RABIES MASS VACCINATION CAMPAIGNS IN TUNISIA:
ARE VACCINATED DOGS CORRECTLY IMMUNIZED?

CHEDIA SEGHAIER, FLORENCE CLIQUET, SALAH HAMMAMI, THOURAYA AOUINA, AIDA TLATLI, AND MICHEL AUBERT

Institut de la Recherche Vétérinaire de Tunisie, Tunis, Tunisia; Agence Française de Sécurité Sanitaire des Aliments, Laboratoire d’Études sur la Rage et la Pathologie des Animaux Sauvages, Maizéville, France

Abstract. Among the 301 dogs vaccinated against rabies in a suburban area of Tunis, 165 were sero-surveyed for 13 months. One month after vaccination, 65% of the dogs less than 1 year of age and 76–84% of the older dogs had significant antibody titers. One month after annual revaccination, these percentages ranged between 92% and 100%. Puppies (less than 3 months old) responded to vaccination with no significant interference by passive maternal immunity. Based on these percentages, a 93% rate of protection may be expected for vaccinated dogs. This study confirms that all dogs (even those less than 3 months of age) must be vaccinated during mass campaigns. The expected protection conferred by locally produced potent vaccines reaches 79–99% based on the age of the dogs. The alleged relative inability of local dogs to respond to vaccination cannot explain the partial success of rabies control in Tunisia.

Canine rabies remains a serious public health problem in Tunisia. A national control program based on the epidemiological surveillance of the disease in animals was initiated in 1982, and mass campaigns of parenteral vaccination of dogs have been carried out since then every 2 years. As a result, the number of rabies cases in animals decreased from an average 256 per year before 1982 to 60 per year during the period 1983–1988. In parallel, the mean annual number of human cases during this same period decreased from 16 to 3, with no human rabies cases recorded in 1985 and 1988.1 Unfortunately, since 1988, these promising results have not been maintained. In 1992, 25 human rabies cases were recorded and in 1996, 36,196 human rabies post-exposure treatments were recorded.2,3 The relative lack of efficiency of the initial campaigns led to the conclusion that the rapid dog population turnover required that vaccinations be carried out every year. Therefore since 1992, mass country-wide vaccination of dogs has been carried out for 2-month periods on a yearly basis.

For assessing the adequacy of these campaigns, the Veterinary Research Institute of Tunisia has set up a research program with the objective of answering the following 2 questions. 1) What is the proportion of the owned dog population that can be vaccinated every year? 2) Are the dogs that are being annually vaccinated actually protected against the disease? Only the second question will be addressed in this paper.

Although in many developing countries the mass rabies vaccination of dogs is regarded as a major part of rabies control programs,4 no experimental study has been carried out for evaluating the level of protection conferred by the vaccines to the local common dogs. Most pharmacopoeia of European and North American countries require that vaccines for dogs be tested for potency on this species, but only dogs reared for laboratory purposes are used for such tests. This could lead to the overestimation of the protective activity of vaccines when used for common local dogs, which are generally parasitized and likely to be poorly fed. Dogs reared in developing countries generally produce lower antibody titers than dogs in Europe, while the latter produce lower titers than laboratory dogs.5,6

Moreover, the first Tunisian vaccine campaigns were carried out using imported tissue culture vaccines. Since 1988, they have been done using a vaccine produced locally from lamb brains, but it has never been tested for potency on dogs. For this reason, we were first confronted with the need to organize virus challenge trials on Tunisian dogs vaccinated with this locally produced vaccine. For practical as well as ethical reasons, a serologic survey of a recent mass vaccination of dogs carried out in a test area comprised of a suburban area near Tunis was considered the most appropriate means to provide data to answer the question whether vaccinated dogs in Tunisia are actually immunized. Many studies have amply demonstrated that dogs parenterally vaccinated according to any vaccination schedule are protected against a challenge by the rabies virus provided that they have produced detectable neutralizing antibodies.7

MATERIALS AND METHODS

Study area. The study area (0.38 km²) was a typical suburban area of Tunisia (Sanhaja), a locality 10 km northwest of Tunis (Gouvernorat of Ariana). This is an aggregate of 830 one-story individual houses separated by fields. The area was studied from a detailed map (1/5000 scale) that was updated with aerial photographs (1/20,000). Each house was systematically visited, inhabitants were interviewed, and dogs were marked and vaccinated.

