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To cite this version:
Philippe Vuillaume, Virginie Bruyere, Michel Aubert. Comparison of the effectiveness of two protocols of antirabies bait distribution for foxes (Vulpes vulpes). Veterinary Research, BioMed Central, 1998, 29 (6), pp.537-546. <hal-00902546>

HAL Id: hal-00902546
https://hal.archives-ouvertes.fr/hal-00902546
Submitted on 1 Jan 1998

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Comparison of the effectiveness of two protocols of antirabies bait distribution for foxes (*Vulpes vulpes*)

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(Received 6 April 1998; accepted 16 July 1998)

Abstract – In a plateau and hill region of France (the Doubs), two protocols of rabies vaccine bait distribution targeted at foxes were compared: helicopter distribution of vaccine baits alone (control zone) and a combined aerial distribution by helicopter with an additional deposit of vaccine baits at fox den entrances by foot (test zone). In the test zone covering an area of 436 km\(^2\), baits were distributed by helicopter at a rate of 13.4 baits/km\(^2\). Additionally, an average of 11.4 vaccine baits at the entrances of 871 fox dens were terrestrially distributed by 110 persons (9,964 baits). In this test zone, 90\% of the young foxes were marked with tetracycline which permitted estimation of the bait consumption; however, only 38\% had significant titre of rabies antibodies and less than one fox cub per 2.4 of those having consumed at least one bait were immunized. In the control zone, these percentages were significantly lower: respectively, 35 and 17\% and one fox cub per 4.2. The relative lack of benefit between bait uptake and rate of immunological response may be due to maternal immunity which could have interfered with fox cub active immunization. A booster effect following a second distribution of baits by foot may be suggested in both adult foxes and their offspring. That these baits needed to be terrestrially distributed in order to obtain a booster effect is uncertain. Terrestrial distribution at fox den entrances is difficult to do and entails additional expenses not incurred in aerial distribution. The cost of terrestrial vaccination is 3.5 times higher than classical aerial vaccination and takes 63.5 times longer. A cost effective analysis of this type of supplementary terrestrial intervention determined that bait deposit at den entrances can be recommended for restricted areas, where residual focii exist, as a complement to the aerial distribution of baits. © Inra/Elsevier, Paris.

rabies / oral vaccination / fox cub / fox den / field trial

Résumé – Comparaison de l’efficacité de deux protocoles de repartition d’appâts vaccinaux antirabiques destinés au renard (*Vulpes vulpes*). Dans un département français de collines et plateaux (le Doubs), deux protocoles de distribution d’appâts vaccinaux antirabiques destinés aux renards ont été comparés : un largage aérien par hélicoptère seul dans la zone témoin et un largage aérien par hélicoptère associé à une distribution réalisée à pied devant les terriers en zone test. La zone test...
couver une surface de 436 km², sur laquelle l'hélicoptère a d'abord permis de déposer 13,4 appâts vaccinaux/km² puis 110 personnes ont distribué en moyenne 11,4 appâts vaccinaux à l'entrée de 871 terriers (9 964 appâts). Dans cette zone, 90 % des renardeaux étaient marqués à la tétracycline (ce qui permet d'évaluer la consommation d'appâts) mais seulement 38 % ont présenté un titre en anticorps antirabiques significatif, soit un renardeau pour 2,4 ayant consommé au moins un appât. Dans la zone témoin, ces proportions étaient significativement plus faibles, respectivement 33 et 17 % et un renardeau pour 4,2. La perte d'efficacité relative entre le taux de prise d'appâts et le taux d'immunisation des renardeaux peut être due à une interférence entre immunité passive et immunité active, la plupart des renardeaux étant nés de renardes déjà vaccinées. La seconde distribution d'appâts réalisée aux terriers peut avoir induit un effet de rappel vaccinal chez les renards adultes et les renardeaux. Cependant cet effet rappel n'est pas nécessairement lié à la méthode de distribution terrestre des appâts vaccinaux. Le dépôt d'appâts vaccinaux à l'entrée des terriers est une méthode lourde et onéreuse, qui a coûté 3,5 fois plus cher que la vaccination classique par hélicoptère et a nécessité 63,5 fois plus de temps. Une étude coût – bénéfice de cette intervention supplémentaire devant les terriers indique que cette méthode devrait être réservée exclusivement à des situations bien précises : petites surfaces où persiste un foyer résiduel de rage et seulement en complément d'une distribution aérienne d'appâts vaccinaux. © Inra/Elsevier, Paris.

