CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

First trials of oral vaccination with rabies SAG2 dog baits in Morocco

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Purpose: Canine rabies is a serious health problem in Morocco and about 22 human deaths are reported yearly. Following the World Health Organization (WHO) recommendations, Moroccan authorities evaluated oral rabies vaccine baits specially designed for dogs.

Materials and Methods: The study was performed in Tiflet area. The vaccine strain was SAG2, a modified live oral rabies vaccine strain. Each bait contained an aluminium/PVC capsule filled with a liquid. Two kinds of baits were used: placebo baits containing methylene blue as a topical marker and vaccine baits containing vaccine suspension. The study was performed according to recommended WHO strategies, i.e., door to door model (DDDM), hand-out and wildlife immunization model (WIM). The DDDM was performed in the rural area of Tiflet on 60 owned dogs. The hand-out strategy was tested on 15 stray dogs. The WIM was performed on 4 transects lines near Tiflet slaughterhouse and near the weekly traditional market location.

Results: Using the DDDM, 100% of owned dogs were attracted by the baits and 77% ate the bait. Using the hand-out model, 100% of dogs showed interest in baits and 46.7% took the baits. Using the WIM in stray dogs, up to 73% of baits disappeared and 68% of the capsules containing the SAG2 vaccine were found pierced, depending on the sites of distribution.

Conclusion: This pilot study showed that baits have a good palatability and that oral vaccination of both owned and stray dogs is feasible with baits specifically developed for dogs and with adapted strategy of distribution.

Keywords: Rabies, Dogs, Oral administration, Vaccination, Morocco

Introduction

Although rabies is a reportable disease, the true incidence of the disease is greatly underestimated due to a lack of systematic surveillance and reporting by most developing countries [1]. In news published in September of 2011, the Global Alliance for Rabies Control estimates that 70,000 people die of rabies every year (http://rabiesalliance.org/media/news/annual-number-of-deaths-from-rabies-hits-70000-worldwide), mostly in developing countries from Asia (58%) and Africa (42%) [2]. Rabies is a vaccine-preventable disease. Since dogs are the main reservoir and source of infection for humans, a better control of rabies could be achieved through vaccination of dogs [3]. In Latin America, the mass vaccination of the dog population has led to a significant drop in the number of human and canine rabies cases [4].

Despite several mass campaigns of parenteral vaccination of dogs, canine rabies re-

mains a serious health problem in Morocco and about 22 human deaths are reported every year [5]. Parenteral vaccination is the method of choice for owned dogs (i.e., dogs with a person that claims responsibility, according to the World Organisation for Animal Health (OIE) definition [6]), since approximately 70% of dogs in Morocco are considered accessible [7]. However, a proportion of dogs are stray (i.e., free-roaming with or without owner, or feral, according to the OIE definition [6]), and owned dogs may also be aggressive and difficult to handle, therefore oral vaccination could be helpful [8]. Rabies has been successfully controlled in several European countries through oral vaccination of wildlife with vaccine baits and considerable success has been achieved in the control of Arctic fox rabies [9] and raccoon rabies in Canada and in the United States [10]. International guidelines for rabies control in dogs and implementation of field trials using oral vaccines are available [11,12].

Moroccan authorities opted for evaluating oral rabies vaccine strain SAG2 in field conditions. The present study is a first trial undertaken in the region of Rabat (Morocco) to evaluate the acceptance of baits specifically designed for dogs.

Materials and Methods

Study zone

The field study was carried out in the rural area of Tiflet city located 40 km from east of Rabat (capital of Morocco) (Fig. 1). The slaughterhouse activity and the weekly regional traditional market (souk) of Tiflet are attractive for both the population and dogs from neighbouring rural municipalities. The dog population of the area was estimated in 2010 and the results are reported in Table 1 [13].

Vaccine strain

The tested vaccine strain was SAG2, a modified live attenuated rabies virus. SAG2 is one of the two rabies vaccine strains

