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THE ORAL VACCINATION OF FOXES AGAINST RABIES AN EXPERIMENTAL STUDY

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Résumé

VACCINATION DES RENARDS CONTRE LA RAGE PAR VOIE ORALE. — L'efficacité de la vaccination contre la rage par voie orale est étudiée chez 99 renards roux (*Vulpes vulpes*). Les deux vaccins vivants utilisés, souche Flury H.E.P. et souche S.A.D., sont entièrement inoffensifs chez le renard. Le dépôt direct dans la bouche entraîne la formation d'anticorps et protège contre une épreuve par voie intra-musculaire avec la souche sauvage, épreuve fatale chez les témoins. Le pourcentage de renards présentant une réponse sérologique, et protégés contre l'épreuve, varie dans chaque groupe, avec la concentration du vaccin utilisé. Ainsi pour protéger 50 p. 100 des renards, le vaccin souche Flury H.E.P. doit présenter un titre de $10^{5,9}$ DL 50 - souriceau. Toutefois, ce titre diminue considérablement après incorporation dans les appâts offerts aux renards (têtes de poule, souriceaux) ; de plus ces appâts ne permettent pas un contact suffisant entre le virus vaccinal et la muqueuse buccale. Ces difficultés, ajoutées à la persistance d'une pathogénicité résiduelle de ces vaccins viraux pour certaines espèces sauvages autres que le renard (les rongeurs, en particulier), continuent à limiter les applications de la vaccination par voie orale comme méthode de prophylaxie de la rage chez les renards.

The steady spread of rabies across Western Europe after the second World War and across France after 1968 necessitated the mobilisation of very substantial prophylactic measures against rabies in foxes which remains the principal vector of the disease.

The prophylaxis is at present based on the reduction of the fox population with the aim of reducing the viral pollution of the affected areas and delaying, if possible, the invasion of new territory. However, the methods

employed for this, need, to be effective, to be applied very precisely, and this is seldom achieved : the effectiveness is thus contested.

For this reason, following the recommendation and with the support of the W.H.O., means of biological prophylaxis have been explored in numerous countries, amongst the most important is the vaccination of foxes.

Research carried out in this domain in the United States (Baer *et al.*, 1971-1975-1976 ; Bell *et al.*, 1971 ; Debbie *et al.*, 1972-1974-

1975 ; Winkler, 1975 ; Winkler *et al.*, 1975-1976), in Canada (Black *et al.*, 1970-1973 ; Johnston, 1975), in West Germany (Forster, 1975 ; Jaeger, 1974 ; Mayr *et al.*, 1972 ; Wachenforfer, 1976) and in Switzerland (Wandeler, 1975) concluded that oral vaccination of foxes against rabies was possible.

The work carried out at the Centre National d'Etudes sur la Rage in 1974, 1975 and 1976-1977, the subject of this report, contributes to the study of this type of vaccination by using the two attenuated strains of rabies best known at the present time : the Flury H.E.P. strain and the S.A.D. strain.

The objectives of this study, following through the objectives chosen in other countries, were to determine the *theoretical* doses of vaccines necessary to immunize the foxes adequately and to assess their true *practical* effectiveness after incorporation into baits.

Materials and methods

1. MATERIALS

In addition to the basic material necessary for this type of experiment, we used :

1.1. The foxes

All the foxes employed were obtained by digging out cubs from earths in rabies-free zones. They were then raised in the Experimental Station at Atton situated 30 km from Nancy, to the age of 18-24 months, when they are usable.

The housing of these foxes consisted of individual mobile wire cages (2 × 1 × 1 m) grouped within an enclosure surrounded by a double security barrier (Fig. 1). The cages were regularly moved over the grass or, better, on boards to avoid infections to which housed animals are prone (in particular, foot lesions). They were equipped with a box of insulating material (Mussicastor) to serve as an « earth ».

Therapeutic attention was rarely given because of a systematic treatment against external (Lindane at 0.25 per cent twice with a 10 day interval) or internal parasites (Dichlorophos and Dichlorophene), and the three main infectious diseases of the fox : Carre and Rubarth diseases, and Leptospirosis (vaccination).

The diet of the unweaned cubs consisted of reconstituted milk (« Welpi » or « Milkodog »)

supplemented by trace elements and vitamins (Vitapaulia). This was progressively replaced by the adult diet (on average 60 hen heads and 3 apples per individual per week). Overall, this type of management seemed appropriate to the foxes since breeding has been possible under these conditions.

