One Health 19 (2024) 100877

Contents lists available at ScienceDirect

One Health

journal homepage: www.elsevier.com/locate/onehlt

Oral rabies vaccination of foxes in Türkiye, 2019–2022

Orhan Aylan [a], Bayram Sertkaya [a], Anıl Demeli [a], Ad Vos [b], Sabri Hacioglu [c], Yeşim Tatan Atıcı [c], Deniz Acun Yıldız [c], Thomas Müller [d], Conrad M. Freuling [d,*]

[a] Ministry of Agriculture and Forestry, General Directorate of Food and Control, Animal Health and Quarantine Department, Eskişehir, Yolu Üzeri 9.km. Lodumlu, Ankara, Turkey

[b] Ceva Innovation Center, 06861 Dessau–Rosslau, Germany

[c] Etlik Veterinary Control Central Research Institute, A.S.Kolayli Cad. No.23, Etlik-Kecioren, 06020 Ankara, Turkey

[d] Friedrich-Loeffler-Institute, (FLI), Institute of Molecular Virology and Cell Biology, Greifswald - Insel Riems, Germany

ARTICLE INFO

Keywords:
Rabies
Red fox
Türkiye
Oral vaccination
SAD-B19

ABSTRACT

Background: Rabies in Turkey is maintained by dogs, but following a sustained spill-over, red fox mediated rabies had spread from the Aegean region to the central part of Türkiye. During the past four years from 2019 to 2023 large scale efforts used oral rabies vaccination (ORV) to control rabies in red foxes. Here, we present the results of the largest ORV campaign on the Asian continent.

Methods: ORV campaigns were carried out twice a year in spring and autumn with a targeted bait density of 20–23 baits/km^2. Monitoring of ORV campaigns included the GIS-based analyses of bait distribution, the assessment of bait uptake through biomarker detection and the determination of seroconversion (sero-positivity in ELISA) in the target species collected within the vaccination area. For determination of fox rabies incidence in vaccination areas as the main indicator of the performance of the ORV campaigns, epidemiological data was obtained from the national passive surveillance program.

Results: Aerial bait distribution was highly accurate, with >99 % of baits being recorded from targeted zones, thus meeting the desired bait densities. Although the overall bait uptake (28.1 %; 95 %CI: 23.2–32.8) and seroprevalance (36.3 %; 95 %CI: 30.0–43.2) were low, rabies incidence drastically decreased in ORV areas and rabies was eliminated from western and central parts of Turkey, with no reported cases in foxes from ORV areas in 2022 and 2023.

Conclusions: A large-scale ORV campaign against fox rabies using high quality vaccine baits and the GIS-aided and monitored bait distribution was able to control fox mediated rabies in the western and central parts of Türkiye. Rabies control both in dogs and foxes should be expanded to cover also the eastern parts of Türkiye, to become eventually rabies free.

1. Introduction

Rabies has been present in what is now modern Türkiye ever since antiquity [1], with dogs (*Canis lupus familiaris*) being the main reservoir. Control measures in recent decades had reduced the incidence of dog-mediated rabies to a relatively low level, with only a few foci persisting, particularly in urban areas [2]. As in most other Middle Eastern countries [3], rabies cases in wildlife in Türkiye were rarely reported, with a share of 1.6 %, mainly in red foxes (*Vulpes vulpes*) and golden jackals (*Canis aureus*) [4]. In Türkiye, a well-established surveillance network detected the emergence of fox rabies in the Aegean province of Izmir in 1999 [2,5–7]. In the absence of an active wildlife rabies control program, the epizootic spread eastward and southward within the fox population in subsequent years (Fig. 1) [5]. Phylogenetic studies revealed that the rabies virus (RABV) isolated from these foxes was closely related to the strain circulating in dogs from this region, indicating a sustained cross species transmission (CST) with foxes now maintaining dog rabies variants in the region [8,9]. Large parts of central Anatolia had not reported any rabies case for many years prior to the arrival of the new fox epizootic, although hundreds of animals had been submitted for rabies diagnosis. Upon incursion of fox rabies also several rabid dogs were reported from this area, which were proven to be re-introductions of the RABV variant circulating in foxes, as whole genome sequencing data showed [9].

* Corresponding author.
E-mail address: Conrad.Freuling@fli.de (C.M. Freuling).

https://doi.org/10.1016/j.onehlt.2024.100877
Received 16 April 2024; Received in revised form 31 July 2024; Accepted 13 August 2024
Available online 28 August 2024

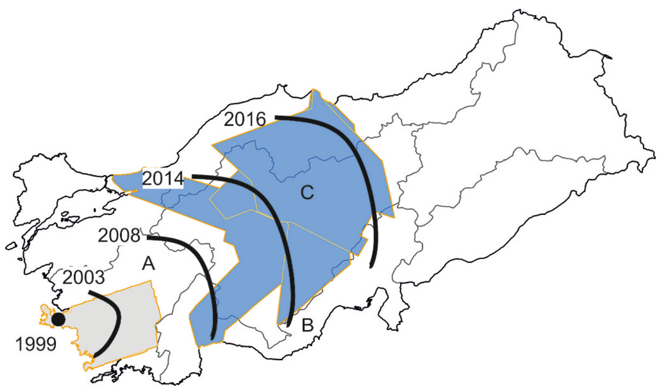

Fig. 1. Map showing major geographical regions of Türkiye, with the Anatolian Region (A), the Mediterranean Region (B) and the Central Anatolian Region (C) indicated. The black dot indicates the location of the first reported cases of fox rabies in 1999, while the black lines indicate the gradual eastward spread of the epidemic fox rabies front. Areas in grey and blue represent ORV areas from 2008 to 2010 and from 2014 to 2016, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

As oral rabies vaccination (ORV) has been proven an effective intervention tool for rabies control in terrestrial wildlife in Europe [10,11] and North America [12,13], the first ORV program for wildlife rabies control was implemented in Türkiye in 2008, approximately a decade after the start of the outbreak. As a result, fox rabies was successfully eliminated from a selected area in the Aegean region covered by ORV between 2008 and 2010 [5]. Because the entire affected area could not be treated at the time, reinfection of the formerly freed area from the neighboring eastern regions occurred after ORV campaigns ended, as well as a continued spread towards previously rabies-free Central Anatolia and beyond. The latter threat prompted the establishment of a vaccination corridor from the Black Sea to the Mediterranean Sea as part of a second round of ORV campaigns from 2014 to 2016 to prevent further spread to the east (Fig. 1). Although cases of fox rabies were observed east of the corridor, the causative RABVs were genetically distinct from those originating from the Aegean region and found in foxes west of the corridor suggesting that the emergent Aegean fox clade had not spread eastwards beyond the vaccination corridor but was about to meet a different fox RABV variant prevailing in Eastern Anatolia [9]. Unfortunately, the vaccination efforts could not prevent the re-emergence among others in areas treated in the period 2014–2016 (Fig. 1). Therefore, a new strategic approach to rabies control, i.e. large-scale ORV campaigns became necessary if fox rabies was to be eliminated in the Aegean Region, the Mediterranean Region and the Central Anatolian Region. This paper describes the planning, implementation, and evaluation of the largest EU-supported ORV program outside Europe in light of the prevailing epidemiological situation in Türkiye.

