Rabies.
New developments in vaccination*

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Summary: Among recent developments with respect to vaccination of domestic animals, the following are noted: mastering of the production of cell culture vaccines, mastering of the antigenic value of the vaccines, increase and duration of the humoral reaction, antigenic associations, identification of the antigenic factors of the virus, studies on the level and duration of immunity.

Vaccination of wild animals remains at the stage of research and does not appear to be recommendable with current knowledge, although important progress has been made: pilot tests in the field, study of new modified virus-vaccines and presentation procedures, vaccination trials using inactivated virus.

Advances made in the control of inactivated virus vaccines (suggested new potency tests, modification or adaptation of the N.I.H. test, harmonisation of reference preparations) and in the assessment of immunity (in particular the ELISA test) are reviewed.

Finally, the techniques among which a choice could be made, concerning the production of vaccines for domestic animals, the methods for their vaccination and the desirable controls to be carried out in both fields, are discussed.

Despite the fact that rabies is one of the oldest diseases known to Man and the rabies vaccine one of the first to be discovered, research in vaccination against this disease has continued practically uninterrupted for a century. In recent years this research has fostered considerable technical progress and has posed new problems.

Recent development will be studied in this report, with regard to preventive rabies vaccination of domestic or wild animals, emphasising the positive elements brought about by these developments and exposing the problems, whether old or new, which remain to be solved.


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I. — REMINDERS ON RABIES VACCINATION

« Vaccination against rabies consists of the administration into the organism of a rabies antigen capable of conferring subsequent resistance towards this disease. »

Using such a definition as a basis, one may recall a few points which might involve variations and, sometimes, considerable confusion.

1. The administration of the rabies antigen may be performed using different routes, parenteral or not.

The subcutaneous route is presently the most commonly used parenteral route, despite the fact that it has been proved that when using vaccines without adjuvants, the intramuscular route is preferable and possibly the intradermal route as well. The oral route may only be used if the antigen penetrates to the buccal or intestinal mucous level; which, to date, has only been successful when using modified virus vaccines.

2. The administration of the rabies antigen may be performed at different stages, either pre or post exposure. In the latter case, which is rather exceptional, it is called « curative vaccination » or « post-exposure treatment » and is strictly reserved to Man, therefore out of our context. However, it often gives rise to undesirable confusion, i.e. :

— the booster carried out on the exposed animal (within the framework of certain regulations) to which protective measures are applied : this injection is only a safety antigenic booster and not treatment, therefore can only apply to one subject (dog or herbivore) which has been previously vaccinated;

— requirements for vaccinal antigen : the requirements differ according to whether a curative vaccine is used and injected at short intervals (as for Man) therefore of a very high antigenic value (concentration) and highly purified or whether a preventive vaccine is used whereby the animal is injected once a year at the most.

3. The rabies antigen used should be prepared so as to produce an immune reaction capable to ensuring resistance to the challenge. The use of modified virus vaccines is the oldest and remains the most efficient method. The in vivo virion multiplication of this virus ensures the production of all the antigenic factors of the virus vaccine.

Inactivated virus vaccines need to be prepared in a way that protects the antigenic structures of the virus, i.e. its glycoprotein in particular. In both cases the choice of vaccinal strains is of great importance due to the genetically coded differences which can occur from country to country. This concept, which is not new, is now again being used due to the recent application of the analysis by monoclonal antibodies to the rabies virus (see infra).
It is important to note that, despite this possible genetic variability, vaccine strains presently recommended by the W.H.O. confer in practice and as a general rule, excellent protection of vaccinated animals (4).

4. Resistance to the challenge may be measured by experimental exposure using a fixed or, better, a field strain. This exposure should preferably involve the « target » species for vaccination, despite the fact that in certain cases (animal species of great value) it can only be used on laboratory animals but in these cases, resistance may also be evaluated with regard to the humoral immunity; the cell-mediated immunity test having given disappointing results (see infra).

5. « Vaccinal rabies » may be brought about by using insufficiently attenuated modified viruses, on the vaccinated species or poorly inactivated viruses. Furthermore, the use of perfectly inactivated viruses may induce « early mortality » among vaccinated animals by an immuno-pathological phenomenon of cellular lesions. Active research work is still being undertaken in this respect (38). However, this phenomenon does not play an important role in preventive vaccination of the animal.

II. — VACCINATION OF DOMESTIC ANIMALS

Several points on preventive vaccination of domestic animals against rabies will be taken into account:

— Rabies vaccines currently available : nature, characteristics;
— Rabies vaccination : regulations, requirements, techniques;
— Recent developments in rabies vaccines or vaccination.

A. RABIES VACCINES

It is now more than a century ago that Victor Galtier, a veterinary surgeon (25 August 1879), then Louis Pasteur (25 February 1884) disclosed results obtained on the preventive vaccination of animals against rabies. These initial vaccinations were successful, in the first case by using a « specific route » (vaccination of sheep by the i.v. route) and, in the second, by modifying the virus by successive passages in monkeys (vaccination of dogs). The major steps which followed this primary work was the vaccination with a strain modified (« fixed ») by serial passage in rabbits, the virulence being attenuated by drying and autolysis; it was applied by Pasteur with success to the curative vaccination of Man (29).

Then, in 1911, Semple’s demonstration that a virus vaccine produced in vivo and totally inactivated by phenol (37) could remain efficient. It was only thirty years later that rabies viruses adapted to in ovo culture could be used (23), or twenty years later that in vitro on cells were used and the proposal made to use the now modified virus as the vaccine for certain animal species, whether these viruses were inactivated or not.
From all these vaccines, only the most efficient and the least dangerous exist today (the list is regularly updated by specialists in the World Health Organization (see: Appendix 1, updated on 10.3.1980). Here, we shall only recall the principal types:

1. Modified non-inactivated virus vaccines.

Two strains of modified viruses (or their derivatives) are generally used throughout the world: Flury and S.A.D. (the Kissling and Kelev strains being scarcely used outside their countries of origin).

a) Vaccines produced from the Flury strain.

This strain was isolated in 1939 from the young Miss Flury who had been infected by a dog. The strain was adapted to the embryonated egg and was proposed as a virus vaccine after 50 more passages (23), named Flury Low Egg Passage (L.E.P.); after 130 passages (24), it was named Flury High Egg Passage (H.E.P.).

This strain is currently used as a modified virus vaccine in numerous countries after replication in eggs or in cell culture. (The L.E.P. strain has assisted in controlling rabies in many countries, notably Japan.) Despite the fact that accidents, in the past or recently (32), have limited or even forbidden the use of this strain in certain conditions, it still remains very useful when correctly produced, conserved and applied as it is economical to produce and confers good immunity.

b) Vaccines produced from the S.A.D. strain.

This strain was isolated in 1935 from a dog which had died from street rabies in Alabama (hence its name Street Alabama Dufferin) and was subjected to a series of passages in mice, in hamster kidney cells, embryonated eggs, pig kidney cells, etc.