1.2. The vaccines

1.2.1. Flury H.E.P. vaccine

This vaccine consisted of the freeze-dried supernatant of the « avianized » Flury « High Egg Passage » (H.E.P.) strain in B.H.K. 21 (C 13) cells. It was provided by the Pasteur Institute in Paris.

In 1974, the batch employed (No. 040) had a titre of 6×10^8 plaque forming units (P.F.U.) per ml. It was administered either directly *per os* (1 ml deposited on the tongue) or after incorporation into the hen head bait. In this case 1 ml of vaccine was introduced, by syringe, partially inside the skull and partially into the comb : the head was offered to the fox two hours later and was usually rapidly consumed.

In 1975, the batch employed (No. 055) had a titre of 5×10^6 P.F.U. per ml. It was used to inoculate 4 day-old suckling mice (0.2 ml of the pure vaccine intracerebrally).

The bait containing the viral vaccine given to each fox then consisted of :

either the brains of these suckling mice, homogenized in Hanks solution (1:5), incorporated into wax coated gelatin capsules (1 ml) introduced under the skin of dead mice : two of these mice were given to a fox which thus

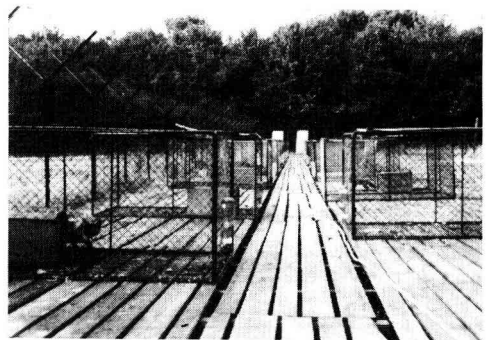


Fig. 1. - The experimental station of Atton : general view.

ingested a total of about $10^{8.1}$ suckling mice LD50 per bait sample,

or by a bundle of 6 whole baby mice : in this case the fox ingested $10^{10.4}$ suckling mice LD50 per bait sample¹.

In 1976-1977 the batch employed (No. 074161) had a titre of 6×10^7 P.F.U. per ml. It was given directly, *per os* (1 ml), either pure or diluted 1/10 or 1/100. The infective capacity of this vaccine in suckling mice was titred using the inoculum which had just been ingested by the foxes. The amount of virus ingested was $10^{6.2 \pm 0.71}$ LD50 for the pure vaccine, $10^{4.9 \pm 0.28}$ LD50 for the vaccine diluted 1/10, and $10^{3.9 \pm 0.26}$ LD50 for the vaccine diluted 1/100, which signifies quite a good viral stability through the manipulations.

1.2.2. S.A.D. vaccine

This vaccine consists of the freeze-dried supernatant of the strain S.A.D. grown in canine renal cells (provided by W.G. Winkler via the Pasteur Institute in Paris).

The infectivity of this vaccine after intracerebral introduction into mice was $10^{5.2 \pm 0.3}$ LD50 per ml before administration of one milliliter by either direct deposition in the mouth or via hen heads. The infectivity was not redetermined after incorporation of one milliliter into the hens heads.

1.3. The challenge virus

The virulent challenge suspension consisted of an homogenate of salivary glands of foxes dead from rabies naturally. The salivary glands, with the capsule removed, were homogenized² and the material separated into 1 ml ampoules which were then conserved at low temperature (at least - 30° C).

The titre of the ampoules contents was determined by intracerebral inoculation of mice. The titre varied ($10^{4.98 \pm 0.21}$, $10^{5 \pm 0.45}$ and $10^{5.28 \pm 0.22}$ in 0.03 ml) following the experiment.

Consequently in order to obtain an inoculum of one milliliter with a titre of 3000 mouse

LD50, previously shown to be consistently fatal in foxes, this suspension was diluted to : $10^{-2.95}$, 10^{-3} and $10^{-3.33}$. This challenge dilution was kept in ice and protected from light during the inoculations.

Note : The virulent suspension suffers some inactivation during the manipulations in spite of these precautions. Its titre, as shown at the end of operations, can thus decrease from 3000 mouse LD50 (the initial theoretical titre) to 500 mouse LD50 (final titre).

2. METHODS

2.1. Techniques

All procedures carried out on live foxes were performed without anaesthesia or pre-anaesthesia but observing the essential precautions against bites and scratches (Fig. 2) and ensuring the firm restraint of the muzzled animal in ventral recumbancy. (Fig. 3).

