# THE FEASIBILITY OF ORAL VACCINATION OF DOGS IN TURKEY - A EUROPEAN UNION SUPPORTED PROJECT

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# TÜRKİYE'DEKİ KÖPEKLERİN ORAL YOLLA AŞILANMASININ UYGULANABİLİRLİĞİ - AVRUPA BİRLİĞİ DESTEKLİ PROJE

**ÖZET**

Köpeklerin kuduz hastalığı temel rezervuarı olduğu ülkelerde (Afrika, Asya, Latin Amerika, Türkiye), başıboş ve gözetim altında tutulamayan köpeklere ulaşılamamasından dolayı parenteral aşılama ile kuduzun kontrolu başarılı olmamaktadır. Yıllardır bu gibi populasyonların imhası ve azaltılması için çaba gösterilmiş, fakat çok az başarı sağlanmıştır. Köpekler ve insanlar arasındaki yakın ilişki dolayısıyle köpek orijinli kuduzdan kaynaklanan insan kuduzu vakaları çoğunlukla yüksektir. Bu nedenle enfeksiyon zincirinin diğer hayvanlara, özellikle de insanlara naklini engelleyecek köpeklerin oral yolla aşılanma metodunu kullanan bir proje tasarlanmış ve aşağıdaki sonuçlar elde edilmiştir : Orta Avrupa'da Tilkilerin kuduza karşı oral yolla aşılanmasında yoğun olarak kullanılan aşı virusu (SAD B19) Türkiye'deki köpeklere ayarlanmıştır. Saha testlerinde aşının köpeklerce kabulu için uygun bir bait bulunmuştur. Yürürlükte uygulanan bir aşı dağıtım sistemi geliştirilmiş, bunun oral ve parenteral kombine uygulanabilirliği test edilmiştir. Bununla birlikte metodun iki kritik noktası gözlenmiştir. SAD B19'un yüksek aşı titresi çok masraflıdır. En çekici bait olan köfte bait'i zaman zaman kapsülle birlikte yutuluyordu. Bu nedenle aşı, ağız boşluğu lenfatik sistemi ile bir bağışıklık oluşturmuyordu.

**SUMMARY**

Rabies in countries where dogs are the main reservoir of the disease (Africa, Asia, Latin America, Turkey) has not been successfully controlled with parenteral vaccination due to the inaccessibility of stray or unsupervised dogs. Destruction or reducing of such dog populations has been attempted for many years but with only transient success. Due to the close relation of dogs and man, human rabies cases in countries with dog-mediated rabies are mostly high and, therefore, a project was designed using the method of oral vaccination of dogs against rabies which could interrupt the chain of infection to other animals and especially to man. There were the following results: A vaccine virus (SAD B19) already used extensively for oral vaccination of foxes against rabies in central Europe was adjusted for use in dogs in Turkey. In field tests a suitable bait was found to deliver the vaccine to the dogs. Under the prevailing administrative set-up a vaccine bait delivery system has been developed and tested for its practicability in a combination of oral and parenteral vaccination. However, two critical points of the method were experienced: i. the high vaccine titre of SAD B19 needed is costly and ii. the most attractive bait, the Köfte-bait, as well as the chicken head bait were at times swallowed with the intact capsule and, therefore, the vaccine could not induce a seroconversion via the lymphatic system of the oral cavity.

**INTRODUCTION**

The concept of rabies elimination in countries with dog-mediated rabies has been and still is: dog movement restriction, elimination of strays and mass vaccination. The latter can be successful if effectively applied reaching approximately 70% of the dog population by parenteral vaccination (WHO, 1992). Nevertheless, in spite of the availability of improved methods of surveillance and control, national programmes have failed over the last years to greatly improve the rabies situation. One reason may be an increase of stray dog populations, another an increase of unsupervised dogs, a third reason the availability of more waste and food to maintain uncontrolled dog populations or a combination of all three reasons.

The principal objective of the project was the assessment of the method of oral vaccination of dogs against rabies. It was intended from the beginning to use the SAD B19 vaccine strain which was developed in Tübingen, Germany, for the oral vaccination of foxes. To date, many millions of doses have been used by countries of West- and Central-Europe. However, in a safety trial, it was shown that for an efficient seroconversion dogs needed a higher vaccine titre than foxes. It was expected that a new type of bait had to be found for dogs and a system to deliver it efficiently.

The epidemiology of rabies in Turkey is a typical example for dog-mediated rabies. Approximately 75% of all animals affected by the disease are dogs.

approximately 20% are other domestic animals and the remaining are wild animals compared to approximately 75% foxes, 5% other wild animals and 20% domestic animals in fox-mediated rabies countries. Human deaths caused by rabies are high compared to countries with wildlife rabies of western and central Europe (Müller, 1996).

Next to a good vaccine and an efficient bait it is especially important to obtain knowledge on the dog population of a country. The results of these partially externally financed studies have been written up elsewhere in this special journal edition (Matter et al., 1998). In this paper the laboratory results of the WHO Collaborating Centre for Rabies Surveillance and Research, Tübingen and the Etlik Veterinary Control and Research Institute, Ankara, are presented as well as field work which was mainly carried out in İstanbul Province, Turkey. The results of the Turkish laboratory and field-studies presented here, were collected between 1991 and 1993.

