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Emmanuelle Robardet, Anna Zdravkova, Darinka Ilieva, Enel Hakmann, Ioanna Georgopoulou, et al.. Retrospective analysis of sero-prevalence and bait uptake estimations in foxes after oral rabies vaccination programmes at European level: Lessons learned and paths forward. *Veterinary Microbiology*, 2024, 288, pp.109917. 10.1016/j.vetmic.2023.109917 . anses-04485072

HAL Id: anses-04485072

<https://anses.hal.science/anses-04485072>

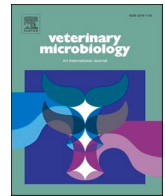
Submitted on 1 Mar 2024

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Retrospective analysis of sero-prevalence and bait uptake estimations in foxes after oral rabies vaccination programmes at European level: Lessons learned and paths forward

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ARTICLE INFO

Keywords:

Rabies
Red fox
Oral vaccination
Bait uptake
Seroconversion
Monitoring

ABSTRACT

Rabies caused by the Classical Rabies Virus (*Lyssavirus rabies* abbreviated RABV) in the European Union has been close to elimination mainly thanks to Oral Rabies Vaccination (ORV) campaigns targeting wildlife (primarily red foxes). ORV programmes co-financed by the European Commission include a monitoring-component to assess the effectiveness of the ORV campaigns at national level. This assessment is performed by a random collection of red foxes in the vaccinated areas with control of antibodies presence by serological analysis and control of bait uptake by detection of biomarkers (tetracycline incorporated into the baits) in the bones and teeth. ORV programmes aim to a vaccine coverage high enough to immunize (ideally) 70 % of the reservoir population to control the spread of the disease. European Union (EU) programmes that led to almost elimination of rabies on the territory have been traditionally found to have a bait uptake average of 70 % (EU countries; 2010–2020 period) while the seroconversion data showed an average level of 40 % (EU countries; 2010–2020 period). To better understand variations of these indicators, a study was been set up to evaluate the impact of several variables (linked to the vaccination programme itself and linked to environmental conditions) on the bait uptake and the seroconversion rate. Thus, pooling data from several countries provides more powerful statistics and the highest probability of detecting trends. Results of this study advocate the use of a single serological test across the EU since data variation due to the type of test used was higher than variations due to field factors, making the interpretation of monitoring results at EU level challenging. In addition, the results indicates a negative correlation between bait uptake and maximum temperatures reached during ORV campaigns questioning the potential

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<https://doi.org/10.1016/j.vetmic.2023.109917>

Received 1 August 2023; Received in revised form 9 November 2023; Accepted 17 November 2023

Available online 25 November 2023

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impact of climatic change and associated increase of temperatures on the ORV programmes efficiency. Several hypotheses requesting additional investigation are drawn and discussed in this paper.

1. Introduction

Rabies virus (*Lyssavirus rabies* abbreviated RABV) is a virus of the genus *Lyssavirus* of the *Rhabdoviridae* family and the most common causative agent of rabies disease (ICTV, 2023). Rabies is a viral neurotropic infection of mammals affecting the central nervous system. This zoonotic disease is transmitted mainly by direct contact via the saliva of an infected animal to a new host through damaged skin or mucous membranes, the most classical route of infection being the bite of an infected animal (WHO, 2018). Moreover, the disease is almost always fatal as soon as its clinical symptoms (abnormal behaviour, hallucinations, insomnia, paralysis, etc.) appears. Rabies virus (RABV) has been found on all continents except in Antarctica but nowadays affects almost exclusively people from developing countries. The post-exposure prophylaxis (PEP), if administered rapidly, is effective, but still an estimated 60,000 people die every year due to lack of PEP access (Hampson et al., 2015). To eliminate the disease, various studies have shown that addressing the source of human infection by eliminating rabies from the animal reservoir is most cost-effective than to achieve human post-exposure treatments only (Shwiff et al., 2013; Mindekem et al., 2017). Among different proposed control strategies, mass vaccination of reservoir species has been shown to be the most efficient way of reducing the disease incidence (WHO, 2018).

In Europe, rabies caused by the rabies virus (RABV) has a sylvatic form since a fox adapted variant appeared in the late 1930s (Anderson et al., 1981). This sylvatic wave spread westwards and southwards from the Russian–Polish border to the rest of Europe with a 20–60 km/year speed to reach its maximum westward expansion in the 1970s (Aubert, 1992). The maximum of recorded cases was reached in 1980s (Freuling et al., 2013). More than eighty percent of reported cases were detected in red foxes (*Vulpes vulpes*) devoting this species to be considered as the main reservoir as well as the main vector of rabies in the European continent. To immunize red foxes against this infection, an original method of oral rabies vaccination (ORV) was developed experimentally in the 1970s in the USA using attenuated rabies virus introduced in palatable baits (Black and Lawson, 1973; Baer et al., 1971). The first ORV field trial was conducted in 1978 in Switzerland (Steck et al., 1982) and was followed by a succession of field trials in the 1980s in neighbouring countries (Aubert et al., 1994).

This unique wildlife vaccination technology has proven to be effective, the maximum efficacy being reached when vaccinating entire infected areas over long periods (Baker et al., 2019; Robardet et al., 2016). Thus, by conducting regular ORV campaigns, covering large areas, it became possible to drastically reduce the number of rabies cases in Western and Central Europe to reach as low as less than 10 cases per year at the approach of 2020 (Robardet et al., 2019). Since 1990, the European Union (EU) has been co-financing ORV programmes to eliminate this disease from EU member states. The ORV programmes are harmonised and based on scientific standardised guidelines (European Commission, 2002, 2015; EFSA AHAW Panel, 2015). Typically, the ORV campaign is performed twice a year, in spring and in autumn, using fixed-wing aircraft with a bait density of 20–30 baits per km². Baits are distributed in a regular pattern with no more than 500 m between flight lines.

