IS IT POSSIBLE TO VACCINATE YOUNG CANIDS AGAINST RABIES AND TO PROTECT THEM?

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1 INTRODUCTION.

Two canid species, the dog and the fox, are the most important reservoirs of rabies in terrestrial mammals. When considering human health, dogs remain the most important host and vector of rabies. They are responsible for estimated 35000 to 50000 human deaths due to rabies worldwide.

Control of the disease is generally achieved by vaccination. In countries where sylvatic rabies exists, oral vaccination campaigns conducted with safe and efficient baits make possible the eradication of the disease. In countries where canine rabies is endemic, WHO recommends mass parenteral vaccination campaigns of dogs and promotes, for dogs impossible to reach, the use of oral immunisation (WHO, 1993). WHO has made recommendations on safety of anti-rabies oral vaccines.

An important part of these populations is constituted of young animals. Vaccination schemes generally indicate that the first vaccination should not be done in dogs younger than 3 months because of the possible presence of blocking levels of maternal antibodies that might interfere with the active immunisation due to vaccination (Povey, 1997).

If the immunology of young dogs is well-known, very little is known of the immunity of the young fox. This paper gathers results obtained during studies of the response to anti-rabies vaccination of young dogs and foxes.

2 RESPONSE OF YOUNG DOGS FOLLOWING VACCINATION.

2.1 Mass parenteral vaccination campaigns in Tunisia.

2.1.1 Surveillance protocol.

This study was conducted near Tunis (Seghaier et al., 1999). From 1982 till 1992 the Tunisian programme for rabies control included mass parenteral vaccination campaigns of dogs every two years. This led to a decline in animal and human rabies cases. However since 1988, the number of human rabies cases has increased and 25 human deaths occurred in 1992. Therefore, since 1992, mass parenteral vaccination campaigns were carried out every year.

A serological survey was conducted in a suburban area close to Tunis (Sanhaja) to study the ability of dogs to respond to rabies vaccination.

The vaccine used for parenteral vaccination (Rabirabta) is produced locally by the Veterinary Research Institute in suckling lambs inoculated with CVS.Brains are harvested when animals are para-

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lysed and the suspension is then inactivated with \( \beta \)-propiolactone and adjuvanted with aluminium hydroxide. All batches are tested for potency in Nancy.

During the study 1ml dose of vaccine was administered subcutaneously to dogs at day 0 and one year later (D365). Two different batches of vaccine titrating 8 and 8.8 IU/ml were used during the study. Blood samples were collected at day 0, 30, 210, 365 and 395 (i.e. 1 month after the second vaccination). The rabies virus neutralising antibody titres were determined by using a modified version of the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973; Zalan et al., 1979).

### 2.1.2 The dog population.

Data related to the demography of the dogs (301 dogs at beginning of the survey) were based on owners information. Twenty three per cent of houses have a dog (348 animals), the dog:human ratio is 1:15.6.

The average ages of dogs was 2.6 years; the annual turnover rate per year was estimated to be 37%.

Thirty six per cent of the dogs were less than 1 year old (figure 1) and 16 % of these dogs were puppies (less than 3 months). The male/female ratio is 2.03. The percentage of dogs less than 3 months old in this study is not significantly different from that reported in other parts of the world (Table 1).

**Figure 1: Structure of the dog population in Sahanja.**

![Chart showing the proportion of dogs by age and gender](image)

**Table 1: Field data related to the proportion of owned dogs younger than 3 months.**

<table>
<thead>
<tr>
<th>Study area</th>
<th>Percent of dogs less than 3 months</th>
<th>1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cleaveland S., 1996</td>
<td>Tanzania (Serengeti)</td>
<td>12</td>
</tr>
<tr>
<td>Matter et al., 1998</td>
<td>Tunisia (El Bassatine)</td>
<td>7.4</td>
</tr>
<tr>
<td>Matter et al., 1993</td>
<td>Turkey</td>
<td>7-20</td>
</tr>
<tr>
<td>Chomel et al., 1988</td>
<td>Peru (Lima – Calla)</td>
<td>7-20</td>
</tr>
<tr>
<td>Matter et al., 2000</td>
<td>Sri Lanka</td>
<td>1.7 – 11.1</td>
</tr>
<tr>
<td>Matter et al., 2000</td>
<td>Kenya (Masaï Mara)</td>
<td>21.5</td>
</tr>
</tbody>
</table>

