THE DELIVERY OF BAITS TO DOGS: CLENBUTEROL AS BAITMARKER

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BAİT İŞARETLEYİCİ OLARAK CLENBUTEROL: KÖPEKLERE BAİT'LERİN VERİLİŞİ

ÖZET

Vahşi hayvanların kuduza karşı ağız yoluyla aşılanmasında kullanılan baitler işaretleyici olarak tetrasiklini içerir. Bu işaretleyici kopeklerin ağız yoluyla işaretlenmesi için uygun değildir. Bu çalışmada anabolik β2 adrenoceptörü agonisti olan Clenbuterol'un bait işaretleyici olabileceği test edilmiştir. Önceki çalışmalarda işaretleyicinin oral uygulamadan sonra kıl uzaması boyunca kıl teline nüfuz ettiğini göstermiştir. Bu işaretleyicinin kinetiği laboratuvar denemesi olarak: 0.5 mg Clenbuterol bir bait içine enjekte edilmiş ve 18 adet köpeğe verilmiştir. Clenbuterol uygulanmasından 28 ve 56 gün sonra Clenbuterol, köpeklerin kıl numunelerinde tesbit edilmiştir. Buna ilaveten elde edilen sonuçlar numunelerdeki Clenbuterol konsantrasyonu ile kıl pigmentasyonu yoğunluğu arasındaki ilişkiyi belirgin şekilde ortaya koymuştur. Bait dağıtım sisteminin kırsal alanlarda seçilen bölgelerde verilerek fizibilitesinin ölçülmesinde Clenbuterol ve 1.5 x 10^8 FFU SAD B19 aşı virusu ile doldurulmuş aşı container'ı içeren 150 adet bait İstanbul'da Ferhatpaşa'da kırsal bölgede yol boyunca konulmuştur. Ertesi sabah konulan baitlerin % 93'ü yerinde bulunamamıştır. Bait dağıtımından önce ve sonra sahipsiz 31 adet köpek yakalanarak kanları alınmış ve bait dağıtımından sonra kıl numuneleri de alınmıştır. Elde edilen sonuçlar baitlerin seçilen alanlarda yerleştirilmesinin sahipsiz köpek populasyonuna ulaşmada etkin bir yol olmadığı şeklinde sonuçlanan saha çalışmalarını desteklemektedir. Şöyleki yakalanan sahipsiz köpeklerin sadece %23'de 0.5 IU/ml eşik değerlik kuduz nötralizan antikoru tesbit edilmiştir.

SUMMARY

Baits used for oral vaccination of wildlife against rabies contain the marker tetracycline. This marker is not suitable for oral vaccination of

dogs. In this study the possibility of the anabolic β2-adrenoceptor agonist Clenbuterol as bait marker was tested. In previous studies it was shown that the marker is incorporated into the hair fibre during hair growth after oral application. The kinetics of this marker was studied in a laboratory experiment: 0.5mg Clenbuterol was injected in baits and offered to 18 dogs. 28 and 56 days after application Clenbuterol could be detected in hair samples of the dogs. Furthermore, the results obtained showed clearly the relationship between the concentration of Clenbuterol in the samples and the intensity of hair pigmentation. To test the feasibility of the bait delivery system by placing baits at selected sites in urban areas, 150 baits containing Clenbuterol and a vaccine container filled with 1.5×10^8 FFU SAD B19 vaccine virus were distributed along roads in the urban neighbourhood Ferhatpaşa in İstanbul. The next morning 93% of the baits had disappeared. Before and after bait distribution 62 and 31 'ownerless' dogs were caught and recaptured and bled on these occasions, respectively, during the latter also a hair sample was taken. The results supported previous field-studies that placing baits at selected sites is not a very efficient way of reaching the ownerless dog population. Only 23% of the recaptured ownerless dogs had a rabies neutralizing antibody titre above the threshold of 0.5 IU/ml.

