EFFICACY STUDIES WITH SAD B19 IN TURKISH DOGS

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SAD B19'UN TÜRK KÖPEKLERİNDEKİ ETKİNLİK ÇALIŞMALARI

ÖZET

Yerli ırk Türk köpekleri SAD B 19 canlı modifiye virus aşısının farklı konsantrasyonları kullanılarak ağız yolu ile aşılanmıştır. İmmun yanıtın izlenmesi için düzenli aralıklarla kan örnekleri alınmıştır. 1 x 10^8 FFU'nun üzerinde doz ile kabul edilebilir serum dönüşüm düzeyleri elde edildiği gözlenmiştir. 10 köpekten 9'u aşılamadan 479 gün sonra 1.5 x 10^8 FFU luk eprüvasyona (direkt olarak ağız boşluğuna akıtma) direnç göstermiştir. 2.1 x 10^7 FFU ile doldurulmuş aşı kapsülü içeren bait verilerek aşılanmış 16 köpekten sadece 3'ünde serum dönüşümü gözlenmiştir. Bununla birlikte hiç kuduz nötrölizan antikoruna sahip olmayan birkaç köpek aşılamadan 74 gün sonraki eprüvasyona direnç göstermiştir.

SUMMARY

Local Turkish dogs were vaccinated orally with the live modified virus vaccine SAD B19, using different concentrations. To monitor the immune response bloodsamples were taken at regular intervals. Among others, dogs were vaccinated with 2.1 x 10^7 FFU orally (bait) but developed no detectable level of rabies virus neutralizing antibodies. Several of these dogs, however, resisted challenge 74 days after vaccination. It was shown that acceptable levels of seroconversion were achieved with dosage of more than 1 x 10^8 FFU SAD B19. Nine out of 10 dogs resisted challenge 479 days after vaccination with 1.5 x 10^8 FFU SAD B19 by direct instillation into the mouth cavity.

INTRODUCTION

Oral immunization of wildlife against rabies with the live modified rabies vaccines has been shown to be effective (Müller & Schlüter, 1998). The success obtained from vaccination campaigns with SAD B19 and other oral vaccines led to the decision to test the feasibility of oral vaccination of

dogs against rabies. Previous safety studies with oral vaccines on dogs as nontarget species indicated that dogs need a higher concentration of the vaccine than foxes to show detectable rabies antibody levels (Perry & Wandeler, 1993; Müller et al, 1998). Also, data on Turkish dogs revealed a lower seroconversion response than German dogs vaccinated with similar concentrations of SAD B19 (Müller et al., 1998). One reason for this could be a result of the poor health conditions of the Turkish dogs examined. The target population for oral vaccination in Turkey, the free-roaming dogs, are often full of parasites, malnourished and are exposed to all sorts of diseases.

The purpose of this paper is to report on the results of different field and laboratory efficacy studies on Turkish dogs after oral vaccination of SAD B19. These tests were performed over the last three years and consisted of successive studies in order to demonstrate the short and long term immunogenicity for dogs of SAD B19. During the various tests, different vaccine doses were tested in order to determine the minimum immunogenic dose.

MATERIAL & METHOD

SAD B19 was produced from MSV SAD B19 (05.04.82) at IDT, Germany. This live modified rabies virus strain is adapted for oral vaccination of dogs on BSR Cl.13 - cells. Depending on the test, the vaccinations were performed either by direct instillation of the viral suspension in the dogs mouth or by ingestion of a Köfte-bait (local minced meat mixed with bread crumbs) containing a capsule filled with the vaccine.

Sera in this and the following trials were tested for rabies neutralizing antibodies using the rapid fluorescent focus inhibition test (RFFIT) at the Veterinary Control and Research Institute in Etlik (VCRI), Ankara. The antibody titres are expressed in International Units (IU). Also, the efficacy of different vaccine doses (Test 3 & 4) was tested by resistance to virulent challenge with 1 ml of challenge virus at VCRI. The challenge virus was isolated from the paired submaxillary salivary glands of a naturally infected rabid coyote *(Canis latrans)* from the State of Texas. USA, in 1994 (CDC, Atlanta) The challenge virus was administered intramuscularly in the *M. masseter* of the tranquillized dogs (1.5 ml of a mixture of ketamine hydrochloride - xylazine, 1:1 ratio). Brain material of dogs who died after the challenge, or which resisted challenge and were euthanized, was examined for the presence of rabies antigen by immunoflourescence (FAT).