Vaccine and vaccination. In the study area, dogs more than 1 year of age had been previously vaccinated during the course of mass campaigns. The Rabisin® vaccine (Mérial, Lyon, France) was used until 1987. This vaccine was produced with the Pasteur PV 11 strain on a hamster embryonic cell line and inactivated with β-propiolactone with aluminum hydroxide as the adjuvant. From 1988 on, the Rabirabta vaccine produced by the Veterinary Research Institute of Tunisia was used.8 This vaccine, which is 1 of the 2 veterinary vaccines manufactured in Tunisia for current mass campaigns, is produced on suckling lamb brains inoculated with a challenge virus strain (CVS strain), inactivated with β-propiolactone with aluminum hydroxide as the adjuvant, and kept in liquid form for use within 1 year. All batches are tested for potency by the World Health Organization Collaborative Center (Nancy, France) according to the European pharmacopoeia test guidelines.9

During the mass vaccination campaigns carried out by
community veterinarians, dogs received 1 dose (1 ml) by the subcutaneous route. In the study area, the last vaccination campaign took place in May 1993. Approximately 1 year later (from May 10 to June 7, 1994), all dogs were subcutaneously given 1 dose (1 ml) of the Rabirabta vaccine (batch 72, 8 IU/ml). This later vaccination day was considered as day 0 (D0) for each dog. A color photograph of the dog was taken, and the dog was described in collaboration with its owners in terms of size, color, sex, age, and rearing status (i.e., was the dog usually restricted to the property or released?). Vaccinated dogs were marked with a nylon collar. Collars of different colors were used for dogs belonging to the same family and owners were given a vaccination certificate. At every following observation, the information recorded for each dog was updated and the collars were replaced if lost. Every family was interviewed, and asked whether they owned a dog or not. One year after the beginning of the survey (day 365), the dogs were given another vaccination with 1 dose of the Rabirabta vaccine (batch 87, 8.8 IU/ml).

**Serologic survey.** On day 0, 301 dogs were vaccinated and marked. Additionally, whenever possible, the dogs were blood sampled at D0 and 1, 7, and 12 months after the first vaccination, and 1 month after the second vaccination (i.e., 13 months after the first one). Among the 301 dogs in this survey 95%, 88%, 72%, 60%, and 54% were sampled at D0 and 1, 7, 12, and 13 months, respectively, after the initial vaccination. Forty-seven other owned dogs were very aggressive towards everybody, including their owners. After several attempts, 8 of these dogs were vaccinated, but none were ever bled or marked. Rabies virus neutralizing antibody titers (in IU/ml) were determined according to the rapid focus fluorescent inhibition test (RFFIT) adapted on 96 microplates.10,11

**Statistical analysis.** To study the kinetics of rabies antibody levels in the dogs that were sampled at various intervals, we verified that the individual antibody titers expressed by the decimal logarithm of the number of IU/ml was a normal variable. We studied the simultaneous effect of age, sex, house-restriction, and the delay of sampling after vaccination by multiple analyses of the variance of the individual titers and the variation of the individual titers. To study the influence of age, the dogs were classified according to their age at D0: group A was composed of puppies ≤3 months of age; group B was composed of dogs >3 months to ≤1 year of age; group C was composed of dogs >1 year to ≤3 years of age; and group D was composed of dogs >3 years of age. Multiple comparisons of the means were performed according to the Newman-Keuls method.12 The variations of the titer of each individual at the different intervals were studied using the paired-sample Student t-test. Data processing was done using Statistica (Tulsa, OK) 5.1® Statsoft.

**RESULTS**

**Census and interview of owners.** Census, labeling, and blood sampling of dogs. A total of 5,418 inhabitants (6.5 persons per house) and 348 dogs were included in this study. Ratios were 1 dog per 15.6 inhabitants and 2.4 houses, respectively. When only the area where houses were built was considered, the dog density was 916/km². Only 194 houses (23%) owned 1 or more dogs. In houses with dog(s), the mean number of dogs was 1.79: there was 1 dog in 65% of these houses, whereas 6–11 dogs were kept in less than 5% of these houses.