It is currently used in a large number of countries as a vaccine after culture in hamster, dog, bovine, pig kidney cells. When it was adapted to the latter system by the Connaught laboratories in Canada (1) it was rechristened E.R.A. (for E. Gaynor, Rokitniki and Abelseth) and during the selection of one of its temperature sensitive mutants in the U.S.S.R. (36) it was called « Vnukovo 32 » (from the name of the Russian airport and the optimal temperature for the replication of the clone).

c) General characteristics of non-inactivated virus vaccines.

The two main characteristics of a vaccine may be defined as follows for non-inactivated rabies vaccines*:

* It is most important to note that certain vaccines called « with virus inactivated by phenol » still retain residual virulence (indispensable for potency) which should put them in the non-inactivated virus vaccine group. These vaccines which are still fairly widespread throughout the world, are, by definition, of very variable or even uncontrollable safety and potency and called « Fermi type vaccines ».
— their safety depends on the genetic stability of the strain, but also on the way they are applied (vaccine dose, age, sex or immunity status of the subject vaccinated...);

— their potency (the antigenic value and duration of immunity conferred) may vary according to the nature, antigenic structure and level of replication of the strain in vivo (importance of the virus titre inoculated). The immunity conferred can be up to 2-3 years in good conditions. This type of vaccine is freeze-dried and, generally, disassociated from other antigens and adjuvant is not added. It is injected in the same dose, regardless of the species of animal.

2. Inactivated virus vaccines.

This type of vaccine allows for much more diversified production than that of the non-inactivated virus vaccines. It permits the use of a much greater number of vaccinal strains, as well as various substrates for in vivo or in vitro viral replication.

In fact, as the attenuation in pathogenicity of the strain for the « target » species is no longer necessary, strains and substrates may vary from one laboratory to another.

However, one can still distinguish two large groups amongst these vaccines (for greater detail see Appendix 1).

a) Vaccines for which the virus is replicated in vivo.

This type of vaccine remains very widespread due to the relative facility to produce the virus. The latter may be obtained by inoculating adult or young animals:

— adult animals: sheep, goats, rabbits, rats, mice are the most commonly used to obtain a substantial harvest of encephalons, but the viral titre is low;

— young or new-born animals: kids, young rabbits, young rats and young mice are most often used to obtain a viral titre 10-100 times higher than that obtained in the adult animal and to produce a vaccine without the « neuro-allergenic » sensitising factor.

b) Vaccines for which the virus is replicated in vitro.

Quite a large variety of this type of vaccine (see Appendix 1) exists, according to the cell substrate used for the replication of the virus or the strain:

— Baby hamster kidney cells or diploid cell lines are most often used, but also chicken fibroblasts, kidney cells of dogs, pigs, etc.

— Strains: with the exception of the Flury strain, most are derived from the « Pasteur » strain. The latter was isolated on 19 November 1982 from a bovine and since, has been subjected to very varied passages in various animal species or cells and called « Challenge Virus Standard » (C.V.S.); « Pitman-Moore » (P.M.), « Pasteur Virus 11th passage » (P.V. 11), « Kiss-
ling », etc. Although the W.H.O. recommends the use of a single fixed rabies virus strain for the production of vaccines, some manufacturers (in Latin America) use a mixture of three strains, two of which were isolated from bats. The possible advantages of this mixture will be discussed later.

c) General characteristics of the inactivated virus vaccines.

As before, two main characteristics are to be noted:

— Safety of the vaccine is almost complete when the vaccine is correctly produced, i.e. « vaccinal rabies » cannot exist with this type of product. However, true inactivation must be checked by reliable and sensitive tests. Sensitivity can be increased by adjusting virus concentrations or by using highly sensitive methods (cells + DEAE for example).

Accidents due to sensitisation (hypersensitivity of a retarded or anaphylactic nature) are rarely observed as vaccinations are widely spaced apart.

— Potency i.e. the level and duration of immunity conferred, mainly depending on the antigenic structure of the strain, the method of growing the virus, the inactivating agent and the final antigenic value, which should not be lower than 0.3 I.U. per dose (32) but not necessarily too high (8).

The duration of immunity conferred may, as with non-inactivated vaccines, be two to three years in good conditions (41). The vaccine may be in the freeze-dried form (stable for at least 18 months) or liquid (stable for at least 12 months). The addition of adjuvants is often practised for these types of vaccine, particularly in those obtained in cell culture, mainly using aluminium hydroxide (dogs, horses) or saponin (cattle). These adjuvants increase the level and duration of the humoral response of the target species.

The association with other antigens is also possible and is becoming common practice especially in dogs (leptospirosis, canine distemper), cats (panleucopaenia) or cattle (foot-and-mouth disease).

B. RABIES VACCINATION

Rabies vaccination as such must only be carried out under certain specific conditions, most of which are covered by national or international regulations or recommendations. We will account for them as follows:

1. Regulations for rabies vaccination.

In most cases vaccination is carried out according to the general recommendations of the international organizations responsible for co-ordinating rabies control (the World Health Organization and the Office International des Epizooties, in particular) and in application of regulations drawn up at national level.

These general recommendations are regularly updated to take into account the development of rabies throughout the world and the results of research in different fields (epizootiology or vaccine production and testing technology,
for example). They have already been listed (31) or will be in the course of this Session.

Specific regulations for each country are laid down by national authorities and are not therefore covered by this report. However, the general characteristics of suitable regulations with respect to rabies vaccination are, in our opinion, as follows:

— They must ensure the usefulness of vaccination within the country by carrying out a comprehensive cost-benefit study and co-ordinating with essential related quarantine and slaughter measures.
— They must ensure the safety and potency of the vaccines, i.e., they must provide for the best vaccine for local conditions (within a given price range) to be selected, supervised and applied under suitable conditions (see paragraph B.2.).
— Finally, they must ensure that the prescribed vaccination is actually performed, by foreseeing appropriate incentives or constraints, failing which the entire range of control measures run the risk of being ineffective.

2. Rabies vaccination requirements.

The general conditions under which individual animals of different species must be vaccinated are as follows:

Species: Each species must receive the vaccination which suits it best. The manufacturer states the indications and counter-indications concerning his vaccine, and these can be very important (for example, cats must not be vaccinated with Flury L.E.P. or E.R.A. vaccine, not all adjuvants can be used with all species, etc.).

Individual animals: The individual reaction of the vaccinated animal depends on a series of very important factors, including:

— The age of the animal. With most species, animals less than three months old are difficult or impossible to immunize because of the presence of maternal antibodies (32, 41). If the vaccination of these young animals cannot be avoided (in the course of « mass vaccination » for example), it is essential that they quickly receive a second vaccination as a safeguard, even if it is accepted that they may be protected without detectable antibodies.