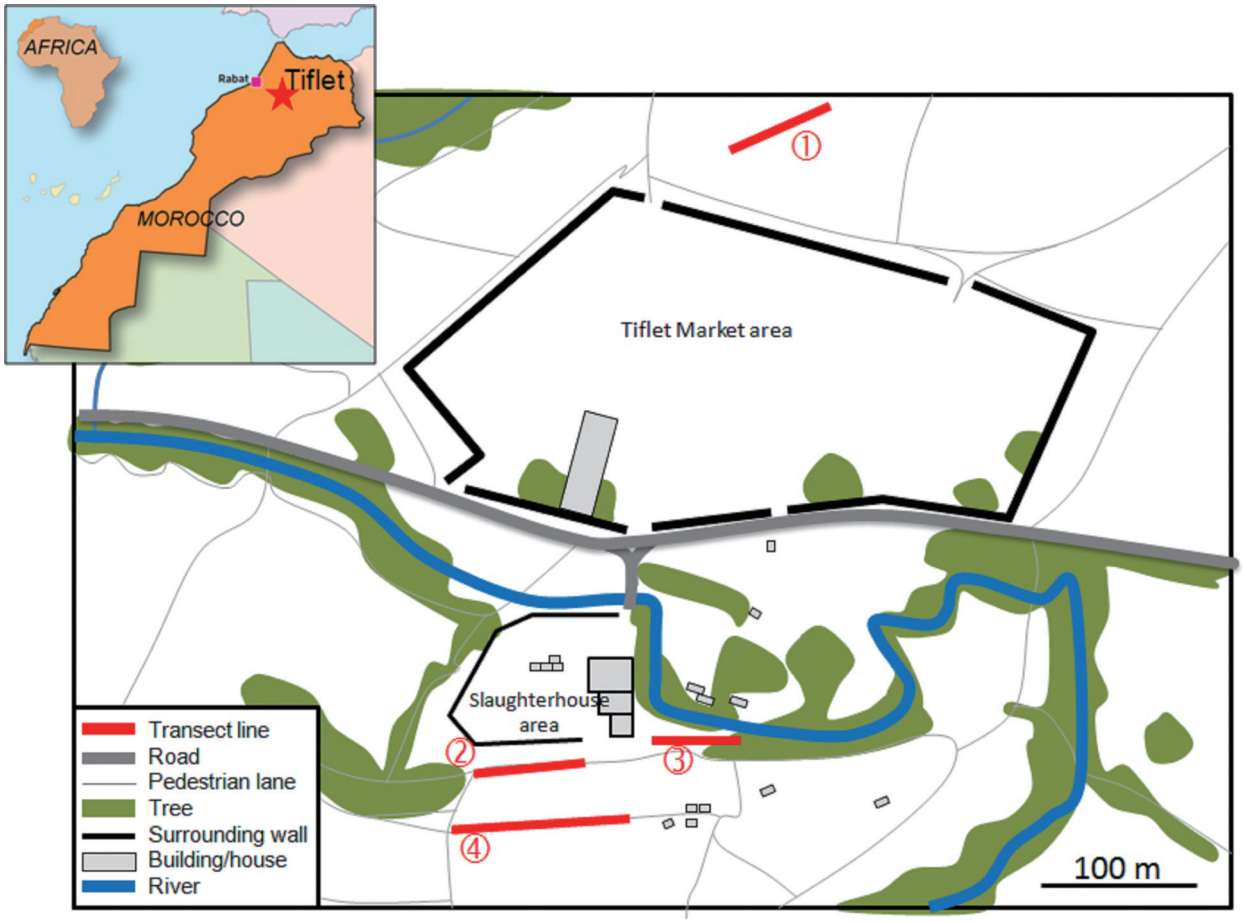

Fig. 1. Location of the transect lines for the distribution of baits. SAG2 baits were distributed in the evening along transect lines used by free-roaming dogs to go to the slaughterhouse: 1, beside the market area; 2 and 3, near the surrounding wall of the slaughterhouse; 4, along an access path to the district.

Table 1. Dog population characteristics in Tiflet area in 2010

Area	Counted dogs	Owned difficult to handle dogs (%)	Free-roaming owned dogs (%)	Unvaccinated dogs (%)
Tiflet	1,167	62	73	79

Percentages were calculated according to the responses given by the dog owners in the questionnaires.

recommended by World Health Organisation (WHO) to be used in dogs by the oral route [11,12].

SAG2 strain was constructed by two successive mutations from the rabies strain SAD Bern using neutralizing monoclonal antibodies. The mutations are on the position 333 of the antigenic site III of the rabies virus glycoprotein. In this position (333) SAG2 has GAA which codes for glutamic acid and SAD Bern strain has AGA which codes for arginine, resulting in a double avirulent mutant [14].

The genetic stability of SAG2 was largely demonstrated *in vitro* and *in vivo* (in mice) [14].