Of the blood sampled dogs, 67% were males (sex ratio = 2.03). The average ages of the females and the males was 2.2 and 2.8 years, respectively (overall mean age = 2.6 years). Twenty-eight percent of the dogs were less than 1 year of age (Figure 1). All dogs with the exception of 1 were of the local mongrel breed. According to the declarations of the owners (which were verified whenever possible), the number of dogs leashed at all times was 194 (65%) (Figure 1). Based on owner declarations during the survey period, 26% of dogs died, 4.8% were lost, and 6.2% were given away to persons who lived outside the study area.

Dogs not present in the study area at the beginning of the survey were not included in the census after the start of the survey. It was therefore not possible to assess the dynamics of the total dog population. If we assume that the dog population was stable, we can, however, estimate a turnover rate of 37% per year from the number of dogs that died, disappeared, or were removed during this study.

**Antibody titers.** Comparison between dogs (whether or not they were followed during the total duration of the survey). In this analysis, we considered all individual antibody titers of dogs that we were able to sample at intervals, including the dogs that were not always present during the total duration of the survey.

Overall antibody titer kinetics are shown in Table 1. At D0, the mean dog population titer was 0.28 IU/ml and reached 1.06 IU/ml 1 month after vaccination, before decreasing to 0.47 IU/ml. The dog population titer remained at this level from month 7 to month 12. The booster vaccination at month 12 provoked a new increase of the mean titer to 2.54 IU/ml at month 13.
The sex or rearing status of the dogs had no influence on the titer. However, analysis of variance revealed a significant effect of age on the titer (at D0, month 1, and month 7). At D0, the differences in the titer according to the age group were highly significant. Dogs in group D showed a significantly higher titer than dogs in all other groups ($F_{3/150} = 4.8, P = 3 \times 10^{-5}$, by Newman-Keuls test: B A C D). At month 1, the titer of all groups of dogs increased, but the dogs in group D had a titer significantly higher than those in the other groups ($F_{3/238} = 4.8, P = 3 \times 10^{-5}$, by Newman-Keuls test: B A C D). At month 7, despite the general decrease in titer, the same significant differences were maintained between age groups; moreover, group B dogs had a significantly lower titer than those in group C ($F_{3/150} = 8.3, P = 3 \times 10^{-5}$, by Newman-Keuls test: B A C D). From D0 until month 7, group A dogs had a higher mean titer than group B dogs, but these differences were not statistically significant.

At month 12, all titers were similar with no statistically significant differences between age groups. One month after the booster vaccination (month 13), all titers increased but again no difference between age groups was observed.

Variation of titer in the same dogs sequentially sampled at intervals. The detailed study on individual variations of titer in relation to age, sex, and rearing status of the dogs demonstrated that the rearing status of the dogs had no significant influence on titer differences from one date to another. One month after vaccination, the increase in antibody titer was highly significant in all dogs (the mean titer was multiplied by $3.8$). During the 11 months following the initial vaccination, the titer decreased significantly in all age groups. This decrease was significantly more intense in dogs in groups C and D (mean titers were divided by 3 and 3.6, respectively) than in dogs in group B (mean titer divided by only 1.2) ($F_{3/136} = 2.9, P = 0.04$, by Newman-Keuls test: B A C D). The variation in titer from D0 to month 12 was dependant on age ($F_{3/136} = 2.9, P = 0.04$, by Newman-Keuls test: D C A B). Only the oldest dogs (group D) did not have a statistically significant higher titer at month 12, in contrast to the dogs in groups C, A, and B whose titers were multiplied by 1.4, 1.6, and 2.7, respectively (the paired sample Student test (1-tailed) allowed us to verify that the increase in titers of dogs in groups A, B, and C was significant: group A: $t = 1.8$, degrees of freedom [df] = 20, $P < 0.05$; group B: $t = 5$, df = 52, $P = 7 \times 10^{-6}$; group C: $t = 1.7$, df = 57, $P < 0.05$; group D: $t = 0.2$, df = 42, $P = 0.59$).