Confirmation of the ORV programme successful implementation, based on field data is one of the components of so-called “EC eradication co-financed programmes”, programmes set up to eliminate rabies from EC territory. While oral vaccine batches are released by Official Control Authority Batch Release (OCABR) before being used in the field, the achievement of ORV campaigns is confirmed principally through 3 control measures. These control measures are the rabies surveillance to

assess the epidemiological trend of the disease (European Commission, 2002, 2015), assessment of the vaccine bait distribution, including coverage and vaccine bait density, by analysing bait’s geolocations (European Commission, 2015; EFSA AHAW Panel, 2015), and the “ORV monitoring” (seroconversion and bait uptake occurrence in the targeted population) to evaluate the effectiveness of the vaccination in the field (European Commission, 2002, 2015).

Regarding ORV monitoring, measuring the proportion of seroconverted animals in the target population is a common way for evaluating the success of the implementation of mass vaccination campaigns. Rabies antibodies titer peak is reached approximately 2–4 weeks after the vaccination (Shiraishi et al., 2014; Sugiyama et al., 1997). However, collecting blood samples from hunted animals in the field is often technically difficult, resulting most of the time in the collection of thoracic fluids rather than proper blood samples (Bedekovic et al., 2016; Wasniewski et al., 2014a). For all these reasons, evaluation of seroconversion after ORV does not always reflect the accurate proportion of vaccinated animals. Nevertheless, if assessed regularly and based on the same protocol, seroconversion evaluations can provide a valuable relative indicator of vaccination that provides trends of ORV outcomes. Follow-up of seroconversion in vaccinated areas is an indicator of ORV effectiveness. It allows to assess if sufficient individuals, by reference to the target of 70 % vaccinated population to eliminate rabies, have consumed the oral vaccine and produced enough quantity of neutralising antibodies.

As recommended by international institutions and depending of national regulation in countries, oral rabies baits contain a biomarker to allow bait uptake evaluation after ORV campaigns (WHO, 2018; EFSA AHAW Panel, 2015). In the EU, all oral vaccines used the last ten years (Lysvulpen® (SAD Bern-SAD B19 “like” strain) from Bioveta, Rabigen® (SAG2 strain) from Virbac, Fuchsoral® (SAD B19 strain) and Rabitec® (SPBN GASGAS) from CEVA) include tetracycline in their composition. Tetracyclines are a broad-spectrum antibiotic commonly used as biomarker of oral rabies vaccines bait uptake. After its consumption, its molecules are incorporated into the bones and teeth permanently and can be detected using epi-fluorescence microscopy (Brochier et al., 1991). It thus allow estimating the proportion of individuals having consumed a bait after a vaccination campaign.

Thus, after each ORV campaign, a random sampling (hunting) is carried out on the target species aiming to sample 4 individuals per km². This sampling has been recommended to be carried out, ideally, one month after the vaccination campaign, which is the best period for detecting the seroconversion (Cliquet et al., 2010).

The purpose of this study was to assess the impact of different factors (biological and environmental) on both seroconversion and bait uptake rates in foxes collected after ORV campaigns performed in the EU between 2010 and 2020.

2. Material and method

2.1. Data collection

Eight countries from Central and Eastern Europe implementing national EU co-financed ORV programmes conducted between 2010 and 2020 volunteered to participate in the study: Bulgaria, Croatia, Estonia, Finland, Greece, Latvia, Lithuania, and Romania (Fig. 1). Thus, gathering data from several countries made it possible to provide more powerful statistics and the highest probability to detect tendencies. The collection of harmonised ORV and ecological data was performed in 2021 using purpose-designed questionnaires. All countries followed a standardised programme of ORV i.e. two oral rabies vaccination

campaigns performed per year (except one campaign per year performed in Finland), by aerial distribution, one in autumn and one in spring with a density of 20–25 baits per km² (European Commission, 2002, 2015). All participating countries, except Croatia and Greece which respectively initiated ORV in 2011 and in 2013, were involved in ORV programmes before 2010, which is the start date of the study period. Foxes analysed during the study were collected in the framework of ORV national veterinary programmes conducted within the EU, for the eradication, control and surveillance of rabies National Veterinary Programmes (europa.eu), meaning these foxes were collected by hunting, randomly, within the vaccinated area with a target of 4 foxes per 100 km² per campaign. Number of foxes finally collected and analysed per country and per year varied from 0.11 to 4 foxes per 100 km² (mean = 0.95, standard deviation = 0.76) for serological samples, and varied from 0.13 to 5 foxes per 100 km² (mean = 1.21, standard deviation = 0.93) for mandible samples. Hunted foxes in the vaccination areas were used for the evaluation of the vaccination sanitary programme developed and launched by the ministry of each country. These sampling processes were realised in compliance with the legislation of each country and under the recommendations of international institution (WOAH (WHO, 2018), EFSA (EFSA AHAW Panel, 2015)). The programmes and sampling of foxes were achieved and co-financed according to the Regulation (EU) No 652/2014 (The European Parliament and of the Council, 2014).

2.2. Studied factors

Tetracycline biomarking and seroconversion can be considered as permanent marking (apart from primary vaccination for serology) and the life span of foxes in nature is generally considered to be globally 2–4 years (Meia, 1996). To limit the impact of such long marking on the multivariable analysis, seroconversion and bait uptake data were not considered by campaign (autumn and spring) but by year.

The responses variables considered were the number of blood /fluid samples of foxes with and without evidence of neutralising antibodies up to a threshold level (i.e. seropositive and seronegative samples) in a first

modelling and the number of foxes 'mandibles with and without evidence of biomarker (Tetracycline) in a second one. The technique used to detect the tetracycline biomarker in teeth and bones of fox mandibles is based on an harmonised procedure shared within the EU network by the European Union Reference Laboratory for Rabies. Since 2011, proficiency testings to evaluate laboratories performances on tetracycline detection are regularly organised (Robardet et al., 2012), they ensure an EU analysis capacity with comparable performances between countries and over time.

To be able to detect reasonable-size effects with reasonable power, enough observations per parameter estimated are needed (Harrell, 2015). Because our database counted 79 observations, and to get a ratio of 12 observation per covariate analysis was performed by selecting seven responses variables hypothesised as the most potentially affecting the explanatory variables.

