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Oral vaccination of foxes against rabies in Turkey between 2008 and 2010

Orale Immunisierung von Füchsen gegen die Tollwut in der Türkei zwischen 2008 und 2010

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Summary

Following a sustained spill-over event from dogs to foxes, fox rabies spread rapidly in the Aegean region, Turkey. In order to control the outbreak a program of oral vaccination of foxes against rabies was introduced. In the selected vaccination area three annual campaigns between 2008 and 2010 were undertaken during the winter months whereby the vaccine baits were distributed exclusively by plane using a density of 18 baits per km². Subsequently, fox rabies cases were reported only from locations bordering the non-vaccinated areas. Hence, it was shown that fox rabies control by means of oral rabies vaccination is feasible in Turkey. However, for the progress towards the elimination of fox-mediated rabies in Turkey to be maintained, it is necessary that political and financial support is secured to extend oral vaccination where infected foxes remain.

Keywords: Aegean region, vaccine bait, elimination

Zusammenfassung

Nachdem die Tollwut erfolgreich die Speziesbarriere vom Hund zum Fuchs überschritten hatte, kam es zu einer raschen Ausbreitung der Fuchstollwut in der Ägäischen Region der Türkei. Um diesen Ausbruch unter Kontrolle zu bringen, wurde die Möglichkeit der oralen Immunisierung von Füchsen untersucht. Drei jährliche Impfkampagnen (2008–2010) jeweils während der Wintermonate wurden in dem ausgewählten Impfgebiet durchgeführt. Die Impfköder wurden ausschließlich mittels Flugzeug mit einer Dichte von 18 Köder pro km² ausgelegt. Tollwutfälle beim Fuchs traten nur noch am Rande zu nicht-geimpften Gebieten auf. Die Ergebnisse haben gezeigt, dass die Bekämpfung der Fuchstollwut mittels oraler Immunisierung auch in der Türkei möglich ist. Jedoch ist für die erfolgreiche Tilgung der Fuchstollwut eine dauerhafte politische und finanzielle Unterstützung erforderlich.

Schlüsselwörter: Ägäische Region, Impfköder, Tilgung

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Introduction

Turkey is the only European country in which the principal vector for rabies transmission is the domestic dog (*Canis lupus domesticus*) rather than wildlife species. Rabies in wildlife species including jackals (*Canis aureus*), wolves (*Canis lupus*) and red foxes (*Vulpes vulpes*) have only been reported infrequently from Turkey with approximately 2% of the total number of cases reported (source: Rabies Bulletin Europe). Between 1990 and 1999 only 35 rabies cases in wildlife were reported. Recent rabies control programmes have reduced the rabies incidence to relatively low levels but foci of disease persist in certain areas. One focus of infection is the area in and around the third largest city in Turkey, Izmir. In the early 1990s the disease was almost eliminated from Izmir and the surrounding Aegean region. However, an increase in dog rabies cases was observed in the provinces Izmir and Manisa in 1997. The first rabid fox during this re-emergence was recorded in 1999 in the town of Urla to the west of the city of Izmir. Although the number of cases in dogs began to decline after 2000, the number of reported rabies cases in foxes started to increase (Johnson et al., 2006, 2010; Vos et al., 2009). The increase in rabid foxes, the steady spread of the disease, increasing numbers of cases in domestic cattle and a peak in the winter period all suggested that an independent endemic fox-mediated cycle of rabies in this area had been established (Vos et al., 2009). The fox epizootic spread further north-, south- and eastwards in the following years (Vos et al., 2009). The situation has prompted the Turkish Government to introduce oral vaccination of foxes against rabies in this region with financial and technical support of the European Union. This paper evaluates the first oral rabies vaccination (ORV) campaigns targeted at foxes in Turkey between 2008 and 2010.

Material and Methods

Vaccination area

The vaccination area (36 847 km²) is part of the Aegean region located in the western part of Turkey (Fig. 1). During all three campaigns the same area was baited. The region is dominated by a narrow coastal plain flanked by mountain ranges. Most of the human population is concentrated in urban centres such as the cities of Izmir, Manisa and Aydin. The climate is mainly Mediterranean with hot and dry summers (average maximum temperatures during July and August: > 35°C) and mild to cool wet winters. The mountain ranges (up to 1517 m above sea level) run west to east with large valleys running perpendicular to the coastline, permitting the Mediterranean Sea climate to reach the inner parts of the region. Some provinces further inland have a more arid continental climate. Agriculture plays an important role in the region's economy, especially in the deforested valley lowlands. Other important economic activities in the region are industry around urban centres, especially Izmir, and tourism along the coast.