INTRODUCTION

In many countries with dog mediated rabies, mass vaccination of these animals remains the most important method to control rabies. This requires the immunization of a large proportion of the dog population, to reduce possibilities of virus transmission by a rabid dog to susceptible ones. The WHO recommends that at least 70% of dogs in a population should be vaccinated to eliminate or prevent outbreaks of rabies (Coleman & Dye, 1996). Especially, the high proportion of ownerless and poorly supervised owned dogs is one of the major obstacles to effective dog rabies control by parenteral vaccination. Oral vaccination may offer additional approaches to reach these, for parenteral vaccination, inaccessible dogs, permitting a significant increase in the vaccination coverage of the overall dog population. Successful oral vaccination against rabies depends on a number of factors. Very important are the efficacy and safety of the oral vaccine candidate and a bait well accepted by the target population. During bait-preference trials in Turkey, it was shown that the so-called Köfte-bait (minced meat mixed with bread crumbs) was extremely well accepted by dogs (Schuster et al., 1998).

Another important precondition for oral vaccination is an effective bait delivery system. Several bait distribution techniques have been suggested, e.g. distribution of baits at selected sites (Linhart, 1993). This system is directed at ownerless and poorly supervised owned dogs. To test the efficiency of this method, Köfte-baits were placed overnight at selected sites

in urban areas of İstanbul, Turkey, to determine bait uptake (Vos & Şanlı, 1998). However, the actual vaccination-rate is often lower than the observed bait uptake-rate, due to several factors. Dogs remove the vaccine container from the bait or swallow the vaccine container without puncturing it with their teeth. Hence, the vaccine is not released in the mouth cavity. Also, dogs can puncture the vaccine container but most of the vaccine is spilled on the ground. Therefore, it was decided to assess the actual vaccination-rate of this bait delivery system. Hence, it was necessary to incorporate a biomarker in the baits. Unfortunately, the marker tetracycline, used in baits for oral vaccination of wildlife against rabies, can not be used for dogs. For these animals, a biomarker should be used that can easily be sampled and measured without sacrificing the dogs. Gleixner et al. (1996a,b) showed that the β2-adrenoceptor agonist Clenbuterol is accumulated in hair of some animal species. A hair sample of the animal was used to detect the ingestion of this β2-agonist. To test if Clenbuterol could also be used as bait-marker for oral vaccination, dogs kept at Veterinary Control and Research Institute in Etlik, Ankara (VCRI), were offered a bait containing Clenbuterol. Furthermore, to assess the feasibility of Clenbuterol as bait-marker under field conditions baits containing this marker were placed in the low-income, suburban area, Ferhatpaşa, in İstanbul. The results of these two studies showed that Clenbuterol is a very effective bait-marker, that can even be detected months after a single application.

MATERIAL & METHOD

Laboratory Study

18 free-roaming dogs were caught in different neighbourhoods by the local municipality in Ankara as part of a stray dog control programme. The dogs were kept in groups of 2-3 animals and were observed daily at VCRI, Etlik. All dogs were offered a Köfte-bait, injected with a 0.1 ml Clenbuterol solution (Clenbuterol-hydrochloride, Sigma Chemical Co., St. Louis, USA), containing 0.5 mg Clenbuterol. The single dosing of Clenbuterol was derived from a ten day therapeutic dosing of livestock (daily 1.6 μg Clenbuterol / kg bodyweight), and not harmful to the animals. A hair sample (E0) was taken prior to Clenbuterol administration. All hair samples were taken with a trimmer or razor blade. 28 days after offering the animals a bait containing Clenbuterol two hair samples were collected from all dogs. One sample (E1) from the same spot as E0 was taken, and one sample (N1) from a completely different area. Four weeks later, day 56, again hair samples were obtained from the dogs, one sample (E2) from the same area as sample E0 and E1, and one (N2) from an area previously not sampled. While the content of Clenbuterol in hair depends on the intensity of hair

pigmentation (Gleixner et al., 1996a,b), samples were taken, if possible, from the darkest pigmented areas of the dogs.

Table 1. Concentration of Clenbuterol (ng/gr) in the hair of the dogs, see text for explantion of samples

	Sample E1	Sample E2	Sample N1	Sample N2
Average	12.76	3.44	6.89	5.72
Stand. deviation	11.16	2.43	7.71	4.20
Sample size	17*	18	18	18
Minimum value	3	1	2	2
Maximum value	38	11	34	17

* hair sample of one dog was missing.