2. Material & Methods

2.1. Vaccine baits

The selection of vaccine baits for the ORV program followed a rigorous tender procedure that required vaccine bait candidates to meet a set of minimum requirements in accordance with the tender specifications issued by the Ministry of Agriculture and Forestry (MoAF) in Ankara, Türkiye.

The choice was Fuchsoral (SAD B19), a vaccine strain widely used in Europe since 1983 [10,14,15] and had proven successful during previous campaigns in Türkiye [5]. Each vaccine batch had to pass a release assay at the National Rabies Reference Laboratory at the Etlik Veterinary Control and Research Institute (VCRI) confirming that the vaccine virus titre was above the minimum effective dose ($\geq 10^{6.5}$ FFU/ml). Before shipment to Türkiye, the vaccine batches were also tested for potency by an independent laboratory (WOAH Reference Laboratory for Rabies at the Friedrich-Loeffler Institute, Greifswald – Insel Riems, Germany) including a stress test whereby the titre of the vaccine was determined after baits were exposed to +25 °C for 7 days, and a bait stability test by exposing baits for one hour at +40 °C in accordance with requirements of the EU [16].

2.2. Vaccination campaigns

In general, planning, implementation and evaluation of ORV campaigns followed several guidance documents but were adapted to the prevailing conditions in Türkiye [16,17]. ORV campaigns were carried out twice a year in spring and autumn from 2019 to 2022.

2.2.1. Selection of vaccination areas

The primary parameter for the selection of ORV areas was the epidemiological situation, i.e. areas where foxes and cattle rabies cases were recorded during the year prior to the planned campaign. The resulting size of the area to be covered by ORV was then determined based on number of baits available and targeted bait density. Due to the predetermined number of baits available per campaign, it was obvious that the entire western part of the country could not be covered. Prior to each follow-up campaign, the situation was re-assessed and if deemed necessary small adaptations were implemented. Within the vaccination area, target and non-target areas (among others; urban areas, water surfaces, areas 1500 m above sea level and areas with a no-fly zone [like military areas]) were identified using available geospatial data and omitted from aerial bait distribution. During the ORV campaigns in spring and autumn, vaccine baits were distributed from February to April and October to December, respectively. There was no hand distribution of baits carried out in any of the ORV campaigns.

2.2.2. Aerial distribution

Fixed-wing aircrafts, i.e. Socata TBM 850 planes, were used for aerial distribution of baits, flying at 1000 ft altitude with an average speed of 170–180 km/h. Different airports were selected and used to optimize cost-effectiveness. For bait distribution a fully automated aerial baiting system (SURVIS) was used consisting of a Global Positioning System (GPS) receiver connected to a computer that controls the automatic process of releasing the vaccine baits from the plane by considering the specified bait density, position and speed of the aircraft [18]. Furthermore, the GPS-receiver allows the pilot to navigate with maximum precision along the pre-set flight routes. For the determination of flight routes special software is used considering the most cost-effective flight path within the vaccination area taking e.g., load capacity, distance between flight lines (1000 m), total flight distance and return flight without pay load into account. Upon receiving a signal from the control unit based on the targeted bait density (20–23 baits/km^2), position and speed, a bait is dropped from the drop machine, which is equipped with a sensor to register the droppings (position and time) from the plane through the drop pipe. The data is encrypted and therefore free from manipulation by the crew or third parties.

2.2.3. Transport and storage of vaccine baits

Vaccine baits were transported by cold trucks from the manufacturer to Etlik-VCRI in Ankara, Türkiye. After temporary storage at this facility, baits needed for the vaccination area covered by a particular airport were brought to the airport in cooling trucks (−20 °C), which remained there as cold storage until bait distribution in the target area was completed. Storage and transportation conditions (<−20 °C) were monitored through temperature data loggers as recommended [16,17].

2.3. Post-campaign monitoring

2.3.1. Evaluation of aerial distribution

For data analysis, the data generated by SURVIS were transferred to the AERIAL FLIGHT MANAGEMENT software (GeoFly GmbH, Magdeburg - Germany), a newly developed, ISO-certified system for high-resolution qualitative evaluation of individual bait distribution per flight activity. After deciphering, the GeoFly system automatically analysed flight data by generating maps depicting individual flight routes and bait drops, with achieved bait density subdivided in subgroups [0–4, 5–9, 10–15, 16–25, 26–30, >30 baits/km^2], as well as bait drops in predetermined targeted and non-targeted areas. Simultaneously, graphs of bait distribution over time, bait density distribution, and distance (m) between two consecutive bait drops were generated.

2.3.2. Monitoring of ORV campaigns

Monitoring of the effectiveness of ORV campaigns included assessment of bait uptake through biomarker detection and determination of seroconversion in the target species collected within the vaccination area in a fixed period after the campaign following EU guidelines [16,17]. For this purpose, special permission was obtained from the Ministry of Agriculture and Forestry, General Directorate of Nature Conservation and National Parks to collect foxes during a predetermined period approximately one month after the campaign was finished. Foxes in ORV areas were shot at random by local hunters and collected by Provincial Veterinary Authorities and then sent frozen to Etlik VCRI.

Age determination (young vs adult foxes) was carried out by visual inspection of incisival tooth wear and/or by examining the width of the pulp cavity of the canines [19]. Detection of the bait marker, tetracycline (TC), in the teeth or mandibles of control foxes was conducted by examining thin sections using a fluorescence microscope as described before [20]. To establish seroconversion, rabies specific binding antibodies in fluids from the thoracic cavity were detected using a commercially available ELISA (BioPro Rabies ELISA, Czech Republic) as described [21,22]. A 40 % inhibition of the test serum compared to the negative control was considered as the cut-off for seropositivity, provided that the internal validity criteria indicated in the instruction of the manufacturer were met.

2.3.3. Rabies surveillance

For determination of fox rabies incidence in vaccination areas as the main indicator of the performance of the ORV campaigns, epidemiological data was obtained from the national passive surveillance program. Besides a spatial and temporal analysis of all rabies positive animals, also the data from all rabies negative animals originating from the vaccination area and submitted for rabies diagnosis were collected. Routine rabies surveillance and diagnosis as established at the eight provincial rabies diagnostic laboratories included antigen detection by fluorescent antibody test (FAT) on brain impression and smears [23] using a commercially available FITC-labeled anti-rabies hyperimmune serum (Fujirebio Diagnostics, United States). For indeterminant results, brain samples were also investigated for the presence of RABV specific RNA using RT-qPCR [24].

3. Results

3.1. ORV campaigns

A total of 120 batches of Fuchsoral were used from 2019 to 2022. The mean titres at the FLI were 6.98 FFU/ml (95 % CI 6.95–7.02) at arrival and 6.50 FFU/ml (95 % CI 6.45–6.53) after the stress test. All bait casings remained intact for one hour at +40 °C. A total of seven ORV campaigns were carried out during the program from 2019 to 2022 with vaccination areas roughly between 200,000 and 250,000 km^2 per campaign covering approximately 25–30 % of the total land mass of Türkiye (Table 1). Adaptations in the areas to be covered in subsequent ORV campaigns were made based on the available epidemiological data (Fig. 2).

Table 1
Overview of the seven ORV campaigns conducted from Autumn 2019 to Autumn 2022, Türkiye.