### 2.1.3 Serological surveillance.

All dogs were not always available during the different periods of the blood samplings. Among the 301 dogs initially included in this survey, 95%, 88%, 72%, 60% and 54% were sampled at D0, D30, D210, D365 and D395 respectively.
Figure 2 shows that one month after the vaccination, the percentage of dogs with titre ≥ 0.5 IU/mL was significantly increased in all age groups. Then it decreased during the following months. Twelve months after the first vaccination only 29 to 44% of the tested animals were ≥ 0.5 IU/mL. One month after the booster this proportion reached 92 to 100 %, depending on the age group. The percentages observed for the puppies and for the dogs younger than 1 year were not significantly different from the rest of the population all along the study.

Twenty six per cent of the puppies (n=39) had neutralising antibody titres greater or equal to 0.5 IU/mL at Day 0. These antibodies were considered of maternal origin. In figure 3 two groups of animals have been done, those having undetectable antibodies at day 0 (n=29) and those having titres of at least 0.5 IU/mL. One month after the first vaccination, puppies that showed titres ≥0.5 IU/mL at Day 0 had a significantly higher neutralising titre (t=2.68, df=23, p=0.011). Thereafter, the kinetic of rabies antibodies responses at months 7, 12 and one month after the booster were similar in the two groups.

Figure 2: Percentage of dogs within each age group with a neutralising antibody titre of at least 0.5 IU/mL.

During this survey, no dog died of rabies or showed clinical symptoms of rabies. Therefore the rabies neutralising antibodies were the result of an immunological response to vaccination and were not due to rabies infection.

2.1.4 Conclusion.

The results of this study confirm the necessity to organise yearly parenteral vaccination campaigns to maintain a minimal protection in the dog population. The serological survey of puppies showed that maternal antibodies do not seem to hamper seroconversion of puppies and the neutralising activity of their serum.

2.2 Other serological surveillance of puppies vaccinated against rabies.

Only few data have been collected on rabies vaccination protocols aiming to define the best period to vaccinate young dogs.

Chappuis (1998) vaccinated puppies born of bitches immunised with an inactivated adjuvanted antirabies vaccine (Rabisin). The vaccination was made on 14 days old animals that presented neutralising antibodies, three vaccines have been used (rabisin, two doses of a canary pox recombinant). All
the animals were challenged with New York strain at the age of 115 days. Figure 3 shows the kinetics of neutralising antibodies level of puppies after vaccination. Whatever the antibody titre at two weeks after vaccination, all animals had a low level of antibodies when challenged, the result of the challenge is given in Table 2.

The survival after challenge was correlated to the immunogenicity of the vaccine used. Although obtained with a limited number of animals these data confirm that rabies antibodies (or at least the level of these antibodies) are not the only factor that protects against rabies.

Figure 3: Kinetics of antibodies of puppies vaccinated at the age of 14 days and challenged at 115 days.

Table 2: Result of the challenge of 16 vaccinated puppies born of vaccinated bitches.

<table>
<thead>
<tr>
<th>Groups of animals</th>
<th>Survival rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>0 out of 4</td>
</tr>
<tr>
<td>Canary pox recombinant 10^8.5</td>
<td>2 out of 4</td>
</tr>
<tr>
<td>Canary pox recombinant 10^7</td>
<td>4 out of 4</td>
</tr>
<tr>
<td>Rabisin</td>
<td>4 out of 4</td>
</tr>
</tbody>
</table>