## MATERIAL AND METHOD

### SAD B19 vaccine strain and titration

The SAD B19 vaccine strain is a variant of the SAD (Street Alabama Dufferin) virus. It has been described by Schneider & Cox (1983). The virus titration is carried out on BHK cells. The focus forming units are calculated per ml (FFU/ml) by counting fluorescing foci in Lab Tek chambers.

### Fluorescent Antibody Technique

For the Fluorescent Antibody Technique (FAT) a commercially available conjugate (Centocor, USA) was used.

### Safety tests

The survival of the live vaccine virus (SAD B19) has been monitored by attempting to isolate it in the saliva after application in test animals or by using FAT to examine various tissues for the presence of viral antigen after euthanizing the animals.

In areas of oral vaccination in foxes thousands of rabies positive field samples of wild and domestic animals have been examined using monoclonal antibodies to distinguish between vaccine and field virus in order to exclude vaccine virus induced rabies.

### Serum neutralization test

In all experiments and in some field tests the seroconversion was evaluated after vaccine virus application. The method used was the Rapid Fluorescent Focus Inhibition Test (RFFIT) described by Cox & Schneider (1976).

### Baits

In the beginning the so-called 'Tübingen-bait' developed for the oral vaccination of foxes was used. This is a machine-made bait consisting of a mixture of fat and fishmeal covering a capsule containing the vaccine. Later the so-called 'Köfte-bait' was used, a minced meat mixed with bread. For economical reasons chicken heads were tested, the same method as previously used in Switzerland and Germany for foxes.

### Delivery system

Parenteral vaccination is, at least at this point, cheaper than oral vaccination. It can be practised faster when well organized. In Turkey there are the following facilities for vaccinating dogs parenterally: clinics of private veterinarians, of municipalities and of the veterinary department (district and province). Field campaigns can be organized by veterinarians of the municipality and/or the veterinary department.

Having evaluated the veterinary administrative set-up and the private veterinary practice in Turkey a possible system to combine parenteral and oral vaccination was suggested at the end of the project in 1993.

## RESULTS

### Safety

From the beginning when the project was drawn up it was considered to make use of the experience gained in Central Europe on the oral vaccination of foxes against rabies with the vaccine strain SAD B19 developed in the laboratory of BFAV. Extensive safety tests had been carried out on the fox as target species and on wild and domestic animals other than the fox as nontarget animals.

The dissemination of the vaccine virus in white mice, white rats, muskrats, foxes, cats and pigs had been tested following oral application by examining the saliva for residual virus and certain tissues for antigen after sacrificing the animals. The results showed that the virus could be found in the saliva for only a few hours after application. There was no antigen in the tissues examined (i.e. brain, spinal cord, salivary glands, brown fat, tonsils, lymph nodes, etc.). The following animals were tested for safety and had shown various levels of seroconversion: foxes, raccoon dogs, raccoons, jackals, wolves, wild boars, muskrats, laboratory dogs, cattle, pigs and cats.

In 1991 an incident occurred when four baboons in Zimbabwe taken from the wilderness into captivity were orally tested for safety using the SAD Bern strain from Switzerland (Bingham et al., 1992). Two of the baboons succumbed to the vaccine strain. In discussing the issue at the '3rd Consultation on Oral Immunization of Dogs against Rabies', July

1992, Geneva, considering that dogs are very closely associated with man, especially with children, WHO and OIE recommended to include primate safety tests on candidate live vaccines. Therefore, in September 1992, 10 chimpanzees were orally vaccinated with the SAD B19 vaccine using 1,5 x $10^8$ FFU/ml. All animals remained healthy and developed protective antibody titres (WHO, 1993).

Other safety tests in connection with the oral vaccination of foxes were the examination of 1378 rodents of different species in oral vaccination areas for the presence of vaccine virus with negative results. Thousands of rabies positive field samples originating from oral vaccination areas were also examined for vaccine virus by distinguishing vaccine and field virus using monoclonal antibodies. Animals examined were foxes, deer, badgers, other mustelids, wild boars, other wild and domestic animals. No case of vaccine virus induced rabies was observed.

In Turkey, the considerable number of stray cats have to be considered as nontarget animals. Therefore, 10 stray cats were adjusted to laboratory conditions and the vaccine was applied orally. None of these cats died.

**Efficacy**

An initial oral vaccination trial with dogs using the SAD B19 strain had been carried out at the BFAV-Tübingen. Using a vaccine titre of 2-4 x $10^7$ FFU/ml, 17 dogs developed durable antibody titres (Table 1). In contrast, foxes could be immunized with a titre of 1 x $10^6$ FFU/ml.

To evaluate the influence of different vaccine titres an experiment was carried out on 15 Beagles by the Behringwerke in Marburg/Lahn, Germany. It became obvious that dogs need a higher vaccine titre than foxes in order to seroconvert (Table 1).