The seven explanatory variables assessed were the following:

- Type of oral vaccine used (Fuchsoral; Lysvulpen; Rabigen; Rabitec) named "vaccine_used".
- Size of the vaccinated area (continuous variable) /10000 named "areascald".
- Bait density (number of baits dropped within the year/total km² vaccinated within the year) named "density".
- Indicator for red fox population abundances (n hunting bags of national level data (number of foxes killed during hunting season in the country)/area of the country x10) named "fox_pop". One country provided snow-tracking indexes for red fox population relative densities. This data was removed from the analysis as snow tracking and hunting bags are not comparable indicators.
- Indicator for wild boar population abundances (n hunting bags of national level data/area of the country x10) named "wildboar_pop". Although the vaccine baits are intended for foxes, different non-target wild animals can also consume them. Among wild animals involved in vaccine bait consumption, the most majority in Europe are wild boars (Vengušt et al., 2011; Dascalu et al., 2019). Index of

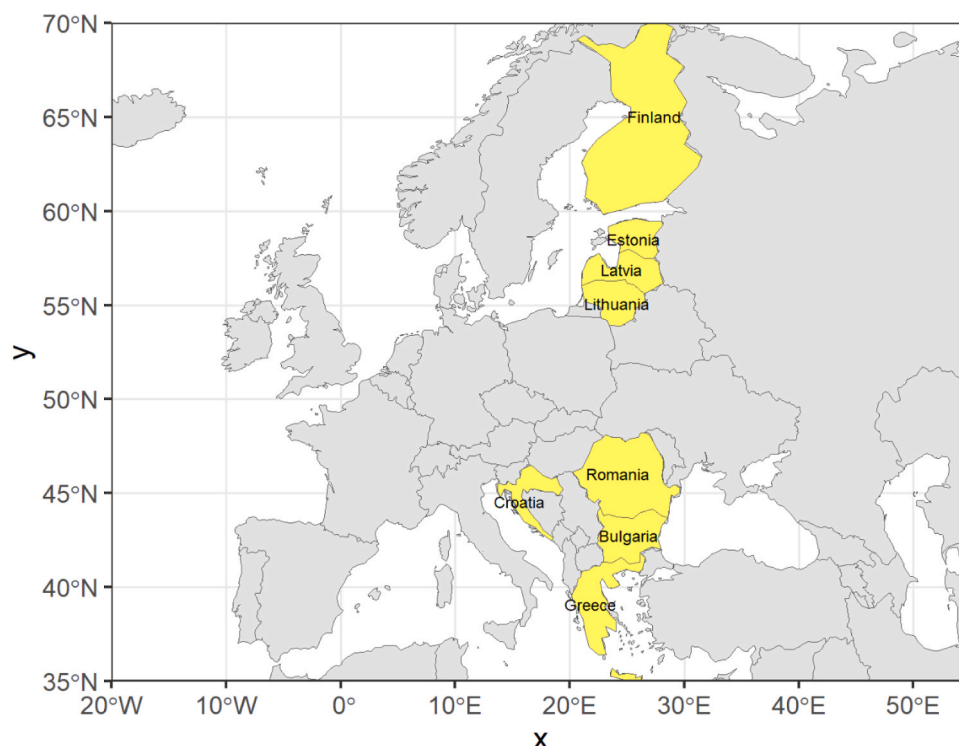


Fig. 1. Geographical distribution of countries involved in the present study. The eight participating countries are indicated in yellow colour.

abundance of this species has consequently been included in the modelling.

- Maximum temperature (regional meteorological data) observed during ORV campaigns, from Day 1 of the ORV to one month after the start (continuous variable) named “Tmax”. In EU programmes, red foxes sampling is performed almost one month after the ORV programmes. Considering this sampling scheme, baits considered in the study (and consumed or not by foxes analysed), were exposed to meteorological conditions maximum 1 month before their consumption by sampled foxes. One month post vaccination was consequently set up as the duration for temperature monitoring.
- Type of serological test used (for the analysis of serological samples only) (ELISA Bio-Rad with cut-off 0.3 EU/mL, ELISA Bio-Rad with cut-off 0.5 EU/mL, ELISA BioPro 40 %PB, FAVN, RFFIT) named “sero_type”.

Some factors for which data were collected were not considered in the analysis. The proportion of compliant titers in vaccine batches was not taken into account as 100 % of vaccine batches tested fulfilled the titer criteria. The adequacy of bait densities (homogeneity of the distribution within the vaccinated area) was not considered in the analysis as 97 % of responses indicated that bait distribution was evaluated by GPS analysis and considered adequate. Blood sample quality was also not included as an explanatory factor because no specific trends in the evolution of blood sample quality during the study period were highlighted by respondents. This factor was also considered to be too subjective to allow a proper and harmonised use in the dataset. The variable “Year” was not considered in the analysis as this correlated with the variable “Tmax”. The age of foxes (adult or juvenile) was not considered in the study as all the participating countries couldn't provide age category of foxes sampled.

2.3. Statistical analysis

Statistical analyses were carried out using R software (R Core Team, 2021). Generalised linear models (GLMs) including the matrix “number of positive samples and number of negative samples” as a response variable were used to study the variations in vaccination coverage after ORV in red fox populations. Since Generalised linear mixed models (GLMMs) in which the linear predictor contains the variable ‘country’ or ‘year’ as random effects did not converge only GLM were used. Both seroconversion rate (samples positive for rabies antibody content) and bait uptake (samples positive for tetracycline in bones and teeth) were analysed using logistic regressions. The explanatory variables included the factors are presented above.

For each GLM analysis, models of all possible combinations of variables were compared using the information-theoretic method outlined by Burnham and Anderson (Burnham and Anderson, 2002). Differences in the Akaike second-order information criterion (AICc) between the best model and all other considered models were calculated to determine the relative ranking of each possible model. The model with the lowest AICc represented the best compromise between residual deviance and the number of variables. When Δ_i was below two (Δ_i = difference between AICc and the lowest AICc value), the most parsimonious model (i.e. the one with the fewest variables) was selected. The goodness of fit of the selected model was assessed with graphical exploration (normality, homoscedasticity and linearity of residuals) (Davison and Snell, 1991). Over-dispersion in models was assessed by analysing standardised GLM residuals using DHARMA package (Hartig, 2022). When over-dispersion was identified, an ad hoc Williams correction was performed to minimize Type 1 error (Williams, 1982). Over-dispersed binomial logit models were consequently estimated using a quasi-binomial distribution (quasi-likelihood approach). The odds ratio of each variable and its confidence interval were computed by calculating the exponential of the corresponding coefficient parameter.