Nr	From	Till	Duration (days)	Number of flying days	Area (km^2)	Nr. of baits*
1	19/09/2019	03/11/2019	45	35	225,000	4,454,950
2	23/02/2020	26/04/2020	63	39	225,000	5,505,952
3	06/10/2020	21/11/2020	46	40	225,000	5,503,519
4	20/02/2021	29/04/2021	68	39	236,679	5,509,591
5	09/10/2021	24/12/2021	76	41	244,029	5,498,289
6	15/02/2022	29/04/2022	73	35	198,583	4,385,692
7	19/10/2022	09/01/2023	82	49	207,142	4,505,456

* - number of baits dropped as counted by the SURVIS system.

Because of bad weather conditions, planes could not always fly, so vaccine baits had to be distributed over a longer period of time than anticipated. This was predominantly a problem during the last two autumn campaigns: The 5th and 7th ORV campaign lasted till the end of December 2021 and early January 2023, respectively (Fig. 3).

3.2. Evaluation of aerial distribution

More than 99 % of the baits distributed were dropped within predetermined target areas during all seven campaigns, and most of the baits dropped in non-target areas were released in mountains 1500 m above sea level (Table 2, Supplementary Fig. 1). While the average bait density per km^2 was 22.23, the percentage of individual 1 km x1km grid cells within ORV areas with bait densities higher than 16 and 26 baits/km^2 ranged between 94.7 and 95.7 % and 47.8 and 65.9 %, respectively (Fig. 3, Supplementary Fig. 2).

3.3. Monitoring of ORV campaigns

From 2019 to 2022, a total of 328 control foxes were submitted for testing to Etlik VCRI; on average 47 animals per campaign (range: 28–64 animals per campaign). The overall bait uptake (detection of TC in teeth) and seroprevalance (sero-positivity in ELISA) was 28.1 % (95 % CI: *23.2–32.8)* and 36.3 % (95 %CI: *30.0–43.2)*, respectively. However, there were large fluctuations in both parameters during the different campaigns (Table 3). Only for the Autumn 2022 campaign, there was significant difference ($p = 0.009$; Fisher's [exact] test) between the proportion of foxes that seroconverted (55.6 %), compared to the proportion of foxes that tested positive for the presence of the bait marker (16.1 %).

3.4. Rabies surveillance

In Türkiye, between 2013 and 2022 rabies in animals declined from a peak in 2014 with 728 reported cases to 151 rabies cases reported in 2021. In 2022, the number of rabies cases reported increased to 277. Rabies cases in foxes also peaked in 2014 and declined afterwards with a single case reported in 2021, followed by 5 reported cases in 2022 (Fig. 5A).

From 2019 to 2022, a total of 1026 animals from ORV areas (Fig. 2) were submitted in the frame of rabies routine surveillance. As the vaccination area was not fixed during the entire period, the allocation 'within vaccination area' was based on the day the case report was filed and the vaccination area treated preceding this date (except for 2019 as

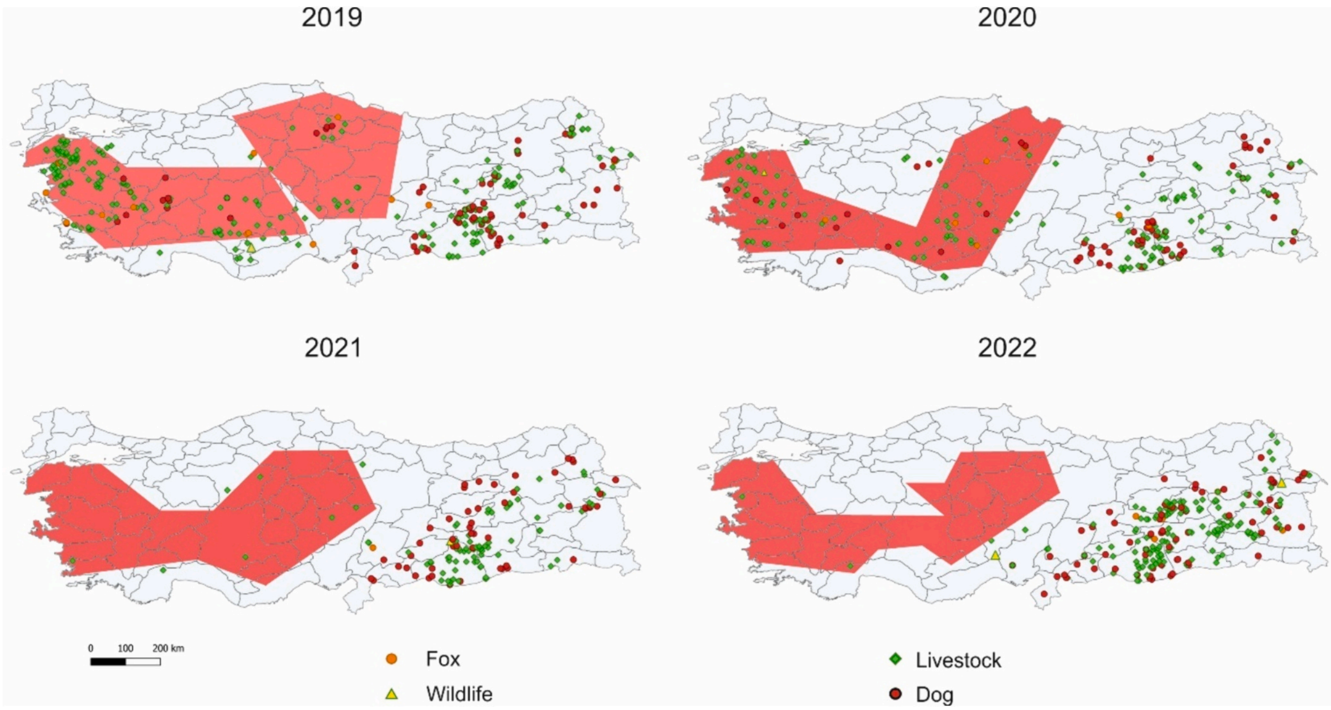

Fig. 2. The vaccination area (red marked) and the reported rabies cases of 4 subclasses (fox, other wildlife, dogs, livestock) per year, 2019–2022. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

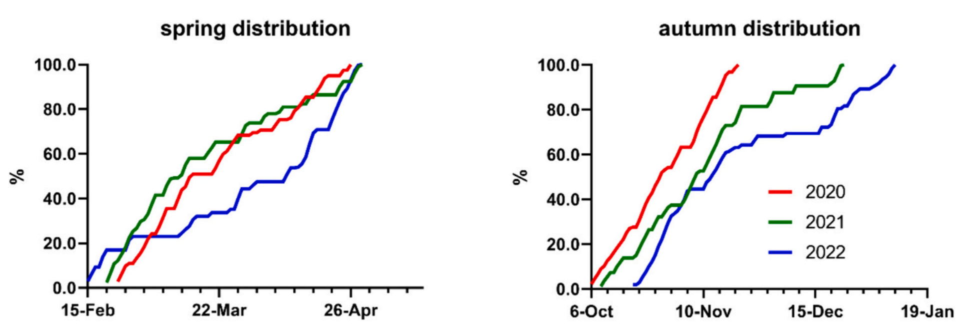

Fig. 3. Temporal bait distribution of vaccination campaigns, 2020–2022 (1st campaign Autumn 2019 not included as no information on daily number of baits dropped was available): cumulative percentage of baits dropped during spring and autumn campaigns. The Autumn campaign in 2022 continued till January 9th (x-axis: date [dd/mm]).

