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## Safety evaluation of the SAG2 rabies virus mutant in Tunisian dogs and several non-target species

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**Abstract** – The safety of the SAG2 rabies virus, a highly attenuated mutant of the SAD strain intended to vaccinate dogs by the oral route, was evaluated in local Tunisian dogs and in five other local species likely to consume vaccine baits. These species were the domestic cat (*Felis catus*), the jackal (*Canis aureus*), the jerboa (*Jaculus orientalis*), the merion (*Meriones* sp.) and the gerbil (*Merbillus campestris*). The vaccine was administered orally to 21 dogs, 11 cats and eight jackals and orally or intramuscularly to 62 wild rodents of the above-mentioned species. Seven dogs, one cat, five jackals (all juvenile and with poor health status) and two rodents died for intercurrent causes. The others were observed for 60–180 days. No animal showed any rabies symptom. Seroneutralizing antibodies were observed in all experimental groups, only after vaccination, with the highest rate being observed in jackals and rodents. The rabies virus was detected in the oral cavity of three cats 6 h after oral instillation, but was not isolated later either in saliva or in salivary glands. Tissue samples (brain and salivary glands) from dead or euthanized animals were examined for the rabies virus antigen by a fluorescent antibody test. No rabies antigen was detected. These trials confirm the safety of the SAG2 strain on the Tunisian species already demonstrated by other authors on many other target and non-target species. © Inra/Elsevier, Paris.

**rabies / SAG2 / innocuity / oral vaccine**

**Résumé** – Évaluation de l'innocuité du virus rabique mutant SAG2 chez des chiens tunisiens et plusieurs espèces non-cibles. L'innocuité de la souche SAG2 du virus de la rage, un mutant hautement atténué de la souche SAD pour vacciner le chien par voie orale, a été étudiée sur des chiens tuni-

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siens tout venant et sur cinq autres espèces locales susceptibles de consommer des appâts vaccinaux. Ces espèces étaient le chat domestique (*Felis catus*), le chacal (*Canis aureus*), la gerboise (*Jaculus orientalis*), le méridien (*Meriones* sp.) et la gerbille (*Gerbillus campestris*). Le vaccin a été administré par voie orale à 21 chiens, 11 chats et 8 chacals, et par voie orale ou musculaire à 62 rongeurs sauvages appartenant aux espèces citées. Sept chiots, un chaton, cinq jeunes chacals (tous en mauvais état de santé) ainsi que deux rongeurs sont morts pour des causes intercurrentes. Les autres animaux mis en observation pendant 60 à 180 j n'ont présenté aucun symptôme particulier. La présence d'anticorps a été observée dans le sérum de tous les groupes expérimentaux uniquement après administration du vaccin. Les pourcentages les plus élevés ont été observés chez les chacals et les rongeurs. Le virus de la rage a été détecté dans la cavité buccale de trois chats, 6 h après instillation orale, mais pas au-delà de ce délai et pas dans les glandes salivaires de ces animaux après euthanasie. Des échantillons de tissus (cerveau et glandes salivaires) prélevés sur les animaux morts ou euthanasiés ont été examinés par la technique d'immunofluorescence : sur aucun organe on n'a mis en évidence l'antigène de la rage. Ces essais confirment sur des espèces tunisiennes l'innocuité de la souche SAG2 déjà démontrée par d'autres auteurs sur de nombreuses autres espèces cibles et non-cibles. © Inra/Elsevier, Paris.

### **rage / SAG2 / innocuité / vaccin oral**

## **1. INTRODUCTION**

Canine rabies remains a serious public health problem in Tunisia even though a national control programme was initiated in 1982. The national control programme has included the epidemiosurveillance of the disease in animals, mass campaigns of parenteral vaccination of dogs, the elimination of stray dogs and public education.

These measures, and in particular the annual vaccination of dogs throughout the entire country, has significantly decreased cases of rabies in animals as well as in humans. In 1985 and 1988, the number of recorded cases of rabies in animals was 60 and 48, respectively, with no human rabies cases being recorded [4]. Unfortunately, these promising results were not repeated in the following years, and in 1992, 25 rabies cases were recorded in humans. Post-exposure treatments need to be maintained at very high levels, reaching 35 159 in 1996 which was not sufficient for preventing rabies in seven humans this year [11, 12].