3. Results

3.1. Type of serological test used identified as the only variable explaining proportions of seropositive foxes

The mean seroprevalence observed during the study period (Fig. 2) was 0.41 (standard deviation= 0.16). A first graphical exploration was assessed by considering the proportion of positive serological samples in relation to all the explanatory variables considered in this study. The results are presented in Fig. 3. A comparison of models with combinations (subsets) of fixed-effect terms in the global model finally selected the model that included the variables “sero_type”, “vaccine_used”, “wildboar_pop”, “fox_pop”, and “areascalded” as the best model to explain the variations of seropositive samples frequency (Table 1). As over-dispersion was detected, an over-dispersed binomial logit model was used. An evaluation of model fitting indicated that data fit the model well. Estimated parameters indicated that the serological test used significantly impact the evaluated proportion of positive serological samples (Fig. 4). The Bio-Rad ELISA kit using a cut-off of 0.5 was found to be significantly associated with lower seroconversion rates compared with seroconversion rates using the BioPro kits (used with 40 %BP). No difference were detected between the Bio-Rad used with a cut-off of 0.5 EU/mL and the Bio-Rad used with a cut-off of 0.3 EU/mL. As well, no difference were detected between the the BioPro kits (used with 40 %BP) and the Bio-Rad kit used with a cut-off of 0.3 EU/mL.

3.2. Maximum temperatures reached during ORV programmes found negatively associated with proportions of foxes presenting bait uptake

The mean proportion of foxes positive for bait uptake marker during the study period (Fig. 1) was 0.71 (sd= 0.17). A first graphical exploration was assessed by considering the proportion of bait uptake in relation to the explanatory variables considered in this study. The results are presented in Fig. 5. A comparison of models with combinations (subsets) of fixed-effect terms in the global model finally selected the model that included all the variables meaning “Tmax”, “vaccine_used”, “wildboar_pop”, “fox_pop”, “density” and “areascalded” as the best model to explain the frequency of variations in bait uptake (Table 2). As over-dispersion was detected, an over-dispersed binomial logit model was used. An evaluation of model fitting indicated that data fit the model well. Except for maximum temperatures (variable “Tmax”), none of the factors considered in this analysis were detected to significantly affect the results. The maximum recorded temperature during ORV campaigns showed a potential significant negative impact on the proportion of bait uptake (Fig. 6). The higher the temperature, the lower the proportion of bait uptake.

3.3. Impact of other factors (vaccine used, red fox and wild boar population indicators)

The efficacy of vaccines in protecting against a fatal rabies infection must be demonstrated in experimental studies in a significant proportion of vaccinated animals prior a marketing authorisation is granted (European Pharmacopoeia, 2014). In the EU, four different oral vaccines have been used in ORV programmes since 2010: Lysvulpen and Fuchsoral containing SAD Bern and SAD B19 strains, and Rabigen and Rabitec containing SAG 2 and SPBN GASGAS strains, respectively. All these are licensed biological products and approved with respect to safety and efficacy criteria. In the present study, four types of bait were used (Fuchsoral, Rabigen, Rabitec, Lyssvulpen) over a ten-year period. No significant differences in bait uptake and seroconversion rates associated with the specific use of an oral vaccine were detected. In our study, the relative abundances of red foxes and wild boars do not seem to have an impact on either the seroconversion rate or bait uptake. However, due to missing data on red fox population, further investigations would be needed to exclude their impact on ORV efficiency.