At VCRI, Ankara 34 stray dogs, supplied by the Ankara municipality, were adjusted to laboratory kennels for a short time and orally vaccinated to determine the seroconversion rate with a titre which was used at the BFAV, Tübingen. It came as a surprise that only 5 seroconverted (Table 1). These results were probably due to the fact that the stray dogs reacted differently from the laboratory dogs in Germany.

In a later trial 14 stray dogs were vaccinated with a vaccine titre of 2 x $10^8$ FFU/ml. Eleven seroconverted with high antibody titres. Three dogs were taken into the test not knowing that they had rabies antibodies. These three animals developed increased titers which is indicative of a booster reaction. The experience of the latter test showed the importance of having a threshold titre of at least 1 x $10^8$ FFU/ml. All dogs in the laboratory were offered the vaccine baits first in the kennels for oral up-take. If they refused to take them readily or the vaccine container was not penetrated, the vaccine was administered onto the mucous membrane of the muzzle by syringe without a needle.

**Table 1.** Results of different efficacy tests in dogs with SAD B19 (B'werke - Behringwerke Marburg/Lahn in Germany).

| **Animals** | Test dogs | Beagles | Beagles | Beagles | Stray dogs | Stray dogs |
|---|---|---|---|---|---|---|
| **Institute** | BFAV | B'werke | B'werke | B'werke | VCRI | VCRI |
| **Titer (FFU/ml)** | 2-4 x $10^7$ | 1.4 x $10^6$ | 7.5 x $10^7$ | 1 x $10^8$ | 3.2 x $10^7$ | 2 x $10^8$ |
| **Seroconversion** | 17 / 17 | 0 / 5 | 4 / 5 | 5 / 5 | 5 / 34 | 14 / 14 |
| **Rate** | 100% | - | 80% | 100% | 14% | 100% |

**Bait studies**

The chicken head as a fox bait in Central Europe was replaced in 1985 by the 'Tübingen bait', a mixture of fat and fish-meal which could be mass produced. The 'Tübingen - bait' also resulted in an improvement in the seroconversion rate in the field. Therefore this bait was first tried regarding dog acceptance.

After observing how laboratory dogs accepted the bait, 135 dogs were tested in house-to-house visitations by the staff of VCRI, Ankara. The 'Tübingen - bait' surrounded a capsule which was filled with a placebo liquid. The capsule has a plastic container which is sealed by an aluminium foil. The latter is penetrated by the teeth and the vaccine is released onto the mucous membrane of the muzzle. Between 60% and, on certain days of observation, up to 85% of all dogs accepted the 'Tübingen-bait' and between 50 and 60% penetrated the capsule, a prerequisite for access to the vaccine. A liquid-proof bag instead of the capsule was tested but found unsuitable as 12 dogs out of 37 swallowed the bait plus vaccine bag.

It was hoped that the 'Tübingen-bait' would be better accepted by stray dogs than by the restricted dogs of the above experiments. Approximately 1000 'Tübingen-baits' were placed in Büyük-Çekmece district of rural İstanbul province in areas with reports of stray dogs. The baits were placed overnight between resting places of dogs during the day and possible food sources at night in the villages. It was interesting that the information given by the villagers regarding stray dog movement was nearly always correct. The results of the follow-up on bait uptake the next day were acceptable, but aside from the penetrated capsules it was found that many vaccine baits had disappeared altogether. The latter were possibly swallowed. During the above field work the experience was made that dogs not accessible for parenteral vaccination needed a different approach to be baited (vaccinated). There were on the one hand the feral dogs, unowned, outside villages and in suburban areas, hiding during the day, or dogs in big garbage dumps, which could only be reached by overnight baiting. On the other hand there were dogs in villages and urban areas, not restricted,

ownership unclear, relatively docile, which could easily be orally vaccinated by the hand-out method.

For the hand-out method in Greater İstanbul the 'Tübingen-bait' was not satisfactory, obviously due to the different eating habits of the dogs in an urban environment. A new bait, a minced meat mixed with bread covering the capsule was tested. The bait was called 'Köfte-bait'. During the baiting the following was observed:

- the 'Köfte - bait' could be offered to individual animals with great safety and a near 100% acceptance,
- the capsules were well chewed and thoroughly penetrated,
- at times though the bait with the capsule was swallowed,
- the price comparison with the 'Tübingen-bait' was five times more favourable for the 'Köfte-bait',

In regard to the economical consideration a further bait, the chicken head, formerly used for fox baiting in central Europe, was tested for acceptance. In house-to-house visitations in İstanbul 20 of 24 dogs (83%) accepted the bait on sight and penetrated the vaccine-capsules. Of 65 chicken head baits with capsules placed at the outskirts of İstanbul overnight 64 (96%) had been taken the next day. The same experience of high acceptance with the chicken head bait was made by Matter et al (1995) in Tunisia, but tested in a garbage dump only (see remarks above).