1. INTRODUCTION

The oral vaccination of foxes against rabies began in France in 1986 as part of an international experiment on a land area of 720 km² [2]. Distribution methods involved many participants, the majority being hunters. As vaccine surface areas increased, helicopters were used starting in 1988, with distribution occurring in spring and autumn at a rate of 13 baits per km² [4]. This method globally reduced the incidence of rabies by 99 % over 6 years [6]. However, the complete eradication of rabies faces two major roadblocks – the persistence of the disease in some areas even after five consecutive vaccine campaigns or more (which has led to the creation of residual rabies infested zones), and the risk of reinfection of zones already rabies controlled. The risk of reinfection is due to an increase in fox population already noted in several regions of France [5, 26] and elsewhere in Europe [22].

For these reasons, two different protocols have been tested to improve classical fox vaccination. A vaccination campaign of foxes was undertaken in the summer of 1992 with the vaccine Raboral VRG. The goal being to vaccinate young foxes earlier than that permitted by bait distribution in the autumn. Indeed, after a spring vaccination campaign, only 20–30 % of young foxes carried traces of tetracycline (the biological marker incorporated into baits) [20]. However, fox cubs accounted for at least two thirds of the total fox population. The increase in young fox population density has been the main reason behind the continued problem of rabies in the Jura region of Switzerland [12].

A second adaptation to traditional methods has been the vaccination of some zones in two aerial passages separated by a period of 15 days in order to provoke a booster effect in the adult fox population [7]. A third adaptation has been to increase the number of vaccine baits distributed in the course of the same passage, a method practised in France since 1996 [16, 21] as well as in other countries [14].

Finally, a vaccination campaign depositing vaccine baits at the entrances of fox dens aiming to improve the vaccinal cover of young foxes was begun in France in 1995 [26] and continued in 1996 [3]. This method has likewise been used in Switzerland since 1994 [11], in Germany since the summer of 1995 [25] and in Belgium since June 1995
In the spring of 1995 this method, in an experimental stage, was begun in a region of the Doubs where rabies has persisted despite the carrying out of nine oral vaccination campaigns by helicopter. Preliminary results have been encouraging [26], and this campaign has been extended to neighbouring areas where rabies has been a constant problem.

Herein are reported the results of two protocols comparing the distribution of rabies vaccine baits solely by helicopter to their manual distribution at the mouth of fox dens complemented with aerial vaccine distribution by helicopter.

2. MATERIALS AND METHODS

2.1. Field study areas

An area of 2,977 km\(^2\) received a distribution of vaccine baits (SAG2) in the department of the Doubs (France) in the spring of 1996 by helicopter (figure 1) according to a method previously described [4]. This region is made up of bordered plateaux intersected by two rivers: the Doubs and the Dessoubre. These rivers descend from 600 to 350 m in altitude. The gorges created by these rivers give birth to the resulting uneven land which fluctuates between 200 and 400 m. A sub-area of 436 km\(^2\), defined as the test zone, additionally received vaccine deposits (VRG) at the entrances of fox dens in the beginning of June 1996. This test zone was in conjunction with a zone located in Switzerland which had been vaccinated in like manner in 1994, 1995 and in 1996 ([12] and pers. comm.). The remaining area of 2,541 km\(^2\), not receiving the additional terrestrial vaccine deposit, was defined as the control zone.

2.2. Vaccine baits

The bait Oral Rabifox (Laboratory VIRBAC – Nice, France), an attenuated rabies virus obtained by double mutation (SAG2), was cased in a plastic polyvinyl chloride capsule that measured 4.3 x 5 x 1.5 cm and weighed 27 g.