Field Study

Before the Köfte-baits containing Clenbuterol were placed at selected marked sites in Ferhatpaşa, 62 free-roaming dogs were caught and bled for rabies antibody determination. Sera were tested by Rapid Fluorescent Focus Inhibition Test (RFFIT), and titres expressed in International Units (IU/ml). In order to mark the animals for identification purposes, a part of the skin was shaved and this area was coloured with a long lasting stock marker (Sprayline Stock Marker, Ritchey Tagg Ltd., Masham, North Yorkshire, UK). Afterwards, the dogs were photographed. During the evening, of 20 May, 1996, 150 Köfte-baits containing Clenbuterol and a capsule filled with 1.5 x 10^8 FFU SAD B19 vaccine virus were distributed at selected marked sites along the roads in this area. In previous studies, it was shown that dogs could be immunized with this dosage and developed durable antibody titres (Aylan & Vos, 1998). Bait density was 183 baits/km^2. 20 and 130 baits contained 1 and 0.42 mg Clenbuterol, respectively. 5-6 weeks later, 31 dogs were recaptured in the same area. From all dogs, a blood- and hairsample were collected. Hair samples were obtained by using a razor blade. For every dog a different razor blade was used to prevent cross contamination.

All hair samples were stored at -20°C till transportation to Germany. Gleixner et al. (1996b) described the method for the preparation and extraction of the hair samples in detail. For the detection of Clenbuterol the Clenbuterol-enzyme immunoassay was used (Meyer & Rinke, 1991; Gleixner, 1996). The extraction protocol for hair analysis of Clenbuterol was validated for humans (Gleixner et al, 1996a) and cattle (Gleixner et al.,

1996b) before. For hair samples of dogs, a validation with externally spiked samples was carried out (Gleixner et al., unpublished data).

RESULTS

Laboratory Study

The concentrations of Clenbuterol (ng Clenbuterol/gr hair) in the hair samples of the dogs after baits were offered are summarized in table 1. All E0-samples were below the limit of detection (1 ng/gr). The results show that Clenbuterol is strongly accumulated in the hair of the dogs after a single application. Even eight weeks after Clenbuterol administration, high levels of Clenbuterol could be detected in the N2-samples. Also, in hair grown between 26 and 58 days after Clenbuterol application (E2-sample), the marker was clearly detectable.

Generally, the darker the hair of the dogs, the higher the concentration of Clenbuterol detected (Table 2). Unfortunately, the sample size was too small for statistical analysis. Only in the E1-sample, the concentration of Clenbuterol detected in the 'brown' hair samples were significantly higher than that in the white and grey hair samples (Student's t-Test).

Field Study

On average, 37 days after the baits were placed sixteen recaptured dogs did not have a detectable titre of rabies virus neutralizing antibodies and concentrations of Clenbuterol in the hair samples above the limit of detection (1 ng/gr). Indicating that 52% of these dogs did not locate and consume a bait. The results of the other 15 dogs are listed in table 3. Only one dog (F49) developed detectable rabies antibody and Clenbuterol levels. Seven dogs had Clenbuterol concentrations above the detectable limit but did not seroconvert. On the other hand six dogs had an (increased) rabies antibody titre but no detectable concentration of Clenbuterol in the hair samples taken.

DISCUSSION

The laboratory trial provided clear evidence on the accumulation of Clenbuterol in the hair of the dogs, although Clenbuterol was only applied once. The data obtained support the observation that the Clenbuterol concentration in hair depends on the intensity of hair pigmentation (Gleixner et al., 1996a,b). Even in the hair-samples taken 56 days after Clenbuterol application of previously shaved areas (E2-sample) comparatively high concentrations were detected. Although, the samples E1 and E2 were not always easy to collect. The rate of hair growth showed considerable differences; some dogs hardly showed any hair growth on previously shaved areas. Although the field-study was aimed at ownerless dogs, some of the

Table 2. The average concentration of Clenbuterol (ng/gr) in the hair of dogs, based on the intensity of hair pigmentation

Hair colour	Number of dogs	Sample E1	Sample E2	Sample N1	Sample N2
White	4	4:00*	3.25	2.50	3.25
Grey	6	6.00	2.83	4.50	4.53
Brown	7	20.29	3.57	10.43	7.29
Black	1	27.00	7.00	14.00	13.00

* hair sample of one dog was missing.