— The animal’s environment. This term can be used to cover all the factors likely to modify the animal’s reaction to the vaccination, whether such factors be of genetic origin (as is the case with certain dogs that, congenitally, have a low or no humoral reaction to the injection of rabies antigen), of pathological origin (intercurrent diseases* which modify the immune reaction) or of therapeutic origin (immunodepressant treatment), etc.

* We naturally exclude the case of animals infected with rabies for which all « treatment » is forbidden. A booster injection only is authorized, in the case of an animal whose vaccination is still valid at the time of its becoming infected.
However, the importance of these factors should not be overestimated, especially with respect to treatment by certain corticoids which seem to have no effect on rabies vaccination (11). It has also never been proved that vaccinated animals can re-excrete vaccinal virus or a field strain of virus which might have infected them (32).

3. Vaccination techniques.

Vaccination of domestic animals is traditionally carried out by the parenteral route although the oral route (the only one possible in the case of wild animals) has been suggested for stray dogs and could perhaps be used in the future once the method has been perfected (6). The injection could be carried out in three ways:

— **intramuscular injection**: this gives an excellent immune reaction, superior to that obtained by the subcutaneous route (in the case of vaccines that do not contain any adjuvants) and an even clearer one inasmuch as the vaccine has a lower antigenic value (40). It is compulsory for modified virus vaccines;

— **subcutaneous injection**: although this does not give such a good reaction as the preceding route in the case of vaccines using adjuvants, it is less painful which means that it is chosen in the majority of cases;

— **intradermic injection**: this route, which is recommended for Man, gives a humoral reaction, with reduced doses of vaccine, that is more or less equal to that obtained with a full dose (28). Tests are underway to discover exactly what happens with dogs, where the phenomenon has already been noted (30). This technique could make it possible to economise vaccine, as long as it was injected with a system similar to the «Dermo-jet» system, without using a needle, which is the only way to ensure that the vaccine actually penetrates the derm (see paper presented by Professor B. Toma *et al.* during this Session).

C. NEW DEVELOPMENTS

Recent developments with respect to rabies vaccination of domestic animals have necessarily been limited in view of the considerable technical advance obtained previously in the study of the rabies virus, about which Professor P. Lépine wrote in 1975: « that it was probably the best known of all viruses ».

In our opinion, the most important progress made recently, which has or might have important practical implications, concerns the following:

— **Mastering of the production of cell culture vaccines**: The general technical progress achieved in the field of cell culture (high capacity fermentor, «micro-carriers», automatisation, etc.) has given excellent yields, both with respect to quantity and quality. The perspective of being able to use the *Escherichia coli* bacteria as the productive cell of the rabies antigen
(after forced introduction of the viral genetic message in the bacterial genome) has even existed since 1980 (33).

— **Mastering of the antigenic value of the vaccines** : This problem has been almost completely mastered thanks to the combining of three techniques:

- **Concentration** of the viral antigen by zonal ultrafiltration or ultracentrifugation.
- **Purification** of the harvest, leading to a reduction in the level of non-viral proteic nitrogen, a component of no use, and to a consequent increase in the quantity of specific viral antigen.
- **Precise titration** of the vaccinating antigen by passive haemagglutination, measurement of the binding to specific antibodies, etc.

The simultaneous use of these three methods allows precisely the most appropriate quantity of purified antigen to be yielded. However, in spite of the degree of purification achieved, few industrially produced vaccines use the isolated viral glycoprotein or chemically defined antigenic « sub-units ».

— **Increase and duration of the humoral reaction** : This was obtained by increasing the quantity and quality of the harvested antigen, in association with addition of immunising adjuvants. The latter is essential in the case of inactivated virus vaccines for use on ruminants, equidae and pigs. But progress remains slow in this field, since the actual effect of these adjuvants can only be measured on the « target » species for the vaccine, and not on laboratory animals in the course of long term experiments. Nevertheless, experiments have now demonstrated, with a virulent challenge, the persistence over two to three years (after the first annual booster or even after a single injection of primo-vaccination) of solid immunity in cattle (41), dogs (18, 39) and cats (42) that have received an inactivated virus vaccine with added adjuvants. These findings are in the course of being confirmed on animals of various origins in practical conditions.

— **Antigenic associations** : The obtention of inactivated virus rabies vaccines, concentrated and purified, made it possible to associate them with other viral antigens, and thus to carry out simultaneous vaccination against various diseases of domestic species. This association was obtained without reducing the efficacy of the rabies humoral response. The development of these « associated », vaccines led to the rabies vaccine being presented in liquid (instead of freeze-dried) form which reduced its period of conservation, although this is longer than that of modified virus-vaccines (32).

— **Identification of the antigenic factors of the virus** : It was proved a long time ago that, although the « classic » rabies virus appeared to have a single antigenic structure whatever its origin, certain African strains belonging to the « rabies » group of rhabdoviruses were exceptions to this common « 1 » serotype and were therefore grouped together in the « 2 » (Lagos Bat), « 3 » (Ibadan, Mokola), « 4 » (Duvenhague) or other (Kotonkan, Obo-dhiang...) serotypes.
These distinctions were based on cross-neutralisation or cross-protection tests, indicating a variability in the viral glycoprotein. The work carried out recently by T.J. Wiktor* (48) and the laboratories involved in his study, confirmed, listed and extended these differences to many other strains, as well as allowing the discovery of an even greater number of variations concerning the structure of the nucleocapsid.

This latest discovery has now made it possible to tell one strain from another thanks to their nucleocapsid «marker» and to give each one of them an identity card which is very useful for the vaccine producing laboratories (Appendix 2).

Nevertheless, at the present stage of research, it is not yet possible to determine whether current vaccines give protection against all the existing rabies strains. The existence of natural (48) or artificial (47) variants and the absence of cross-protection in mice leads to the belief that this is not always the case, especially against certain African viruses or viruses isolated from bats (35). This fact would justify, with hindsight, the addition of native strains to the fixed strain in certain South American vaccines (see supra) or, better still, research to find a vaccinal strain with a large antigenic spectrum adapted to each user country.

With respect to Europe it would appear that the vaccines currently used, for the most part derived from the French strain isolated by L. Pasteur, offer perfect protection against the field virus of the present vulpine rabies enzootic (11, 35).

— Study concerning the level and duration of immunity: Several studies are underway concerning the actual duration of the immunity conferred by the new inactivated virus vaccines, which appears to be greater than was expected, especially when adjuvants are added to these vaccines. Studies are also being carried out with respect to the efficacy of the intramuscular, intradermic and oral routes in the administration of vaccine with a view to increasing the efficacy of the vaccination or to facilitate it.

III. — VACCINATION OF WILD ANIMALS

Although it has been planned for twenty years, the vaccination of wild animals is still at the research stage for the time being. It poses a totally different problem to that of the vaccination of domestic animals, and we will examine this point before relating the new developments in this field.