Aghomo et al. (1990) studied the response to vaccination with a Flury LEP vaccine of dogs born from vaccinated and unvaccinated bitches. The first vaccination was performed on 4, 6, 8, 10 and 12 weeks old animals, two boosters were administered at the age of 12 and 26 weeks. Figure 4 shows the observed kinetics. There is a clear interference when the first vaccination was done at 4, 6 or 8 weeks of age, puppies born of vaccinated bitches have always a lower titre than the controls born of unvaccinated bitches, even after the booster vaccinations. When the first vaccination is made on 10 weeks old animals, the interference phenomenon is less clear, puppies born of vaccinated vixens have a lower titre till the second booster. When the first vaccination is made on 12 weeks old animals, there is no difference between the two groups of animals. In this study, maternal antibodies (or the interference-inducing factor) are active up to the age of 3 months. But animals have been vaccinated with a live vaccine that may also induce cell-mediated immunity and without challenge, it is difficult to consider that the neutralising antibody level characterises the protection to infection conferred by the vaccine which is the key element in rabies control through vaccination.

A previous study on laboratory dogs demonstrated that passive antibodies of maternal origin interfere with active immunisation (Précausta et al., 1985) this work compared the level and the kinetics of the humoral response in puppies born of immune and non-immune dams and vaccinated against rabies with an inactivated vaccine. The results show that puppies born of non immunised dams and vaccinated against rabies at 1 month of age responded to vaccination with neutralising titres similar to puppies vaccinated at 7 months of age. However, the antibody synthesis of immunised puppies born from dams and vaccinated at 1 month of age was inhibited by the persistence of maternal antibodies.

Similar results were recorded in a study (Van Kampen, 1999) reporting solid protection against lethal challenge of 2 week-old and 6 week-old puppies still having maternal antibodies and vaccinated with a single injection of recombinant Canary Pox rabies vaccine or vaccinia recombinant vaccine (Raboral VRG).
Figure 4: Kinetics of antibodies following Flury LEP vaccination of puppies born of vaccinated or unvaccinated bitches at the age of 4 weeks (panel a), 10 weeks (panel b) and 12 weeks (panel c). Boosters were administered at the age of 12 and 26 weeks.

3 ORAL VACCINATION OF YOUNG FOXES (*VULPES VULPES*).

The control of rabies in fox populations must address different problems: the increase of fox populations, the persistence of residual foci, the risk of re-infection of freed areas, the age structure of fox population. Figure 5 shows the evolution of a fox population orally vaccinated against rabies during a three year period. The impact of oral vaccination measured by the bait uptake in the adult portion of the population is clearly different from the one observed in the "cub" part. After the third campaign, 75% of adults are vaccinated and even with an increasing population, the proportion of vaccinated animals does not decrease. The observation of the "cub" part of the population shows a global increase of the population, more important than the one observed in adults. The proportion of vaccinated animals remains relatively low (20 to 50%) and constant in this group, which means that there is an increasing number of unvaccinated receptive cubs, leading to an important decrease in the impact of oral vaccination in the fox population.
Figure 5: Evolution of an infected red fox population after oral vaccination campaigns.

In spring cubs constitute two thirds of the fox population, their vaccination is then crucial to maintain the vaccination coverage. In areas that have been vaccinated several times, cubs are born from immunised vixens; maternal antibodies may hamper active immunisation of fox cubs during the spring campaigns.

Different parameters have been monitored: duration of the maternal antibodies, age of immunocompetency, protection against challenge conferred by oral vaccination. The results presented here have been obtained during a project financed by the European commission (contract FAIR CT97-3515: "Rabies vaccination in emergency - Wildlife vaccination rabies in difficult and emergency situations and its potential impact on the environment") and more precisely in the phase dealing with the study of immunisation of fox cubs with oral vaccines.

The vaccines that have been studied over the two-year experiment are the two oral vaccines that fulfil the requirements of WHO, a vaccinia recombinant expressing the glycoprotein of rabies virus (V-RG) and a double mutant from the SAD Berne strain (SAG2 vaccine).