Dog vaccinated in the field may have antibody titres lower than those in dogs kept in the laboratory under 'ideal' conditions (Precausta et al., 1985). Therefore , the animals kept at VCRI were not treated against parasites or vaccinated against other infectious diseases, other than rabies, in order to reflect better the conditions of dogs in the field.

Test 1: Twenty free-roaming dogs were captured by the municipality of Ankara, divided into two groups of ten animals and housed at VCRI in May 1994. All animals were bled before vaccination. The dogs were vaccinated by instillating SAD B19 directly into the oral cavity of each unanaesthized animal; one group received 3 x 10^7 FFU and the other 1.5 x 10^8 FFU. Bloodsamples were taken on days 28, 42 and 57 after vaccination for rabies antibody determination. The animals were observed daily.

Test 2: Between May and December 1994, owned dogs in the Anatolian urban parts of İstanbul were offered a Köfte-bait containing a capsule with 3 x 10^7 FFU SAD B19. Serum samples were obtained directly after a bait was offered, and a second bloodsample was taken from relocated dogs on average 42 days after vaccination. The dogs were photographed and a brief physical description of the dogs was noted for identification purposes.

Test 3: Sixteen free-roaming dogs were caught by the municipality in different neighbourhoods of Ankara and brought to the dog enclosure at VCRI, Etlik in April 1996. A single Köfte-bait was placed in front of each dog, which for this purpose had been separated from the other dogs to avoid distraction. No dog included in this study had detectable rabies antibody levels prior to vaccination. In every Köfte-bait a vaccine container with SAD B19 (2.1 x 10^7 FFU) was hidden. Further bloodsamples were taken 54 and 74 days post vaccination. The animals were observed daily. On average 74 days postvaccination all dogs were challenged.

Test 4: Bloodsamples from ownerless and owned dogs were collected in the Anatolian urban part of İstanbul on several occasions. The dogs were photographed and a brief physical description of the dogs was noted. Free-roaming animals were additionally eartagged for identification purposes. 1.5 x 10^8 FFU SAD B19 was given orally to the dogs (n=123). Several of these dogs (n=17) were offered a Köfte-bait containing a vaccine capsule. The other dogs were vaccinated by direct application into the oral cavity using a needleless syringe. A bloodsample was taken from 122 of these dogs directly after vaccination. On average 22, 163 and 400 days after vaccination bloodsamples were taken from 99, 45 and 26 relocated dogs, respectively. On average, 460 days after vaccination 10 relocated dogs were brought to the dog shelter at VCRI in Etlik, Ankara, and challenged on average 479 days after vaccination. Before administration of the challenge virus a bloodsample was taken. 69 days after challenge all surviving animals were euthanized and bled for serum. From a neighbouring

area in İstanbul six juvenile ownerless dogs were caught and used as controls.

RESULTS

Test 1: Serological results are summarized in tables 1 and 2, dogs with detectable seroneutralizing antibodies before vaccination were omitted from these tables. Most animals vaccinated with 1.5 x 10^8 FFU responded with antibody levels above the threshold of 0.5 IU/ml. However, only 2 out of 6 dogs that received 3.0 x 10^7 FFU seroconverted (>0.5 IU/ml). The arbitrarily defined level of 0.5 IU/ml is used here, while in humans it is considered indicative of successful rabies immunization (Brochier et al., 1989).

Table 1. Rabies antibody titres in dogs vaccinated directly into the mouth cavity with 1.5 x 10^8 FFU SAD B19, by time after vaccination. Only dogs with no rabies neutralizing antibodies before vaccination are included.

Sample	Days after	Number	Number of dogs with indicated titre (IU/ml)			
	vaccination	of dogs	<0.5	<1.0	<5.0	≥5.0
1	28	7	1	-	1	5
2	42	7	1	-	1	5
3	57	7	3	-	2	2

Table 2. Rabies antibody titres in dogs vaccinated directly into the mouth cavity with 3.0 x 10^7 FFU SAD B19, by time after vaccination. Only dogs with no rabies neutralizing antibodies before vaccination are included.