Epidemiological and surveillance data

Epidemiological data was obtained from surveillance information recorded by the Bornova Veterinary Control Institute (BVCI) in Izmir with support of the National Rabies Reference Laboratory at the Etlik Veterinary Control Central Research Institute (EVCCRI) in Ankara. For the monitoring of the ORV campaigns the World Health Organization recommends examining at least four foxes per 100 km² (WHO, 2005). This would mean that almost 1500 foxes would need to be sampled from the vaccination area. Following discussions with the Ministry of Environment and Forestry, it was decided to reduce this number to 500 animals. The control foxes would be examined for rabies and the presence of rabies virus neutralizing antibodies. For rabies diagnosis, the brains of the animals were examined for viral antigen using the fluorescent antibody test (FAT) (Dean et al., 1996). Blood samples were tested by the rapid fluorescent focus inhibition test (RFFIT) (Smith et al., 1973), with modifications described by Cox and Schneider (1976).

Communication, public awareness and training

These activities were undertaken as part of the EU-funded project (TR 503.06/001 Technical Assistance for control of rabies disease). Three separate staff training courses in the provinces of Aydin, Denizli and Izmir were performed prior to the first ORV campaign with 250 participants from the provinces where baits were to be distributed. The participants were selected from the provincial directorates of Agriculture, Health and Environment, local municipalities, BVCI, NGOs and private veterinarians. The twelve pilots and twelve technicians responsible for the aerial distribution of the baits were trained separately prior to the first campaign. Information on the ORV campaigns were prepared and delivered to all relevant parties. The information included posters, leaflets, local radio and television spots and audio cassettes.

ORV campaigns

Due to the climatic conditions and human agricultural activities the timeframe available for the distribution of the vaccine baits was limited to the winter months. Approx-

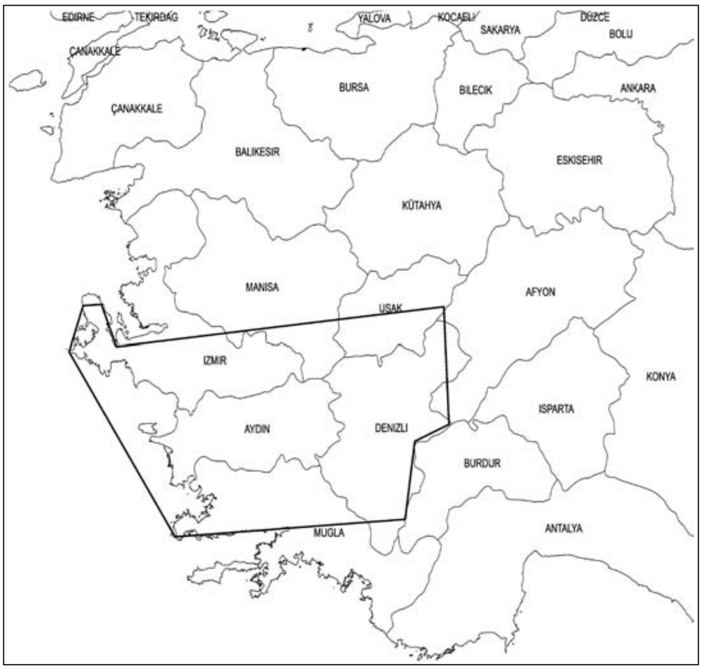

FIGURE 1: *The Aegean region, Turkey, with the individual provinces and the outlined vaccination area (36 847 km²).*