One possible cause limiting the efficiency of mass vaccination may have been an insufficient coverage of the dog population. This mass vaccination inefficiency was due in part to the inaccessibility of unrestricted or aggressive dogs to parenteral vaccination.

To address this problem, several authors investigated the possibility of using oral vaccination as a complementary measure. The SAD Bern strain given orally to Tunisian dogs has been shown to confer protection against rabies [16] but, for safety reasons, the very close relationship between dogs and humans requires that the most attenuated strains be selected; this was not the case with the SAD Bern strain which remains pathogenic by oral route for rodents and other species including non-human primates [5]. We chose the SAG2 vaccine because of its innocuousness proven even at very high doses on non-human primates [6]. Using the SAG2 vaccine, three areas of research have been explored: a) the possibility of conferring immunity to dogs by an oral avenue [13, 23]; b) the safety of the SAG2 candidate vaccine for target and local non-target species [6, 7, 14, 18, 21]; and c) the possibility of increasing vaccination coverage of the dog population by using baits and bait distribution systems [15, 17, 20, 21, 24].

The objective of this present study was to evaluate the safety of the SAG2 rabies virus mutant in local Tunisian dogs and several non-target species commonly found in Tunisia in view of its future use in the immunization of dogs in this country.

## 2. MATERIALS AND METHODS

### 2.1. Animals

Twenty-four dogs and 12 cats of local breeds were donated by their owners in the region of Tunis for this study. According to the owner's reports, these animals had never been vaccinated against rabies. For the younger dogs this was true as they were collected prior to the start of the annual national rabies vaccination campaign, and for cats as they had never been included in such mass rabies vaccination campaigns. On the day of arrival, dogs and cats were individually described, fitted with a numbered collar, clinically examined and individually caged. Cats and dogs were housed separately and cage sizes were in accordance with guidelines for the care and use of laboratory animals of the National Institute of Health. Animals were fed daily with a commercial dry cat or dog food and provided water at will. Additionally, dogs were vaccinated against distemper, hepatitis, leptospirosis and parvovirus (Tetradog<sup>®</sup>, Lyon, France), while cats received no vaccines. After vaccination, animals were observed for 1–4 months (they were collected at various dates) before being given the SAG2 virus orally. Three dogs and one cat died from unknown causes during the acclimatization period. Twenty-one dogs survived until the beginning of the experiment: eight adults (> 1 year), three juveniles (3 months–1 year) and ten puppies (< 3 months). Among these dogs, 13 suffered from a more or less severe depilation, scars or other cutaneous lesions. Nine dogs were heavily infested with fleas, while two had a light pyoderma and one a light demodectic mange, two had swelled sub-maxillary nodes, two other dogs suffered from diarrhoea and another one from anaemia. Only one dog had none of the above-mentioned symptoms. None of the 11 cats (six adults and five kittens) had any apparent health problems except that they were very thin at the beginning of the experiment which is normal for street cats in Tunisia.

Fourteen golden jackals (*Canis aureus*) were captured in the wild in Tunisia, identified and individually caged, fed daily with a commercial dry dog food, and provided water at will. Many individuals, mainly the adults, were highly stressed and agitated. Some did not feed and died within a few days. Many difficulties were experienced in keeping the jackals in good health despite efforts to provide the best possible captivity conditions. Jackals were kept in large individual cages in an animal house free of distur-

bances except for care visits provided by two specially trained and selected technicians. Therefore, in order to shorten the captivity period, the acclimatization phase was kept as short as 1 week for the last individuals captured. Six jackals died before receiving the SAG2 virus orally, and in total three adults and five juveniles were orally vaccinated. Of these eight animals, two adults were emaciated, one of which was stressed owing to having its left hind leg wounded after trapping. The third adult had a similar wound but appeared in the same good health as the five juveniles.

All dogs, cats and jackals were blood sampled 1 day before the start of the experiment to verify that no rabies-neutralizing antibodies were present.

Sixty-two wild rodents of different species (*Meriones* sp., *Gerbillus campestris* and *Jaculus orientalis*) captured in Tunisia were housed in individually identified cages, fed with dry food for laboratory rodents and provided water at will. Cages were kept in a rodent-proof shelter with no artificial heating system. These rodents went through an acclimatization period from 1.5–3 months.