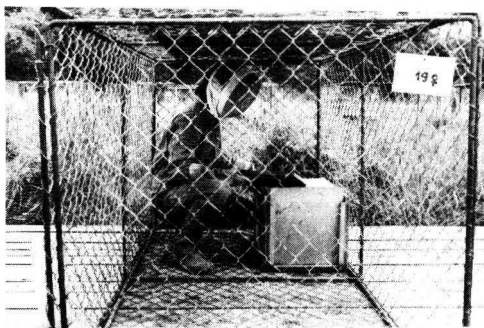


Fig. 2. - Handling of a fox.



Fig. 3. - The inoculation of the challenge rabies virus in the temporal muscle of a vaccinated fox.

¹ This amount may seem high, but we have nevertheless been able to reproduce it by repeating the same experiment with another batch of the same vaccine.

² Virtis 45 blade homogenizer fitted with an anti-aerosol device, turning at 45,000 r.p.m.

Table 1 : General organisation of the vaccination experiments carried out on 99 foxes over the four years 1974-1977 (number of foxes at the beginning of the experience).

| Year | No vaccination control group | Type of vaccination | | | | | | Vaccination by « Flury H.E.P. » | | | | |
|-----------------|------------------------------|---------------------------|-------------|------------------------|---------------------------------|-------------|----------------|---------------------------------|-------------------------|--------------------------|----------|---------------|
| | | Vaccination by « S.A.D. » | | | Vaccination by « Flury H.E.P. » | | | Vaccination by « Flury H.E.P. » | | | | |
| | | Intra-muscular | Direct oral | Oral via hen head bait | Intra-muscular | Direct oral | Oral via baits | Pure vaccine | Vaccine diluted by 1/10 | Vaccine diluted by 1/100 | Hen head | Suckling mice |
| 1974 | 5 | 5 | 5 | 10 | 5 | 5 | - | - | - | 10 | - | - |
| 1975 | 5 | - | - | - | - | - | - | - | - | - | - | 14 |
| 1976-1977 | 5 | - | - | - | - | 10 | 10 | 10 | 10 | - | - | - |
| Total per group | 15 | 5 | 5 | 10 | 5 | 15 | 10 | 10 | 10 | 10 | 10 | 14 |

2.1.1. Vaccination

The vaccines were administered in three ways :

either *intramuscularly*, essentially with the object of checking their harmlessness and at the same time to compare their effectiveness to oral vaccination. 1 ml was given deep in the thigh muscle using a Yale-BD plastic syringe with a 31 1/2 G needle.

or *orally* by the deposition of 1 ml on the surface of the tongue using a syringe without needle prolonged by 5 cm with 11/10 micro-perfusion tubing.

or *incorporated into the bait* : the baits consisted of hens heads or of mice containing gelatin capsules filled with an homogenate of suckling mice, or by whole baby mouse cadavers (see above). In all these forms with the sole exception of one of the hens heads, the bait was taken up without any difficulty, possibly too rapidly in the case of the suckling mice.

2.1.2. Challenge

The challenge was made by inoculation, deep into a temporal muscle, with 1 ml of the dilution made to contain 3000 mouse LD50.

2.1.3. Sampling

Several types of sample were taken from the foxes, *in vivo* and *post mortem* :

Serum was obtained by centrifugation of blood obtained from either the jugular vein (live animal) or from the heart (*post mortem*), both before and at different times after the vaccination.

Brain, Salivary glands : These organs were removed by the techniques recommended by the W.H.O. (« La Rage, Techniques de Laboratoire » O.M.S. Geneve edition, 1974).

Saliva : obtained by swabbing the mouth. A 1 in 5 dilution of the quantity collected with an antibiotic-containing Hanks solution, subsequently decanted for 2 hours at 4°C, was used to inoculate mice.

Greater dilutions are occasionally necessary to avoid the early death of the mice from bacterial encephalitis.

2.1.4. Titre

All the viral and serum titres were determined using the W.H.O. techniques (see above). The viral titre is expressed in doses 50 per cent lethal (LD50) in mice (strain S.A.D.) or suckling mice (Flury H.E.P. strain). The titres of the sera are expressed in International Units (IU) determined by comparison with a standard

serum (titre 65 IU/ml) furnished by the Pasteur Institute in Paris.