To reach a high degree of bait-acceptance and to be as economical as possible the chicken head and 'Köfte-baits' were combined. During day baiting with the hand-out method the chicken head was first offered, and in approximately 1/3 of cases readily accepted, and if not taken the 'Köfte-bait' was offered. For overnight baiting of the feral dogs the chicken head bait was used only and was very efficient. In the combination of these two baits a near to 100% acceptance was achieved. However, there remains one problem which hampers the method - up to 1/4 of the vaccine baits offered are being swallowed. It was observed though that many of the vaccine baits were properly chewed before swallowing, and thus vaccine does reach the mucous membranes. If not chewed, though, and the capsule is not penetrated the vaccine can not develop its effect. Tests to enlarge the capsule did not bring an improvement.

**Delivery of vaccine baits**

The vaccine virus in a capsule, used with the chicken head or 'Köfte-bait' or in combination was found to be the optimal vaccine bait delivery system for oral vaccination. The parenteral vaccination was, at least at that point, the cheaper method. Considering the governmental and private veterinary practices a procedure was needed to include the oral vaccination

in order to reach the dogs inaccessible for parenteral vaccination. The following procedure was finally adapted when oral vaccination was indicated:

| | |
|---|---|
| (i) Decision on area to be vaccinated | - provincial veterinary office |
| (ii) (poisoning) | - municipality |
| (iii) publicity | - muhtarlık, mosque, school (newspaper, information leaflets, dates of campaigns, etc.) |
| (iv) parenteral vaccination | - district veterinary office |
| (v) oral vaccination | - provincial veterinary office |

To (ı). With the decision on an area to be freed of rabies the financial situation needs to be considered and a strategy developed.

To (ii). The elimination of stray dogs is decreed by law and has been hitherto used for rabies control. If it is practised it needs to be done first, so that orally vaccinated animals are not removed.

To (iii). The muthar's (headman/mayor) office (muhtarlık) is considered the key for the participation of the public. His means of spreading the message is better than newspaper and television though they should be used as well.

To (iv). The district veterinary officer organizes the parenteral vaccination campaigns, best using a mobile unit and those dogs vaccinated should get a collar.

To (v). The oral vaccination has been practised street by street using the hand-out method and reaching this way the more docile dogs and by baiting overnight to reach the feral dogs. The hand-out method works best when the person, after offering the vaccine bait, steps back. Dogs usually feel undisturbed at a distance of 6 to 10 meters.

A typical example how the method was tested in an urban area of İstanbul with a mock vaccine is shown in table 2. It can be seen that from 168 vaccinated dogs, 138 (104 capsules penetrated by oral vaccination and 34 vaccinated parenterally) animals or 82.1% could be considered to be protected (seroconverted). It can also be seen that in 41 cases (of 134 orally vaccinated animals) the chicken head bait was readily accepted (30.6%). Unfortunately, 30 dogs of 134 (22.4%) swallowed the capsule and bait or refused them altogether.

During the project 306 out of 1089 vaccine baits (with chicken head or Köfte) were swallowed (28.1%). Therefore, the combination of parenteral and oral vaccination is a condition sine qua non to achieve a high level of vaccination coverage. However, it can be assumed that some of the swallowed vaccine baits do initiate seroconversion (see next section)

**Table 2.** The results of a 'vaccination campaign' in an urban area of İstanbul according to the suggested delivery system (comments in text).

| | capsule penetrated | | capsule sallowed | | baits not accepted | | parenteral vaccination |
|---|---|---|---|---|---|---|---|
| | CH* | K** | CH | K | CH | K | |
| **Day 1** | 11 | 24 | 1 | 9 | 3 | | |
| **Day 2** | 8 | 19 | 7 | 7 | | 2 | |
| **Day 3** | 7 | 11 | | | | | 25 |
| **Day 4** | 7 | 17 | | 1 | | | 9 |
| **Total** | **33** | **71** | **8** | **17** | | **5** | **34** |

**Seroconversion with oral vaccination under field conditions**

While experiments regarding the technique of oral vaccination (with baits and the delivery technique) were carried out with mock vaccine, one test at the end of the project was to simulate field conditions with the potent SAD B19 vaccine.

To collect serum samples the hand-out method was changed for a certain time to house-to-house visitations in 1993. The owners were asked whether or not their dogs were vaccinated parenterally and when not, if they would volunteer that the dogs were bled, vaccinated by offering first the chicken head and, if not taken, the 'Köfte-bait' and bled again 3 to 6 weeks later. All these dogs were restricted and could easily be identified. It was recorded whether or not the animals had penetrated or swallowed the capsules. Of 56 owners approached, 41 of their dogs could be evaluated. All four dogs offered a chicken head penetrated the capsule and seroconverted. 37 dogs were offered a 'Köfte-bait', 21 of these dogs sero-converted (19 dogs penetrated the capsule and 2 dogs swallowed it). 16 dogs did not seroconvert (2 dogs penetrated the capsule and 14 swallowed it). If these results can be used to simulate the field conditions approximately 61% of all dogs (25 of 41) would show a seroconversion, in two cases even though the vaccine baits were swallowed (see previous section). The figure 61% though refers only to animals vaccinated by the oral route. This does not take into account the free-roaming owned animals which are often vaccinated. A better seroconversion rate than 61% could also be expected from night active feral dog populations. The latter are hungry and not choosy as is the case with owned restricted dogs.