imately 1.5 ml SAD B19 vaccine virus (≥ $10^{6.5}$ FFU/ml) was filled in a polyvinylchloride blister sealed with aluminium foil cover and subsequently incorporated in the so-called Tuebingen-bait, a mixture of among others fish meal, coconut fat and paraffin (Neubert et al., 2001). Vaccine baits were shipped prior to every campaign from the manufacturer to BVCI and stored frozen (–20°C) until distribution. The cold chain was confirmed by data analysis of data loggers shipped and stored together with the baits. The titre of the vaccine virus was determined upon arrival at BVCI by the National Rabies Reference Laboratory at EVC-CRI using the titration protocol of the vaccine bait manufacturer confirming that virus titre of the vaccine bait batches used was above minimum effective dose claimed (≥ $10^{6.0}$ FFU/ml). Bait consumption and subsequent immune response was confirmed by seroconversion in animal samples from the vaccination area after bait distribution. For the preparation and implementation of the aerial distribution of the baits the SURVIS-system was used (Müller et al., 2012), no additional hand distribution of baits was considered. A total of approximately 610 000 baits were distributed per campaign using planes flying at 1000 feet altitude with an average speed of 170–180 km/hr. Vaccine baits were distributed at a density of 18 baits/km² with flight lines 1000 m apart. During the first campaign the baits were dropped from five Antonov and one Cessna 182 planes and in the following two campaigns only Cessna 182 planes were used.