2.1.5. Rabies diagnosis

In order to verify the true etiology of the lesions in the samples, the rabies virus was sought by the analytical techniques recommended by the W.H.O. (immunofluorescence, inoculation of mice¹, presence of Negri bodies). As an additional measure the rabies antigen was shown up by immunofluorescence in frozen sections by the technique of Beauregard *et al.* (1969).

2.1.6. Statistical analysis

The titres and their 5 percent confidence limits were calculated by the statistical methods of Lichtfield *et al.* (1969) or, for small samples, with the help of the tables of Saint-Pierre *et al.* (1976).

2.2. Overall Experimental Protocol

In view of the restricted number of experimental animals that is was possible to use simultaneously, the experiments were carried out over four years corresponding to three distinct series : 1974, 1975 and 1976-1977. Experiments spread out over the time allowed the benefit of knowledge from the previous experiment to be use for the following set.

The successive aims were :

to make a choice based on effectiveness and safety between the Flury H.E.P. strain and the S.A.D. strain (1974).

the choice having been made, to check whether when incorporated into baits the vaccine would remain effective (1975) and what dose was effective by the direct oral route (1976-1977).

to facilitate comparison of the modalities and final layout of these three experimental series an overall view is shown in Table 1.

Results

1) THE 1974 EXPERIMENTAL SERIES

The results are seen in table 2 showing, for each group of foxes, the evolution of antibody titre (after vaccination and after challenge), the mortality rate post-challenge and the results of the examination of the salivary glands and the saliva for the rabies virus.

¹ Specific pathogen free strain O.F.I., Centre de Recherches et d'Elevage des Oncins, 69210 Saint-Germain-sur-l'Arbresle (France).

Table 3 shows the changes in antibody levels after vaccination and challenge, of which some examples of the four groups effectively vaccinated are plotted in Figure 4.

2) THE 1975 EXPERIMENTAL SERIES

The results are grouped in Table 4 which illustrates the same information as that collected in 1974. The changes in antibody levels could not be established since on all the occasions when serum samples were taken (before vaccination and 48 days and 97 days after) the titre was always zero. Post-challenge the titres increased to :

0.82 ; 0.86 ; 2.06; 3.26 and 3.36 IU for 5 of the 7 foxes of the batch vaccinated by gelatin capsules.

3.49 ; 4.60 and 14.56 IU for 3 of the 7

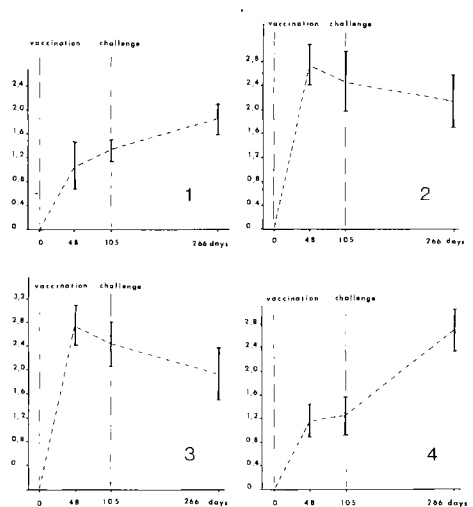


Fig. 4. - The evolution of neutralising antibody levels in foxes having received S.A.D. or Flury H.E.P. vaccines intramuscularly (graphs 1 and 3) or by direct oral deposition (graphs 2 and 4). The titre of neutralising antibody (ordinate) expressed as the logarithm to the base ten of the 50 per cent effective dilution.

1. One fox vaccinated intramuscularly with 1 ml of S.A.D. vaccine.
2. One fox vaccinated by direct oral deposition of 1 ml S.A.D. vaccine.
3. One fox vaccinated intramuscularly with 1 ml of Flury H.E.P. vaccine.
4. One fox vaccinated by direct oral deposition of 1 ml Flury H.E.P. vaccine.

Table 2 : Experiments carried out in 1974 Flury - H.E.P. and S.A.D. vaccines administered by various routes