Baits

The baits were provided by Virbac Laboratories (Carros, France). The baits were cylindrical with 44 mm diameter, 16 mm height and 23 g weight for both baits types (vaccine baits and placebo baits). The colour of the baits was "cement/dirt" and the baits were "chicken liver" flavoured. The cylindrical shape, the colour and the chicken liver flavour were modifications to adapt the fox bait [15] (49 mm×44 mm×15 mm and 28 g, chocolate colour and fish flavour) to the Moroccan dog. The baits contained an aluminium/PVC sachet filled with a liquid. Vaccine baits and placebo baits were provided by the manufacturer. The baits were stored at -20°C until use.

Vaccine baits

Each bait contained a liquid suspension ($10^{7.8}$ $DICT_{50}$) of SAG2 vaccine strain. Its safety and efficacy have been demonstrated in fox, raccoon dog and dog [12,15-17] and the innocuity in numerous wild non-target species [16,18-22].

Placebo baits

Placebo baits contained a capsule with methylene blue as a topical marker. The bait uptake and perforation of the capsule could be evaluated visually. This aimed the monitoring of the bait uptake as recommended by the WHO [11,12].

The placebo bait casing had the same dimensions and composition as the vaccine baits.

Dogs

A total of 60 owned dogs, mainly adult males, living in villages located around Tiflet and 15 stray dogs living close to the slaughterhouse of Tiflet were included in the study.

Study strategy

Different strategies of distribution were tested for dogs according to directions of WHO [11].

First trial

This trial was conducted in the rural area of Tiflet according to the "door to door" oral vaccination model or door to door model (DDDM) [11] for owned dogs to assess the attractiveness and palatability of the bait casing. Each of 60 dogs was offered one placebo bait (Fig. 2A).

Second trial

This trial was conducted with stray dogs in two steps. First, baits were offered to 15 dogs present on the site to evaluate their attractiveness according to the WHO "hand-out" model [11]. Secondly, a total of 30 SAG2 baits were distributed in the evening along 4 transect lines used by stray dogs to go to the slaughterhouse (Fig. 1) [13]. The distribution was carried out according to the "wildlife immunization model" or wildlife immunization model (WIM) [11]. The targeted sites were known to be visited by free ranging dogs [13]. The vaccine baits were left overnight. The next morning, remaining baits were removed.

Results

First trial

All dogs smelled the bait and most of them (77%) ate it in less than 3 minutes (Fig. 2B). The capsule was pierced by 62% of the dogs, i.e., 80% of dogs which consumed the bait (Fig. 2C). Although the mouth could not be examined in all dogs, the tongue was coloured in blue in at least 25 dogs, i.e., 67% of dogs which ate the bait (Fig. 2D). Some dogs, probably disturbed by the presence of strangers, did not eat the bait, but when they were left alone, half of them ate the bait within few minutes.

Second trial

For baits presented according to the hand-out model, although the environment was filled with meat odours, all 15 dogs smelled the baits. Four dogs ate the bait immediately and 3

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Fig. 2. Uptake of the bait shown by the blue colour of the tongue. (A) Bait. (B) Dog consuming a bait. (C) Capsule pierced. (D) Tongue coloured by methylene blue.

Table 2. Attractiveness and palatability of SAG2 dog baits in free-roaming dogs

Transect line	No. 1	No. 2	No. 3	No. 4
No. of baits distributed	8	5	5	12
No. of baits eaten	0	4	2	10
No. of capsules found	-	4	2	10
No. of capsules empty	-	3	2	10

dogs took the baits to bury them (46.7%).

For baits distributed according to the WIM, none of them deposited along line 1 were taken. In contrast, a high proportion of baits distributed in lines 2, 3 and 4 (16 out of 22, i.e., 73%) had disappeared overnight and 68% of the capsules were found perforated (Table 2).

Discussion

Only two vaccines strains (SAG2 and V-RG) are currently recommended by WHO for oral vaccination of dogs [12]. SAG2 has been widely used in Europe and led to wildlife rabies elimination in several European countries [23]. It should be noted that no vaccine-induced rabies cases were reported in Europe after the distribution in the environment of more than 20 millions of SAG2 baits. This vaccine is registered for the control of canine rabies in India [16] and has been mainly evaluated in Tunisia [22], in Mexico [24], in South Africa [25] and in Indonesia [26], demonstrating its efficacy for dog vaccination in the field [27].

