SAFETY TESTS OF SAD B19 IN TURKISH DOGS

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TÜRK KÖPEKLERİNDE SAD B19'UN ZARARSIZLIK TESTLERİ

ÖZET

Dünya Sağlık Örgütü'nün isteklerine göre, köpeklerin kuduza karşı ağız yolu ile aşılanması için örnek aşı virusu SAD B19, yerel Türk köpeklerinde zararsızlık yönünden test edildi SAD B19'un 10 haftalıktan küçük yavru köpeklerde oral ve parenteral uygulamadan sonra tamamen zararsız olduğu gösterilmiştir. SAD B19 ile intracerebral aşılanan ergin köpeklerde 21 günlük gözlem süresince kuduza ait hiçbir klinik belirti görülmemiştir. SAD B19 ile oral, intracerabral veya parenteral yollarla aşılandıktan sonra, farklı zamanlarda salya numuneleri toplandı. Yetişkin ve yavru köpeklerden alınan salya swablarında virus tespit edilemedi.

SUMMARY

The candidate vaccine-virus SAD B19 for oral vaccination of dogs against rabies was tested in local Turkish dogs in respect of safety, according to the requirements of the World Health Organization. It was shown that SAD B19 was completely innocuous for puppies less than 10 weeks of age after oral and parenteral application. Adult dogs vaccinated *i.c.* with SAD B19 did not show any clinical symptoms of rabies during the observation period of 40 days. Also, no excretion of vaccine-virus could be detected in saliva swabs taken from adult dogs and puppies. The saliva swabs were collected at different intervals after the vaccine virus SAD B19 was administered orally, intracerebrally or by the parenteral route.

INTRODUCTION

Lately, attention has been focused on the development of oral rabies vaccines for dogs to increase the vaccination coverage. One of the candidate vaccines is the live modified rabies virus vaccine SAD B19. It is important to stress that oral vaccination is not a replacement of parenteral vaccination but an additional method to increase the effectiveness of existing vaccination

campaigns. However, the method used for oral vaccination of wildlife can not be copied for dogs without adaptations. Dogs are very closely associated with humans, especially children. The likelihood of direct exposure and of passive vaccine virus transmission to humans is considerably higher for oral dog vaccination than for wildlife vaccination programmes. The World Health Organization (Blancou &° Meslin, 1996) has therefore proposed detailed safety requirements for rabies vaccine candidates for oral vaccination of dogs. For example, considering that puppies may form an important part of the dog population and the high probability of contact between young children and puppies, candidate vaccines should not produce disease in dogs less than 10 weeks of age. Also, the possibility of excretion of vaccine virus in the saliva of vaccinated animals should be examined. Exposure of humans to the vaccine virus may occur through contact with a freshly vaccinated dog (e.g. by licking).

This paper describes the results of different safety tests with SAD B19 in local Turkish dogs conducted at the Veterinary Control and Research Institute (VCRI) in Etlik, Ankara; possible reversion of vaccine virus to virulence, presence of vaccine virus in saliva and safety tests in puppies.

MATERIAL & METHOD

The live modified rabies virus vaccine SAD B19 is produced at IDT, Germany, and is adapted for oral vaccination of dogs on BSR Cl.13-cells. All dogs used in the studies presented here were free-roaming cross-breeds, captured by the local municipality in different neighbourhoods of Ankara, Turkey. The animals were placed in a protected closed environment for the entire duration of each of the tests and were observed daily at VCRI, Etlik.

Test 1. Innocuity of SAD B19 in puppies

All puppies were aged as less than 10 weeks old at the time of vaccination. The mother animals were free of antirabic antibodies (RFFIT). Two concentrations, 2.4×10^7 and 4.2×10^8 FFU, were administered orally by a single instillation of the viral suspension in the dogs' mouth (n=20) using a needleless syringe. Furthermore, two groups of two and six puppies were administered 2.4×10^7 and 1.0×10^8 FFU by the parenteral route, respectively. At the end of the observation period the animals were euthanized and the brains examined for rabies virus by the fluorescent antibody test (FAT). Also, all puppies that died during the observation period were examined (FAT). Of one group of puppies also the serum of the animals was examined by seroneutralization on cells (RFFIT).

Test 2. Reversion of SAD B19 to virulence in dogs

The vaccine virus SAD B19 was re-isolated from the injection site of the brain after the 4th-passage *i.c.* in foxes *(Vulpes vulpes)*. The 4th-passage

with a titre of 2.5 x 10^7 FFU SAD B19 was administered *i.c.* in four juvenile dogs (approximately 6 months old). All dogs tested negatively for rabies neutralizing antibodies prior to vaccination. Bloodsamples were taken 6, 21 and 40 days after inoculation. Sera samples were tested by RFFIT for the presence of rabies neutralizing antibodies. Furthermore, saliva swabs were taken 1, 3, 5, 8 and 16 days after inoculation. The saliva swabs were examined as described in the section 'presence of SAD B19 in saliva of vaccinated dogs'. Forty days after inoculation all animals were euthanized.