The bait Raboral (Laboratory Merial – Lyon, France), a recombinant vaccinia virus expressing the glycoprotein of the rabies virus (VRG), was cased in a polyethylene sachet measuring 3.2 x 2 x 2 cm that weighed 40 g.

![Figure 1. Periods and methods of antirabies bait distribution in test and control zones in spring 1996.](image-url)
Baits were composed of an appealing coating made from fish flour and animal grease surrounding a capsule or sachet which contained a 2 mL viral suspension. In both types of bait the outer-coating contained 150 mg of tetracycline that served as a biological marker.

2.3. Preparation procedures

Guidelines for aerial distribution were made according to procedures formalized in France since 1991.

The proper preparation of terrestrial operations was essential in order to mobilize the volunteer efforts of hunters in the region. Preparation undertaken in 1996 alone will be described having been simplified thanks to experience gained in 1995 [26].

The test zone included 53 public and ten private hunting clubs. Their representatives were gathered 2 months before the expected date of vaccine bait distribution where operation methods, procedures and preliminary results were shared. Participants at this time received a map at a scale of 1/25 000 on which known occupied and un-occupied dens were reported. Thereafter, 30 days in advance of initiation of bait depositing, a calendar outlining detailed plans was sent to teams (teams were composed of a local hunter and one or two technician agents responsible for vaccinal bait deposition).

2.4. Bait distribution

Aerial distribution of baits by helicopter was insured by flights totalling 4 h 20 min in the test zone and 30 h in the control zone (figure 1). Classical helicopter distribution of baits in France usually deposits on average 13 vaccine baits per km². In this study, 13.4 baits per km² were distributed by helicopter in both the control and test zones.

Terrestrial distribution at den entrances was carried out over an 8-day period. Baits were thus laid down by approaching the den as discreetly as possible. The den was observed to determine if it was occupied or not. If occupied, ten vaccinal baits were deposited in front of the main entrances. For an occupied den having three or four entrances, five supplementary baits were placed in front of a secondary entrance. When a den had seven or more entrances, a second series of five supplementary baits were left. If a den appeared un-occupied, six baits were left regardless of the number of entrances. For each den the following information was noted: number of entrances, occupation status, description of the site (i.e. on the slope, hillside or plateau) and substrate foundation (i.e. was den dug in the earth or in the rocks).

2.5. Follow-up after vaccine deposition

A follow-up of the disappearance of baits at 7 days post-distribution was asked of hunters for one den in eight. Hunters were given a pre-established form which indicated the location, number of deposited baits, and the name of the distributor for each den.

Game keepers as well as hunters were solicited to collect adult and young foxes in the two zones 10 days after the deposit of the last vaccinal baits. Fox sampling needed to be completed prior to the beginning of the autumn vaccination campaign that was carried out by helicopter (23 September 1996).

A total of 100 foxes were collected in the test zone and 152 foxes in the control zone. Of foxes collected, 94 % were killed in the course of night shootings in accordance with methods described by Roboly [24]. The remaining 6 % were found dead or killed due to their suspicious rabies behaviour. All foxes were diagnosed for rabies [8] and whenever available their canines were cut for determination of tetracycline presence [19] and their sera were titrated for rabies antibodies by an ELISA technique. The ELISA test used plates of 96 wells in polystyrene sensitized to the rabies glycoprotein (Diagnostics Pasteur, France). Sera were diluted to 1/100 and tested in double. Control sera with or without antibodies were tested in like-manner. Positive threshold was determined from a sample of 40 fox sera collected from foxes in rabies-free zones and was fixed as equal to or superior to the non-specific optical density (+ 2 standard deviations). Herein are presented the results obtained from foxes with both presence of tetracycline and titration in order to have the same samples used in the different studies (i.e. 50 and 60 cubs, 31 and 77 adult foxes, respectively, in the test and control zones).