* This work is based on the production and use of monoclonal antibodies. These are produced in vitro by the fusion of a lymphocyte of a mouse vaccinated against rabies and a cancerous cell (mouse myeloma). The resulting hybridomes are cloned and multiplied: each clone produces therefore perfectly monospecific antibodies with a single antigenic factor.
A. THE GENERAL PROBLEM

Rabies vaccination is one of the possible weapons in the control of the enzootic disease, when it is proved that it is in fact authentic wildlife rabies.

But there exists a gap which is difficult to breach between the possibility of using this weapon and the practical problems related to its use in the field.

This is because such a vaccination, which had been contemplated mainly in the case of vulpine rabies, poses a multitude of questions of which the main ones are as follows:

1. **Is it worth vaccinating wild animals?**

   This first question is already the subject of an important controversy. It has been answered by a certain number of favourable arguments:

   — Sanitary control measures can only be effective under well-defined conditions (34) that it is not always possible to meet for financial (cost), technical (inadequate geographical areas), biological (« leak » of wild vectors) or psychological reasons (fear of a genocide which would disturb the natural balance). The alternative, or the addition of vaccination would partially solve this problem.

   — « A vaccinated fox is better than a dead fox », it has been said, since an immunized population would appear to be better equipped to halt the epizootic than a sparse population that is still susceptible.

   — Vaccination avoids the unbalancing effect of the possible total destruction of the vector, which would favour a compensatory multiplication of its competitors for food or of its prey.

   But this same question is sometimes answered by the following unfavourable arguments:

   — Vaccination can only be effective under well-defined conditions (9), which are just as difficult to meet for the same financial (cost), technical and biological (see chapter A.2.) and psychological reasons (fear of increased multiplication of the vectors).

   — A poorly vaccinated population would only constitute a partial brake on the epizootic, which it could transform into a permanent enzootic.

   — Vaccinating the vector, like destroying it, could create an imbalance whose immediate advantages could hide long-term disadvantages, especially for « non target » species (see infra), or whose effects could rule out a possible genetic selection of naturally resistant animals.

   In the absence of experimental evidence in either case, it is difficult to take a stand. The only certitude is that the supporters of one or the other solution recognize that the two control systems should be complementary and therefore applied simultaneously, which would appear to be both costly and, in a certain respect, illogical and contradictory.
2. What are the possible vaccination techniques?

It is possible to vaccinate a wild animal in two ways: by parenteral or oral route.

The **parenteral route** was tried in 1976 in Switzerland by capturing and vaccinating fox cubs, and in the United States in 1961 and 1968 by « self-vaccination » of adult foxes either with an inactivated virus hidden in the bait which « exploded » in their mouth, or by automatic injection of the vaccine while the animal was caught in a trap: these trials were abandoned in view of their cost and the poor results obtained.

The **oral route**, which meant that the vaccine could be distributed *ad libitum* throughout the countryside, appeared to be more effective and gave rise to more than fifty publications in the United States (49, 50), in Canada and in Europe, which have recently been reviewed (6, 9, 34). In practice, these tests used only non-inactivated modified viruses (mainly S.A.D. and Flury strains) administered directly or in various baits.

They enabled the following conclusions to be drawn:
— that it was possible to solidly immunize the fox by this route, the virus generally penetrating through the buccal mucosa, except in the case of « enteric » capsules passing through the stomach layer;
— that existing vaccinal strains were not dangerous for the fox but that they could be for other species (stoat, weasel, ferret, hedgehog, vole, field mouse, musk rat...) likely to ingest the virus spread around the countryside.

It is this absence of innocuity on the part of the virus-vaccines and the fact that they are accessible to other « non target » species, contrary to parenteral injections of the same strains to domestic animals, which led the W.H.O. to recommend the « greatest care » in possible field tests (32). Taking such caution is all the more necessary in countries where more than one wildlife species may be a rabies vector.

3. What are the possible vaccination procedures?

The vaccine to be administered by the oral route, protected by a stabiliser and enclosed in a plastic envelope or in pill form, can be placed in many different types of bait that have been tested to check their palatability. The use of chicken heads and meat balls seems to have given the best results (25). After examination of the incorporation of coloured indicators (tetracycline, rhodamine) in the bones or the teeth of foxes living in the experimental zones, it would appear that 60% of these animals would ingest this bait if 20 to 80 were distributed per square kilometer. This could be carried out by plane (Canada) or by manual distribution (Switzerland: see *infra*).

In reply to these principal questions, it would therefore appear possible to say that, technically, rabies vaccination of wild animals is possible. The only problem yet to be solved and which creates a dilemma is that of the safety of this vaccination:
— either the risk is taken to use these vaccines, which could replace a known danger (a wildlife rabies epizootic) by an unknown danger (« laboratory rabies » in other species);
— or the decision is taken to wait until a new, completely safe vaccine or vaccination method is found.

Recent developments in this field give some hope.

B. NEW DEVELOPMENTS

Several new routes have been found recently with respect to the vaccination of wild animals. Although they do not yet provide a final solution to the problem, the experimental information obtained constitutes an appreciable step forward.

1. Direct tests in the field

have been carried out in Switzerland, in an effort to immunize foxes with the S.A.D. strain (43). 21,219 doses of vaccine enclosed in plastic sachets and chicken heads were distributed from 1978 to 1980 in the Valais region, at a rate of 12-20 per square kilometer. Examination of foxes killed in this region (indicated by means of the tetracycline in the bait), indicates that 60% of them appear to have eaten these baits and that the rabies epizootic has not spread within the treated area, whereas it did spread in the surrounding areas.

Trial vaccination was continued in 1981 and extended to neighbouring areas.

Simultaneous control of the possible spread of the vaccinal strain within the treated area gave a negative result which does not, of course, exclude the possible long-term danger of these tests.

2. New modified virus strains.

Several new modified virus strains are under study in vitro and in vivo which could possibly provide vaccines that are less pathogenic than the S.A.D. or Flury strains for « non target » species. These are cloned strains « H.E.P. - 675 »* whose pathogenicity appears to be far less than that of the original Flury strain (46); « G S/C » derived from a vulpine strain (9); « Ts 0 55 » thermosensitive C.V.S. mutant (13); « Av 01 », derived from the C.V.S. strain by mutation in the presence of monoclonal antiglycoprotein antibodies (17). But the in vivo tests, especially in the case of the latter three strains, are still insufficient for it to be possible to envisage their use in the field.

* According to J.W. Frost et al., G. Wachendorfer and A. Rojahn (in litteris) the fox can be perfectly immunised against experimental challenge.
3. New methods of presentation of the vaccine

have been studied, using either capsules or tablets with a diameter of less than 1.5 mm, « enteric », which only dissolve after passing through the stomach and therefore immunise through action on the intestinal and not the buccal mucosa. One of the advantages of the penetration of the modified virus by this route is that it appears to be less pathogenic, under these conditions, for « non target » species (6).