Table 2
Number of baits dropped within target (with percentage in parenthesis) and non-target areas.

Campaign	Target areas	Average bait density per km^2	Non-target areas				
			urban	water	outside	mountain	total
Autumn 2019	4,438,790 (99.6 %)	19.0	98	867	15,195*		16,160
Spring 2020	5,489,101 (99.7 %)	23.2	101	1,303	12,894*		16,851
Autumn 2020	5,486,770 (99.7 %)	23.6	1,144	368	5135*		16,749
Spring 2021	5,494,811 (99.7 %)	23.2	997	845	1,269	11,669	14,780
Autumn 2021	5,481,451 (99.7 %)	22.9	1,516	1,316	2,942	11,604	16,838
Spring 2022	4,378,115 (99.8 %)	22.0	1,265	1,168	622	4,525	7,580
Autumn 2022	4,496,796 (99.8 %)	21.7	1,571	1,465	650	4,966	8,660

* during the campaign baits dropped in areas 1500 m above sea level were included in the group 'outside'.

only one campaign took place). Also, the exact location of the animal was not always available, in this case the capital of the province involved was selected. Of those, 30 % (n = 306) tested rabies positive.

Generally, rabies cases in the ORV area sharply declined, from 223 cases in 2019 to 93 cases in 2020, 9 cases in 2021 and only 3 cases in 2022. Fox rabies cases were only detected in 2019 (n = 6) and 2020 (n = 7), whereas between 59 and 87 fox samples were tested for rabies per years from 2019 to 2022 (Fig. 5B).

Spatially, while rabies was present in most parts of the country in 2019 and 2020, there were only sporadic cases in the Central Anatolian Region of Türkiye and the Aegean Region in 2021. In 2022, only two cases were reported from the Aegean region while there were no cases

Table 3
Summary of the control foxes submitted after each vaccination campaign, incl. Sampling period, serology (ELISA) and presence of bait marker (TC) in teeth sections (n/N: number of samples testing positive (n) and number of samples tested (N)). *P*-value of Fisher's test comparing the proportion of foxes that seroconverted with the proportion of animals positive for the bait marker.

Campaign	Sampling period		Seroconversion			Bait marker (TC)			Fisher's test
	from	till	n/N	%	95 % CI	n/N	%	95 % CI	p-value
Autumn 2019	20/12/2019	18/02/2020	09/62	14.5	7.8–23.5	6/63	9.5	4.4–19.3	0.423
Spring 2020	12/07/2020	18/08/2020	07/31	22.6	11.4–39.8	8/43	18.6	9.7–32.6	0.772
Autumn 2020	13/01/2021	09/03/2021	16/32	50	33.6–66.4	15/51	29.4	18.7–43.0	0.067
Spring 2021	25/.06/2021	09/07/2021	10/20	50	29.9–70.1	17/53	32.1	21.1–45.5	0.182
Autumn 2021	09/02/2022	07/03/2022	16/31	51.6	34.8–68.0	29/55	52.7	41.5–67.3	>0.999
Spring 2022	23/06/2022	07/07/2022	05/07	71.4	35.9–94.9	11/28	39.3	23.6–57.6	0.208
Autumn 2022	24/01/2023	28/02/2023	10/18	55.6	33.7–75.4	5/31	16.1	7.1–32.6	0.009
Total			**73/201**	**36.3**	**30.0–43.2**	**91/324**	**28.1**	**23.2–32.8**	**0.053**

Of 328 control foxes 201 combined data sets on seroprevalence and bait marker was available (Supplementary Table 1). Most animals (58.2 %, $n = 117$) tested negative for both parameters, only 21.9 % ($n = 44$) had evidence of antibodies and bait marker.

reported in the central part of Türkiye, while most cases were notified from the southeastern parts of the country (Fig. 2). In 2023, rabies cases occurred exclusively in the latter region (Fig. 6).

4. Discussion

Spillover rabies infections have been reported many times but most often these involve single cases or a limited number of onwards infections [25,26]. The emergence of fox mediated rabies in the Aegean Region of Türkiye as a result of a spillover from dogs was monitored in near realtime [4,6,8]. The subsequent host shift highlights the fact that cross-species transmission from dogs to foxes is more likely to occur due to the generally high susceptibility of foxes for dog-adapted rabies viruses [27,28], especially when canine rabies is endemic or dog rabies control is not consistently followed through.

As fox-mediated rabies was a new phenomenon in Türkiye, the existing rabies control measures traditionally targeting dog-mediated rabies prevented the re-establishment in the original reservoir in Central Anatolia, but were inadequate to control this outbreak in foxes. Because of promising results from initial ORV campaigns [5], a large EU-supported ORV program was conducted in Türkiye from 2019 to 2022.

Ad hoc analyses using state of the art GIS technology demonstrated a high precision of bait distribution using fixed-winged aircraft, with more than 99 % of baits being distributed in previously selected areas (Table 1, Supplementary Fig. 1). Bait dropping in other areas can be considered irrelevant and are often a result of slight deviations from the flight line due to wind turbulences. Within ORV areas, most square kilometres were actually baited with the recommended and planned bait density (Fig. 4). As a result, while monitoring indicated relatively low bait uptake and seroconversion in foxes (see discussion below), the rabies incidence as the ultimate measure of the campaigns' impact [11] demonstrated a success, both in the decrease of cases reported and the area affected by rabies (Figs. 2, 5–6). Actually, no rabies cases in foxes and other animal species, including dogs, were reported in the targeted area at the end of the campaign series. The fact that also no cases in dogs were reported in the targeted areas (Figs. 4, 5) indicate that there was no sustained dog-to-dog transmission in the ORV areas and these cases in dogs were spill-over events from foxes. Such decline of rabies cases in target (i.e. foxes and raccoon dogs) and other species was also seen in successful European ORV programmes [11]. However, dog vaccination campaigns using parenteral vaccines were carried out at the same time in Türkiye and may have also contributed to the overall reduction of cases (Fig. 5A).

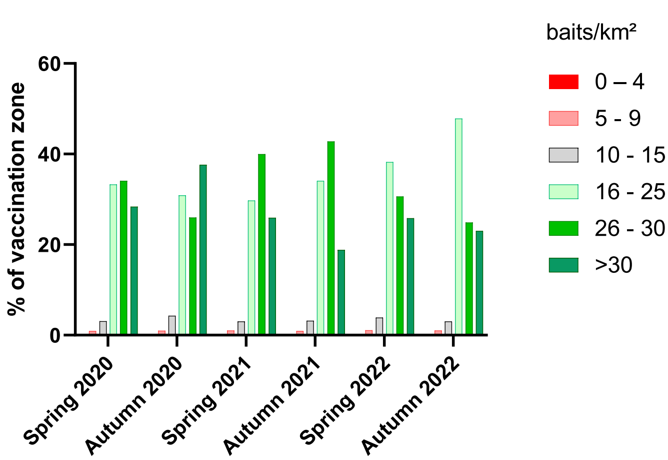

Fig. 4. Proportion of different bait densities in the ORV areas per campaign.