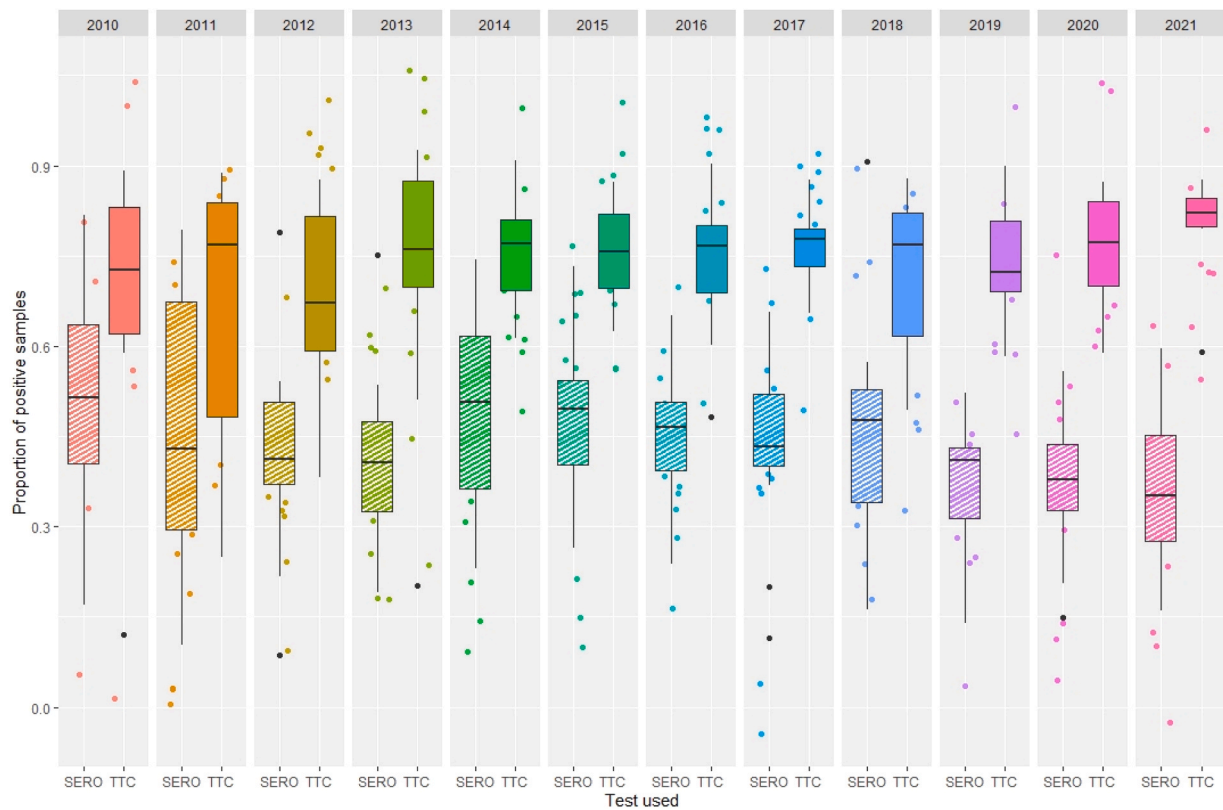


Fig. 2. Distribution and related box plots of the annual seroprevalence named “SERO” (hatched bar) and annual bait uptake estimation named “TTC” (open bars) of foxes collected after ORV in the eight participating countries and over the study period.

4. Discussion

4.1. Variety of serological tests used for seroconversion evaluation challenge ORV data interpretation at EU level

Both cell-mediated and humoral immune system responses are important in combatting rabies infection (Dietzschold et al., 2008) but the humoral response receives greater attention with regard to rabies prevention. Neutralising antibodies are indeed recognised as a reliable and easily measurable parameter for assessing the efficacy of vaccination as they are closely linked to the protection against infection. To date, several tests are available to assess their level in blood samples. The type of test used is known to influence the probability of detecting antibodies as each test varies in sensitivity and specificity. Based on laboratory evaluation and Bayesian modelling, serum neutralisation tests such as the FAVN test and RFFIT have been shown to vary within a range of 82–91 % for sensitivity (Se) and 94–100 % for specificity (Sp) (Smith et al., 1973; Cliquet et al., 1998; Crozet et al., 2023).

In previous studies, some ELISAs have been shown to give a comparable performance (≈ 95 % of concordance) to the FAVN test (Cliquet et al., 2000; Wasniewski et al., 2013; Wasniewski and Cliquet, 2012), however, ELISA performances have also been proven in later a stage to be highly variable according to the type of ELISA kit used (Wasniewski et al., 2014b; Knoop et al., 2010; De Benedictis et al., 2012).

Several more recent studies have shown that the BioPro kit allows a good assessment of ORV campaigns efficiency in target species even from poor quality samples (body fluids rather than blood samples) that are quite common in wildlife sampling performed after ORV campaigns (Bedekovic et al., 2016; Wasniewski et al., 2013). It has been suggested that, when used for research purposes, the threshold for the Bio-Rad ELISA — that was found to be less sensitive — should be set at 0.3 EU/mL instead of 0.5 EU/mL to increase the limit of positivity and therefore its sensitivity (Wasniewski et al., 2013).

For many years, ELISA kits have been routinely used to monitor the effectiveness of ORV campaigns targeting red foxes at national levels. In 2020, for example, 85 % of the countries involved in ORV campaigns used commercial ELISA kits to assess seroconversion rate in ORV target populations (Robardet and Cliquet, 2021). However, when using these kits, although all the laboratories used the same reagents and followed the instructions provided with the kits, a recent inter-laboratory study highlighted variations in the results obtained, indicating that the use of ELISAs is not well standardised throughout the EU NRLs (Wasniewski and Cliquet, 2020). This study also clearly demonstrates that the limit of detection obtained with the BioPro ELISA kit was lower than that obtained with the Bio-Rad ELISA kit, this difference being certainly due to the different threshold of positivity recommended by the manufacturers for the two tests (i.e. 40 % percentage of blocking (PB) vs 0.5 EU/mL). Such findings have also been observed in a field study where decreasing the threshold values of the Bio-Rad ELISA kit to 0.125 EU/mL significantly impacted the seroconversion assessment, providing higher estimates than those obtained when using BioPro kits (Robardet et al., 2016).

In the present study, at least four different types of tests were used on thoracic body fluids, including the Bio-Rad ELISA kit with two different threshold values (0.5 EU/mL and 0.3 EU/mL). The Bio-Rad ELISA kit using a cut-off of 0.5 was found to be significantly associated with lower seroconversion rate compared to BioPro kit. No difference were detected between the Bio-Rad used with a cut-off of 0.5 EU/mL and the Bio-Rad used with a cut-off of 0.3 EU/mL. The variety of tests used associated with variation in sensitivity and specificity and the different threshold value used for the same kit undoubtedly makes the comparison of ORV results between countries difficult to assess. To allow pooling and to facilitate use and interpretation of seroconversion data at regional/EU level, a single serological test with a common threshold value would be recommended to be used. It should be noted that the significant effect of the factor ‘Type of test used’ could be hiding the effects of other factors,

