines available for immunising foxes and other wild animals, with reference to modified strains of rabies virus and recombinant vaccinia-rabies virus. Field trials conducted in Europe and North America are summarised.

KEYWORDS: Fox - Immunisation - Live vaccines - Oral administration - Rabies - Wild animals.

INTRODUCTION

Rabies is an encephalomyelitis of viral origin which affects homoiothermic animals, including man. Species of domestic animals are just as receptive to infection as wild animals, but they have suffered heavier losses from disease than domestic animals which have benefitted from the protection provided by control measures.

According to the WHO Collaborating Centre for Rabies Surveillance and Research at Tübingen, 40,964 domestic animals died from rabies in Europe during the past decade, while the disease was diagnosed by laboratory testing in 150,894 wild animals. Such testing covers only 10-20% of the animals which have actually died in the wild, but it provides some indication of the impact of the epidemic on wildlife.

The principal vector of the current epidemic of sylvatic rabies in Europe is the red fox (6, 15, 43), but similar situations exist in South Africa (rabies in yellow mongoose), North America (rabies in skunk and raccoon) and Central and South America (rabies in vampire bats). Here the term vector means the animals most susceptible to rabies in a region at a given time, solely responsible for maintaining the infection. All other species are victims, even if they are capable of transmitting rabies. Consequently, their destruction or immunisation has no effect on the disease cycle.

Whereas rabies among domestic animals can be controlled by the appropriate prophylactic measures, it poses a bigger problem in wildlife, and until 1960 the only possible means available was considered to be the reduction of vector populations.

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This has been long recognised as barely effective or even dangerous for the ecological balance (6, 15). For this reason, there have been attempts to interrupt the cycle of sylvatic rabies by immunising vector species.

A method of vaccinating wild animals against rabies was developed first in the USA (78, 9, 32, 89, 90) and then in Europe (1, 16, 56, 66, 84), and used for the first time in the field in October 1978 in Switzerland (75, 76). Since then, the method has been recognised as extremely effective in several countries, and will probably be extended to many others (16, 65). There has been much recent research on technical adjustments, particularly the type of vaccine used (1, 44).

The purpose of this article is to review the current situation in the various countries concerned, and the prospects for further development of the method.

VACCINATION OF WILD ANIMALS IN EUROPE

Vaccination of wild animals against rabies has been developed most extensively in Europe. Since the beginning of the seventies, the World Health Organization (WHO) has supported, organised and coordinated the work of scientific teams involved in studying this method. The scientific meetings organised almost every year by the WHO in Geneva, Frankfurt, Nancy, Tübingen, etc. from 1970 to 1988 provided the turning point for progress in the use of the method in the field (91, 92). Two stages in this development are research in experimental stations and field trials.

RESEARCH

For a long time, most research concentrated on the parenteral or oral administration of vaccines already widely used in domestic animals, particularly strain SAD ("Street Alabama Dufferin") of rabies virus. During recent years there have been remarkable developments in oral immunisation with vaccines derived directly or indirectly from genetic manipulation. These two aspects of research will be treated separately.

Research with rabies vaccines already used in domestic animals

Experiments conducted in Europe have involved essentially the red fox, *Vulpes vulpes*. The two approaches investigated have been parenteral vaccination of captured foxes (58, 77), and oral vaccination.

There have been few attempts to immunise foxes parenterally, after capture within their dens. Field trials were conducted in Switzerland in 1976, and subsequently in the Federal Republic of Germany (74), with inactivated vaccine, but it proved impossible to immunise more than 40% of the population, and attempts were soon abandoned. Furthermore, the cost of this procedure was prohibitive.

Research then focussed on oral vaccination, the only procedure truly applicable in the field. There have been numerous experiments and attempts at immunisation. Procedures varied according to the type of vaccine and the type of bait used to contain the vaccine. This paper concentrates on these experiments and attempts.