Test 3. Presence of SAD B19 in saliva of vaccinated dogs.

The puppies were aged as less than 10 weeks at time of virus administration. The mother animals were free of antirabic antibodies (RFFIT). The adult dogs were kept in groups of 2-4 animals in one cage (2x4x2.5m) and were free of antirabic antibodies at the time of vaccination. Details of this test are listed in table 1.

Table 1. Test on virus excretion in the saliva of dogs vaccinated with SAD B19 (Saliva Test - test used to detect residual vaccine virus).

Group	Age	Number of animals	Inoculation date	Vaccination Method	Dose (FFU)	Saliva Test
1	puppy	5	15.08.95	oral/direct	4.2 x 10^8	RFFIT
2	puppy	6	19.09.95	oral/direct	4.2 x 10^8	RFFIT
3	puppy	6	18.04.96	parenteral	1.0 x 10^8	MIT
4	adult	5	26.03.96	oral/bait	4.0 x 10^7	MIT
5	adult	1	26.03.96	oral/direct	4.0 x 10^7	MIT

Saliva of the dogs vaccinated orally was collected 2, 24, 48 and 72 hours after vaccination. Of the puppies vaccinated parenterally, saliva was collected 4, 14 and 19 days after vaccination. Saliva was collected through chewing a cotton wool cylinder by the animal and by swabbing of the oral cavity for 1 - 1.5 minutes. Afterwards the cotton wool cylinder was placed in the inner holding tube of the Salivette® (Sardstedt). 1 ml of the following medium was added: 1 ml Gentamycin and a mixture of 100ml MEM / SNT - 10 ml New Born Calf Serum (MEM - Minimal Essential Medium, SNT - Sera Neutralisation Test). Saliva was extracted by centrifugation (2000 rpm for 10 minutes) and collected. The samples were evaluated by the Rapid Fluorescent Focus Inhibition Neutralisation Test (RFFIT) and/or by Mouse Inoculation Test (MIT). In case of the MIT, for every saliva sample four mice were needed. The extracted saliva was administered to every mouse (0.5 ml *s.c.*). After 14 days the mice were bled. The bloodsamples from the mice

inoculated with the same saliva sample were pooled. The bloodsamples were centrifuged at 3000 rpm for 25 minutes. Serum was separated and stored at -20°C until the RFFIT.

Also, saliva swabs were collected during the test to examine possible reversion of SAD B19 to virulence in dogs. However, during this test the method of examination was different from the above-mentioned method description. Here, saliva secretions were collected by swabbing of the oral cavity for 1 - 1.5 minutes. Afterwards the cotton wool cylinder was placed in the holding tube, Cultiplast®. 2 ml of the following medium was added: MEM / SNT (MEM - Minimal Essential Medium, SNT - Sera Neutralisation Test) plus antibiotics (Gentamycine [50 mg/l] and Amphotericine B [2.5 mg/l]). The suspension was centrifuged for 5 minutes at 4000 rpm. The liquid phase was removed for further testing. For every sample one microflask (25 cm^2) was used. Every microflask contained 10 ml MEM / TW (BSR Cl 13, 3 x 10^5 cells/ml) plus 10% serum. Of every sample 0.5 ml was added to the microflask. The microflasks were incubated at 35°C for 6 days. At the fourth day the medium was changed with MEM / TW plus 1 % serum. Subsequently, two cavities of an 8-chamber-slide (Lab-Tek® , Nunc, Inc.) were filled with 0.5 ml liquid phase from the microflasks and 0.1 ml cellsuspension MEM / SNT (BSR Cl 13, 1 x 10^6 cells/ml) plus 10% serum material. The slides were put into an incubator (35°C, 5% CO2) for 48 hours. After draining of medium the cells were fixed with 80% acetone for 30 minutes at room temperature. The chamber-slides were drained again and dried. FITC-marked antirabies monoclonal-antibodies (Centocor) were added. The chamber-slides were stained for 30 minutes at 37°C. The chamber-slides were analysed for rabies virus by the fluorescent antibody test (FAT).

RESULTS

Test 1. Innocuity of SAD B19 in puppies

In table 2 the number of days that the individual puppies were observed is shown. The average overall observation period for puppies 'vaccinated' orally and parenterally was 57.0 (n=20, s.d.=20,3) and 53.8 days (n=8, s.d.=21.1) respectively. Although, hyperimmunsera against distemper, parvo and hepatitis was administered regularly, several puppies died as a result of parvo-enteritis. All puppies that died during the trials or were euthanized afterwards tested rabies negative (FAT). In table 3 the results of the RFFIT (litter E) are shown. Hence, the vaccine virus SAD B19 was completely innocuous for puppies less than 10 weeks of age.