Both experiments followed the same general protocol. The animal model used in this study was the silver fox, which belongs to the same species as the "wild" red fox, *Vulpes vulpes*. All silver fox cubs used were born in the experimental farm of our laboratory. One to 4 years old males and 2 to 9 years old females were purchased at least one month before the beginning of the reproductive activity of the vixens from the Norwegian Fur Breeder’s Association (Oslo, Norway). On arrival, all animals were treated with anthelmintics (Droncit®, Bayer Pharma, France and Ivomec®, Merial SAS, France) and were vaccinated against canine distemper, viral hepatitis, parvovirus infection, infectious tracheobronchitis and leptospirosis with Canigen® CHPPi/L (Virbac, France). They were kept in individual cages, fed daily with a commercial dry food for adult dogs and water was provided *ad libitum*. All animals were observed daily.

Vixen sexual cycles were monitored using both vaginal resistivity and keratinisation of epithelial cells in vaginal smears. Once a vixen was determined receptive, a male was co-housed with it for one day. After this, detection of spermatozoa was performed by examination of vaginal smears to assess the covering. In this way it was possible to determine precisely the beginning of the gestation period and the estimated parturition time (mean gestation period is 52 days). Covered vixens were then transferred to maternity cages and pregnancy was verified 30 days later by echography and trans-abdominal palpation.

When cubs of the litter were 8 to 9 weeks old, the dam was removed and the cubs were re-caged usually in pairs. They were fed daily with commercial dry food for young dogs with drinking water *ad libitum*. According to their size (generally when 3 to 4 months old), young foxes were placed in individual cages. At this time they were also treated with anthelmintics (Droncit® and Ivomec®) and vaccinated with Canigen® CHPPi/L (two injections, 4 weeks apart).
3.1 Protection conferred to fox cubs by V-RG

Seventeen males and 40 females were included in the protocol. Twenty one pregnant vixens were divided in six groups as shown in Table 3. Twelve vixens vaccinated with V-RG at thirty days of pregnancy and nine vixens kept as controls. In both groups, litters were divided in 3 sub-groups: cubs vaccinated when 30 days old (groups E and H), cubs vaccinated when 3 months old (groups F and J) and non-vaccinated cubs (groups G and K). Both adult and young foxes received the V-RG vaccine by direct instillation into the oral cavity (2.7 ml corresponding to the average dose of the batch used in the test). All young foxes were blood sampled when 30 days old and thereafter at 2 weeks intervals until 5 months of age. When 5 months old, cubs were challenged intramuscularly with $10^3$ MICLD$_{50}$ of a street strain isolated from sub-maxillary glands of a naturally rabies infected fox.

Table 3: Groups of fox cubs vaccinated with V-RG and calendar of the experiment.

<table>
<thead>
<tr>
<th>Litter codes</th>
<th>Vixens vaccinated at 30+/−2 days of pregnancy</th>
<th>Unvaccinated vixens</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of cubs</td>
<td>E1 E2 E3 E4 F1 F2 F3 F4 G1 G2 G3 G4 H1 H2 H3 H4 J1 J2 J3 K1 K2</td>
<td></td>
</tr>
<tr>
<td>Vaccination</td>
<td>D30 D90 no vaccination</td>
<td>D30 D90 no vaccination</td>
</tr>
<tr>
<td>Serological survey</td>
<td>titration of neutralising antibodies from 1 month to 5 months at 2 weeks intervals</td>
<td>at 5 months of age</td>
</tr>
<tr>
<td>end of the experiment</td>
<td>65 days post-challenge</td>
<td></td>
</tr>
</tbody>
</table>

The disappearance of maternal antibodies in fox cubs born from vaccinated vixens and allocated to the groups F (vaccinated at 90 days old) and G (unvaccinated) is shown in Figure 8b; litters of group K. Individual levels of rabies neutralising antibody were highly variable.

Figure 6: Distribution of the mean neutralising titre of a litter according to the neutralising titre of the vixen.