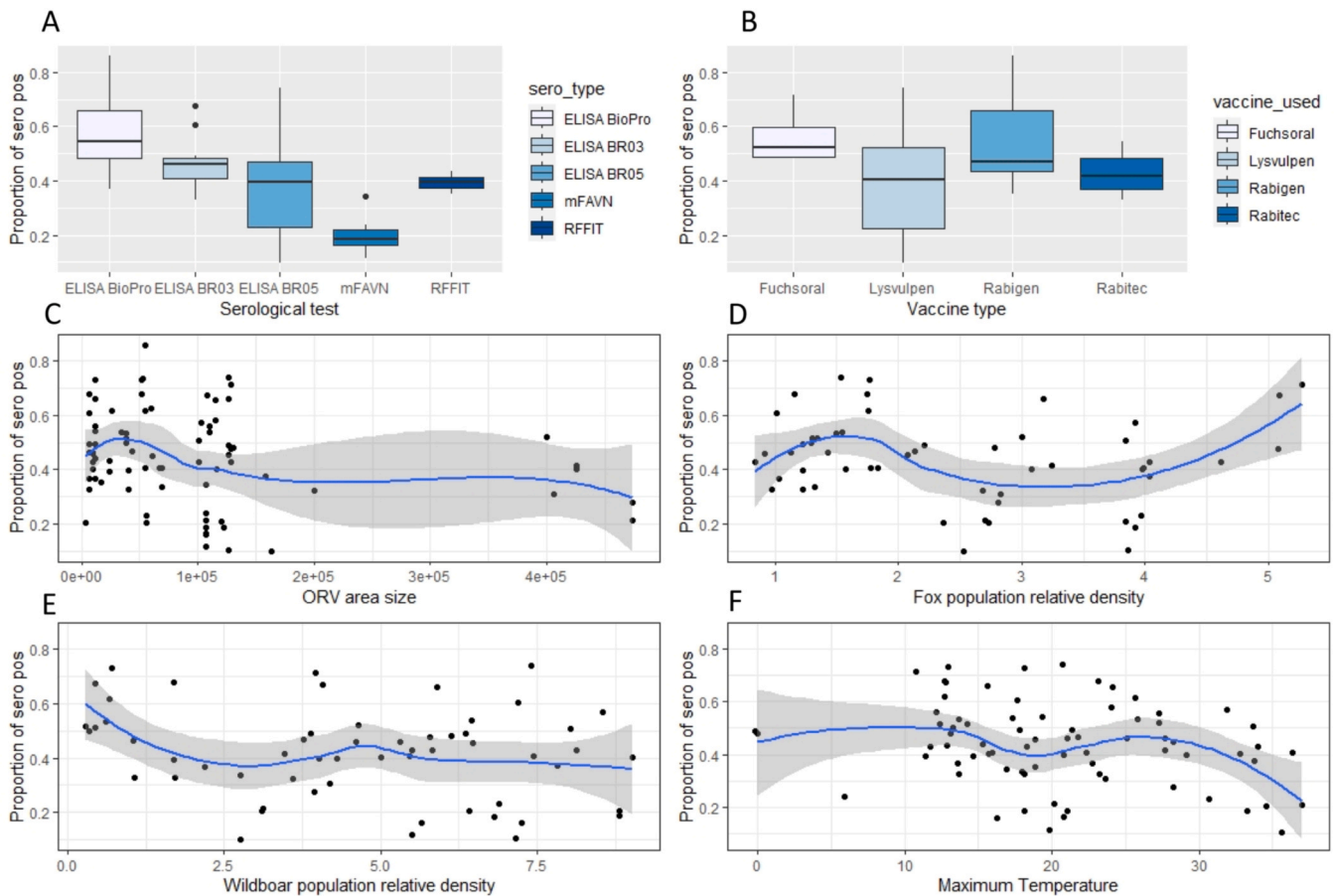


Fig. 3. Proportions of positive serological samples in relation to the different explanatory variables investigated in the study. All the data were collected annually over the period 2010–2020 and per participating country. Graph A describes the proportion of positive serological samples observed according to the type of serological test used during the ORV programmes. Graph B describes the proportion of positive serological samples observed according to the type of vaccine used during the ORV programmes. Graph C describes the proportion of positive serological samples observed according to the area size of the ORV programmes. Graph D and E describe the proportion of positive serological samples observed during the ORV programmes according to the number of hunting bags for 10 km² (used as indicator of red fox population abundances) for foxes and wild board respectively. Graph F describes the proportion of positive serological samples observed in ORV programmes according to maximum temperature recorded during the period of the programme. NB: FAVN, RFFIT and ELISA BR03 tests are each represented by only one participating country.

Table 1

AICc-based comparison of the statistical models used to explain variability of the proportion of seropositive foxes sampled after ORV programmes. Only the 10 most parsimonious models classified in decreasing order of their Akaike weights are shown. The selected model is shown in bold.

Model id	areascaled	density	fox_pop	sero_type	Tmax	vaccine_used	wildboar_pop	df	logLik	AICc	delta	weight
110	-0.0136		-0.1427	+		+	0.0624	9	-748.11	1518.8	0.00	0.470
112	-0.0139	-0.0034	-0.1434	+		+	0.0619	10	-746.83	1519.4	0.60	0.348
126	-0.0134		-0.1423	+	-0.0008	+	0.0638	10	-748.03	1521.9	3.02	0.104
128	-0.0136	-0.0036	-0.1430	+	-0.0013	+	0.0640	11	-746.64	1522.4	3.57	0.079
62	-0.0166		-0.0863	+	0.0069	+		9	-781.94	1586.5	67.66	0
64	-0.0167	-0.0033	-0.0867	+	0.0065	+		10	-780.76	1587.3	68.47	0
48	-0.0153	-0.0043	-0.0727	+		+		9	-786.46	1595.5	76.69	0
46	-0.0150		-0.0710	+		+		8	-788.53	1596.7	77.81	0
96	-0.0118	-0.0075	-0.1157	+	-0.0153		0.0826	8	-802.67	1624.9	106.10	0
106	-0.0155			+		+	0.0176	8	-805.62	1630.8	112.00	0

model id: model number; areascaled: size of ORV; density: bait density; fox_pop: relative index of fox densities; sero_type: type of serological test used; Tmax: maximum temperature that occurred during the ORV program; vaccine_used: type of vaccine used; wildboar_pop: relative index of wildboar densities. df: number of parameters in the model; logLik: Log-likelihood; AICc: bias corrected Akaike’s Information Criterion for fitted models; delta: (AIC c) i -(AIC c) min; weight = Akaike weights.

with less variability but just as important to consider for the successful monitoring and completion of ORV programmes.