### 2.2. The SAG2 virus inoculum

The SAG2 virus, selected through the use of monoclonal antibodies, is an escaped mutant of the SAD Bern strain [18]. A glutamic acid has been substituted at a double mutation for arginine in the 333 position of the rabies glycoprotein.

Produced by the VIRBAC Laboratories (Carros, France), with a titre on BSR cells of  $10^{9.4 \pm 0.4}$  TCID<sub>50</sub>-mL<sup>-1</sup>, the SAG2 virus was transported on dry ice to Tunis and stored at -80 °C until use. At the time of administration it was thawed rapidly under tap water. After administration, two 1-mL samples of the inoculum were frozen and sent on dry ice back to the VIRBAC Laboratories for a new titration on BSR cells (results,  $10^{9.5 \pm 0.2}$  TCID<sub>50</sub>-mL<sup>-1</sup>).

### 2.3. Experimental protocol (table 1).

The SAG2 virus was administered orally to dogs, cats, jackals and merions in accordance with the WHO recommendations as the possible contamination of these species could also occur orally. Intra-muscular vaccine adminis-

**Table I.** Experimental protocol of the oral vaccination of several species with SAG2.

Species	Number of animals (route of inoculation)	SAG2: dose per animal (TCID 50)	Volume of SAG2 suspension instilled (mL)	Observation time (days) after vaccination
Dog ( <i>Canis domesticus</i> )	21 (p.o.)	$10^{9.5}$	1	90 or 180
Cat ( <i>Felis catus</i> )	11 (p.o.)	$10^{9.5}$	1	90
Jackal ( <i>Canis aureus</i> )	8 (p.o.)	$10^{9.5}$	1	90
Jerboa ( <i>Jaculus orientalis</i> )	2 (i.m.)	$10^{7.0}$	0.03	60
Merion ( <i>Meriones</i> sp.)	14 (p.o.) 14 (i.m.)	$10^{8.2}$ – $10^{9.2}$ $10^{7.0}$ – $10^{8.5}$	0.05–0.5 0.03–0.1	60 60
Gerbil ( <i>Gerbillus campestris</i> )	32 (i.m.)	$10^{7.0}$ – $10^{8.5}$	0.03–0.1	60

TCID 50: Tissue culture infective dose; p.o.: per os; i.m.: intramuscular.

tration was also used on another group of merions in order to increase the possibility of revealing any pathogenicity of the tested virus. Due to the difficulty in orally vaccinating the smallest species (jerboas and gerbils) without anaesthesia, the intra-muscular route was used instead. All protocols were in accordance with the WHO recommendations for assessing the safety of the rabies vaccines destined for the oral immunization of foxes or dogs [26–31].

In summary, orally vaccinated animals were not anaesthetized. A syringe without a needle was used to drop the virus suspension directly onto the tongue of the dogs, cats, jackals and merions. When used, the intra-muscular inoculation was performed on the thigh.

Carnivores were administered a 1-mL inoculum containing ten times the dose used in the field, and rodents were given volumes adapted to their size at the same virus dilution as that used for carnivores.

#### 2.4. Follow up

Animals were observed daily and special attention was paid to any loss of appetite or behavioural changes. As the incubation periods for rabies in rodents do not exceed 30 days after

an artificial inoculation, rodents were observed for 60 days in order to reveal any possible long incubation periods that may occur with attenuated virus strains. Cats, jackals and dogs were observed for 90 days, except for five randomly selected dogs which were observed for 180 days.

All animals were blood sampled at various moments after the oral administration of the SAG2 virus: rodents on day 60, all dogs, cats and jackals on days 30, 60 and 90, and additionally for the five dogs kept under observation for 180 days on days 120, 150 and 180. Sera were kept at  $-20^{\circ}\text{C}$  and sent to CNEVA Nancy (France) on dry ice for titration of rabies-neutralizing antibodies. Unfortunately the dog sera sampled on days 30, 60 and 90, and the sera of six cats sampled on days 60 and 90 were lost during shipment. Among the 48 sera of rodents that were sent to the CNEVA, only 32 were in sufficient quantity for titration.