Young and adult foxes were distinguished by histological dental examination [19]. Tetracycline was detected by ultraviolet light examination of tiny canine chips by inverse microscopy (model IMT2-RFL, Olympus). A longitudinal
section of canine (cut to a thickness of about 500 μm) was prepared with a cut saw. The deposit, placed on a blade and immersed in oil was observed by microscope (×100). Tetracycline deposits appeared pale yellow on a blue background [19]. Young foxes analysed had lived through one spring vaccination campaign and any detection of tetracycline could therefore be attributed to consumption of vaccine baits distributed that same year. Every stria of tetracycline corresponds to ingestion of a vaccine bait or of a large piece of it, so that the animal may assimilate enough tetracycline to be marked. Two significant ingestions of tetracycline within an interval of 24 h are followed by two different strias that appear clearly at lecture of histological dental examination [19].

According to the size of raw data, analysis of results was carried out by Chi square test or according to the Fisher-exact factorial test [17].

3. RESULTS

3.1. Field results

3.1.1. Number and characteristics of fox dens visited

In total, 871 fox dens were located and visited which meant on average two fox dens per km². Of these 871 fox dens, the entrances of 844 were recorded: 28 % of noted fox dens had only one entrance with the median being three and the average four entrances per den (figure 2). A description of 771 fox dens was noted (table I).

3.1.2. Rate of vaccine bait disappearance

Compared to the control zone, 9,964 vaccine baits were deposited terrestrially at den entrances (11.4 baits per den on average) in the test zone. To assure complete vaccination of the test zone, an additional 4,366 vaccine baits were deposited at fox den entrances overlooked during the census or in freshly cut prairie lands. Therefore in the test zone a total of 46.3 vaccinal baits per km² were deposited by combined aerial and terrestri al distribution.

At the end of 7 days, data were collected on the disappearance of manually laid-down vaccine baits for 158 fox dens, where a total of 1,941 vaccinal baits had been deposited. A total of 129 Raboral vaccine baits were found intact, slightly nibbled or with the sachet containing the vaccine liquid still intact. The rate of disappearance of vaccine baits laid at den entrances was therefore 93.4 %.

![Figure 2](image-url)  
**Figure 2.** Number of entrances for 844 fox dens visited. Among the 871 dens visited, number of entrances was not noted for 27.
Rabifox bait capsules (distributed by helicopter) were found at the entrances of several fox dens opened and crushed by mastication. This observation was noted for approximately one out of every ten fox dens visited, though this information was not systematically noted by all hunters. When noted, crushed capsules were returned for verification.

3.1.3. Estimated operation costs

The cost of aerial rabies bait vaccine distribution (13.4 baits/km²) was approximately 185 F/km² when taking into account costs of personnel, vaccine baits, materials and the helicopter. If the cost of personnel is removed, cost could be reduced to 165 F/km². For terrestrial rabies bait vaccine distribution at den entrances (32.8 baits/km²) costs were, respectively, 652 and 213 F/km². Global costs in the test zone (vaccination by both helicopter and foot) was approximately 837 and 378 F/km².

A man’s work-day permitted the vaccination of 138.5 and 2 km², respectively, by helicopter and terrestrially.

3.2. Laboratory analyses

Of the 100 foxes collected from the test zone, 43 were adult and 57 were young foxes and of the 152 from the control zone, 85 were adults and 67 were young.

The comparisons of the test and the control zones have shown the following: concerning the young fox population, tetracycline was detected in significantly higher proportions in the test zone (table II); of the 66 young foxes biologically marked with tetracycline in the two zones, those from the test zone had significantly more strias (table III, figure 3); a higher proportion of young foxes were immunized in the test zone (table 11). Furthermore it was observed in the test zone that fox cubs who had consumed baits at least twice, more frequently had rabies antibodies than those that had consumed bait only once (58 versus 24 %), whereas this difference was not observed in the control zone (table III). This last result was observed equally for adult foxes in the test zone (86 versus 37 %) (table III). This result may indicate a booster effect from the second bait distribution.