**DISCUSSION**

The vaccine tests revealed that the SAD B19 strain is immunogenic. However, the vaccine virus titre has to be as high as $1x10^8$ FFU/ml and,

therefore, the production costs are high. The bait used to deliver the vaccine is very efficient. The method is hampered by the fact that dogs swallow, at times, bait and vaccine capsule; 306 out of 1089 vaccine baits were swallowed (chickenhead and Köfte). The rate of swallowing is reduced when dogs are not disturbed when taking up the vaccine bait (during overnight baiting or if the flight distance of 6 to 10 meters with the hand-out method is observed). When well organized, a team (1-2 persons) can bait 50 to 100 dogs or more a day. Of course, it is important that at the time of oral vaccination, parenteral vaccination is promoted and facilitated as well to have a greater impact on the total vaccination coverage. The delivery system is no doubt specific for the administrative set-up in Turkey. It would have to be adjusted for other countries.

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# FIELD TRIALS EVALUATING BAIT UPTAKE BY AN URBAN DOG POPULATION IN TURKEY

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# TÜRKİYE'DE KIRSAL KÖPEK POPULASYONU TARAFINDAN BAİT ALIMINA YÖNELİK SAHA DENEMELERİ

## ÖZET

1992 yılında, köpeklerde kuduza karşı oral aşılamanın fizibilite testleri, saha çalışmaları ile İstanbul / Türkiye'de başlatıldı. Köpeklerde oral aşılama programlarının uygulanması için önemli kriterler, köpekler için özel olarak tasarlanmış bait ve aşı muhafazalarının geliştirilmesi ve bait'lerin köpekler tarafından alınmasıdır. Birkaç farklı tipte bait ve aşı taşıyıcıları, saha şartları altında İstanbul'un kentsel alanlarında her bir köpeğin elle beslenmesi yoluyla test edildi. Teste tabi tutulan tüm bait türleri arasında ekmek kırıntısı ile kıyma karışımından ibaret yerel yapım köfte en çok kabul edilebilirlik oranına sahipti (%96). Köpekler tarafından, fabrikasyon bait ve tavuk kafası bait'lere oranla daha fazla kabul edilebilirlik önemli bir durumu ortaya koymaktaydı. PVC aşı muhafazasının çiğnenme oranı ve büyüklüğü arasında ters bir ilişki gözlendi. Kapsülün testere dişli kenarları çiğnenme oranını azaltmadı.

## SUMMARY

In 1992, a field study was initiated to test the feasibility of oral vaccination of dogs against rabies in İstanbul, Turkey. Important criteria for the implementation of a canine oral vaccination programme are the development of baits and vaccine-containers specifically designed for dogs and acceptability of baits by dogs. Several different types of baits and vaccine-containers were tested under field conditions by hand feeding of baits to individual dogs in urban areas of İstanbul. Of all baits tested, the cheap local-made Köfte-bait, minced meat mixed with bread crumbs, had the highest acceptance-rate (96%). It was significantly better accepted by dogs than manufactured baits and Chickenhead-baits. An inverse relationship between size and swallowing-rate of the PVC vaccine-container was observed. Capsules with serrated edges did not decrease the swallowing-rate significantly.

## INTRODUCTION

Oral vaccination of wildlife is currently an effective method of controlling rabies in Europe (Wandeler, 1991). One important aspect of oral vaccination is the development of effective baits, which are readily accepted by the target population under field conditions. During the initial phase of a field-study to evaluate the feasibility of oral vaccination of dogs against rabies in Turkey, it was shown that the Tübingen-bait (fishmeal polymer bait), used for oral vaccination of foxes in Europe, was not accepted satisfactorily by dogs, especially in urban areas (Müller et al., 1998). Also the Chickenhead-bait was not accepted well under all circumstances. Therefore, it was partly replaced by the hand-made Köfte-bait; a mixture of local available minced meat and bread crumbs (Müller et al., 1998). Unfortunately, the Köfte-bait was often completely swallowed without being chewed on, including the vaccine-container used. This would result in an unsuccessful 'vaccination-attempt'; since gastric contents are sufficiently acidic to inactivate most attenuated rabies virus vaccine strains (Baer et al., 1975). Therefore, other bait candidates were tested in this study to examine their acceptance-rate in comparison with the Köfte-bait. To investigate a possible influence of size and shape of the vaccine-container on the swallowing-rate, different types of containers were used.