4. Immunisation trials with inactivated virus

have been carried out simultaneously in Europe (27, 45) and Canada (25). According to the authors, these trials appear to have led to seroconversions in 10 to 100% of cases depending on the species, after forced oral introduction of the vaccine in capsules. The confirmation of such results would certainly constitute a marked progress since it would eliminate the lack of safety presently represented by the distribution of modified rabies virus in the countryside.

Comment :

Although all the efforts made to immunise wild animals by the oral route have not yet resulted in it being possible to use this method without restriction to this end, at least they have had one undoubted indirect advantage: they have stimulated research with respect to vaccination by the oral route against rabies. This technique could prove to be very useful in campaigns to control rabies in stray dogs, that prove to be impossible to capture or that belong collectively to an urban or village community that protects them and would not accept their being destroyed.

IV. — VACCINE AND IMMUNITY CONTROLS

The control of rabies vaccines and of the immunity they confer is probably, once the problem of production has been solved, one of the most important problems posed with respect to the immuno-prophylaxis of rabies.

Success in eradicating the disease depends on the efficacy of this control, as does the trust of Public Health Authorities in Animal Health Authorities and the possibility of actively encouraging the vaccination of domestic animals, thus avoiding their having to be slaughtered in the event of exposure. We will examine successively the current rabies vaccine and immunity control techniques and the latest developments in this field.

A. VACCINE SAFETY AND POTENCY TESTS

These are carried out in a different way according to whether or not an inactivated virus vaccine is concerned.
1. Inactivated virus vaccine.

These are subject to two tests:

**Safety**: tested by intra-cerebral inoculation into mice and intramuscular injection in at least two animals of the species for which the vaccine is destined.

**Potency**: the « Habel Test » which was used for a long time, is being increasingly abandoned due to its imprecision (3, 13).

It consisted of challenging mice that had or had not been vaccinated, with increasing doses of virus and quantifying the gap between the lethal dose 50% existing between one and the other groups: this gap should be at least 1 to 1 000. The National Institutes of Health (N.I.H.) test is currently the most widely used. It consists of challenging, with the same dose of virus, mice having received two intraperitoneal injections of various dilutions of the vaccine to be tested. The efficacy of the vaccine is quantified by the final dilution that offers protection at 50%, which has the advantage of being able to be expressed in international units thanks to the simultaneous use of an international reference preparation or its sub-standard (21). A threshold of 0.3 to 1 international unit per dose is currently required by national and international regulations.

This test, although far from reproducing natural immunisation and challenge conditions, seems to reflect well the actual potency of rabies vaccines (4). It is currently the « least bad » of the existing tests, but several other techniques have recently been developed which could complete it, and perhaps even replace it (see chapter C).

2. Non-inactivated modified virus vaccine.

Two specific controls must be carried out:

**Safety**: This can be checked through muscular inoculation in at least 20 guineapigs and 2 animals of the most receptive species for which the vaccine is destined (12).

**Potency**: This is tested by determining the virus vaccine titre and by muscular inoculation in at least 10 guineapigs of which 70% must be resistant to the virulent challenge, while 80% of the control animals die (12).

B. IMMUNITY ASSESSMENT

In animals, post-vaccinal immunity can be assessed directly or indirectly.

1. Direct test.

This consists of comparing the resistance of vaccinated animals with that of non-vaccinated animals with respect to the challenge of natural or experimental exposure:

* We will not examine here the case of vaccines destined for wild animals. The fact that, in this case, many « non target » species have access to the virus makes many, unregulated controls necessary for which the W.H.O. (34) recommends at least inoculation with a tenfold vaccinal dose of 10 animals of wild species « representative of the area ».
Challenge from natural exposure: a detailed study of the number of rabies cases in an enzootic area where the animals are or are not submitted to vaccination, often gives statistically significant results. Such a study can, in particular, permit a rapid evaluation of the effects of « mass campaigns » (see Appendix 3: development of rabies cases in cattle before and after vaccination).

Challenge from experimental exposure: this completes and should precede the above challenge. Although it is technically delicate due to the scarcity of « good » field challenge strains, and costly it should not be completely abandoned in favour of indirect tests as the W.H.O. frequently repeats (32). It is the only technique whereby it is possible to judge, with certitude, the quality and the duration of the immunity conferred by a rabies vaccine.

2. Indirect assessment.

This type of control has the advantage of being simpler and less costly, and is therefore increasingly applied and on a larger scale. It can be carried out by studying serum from the vaccinated animals (humoral immunity) or by examining reactions of cell-mediated immunity.

— Humoral immunity study: This consists of titrating the gammaglobulin antibodies of the serum, either on the basis of their neutralising properties or on the basis of various other properties. The neutralising properties are assessed according to the capability of increasingly diluted serum to neutralise a lethal dose of virus, this neutralisation being established either by mouse inoculation (« neutralisation test in mice », the current reference method) or by inoculation of susceptible cell cultures (« plaque technique » or « rapid fluorescent focus inhibition test ») (21). These properties are very well correlated, in the majority of cases, to the resistance of animals to a virulent challenge. They can be expressed in « international units » thanks to the simultaneous titration of a W.H.O. reference serum (21).

The other properties are assessed by the appropriate techniques, which are indicated elsewhere (2), such as the complement fixation test, passive haemagglutination test, haemagglutination-inhibition test, interference inhibition test, counter immuno-electrophoresis test, immuno-enzymatic assay, etc. which have recently made it possible to develop quicker and less costly methods (see paragraph C). However, these methods do not yet provide such precise results as those obtained through measurement of the neutralising properties of the serum and, above all, they are less directly linked to protection as are the latter methods.

— Cell-mediated immunity study: This type of study, whose results should logically have completed those obtained by humoral immunity measurement, is not yet as satisfactory. Whether it be a question of the lymphoblastic transformation test, the leucocyte migration inhibition test, or delayed hypersensitivity tests, the results obtained seem to have a poor correlation to the resistance to the challenge.
C. NEW DEVELOPMENTS

Over the past few years several improvements have been made or suggested with respect to vaccine and immunity assessment.

1. Vaccine assessment.

Several advances have been made concerning the control of inactivated virus vaccines.

— **New potency tests** have been suggested, including the measurement of antibodies in vaccinated mice (correlated to their resistance to the challenge) and the Antibody Binding Test. The latter appears to be more reliable than the N.I.H. test and could be used to complete it (7).

— **Modification or adaptation** of the N.I.H. test has been suggested. The antigenic value of the vaccines, determined by N.I.H. tests, seems poorly correlated to the antibody titre obtained in the vaccinated animal (7, 8, 15). A modified test, using only one injection of dilutions of the vaccine instead of two (cf. p. 945) could make it possible to obtain better results (33).

In addition, a « simplified test » using only one dilution of vaccine would make it possible to measure the quality, that is, to determine whether a vaccine is acceptable or not, using a simpler and less costly technique (5).

— **Harmonization of reference preparations**: The international reference preparation, which makes it possible to determine the exact titre, in international units, of inactivated virus vaccines, was only available in very small quantities, which favoured the creation of many sub-standards. A European reference preparation, which is in the process of being calibrated (with one or two injections) by several official laboratories, will soon be made available to control laboratories by the European Pharmacopoeia.