Type of vaccine

Some inactivated vaccines have been used (22), but in most cases an attenuated strain has been propagated in cell culture or in the brain of mice. The strains used most often are Standard SAD ("Street Alabama Dufferin") (8, 12, 13), strain SAD B19 (65, 67, 69), a variant of SAD (68), the derived strain ERA (from the names E. Gaynor, Rokitniki & Abelseth), Flury low egg passage (LEP), Flury high egg passage (HEP) or its variant strain "675" (39, 47, 53).

Other strains of virus (thermosensitive strains, mutants of strains GS, CVS and SAD) and recombinant vaccinia-rabies virus will be dealt with later.

Type of bait

In early experiments on immunisation, the vaccine strain was administered either directly into the mouth of the animal, or after incorporation into a bait. Research on the type of bait has become just as important as that on the type of vaccine strain.

Various baits have been tried by different laboratories, including young mice, chicken heads or, more frequently, baits manufactured from fats and proteins, moulded by heat.

The final choice depends on compatibility between bait and vaccine, stability in the environment, ease of handling and storage, attractiveness to the target species and, of course, cost.

Vaccine may be incorporated in the bait directly or, more frequently, within a plastic capsule containing 1-2 ml of fluid vaccine.

The last-named method has been chosen for use in Europe, using chicken heads (69, 76) or a manufactured bait. Only in the USSR has the vaccine been injected directly into chicken heads (72).

Results of the experiments

The results of trials in experimental stations have been evaluated by the titre of rabies antibodies after vaccination and/or challenge infection with virulent rabies virus (17, 79). It is beyond the scope of this article to present detailed results of research conducted during the past ten years, but the general conclusion is that only strain SAD, the derived strain SAD B19 (developed in the Federal Republic of Germany) and perhaps strain Vnukovo 32 (developed in eastern Europe) are capable of protecting 100% of foxes without prior concentration of the cell culture fluid.

The titre of these strains of virus may easily exceed 10^7 ID_{50} per ml of harvested fluid, which is the dose needed to immunise a fox. This is not the case with other strains, such as the Flury strain and its derivatives. In no case has an orally administered inactivated vaccine elicited an immune response capable of protecting an animal (3, 4, 57). Hence there has been a unanimous choice of strain SAD for the initial field trials.

American research (16) has shown that foxes can be immunised with live vaccine by two routes: oropharyngeal (in which case the vaccine virus is recovered solely from the tonsils) and/or intestinal. The oropharyngeal route requires direct contact between the mucosa and the vaccine. Conversely, the intestinal route requires rapid transit

through the upper digestive tract, hence the use of small pills of coated virus. However, the manufacture of such pills is difficult and dangerous (formation of virus aerosol during coating) and for this reason it has been abandoned (5).

The main shortcoming of strain SAD is its lack of complete harmlessness for non-target species which might eat the vaccine (particularly certain members of the families Muridae, Mustelidae and Felidae), which has raised concern among some writers (11, 14, 30, 31, 84).

However, it has proved impossible to passage this virus serially in such species, and it is not excreted by animals which develop vaccinal rabies (2, 75, 85, 86). This strain can be distinguished from wild strains by using monoclonal antibodies (87).

The lack of safety of strain SAD for certain non-target species has resulted in research being undertaken, even after the initial field trials commenced, to find another vaccine that is effective as well as perfectly safe.

THE SEARCH FOR NEW VACCINES

While a recombinant vaccinia-rabies virus now seems to offer an excellent alternative to the attenuated SAD strain of rabies virus, the early research concentrated on obtaining new attenuated strains, and this research is still in progress.

New attenuated strains of rabies virus

New strains which have been tested are GSC, CVS and mutants derived from them (thermosensitive-ts, AVO_1, etc.), also the SAG mutant derived from SAD (Bern). The main problem to be solved is enhancement of innocuity.