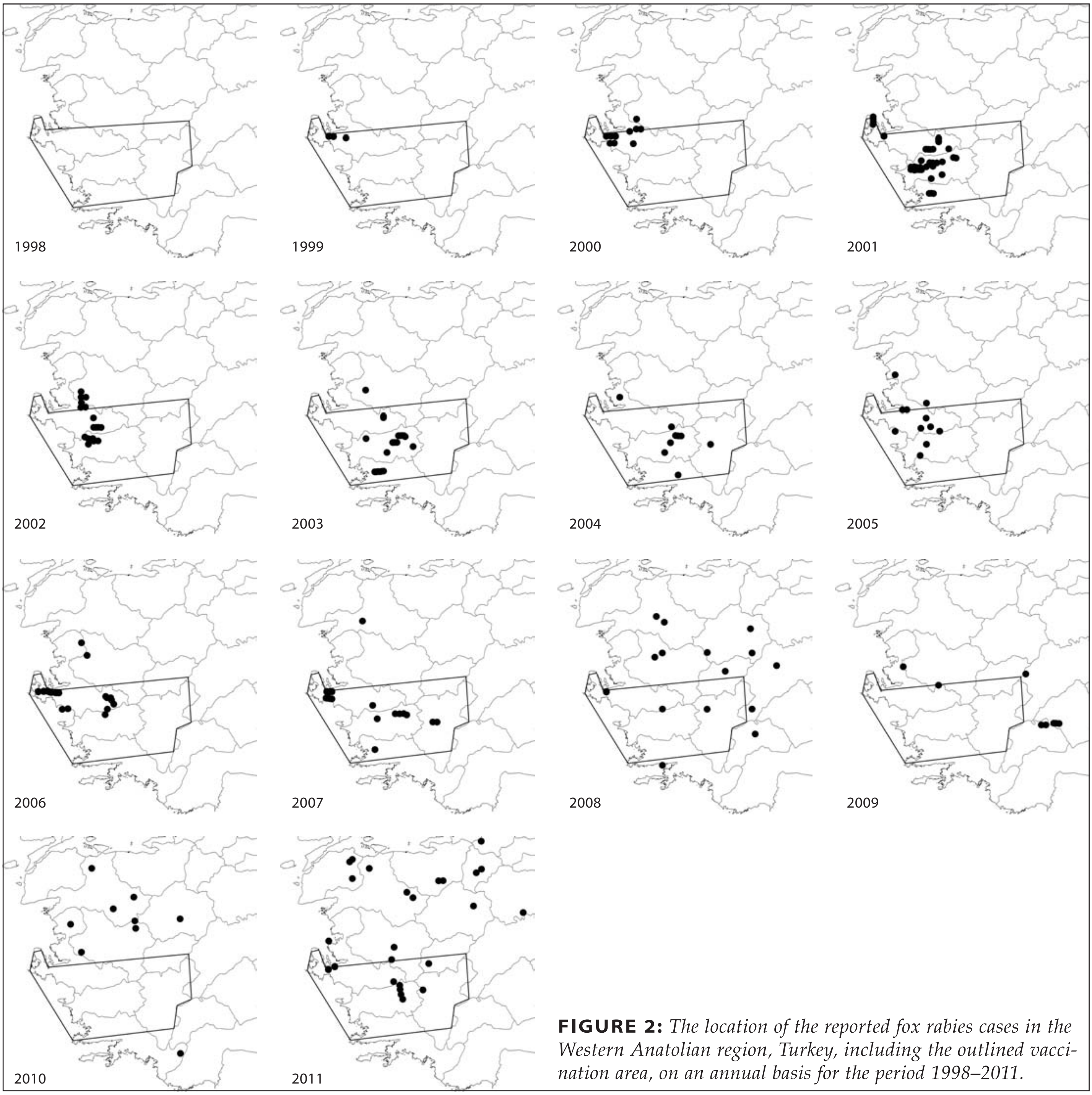

FIGURE 2: *The location of the reported fox rabies cases in the Western Anatolian region, Turkey, including the outlined vaccination area, on an annual basis for the period 1998–2011.*

Results

The spatial and temporal fox rabies incidence between 1998 and 2011 is shown in Figure 2. The number of rabies cases increased rapidly and the disease spread pre-dominantly in a southeastly direction. Prior to the first campaign, rabies within the fox population appeared to have become endemic in the region. The first campaign took place during a twelve day period in February–March 2008.

As a result of baits only being distributed by plane it cannot be assumed that all areas were covered due to the restrictions associated with this approach. Baits could not be dropped close to human settlements and certain areas were also no-fly zones such as military areas and power plants.

While the Ministry of Environment and Forestry did not issue official permission for the collection of control foxes from the vaccination area through active surveillance as agreed, control foxes only became available through animals handed in for rabies diagnosis. Only 13 control foxes were collected after the first campaign in 2008. These control animals originated from the following provinces; Uşak (n = 1), Denizli (n = 1), Izmir (n = 3), Manisa (n = 1) and Mugla (n = 7). All of these animals tested rabies negative by the fluorescent antibody test (FAT). Serum was collected from 11 of these 13 animals and 73% (n = 8; 95% C.I.: 43.6–92.1%) of these animals had detectable levels of rabies virus neutralizing antibodies (VNA) indicating bait uptake and subsequent contact with the vaccine virus. Rabies VNA titres ranged between 1.5 and 10.3 IU/ml. The confidence interval of the percentage animals with a measurable rabies VNA clearly showed that the sample size was too small to determine a reliable estimate of the seroconversion rate.

A considerable improvement in the rabies situation was observed within months of the first campaign (Fig. 3). In May 2008, the last fox case was reported within the core vaccination area (Baharli Köyü, Mugla). In August of the same year another rabid fox was reported from Urla on the peninsula to the west of the city of Izmir, at the border of the vaccination area. Also, vaccination campaigns targeted at cattle reduced overall rabies numbers considerably. However, no additional vaccination campaigns aimed at domestic dogs, the other rabies reservoir in the area, were implemented during the period of aerial distribution of baits. Prior to the start of the first vaccination campaign it was known that fox rabies had moved beyond the borders of the selected vaccination area (Fig. 2, 2008). Due to contractual obligations it was not possible to increase the number of baits and consequently the size of the vaccination area. It was therefore decided not to change the vaccination area in order to incorporate newly infected areas but concentrate on the original selected area. This would allow evaluation of the ORV campaigns and determine if the selected baiting strategy was able to eliminate fox rabies from the originally infected area.

The second and third campaigns were undertaken between February and March 2009 and again in December 2009 and February 2010, respectively. In the core vaccination area no further fox rabies cases were observed in 2009 and 2010 and also the overall incidence of rabies lowered (Tab. 1). In 2009, two cases of fox rabies were reported in the northern border region of the vaccination area (Ödemis – Izmir; Türkmen Köyü – Manisa). In 2010, again a rabid fox was reported from the peninsula west of the city of Izmir (Germiyan Köyü – Izmir). However, the spread of fox rabies beyond the vaccination area in eastern and northern directions continued. Presently it has reached the Marmara Sea to the North and the first cases have been reported from Konya in Central Anatolia (Fig. 2).