Sample	Days after	Number	Number of dogs with indicated titre (IU/ml)			
	vaccination	of dogs	<0.5	<1.0	<5.0	≥5.0
1	28	8	6	-	1	1
2	42	8	5	-	1	2
3	57	8	5	-	3	-

Test 2: 17 (74%) out of 23 dogs vaccinated with 3.0 x 10^7 FFU SAD B19 did not have detectable levels of rabies virus neutralizing antibodies

(<0.5 IU/ml), on average 42 days after vaccination, including dogs that swallowed the vaccine container .

Test 3: The results are shown in table 3. The dogs in this test received the lowest dosage of SAD B19 (2.1 x 10^7 FFU). Only three dogs had a rabies neutralizing antibody titre of ≥0.5 IU/ml at the time of challenge. Eight out of fifteen dogs were protected against challenge despite the absence of demonstrable rabies antibodies.

Table 3. Rabies antibody titre of dogs kept at VCRI, Etlik, that were offered a bait containg a capsule filled with 2.1 x 10^7 FFU SAD B19 (P - vaccine container punctured and discarded, SW - vaccine container swallowed and days - number of days after vaccination)

Dog	Capsule	Bloodsample 1		Bloodsample 2		Challenge Test
		IU/ml	days	IU/ml	days	(FAT)
1	SW	(-)	57	(-)	77	pos.
2	SW	(-)	57	(-)	77	neg.
3	P	(-)	57	(-)	77	pos.
4	P	(-)	57	(-)	77	neg.
5	P	(-)	57	(-)	77	neg.
6	P	(-)	57	(-)	77	neg.
7	P	(-)	57	(-)	77	pos.
8	P	(-)	50	(-)	70	neg.
9	SW	(-)	50	(-)	70	pos.
10	P	(-)	50	(-)	70	pos.
11	P	(-)	50	(-)	70	pos.
12	SW	(-)	50	(-)	70	pos.
13	P	1.1	50	0.6	70	neg.
14	P	0.4	50	(-)	70	neg.
15	SW	3.3	57	0.6	77	neg.
16	P	3.3	50	3.3	70	neg.

Test 4: On average 21, 162 and 399 days after vaccination by direct application into the oral cavity; 92%, 83% and 81% still had an antibody titre above the threshold of 0.5 IU/ml, respectively (Table 4). There was no significant difference in rabies neutralizing antibody titre of the first

bloodsample taken after vaccination between the two vaccine delivery methods; bait-vaccine system and squirting the vaccine directly in the mouth (Mann-Whitney U-Test). However, the titres of the dogs vaccinated by offering the animals a bait dropped rapidly; all dogs had a titre of <0.5 IU/ml during the second and third bloodsample (Table 5).

The results of the challenge test with ten dogs vaccinated by direct application into the mouth cavity are shown in table 6. All of these dogs had no rabies neutralizing antibodies prior to vaccination. On average 479 days after vaccination nine out of ten dogs survived the challenge, one dog died from rabies (FA-positive), although the animal had a detectable level of rabies neutralizing antibodies at the time of challenge. All six control dogs died from rabies, on average 24 days after the challenge virus was administered.

Table 4. Rabies antibody titres of dogs vaccinated directly into mouth cavity with 1.5 x 10^8 FFU SAD B19, by time after vaccination. Only dogs with no rabies neutralizing antibodies before vaccination are included.

Sample	Days after vaccination	Number of dogs	Number of dogs with indicated titre (IU/ml)			
			<0.5	<1.0	<5.0	≥5.0
1	21	78	6	-	2	70
2	162	35	6	-	17	12
3	399	21	4	1	4	12

Table 5. Rabies antibody titres in dogs, by time after vaccination, dogs were offered a bait containing a capsule with SAD B19 (1.5 x 10^8 FFU), only dogs with no rabies neutralizing antibodies prior to vaccination are included.

Sample	Days after vaccination	Number of dogs	Number of dogs with indicated titre (IU/ml)			
			<0.5	<1.0	<5.0	≥5.0
1	23	11	2	1	1	7
2	166	5	5	-	-	-
3	404	2	2	-	-	-

DISCUSSION

The rabies serologies of these studies demonstrate that SAD B19, when orally administered, can elicit seroconversion in dogs. It seems that

the minimum dosage to achieve an acceptable seroconversion rate is 10^8 FFU. Nine out of ten dogs vaccinated with 1.5×10^8 FFU resisted challenge more than 15 months after vaccination. However, one dog was not protected despite the presence of demonstrable rabies antibodies. The reason for this remains unclear. All dogs captured were free-roaming, often these animals are exposed to all kinds of infectious diseases that can influence the immune response. Also, at the time of challenge several dogs from test 3 with no detectable circulating antibodies present resisted the challenge. Hence, it seems that post vaccinal immunity to rabies in Turkish dogs is not always detectable by the presence of neutralizing antibodies.