Strain GSC was isolated from a rabid fox at the National Rabies Centre at Malzéville (France) and was subsequently propagated in CER or BHK-21 cell lines in the USA and in France. In tests on 30 foxes, its efficacy was similar to that of the SAD and Flury HEP strains, but its innocuity for non-target species has not been examined (17, 18, 15).

The unmodified CVS strain was tried only once by Baer *et al.* (8) in 1963, who administered the virus by stomach tube to five foxes, only one of which formed antibodies and became resistant to challenge infection.

Since then, numerous apathogenic mutants have been obtained from this strain. The first mutant was a thermosensitive (ts) strain which was soon abandoned because it was not immunogenic in the concentrations employed (29). Other mutants have since been obtained (33, 34, 42, 45, 80) in which a single nucleotide in the glycoprotein gene was substituted, resulting in the replacement of arginine at position 333 by a leucine, isoleucine, glutamine, glycine or serine residue (34, 35, 38, 43, 71). All mutations based on arginine 333 substitution rendered the virus apathogenic for mice, foxes, dogs and six species of wild rodents (62).

Unfortunately, administration of mutants such as AVO_1 has failed to protect sufficient numbers of animals. Consequently, their use could not be considered.

Since then, similar mutants (SAG) have been obtained from the Bern SAD strain, and seem to give more promising results (45, 55).

Recombinant vaccinia-rabies virus

The problem of innocuity of ordinary attenuated vaccine strains of rabies virus could be solved by obtaining apathogenic mutants, although unfortunately all such mutants showed poor efficacy until 1987. A recombinant vaccinia-rabies virus (VVTGgRAB) has been tested recently in an attempt to find a vaccine which is both perfectly safe and highly effective. A gene coding for the glycoprotein of strain ERA of rabies virus has been inserted into vaccinia virus. In cultures of Vero cells, the virus reaches a titre of 10^8 $CCID_{50}$ per ml. Insertion of a rabies virus glycoprotein gene which codes for the thymidine kinase (TK) of vaccinia virus (52) confers the recombinant with the phenotype TK^-. Buller *et al.* (28) have shown that recombinant TK^- viruses, obtained by inserting coding sequences into the TK gene of attenuated strains of vaccinia virus, are perfectly safe (49).

The efficacy of this recombinant virus has been demonstrated in both adult and young foxes (20, 21, 27).

Oral, intradermal or subcutaneous administration of VVTGgRAB elicits high titres of neutralising antibody and confers long-lasting protection against challenge infection. It disappears from the tonsils 48 hours after oral administration (79) and cannot then be transmitted from a vaccinated to an unvaccinated animal. Administration during the incubation period of rabies does not lead to the asymptomatic carrier state (26).

Its innocuity has been tested in foxes and in numerous other domestic and wild species, such as sheep (73), cattle, dog, cat, mouse, rabbit, ferret (*Mustela furo*), wild boar (*Sus scrofa*), badger (*Meles meles*), carrion crow (*Corvus corone*), magpie (*Pica pica*), jay (*Garrulus glandarius*), buzzard (*Buteo buteo*), kestrel (*Falco tinnunculus*), wood mouse (*Apodemus sylvaticus*), yellow-necked mouse (*Apodemus flavicollis*), common vole (*Microtus arvalis*), field vole (*Microtus agrestis*), water vole (*Arvicola terrestris*) and bank vole (*Clethrionomys glareolus*) (24, 25).

The results obtained have confirmed the complete harmlessness of this virus for the species tested, and there is no evidence of transmission of virus from a vaccinated animal to an unvaccinated animal in contact. Other similar recombinants have also been prepared (40).

APPLICATIONS IN THE FIELD

Attenuated strains of rabies virus

Following selection, firstly in the laboratory and then in experimental stations, the least dangerous and most effective live virus vaccines were used for field trials in Europe.

