INNOCUITY TESTS OF SAD B19 IN TURKISH NONTARGET SPECIES

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SAD B19'UN TÜRKİYE'DE HEDEF DIŞI TÜRLERDEKİ ZARARSIZLIK TESTLERİ

ÖZET

SAD B19 canlı modifiye virus suşunun, köpeklerin ağız yoluyla aşılanması için güvenirliği Türkiye'de iki ana bait alıcısında değerlendirilmiştir: bunlar evcil kedi ve karga vb. SAD B19 aşı suşunun oral uygulamadan sonra evcil kediler ve saksağanlar için tamamen zararsız olduğu gösterilmiştir. Ayrıca toplam 81 adet yerel kemirgen (17 *Mus musculus*, 9 *Rattus norvegicus*, 13 *Microtus epiroticus*, 29 *Apodemus sylvaticus* ve 13 *Apodemus agrarius*) SAD B19 attenue kuduz aşı virusu ile inokule edildi. Elde edilen sonuçlar ekstrem laboratuvar şartlarında oral uygulamalarda SAD B19'un kalıntı seviyesinde patojenitesinin olduğunu teyit etmiştir. Bu kemirgenlerden toplam 5'i kuduzdan ölmüştür. Bununla birlikte bu rodentlerden 3 tane fare, aşı uygulamasından önce ether, ketamin hydrochloride ile anestezi edilmiştir. Aşı virusu muhtemelen direkt olarak solunum yolu organlarına uygulanmış olabilir. Aşı virusunun aynı türdeki inokule hayvanlardan kontrol hayvanlarına nakli gözlenmemiştir. Bu da SAD B19'un kemirgen populasyonlar içinde yayılım eğilimi olmadığını göstermektedir.

SUMMARY

The safety of the live modified vaccine virus SAD B19 for oral vaccination of dogs was evaluated in the two main bait competitors in Turkey; domestic cats and corvine species. It was shown that the SAD B19 vaccine virus was completely innocuous for domestic cats and magpies (*Pica pica*) after oral application. Furthermore, a total of 81 local Turkish rodents (17 *Mus musculus*, 9 *Rattus norvegicus*, 13 *Microtus epiroticus*, 29 *Apodemus sylvaticus* and 13 *Apodemus agrarius*) were inoculated orally with the attenuated rabies vaccine virus SAD B19. The obtained results confirm that under laboratory conditions a residual pathogenicity of SAD B19 for rodents exist when administered orally. A total of five rodents died from rabies. However, of these animals three *M. musculus* were anaesthetized

with ether and ketamine-hydrochloride before vaccine administration. Partly, the vaccine virus was probably directly applied in the respiratory organs. Transmission of vaccine virus from inoculated to control animals of the same species was not observed, indicating that there is no tendency for SAD B19 to spread within rodent populations.

INTRODUCTION

Oral vaccination of dogs against rabies to control the virus disease has been suggested by many authors (Beran, 1991; Perry & Wandeler, 1993). In Turkey, the only European country with dog mediated rabies, control programmes have not been successful to eradicate the disease completely. Due to a significant proportion of dogs not accessible for parenteral vaccination against rabies, it is usually not possible to reach an appropriate vaccination coverage. Therefore, oral vaccination of dogs with the live modified rabies virus vaccine SAD B19 as a supplementary method to parenteral vaccination is under investigation in Turkey since 1992. When baits containing a capsule filled with vaccine are placed at selected sites, not only the target population can locate and consume the baits. During field trials in İstanbul, the domestic cat and corvine species were identified as the major bait competitors (Vos & Şanlı, 1998). Therefore, it was decided to conduct safety tests with SAD B19 in these local animal species. Since SAD B19 virus vaccine has some residual pathogenicity for a variety of rodent species (Schneider & Cox, 1983; Artois et al., 1992), it was decided to test the pathogenicity of SAD B19 for local Turkish rodents according to the safety recommendations of the World Health Organization (Blancou & Meslin, 1996). It may also be necessary to investigate possible virus vaccine transmission from vaccinated to unvaccinated rodents. In this paper we summarize the results of laboratory studies on the effect of the SAD B19 vaccine virus in domestic cats, magpies (*Pica pica*) and five selected local Turkish rodent species.