| Results | Type of vaccination | | | | | | |
|-----------------------------------------------------------------------------------|-------------------------------------------------|----------------------------|--------------------------|---------------------------------------------------|----------------------------|--------------------------|---------------------------------------|
| | S.A.D. vaccine (10 ^{5.2} mouse L.D.50) | | | Flury H.E.P. vaccine (6 × 10 ⁸ P.F.U.) | | | |
| | No vaccination : control animals | Intramuscular route (1 ml) | Direct oral route (1 ml) | Oral route via bait : hen head (1 ml) | Intramuscular route (1 ml) | Direct oral route (1 ml) | Oral route via bait : hen head (1 ml) |
| Mean increase in antibody titre per ml serum 48 days after vaccination | nil | 0,8 I.U. | 3,4 I.U. (1) | nil | 2,4 I.U. | 0,7 I.U. | nil |
| Same increase 150 days after the challenge (or day of death) | not measured | 1,2 I.U. | 1,3 I.U. | not measured | 0,9 I.U. | 4,3 I.U. | not measured |
| Number of foxes dead from rabies (2) after the challenge by 525 to 750 mouse LD50 | 5/5 | 0,5 (3) | 1/5 | 10/10 | 0/4 (3) | 0/5 | 8/8 (4) |
| Presence of rabies virus in : | | | | | | | |
| Salivary glands | 5/5 | 0/5 | 0/5 | 10/10 | - | all survived | 8/8 |
| Saliva | 4/5 | - | 0/5 | 2/10 | - | survivors | 4/8 |

(1) In this group one of the animals showed no serum response before the challenge, which proved fatal.

(2) Death from rabies was confirmed by immunofluorescence, mouse inoculation, presence of Negri bodies ; good agreement between techniques in most cases.

(3) In this group one of the animals died from an intervening disease (rabies virus was not found).

(4) Out of the ten animals in this group, 2 were eliminated, one having refused the bait, the other having died of an intervening disease.

(5) The symptoms were sometimes quite rough, the most common (14 out of 24) being a prominence of the nictating membrane, associated with a mydriasis on the side the challenge inoculation was given. Other symptoms were essentially modifications of behaviour, of the voice with an increase in cries and complaints from the patients, often suffering from paresis and posterior paralysis. One of the first signs of rabies remains the loss of appetite.

foxes of the batch vaccinated by bundles of suckling mice.

0.15 ; 1.92 and 13.58 IU for 3 of the 5 control foxes.

3) THE 1976-1977 EXPERIMENTAL SERIES

The results grouped in tables 5 and 6 give the same information as that gathered in previous years. It was possible to follow antibody changes only in the two survivors : the changes are plotted in figure 5.

4) OBSERVATIONS ACCUMULATED OVER THE FOUR YEARS

During the four year series of experiments it

was possible to pick out a number of points concerning :

a) The interval between the challenge inoculation and the death of the animal : over the years 1974 to 1977 the interval did not vary significantly from one year to another nor from one group to another. This indicates the similarity of the challenge dose received (according to Sikes, 1962) and the weak effect of a possible immunity on the interval before death, at least at the challenge dose employed. Out of the 70 rabic foxes, one was dead on the 14th day following the challenge (D14) ; 1 at D15 ; 6 at D 16 ; 10 at D17 ; 15 at

Table 3 : The evolution in the titre of neutralising antibodies (International units/ml) in the animals vaccinated in 1974

| Experimental groups (5 foxes per group) | Day of vaccination | Post vaccination interval | | |
|----------------------------------------------------------------------|--------------------|-------------------------------------------------------------------|-----------------------------------------------------|--------------------------------------------------------------------|
| | | 50 days after vaccination (55 days before the challenge) | 105 days after vaccination (day of challenge) | 260 days after vaccination (155 days after the challenge) |
| Vaccinated by intra- muscular S.A.D. (1 ml) | Titre nil | 0,17 | 0,32 | 1,04 |
| | | 0,32 | 0,16 | 1,62 |
| | | 0,53 | 0,64 | 0,57 |
| | | 1,04 | 0,93 | 0,85 |
| | | 1,94 | 2,13 | 2,13 |
| Vaccinated by direct oral deposition of S.A.D. (1 ml) | Titre nil | 0,89 | 1,47 | 1,04 |
| | | 11,7 | 6,28 | 3,01 |
| | | 0 | 0 | 0* |
| | | 4,15 | 1,02 | 1,77 |
| | | 0,27 | 0,60 | 0,46 |
| Vaccinated by intra- muscular Flury H.E.P. (1 ml) | Titre nil | 0,16 | 0,15 | 0,17 |
| | | 0,07 | 0,19 | 0,17 |
| | | 0,32 | 0,25 | 0,33 |
| | | 8,28 | 4,25 | 1,25 |
| | | 3,15 | 0,32 | 2,87 |
| Vaccinated by direct oral deposition of Flury H.E.P. (1 ml) | Titre nil | 0,47 | 0,57 | 15,42 |
| | | 0,74 | 0,47 | 1,94 |
| | | 0 | 0,14 | 2,39 |
| | | 0,11 | 0 | 0,29 |
| | | 1,94 | 2,18 | 1,65 |