This high inter-individual variability in neonates has been already hypothesised (review by Chappuis, 1998) as a possible consequence of the rank of birth (therefore a greater possibility to consume colostrum for those born first), the size of the litter, and the rates of antibodies in vixens. In this study however, no significant correlation was found between the levels of antibodies in cubs and those of their respective mothers (Figure 6).
Figures 7 and 8 describe the kinetics of acquired humoral immunity of fox cubs born from vaccinated or non-vaccinated mothers and vaccinated at 30 days (groups E and H) and at 90 days of age (groups F and J) respectively compared to control litters (group K, unvaccinated cubs born of unvaccinated vixens). As early as fifteen days after oral administration of the vaccine, mean titres were significantly increased for all groups of cubs whatever their immune status before vaccination. Furthermore, mean antibody titres did not differ significantly during the study whether cubs were born from vaccinated vixens or from non-vaccinated vixens (0.059 < p < 0.451 for cubs vaccinated at 30 days of age; 0.063 < p < 0.897 for cubs vaccinated at 90 days of age). Then mean antibody titres remained stable until at least five months of age, when all cubs were submitted to a virus challenge.

Figure 7: Kinetics of neutralising antibodies of 30 days old fox cubs orally vaccinated with V-RG. Unvaccinated litters K1 and K2 born of unvaccinated vixens were used as controls.

No significant difference was observed between mean antibody titres of fox cubs whether or not they were issued from vaccinated or non vaccinated vixens and whether they were vaccinated at 30 days or at 90 days of age (0.144 < p < 0.935). Following the rabies challenge, whatever the immune status of the vixens, all vaccinated young foxes (n = 65) resisted challenge and all unvaccinated controls (n = 29), except one, died of rabies.

This study demonstrated that vixens orally vaccinated with V-RG during pregnancy transfer neutralising antibodies to their offspring. However no correlation was found between titres of cubs and those of their mother, contrary to the results reported by Müller et al. (1999). We hypothesise that this absence of correlation may be related to individual differences in colostral absorption and difference in antibody concentration in colostrum.
Figure 8: Kinetics of neutralising antibodies of 90 days old fox cubs orally vaccinated with V-RG. Unvaccinated litters K1 and K2 born of unvaccinated vixens are used as controls.

The duration of passive immunity ranged between 45 and 75 days after birth (Figure 8a) was depending upon the levels of antibodies reached at 30 days of age and this period. It should be noted that the kinetics of disappearance of antibodies of maternal origin was similar for all litters.

3.2 Protection conferred to fox cubs by SAG2. (preliminary results)

This part of the programme was made during the next year and the final interpretation of the results obtained is in process. To assess the reproducibility of the results between these two sets of serological analyses, 5 sera already tested during year 1 have been retested blindly during year 2.

Thirty-one males and 70 females were included in the experiment. The general arrangement of the 27 litters (122 cubs) obtained is summarised in Table 4.

Table 4: Groups of fox cubs vaccinated with SAG2 and calendar of the experiment.

<table>
<thead>
<tr>
<th>Litter codes</th>
<th>number of cubs</th>
<th>Vaccination</th>
<th>Serological survey</th>
<th>end of the experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vixens vaccinated at 30+/2 days of pregnancy</td>
<td>Unvaccinated vixens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L1 L2 L3 L4 L5</td>
<td>4 1 6 7 5</td>
<td>D30</td>
<td>titration of neutralising antibodies from 1 month to 5 months at 2 weeks intervals</td>
<td>65 days post-challenge</td>
</tr>
<tr>
<td>M1 M2 M3 M4</td>
<td>5 5 4 4</td>
<td>D90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N1 N2 N3 N4 N5</td>
<td>5 2 5 3 5</td>
<td>no vaccination</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1 P2 P3 P4</td>
<td>1 4 5 5 6</td>
<td>D30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Q1 Q2 Q3 Q4</td>
<td>5 3 3 5</td>
<td>D90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>R1 R2 R3 R4 R5</td>
<td>5 3 3 3</td>
<td>no vaccination</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Serum samples collected during the previous experiment were blind tested during these tests to assess the correlation between the two groups of tests.