MATERIAL & METHOD

The SAD virus (Street Alabama Dufferin) was originally isolated from a dog. The attenuated SAD B19 rabies vaccine strain was adapted for oral vaccination for dogs on BSR Cl. 13 cells and produced at IDT, Germany. The vaccine administered to the nontarget species was directly extracted from frozen vaccine containers (-20°C) used for field tests of oral vaccination of dogs.

Magpies

All magpies (n=10) were birds captured on the premise of the Veterinary Control and Research Institute (VCRI) in Etlik, Ankara, by the laboratory staff of this institute using a special constructed cage. All

animals were aged as juveniles at the time of vaccination. The birds were clipped (one wing) and were kept together in one room after virus vaccine administration at VCRI, Etlik. Due to the poor condition of the birds, they were moved to another cage after one week. The vaccine was administered orally by a single instillation of 6.3×10^7 FFU SAD B19 per bird using a needleless syringe. The birds were daily observed and were euthanized at the end of the observation period, on average 43 days after inoculation. At the end of the observation period the brains of all animals were analysed for rabies virus by the fluorescent antibody test (FAT), and the sera of the animals were examined by seroneutralization on cells (RFFIT). The brains of the animals that died during the observation period were also analysed for rabies virus (FAT).

Cats

All cats (n=12) were free-roaming domestic animals captured by the municipality in different neighbourhoods of Ankara. The animals were aged as older than 4 months at the time of vaccination and serological tested negative for rabies before inoculation (RFFIT). Two groups of cats were kept at a different period in one room (3x4x2.5m) at VCRI, Etlik. The vaccine was administered orally by a single instillation by a needleless syringe. The first five cats received 6×10^7 FFU and were observed for 84 days. The second group (n=7) was inoculated with 3.6×10^6 FFU and observed for 183 days. At the end of the observation period the brains of all euthanized animals were analysed for rabies virus by the fluorescent antibody test (FAT). Of the second group the serum of the animals was also examined by seroneutralization on cells (RFFIT).

Rodents

The small rodents were caught in so-called 'adapted trip-trap cages' (Vantorre, 1994), and checked twice a day. Several *Mus musculus* were caught at the Veterinary Research Institute and the Provincial Veterinary Office in the urban neighbourhoods of İstanbul, Şenliköy and Erenköy, respectively. All other small rodents caught were trapped in the Atatürk Arboretum, Bahçeköy, just outside the city of İstanbul. Also several litters were born in captivity. Rats *(Rattus norvegicus)* were caught in local produced traps in a cellar of an apartment building in the urban district of Ümraniye, İstanbul.

The different species and number of animals used in this study are listed in table 1. In Turkey, it is almost impossible to distinguish *A. sylvaticus* from *A. flavicollis*. Hence, in this study only the name *A. sylvaticus* is used. The animals were kept in a special closed room at the veterinary clinic of the Provincial Veterinary Office in Erenköy, İstanbul. The animals were observed for several days before inoculation to reveal any intercurrent

Table 1. Innocuity tests of SAD B19 vaccine virus in different Turkish rodent species.

Species	Number of inoculated animals	Number of control animals
Mus musculus	17	1
Apodemus sylvaticus	29	20
Apodemus agrarius	13	4
Microtus epiroticus	13	-
Rattus norvegicus	9	-
Total	**81**	**25**

Table 2. Number of animals inoculated with the different concentrations listed

Species	Number of inoculated animals	2.6×10^6 FFU/ml	3.6×10^7 FFU/ml	4.2×10^7 FFU/ml
Mus musculus	17	-	9	8
Apodemus sylvaticus	29	28	1	-
Apodemus agrarius	13	12	1	-
Microtus epiroticus	13	3	10	-
Rattus norvegicus	9	1	3	5

Table 3. Horizontal transmission experiment with Turkish rodents inoculated with SAD B19 vaccine virus. Number of cages (n) with a certain combination of animals (inoculated / control).

Combination	1/0	2/0	3/0	1/1	2/1	1/2	2/2
n	8	15	3	13	2	2	3

diseases. The animals were fed on a daily basis; special commercial rodent food, supplemented by fruits and/or vegetables.