4.2. What potential impact of climatic change and associated increase of temperatures on the ORV programmes efficiency?

In recent decades, the impact of climate change on health, ecological and economic systems has led to many debates and recommendations (Pörtner et al., 2022). Higher temperatures in a system used to certain

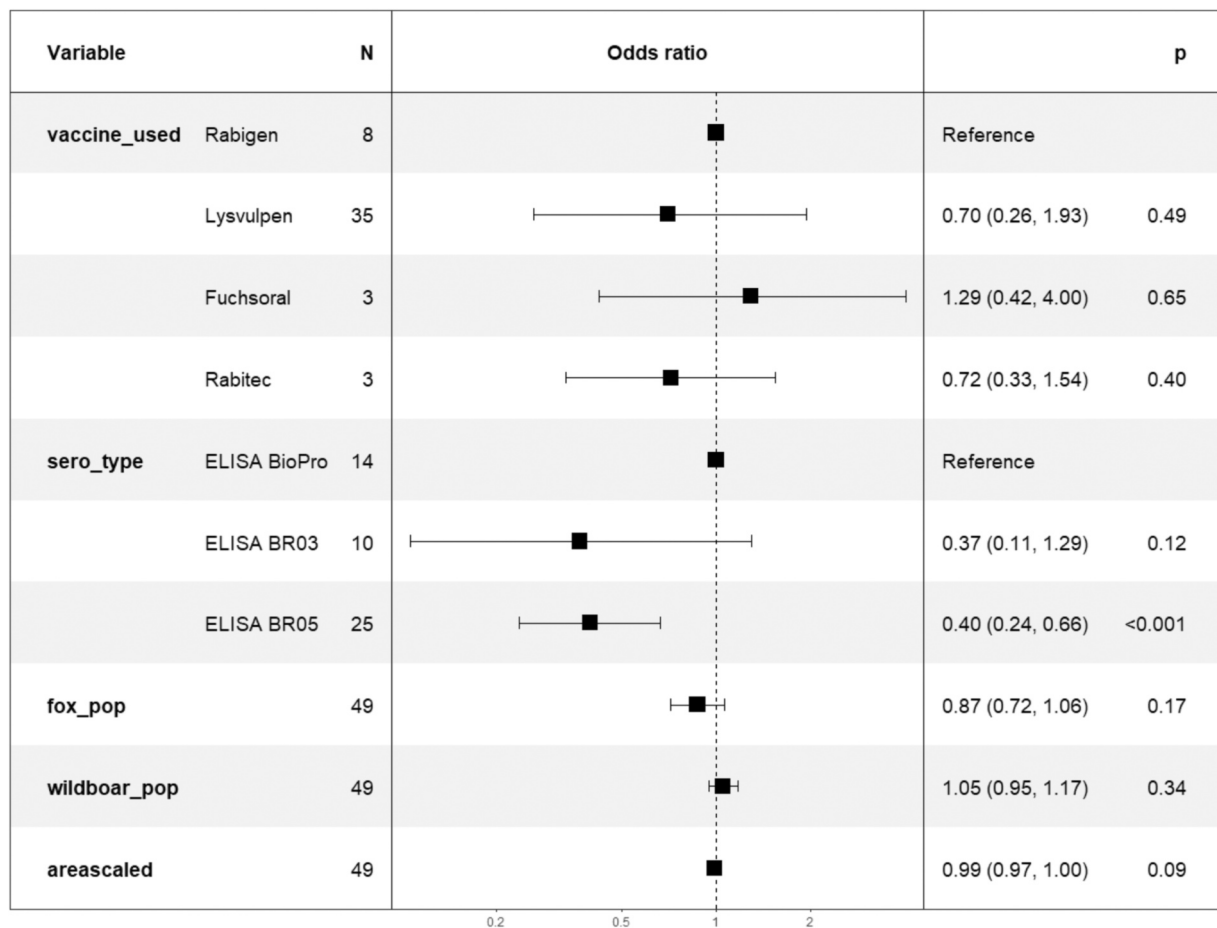


Fig. 4. Parameter estimates of the best model selected to explain variations in the proportion of foxes with an immune response after ORV programmes. All the data were collected per year over and per participating country. “vaccine_used” is the type of vaccine used during the ORV programme. “sero_type” is the type of elisa test used during the ORV programme. ‘ELISA BR03’ is Bio-Rad kit used with 0.3 as the cut-off value. ‘ELISA BR05’ is Bio-Rad kit used with 0.5 as the cut-off value. “fox_pop” is the number of hunting fox bags during the ORV programme for 10 km² (used as indicator of red fox population abundances). “wildboar_pop” is the number of hunting wildboar bags observed during the ORV programme for 10 km² (used as indicator of wildboar population abundances). “areascaled” is indicator of the size of the vaccinated area. Odds ratio > 1 reflects a tendency to positively impact the seroprevalences; Odds ratio < 1 reflects a tendency to negatively impact the seroprevalences.

‘traditional’ temperature values could indeed bring forward issues that were previously unknown. The overall increase in temperatures throughout Europe, which may be accompanied locally by a greater frequency of extreme temperatures, could have a negative impact on medicinal products affected by environmental conditions such as oral baits distributed in the field. In late spring 2022, for example, Europe experienced local temperatures over 30 °C (measures taken in the shade) which are temperatures that are expected in the summer, a season when ORV campaigns are not usually carried out, partly because of these high temperatures. In our study, we included extremely high temperatures observed during ORV campaigns to assess their impact on seroconversion and bait uptake. The result showed a significant relationship with the bait uptake rate but not with the seroconversion rate. As seroconversion variations are ‘masked’ by the serological tests used, it makes a sense to find differences in bait uptake but not in serological results. Extreme temperatures could indeed compromise ORV success by inducing the melting of the bait matrix therefore leading to a reduced palatability and odor quality (even if the matrix is re-solidified when temperature decrease) and, a consequent decrease in vaccine uptake. The bait type is known to affect bait uptake (Matter et al., 1995; Bonwitt et al., 2020; Koepfel et al., 2020). Coating formulas used in the market for bait casing are based on several oils (Palm, coconut, paraffin, beef tallow, etc.) characterised by a specific melting points, and their assemblage variations consequently induce different melting point of

matrixes. With regard to oral vaccination of wildlife, bait casing is indeed a key component as it determines the access to the vaccine and consequently the success of the vaccination. The bait casing should be palatable and attractive for the target species and, as much as possible, selective for the species of interest to avoid bait access competition with other species. The bait also acts as a physical barrier to protect the vaccine from ultraviolet rays from the sun that could impact the stability of the virus vaccine included in the baits. Out of the matrix, vaccine titer itself could also be affected by high temperatures. Also, high temperatures could impact activity of wildlife and reduce consumption probability. All this hypotheses could deserve further attention.