The only other two countries that systematically distributed baits outside Europe and North America are Israel (red fox, golden jackal) and South Korea (raccoon dog) since 1998 and 2000, respectively [29,30]. Reports from other Asian countries on ORV campaigns have been published but it is not known if they are conducted in a systematic way or more ad hoc [31,32].

In Türkiye, the protocol for the implementation of the ORV campaigns largely followed the recommendations made by the EU [17], but needed to be modified in order to consider the local situation. For example, it is recommended that during the Spring campaign baits should be preferably distributed during May or June in order to reach also the young fox population [16]. Unfortunately, climatic conditions in Türkiye during spring are unfavorable for bait distribution due to high environmental temperatures. Hence, it was decided to start bait distribution in February. Also, both, bait density and flight line distance should be linked to the fox population density. Unfortunately, there is only limited data available on the population density of foxes in Türkiye [33,34]. The few available studies indicate a rather broad range from 0.07 to 4.0 foxes/km^2 where in most areas low densities prevail [35,36]. Increasing bait density would limit the area to be vaccinated as the absolute number of available baits was fixed and reducing flight line distance to 500 m would increase operational costs considerably. Therefore, the selected bait density (19–24 baits/km^2) was at the lower end of the recommendations (18–30 baits/km^2) and instead of the suggested maximum distance of 500 m between flight lines, 1000 m was used.

As no further (fox) rabies cases have been reported from the western part of Türkiye including the vaccination area in 2023, it seems that the selected strategy has been successful. In an assessment of ORV programmes in Europe an index (area index, AI) capturing the size and overlap of successive ORV campaigns was identified as one factor with a statistically significant effect on the number of campaigns required to both control and eliminate rabies. Repeat comprehensive campaigns that are wholly overlapping with an AI close to 1 are much more likely to rapidly eliminate infection. For the Turkish ORV programme, the AI was 0.82 and lead to a drastic reduction of the rabies incidence in ORV areas

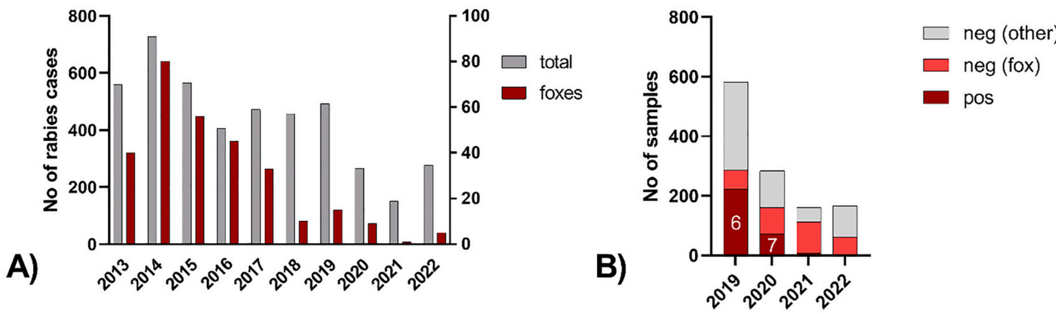

Fig. 5. A) Rabies surveillance data (animals reported as rabies positive) for Türkiye for the years 2013–2022. Fox rabies cases are separately displayed (right y-axis). B) Total number of animals tested positive (pos.) and negative (neg.) for rabies originating from ORV areas (see Fig. 2). The number of rabies positive foxes are indicated within bars.

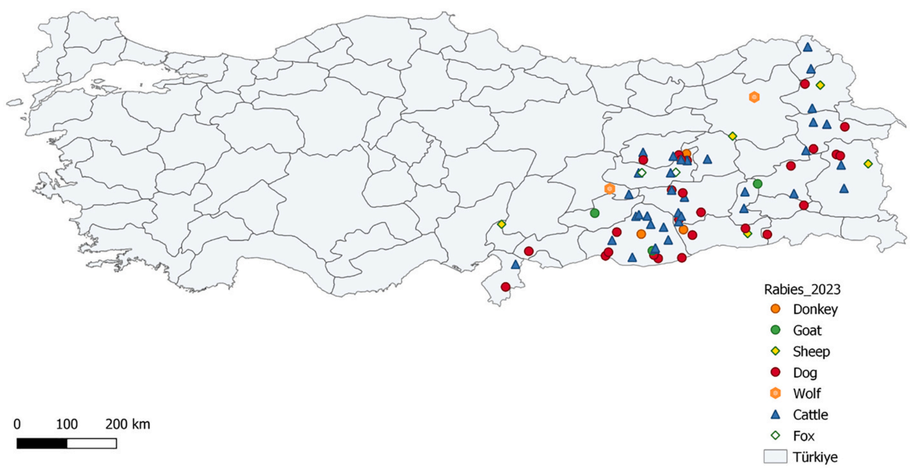

Fig. 6. Rabies cases in Türkiye during the first 6 months of 2023; geographical distribution per animal species (source: Ministry of Agriculture and Forestry).

(Figs. 2, 6) and when contrasted with the cumulative costs showed an immediate decline (Supplementary Fig. 3) compared to other European countries [11]. However, this apparent success should still be seen with caution; the absence of reported rabies cases in foxes in this relatively short period post vaccination is not sufficient evidence of elimination of fox rabies. Vaccination campaigns should continue for at least two years after the last confirmed case of rabies [17,37]. This is partially true for areas with overlapping vaccination areas, but not for others (Fig. 2). Even after this period elimination is not always guaranteed. For example, re-emergence of fox-rabies in Ontario in 2016 was originally thought to be a result of a re-infection from the north. Phylogenic studies, however, showed that the re-emergence was most likely a result of the resurfacing of undetected foci. Despite ongoing passive surveillance, the persistence of wildlife rabies went undetected in the affected area for almost three years [38]. Although the rabies situation in the western part of Türkiye looks promising in 2023 (Fig. 6), rabies surveillance in this region must continue at a high level to prove success for the entire treated area.

Beside quality of the vaccine and bait distribution, post-campaign monitoring usually also includes the detection of indirect (bait marker) and direct (antibodies) markers of vaccination to obtain information on bait uptake and serology in the target animal, respectively. The latter can provide information on vaccine immunogenicity in an individual (level of antibodies) and vaccine coverage across a population (proportion of foxes testing sero-positive) [17]. For a long period of time this has been considered standard practice for evaluating the success of ORV campaigns in wildflife target species in the EU and North America. However, biases in sampling, different quality of samples and the use of different serological tests made it not only difficult to compare results between different campaigns both temporally and geographically [22,39], but often had contradicting results as compared to the reduction of rabies cases. Therefore, only at the beginning of ORV campaigns bait uptake and seroconversion were regarded as valuable [17].

Our results corroborate this experience made at an international level. Given the sheer size of the vaccination areas (Table 1), with an average of 0.025 fox/km^2, the sample size of the control foxes was rather small and therefore much lower than recommended [17]. However, if this recommendation (4 animals per 100km^2 and year) was to be met, 4000 control foxes should have been collected after each ORV campaign which is unrealistic based on the remoteness and the low fox density in Türkiye [34]. As a result, vaccination coverage and bait uptake as biological parameters clearly showed an effect of the ORV campaigns on the fox population, but was below expectations (Table 3). The fact that more animals were tested for the bait marker than for antibodies is consistent with other experiences and can be explained by the fact that it was often not possible to take serum samples from fox carcasses upon arrival at the laboratory or the quality of the serum samples no longer permited testing [40,41].