One month after the second vaccination, i.e., from month 12 to month 13, the antibody titer increased in all dogs regardless of their age group. The mean titer was multiplied by an average of 5.4.

Percentage of dogs with titers $\geq 0.5$ IU/ml. For all age groups, the percentage of dogs with titers $\geq 0.5$ IU/ml increased 1 month after vaccination and decreased during the following 11 months (Table 2). At D0, month 1, and month 7, this percentage was significantly higher for dogs in group B (mean titers were divided by 3.8) than in dogs in group D (mean titers were divided by 3 and 3.6, respectively). One month after the second vaccination, more than 90% of the dogs had titers $>0.5$ IU/ml, regardless of the age group.

Comparison between puppies with or without a significant antibody titer at D0. Ten of 39 puppies had titers $\geq 0.5$ IU/ml (n = 10, mean = $-0.05$, SD = 0.10); 29 puppies had no

### Table 1
Neutralizing antibodies titer (IU/ml) in sera of owned dogs in Sahanja, Tunisia where mass vaccination of dogs is organized yearly

<table>
<thead>
<tr>
<th>Age of dogs at Day 0</th>
<th>Day 0</th>
<th>Month 1</th>
<th>Month 7</th>
<th>Month 12</th>
<th>Month 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.22</td>
<td>0.85</td>
<td>0.34</td>
<td>0.39</td>
<td>2.45</td>
</tr>
<tr>
<td>≤3 months</td>
<td>(0.04–1.1)</td>
<td>(0.15–4.8)</td>
<td>(0.1–1.7)</td>
<td>(0.1–1.8)</td>
<td>(0.5–12)</td>
</tr>
<tr>
<td>B</td>
<td>0.19</td>
<td>0.72</td>
<td>0.27</td>
<td>0.56</td>
<td>3.2</td>
</tr>
<tr>
<td>3 months to ≤1 year</td>
<td>(0.03–1)</td>
<td>(0.14–3.8)</td>
<td>(0.06–1.2)</td>
<td>(0.1–3.1)</td>
<td>(0.6–17)</td>
</tr>
<tr>
<td>C</td>
<td>0.26</td>
<td>1.1</td>
<td>0.48</td>
<td>0.38</td>
<td>2.68</td>
</tr>
<tr>
<td>1 year to ≤3 years</td>
<td>(0.04–1.6)</td>
<td>(0.2–6.5)</td>
<td>(0.1–2.5)</td>
<td>(0.1–2)</td>
<td>(0.4–18)</td>
</tr>
<tr>
<td>D</td>
<td>0.51</td>
<td>1.7</td>
<td>0.84</td>
<td>0.46</td>
<td>1.99</td>
</tr>
<tr>
<td>&gt;3 years</td>
<td>(0.8–3.4)</td>
<td>(0.3–10)</td>
<td>(0.1–5.1)</td>
<td>(0.1–2.4)</td>
<td>(0.4–9.4)</td>
</tr>
<tr>
<td>All ages</td>
<td>0.28</td>
<td>1.06</td>
<td>0.47</td>
<td>0.45</td>
<td>2.54</td>
</tr>
<tr>
<td>(0.04–1.8)</td>
<td>(0.2–6.3)</td>
<td>(0.1–2.7)</td>
<td>(0.1–2.3)</td>
<td>(0.5–14)</td>
<td></td>
</tr>
</tbody>
</table>

*Values are the percentage of dogs (total number of individuals tested).