The first animals caught, all *M. musculus*, were anaesthetized with ether followed by ketamine-hydrochloride (Ketavet 110 mg/ml, Parke-Davis GmbH, Berlin - Germany) before inoculation. Two out of five animals did not survive the anaesthesia. Therefore, it was decided to administer the vaccine directly into the mouth cavity of the small rodents. When offered directly, most rodents licked the vaccine without problems from a plastic syringe. *R. norvegicus* were anaesthetized with ether before inoculation. The small rodents and *R. norvegicus* were inoculated with approximately 0.05 and 0.1 ml SAD B19 vaccine virus, respectively (Table 2).

The nine *R. norvegicus* were divided over eight cages, in one cage two animals were kept together. To test a possible transmission of the vaccine virus from rodent to rodent (inter-individual transmission), inoculated animals were placed together with control animals of the same species; up to a maximum of 4 animals in one cage (Table 3). All animals that died during the observation period were necropsied and the brains were sampled and tested for rabies by the fluorescent antibody test (FAT) at VCRI, in Etlik. The animals were observed daily for an average of 34 days. At the end of the observation period all animals, inoculated and control, were euthanized and their brains were examined for rabies virus (FAT).

RESULTS

Magpies

SAD B19 vaccine virus was completely innocuous for domestic magpies after oral application. No rabies virus was detected in the brains of birds that died during the observation period or that were euthanized afterwards. Also no antirabic antibodies could be detected in bloodsamples of the birds euthanized. The results of the individual birds are shown in table 4.

Cats

None of the cats showed any sign of sickness during the observation period, and all survived and were rabies negative (FAT). Also, in the second group of animals no antirabic antibodies could be detected at the end of the observation period (RFFIT).

Rodents

The results of the inoculation test are summarized in table 5, a total of five animals died from rabies; three *M. musculus* (after 11, 12 and 20 days), one *M. epiroticus* (after 19 days) and one *A. sylvaticus* (after 16 days). All three *M. musculus* that died from rabies were anaesthetized with ether

Table 4. Results of the innocuity test of SAD B19 virus vaccine in local magpies *(Pica pica)*.

Bird	Date of capture	Date of inoculation	Observation period (days)	FAT	RFFIT	Remark
1	04.07.96	05.07.96	died	neg.		died 22.07.96*
2	04.07.96	05.07.96	died	neg.		died 26.07.96*
3	04.07.96	05.07.96	47	neg.	(-)	
4	04.07.96	05.07.96	47	neg.	(-)	
5	04.07.96	05.07.96	47	neg.	(-)	
6	07.07.96	07.07.96	44	neg.	(-)	
7	07.07.96	07.07.96	44	neg.	(-)	
8	07.07.96	07.07.96	died	neg.		died 28.07.96*
9	16.07.96	17.07.96	36	neg.	(-)	
10	16.07.96	17.07.96	36	neg.	(-)	

* - the cause of death could not be determined, it was probably a result of the poor condition of the birds.

Table 5. Results of the innocuity test of SAD B19 vaccine virus in Turkish rodents.

Species	Pre-inoculation treatment	Number of animals inoculated	Rabies positive (FAT)	Rabies negative (FAT)
Mus musculus	ether & Ketavet	3	3	-
	ether	1	-	1
	direct	13	-	13
Apodemus sylvaticus	direct	29	1	28
Apodemus agrarius	direct	13	-	13
Microtus epiroticus	direct	13	1	12
Rattus norvegicus	ether	9	-	9
Total		**81**	**5**	**76**

followed by ketamine - hydrochloride, suggesting that the administered vaccine entered also the respiratory organs. None of the 25 control mice died from rabies. Furthermore, in three cages where one of the inoculated animals died from rabies, all other animals placed in the same cage survived and were rabies negative. In three cases, an inoculated animal gave birth: all young survived and were rabies negative. Four animals, diagnosed rabies negative (FAT), died before the end of the observation period; two *M. musculus* (after 10 and 14 days), one *A. sylvaticus* (after 5 days) and one *R. norvegicus* (after 19 days).

DISCUSSION

The SAD B19 vaccine virus has been successfully used for oral vaccination of wildlife in Europe since 1983. In 14 years millions of doses have been distributed in Central Europe by hand and aerial distribution without any incidents. A major drawback when baits are distributed by placing them at selected sites is the bait competition between the target species and other animal species. Therefore, extensive safety tests with SAD B19 in nontarget species, domestic and wildlife, have been carried out during the 1980s in Europe: e.g. red fox *(Vulpes vulpes)*, badger *(Meles meles)*, raccoon dogs *(Nyctereutes procyonoides)*, jackal *(Canis aureus)*, stone marten *(Martes foina)*, wild boars *(Sus scrofa)*, cows, dogs, cats (e.g. Schneider & Cox, 1983; Müller et al., 1998).