The relatively low overall seroconversion rate (36.3 %) determined in control foxes submitted is consistent with results obtained in Kosovo [40], where the same highly sensitive ELISA test was used [22]. This seems to contradict the reduction of reported (fox) rabies cases in the vaccinated areas to zero. Generally, a threshold of 70 % vaccination coverage is considered necessary to prevent spread of the disease in the fox population [16]. This estimated threshold was based on a fox population model [42] and corresponds with the vaccination coverage claimed to be needed for dog rabies control as well [43]. However, is this required 70 % vaccination coverage justified as a general threshold to successfully eliminate fox rabies? From a theoretical perspective, it can be argued that a disease like rabies with a low effective reproductive ratio (R_{eff}) would need relatively low levels of vaccination to eliminate rabies. For fox rabies, R_{eff}-estimates range between 1 and 2 [44,45]. These low values suggest that a much lower vaccination coverage (20–40 %) is needed to interrupt the transmission cycle [45,46]. However, most likely a higher vaccination coverage after each campaign is required to compensate the high population turnover, especially after spring when many susceptible fox cubs enter the population [44]. Also, the model estimating the threshold of 70 % vaccination coverage was based on a population density of 3 foxes/km^2 [42]. Model estimates indicate that lower fox densities - most likely also applicable for Türkiye - needed lower vaccination coverages to eliminate fox rabies [47]. The situation in Turkey also confirms other model assumptions that even low levels of immunity could result in high probabilities of elimination under ecological conditions of small populations, low infectivity and low initial incidence [48]. Given that the presence of VNAs may not always be a reliable parameter as an indicator of a successful ORV campaign [49], their use is controversial [29]. So it remains open for discussion, if a higher seroconversion rate would have achieved a more rapid elimination.

Counterintuitively, often the proportion of animals with bait marker was lower than with antibodies as the overall bait uptake of 28.1 % (CI 95 %, 23.2–32.8 %) shows (Table 3). This most likely is a result of a reduced sensitivity of the assay preparing and detecting TC in the tooth samples collected [20]. The detection of TC in bones, e.g. mandible or femur, would probably have been a better alternative [50,51]. Interestingly, a review of bait-uptake rates in ORV areas in Europe revealed large discrepancies, which ranged from 12 to 91 % [52]. Although the number of animals tested for bait markers and seroconversion varied (Table 3), there were no significant differences in seroconversion and bait uptake rates except in August 2022.

Conclusively, bait uptake and serology results from control foxes with such small sample sizes should be carefully interpreted as indicators of success as they can be influenced by many confounding variables [29] particularly the large geographical bias that can be assumed. As rabies prevalence is the best indicator of control success, the monitoring of ORV campaigns has generally become less important, at least in Europe [53]. The low number of foxes submitted for testing was a general limitation, also in terms of passive rabies surveillance as it hindered a detailed assessment of the epidemiological situation, especially the spatial distribution of fox-rabies. Between two and ten foxes originating from the ORV area were submitted for rabies diagnosis on an annual basis during this ORV program. But spatial occurrence of rabies in cattle was used as supportive information to determine the area affected by fox-mediated rabies, since it has been shown before that rabies cases in cattle were indicative for the presence of fox-mediated rabies [54].

5. Conclusion

Thanks to funding from the European Union, Türkiye implemented a large-scale ORV campaign against fox rabies and modified the strategy to fit regional circumstances. The high quality of vaccine baits and the GIS-aided and monitored bait distribution during the seven ORV campaigns seemed to be able to control fox mediated rabies in the western and central parts of Türkiye. However, the apparent elimination of fox-mediated rabies in the area should not lead to a discontinuation of control efforts, as vaccination campaigns should continue for at least two years after the last confirmed case of rabies [37,55]. Furthermore, in a concerted action, rabies control both in dogs and foxes should be expanded to cover also the eastern parts of Türkiye, to become eventually rabies free.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Funding

The project was funded by the EU (EuropeAid/138318/IH/SUP/TR, contract No: TR2015/AG/08/A2–01/001). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

CRediT authorship contribution statement

Orhan Aylan: Writing – review & editing, Validation, Project administration, Conceptualization. **Bayram Sertkaya:** Writing – review & editing, Formal analysis. **Anıl Demeli:** Writing – review & editing, Formal analysis. **Ad Vos:** Writing – review & editing, Writing – original draft, Supervision, Investigation, Formal analysis. **Sabri Hacioglu:** Writing – review & editing, Formal analysis. **Yeşim Tatan Atıcı:** Writing – review & editing, Formal analysis. **Deniz Acun Yıldız:** Formal analysis. **Thomas Müller:** Writing – review & editing, Writing – original draft, Supervision, Investigation. **Conrad M. Freuling:** Writing – review & editing, Writing – original draft, Supervision, Formal analysis, Data curation.

Declaration of competing interest

A.V. is a full-time employee of Ceva Innovation Center, formerly IDT Biologika, Germany, a company manufacturing oral rabies vaccine baits. T.M. and C.M.F. from the Friedrich-Loeffler-Institute received funding from IDT Biologika / Ceva for research into mechanisms of oral rabies vaccination and serological response. All the other authors declare no competing interests.

Data availability

All data are included in this published article. The raw datasets are available from the corresponding author on reasonable request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2024.100877.

References

[1] N. Akkoca, P. Economics, G. Maksoud, M. Mestom, Rabies in Turkey, Cyprus, Syria and Lebanon, in: A.A. King, A.R. Fooks, M. Aubert, A.I. Wandeler (Eds.), Historical Perspective of Rabies in Europe and the Mediterranean Basin, OIE, Paris, 2004, pp. 157–169.

[2] N. Johnson, H. Un, A.R. Fooks, C. Freuling, T. Müller, O. Aylan, A. Vos, Rabies epidemiology and control in Turkey: past and present, Epidemiol. Infect. 138 (2010) 305–312, https://doi.org/10.1017/S0950268809990963.

[3] O. Aylan, A.F.M. El-Sayed, F. Farahtaj, A.R. Janani, O. Lugach, O. Tarkhan-Mouravi, G. Usluer, R. Vodopija, N. Vranjes, N. Tordo, B. Dodet, Report of the first meeting of the Middle East and Eastern Europe rabies expert bureau, Istanbul, Turkey (June 8-9, 2010), Adv. Prev. Med. 2011 (2011) 812515, https://doi.org/10.4061/2011/812515.

[4] A. Vos, C. Freuling, S. Eskiizmirliler, H. Un, O. Aylan, N. Johnson, S. Gurbuz, W. Muller, N. Akkoca, T. Müller, A. Fooks, H. Askaroglu, Rabies in foxes, Aegean region, Turkey, Emerg. Infect. Dis. 15 (2009) 1620–1622.

[5] H. Ün, S. Eskiizmirliler, N. Ünal, C. Freuling, N. Johnson, A.R. Fooks, T. Müller, A. Vos, O. Aylan, Oral vaccination of foxes against rabies in Turkey between 2008 and 2010, Berl. Munch. Tierarztl. Wochenschr. 125 (2012) 203–208.

[6] N. Johnson, H. Un, A. Vos, O. Aylan, A.R. Fooks, Wildlife rabies in Western Turkey: the spread of rabies through the western provinces of Turkey, Epidemiol. Infect. 134 (2006) 369–375, https://doi.org/10.1017/S0950268805005017.