### Table 2
Proportion of dogs with a rabies neutralizing antibody titer equal to or more than 0.5 IU/ml

<table>
<thead>
<tr>
<th>Age at Day 0</th>
<th>Day 0</th>
<th>Month 1</th>
<th>Month 7</th>
<th>Month 12</th>
<th>Month 13</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>26 (39)</td>
<td>64 (44)</td>
<td>22 (27)</td>
<td>32 (28)</td>
<td>95 (21)</td>
</tr>
<tr>
<td>≤3 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>19 (81)</td>
<td>65 (68)</td>
<td>20 (49)</td>
<td>44 (54)</td>
<td>100 (43)</td>
</tr>
<tr>
<td>3 months to ≤1 year</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>29 (93)</td>
<td>76 (86)</td>
<td>40 (81)</td>
<td>29 (58)</td>
<td>92 (53)</td>
</tr>
<tr>
<td>1 year to ≤3 years</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>55 (74)</td>
<td>84 (69)</td>
<td>62 (60)</td>
<td>37 (43)</td>
<td>94 (48)</td>
</tr>
<tr>
<td>&gt;3 years</td>
<td>32 (287)</td>
<td>73 (267)</td>
<td>39 (217)</td>
<td>36 (183)</td>
<td>95 (165)</td>
</tr>
</tbody>
</table>

*Values are the percentage of dogs (total number of individuals tested).
detectable antibody at D0 (n = 29, mean = −0.86, SD = 0.24) (Figure 2). At month 1, the mean antibody titer was significantly higher (t = 2.68, df = 23, P = 0.011) for puppies that showed titers ≥0.5 IU/ml at D0. At months 7, 12, and 13 after the first vaccination, all antibody titers tended to be similar, regardless of titers measured at D0.

DISCUSSION

Our repeated visits and systematic surveillance of the studied area using a detailed map and aerial photographs led us to assume that all owned dogs in the study area were accounted for. However, the proportion of dogs that were impossible to vaccinate was still very high (11.5%) and may be of major importance in the transmission of rabies: those dogs are difficult to vaccinate and prone to bite humans or other dogs.

The dog density observed in Sanhaja falls within the range of 700–1,000 dogs/km² recorded in other Tunisian urban and suburban zones (review by Wandeler and others). Our ratios are situated at the limits described by these investigators: percentage of households keeping dogs =10–20% (23% in our study), number of dogs per inhabitant = 1/16–46 (1/15.6 in our study).

The sex ratio observed in Sanhaja (2.03) was similar to that calculated by Matter in Maghra, a Tunisian village in a rural area (1.92). These results indicate that the studied area is quite representative of Tunisian suburban areas and are consistent with the results described by Wandeler and others, who observed surprising uniformity in the structure and dynamics of the Tunisian dog population in 8 urban or suburban study sites.

The antibody titer of Tunisian dogs is highly variable between individuals: in this study, 1 month after vaccination, the titer ranged from 0 to 7 IU/ml (1 vaccination) or to more than 100 IU/ml (several vaccinations). Titers obtained after a similar schedule of vaccination commonly ranged from 0 to 20 or more IU/ml. This high inter-individual variability has already been described, even in standardized laboratory conditions.

Data from owned dogs in Thailand and Java have shown that neutralizing antibody titers decrease very rapidly 60–120 days after vaccination to levels 5–25-fold less than the highest level reached. Our study confirms the benefit of yearly booster vaccinations: the oldest dogs (group D), who had the benefit of several vaccinations before the survey, showed the highest titers, which lasted 1 year after vaccination. The higher level of neutralising antibodies obtained after several vaccinations has also been described by Sasaki and Gooch. In Sanhaja, the oldest dogs experienced the most intense decrease in titer 1 month after the booster vaccinations, and were also the only age group to show no increase in titer at a 1-year interval. These results indicate that the antibody response of these dogs was already maximal. In contrast, young dogs (group B) showed the greatest benefit from vaccination: they showed the smallest decrease in titer after the first increase in antibody level compared with dogs in other age groups, and they showed the greatest increase in antibody titers 1 month after the second vaccination. After 2 vaccinations, all age groups showed similar antibody levels.

The highest titers observed in the oldest dogs may be correlated not only with the number of previous vaccinations, but also with a more mature immunocompetency. A survey on owned dogs in France demonstrated that after a primary vaccination the antibody response level was positively correlated with dog age. Given the poor health status of dogs in Tunisia, the superior response of older individuals to vaccines could be related to the selection of individuals with a more mature immune system.