#### 2.5. Rabies virus detection in saliva

Saliva swabs were taken from the dogs, cats and jackals 6 h and 3 days after the SAG2 virus was given orally. Saliva swabbing was performed with special care according to previously described methods [1] to avoid, as much as pos-

sible, the neutralization and dilution of the virus potentially present in the saliva. The quantity of saliva taken from the dogs, cats and jackals was species independent and ranged from 0.06 to 1.86 g (mean = 0.37 g). Saliva extracts were kept at  $-80^{\circ}\text{C}$  and sent on dry ice to CNEVA Nancy (France) for virus isolation on N2a cells (268th passage). The mean detection threshold of the method was lower than  $10^{1.4}$  TCID<sub>50</sub>-mL<sup>-1</sup>. Unfortunately the dog saliva swabs were partially destroyed during shipment: a total of 11 saliva swabs (seven adults, two juveniles and two puppies) taken 6 h after inoculation arrived at CNEVA Nancy.

## 2.6. Post-mortem examination

At the end of the observation period all surviving animals were blood sampled and euthanized with an intravenous injection of a saturated solution of magnesium sulphate. Autopsies were performed following euthanasia or death during the observation period. The encephalon and the sub-maxillary salivary glands of all animals were removed and laboratory tested immediately for the rabies virus or antigen presence [3, 10]. Additionally, the aetiology of intercurrent deaths was looked for by anatomical examination and, whenever possible, by laboratory diagnosis of other pathogens.

The encephalon and salivary glands of all animals were tested for the presence of the rabies virus or its nucleocapsid. For rodents, the entire encephalon was used; for dogs, cats and jackals the Ammon horn, the bulb and the cerebellum were tested separately. Additionally, sub-maxillary and parotid lymph nodes were rabies diagnosed in dogs, cats and jackals. On all samples tested the following techniques were used simultaneously:

- direct immunofluorescence on impression smears from organs stained with a fluorescent conjugate specific for rabies nucleocapsid (Sanofi-Pasteur Ref. 72112) [10];
- inoculation of cell culture (neuroblastoma 2a line ATCC no. CCL131) [3].

## 2.7. Titration of rabies-neutralizing antibodies in the serum

Serum samples from all animals (rodents included) were individually titrated. Titration was carried out by using a seroneutralization test

on BHK21 cells in 96-well microplates according to the fluorescent antibody virus neutralization test (FAVN) [2]. Briefly, sera to be tested as well as positive and negative controls were serially diluted four times. After the addition of the challenge virus (CVS 11 strain ATCC VR 959), the microplates were incubated for 48 h at  $37^{\circ}\text{C}$  in a humidified incubator with 5 % CO<sub>2</sub>. The microplates were then washed and fixed before the addition of a fluorescein isothiocyanate-conjugated antirabies serum to each well. The fluorescence reading was assessed microscopically by using an 'all or nothing' method and 50 % endpoint dilution determination. The calculation of titres was performed by comparing the values obtained from the tested sera and the value given by the positive control with a known titre (in IU·mL<sup>-1</sup>). A study of both the sensitivity and the specificity of the FAVN test was evaluated with reference to tests already described which showed that the positive threshold of the test was 0.24 IU·mL<sup>-1</sup> [9].

## 3. RESULTS

### 3.1. Mortality

One puppy fled from its cage and disappeared at day 7. It was found drowned and no rabies diagnosis was possible. One juvenile dog and five other puppies died on days 2, 9 (two dogs), 13, 15 and 24, respectively, without showing any clinical symptoms of rabies (the rabies diagnosis was negative). These dogs were all very thin and necropsy examination of the puppy who died on day 24 revealed a heavy sudden tick infestation and an advanced anaemia.

One kitten died suddenly on day 14; the rabies diagnosis was negative. This kitten displayed acute lesions of the respiratory organs and *Streptococcus β hemolytica* was isolated from its lungs.

A high mortality rate was observed for jackals: one died on day 3, three on day 6 and one on day 12. These five belonged to the juvenile group. The first four jackals that died tested negative for rabies, whereas the cadaver of the jackal that died on day 12 was cremated by mistake before being autopsied and sampled for diagnosis.

*Salmonella typhimurium* was isolated from the liver and intestines of the four other jackals (and from the spleen of three of them). Three of the jackals suffered from severe dehydration, diarrhoea and anaemia. In fact, these five animals, which we were never able to calm down, most likely died from the stress of capture and caging.

All rodents remained in good health during the observation period with the exception of two merions out of a group of 14 given the SAG2 virus by muscular route. These two merions died on days 11 and 26, without showing any rabies symptoms. The results of the rabies diagnostic test were negative for both merions and *Pasteurella hemolytica* was isolated from the lung of the merion that died on day 26.