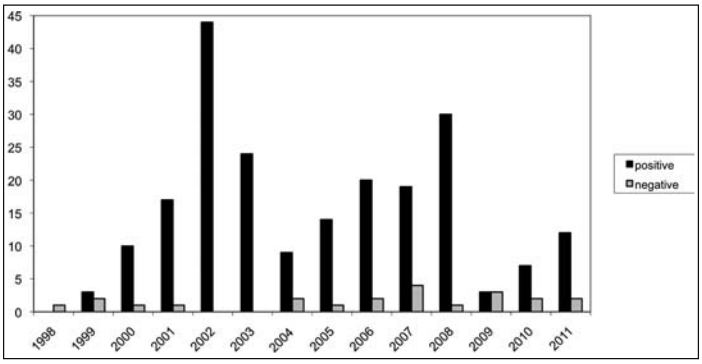

FIGURE 3: *Number of fox rabies cases reported in the vaccination area between 1998 and 2011 (Source: BVCI, Izmir and EVCCRI, Ankara).*

Discussion

Sustained host-switches of rabies virus are rare events and the underlying mechanisms are still not completely understood. The most recent documented sustained spill-over event occurred in Arizona, USA, where a rabies virus variant from bats is now circulating in striped skunks (*Mephitis mephitis*) (Leslie et al., 2006). In the Aegean region, the urbanization and agricultural development together with the high number of unrestricted dogs offered increased possibilities of contact between this domestic rabies vector species and wildlife species including the red fox. During the initial phase of the outbreak among foxes in the Aegean region it was not clear if this would be a locally restricted self-limiting spill-over infection from dogs to foxes or would develop into an independent sustained transmission

TABLE 1: *The number of rabies positive (pos) and negative (neg) cases in the Aegean region between 1998 and 2011 (Aegean region: provinces Afyon, Aydin, Denizli, Izmir, Kütahya, Manisa, Mugla, and Usak)*

Year	Fox		Dog		Cattle		Others		Total	
	pos	neg	pos	neg	pos	neg	pos	neg	pos	neg
1998	0	1	68	101	3	4	6	70	77	176
1999	3	2	78	82	13	5	12	82	106	171
2000	10	1	39	162	7	1	16	125	72	289
2001	17	1	39	136	66	18	20	109	142	264
2002	44	0	30	127	234	28	29	97	337	252
2003	24	0	11	151	100	13	18	100	153	264
2004	9	2	12	126	49	27	7	97	77	252
2005	14	1	17	134	54	18	10	97	95	250
2006	20	2	18	127	39	12	13	107	90	248
2007	19	4	26	134	37	37	6	124	88	299
2008	30	14	18	132	31	69	11	114	90	316
2009	4	3	4	114	10	69	6	110	24	296
2010	7	10	4	58	8	117	6	71	25	248
2011	12	2	6	93	28	170	9	90	55	355
total	213	22	370	1677	679	588	169	1393	1431	3680

cycle among foxes. Such small clusters of wildlife cases have been observed previously in Turkey but these cases were not able to establish an independent wildlife chain of infection. Hence, the initiative in 2001 to implement ORV campaigns was postponed until further evidence that the outbreak would continue became available. Unfortunately, when the first baits were distributed in February 2008 the infected area was much larger than originally anticipated (Fig. 2) and the number of available baits was not sufficient to cover the whole affected region. The selection of the vaccination area was further complicated through the low number of foxes handed in for rabies diagnosis. Therefore, it was not exactly known how far fox rabies had already spread. While no information was available on the population density and the behavioural ecology of the fox population in the Aegean region, the selected bait density and flight line spacing were based on an assumed relatively low fox density (< 1 adult fox /km^2). Hence, these ORV campaigns represent proof-of-principle and not an attempt to eliminate fox rabies from the Aegean region per se. Experience with ORV of wildlife in the Middle East region is only available from Israel (Yakobson et al., 2006). Fox rabies control is also feasible in Turkey as shown by these ORV campaigns in the Aegean region but will require sustained efforts to succeed in the future. These efforts include most importantly continued political and financial support for fox rabies control and improvement of surveillance and diagnostics. Furthermore, without an effective dog rabies control program possible spill-over infections from dogs to foxes remain a continuous threat for sustainable eradication of fox rabies from the area.