## MATERIAL AND METHOD

### Test 1. Acceptance of different bait types.

In table 1 several characteristics of the baits used are summarized, the Chickenhead-bait (CH) is excluded from this summary while it speaks for itself. Previous bait-trials showed that the Köfte-bait was extremely attractive to dogs (Müller et al., 1998). We were interested if the other bait candidates would be just as readily accepted by the dogs as the Köfte-bait. Hence, it was decided to offer first one of the other bait candidates and afterwards, as a reference, the Köfte-bait. However, in case of the Softcheese-bait, no Köfte-bait was offered afterwards. To determine Köfte-bait acceptance, Köfte-baits were offered directly to dogs on different occasions and in different study-areas. In this case, no reference bait was offered. The previous observed low acceptance-rate of the Chickenhead by dogs in İstanbul, Turkey (Müller et al., 1998), could be a result of the texture of this bait. Therefore, it was decided to grind the chickenheads, so the baits would have the same size and texture as Köfte-baits. Also chickenlegs are cheap local available bait materials. The legs were ground and mixed with bread crumbs so they resembled the Köfte-bait (texture and size). Local available softcheese mixed with bread crumbs was chosen as baitmaterial; it was assumed that the tenacious-sticky texture of the bait could reduce the swallowing-rate of the vaccine-container. While the minced-meat used for the Köfte-bait is a mixture of sheep - and beef meat (inferior quality), different manufactured baits based on sheep - (S-bait) and beef meat (D- and E-bait) were developed. For this, it was necessary to boil

**Table 1.** Several parameters of the baits tested ( h - height, d - diameter, w - width, l - lenght [cm]).

| Type | Abbrev. | Ingredients | Shape | Size | Texture | Production |
|---|---|---|---|---|---|---|
| **E-bait** | E | 32% rumen contents (boiled) and 21% corn | cylindrical | h: 8<br>d: 4 | solid | machine-made |
| **D-bait** | D | 50% meat - rumen (boiled) and 25% corn | cylindrical | h: 8<br>d: 4 | solid | machine-made |
| **S-bait** | S | 30% sheep meat (boiled) | half cylindrical (longtidudinal section) | h: 8<br>d: 4 | solid | machine-made |
| **Chickenhead köfte-bait** | CHK | ground chicken-head with bread crumbs | rectangular-oval | l: 6-7<br>w: 4<br>h: 2-2.5 | kneadable | hand-made |
| **Chickenleg köfte-bait** | CHL | ground chicken-legs with bread crumbs | rectangular-oval | l: 6-7<br>w: 4<br>h: 2-2.5 | kneadable | hand-made |
| **Softcheesebait** | SC | softcheese with bread crumbs | rectangular-oval | l: 6-7<br>w: 4<br>h: 2-2.5 | kneadable | hand-made |
| **Köfte-bait** | K | minced meat with bread crumbs | rectangular-oval | l: 6-7<br>w: 4<br>h: 2-2.5 | kneadable | hand-made |
| **Köfte-bait (boiled)** | KB | boiled minced meat | rectangular -oval | l: 6-7<br>w: 4<br>h: 2-2.5 | kneadable | hand-made |

the meat before it could be used for the manufactured baits. To test if the boiling procedure would influence bait-acceptance, the raw minced meat used for the Köfte-bait was boiled for 10 minutes. All baits, except the boiled Köfte-bait, contained a (placebo) vaccine-container. All bait trials were carried out in urban low-income areas in the Anatolian part of İstanbul. Dogs encountered by driving around in the different study-areas during daytime were offered a bait. No distinction was made between dogs chained up or dogs moving around unrestricted. If a bait could not be placed in front of the dog, it was thrown to the animal (3-10m). It was assumed that this later technique did not have any effect on bait-acceptance in comparison with the first method. Young dogs, less than three months of age, were excluded from these bait-trials. A bait was recorded as 'accepted' when the dog actually (partly) consumed the bait. When the animal only licked or sniffed at the bait, it was recorded as 'not-accepted'. Every dog was only once offered a bait or a combination of two baits. However, it can not be excluded that a free-roaming dog was encountered twice or more often on different occasions. To avoid this as much as possible, every neighbourhood was only visited once during these field trials.

**Test 2. Swallowing-rate of different vaccine-containers.**

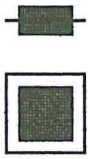
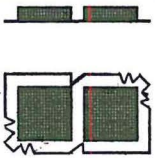
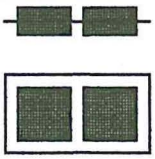
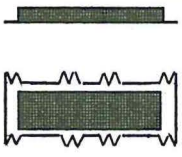
As previously stated, it was observed that many dogs swallowed the vaccine-container, increasing the possibility that the container was not punctured and consequently the 'vaccine' was not released in the mouth-cavity. Therefore, we decided to test different types of vaccine-container (size and shape) incorporated in Köfte-baits, to find a vaccine-container with a low swallowing-rate (see Figure 1).