2. Immunity assessment.

The concordant results of studies concerning the existing correlation between seroneutralisation tests in mice and those relating to plaque reduction or, above all, the rapid fluorescent focus inhibition mean that far quicker titration is possible. Former tests (haemagglutination inhibition) or recent ones (Enzyme Linked Immunosorbent Assay : ELISA*) have also been studied, and their correlation to seroneutralisation in mice is satisfactory, except for sera having few antibodies.

Although they are quicker and less costly, these various tests are not always accepted as an official equivalent to the reference method, but they make it possible to quickly check, in a very large number of sera, the post-

* This test consists of measuring, by a coloured immuno-enzymatic reaction, the quantity of serum specifically linked to the rabies antigen adsorbed on a polystyrene cupule. The advantage of this technique is that it makes it possible to titrate the antibodies by automatic reading with a spectrophotometer, without having to carry out serum dilutions. The antibody can be identified either by a specific gamma-globulin or by « A protein » (2).
vaccinal rabies immunity, which can prove useful in the case of mass campaigns.

V. -- SELECTION OF TECHNIQUES

In this report we will not examine the choice that might have to be made between immunoprophylaxis and sanitary police measures, or their respective role in national or international strategies for the control of rabies, a choice which is clearly defined by national and international organizations. Nor will we examine the choice of techniques to be used with respect to wild animals in liberty which remains the concern of specialised research groups.

The only techniques subject to a possible choice are therefore those concerning the production of vaccines for domestic animals, the method of vaccination, and the respective monitoring that is desirable in both cases.

A. THE VACCINES

It is accepted that any of the vaccines currently available (cf. Appendix 1), as long as it is correctly tested and applied, can provide satisfactory immunity in all species (32).

Nevertheless, several choices remain to be made by those responsible for national medical control, concerning:

1. The production of vaccines.

This can either be carried out in the user country itself (better supervision of production quality, quantity and costs) or left to exporting countries (possible advantage of better technology). This choice naturally depends on factors such as the technological development, financial resources, international relations of the country concerned, etc. But it remains very important and can, to the same degree as purely technical selections, determine the success or the failure of a vaccination campaign. The following elements can help in making a decision in this respect.

2. The most appropriate types of vaccine.

The main selections must be made between inactivated or non-inactivated virus vaccines, and vaccines produced in vivo or in vitro. We will therefore state their respective characteristics, advantages and disadvantages:

a) Non-inactivated modified virus (M.L.V.) vaccines.

For a long time they presented the advantage of granting a longer lasting immunity than inactivated virus vaccines, and of being able to be produced in ovo in laboratories not equipped for cell cultures. But it has now been clearly
proved that inactivated viruses produced in appropriate conditions (either *in vivo* or *in vitro*) can provide an equally long immunity (2 to 3 years).

They have several disadvantages mainly concerning their possible lack of safety in certain species* or individual animals in a state of immunodepression, or the possible presence of pathogenic contaminants (31). But they also have a lesser thermal stability since any drop in titre during a break in the cold chain leads to an uncontrollable drop in potency, and since, once the vaccine is reconstituted, its conservation period is even shorter (problem in tropical countries).

b) *Inactivated virus vaccines.*

These present the advantage of safety, with the exception of very rare accidents. The presence of neuro-allergenic factors is reduced by purification (*in vitro*) or by the use of new-born animals (*in vivo*). They can be presented in liquid or in freeze-dried form, associated with adjuvants or other antigens, and produced and stored in large quantities since they have a remarkable thermostability even after reconstitution of the freeze-dried product.

They have the disadvantage of necessitating very modern production facilities (when they are produced *in vitro*) and of therefore being expensive and requiring technology which is difficult to transpose.

c) *Vaccines produced* in vitro.

These have the advantage (whether the virus is inactivated or not) of being able to be produced on a large scale under perfectly defined technical conditions (sterility, small number of foreign proteins, titration, etc.) but also the corresponding disadvantage of their cost and the difficulty of transposing them to less well equipped laboratories, especially in less developed countries. This last disadvantage can sometimes be avoided by installing regional inter-country laboratories to supply several States, which leads to a reduction in the cost price.

d) *Vaccines produced* in vivo or in ovo.

These have the advantage of being able to be produced by more modestly equipped laboratories, in fairly large quantities (up to 10 million doses a year). The use of suckling mice, baby rats and rabbits, or better still of new-born lambs or kids, makes it possible to prepare excellent quality inactivated virus vaccines at a low cost, since they do not involve the use of costly material or sophisticated techniques (21).

They present the disadvantage of being less purified than vaccines produced *in vitro*, which is not a major problem in animal disease control.

* The L.E.P. Flury strain is forbidden in the dog and the cat, and E.R.A. in the cat, in certain countries. The Flury (H.E.P.) and E.R.A. strains are still widely used in dogs and cattle in many other countries. Captive wild animals, whose specific sensitivity is unknown, should only receive inactivated virus vaccines.
On the basis of all these characteristics, it presently appears logical, with the exception of special cases, to select inactivated virus vaccines, prepared in vitro or in vivo according to the specific conditions of each country, with added adjuvants.


Vaccine assessment should be carried out systematically on each batch, using N.I.H. type tests with one or two injections, and abandoning the Habel test. This type of test presents the advantage of being more precise and relatively easy to reproduce, and of indicating the antigenic value of vaccines in international units. A minimum antigenic value of 1 international unit per dose of vaccine should be required. In countries where problems of costs and the technical difficulty of monitoring exist, the simplified N.I.H. test could be extremely useful. All the other tests should, for the time being, be reserved for research laboratories and large-scale industrial production.

B. VACCINATIONS

With respect to vaccination, the selection to be made can concern the areas where it should be carried out, the species and individual animals to be vaccinated, the vaccine injection route, the vaccination incentive methods, the monitoring of the vaccination, etc.

Few new developments have been made in this respect:

— Vaccination areas.

The delimitation of these areas is generally carried out by national authorities according to epizootiological data that they must collect beforehand. But it is very important that these delimitations take into account the epizootiological situation of neighbouring countries and measures that are taken there. Hence the importance of international cooperation and concertation (O.I.E., W.H.O., F.A.O., etc.). W.H.O. experts, at a meeting in March 1980, stated that « they consider preventive rabies vaccination of dogs to be one of the most effective weapons in rabies control, and urge those countries where infected areas exist to develop vaccination programmes for dogs ».

— Species to be vaccinated.

Dogs should be the first to benefit from vaccination campaigns, especially in the event of canine enzootic rabies. Cats form a frequent source of human exposure and can be vaccinated (but should not benefit from follow-up measures; see infra), particularly in the case of wildlife enzootic rabies, as can cattle, particularly in those countries where wildlife rabies prevails (vampires or foxes).