Maternally derived antibodies may persist for up to 70 days. As observed for V-RG, there is no correlation between the neutralising titre of the female and the one observed in the offspring. Among the 66 cubs born of vaccinated vixens, 57 had no detectable neutralising antibody 2 weeks after oral vaccination. Five of the 9 other cubs belonged to the same litter (N5), 2 to litter L4 and one to litters L3 and N1; their titres ranged between 0.29 and 0.98 IU/ml.

A clear interference exists between this passive immunity and the seroconversion of cubs vaccinated when 30 days old (Figure 9a). Cubs born of vaccinated vixens constantly show a neutralising titre lower than the one observed in litters born of unvaccinated vixens.

Figure 9: Kinetics of neutralising antibodies of 30 days old fox cubs orally vaccinated with SAG2. Unvaccinated litters R2 to R5 born of unvaccinated vixens were used as controls.

No interference was observed when vaccination is made when animals are 90 days old. Response to vaccination (increase in neutralising titre 15 days after vaccination) and kinetics of the neutralising titre are not statistically different between the two groups.

All except two vaccinated cubs resisted challenge, both belonged to the group vaccinated at the age of 30 days. One of them did not show any neutralising activity throughout the study, the other one was serologically negative except on the control performed at 60 days of age (i.e. 0.37 IU/ml, log = -0.43). But it must also be emphasised that such serological responses have also been observed in cubs that resisted challenge.

The very early ability of canids to respond to oral vaccination with the SAG2 has been observed (Schumacher et al., 1997) who tested the innocuity of a highly concentrated SAG2 suspension ($10^6$ TCID$_{50}$) in puppies. Twenty puppies (7 to 10 weeks old) were inoculated either per os or by the intramuscular route. No animal developed clinical signs during the 120 day period of the trial. All dogs rapidly synthesised (7 days after inoculation) rabies antibodies and titres remained stable during the four months observation period (Figure 10).
3.3 Conclusions of the experiment.

The analysis of data collected during the second part of the experiment (SAG2 vaccine) is still in process. However some conclusions may already be drawn.

- One-month-old fox cubs are able to respond to oral vaccination with SAG2 or V-RG, this capacity may exist earlier. This is confirmed by the resistance to challenge of cubs born of unvaccinated vixens and orally vaccinated at the age of 30 days.

- When vixens are vaccinated, no correlation is observed between the neutralising titre of the vixen and that of the offspring. This passive immunity lasts less than 75 days.

- Passive immunity of maternal origin may interfere with neutralising serological response of fox cubs vaccinated at the age of 30 days with SAG2. No interference is observed with V-RG vaccine. No interference has been observed when vaccination is made at the age of 90 days.

- Cubs vaccinated with V-RG at the age of 30 days resist challenge whatever the vaccinal status of the vixen. If the vixen has been vaccinated with SAG2, one-month-old cubs vaccinated orally with SAG2 may not resist challenge.

- On a more general point of view during epidemiological studies, serological data obtained in young animals (less than 3 months) do not necessarily come from the vixen because one month old fox cubs can respond to the stimulation at least of some antigens.

4 Conclusion.

Domestic species are generally immuno-competent at birth. However, an additional maturation of the immune response occurs during neonatal period. The vaccination of young canids must be considered and encouraged for different reasons:

- Dog and fox populations are young populations with a rapid turnover.
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- Rabies control

- Puppies are generally confined and less mobile than adult dogs and therefore they are easier to reach and to handle.
- Puppies are in frequent contact with young children and frequently fondled by people.
- Low amounts of maternal antibodies in neonates may be insufficient to prevent a rabies infection.
- Life expectancy of live-born puppies is very low during the first three months of life.

The lack of interference between maternal antibodies against rabies and active immunisation conferred by vaccination have been observed in young dogs vaccinated with inactivated vaccines given parenterally and in young foxes vaccinated orally with V-RG vaccine.

When annual mass vaccination campaigns of dogs are planned with inactivated vaccines given parenterally puppies must be included whatever the immune status of the bitch. This measure should contribute to a decrease in rabies infections in children. It should be outlined that WHO do not recommend the use of live attenuated vaccines that may be pathogenic in puppies.

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REFERENCES.