The unpatented Standard SAD strain produced by the Virology Laboratory of Bern University (Switzerland) has been used from 1978 until now throughout the infected areas of Switzerland, and also in certain regions of the Federal Republic of Germany (before 1985), Italy (in 1986) and France (in 1987). About one million doses have been distributed within chicken heads, and no accident has been reported, other than the accidental death of a fox cub, a cat and a stone-marten (Wandeler, personal communication). Baits were distributed at a density of 15-20 per km^2 (75)

with progressive and very satisfactory efficacy, for less than 90 rabies cases were reported in Switzerland during 1987, compared with 1,190 at the start of the operations (according to *Rabies Bulletin Europe*).

Strain SAD B19 was first used in 1985 in the Federal Republic of Germany, where it was patented. Austria, Belgium, France, Italy and Luxemburg began to use it in 1986 (23, 46, 49, 50). No accident has been reported after distribution of more than six million baits prepared at Tübingen (baits of meat meal and fat meal made by a process patented in 1987). According to its promoters, the combined efficacy of virus SAD B19 and the particular bait, is slightly greater than that of the chicken-head system (69). Results were judged by the rate of taking of baits and serological conversion in foxes captured within baited areas. By incorporating tetracycline into the bait, the rate of uptake can be assessed from the number of foxes with tetracycline in their bones in vaccinated areas, and it has ranged from 70 to 80%, depending on the type of bait. This rate is usually slightly higher than that of serological conversion.

Another strain, SAD Va, was prepared by using monoclonal antibodies to select avirulent mutants (68), but it has never been used in the field.

The spectacular disappearance of fox rabies from treated areas, such as those in Bavaria, Schleswig-Holstein, Hesse, northern Italy, Luxemburg and certains parts of Belgium and France shows that the results achieved are at least as good as those obtained in Switzerland (59, 65, 86). For this reason, the combination of strain SAD B19 and Tübingen bait, which has the advantage of being produced on an industrial scale – over 40,000 baits a day, stored in frozen form – has met with considerable success in most European countries since 1986.

This system may also be suitable for controlling rabies among raccoon-dogs (*Nyctereutes procyonoides*) in eastern and northern Europe (72). Several thousands of doses of SAG vaccine were used in the field in Switzerland in 1988.

Recombinant vaccinia-rabies virus

Because of its efficacy, complete innocuity from the rabies aspect, and stability, this recombinant virus would seem to offer an excellent alternative to the attenuated strains of rabies virus currently used in the field. The first field trial of vaccination of foxes commenced in Belgium in October 1987 (60, 61). This trial is being conducted in accordance with the WHO recommendations.

The trial is using 600 hectares in a military ground which totals 2,700 hectares. The vaccine is issued in liquid form at a titre of 10^8 $CCID_{50}$ in 1.5 ml. It is packed in hermetically sealed capsules made from aluminium and plastic (provided by Professor Wandeler of Bern University).

Bait of the Swiss type consists of chicken heads each injected with 150 mg tetracycline as a marker of uptake. A total of 250 baits were distributed at 40-50 per km^2. The sites were marked and examined for uptake after 4, 8 and 15 days. After 15 days, 64% of the baits had been consumed, mostly by foxes, but also by some other species (wild boar, mustelids, etc.).

This preliminary trial has confirmed the innocuity of the procedure, but it has not been possible to assess efficacy because of the small area treated.

Another trial is planned in Belgium in October 1988 over a larger area (436 km^2) in order to provide conditions suitable for assessing efficacy.

VACCINATION OF WILD ANIMALS IN NORTH AMERICA

The most important research along with application has taken place in the USA and Canada. In other countries, research still covers limited subjects (5), or techniques developed in Europe and North America are being utilised (51).

RESEARCH

Studies of existing rabies vaccines

Vaccines

Before attempting oral vaccination, certain American teams tried to vaccinate foxes by a trap system which would "explode" into the mouth of the animal, introducing vaccine into its organism. It was not very effective (protecting about half of the animals vaccinated) and was soon abandoned.

Research then turned to the use of virus strains SAD or ERA propagated either in pig kidney cells (the "ERA/PK" of Canadian laboratories) or hamster cells ("ERA/BHK-21" or "Wirab").