Table 3. Results of the blood- and hairsamples of the 'ownerless' dogs captured and recaptured in Ferhatpaşa, İstanbul.

Dog	Hair-colour	Bloodsample 1 (IU/ml)	Bloodsample 2 (IU/ml)	Clenbuterol (ng/gr)
F31	white	(-)	missing	2.5
F10	brown	(-)	(-)	1.5
F30	brown	(-)	(-)	3.1
(-)	grey	(-)	(-)	5.3
F37	grey	(-)	(-)	1.5
F44	black	(-)	(-)	55.0
F47	grey	(-)	(-)	2.0
F50	black	(-)	(-)	3.7
F17	grey	(-)	1.1	<1
F40	black	(-)	2.2	<1
F45	black	(-)	1.1	<1
F48	grey	(-)	1.1	<1
F59	brown	(-)	6.7	<1
F38	white	2.2	6.7	<1
F49	white	(-)	10.0	2.7

captured dogs identified as ownerless were relocated tied up in front of a house. It is extremely difficult to determine the true ownership-status of the free-roaming dogs. People tend to 'decide' arbitrary whether these dogs are owned or ownerless. The field-study showed that placing baits overnight to reach the ownerless dog population is not a very efficient method in urban areas. 93% of the baits placed disappeared in one night, but only seven out of 30 recaptured dogs (23%) had a rabies antibody titre above 0.5 IU/ml. This low vaccination rate is probably a result of bait depredation by the high number of free-roaming owned dogs in this area. The observed bait disappearance rate was higher than in previous field studies in İstanbul (Vos & Şanlı, 1998). The low efficiency of this bait delivery system was also observed in Sarıgazi. Here, 70 Köfte-baits (bait density: 43 per km^2) were placed at selected sites. Bloodsamples were taken from ownerless dogs before and after the baits were placed. Only one out of 22 dogs seroconverted (Vos, unpublished data). The low rate of bait-uptake by dogs was probably principally a result of the small number of baits distributed per dog present. A higher bait density could increase the 'vaccination-coverage' of the ownerless dogs. However, this would be very cost-ineffective. For oral vaccination of foxes against rabies a bait-density of 15-20 baits per km^2 is used (Müller & Schlüter, 1998), although the adult fox density in spring will seldom exceed 2 foxes per km^2 in most areas of Central Europe. Using the same ratio (bait - vs. population density) for oral vaccination of dogs against rabies in urban areas of İstanbul would mean distributing an unrealistic number of thousands of baits per km^2. Therefore, this bait delivery system should be discouraged, except for very restricted areas like waste disposal sites.

It was remarkable, that many dogs showed a detectable concentration of Clenbuterol in their hair-samples but no rabies virus neutralizing anti-bodies could be observed. This could be a result of the fact that dogs removed or discarded the vaccine container intact or that the dogs swallowed the vaccine container without perforating it. One dog (F44) had a very high level of Clenbuterol in its hair (55 ng/gr). This concentration was not even observed in the controlled laboratory trial. Although this dog had black, short hair, it seems reasonable to assume that this animal located and consumed more than one bait. Several ownerless dogs showed a rabies antibody response to the oral vaccination but did not have a detectable concentration of Clenbuterol. This may be an artefact from the method of incorporation of the bait-marker in the Köfte-baits. In baits used for oral vaccination of wildlife the marker tetracycline is mixed homogeneously with the other bait-components. In our study, the Clenbuterol solution was injected in the already prepared Köfte-baits. This means, that the marker is not evenly distributed in the bait, but concentrated in a very small part. Some dogs may not have consumed the complete bait, e.g. during consumption small parts fell off or the dogs could have deliberately rejected the parts containing Clenbuterol (smell, taste). Also, Clenbuterol solution

could have been leaking from the bait between preparation and consumption. Therefore, it is suggested to mix the bait marker more homogeneously with the other bait material substances. Nevertheless, Clenbuterol is a very effective bait-marker, because of its detection-possibilities even months after a single application and that it can easily be sampled without sacrificing the animal.

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