In our survey, 26% of puppies (≤3 months of age) had >0.5 IU/ of antibody/ml at D0. The majority of these puppies were born of immunized bitches, although it was not possible to verify this information for all puppies of this age. When the date of the last mass vaccination performed before D0 was taken into account, all puppies and a majority of dogs <1 year of age had never been previously vaccinated. This means that rabies antibodies detected in these animals were of maternal origin. The fact that at D0 the percentage of titers ≥0.5 IU/ml was higher (but not statistically significant) for puppies (26%) than for young dogs (19%) is consistent with the natural decrease of passively acquired immunity.

Despite the presence of maternal antibodies, puppies and young dogs responded to vaccination. No statistical difference was observed in the levels of antibodies to rabies in puppies at months 7, 12, and 13, regardless of the presence of maternal antibodies at D0. Maternal antibodies did not hamper a rapid antibody response shortly after vaccination (1 month). It is interesting to note that puppies had higher (but not significantly) titers than young dogs at least up to 7 months after vaccination. This suggests that the antibody titer we measured in these animals was the result of passively acquired and actively produced antibodies. In vaccinated puppies, antibodies to rabies of maternal origin did not
seem to interfere significantly with vaccination. If this was the case, dogs in group B, which had the lowest passive immunity, would have developed higher titers more rapidly and in a larger proportion than puppies.

The lack of (or the limited) interference between passive and active immunity is in accordance with previous studies done by Précausta and others on laboratory-reared dogs. It was shown that puppies from non-immune bitches vaccinated at the age of 1 month responded with a similar neutralizing antibody level as puppies that had been vaccinated at 7 months of age. Puppies of immune bitches vaccinated at 1 month of age showed neutralizing antibody levels that decreased with kinetics similar to those of unvaccinated puppies from the same litter.

Précausta and others and Lawson and Crawley detected no trace of maternal antibodies in puppies more than 10–12 weeks of age. In our study, 19% of the dogs between 10 and 12 weeks and 1 year of age were found to have significant antibody titers. This difference may be explained by the larger number of dogs used in our study, by a longer sucking period of Tunisian dogs, and by a lack of precision concerning the age given by the owners (in particular for dogs said to be 1 year of age that could have been vaccinated before the survey). However, this last hypothesis cannot be supported by a detailed analysis of the data. (In group B dogs, there is a similar percentage of dogs with antibody titers ≥0.5 IU/ml in the subgroups age = 1 year of age and 3–11 months of age [9 of 46 and 6 of 35, respectively].)

The influence of the health or rearing status of dogs on the antibody response to rabies vaccination has been previously demonstrated by several investigators. Such an influence has generally been observed in well-differentiated groups: laboratory dogs compared with pet dogs in France, laboratory dogs compared with pet dogs in Tunisia, or among pet dogs entering quarantine. In our study site, the health status of dogs, which were all owned by Tunisian families having a rather homogenous standard of living, seemed rather homogenous as well. Therefore, studying dogs restricted to their house and garden is not appropriate for measuring the intensity of care received from their owners.

This study shows that 1 year after primary vaccination, dogs produce low antibody titers. In Thailand, 42% of the dogs vaccinated subcutaneously had no detectable antibody titers 1 year after vaccination. In Europe, this proportion ranged between 42% and 17%. In Alaska, 33% of the dogs vaccinated intramuscularly had titers <0.5 IU/ml. In contrast, in a large field study conducted in Peru, this percentage was only 3% after a primary subcutaneous vaccination. Differences in dog population and vaccination protocols cannot be solely responsible for explaining the discrepancies of antibody levels determined in these studies. We assume that despite the use of a common technique for the antibody titration (the RFFIT), numerous differences in its application adds variability (Cliquet F, unpublished data). The percentage of dogs with detectable antibody titers 1 year after a primary or booster vaccination was higher in our study area of Sanhaja than in all of the above-mentioned surveys, with the only exception being the result of Chomel and others.