* This animal died after the challenge

D18 ; 10 at D19 ; 13 at D20 ; 8 at D21 ; 3 at D22 ; 2 at D23 and 1 at D24.

b) The presence of the rabies virus in the nerve centres, the salivary glands and in saliva : in the 70 samples from rabid foxes examined the presence was confirmed in 100 per cent of the cases for the Ammon's horn (by immunofluorescence and inoculation of mice) ; in 89 per cent (immunofluorescence) and 60 per cent (mouse inoculation) of the cases for the salivary glands ; in 43 per cent (mouse inoculation) of the cases for saliva.

c) The proportion of cases in which the saliva was virulent : the proportion was 10/25 in the control animals against 19/52 in the vaccinated animals, a statistically significant difference (to a level of 0.05 by χ^2 test). This observation would have important practical consequence if confirmed by other experiments.

d) The antibody levels in foxes dead from rabies : when compared to the animals resisting the challenge given at the same date, the levels showed no statistically significant difference (Mann-Whitney test).

e) The antibody levels of foxes with virulent saliva compared with the animals with non-virulent saliva : here again the levels did not differ significantly (Mann-Whitney test).

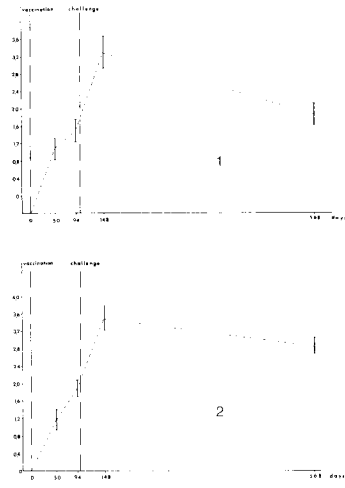


Fig. 5. - The evolution of neutralising antibody levels in foxes having received, orally, pure Flury H.E.P. vaccine (graph 1) or diluted 1/10 (graph 2). The titre of neutralising antibody (ordinate) is expressed as the logarithm to the base ten of the 50 per cent effective serum.

1. One fox vaccinated by direct and deposition of 1 ml of the pure Flury H.E.P. vaccine.
2. One fox vaccinated by direct oral deposition of 1 ml of Flury H.E.P. vaccine diluted 1/10.

Table 4 : Experiments carried out in 1975 - The Flury - H.E.P. vaccine was administered orally pure and via baits consisting of vaccine virus infected suckling mice.

| Results | Type of vaccination | | |
|-----------------------------------------------------------------------|-------------------------|--------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------|
| | No vaccination controls | Flury H.E.P. : vaccine put into mice and given in the form of : | |
| | | Mouse brains in waxed gelatin capsules (total dose : $10^{8.1}$ LD50 suckling mouse) | Bundles of six whole suckling mouse cadavers (total dose : $10^{10.4}$ LD50 suckling mouse) |
| Average increase in antibody titre/ml serum 48 days after vaccination | nil | nil | nil |
| Same increase in foxes dead after challenge, on day of death. | 5 I.U. | 2 I.U. | 7,5 I.U. |
| No. of foxes dead after challenge with 3000 mouse LD50 | 5/5 | 7/7 | 7/7 |
| Presence of rabies virus in : | | | |
| Salivary glands | 5/5 | 7/7 | 6/7 |
| Saliva | 3/5 | 6/7 | 2/6 |

Conclusions - Discussion

1. CONCLUSIONS AND DISCUSSION FOR EACH EXPERIMENTAL SERIES

A. 1974

It is possible to draw a number of conclusions from an examination of Tables 2 and 3.

a. The non-pathogenicity of the vaccines. This seems to be confirmed for the two vaccine strains studied, after both intramuscular and oral administration. However, we have not examined the pathogenicity of these vaccines in any species other than the fox. The S.A.D. strain in particular apparently possesses a residual virulence under certain conditions in diverse species (Bijlenga *et al.*, 1974 ; Forster, 1975 ; Steck, 1975).

Because of this, since the immunogenicity appears similar, we decided from 1975 onwards to abandon the S.A.D. strain in favour of the Flury H.E.P. strain.

b. The effectiveness of the vaccines

After intramuscular administration both the vaccines are effective, judged either by the level of serum response or by the degree of protection afforded against a challenge fatal in the controls.