The other species (equidae, pigs, sheep, etc.) will be vaccinated according to the regional epizootiological situation.
— Individual animals.

All individual animals in the species of more than three months of age should be vaccinated. If the animal is less than three months old, it should only receive an inactivated virus vaccine and receive a booster dose as soon as possible after this age.

The best guarantee of solid and lasting immunization, whatever the species, consists of carrying out a booster injection one year after primary vaccination. This « guarantee period » should always be taken into account in estimating the degree of immunity of the animal population.

— Route of application of the vaccine.

With the exception of individual counter-indications, the intramuscular route should currently be recommended, whatever the vaccine, if it does not contain any adjuvant substances in the dog.

— Vaccination incentives.

Vaccination can be compulsory, in which case it will be paid for by the local authorities, and/or incitative. In either case, these measures can only be made easier by such counterparts as the issue of an internationally recognized certificate (or « passport »), or the authorisation to keep the vaccinated animals, when they are exposed, as long as they receive a booster vaccination. But such counterparts can only be given in the case of individual, indelible identification (tattooing) of the animal. In the case of a « mass campaign », the incentive could consist of the slaughter of non-vaccinated dogs which would have been recognized by the absence of collective identification signs (collar, ear-marking, etc.).

— Vaccination monitoring.

Every vaccination campaign must foresee the means of monitoring the effective immunisation of the animals involved. This monitoring can currently be made easier by:

— the selection of an inactivated virus vaccine, whose thermostability during storage or use is a guarantee for real efficacy;

— the monitoring of selected vaccines, upon receipt and during use, at least by a « simplified test »;

— the monitoring of post-vaccinal immunity, either by random tests (of the ELISA type for example) or by the drawing up of reliable statistics of the cases of rabies before and after the campaign (organisation of a diagnostic laboratory service).

All these measures should make it possible, thanks to the practical application of the latest knowledge or techniques, to ensure the efficacy of vaccinations on a national scale.
CONCLUSIONS

New developments with respect to rabies vaccination that have taken place over the past few years have not greatly changed the recommendations listed in 1977 in the document « Arrangements recommended by the O.I.E. for rabies control », in particular the indication of medical and sanitary prophylaxis measures, respectively.

But these developments can determine, even modify, the practical methods of medical prophylaxis, in particular those concerning vaccination of domestic and wild animals, as well as vaccine and immunity testing.

1. Vaccination of domestic animals.

a) The vaccines.

— Inactivated virus vaccines currently offer a better guarantee of safety than modified virus vaccines. The duration of the immunity that these two types of vaccines can confer on the principal domestic species (dogs, cats, cattle) can be as long as three years, the conferred immunity being considerably strengthened by a booster injection carried out one year after the primary vaccination.

It therefore appears preferable to use inactivated virus vaccines, unless there are specific counter-indications (cost, production difficulties, etc.).

— Inactivated virus vaccines produced in vitro or in vivo offer the same guarantees of safety, and can lead to an immunity of an equivalent duration, but in vitro production gives a better degree of purification. The choice between these two production techniques will therefore be based on their respective cost price and ease of production.

— Inactivated virus vaccines will necessarily contain adjuvants when they are for use on ruminants, equidae and pigs, and preferably also when they are for dogs and cats. They can be associated or combined with other specific vaccines.

— The choice of vaccinal strains has been re-assessed according to their analysis by the monoclonal antibody technique.

b) The vaccinations.

— All species of domestic animals can be solidly immunised against rabies using inactivated or modified virus vaccines. In the latter case, it is still recommended to exclude the Flury L.E.P. vaccine for all animals and the E.R.A. vaccine for cats.

— All animals over three months old can be immunised if they are in good health. Before this age the persistence of maternal antibodies can hinder, or even prevent immunisation, or at least serological conversion.

For inactivated virus vaccines, the injections will preferably be carried out by the intramuscular route in carnivores if these vaccines do not contain adjuvants, and in other cases by the subcutaneous route.
2. Vaccination of wild animals.

This vaccination remains at the stage of research aimed at determining its usefulness, the techniques to be used, application methods and conditions for its safe use.

Although important progress has been made with respect to this research (pilot tests in the field, study of new modified virus-vaccines and presentation procedures, trial vaccination by inactivated virus) the immunisation of wild animals in liberty does not appear to be recommendable with current known techniques.

3. Vaccine and immunity testing.

Effective testing of the safety and potency of rabies vaccines is essential for the success of animal rabies vaccination.

— The type of tests with respect to modified virus vaccines have changed little, but they have benefited from the precise identification, through the monoclonal antibodies technique, of vaccinal strains.

— The testing of inactivated virus vaccines will preferably be carried out by an N.I.H. type test or, at least, by a simplified N.I.H. test.

This can later be completed by other tests such as the Antibody Binding Test (A.B.T.).

The N.I.H. test makes it possible to determine the antigenic value of the vaccine, expressed in international units (I.U.); the minimum threshold required for an inactivated virus should be 1 I.U./dose.

— The assessment of post-vaccinal immunity can be carried out, in the absence of a virulent challenge, by titration of serum antibodies. If the reference technique of this titration remains seroneutralisation in mice, other neutralisation techniques on cell cultures can give equivalent results, also expressed in international units. Other titration techniques can also be useful in view of their rapidity and their lesser cost (example, the ELISA test) but they are less accurate.
Appendix 1

MAIN RABIES VACCINES FOR USE ON ANIMALS USED IN 1980*

1. Non-inactivated modified virus vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>For use in</th>
<th>Dose</th>
<th>Age at time of primary vaccination</th>
<th>Booster dose recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin: embryonated egg « Low Egg Passage », Flury strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td>Origin: canine cell lines « High Egg Passage », Flury strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: pig cells « High Cell Passage », S.A.D. strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>horse</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>goat</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: dog cells « High Cell Passage », S.A.D. strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>after 3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: cattle kidney cell « High Cell Passage », S.A.D. strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: hamster cell lines « High Cell Passage », Kissling strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: hamster cells Vnukovo 32 strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td>1 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>horse</td>
<td>1 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>1 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>goat</td>
<td>1 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
</tbody>
</table>

* Document taken from the Proceedings of the World Health Organization Meeting (10-12 March, 1980 - Lyons) with the kind authorization of this Organization.
### MAIN RABIES VACCINES FOR USE ON ANIMALS
#### USED IN 1980