Test 2. Reversion of SAD B19 to virulence in dogs

None of the animals vaccinated by the intracerebral route showed any sign of rabies during the observation period. All dogs showed a strong

Table 2. Duration of observation period of puppies vaccinated with SAD B19, using different routes and concentrations.

Litter	Date of inoculation	Method	Dose (FFU)	Number of puppies	Number of days observed
A	27.04.95	oral	$2.4x10^7$	4	46,50,51,81
B	03.05.95	oral	$2.4x10^7$	4	40,43,43,43
C	17.05.95	parenteral	$2.4x10^7$	2	27,28
D	19.09.95	oral	$4.2x10^8$	7	50,50,52*,39*,24*,51,49
E	15.08.95	oral	$4.2x10^8$	5	79*,76*,97,97,79
F	18.04.95	parenteral	$1.0x10^8$	6	30,69,69,69,69,69

* - autopsy: parvo-enteritis

Table 3. Results of the bloodsample in IU/ml taken from puppies (litter E) vaccinated with 4.2×10^8 FFU SAD B19.

Days after vaccination	Puppy 1	Puppy 2	Puppy 3	Puppy 4	Puppy 5
31	10.0	(-)	10.0	10.0	6.7
59	10.0	1.1	(-)	6.7	6.7

Table 4. Individual neutralizing titres (IU/ml), determined by RFFIT, for each dog inoculated with 2.5×10^7 FFU SAD B19 by the intracerebral route.

Dog	6 days after vaccination	21 days after vaccination	40 days after vaccination
1	1.2	10.0	20.0
2	3.5	10.0	14.1
3	0.9	8.4	14.1
4	(-)	14.1	7.1

antibody response (Table 4). Furthermore, no excretion of rabies virus particles of vaccinal origin was demonstrated in the saliva swabs taken.

Test 3. Presence of SAD B19 in saliva of vaccinated dogs.

No residual vaccine virus could be detected in the saliva of the dogs taken 2, 24, 48 and 72 hours after being vaccinated orally by using the RFFIT and MIT. Also no rabies antibodies were detected in the bloodsamples of the mice, inoculated with the saliva samples of the 6 puppies vaccinated by the parenteral route, taken 4, 14 and 19 days after inoculation.

DISCUSSION

In case of oral vaccination of dogs against rabies, it is necessary to be extremely carefully with modified live vaccinal strains in terms of innocuity and of virus excretion (Haddad et al., 1994). Even if baits are offered directly to dogs, it can not be excluded that e.g. puppies will come in contact with the vaccine-virus. Many times puppies snatch away the bait when offered to the mother-animal, or the mother-animal will bring the bait to her offspring. In the study presented here, it was shown that the vaccine virus SAD B19 was completely innocuous for puppies less than 10 weeks of age. Also, no residual infectious virus was detected in any of the saliva swabs taken of adults or puppies, irrespective of the route of administration (parenteraly, oraly or intracerebraly), indicating that the vaccine virus is not excreted in any detectable amounts. Even the, for rabies-virus, highly susceptible mouse did not show any sign of sickness or succumbed of rabies after the animals were inoculated with saliva-suspension of the dogs.

After the 5th-passage (*i.c.*) the vaccine virus SAD B19 did not induce any signs of sickness in all four dogs during the observation period of 40 days. However, all dogs developed a strong antibody response. An identical study was carried out with foxes, also here no clinical symptoms of illness were observed in the inoculated animals (Neubert, unpublished data).

In conclusion, oral vaccination of dogs with the live modified vaccine strain SAD B19 appears to be a realistic possibility in view of the good results obtained in terms of safety in dogs.

ACKNOWLEDGEMENTS

The author would like to thank İbrahim Adalı, Serbülent Dündar, Hikmet Ün for their assistance during these studies and especially Ahmet Çelik for looking after the dogs.

REFERENCES

1 - Blancou J, Meslin F-X (1996) Modified live-virus rabies vaccines for oral immunization of carnivores. In: Laboratory techniques in rabies,

4th ed. (eds. Meslin F-X, Kaplan MM, Koprowski H) 324-337. World Health Organization, Geneva.

2 - Haddad N, Ben Khelifa R, Matter H, Kharmachi H, Aubert MFA, Wandeler AI, Blancou J (1994) Assay of oral vaccination of dogs against rabies in Tunisia with the vaccinal strain SAD_{Bern}. Vaccine, 12: 307-309.

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