3.3. Rabies impact

Before the campaign to vaccinate fox dens was undertaken in 1995, rabies was reported throughout the two zones of study. After the vaccination operation in fox dens in 1995 and before those of 1996 only three cases of rabid foxes were reported. The first
case was a tetracycline-free adult fox killed in the test zone on 28 June 1995. Two foxes less than 1 year old were killed on 6 February 1996 and 2 March 1996 at Montécheroux in the control zone (helicopter treated only). The first of these last two foxes was not marked with tetracycline, the second carried a sole stria. From 2 March 1996 to this day (1 June 1998), no other positive symptoms of rabies have been diagnosed over 750 foxes analysed.

### Table II. Comparison of tetracycline and serological results between test and control zones in adult and young foxes.

<table>
<thead>
<tr>
<th></th>
<th>Test zone N°(%)</th>
<th>Control zone N°(%)</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Foxes marked tetracycline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>45/50 (90)</td>
<td>21/60 (35)</td>
<td>(P &lt; 10^{-7})</td>
</tr>
<tr>
<td>Adult</td>
<td>29/31 (93)</td>
<td>62/77 (80)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Foxes with more than one stria of tetracycline</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>24/45 (53)</td>
<td>5/21 (24)</td>
<td>(P = 0.02)</td>
</tr>
<tr>
<td>Adult</td>
<td>21/29 (72)</td>
<td>35/62 (56)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Immunized foxes</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Young</td>
<td>19/50 (38)</td>
<td>10/60 (17)</td>
<td>(P = 0.011)</td>
</tr>
<tr>
<td>Adult</td>
<td>22/31 (71)</td>
<td>50/77 (65)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Test zone: zone vaccinated with Rabifox by helicopter and with Raboral at den entrances. Control zone: zone only vaccinated with Rabifox by helicopter.

### Table III. Number of immunized foxes among individuals with one or more than one stria of tetracycline in 1996 (%).

<table>
<thead>
<tr>
<th></th>
<th>Test zone</th>
<th>Control zone</th>
<th>Statistical significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Young foxes with one stria</strong></td>
<td>5/21 (24)</td>
<td>3/16 (19)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Young foxes with more than one stria</strong></td>
<td>14/24 (58)</td>
<td>2/5 (40)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Statistical significance for young foxes</strong></td>
<td>(P = 0.02)</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td><strong>Adult foxes with one stria</strong></td>
<td>3/8 (37)</td>
<td>17/27 (63)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Adult foxes with more than one stria</strong></td>
<td>18/21 (86)</td>
<td>27/35 (77)</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Statistical significance for adult foxes</strong></td>
<td>(P = 0.02)</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

Test zone: zone vaccinated with Rabifox by helicopter and with Raboral at den entrances. Control zone: zone only vaccinated with Rabifox by helicopter.

4. DISCUSSION

4.1. Accessibility of vaccine baits

The terrestrial deposit of baits at den entrances may be an efficient method in providing antirabies vaccines to fox cubs. It is safe to assume that the majority of dens were found in the study as three out of four dens where baits were distributed were located
dug into hillsides. This confirms the fox habit of making dens in well-drained areas [1]. As well, personnel who distributed baits declared that only 12% of the dens previously located by hunters were occupied only by foxes. This confirms that many dens are really secondary shelters irregularly frequented by foxes [27]. Over 93% of vaccine baits deposited at den entrances (VRG) disappeared within 7 days of deposition. This confirms the fact that foxes found baits rapidly when baits were deposited near the dens [26]. However as many of the aerially distributed vaccine baits (SAG2) were found chewed-up near dens, it is noted that adult foxes are able to carry aerially distributed baits back to fox dens. Thus aerial distributed baits can be made accessible to young foxes in the spring.