### 3.2. Rabies diagnosis after euthanasia

All tests were negative.

### 3.3. Rabies virus detection in saliva swabs

No virus was isolated in saliva swabs from dogs 6 h after virus inoculation, but

the rabies virus was isolated in saliva swabs taken from three of the nine cats 6 h after inoculation. At day 3, though, none of the cats was found to be rabies positive. No virus was isolated from the saliva swabs taken from the eight jackals 6 h or at day 3 after inoculation.

### 3.4. Detection of rabies-specific neutralizing antibodies

Neutralizing antibodies were not detected in the sera of the 21 dogs, the 11 cats and the eight jackals whose blood was sampled at day 0. Antibody titration results on the sera sampled after vaccination are given in *table II*. Five and 6 months after SAG2 administration to dogs, two out of the five dogs had a significantly elevated titre. At 1, 2 and 3 months after vaccination only one cat had a significant antibody titre. The three surviving jackals had a significant antibody titre at least once when tested at 1, 2 or 3 months after oral administration of the vaccine. At 2 months, the number of rodents with significant antibody titres was very high. For the merions the seroconversion rate was 9/14 and 10/12 after oral and parenteral administrations, respectively. Titres

**Table II.** Titration of the rabies virus-neutralizing antibodies in the serum of animals vaccinated with SAG2.

Species	Number of animals (route of inoculation)	Fluorescent antibody virus neutralizing (FAVN) positive/number of available sera (higher individual titres during the survey – IU.mL <sup>-1</sup> )
Dog	21 (p.o.)	2/5 at day 150 and 2 (the same dogs)/5 at day 180 (3.8; 1.0; 0.02; 0.02; 0.02)
Cat	11 (p.o.)	1/10 at day 30 and 1 (the same cat)/4 at day 60 and 90 (2.2; ≤ 0.06..... ≤ 0.06)
Jackal	8 (p.o.)	3/3 at day 30, 60 or 90 (1.0; 0.3; 0.4)
Jerboa	2 (i.m.)	0/1 at day 60 (≤ 0.2)
Merion	14 (p.o.)	9/14 at day 60 (1.7; 1.7; 1.3; 1.0; 1.0; 0.7; 0.7; 0.6; 0.4; ≤ 0.2; ≤ 0.2; ≤ 0.2; ≤ 0.2; ≤ 0.2)
	14 (i.m.)	10/11 at day 60 (6.6; 5; 5; 5; 3.8; 2.2; 2.2; 1.7; 1.7; 1.7; ≤ 0.2)
Gerbil	32 (i.m.)	4/6 at day 60 (5; 5; 2.9; 2.2; ≤ 0.2; ≤ 0.2)

p.o.: per os; i.m.: intramuscular; IU.: international units.

were significantly higher after inoculation than after oral instillation (Mann-Whitney U test significant at the  $P < 0.001$  level).

#### 4. DISCUSSION

The safety of the SAG2 rabies vaccine has been proven on several species known to be rabies vectors: two species of jackals (*Canis adustus* and *Canis mesomelas*) [7], the red fox (*Vulpes vulpes*) [23] and the domestic dog [22, 23]. Numerous other species of domestic and wild fauna in Europe, sub-Saharan Africa and North America [6, 14, 19] not known to be potential reservoirs of rabies, but which may consume vaccine baits, have also been tested. Several of these species are commonly found in Tunisia: domestic dogs and cats, the domestic goat (*Capra hircus*), the Norway rat (*Rattus norvegicus*), the wild boar (*Sus scrofa*), the red fox (*Vulpes vulpes*), and several scavenger and prey bird species: the buzzard (*Buteo buteo*), the red kite (*Milvus milvus*), the tawny owl (*Strix aluco*), the long-eared owl (*Asio otus*) and the barn owl (*Tyto alba*).

Other species which are not found in Tunisia are still very similar to species common to Tunisia: the hedgehog (*Erinaceus europaeus*), the carrion crow (*Corvus corone*) and the rook (*Corvus frugilegus*).

As these species did not include several wild rodent species most common to Tunisia, several tests were performed on merions and gerbils in the present study. Out of the 62 Tunisian rodents tested, 60 remained in good health, while the two deaths were due to intercurrent causes. The high seroconversion rate observed in merions and gerbils and inoculum titration after use, confirm that rodents received a high dose of SAG2. The administration of a large amount of the SAG2 virus confirmed the lack of pathogenicity, or at least the very low pathogenicity of this vaccine on rodents vaccinated orally or parenterally.