Approximately, three weeks after vaccination no significant difference was observed in rabies neutralizing antibody titre between the two vaccine delivery techniques used (Test 4); vaccine placed in baits and direct administration into the oral cavity. However, subsequent bloodsamples showed substantial differences. Wandeler (1991) already mentioned that immunization using a bait is 'less effective' than by direct application into the oral cavity. For instance, part of the vaccine may drip out of the dog's mouth when it is chewing the bait. Also, the punctured capsule may still contain part of the vaccine-dose after the dog discarded it. Furthermore, part of the vaccine-dose may be absorbed by the bait-material decreasing the volume coming into contact with the oral mucous membrane of the dog; a prerequisite for effective oral immunization. This loss of activity can be compensated for by placing a larger amount or a higher concentration of vaccine in the capsule hidden in the bait (Frontini et al., 1992).

Table 6. Results of the Challenge Test, dogs were vaccinated with 1.5×10^8 FFU SAD B19 (days - number of days after vaccination)

Dog	Vaccinated	Bloodsample 1		Bloodsample 2		Result Challenge Test		
	on	IU/ml	days	IU/ml	days	Remark	FAT	IU/ml
1	14.06.95	20.0	21	1.1	478	killed	neg.	7.1
2	14.06.95	6.7	21	2.2	478	died	pos.	
3	14.06.95	20.0	21	10.0	478	killed	neg.	23.8
4	14.06.95	10.0	21	1.1	478	killed	neg.	16.8
5	12.06.95	10.0	21	6.7	480	killed	neg.	10.0
6	12.06.95	>10.0	160	30.0	480	killed	neg.	47.6
7	12.06.95	30.0	21	6.7	480	killed	neg.	14.1
8	12.06.95	5.0	22	20.0	480	killed	neg.	20.0
9	14.06.95	20.0	20	3.3	478	killed	neg.	5.0
10	14.06.95	20.0	20	20.0	478	killed	neg.	11.9

Schumacher et al (1993) determined the immune response in dogs after administration of another oral rabies vaccine candidate, the live modified SAG-2 vaccine-virus. Considering the results of this study and the results presented in this paper (Test 4), it seems that SAD B19 elicit a higher immune response in dogs than SAG-2. The seroconversion rate observed in test 4 by direct instillation of the vaccine virus showed an almost identical development with dogs vaccinated parenterally with a commercial rabies vaccine. Eight to nine weeks after vaccination by the parenteral route with a commercial rabies vaccine 126 out of 130 (96.9%) dogs had a rabies antibody titre of ≥0.5 IU/ml in Finland. Approximately 1 year after vaccination 83% of these dogs still had a titre of ≥0.5 IU/ml (Sihvonen et al., 1995). In a study in Peru, more than 95% of the dogs still had titres of ≥0.5 IU/ml 12 months after vaccination with an inactivated tissue culture vaccine by the parenteral route (Chomel et al., 1988). However, in Thailand 42% of dogs had no detectable rabies antibody titre 360 days after vaccination with an inactivated tissue culture vaccine (Tepsumethanon et al., 1991). Also, in Tunisia only 24% of 29 dogs vaccinated with a similar type of vaccine had titres of ≥0.5 IU/ml (Haddad et al., 1985). Hence, responses to rabies vaccines may vary considerably between dog populations. Another explanation could be poor quality of the vaccine used. In the Netherlands, of six commercially released, inactivated rabies vaccines for veterinary use, two were clearly below the minimal requirements for potency of 1.0 IU (Rooijakkers et al., 1996).

It can be concluded that dogs can be vaccinated orally with SAD B19 under fieldconditions and are protected against a rabies infection over a long time. A large segment of the dog population in countries with dog mediated rabies is inaccessible for parenteral vaccination; ownerless dogs and animals that can not be handled by their owner. Oral vaccination with SAD B19 offers possibilities to reach these inaccessible dogs, permitting a significant increase in the vaccination coverage of the overall dog population

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