The vaccine virus SAD B19 was also not pathogenic for cats and foxes treated with immunosuppressant drugs, corticosteroids (Ciuchini et al., 1986; 1988).

To identify possible bait competitors in Turkey baits were placed at selected sites in urban areas of İstanbul and their fate was controlled by direct observations from a car. Cats and corvine species are very common animal species in urban areas of Turkey. During the day and night cats located 8.6% and 27.3% of the baits placed, respectively (Vos & Şanlı, 1998). During the day the bait-depredation of certain corvine species, especially the hooded crow *(Corvus frugilegus)*, was 30.1% (Vos & Şanlı, 1998). The safety studies presented here, showed that SAD B19 was completely innocuous for these major bait competitors.

In our study it was shown that under laboratory conditions a low residual pathogenicity of the attenuated SAD B19 virus for some Turkish wild rodents exist. Although several rodents died from rabies, the death of the infected rodents could perhaps be explained by the procedure used for virus inoculation in which the virus not only entered the mouth but also the respiratory tract; when the vaccine is administered orally under general anaesthesia it may be refluxed into the nasal passage (Steck et al., 1982). None of the *M.musculus* died from rabies when the virus was inoculated directly without using anaesthetics. Furthermore, all *R. norvegicus* lightly

anaesthetized wtih ether survived. Steck et al. (1982) reported that when the virus is drunk by rodents (replacement of drinking water by virus vaccine) the mortality rate is much lower than 'forced' administration of the virus. During previous laboratory trials with SAD B19 in Germany, 11 out of 240 (4.5%) orally vaccinated NMRI-mice and 2 out of 34 (5.8%) Wistar-rats died from rabies (Schneider & Cox, 1983). Furthermore, none of the highly susceptible rodent, Ondata zibethicus, died during the observation period of 51 (n=13) and 106 (n=9) days and no vaccine virus could be isolated from the brains and salivary glands (Schneider & Cox, 1983). Artois et al. (1992) administered SAD B19 virus orally to four species belonging to the genera *Apodemus*, *Arvicola*, *Clethrionomys* and *Microtus*; of the 22 rodents inoculated two mice died from rabies. The mortality rate of local rodents inoculated orally with SAD B19 virus in this and other studies is lower than the limit of 10 % acceptable for oral vaccines for dogs and wild carnivores (WHO, 1989). However, the safety requirements for the use of a modified live vaccine for oral vaccination of dogs against rabies have recently been adapted. In urban areas there is a much higher probability of human exposure to vaccine virus by both direct and indirect contacts than would occur in the case of oral vaccination of wildlife. Therefore, more stringent safety standards are required for the release of any possible vaccine candidate for oral vaccination of dogs against rabies; at least 10, and if possible 50, of each rodent species should be given the field dose of the vaccine but none of the animals should show signs of sickness or die from rabies (Blancou & Meslin, 1996). However, the important issue here is not the residual pathogenicity of the virus for rodents but the risk of the vaccine virus producing an independent infection-chain leading to the persistence of the virus in the rodent population (Schneider & Cox, 1983). In our study, no rabies virus was found in the control animals, indicating that horizontal transmission of virus from rodent to rodent did not occur, although the control animals had contact with inoculated animals during the complete observation period. Also, no vertical transmission of the vaccine virus from the dam to her offspring could be observed. Wandeler et al. (1982) concluded that a field study showed no evidence that SAD Bern virus became established in the small mammal community (rodents and shrews) on an island. Three further observations support the conclusion that horizontal transmission of SAD vaccine virus from rodent to rodent is a very rare event (Schneider & Cox, 1983): 1) the very low mortality rate of rodents inoculated with the vaccine virus strain under laboratory conditions 2) only on very rare occasions can the vaccine virus be isolated from the salivary glands of the inoculated rodents and 3) in none of the rodents from areas where baits for oral vaccination of wildlife were distributed could the vaccine virus be isolated.

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