[7] N. Johnson, C.M. Freuling, H. Ün, A. Kliemt, O. Aylan, N. Ünal, S. Eskiizmirliler, N. Akkoca, A.R. Fooks, T. Müller, The role of Phylogeography in the control of wildlife rabies in Turkey, in: Introduction to Sequence and Genome Analysis III, iConcept Press, 2013.

[8] N. Johnson, C. Black, J. Smith, H. Un, L.M. McElhinney, O. Aylan, A.R. Fooks, Rabies emergence among foxes in Turkey, J. Wildl. Dis. 39 (2003) 262–270, https://doi.org/10.7589/0090-3558-39.2.262.

[9] D.A. Marston, D.L. Horton, J. Nunez, R.J. Ellis, R.J. Orton, N. Johnson, A. C. Banyard, L.M. McElhinney, C.M. Freuling, M. Fırat, N. Ünal, T. Müller, X. de Lamballerie, A.R. Fooks, Genetic analysis of a rabies virus host shift event reveals within-host viral dynamics in a new host, Virus Evol 3 (2017) vex038, https://doi.org/10.1093/ve/vex038.

[10] T. Müller, C.M. Freuling, P. Wysocki, M. Roumiantzeff, J. Freney, T.C. Mettenleiter, A. Vos, Terrestrial rabies control in the European Union: historical achievements and challenges ahead, Vet. J. 203 (2015) 10–17, https://doi.org/10.1016/j.tvjl.2014.10.026.

[11] C.M. Freuling, K. Hampson, T. Selhorst, R. Schroder, F.X. Meslin, T.C. Mettenleiter, T. Müller, The elimination of fox rabies from Europe: determinants of success and lessons for the future, Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 368 (2013) 20120142, https://doi.org/10.1098/rstb.2012.0142.

[12] C. Fehlner-Gardiner, Rabies control in North America - past, present and future, Rev. Sci. Tech. 37 (2018) 421–437, https://doi.org/10.20506/rst.37.2.2812.

[13] A.T. Gilbert, R.B. Chipman, Rabies control in wild carnivores, in: Anthony R. Fooks, Alan C. Jackson (Eds.), Rabies (Fourth Edition), Fourth edition, Academic Press, Boston, 2020, pp. 605–654.

[14] F.T. Müller, C.M. Freuling, Rabies control in Europe: an overview of past, current and future strategies, Rev. Sci. Tech. 37 (2018) 409–419, https://doi.org/10.20506/rst.37.2.2811.

[15] A. Vos, T. Müller, P. Schuster, H. Schlüter, A. Neubert, Oral vaccination of foxes against rabies with SAD B19 in Europe, 1983–1998: A review, Vet. Bull. 70 (2000) 1–6.

[16] European Commission, The Oral Vaccination of Foxes against Rabies, Report of the Scientific Committee on Animal Health and Animal Welfare, 2002.

[17] EFSA, Scientific opinion - Update on oral vaccination of foxes and raccoon dogs against rabies, EFSA J. 13 (2015) 4164, https://doi.org/10.2903/j.efsa.2015.4164.

[18] T. Müller, C. Freuling, P. Geschwendner, E. Holzhofer, H. Mürke, H. Rüdiger, P. Schuster, D. Klöß, C. Staubach, K. Teske, A. Vos, SURVIS: a fully-automated aerial baiting system for the distribution of vaccine baits for wildlife, Berl. Munch. Tierarztl. Wochenschr. 125 (2012) 197–202.

[19] A. Kappeler, Untersuchungen zur Altersbestimmung und zur Altersstruktur verschiedener Stichproben aus Rotfuchspopulationen (*Vulpes vulpes*) in der Schweiz, Lizentiatsarbeit, 1985.

[20] D.H. Johnston, D.G. Joachim, P. Bachmann, K. Kardong, R.B. Stewart, L. Dix, M. Strickland, I. Watt, Aging furbearers using tooth structure and biomarkers, in: M.J. Novak, J.A. Baker, M.E. Obbard, B. Malloch (Eds.), Wild furbearer management and conservation in North America, 1999, pp. 228–243.

[21] M. Wasniewski, I. Almeida, A. Baur, T. Bedekovic, D. Boncea, L.B. Chaves, D. David, P. de Benedictis, M. Dobrostana, P. Giraud, P. Hostnik, I. Jaceviciene, S. Kenklies, M. König, K. Mähar, M. Mojzis, S. Moore, S. Mrenoski, T. Müller, E. Ngoepe, M. Nishimura, T. Nokireki, N. Pejovic, M. Smreczak, B. Strandbygaard, E. Wodak, F. Cliquet, First international collaborative study to evaluate rabies antibody detection method for use in monitoring the effectiveness of oral vaccination programmes in fox and raccoon dog in Europe, J. Virol. Methods 238 (2016) 77–85, https://doi.org/10.1016/j.jviromet.2016.10.006.

[22] M. Wasniewski, A.L. Guiot, J.L. Schereffer, L. Tribout, K. Mähar, F. Cliquet, Evaluation of an ELISA to detect rabies antibodies in orally vaccinated foxes and raccoon dogs sampled in the field, J. Virol. Methods 187 (2013) 264–270, https://doi.org/10.1016/j.jviromet.2012.

[23] The direct fluorescent antibody test, in: C.E. Rupprecht, A.R. Fooks, B. Abela-Ridder (Eds.), Laboratory Techniques in Rabies, fifthth edition, World Health Organization, Geneva, 2018, pp. 108–130.

[24] B. Hoffmann, C.M. Freuling, P.R. Wakeley, T.B. Rasmussen, S. Leech, A.R. Fooks, M. Beer, T. Müller, Improved safety for molecular diagnosis of classical rabies viruses by use of a TaqMan real-time reverse transcription-PCR "double check" strategy, J. Clin. Microbiol. 48 (2010) 3970–3978, https://doi.org/10.1128/Jcm.00612-10.

[25] P.Y. Daoust, A.I. Wandeler, G.A. Casey, Cluster of rabies cases of probable bat origin among red foxes in Prince Edward Island, Canada, J. Wildl. Dis. 32 (1996) 403–406.

[26] N. Arechiga-Ceballos, A. Velasco-Villa, M. Shi, S. Flores-Chavez, B. BarroN, E. Cuevas-Dominguez, A. Gonzalez-Origel, A. Aguilar-Setien, New rabies virus variant found during an epizootic in white-nosed coatis from the Yucatan peninsula, Epidemiol. Infect. 138 (2010) 1586–1589, https://doi.org/10.1017/S0950268810000762.

[27] J. Blancou, M. Aubert, Transmission of the rabies virus: importance of the species barriers, Bull. Acad. Vet. Fr. 181 (1997) 301–312.

[28] J. Blancou, M. Aubert, J.P. Soulebot, Différences dans le pouvoir pathogène de souches de virus rabique adaptées au renard ou au chien, Annales de l'Institut Pasteur / Virologie 134 (1983) 523–531, https://doi.org/10.1016/S0769-2617(83)80024-0.

[29] B.A. Yakobson, R. King, S. Amir, N. Devers, N. Sheichat, D. Rutenberg, Z. Mildenberg, D. David, Rabies vaccination programme for red foxes (Vulpes vulpes) and golden jackals (Canis aureus) in Israel (1999-2004), Dev. Biol. (Basel) 125 (2006) 133–140.