Baits

North American workers displayed a wide imagination in the selection of baits, for minced meat, smoked sausage, biscuits, eggs, chicks, young mice were all tried (36, 37, 89), but none was developed further than the experimental station stage. The prototype used in the field from 1986 was developed in Canada. It is a polyurethane sponge measuring 36 cm^3, soaked in 14 ml of vaccine and then coated with a layer of wax containing chicken meat extract. Sometimes this bait was placed in a plastic bag containing another attractant substance (liver, minced meat). This failed because the vaccine in the sponge rapidly became contaminated.

Another bait at present being studied is a capsule of vaccine coated with a mixture of wax and meat extract (82).

Results obtained

Initial trials with the CVS and Flury LEP strains, given orally to foxes (*Vulpes fulva*), failed to give conclusive results in the USA (5), and the same applied to a Canadian trial of inactivated vaccine (54). These strains were abandoned in favour of strains SAD and ERA, which gave good results under experimental station conditions, whatever bait was used (13, 54). However, results obtained in the field were not so good (see below).

Research on new vaccines

Experiments on the vectors of North America with the standard attenuated strains of rabies virus have given relatively poor results (5). Certain strains were pathogenic for certain species, such as the striped skunk (*Mephitis mephitis*) (Charlton *et al.*, personal communication), or they were ineffective in certain target species, such as the raccoon (*Procyon lotor*) (63). The research effort then turned to alternative

solutions, such as the use of a carrier virus. Work is in progress on several viruses, including a raccoon poxvirus (41, 78).

As in Europe, however, the first candidate to actually emerge is the recombinant vaccinia-rabies virus, which has proved to be very effective and quite harmless for raccoons (*Procyon lotor*) (88, 63) and striped skunks (*Mephitis mephitis*) (81), the two principal vectors of North America. So far it has also proved to be quite safe for both target species and numerous other non-target animals. The failures associated with attenuated strains under American conditions, and the initial, extremely encouraging results obtained with recombinant vaccinia-rabies virus, indicate that the latter is a serious candidate for field trials.

APPLICATIONS IN THE FIELD

Application of established vaccines

So far only the Canadians have distributed vaccine in the field, after thorough testing of the acceptability of "blank" baits containing only a marker (48). The first trial covered 500 km² of cultivated land near Lake Huron in September 1985. Strain ERA in a sponge bait (see above) was provided by Connaught Laboratories. The trial was repeated in 1986, but it was judged disappointing because only 45% of foxes which had consumed bait developed a detectable serological response. In 1987 the vaccine was distributed in wax-coated capsules, but the rate of serological conversion again remained inexplicably low (32%) in comparison with results obtained in the laboratory, and has led to further research on presentation of the vaccine (82).

There has been no field trial of this type in the USA so far.

Application of new vaccines

Only ERA virus vaccine has been used on the North American continent to date, but many further trials are projected. Requests have been submitted to the competent authorities in the USA to proceed with field trials using recombinant vaccinia-rabies virus. The projects may take place on certain isolated islands along the coastline of the continent (64). If authorisation is obtained, the first trials will be conducted in 1989.

CONCLUSIONS

Since 1960 there has been much lengthy, thorough and costly research in Europe and North America into vaccination of wild animals under laboratory and experimental station conditions. This clearly indicates the extent of scientific interest of such research and also the high stakes involved. In fact it has become a model for testing a new procedure midway between ecology and animal pathology. Should a free-living population be vaccinated rather than destroyed?

Virologists have already given a clear reply to this question: yes, in practice, certain wild species can be successfully vaccinated by the oral route against rabies in the field, provided sufficient quantities of an appropriate live virus vaccine are used.

It seems that mankind is entering into a new relationship with the environment.

The future will be judge of the efficiency and timeliness of this new approach which may well interfere with the natural mechanisms regulating major epidemics (5, 16).

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REFERENCES

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