Considering the results with the Type VIII vaccine-container, it was decided to determine the minimum-size to prevent the dogs from swallowing the vaccine-container. Only the length of the vaccine-container was changed, the width (4.7 cm) and height (0.3 cm) remained the same as Type VIII. Three types were tested; with a length of 4.7 (Type IX), 5.0 (Type X) and 5.5 cm (Type XI). Every dog (restricted or unrestricted) was given only one bait, and the baits were offered directly to the dogs. In all cases where a bait was offered, it was recorded if the (placebo) vaccine-container was discarded intact, discarded but punctured or swallowed. If a dog chewed well before swallowing the vaccine-container, indicating that the container was probably punctured, it was still recorded as swallowed. Several capsules tested had a serrated edge (saw-toothed); it was assumed that the serrated edges when chewed on would provoke irritation and prevent the animal from swallowing the capsule. The vaccine-containers were a thermo-formed PVC-shell sealed with an aluminium sheet. Biomarkers were not used in this study since the baits were offered directly and biomarkers like tetracycline cannot differentiate oral-pharyngeal absorption from intestinal absorption of the biomarker thereby precluding effective assessment of vaccination rates.

September 1995, a completely different capsule was tested; the cylindrical Torpac lock-ring-capsule made out of gelatine (∅ =1.4cm - height = 5.6cm.

**Figure 1.** The different vaccine-containers tested, and the their swallowing-rate (SW - bait including vaccine-container swallowed, A - bait accepted).



| | Type I | Type II | Type II | Type IV | Type V | Type VI | Type VII | Type VIII |
|---|---|---|---|---|---|---|---|---|
| |  |  |  |  |  |  |  |  |
| **Size (cm)** | 3,5 x 5 x 0.7 | 3 x 3 x 0,8 | 3 x 3 x 1,6 | 7 x 3,5 x 0,8 | 7 x 3 x 0,8 | 7 x 3,5 x 1,6 | 6,5 x 3 (2,5) x 0,7 | 6,5 x 4,7 x 0,3 |
| **Ratio SW/A** | 37 / 87<br>0,43 | 38 / 54<br>0,70 | 49 / 100<br>0,49 | 25 / 77<br>0,32 | 29 / 70<br>0.41 | 12 / 38<br>0,32 | 26 / 128<br>0,20 | 0 / 119<br>0 |
| **Study period** | Spring '94 | Spring '94 | Spring '94 | Spring '94 | Spring '94 | Spring '94 | Winter '96 | Autumn '96 |

Torpac Inc., USA). When the Torpac-capsule was filled with liquid (dye water) and incorporated in the Köfte-bait, it became very soft and flexible. In this trial only unrestricted dogs were offered a bait.

## RESULTS

### Test 1.

The overall acceptance rate of the different alternative baits tested are summarized in table 2. The results presented in this table are based on the observations made when a combination of baits was offered to the dogs; Köfte-bait second, alternative first. As mentioned before, no Köfte-bait was offered as a reference in case of the Softcheese-bait. Of 296 dogs offered only a Köfte-bait, 285 (96.3%) animals accepted the bait without hesitation. Hence, the most frequently consumed bait was the Köfte-bait followed by the boiled Köfte-bait (94.1%) and the Softcheese-bait (87.1%). No significant difference was found among the acceptance-percentages of these three baits. However, the proportion of each of these baits accepted was significantly higher than that of the other baits tested (Chi-square test, 2x2 contingency table). The results of the two-choice-food-preference test (Köfte-bait second, alternative first) are shown in table 3. The Köfte-bait was accepted significantly better than the alternative baits, except for the boiled Köfte-bait. In this case the acceptance-rate was identical.

**Table 2.** The overall acceptance rate of the different alternative baits tested on local dogs in İstanbul, Turkey.

| Bait-type | Number of baits offered | Baits accepted | | Test |
|---|---|---|---|---|
| | | n | % | period |
| E | 39 | 3 | 7.7 | July'94 |
| D | 28 | 5 | 17.9 | July'94 |
| S | 76 | 36 | 47.4 | Aug.'94 |
| CH | 137 | 64 | 46.7 | Sept.'94 |
| CHK | 56 | 27 | 48.2 | Sept.'94 |
| CHL | 34 | 18 | 52.9 | Sept.'94 |
| SC | 31 | 27 | 87.1 | Oct.'94 |
| KB | 17 | 16 | 94.1 | July'94 |

### Test 2.

The swallowing-rate of the different PVC-vaccine containers is shown in figure 1. There was an inverse relationship between vaccine-container size (surface) and swallowing-rate (Spearman's Rank Correlation Coefficient, $R_s = -0.82$, $df=6$, $P<0.01$). None of the vaccine containers of Type VIII was swallowed, indicating that there is a threshold-value in size; at a

certain size dogs are not 'capable' of swallowing the capsule. However, a bigger vaccine-container means more bait material; increasing the price of the cost-effective Köfte-bait. Therefore, it is important to determine the minimum-size of the vaccine container that is not swallowed; 8 out of 47 dogs swallowed Type IX. Only 1 out of 44 dogs swallowed Type X and none of the dogs (n=71) swallowed Type XI. The effect of the serrated edges on the swallowing-rate is not clear. The observed differences in the number of vaccine-containers swallowed between Type IV and Type V (serrated) was not significant (Chi - square Test, 2 x 2 contingency table). However, the swallowing-rate of Type VII was significantly lower than Type IV ($\chi^2$=6.58, df=1, P<0.05) and Type V ($\chi^2$=10.06, df=1, P<0.01). Due to the characteristics of the Torpac-capsule, it was difficult to determine if the liquid was released in the mouth cavity or not. The capsule was filled with a solution of neutral water and a green dye. While most unrestricted dogs were difficult to approach, it was often impossible to observe the green staining in the mouth, indicating that the liquid was released in the mouth cavity. Of 39 baits containing the Torpac-capsule; 27 capsules were consumed, 5 capsules were discarded and torn apart and only 1 capsule was discarded intact.