4.2. Fox cub population

Test zone results of the fox cub population indicated an additive effect of terrestrial bait deposit at the den entrances versus a single aerial bait distribution. Fox cubs found baits 4.8 times more often in the test zone. One can hypothesize that vaccine baits terrestrially deposited at fox den entrances alone would have touched at least 55% of the cub population (the difference between 90% efficacy observed in the test zone and the 35% efficacy observed in the control zone) and at the most 90%. In addition, fox cubs that first find a vaccine bait, found a second vaccine bait 3.5 times more often in the test zone compared to the control zone. Therefore the terrestrial distribution of vaccines at den entrances significantly increased fox cub uptake of vaccine baits.

As well, the benefit of a second distribution with another type of vaccine bait 4–5 weeks after the first distribution was demonstrated (SAG2 followed by VRG). However it must be noted that there was a significant increase in the number of vaccinal baits distributed during this second distribution in the test zone (2.4 times more than in the first distribution). We therefore hypothesize that a second distribution of a like number of baits by helicopter would equally have obtained similar results.

However, the expected benefit of easy access of baits terrestrially distributed to cubs was not supported by serological findings. The number of fox cubs with rabies antibodies was only 2.2 times higher in the test zone than in the control zone (38% versus 17%). We should hypothesize that fox cubs could be marked with tetracycline
transmitted by their mother’s milk, which provokes a difference between the amount of tetracycline and the rate of antibodies [15]. However, as at least 55% of fox cubs in the test zone were really marked with tetracycline from consumed baits, it is obvious that there was a real lack of benefit in seroconversion. This may be due to the lower ability of fox cubs to produce antibodies under natural conditions. As this is opposite to the results observed in experimental trials [10, 13], it is most probable that maternal immunity may have interfered with fox cub active immunity, as has been observed in other canine species [9]. Only 17% of fox cubs developed an antibody response following the first distribution and though this increased to 38% after the second distribution, this is still quite low. However, considering that immunity against rabies may also depend on cellular mediation response [18, 23], the production rate of fox cubs may be higher.

4.3. Adult fox population

The additional terrestrial distribution of baits had nearly no effect on the adult fox population. No statistically significant difference was observed between the control and the test zones concerning the rate of bait uptake, the number of tetracycline strias and the presence of rabies antibodies. The dose of tetracycline in one bait is higher than the one which is useful for marking fox teeth. The presence of one stria of tetracycline may correspond to one bait consumption or different close feedings of baits, so the presence and the number of tetracycline strias and the serological results must be associated. The only significant difference for adult foxes can be attributed to a booster effect. Therefore, aerial distribution alone (control zone) was efficient enough in itself to immunize the adult fox population. The additional terrestrial distribution of vaccine baits brought no significant further benefit for the adult fox population.

4.4. Cost effectiveness

Terrestrial bait distribution is expensive and cumbersome. A man’s work-day by helicopter vaccinated a surface area 63.5 times greater than that permitted by terrestrial distribution. Furthermore, the cost per km² for terrestrially distributed vaccines was 3.5 times greater than aerial distribution. This difference is mainly due to the density of baits distributed and the salary of personnel.

4.5. General conclusions

Terrestrial distribution of rabies vaccine baits at fox den entrances following a spring aerial distribution, reaches a much higher proportion of fox cubs than a sole aerial campaign in the spring (90% compared to 33% in this study). However, only 38% of fox cubs have a significant level of rabies antibodies after the second bait distribution. Terrestrial vaccine bait distribution was of no additional value to the adult fox population, except for a booster effect probably due more to the second distribution of baits than to the manner in which the baits were distributed. Though not highly cost-effective, terrestrial vaccine bait distribution can complement aerial distribution for small surface areas, in remnant enzootic focii or where helicopter flights are dangerous or prohibited.

ACKNOWLEDGEMENTS

We would like to thank J.M. Demerson, P.M. Francois, S. Lemoyne, P. Mancho and B. Thenoz for their technical support; game keepers in the Doubs, the Hunter’s Federations in the Doubs, the Lieutenants de louveterie, and the Direction des Services Vétérinaires du Doubs who have given us special help. May these receive our gratitude and sincere recognition for their assistance in this project.
REFERENCE