Several experiments have shown the innocuity of the SAG2 rabies strain for laboratory dogs and cats. Due to the poor health status of common dogs in Tunisia, the SAG2 rabies virus innocuity had to be determined in animals reflecting, as much as possible, the local dog and cat populations. Any mammal population that is multi-parasitized and poorly fed cannot develop a strong immunity against pathogens. This has been observed amongst Tunisian dogs vaccinated against rabies. Tunisian dogs have been observed to develop lower rabies-neutralizing antibody titres than common dogs in France, while the latter develop lower antibody responses than laboratory dogs [8]. The objective of this study was to collect stray or immuno-compromised dogs in order to determine the eventual residual pathogenicity of the SAG2 vaccine to the dog (if any). Several ecological studies have indicated a mean age ranging between 1.8 and 3.3 years for owned Tunisian dogs, which is much lower than the 4.5 years observed in North America and Europe (for review, see [25]). The high proportion of young dogs, which reaches approximately 30 % in the suburbs of Tunis (Seghaier et al., unpublished results), is the reason why three young dogs and ten puppies were included in our experiment. Additionally, WHO rabies experts recommend including dogs less than 10 weeks old in such safety tests because these animals are in frequent contact with children, and are thus at a higher risk of transmitting the rabies virus, whether attenuated or not [27]. It must be noted that the virus was not detected in the saliva of dogs 6 h after oral instillation. The rabies live virus was detected in the saliva of three cats 6 h after oral instillation, but was absent 3 days later, and was never detected in the six other cats. These results do not support the hypothesis of a multiplication of the SAG2 virus in the salivary glands of cats, and may indicate that there is a slow disappearance of a very large amount of the virus that is given by direct instillation.



The poor puppy health status was confirmed by the high mortality rate observed in this group. Because the rabies aetiology was eliminated by the use of several validated diagnostic techniques, the poor health state of puppies indicates that the SAG2 vaccine is not pathogenic for immuno-compromised young dogs.

A significant seroconversion was observed in only one cat. This result may be surprising considering the large amount of the SAG2 virus administered. This result is in accordance with a previous test performed on cats in CNEVA Nancy (France), where only two out of five cats given the same virus orally had a significant antibody titre after 33 days [23].

Due to the difficulty for jackals to cope with captivity, oral instillation of the vaccine was performed before the end of the usual period of acclimatization. The original observed mortality rate of jackals before the beginning of the trial (6/14) continued at the same pace after oral vaccination (5/8). It should be noted that the latter five jackals died within 12 days or less of capture. Once again the rabies aetiology was eliminated by laboratory diagnosis, and the poor health status of the jackals gave more significance to the lack of observed pathogenicity of the vaccine.

Despite the small number of jackals that survived captivity, all seroconverted. This complete seroconversion rate is a new indication of the possible use of the SAG2 vaccine for controlling rabies in areas where the jackal is the main rabies reservoir [7].

The safety of the attenuated SAG2 rabies virus was evaluated in several domestic and wild species that live in Tunisia according to the general protocols recommended for candidates of oral rabies vaccines. The results confirmed those that had already been obtained in many other laboratories using domestic and wild species from other countries and continents. Following oral administration of the strain used in this experiment to dogs, cats, jackals and wild rodents

(merions) no rabies-related pathology was observed, and this strain was observed to be immunogenic. Administered by the intramuscular route to wild rodents (jerboas, merions and gerbils) the SAG2 vaccine was similarly non-pathogenic and immunogenic. These new results obtained on wild and domestic carnivores in difficult health conditions are of special interest for assessing the lack of pathogenicity of the SAG2 vaccine for the oral immunization of stray dogs against rabies.

The vaccinal efficacy of this strain has already been demonstrated in the dog after direct oral administration [13] or by using a bait specific for the dog (Hammami et al., unpublished results). Therefore, its use in the oral immunization of dogs that are difficult or impossible to parenterally vaccinate, seems preferable in Tunisia (and in countries with similar domestic and wild fauna) than the use of other less attenuated rabies strains. This is especially true because of the considerable safety and high genetic stability of the attenuated SAG2 rabies virus.

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