**Table 3.** Results of the test when two baits were offered to the dogs; alternative-bait first, Köfte-bait second (YN - first bait was accepted, but Köfte-bait not, NN - both baits were not accepted, YY - both baits accepted and NY - first bait was not accepted but Köfte-bait was accepted).

| Bait-Type | Number of dogs tested | YN | NN | YY | NY | Fisher & Yates Test |
|---|---|---|---|---|---|---|
| D vs. K | 7 | 0 | 1 | 0 | 6 | P<0.01 |
| E vs. K | 18 | 0 | 2 | 1 | 15 | P<0.001 |
| S vs. K | 59 | 0 | 0 | 25 | 34 | P<0.001 |
| CH vs. K | 133 | 0 | 5 | 63 | 65 | P<0.001 |
| CHK vs. K | 56 | 0 | 2 | 27 | 27 | P<0.001 |
| CHL vs. K | 38 | 0 | 3 | 17 | 18 | P<0.001 |
| KB vs. K | 12 | 0 | 1 | 11 | 0 | n.s. |

## DISCUSSION

An important precondition for oral vaccination is the availability of a bait well accepted by the target species under field conditions. A bait that is poorly accepted by dogs, even if all other requirements are fulfilled, has no use for oral vaccination. A dog offered a bait will first investigate it by sniffing and licking. The dog then 'accepts' the bait and starts consuming it. Interruption is possible at any stage of this sequence. It may occur as a

result of certain characteristics of the bait; like size, shape, taste, odour and texture. However, it can also be induced by external factors; e.g. a subordinate dog is chased away by a dominant animal or the dog is disturbed by pedestrians. Especially, if the investigation and bait-handling phase is very long, the chances that external factors will lead to a unsuccessful vaccination attempt increase. To limit the risks of interruption the acceptance-threshold should be as low as possible; the bait should be immediately attractive to dogs. In Turkey, the acceptance-threshold of the Köfte-bait is extremely low; 96.3 % of the dogs offered a Köfte-bait accepted it without hesitation. The acceptance-rate of the relative expensive Softcheese-bait was also very high (87.1 %). The rather low acceptance-rate of the Chickenhead-bait (48.2 %) is in contrast to that observed in Tunisia; here 94 % of the dogs (n=50) accepted the Chickenhead-bait (Kharmachi et al., 1992). Apparently it is not the texture of the Chickenhead-bait that influences bait-acceptance. Ground Chickenhead-baits resembling the texture and size of the Köfte-baits were accepted just as poorly as the normal Chickenhead-baits. The very low acceptance of the manufactured baits could be a result of lack of familiarity of the dogs with this kind of bait-material. The dogs in this study had largely been fed on households' leftovers and offal. Dogs can be expected to accept those types of foods that are more attractive and with which they have had previous experiences (Perry et al., 1988). Therefore, manufactured baits with a high acceptance-rate by dogs in one area can be refused by dogs in another area, due to different food-preferences and - experiences of the dog populations involved. Furthermore, the manufactured baits tested in this study, when accepted, were often broken into pieces. The vaccine-container incorporated in the baits was 'recognized' by the dogs as an independent structure and was consequently separated from the bait material. It can be concluded that the Köfte-bait meets the criteria for dog vaccine baits as summarized by Linhart (1993). The Köfte-bait is inexpensive, contains locally available products known to be attractive to dogs and can be produced under local conditions. Unfortunately, the 'vaccination-rate' by using the Köfte-bait is much lower than the acceptance-rate, because the vaccine-container is often swallowed without (much) chewing. Hence, increasing the chances that the vaccine virus does not come into contact with the oral mucous membrane; a prerequisite for successful vaccination. It was shown that an increase in vaccine-container size (surface) reduced the swallowing rate; the vaccine-containers Type VIII and XI were apparently too big for dogs to swallow. Even when several dogs were together and offered a bait they did not swallow the vaccine container. Only one dog swallowed Type X (5.0 x 4.7 x 0.3cm), indicating that this would be an acceptable size. A great advantage of the Torpac-capsule tested is that it is not harmful for the dog when it is swallowed in contrast to the PVC vaccine-containers, which could cause irritation in the digestive tract when swallowed. Furthermore, most Torpac-capsules were consumed and therefore, did not 'pollute' the

environment. Unfortunately, the Torpac-capsule is unsuitable for large scale application, because it has to be filled by hand and subsequently becomes 'soft' (storage and transportation difficulties).

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