After direct oral administration, by contrast, the S.A.D. vaccine seemed to give poorer results than the Flury vaccine probably because of too weak a titre of the batch of S.A.D. employed.

After oral vaccination via baits neither of the two vaccines caused a serum response nor gave protection, probably because of the absence of adequate bucco-pharyngeal contact (Baer *et al.*, 1975). The clear-cut correlation between serum response and protection should be noted. All animals showing a serum response before challenge resisted, whatever the level of antibodies. Conversely, all animals showing no serum response succumbed to the

Table 5 : Experiments carried out in 1976-1977 Flury H.E.P. vaccine administered by direct oral deposition at different dose levels.

| Results | Type of vaccination | | | |
|--------------------------------------------------------------------|-------------------------|----------------------|------------------------------|-------------------------------|
| | No vaccination controls | Ingested pure (1 ml) | Ingested diluted 1/10 (1 ml) | Ingested diluted 1/100 (1 ml) |
| Mean increase in antibody titre per ml of serum. | Nil | 0.5 ⁽¹⁾ | 0.5 ⁽²⁾ | Nil |
| 48 days after vaccination | | | | |
| Same increase in the animals dead after challenge on the day death | less than 0.1 I.U. | 4.4 I.U. | 3 I.U. | Nil |
| Number of foxes dead after challenge with 708 mouse LD50 | 5/5 | 4/10 ⁽³⁾ | 8/10 ⁽³⁾ | 9/10 ⁽³⁾ |
| Presence of rabies virus in : | | | | |
| Salivary glands | 5/5 | 3/4 | 6/8 | 6/9 |
| Saliva | 3/5 | 0/4 | 3/8 | 2/9 |

(1) In this group 5/10 animals showed no serum response.

(2) In this group 8/10 animals showed no serum response.

(3) Amongst the surviving animals some showed characteristic rabies symptoms. The salivary excretion of rabies virus was not sought in these animals.

Table 6 : The evolution of neutralising antibody titre in (I.U.) in the animals used in 1976-1977 after vaccination with different doses of Flury H.E.P. vaccine.

| Experimental groups (10 foxes per group) ⁽¹⁾ Dilution of vaccine Flury-H.E.P. (1 ml) | Day of vaccination | Post vaccination interval | | | | |
|----------------------------------------------------------------------------------------------------------|--------------------|---------------------------|----------------------------------------------|-----------------------------|-----------------------------------------|-------------------------------------|
| | | Before challenge | | Post-challenge | | |
| | | 50 days after vaccination | 94 days after vaccination (day of challenge) | Day of death ⁽²⁾ | Surviving 148 days after vaccination | Foxes 568 days after vaccination |
| 1/1 | Titre nil | 0 | 0.01 | | 0.05 | 0.1 |
| | | 0.3 | 0.5 | | 33 | 23 |
| | | 0.2 | 1 | 6 survivors | 44 | 37 |
| | | 1.5 | 0.2 | | 0.5 | 0 |
| | | 0.3 | 0.3 | | 2.5 | 0.2 |
| | | 0 | 0 | | 0.5 | 0 |
| | | 0 | 0 | | 3 | |
| | | 0 | 0 | 5.3 | | |
| | | 0 | 0 | 4.8 | 4 dead | 4 dead |
| | | 0 | 0 | - | | |
| 1/10 | Titre nil | 0.8 | 1.7 | 2 survivors | 136 | 9.2 |
| | | 0.1 | 0 | | 11 | 38 |
| | | 0 | 0 | 0.5 | | |
| | | 0 | 0 | 2.4 | | |
| | | 0 | 0 | 6 | | |
| | | 0 | 0 | - | 8 dead | 8 dead |
| | | 0 | 0 | - | | |
| | | 0 | 0 | - | | |
| | | 0 | 0 | - | | |
| | | 0 | 0 | - | | |
| 1/100 | Titre nil | Titre nil | Titre nil | 1 survivor | 0.3 | 0.1 |
| | | | | 0 | | |
| | | | | 0 | | |
| | | | | 0 | | |
| | | | | - | 9 dead | 9 dead |

(1) In the 5 controls all died after the challenge ; none had antibodies before death. After death the sera from 2 could be measured : each had a titre of 0.1 I.U.

(2) The cases marked with a dash correspond to sera in which it was not possible to determine the titre.

challenge dose, except one vaccinated with Flury H.E.P. at 1/100 dilution (see Table 6).