The selected strategy deviated in certain aspects from the recommendations of the EU (European Commission, 2002). Not only was the bait density used relatively low and flight line spacing larger than usually applied during ORV campaigns in Europe but also baits were only distributed once per year. The often used and recommended concept of bi-annual campaigns during Spring and Autumn is not feasible in Turkey, predominantly because of the climatic conditions in large parts of Turkey. The bait matrix and/or vaccine virus formulation of the commercially available oral rabies vaccine baits cannot withstand prolonged exposure to the high temperatures between Spring and Autumn. Furthermore, bi-annual campaigns would also considerably increase overall costs and scientific evidence for this approach in terms of cost-effectiveness is lacking (Vos, 2003). The concept of a single annual campaign has been successfully applied in different countries including Finland and USA (Texas) (Sidwa et al., 2005; Metlin et al., 2008).

However, there are certain aspects that would need further improvement in subsequent ORV campaigns. Although the serology results indicated that 73% of the foxes in the vaccination area seroconverted, it is evident that no valid evaluation of this parameter is possible with the low number of control foxes examined. In order to assess bait uptake, control animals should be examined for the presence of bait marker and/or virus neutralizing antibodies. The presence of virus neutralizing antibodies as indicator of a successful rabies vaccination attempt may not always be a reliable parameter (Hanlon et al., 2002). The use of seroconversion levels must therefore be treated with caution when used as an indicator of ORV success (Yakobson et al., 2006). However, experimental and field data clearly underscores the validity of this method to assess vaccination coverage in red foxes (Müller et al., 2001). Hence, the reluctance of the Ministry of Environment and Forestry to collaborate must be resolved in order to obtain sufficient control animals in future ORV campaigns. For these control foxes no distinction has to be made between so-called indicator animals (animals found dead or showing abnormal behaviour) and healthy ones. Only the age of the animals need to be determined (juvenile versus adult) especially animals that are born in the period between the vaccination campaign and the actual collection of the control foxes.

An additional problem identified was that of areas not covered by aerial bait distribution. Some of these were of substantial size and could have supported populations of fox that would be inaccessible to aerial bait distribution. In future campaigns, hand distribution of vaccine baits in areas not treated during aerial distribution needs to be considered. For example, the first reported fox case in the Aegean region in 1999 occurred actually in the northern part of the peninsula to the west of the city of Izmir. In the same region a rabid fox was found after the first and third ORV campaigns in May 2008 and October 2010, respectively. These two cases could be a result of separate spill-over infections from dogs but during this period no rabid dogs were reported from this area. Most likely it seems that residual foci of fox rabies could persist in this area. Due to flight restrictions baits were not homogeneously distributed here and this patchy distribution resulted in a vaccination coverage that was not able to interrupt the chain of infection among foxes. Unfortunately, for the moment no detailed protocol for hand distribution has been developed and established for the vaccination area.

Finally, to better identify the area to be treated rabies surveillance among wildlife must be improved. For rabies surveillance only indicator animals should be submitted. The (local) authorities should actively support the submission of animals found dead or animals showing abnormal behaviour. This approach is more cost effective and the vaccination area can be selected much more precisely. The present campaigns have clearly shown this. If the vaccination area is too small and fox rabies has already spread beyond the borders, the positive effects of oral vaccination are only temporary. As a result of the ORV campaigns fox rabies was eliminated from the core vaccination area but it seems that within two years after the last campaign the area is again threatened. However, the favourable results obtained in the vaccination area clearly showed that the selected ORV baiting strategy is adequate and capable of eliminating the disease among the local fox population and that ORV offers the opportunity for Turkey to eliminate fox-mediated rabies.

During 2011, fox rabies has reappeared in the Aegean provinces of Turkey (Fig. 2), either through further spillover from the dog population or reintroduction from northern areas adjacent to the vaccination area. This may have been accelerated by the rapid turnover of the fox population with juveniles now susceptible to infection. Whilst this is a setback for rabies control in Turkey, the analysis of the first ORV campaign suggests that this approach can be successfully deployed to control and eliminate fox rabies in the Mediterranean if applied correctly.

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Conflict of interest: Co-author Adriaan Vos is a full time employee of IDT Biologika GmbH, the manufacturer of the oral rabies vaccine baits used in these campaigns.

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