[30] D.-K. Yang, H.-H. Kim, E.-J. Lee, J.-Y. Yoo, J.-T. Kim, S. Ahn, Rabies immune status of raccoon dogs residing in areas where rabies bait vaccine has been distributed, Clin Exp, Vaccine Res 8 (2019) 132–135, https://doi.org/10.7774/cevr.2019.8.2.132.

[31] A.A. Sultanov, S.K. Abdrakhmanov, A.M. Abdybekova, B.S. Karatayev, P. R. Torgerson, Rabies in Kazakhstan, PLoS Neglect Trop Dis 10 (2016) e0004889, https://doi.org/10.1371/journal.pntd.0004889.

[32] E. Hasanov, S. Zeynalova, M. Geleishvili, E. Maes, E. Tongren, E. Marshall, A. Banyard, L.M. McElhinney, A.M. Whatmore, A.R. Fooks, D.L. Horton, Assessing the impact of public education on a preventable zoonotic disease: rabies, Epidemiol. Infect. 146 (2018) 227–235, https://doi.org/10.1017/S0950268817002850.

[33] K. Johnson, The status of mammalian carnivores in Turkey, Endangered Species Update 19 (2002) 232–237.

[34] H. Ambarlı, A. Ertürk, A. Soyumert, Current status, distribution, and conservation of brown bear (Ursidae) andwild canids (gray wolf, golden jackal, and red fox; Canidae) in Turkey, Turk J Zool 40 (2016) 944–956, https://doi.org/10.3906/zoo-1507-51.

[35] T. Albayrak, G. Giannatos, Kabasakal B, carnivore and ungulate populations in the Beydaglari mountains (Antalya, Turkey): border region between Asia and Europe, Pol. J. Ecol. 60 (2012) 419–428.

[36] Y. Ünal, Yazilikaya'da av - yaban hayatı envanteri, Isparta, Turkey (in Turkish), 2011.

[37] World Health Organization, WHO expert consultation on rabies: third report, WHO Tech Rep Ser 1012 (2018) 195.

[38] S.A. Nadin-Davis, C. Fehlner-Gardiner, Origins of the arctic fox variant rabies viruses responsible for recent cases of the disease in southern Ontario, PLoS Neglect Trop Dis 13 (2019) e0007699, https://doi.org/10.1371/journal.pntd.0007699.

[39] F. Cliquet, C. Freuling, M. Smreczak, W.H.M. Van der Poel, D.L. Horton, A. R. Fooks, E. Robardet, E. Picard-Meyer, T. Müller, Development of harmonised schemes for monitoring and reporting of rabies in animals in the European Union, Scientific Report submitted to EFSA (Q-2010-00078), 2010.

[40] B. Yakobson, I. Goga, C.M. Freuling, A.R. Fooks, V. Gjinovci, B. Hulaj, D. Horton, N. Johnson, J. Muhaxhirif, I. Recica, D. David, R. O'Flaherty, N. Taylor, T. Wilsmore, T. Müller, Implementation and monitoring of oral rabies vaccination of foxes in Kosovo between 2010 and 2013—an international and intersectorial effort, international journal of medical microbiology, IJMM 304 (2014) 902–910, https://doi.org/10.1016/j.ijmm.2014.07.009.

[41] T. Müller, H.-J. Bätza, C. Freuling, A. Kliemt, J. Kliemt, R. Heuser, H. Schlüter, T. Selhorst, A. Vos, T.C. Mettenleiter, Elimination of terrestrial rabies in Germany using oral vaccination of foxes, Berl. Munch. Tierarztl. Wochenschr. 125 (2012) 178–190.

[42] R.M. Anderson, H.C. Jackson, R.M. May, A.M. Smith, Population dynamics of fox rabies in Europe, Nature 289 (1981) 765–771.

[43] P.G. Coleman, C. Dye, Immunization coverage required to prevent outbreaks of dog rabies, Vaccine 14 (1996) 185–186.

[44] L. Baker, J. Matthiopoulos, T. Müller, C. Freuling, K. Hampson, Local rabies transmission and regional spatial coupling in European foxes, PLoS One 15 (2020) e0220592, https://doi.org/10.1371/journal.pone.0220592.

[45] P. Nouvellet, C.A. Donnelly, M. de Nardi, C.J. Rhodes, P. de Benedictis, C. Citterio, F. Obber, M. Lorenzetto, M.D. Pozza, S. Cauchemez, G. Cattoli, Rabies and canine distemper virus epidemics in the red fox population of northern Italy (2006-2010), PLoS One 8 (2013) e61588, https://doi.org/10.1177/0300985812436743.

[46] K. Hampson, J. Dushoff, S. Cleaveland, D.T. Haydon, M. Kaare, C. Packer, A. Dobson, Transmission dynamics and prospects for the elimination of canine rabies, PLoS Biol. 7 (2009) e53, https://doi.org/10.1371/journal.pbio.1000053.

[47] H. Thulke, D. Eisinger, The strength of 70%: Revision of a standard threshold of rabies control, in: B. Dodet, A.R. Fooks, T. Müller, N. Tordo (Eds.), Towards the Elimination of Rabies in Eurasia, Karger, Basel, 2008, pp. 291–298.

[48] J.L. Kotzé, J. Duncan Grewar, A. Anderson, Modelling the factors affecting the probability for local rabies elimination by strategic control, PLoS Neglect Trop Dis 15 (2021) e0009236, https://doi.org/10.1371/journal.pntd.0009236.

[49] C.A. Hanlon, M. Niezgoda, P. Morrill, C.E. Rupprecht, Oral efficacy of an attenuated rabies virus vaccine in skunks and raccoons, J. Wildl. Dis. 38 (2002) 420–427.

[50] K. Stöhr, R. Gebauer, W. Herold, J. Hähn, W. Pietel, M. Kintscher, Fluoreszenzmikroskopischer Nachweis von Oxytetracyclin in Knochen von Füchsen, Monatsh. Veterinarmed. 45 (1990) 88–92.

[51] J. Bingham, R. Matema, A. Kappeler, F.W.G. Hill, Naturally-occurring tetracycline-like fluorescence in sections of femur from jackals in Zimbabwe, Vet. Rec. 135 (1994) 180–182.

[52] E. Robardet, F. Cliquet, Review of the analysis related to rabies diagnosis and follow-up of oral vaccination performed in NRLs in the EU, 2010, Rabies Bulletin Europe (2011) 11–14.

[53] C.E. Rupprecht, T. Buchanan, F. Cliquet, R. King, T. Müller, B. Yakobson, D.-K. Yang, A global perspective on Oral vaccination of wildlife against rabies, J. Wildl. Dis. (2024), https://doi.org/10.7589/JWD-D-23-00078.

[54] A. Vos, H. Un, K. Hampson, K. de Balogh, O. Aylan, C.M. Freuling, T. Müller, A. R. Fooks, N. Johnson, Bovine rabies in Turkey: patterns of infection and implications for costs and control: patterns of infection and implications for costs and control, Epidemiol. Infect. 142 (2014) 1925–1933, https://doi.org/10.1017/S0950268813002811.

[55] European Commission, Guidelines to design an EU co-financed programme on eradication and control of Rabies in wildlife, 2015. SANTE/10201/2015rev1.