Numerous and extensive pathogenicity studies undertaken on more than 30 target and non-target warm-blooded spe-

cies, including 5 from the canine family (of which dog), wild carnivores, rodents and non-human primates, demonstrated that SAG2 is an avirulent rabies vaccine strain [28]. The studies were performed according to the guidelines of WHO for safety testing of modified live-rabies vaccines [29] in species including rodents (mouse, rat, vole, squirrel, gerbil, jerboa, and meriones), carnivores (coyote, ferret, civet, mongoose, badger, and genet), non-human primates (Chacma baboons), other mammals (hedgehog, wild boar, domestic goat, and cow), and diurnal and nocturnal birds (crow, rook, buzzard, kite, and owl) [18-20,22,30]. Different studies showed that the persistence of the SAG2 virus in the oral cavity is very limited or even nil in non target species [14,19-22].

The immunogenicity and efficacy of SAG2 were evaluated in laboratory (mouse model) and in field conditions. Different trials demonstrated the protective effect of SAG2 vaccine bait in controlled laboratory conditions in different wildlife species and in dogs. These studies suggest that SAG2 induce humoral [15-17,31-34] and cell mediated immunity [31].

SAG2 vaccine baits appeared well-adapted to both owned and stray dogs living in Morocco. The shape of the bait is round (diameter=44 mm), facilitating the uptake by dogs of different sizes. The quantity of bait matrix was reduced to facilitate the puncture of the capsule to favour the contact of the liquid vaccine with oral mucosa and tonsils. It should be noted that capsules which were not pierced did not stick enough to the bait. It was observed that when the capsule was pierced before detaching from the bait, dogs tended to take again the capsule and lick the sweet content of the capsule. The baits have "cement/dirt" colour so that it merged with the colour of the soil and it does not attract children's attention. The chicken liver flavour has an attractive smell and a high palatability for Moroccan dogs. Several studies have shown that there were regional preferences for the bait flavours, such as poultry in Guatemala [35], dog biscuits in Mexico [24], chicken heads in Egypt [36] or fish in US Indian reservations (Navajo Nation and the Hopi Nation lands) [37].

Three different methods of distribution (central point, DDDM, and WIM) were previously tested in semi-rural areas in Tunisia with placebo baits containing sulfadimethoxine as a systemic marker [27]. When baits were given to dog owners at mobile vaccination centres with precise instructions for use, 79% of baits were fully consumed by the dogs [27]. The uptake reached 59% in owned dogs after door-to-door baiting and 42% in owned and ownerless dogs after transect line baiting [38]. In another study conducted in South Africa, the DDDM was tested. In total, 77% of the vaccine baits were at least partly consumed [39].

These methods of distribution were tested in this study. The DDDM, which reached primarily owned dogs and the hand-out model, which reached accessible stray dogs [11], were intended to evaluate bait palatability. The WIM adopted to distribute baits to stray dogs around the slaughterhouse of Tiflet gave encouraging results. More than 70% of the baits distributed along lines 2, 3, and 4 disappeared overnight despite the presence of abundant food and familiar smells in the environment. We assume that baits had been consumed by stray dogs, since 68% of capsules were found pierced with different characteristic canine teeth marks from dogs and because there were no feral animals in this area close to human habitations. No bait distributed along line 1 was consumed, probably because dogs could not hide in this fallow land. When this line 1 was identified on the previous year [13], the area was covered with bushes where stray dogs used to stay. This result illustrates that baits should not be distributed at random. Baits could be placed at selected sites where large numbers of stray dogs congregate such as slaughterhouses, garbage dump or public markets (souks) and along ways frequently used by dogs. Although the WIM is not specific and less safe than the DDDM and hand-out method, it allows the access to free-roaming and feral dogs, which constitute a high risk group in terms of rabies transmission [11]. The WIM requires the use of safe vaccines, fulfilling all criteria listed by WHO [11,12].

This pilot study showed that oral vaccination of both owned and stray dogs is feasible with baits specifically developed for dogs and adapted modes of distribution. These data will be confirmed with a larger field trial taking into account results of this study. The necessary 60-70% vaccination coverage could be achieved using vaccine baits to target stray dogs in addition to injectable vaccines used during mass parenteral vaccination programmes. Methods of distribution should be adapted to local conditions. For instance, in Asia, the "hand-out" model can be used because free-roaming dogs accept easily baits even when given by unknown people, whereas in Maghreb dogs wary of strangers because usually people prevent contacts with them by throwing stones in their direction, people worrying of dog bites and diseases (personal observations). Although the cost of oral vaccination is higher than that of the parenteral vaccination, the combination of both vaccination strategies may increase the vaccination coverage in the vector population and lead to rabies elimination. The

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cost of vaccination could be reduced by the use of aerial distribution in certain well defined and restricted areas.

Research on effective field methods for control and elimination of rabies, although less attractive than sophisticated molecular analyses, should remain the priority [40].

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