#### 2. Inactivated virus vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>For use in</th>
<th>Dose</th>
<th>Age at time of primary vaccination</th>
<th>Booster dose recommended</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>On nerve tissue</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Origin: adult animal encephalon</td>
<td>dog</td>
<td>2 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>2 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: new-born animal encephalon</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>3 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td><strong>On cell culture</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Origin: hamster cell lines « High Cell Passage », Kissling strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Pitman-Moore strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 and 15 months</td>
<td>Every 3 years</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>horse</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>and goat</td>
<td>1 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: hamster cell lines Pitman-Moore strain in association with panleucopenia vaccine, leptospirosis vaccines, canine distemper and I.C.H., foot-and-mouth disease vaccine</td>
<td>cat</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>dog</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td>5 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>2 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>and goat</td>
<td>2 ml</td>
<td>4 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: embryonated egg « Low Egg Passage », Flury strain</td>
<td>dog</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td>1 ml</td>
<td>3 months</td>
<td>Annually</td>
</tr>
<tr>
<td>Origin: pig kidney cells « Low Egg Passage », Flury strain</td>
<td>dog</td>
<td>2 ml</td>
<td>3 and 4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cat</td>
<td>2 ml</td>
<td>3 and 4 months</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>cattle</td>
<td>2 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>horse</td>
<td>2 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>sheep</td>
<td>2 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
<tr>
<td></td>
<td>and goat</td>
<td>2 ml</td>
<td>Discretionary</td>
<td>Annually</td>
</tr>
</tbody>
</table>
## Appendix 2

**NUCLEOCAPSID ANTIGEN DETERMINANTS OBTAINED BY THE MONOCLONAL ANTIBODIES TECHNIQUE (35, 43, 45, 46) ON CERTAIN VACCINAL OR FIELD STRAINS**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Antibody No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most common virus vaccines:</td>
<td></td>
</tr>
<tr>
<td>E.R.A. - Pitman-Moore-PV11</td>
<td></td>
</tr>
<tr>
<td>Flury L.E.P. - Kelev</td>
<td>+ + + + + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Flury H.E.P.</td>
<td>+ • • + + + + + • • • + + + + + + + + + + +</td>
</tr>
<tr>
<td>C.V.S. - D.E.V.</td>
<td>+ + + + + + + + + + + + + + • + + + + + + +</td>
</tr>
<tr>
<td>Some field viruses:</td>
<td></td>
</tr>
<tr>
<td>European type 1 (fox)</td>
<td>+ + + + + + + + • + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>European type 2 (fox)</td>
<td>+ + + • + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>African type 1 (Tunisia, Senegal, Gambia, Central Africa)</td>
<td>+ + + + + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Thailand type 1</td>
<td>+ + + + + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>Madagascar</td>
<td>+ + • + + + + • + + • + + + + + + + + + + + +</td>
</tr>
<tr>
<td>U.S.A. (isolated in man)</td>
<td>+ + + + + + + + + + + + + + + + + + + + + + +</td>
</tr>
<tr>
<td>« Lagos Bat » (rabies related virus)</td>
<td>+ + + • + + • + + • + + • + + • + + • + + • +</td>
</tr>
</tbody>
</table>

+ Reaction with the corresponding monoclonal antibody.
• Absence of a reaction with the corresponding monoclonal antibody.
Appendix 3

REDUCTION IN THE INCIDENCE OF CATTLE RABIES AFTER RABIES VACCINATION
(in Inf. tech. Serv. vét., 1978, No. 64-67)

The decrease in the incidence of cattle rabies is in exactly opposite proportion to the increase in the number of rabies vaccinations. It would therefore be extremely ill-timed for these vaccinations to be, as has been the case since 1975, progressively replaced by insurance against rabies death, which while appearing less costly does not lead to any specific protection and places a heavy financial burden on control programmes (post-exposure treatment of exposed people, in particular).

It should be noted that the bearing of cattle vaccination on the number of cattle rabies cases would have been more significative if a logical strategy was followed, with as many vaccinations as possible in areas which are infected or directly at risk.
Appendix 4

50th GENERAL SESSION OF THE O.I.E.
RESOLUTION NO. XIV
RABIES - NEW DEVELOPMENTS IN VACCINATION

Having examined the Report on « Rabies - New developments in vaccination » (document 50 SG/6), Item 1 of the Agenda and having heard the presentation of this Report during the Second Plenary Session

Considering the discussions and conclusions on this Item

THE COMMITTEE

RESOLVES

1. To remind Governments of Member Countries of the need to establish control and/or eradication programmes against rabies of domestic animals by providing adequate political and economic support to enable veterinary services to obtain the necessary human and material means to control the disease.

2. To recommend that Veterinary Services examine the following points when finalising and implementing rabies control programmes:

   a) specific criteria should be defined regarding the choice of rabies vaccine to use on the different species and in the various geographical areas, accounting for the epidemiological characteristics of the region involved, local facilities for vaccine preparation, vaccine quality control, conservation and use of the vaccine in the field;

   b) vaccination should be used only as an auxiliary technique to rigorous animal disease control measures directed to the domestic or wild species responsible for the enzootic. To be effective, vaccination must be on a large scale, involving at least 70% of the population to be protected;

   c) cost-benefit surveys of vaccinations and other control operations to be conducted must be undertaken to determine the animal species to be protected and those which, from an economic point of view, do not merit vaccination. This survey must also take into consideration the consequences of outbreaks of animal rabies on public health;

   d) vaccines prepared from non-inactivated modified virus strains which have shown excellent results are also recommended for vaccination campaigns among cattle in areas affected by rabies transmitted by bats;

   e) preference should be given to the use of vaccines prepared from totally inactivated virus strains after including an immunity adjuvant and possibly combined with other antigens in countries where rabies incidence is moderate in domestic animals or in free countries which are threatened at their borders;

   f) irrespective of the method of preparation, vaccines must be tested for innocuity and potency in accordance with the norms defined by the W.H.O.
The use of the test established by the National Institutes of Health (complete or simplified) is particularly recommended. By expressing the antigenic value of vaccines in International Units (I.U.) per vaccinal dose, immunity conferred can be controlled by titration of neutralising antibodies in the serum of vaccinated animals. For titration, the serum-neutralisation test in mice or alternatively the ELISA test may be used. Antibody titres must be expressed in International Units (I.U.) for the serum-neutralisation test in mice and in Equivalent Units (E.U.) for the F*:ISA test;

g) research using the monoclonal antibody technique must be developed to define precisely the characteristics of prevalent strains in various areas of the world. Results from this work should then be applied to determine the potency of vaccines used in different countries; and

h) on the specific point of vaccination of wild animals within the framework of wildlife rabies control, particular care must be taken when using non-inactivated modified virus vaccines to eliminate all risks of residual pathogenicity and positivity to immunofluorescence diagnostic tests for target and non-target animal species. In this respect, research is recommended to obtain satisfactory immunity by oral administration of inactivated vaccines. If effective oral vaccination of wild species involved in wildlife rabies enzootics becomes feasible, then reduction in the numbers of wildlife would no longer be necessary for rabies control reasons and regulation of wildlife populations would become the responsibility of hunters.

3. To express gratitude to Drs. L. Andral and J. Blancou for the excellent paper they drafted and for their remarkable presentation during the technical discussion on this Item at the 50th General Session of the International Committee of the O.I.E.

(Adopted by the International Committee of the O.I.E. on 29 May 1982)

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REFERENCES

(See p. 927)