B. 1975

Scrutiny of Table 3 shows that despite the elevated titre of vaccine-virus (even after 12 hours) in the suckling mouse baits, the ingestion of the bait led to neither a serum response nor gave protection in the 14 foxes in the experiment. Here again the bucco-pharyngeal contact must have been insufficient, either because the capsule was poorly crushed or because the suckling mice were swallowed whole without mastication.

C. 1976-1977

The experiment above all allowed the precise determination of the regression line expressing the resistance of the foxes to the same challenge dose as a function of the dose of Flury H.E.P. vaccine ingested orally directly. The regression line is shown in Figure 6. Its linearity is highly significant since $\chi^2 = 0.16$ (5% confidence limit = 3.86).

The resistance of the foxes to the challenge is thus directly related to the concentration of the vaccine. An infective power of $10^{5.5}$ LD50, determined on suckling mice, was equal to a 50 per cent protection of foxes in our experimental conditions. The long-term evolution of this protection shown in Figure 4 confirms the stability of the immunity with time, as was reported for the fox by Schmidt *et al.*, 1968, or the dog by Sikes *et al.*, 1971.

2. GENERAL CONCLUSION

The results of the experiments carried out over the four years generally confirm those obtained by other authors and bring the number of tests with foxes to about 200 for the strain S.A.D. and about 70 for the Flury H.E.P. Strain, with reference to the indications of W.H.O. 1972 and 1974.

The lack of pathogenicity of the two strains is thus confirmed for the fox, a species nevertheless reputed to be very sensitive to wild virus strains (Sikes, 1962 ; Atanasiu *et al.*, 1970 ; Fersing, 1973).

Their immunogenicity by the direct oral route is also confirmed and more accurately assessed (for the Flury H.E.P. strain) as a function of the titre of vaccine used. By contrast, the retention of immunogenicity was, under our experimental conditions, demonstrated to be zero when the vaccines were

incorporated into baits. This contradicts the observations of some authors. The difference probably results from the low residual titre of vaccine-virus in the bait at ingestion (in the case of hen heads) or an inadequate contact with the buccal mucosa (in the case of the suckling mice).

In any event, future research should be concentrated on improvement of titre and contact. Simultaneously a better knowledge of the residual pathogenicity of the S.A.D. strain should be urgently acquired in order to reduce this before proposing its usage in the field.

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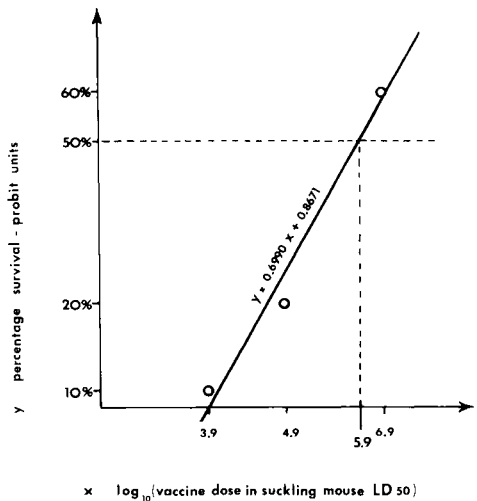


Fig. 6. - Protection conferred by the direct oral deposition of the Flury H.E.P. vaccine as a function of the dose of vaccine.

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Summary

The effectiveness of oral vaccination against rabies particularly by the oral route was studied in 99 red foxes (*Vulpes vulpes*). Two live virus vaccines were tried, one obtained from the strain Flury H.E.P., the other from the S.A.D. strain.

The two vaccines were totally harmless in the fox. Their direct deposition in the mouth provoked the formation of serum antibodies and protected against an intramuscular challenge by the wild strain of the virus fatal in the controls. The proportion of foxes showing a serum response and protected against the challenge varied in each group with the viral titre of the vaccine distributed. Thus the titre of Flury H.E.P. necessary to protect 50 per cent of the foxes was $10^{5.9}$ « suckling mice LD50 ». However, this viral titre suffers a considerable diminution after incorporation into the baits offered to the foxes (hen heads, suckling mice) ; in addition these baits do not permit an adequate contact between the virus vaccine and the buccal mucosa.

These difficulties, added to the persistence of a residual pathogenicity of these virus vaccines in certain wild species other than the fox (rodents in particular), continue to limit the practical application of oral vaccination as a method of prophylaxis of rabies in foxes.

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