In a group of dogs vaccinated against rabies, the survival to a challenge with various strains of the rabies virus is significantly correlated with the percentage of individuals that have rabies antibodies at any time after vaccination and before challenge. A correlation between the survival rate and the seroconversion rate has been drawn from an exhaustive compilation that included the results of many investigators (for bibliographic references see Aubert). For dogs vaccinated with inactivated vaccines, this correlation is $y = 0.34 x + 69$ where the survival rate ($y$) and seroconversion rate ($x$) are expressed as percentages. It should be noted that this relation is independent of the potency of the vaccine, the delay of seroconversion after vaccination or before challenge, the delay of challenge after vaccination, and the dose of virus used for challenge. Therefore, whenever the conditions of use of a rabies vaccine are assumed to fall within the general experimental conditions that prevailed for establishing this correlation, it is preferable for ethical, safety, and practical reasons not to subject vaccinated dogs to a challenge. We assumed that this was the case in our study. From the above formula, using the percentage of dogs that seroconverted at any time after vaccination for all groups of ages, we have estimated the probability of survival of our groups of dogs (Table 3). For puppies, titers at D0 and month 1 were not considered to eliminate the bias introduced by natural antibodies. Overall, the expected protection rate was 93% for the entire population of vaccinated dogs.

Few results have been published reporting the antibody response of dogs vaccinated against rabies in countries where rabies is endemic and where the dog is the main reservoir of the virus. In Tunisia, despite the organization of annual mass dog vaccinations by the parenteral route all over the country, animal and human rabies cases are still reported.

Among the numerous reasons that may explain the lack of success of mass vaccination programs for dogs, this study excluded the alleged relative inability of local dogs to respond to a locally produced rabies vaccines. When these vaccines satisfy the potency requirement, which must be tested in any case, the percentage of dogs of all age groups is high enough so that a theoretical protection rate of more than 90% can be expected. Moreover, this study confirmed that the vaccination of very young dogs (even before 3 months of age) can induce antibodies even when these dogs have maternal antibodies. However, due to the fact that young dogs do not have an optimal antibody response after receiving a primary vaccination, and due to the very rapid turnover of the dog population, as well as the general lack of a good individual identification of dogs, these vaccination
campaigns must be organized every year and vaccines should be applied to all dogs.

The high proportion of owned dogs that are impossible to handle and to vaccinate, and the existence of a stratum of dogs without individually identified owners stress the need to develop oral vaccination of dogs as a complementary measure to mass parenteral vaccination campaigns. Considering the proven efficacy of parenteral vaccination organized with local vaccines and taking into account that this type of vaccination can reach a large proportion of dogs, it is worthwhile to enforce the principal conditions for its efficacy: i.e., mainly the use of vaccines with well-controlled potency, a well-planned organization of the field work, and the active participation of communities in the vaccination of the largest possible proportion of owned dogs.

Acknowledgments: We acknowledge Latifa Bjaoui, Rebah Oueslati, and Fayrouz Hammou for their diligent help in the field survey, and Florence Aouina, and Aida Tlatli, Institut de la Recherche Veterinaire de Tunis, for their help in the titration of rabies antibodies. Myriam Selve and Line Sagne for their help in the titration of vaccines.

Financial support: This research was partially funded by the French Embassy in Tunis and the French Ministry of Foreign Affairs.

Authors’ addresses: Chedia Seghaier, Salah Hammami, Thouraya Aouina, and Aida Tlatli, Institut de la Recherche Veterinaire de Tunis, Rue Djebel Lakhdhar, 1006 La Rabta, Tunis, Tunisia. Florence Aouina, and Aida Tlatli, Institut de la Recherche Veterinaire de Tunis, Rue Djebel Lakhdhar, 1006 La Rabta, Tunis, Tunisia. Florence Cliquet and Michel Aubert, Agence Francaise de Securite Sanitaire des Aliments, Laboratoire d’Etudes sur la Rage et la Pathologie des Cliquet and Michel Aubert, Agence Francaise de Securite Sanitaire des Aliments, Laboratoire d’Etudes sur la Rage et la Pathologie des Aliments, Rue Djebel Lakhdhar, 1006 La Rabta, Tunis, Tunisia. Florence Cliquet and Michel Aubert, Agence Francaise de Securite Sanitaire des Aliments, Laboratoire d’Etudes sur la Rage et la Pathologie des Aliments, Rue Djebel Lakhdhar, 1006 La Rabta, Tunis, Tunisia.

REFERENCES