4.3. Considering red fox and wild boar abundances in ORV programmes organisation

Across Europe, several studies have reported the steady growth of wild boar populations since the 1980s (Bieber and Ruf, 2005; Keuling et al., 2013; Massei et al., 2015). While the wild boar is a non-target species of ORV, it remains a potential competitor for the consumption of oral baits due to its omnivorous diet and the size of the animal (and consequently the size of the mouth) adapted to the size of the bait. Anti-rabies antibody surveys in wild boar populations from vaccinated areas have indeed found a non-negligible proportion of positive samples in different countries (Dascalu et al., 2019; Cerovšek et al., 2011). A

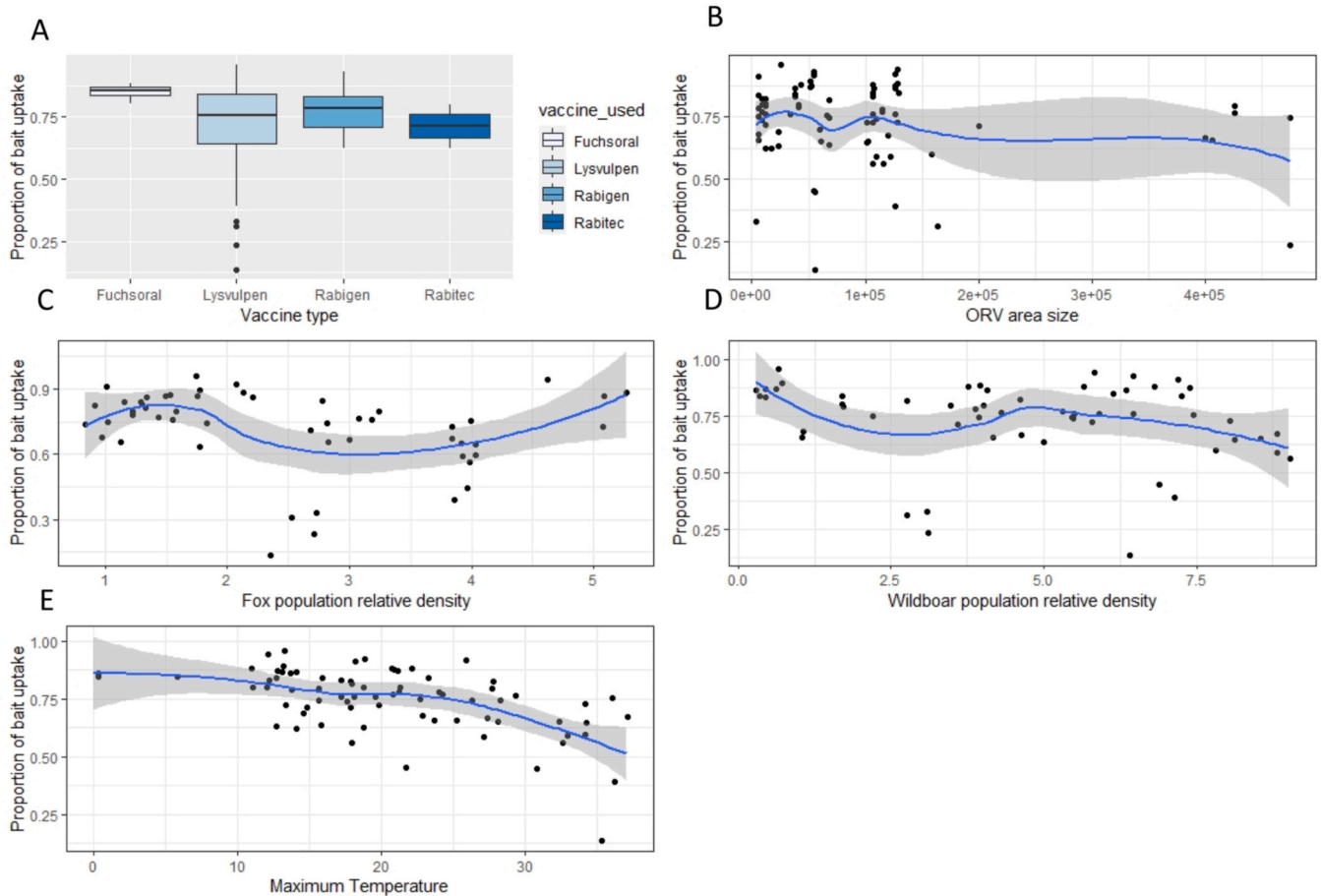


Fig. 5. Proportions of positive bait uptake in relation to the different explanatory variables investigated in the study. All the data were collected per year over the period 2010–2020 and per participating country. Graph A describes the proportion of bait uptake observed according to the type of vaccine used during the ORV programme. Graph B describes the proportion of bait uptake observed according to the size of the ORV programme. Graph C and D describe the proportion of bait uptake observed according to the number of hunting bags during the ORV programme for 10 km² (used as indicator of red fox population abundances) for foxes and wild board respectively “. Graph E describes the proportion of bait uptake observed in ORV programmes according to maximum temperature recorded during the period of the programme.

Table 2

AICc-based comparison of the statistical models used to explain variability of the proportion of foxes with bait uptake after ORV programmes. Only the 10 most parsimonious models classified in decreasing order of their Akaike weights are shown. The selected model is shown in bold.

model id	areascaled	density	fox_pop	Tmax	vaccine_used	wildboar_pop	df	logLik	AICc	delta	weight
64	-0.0020	-0.0251	0.0562	-0.0315	+	-0.0906	9	-1356.55	2735.7	0.00	0.809
63		-0.0247	0.0543	-0.0349	+	-0.0786	8	-1359.51	2738.6	2.89	0.190
60	-0.0018	-0.0238		-0.0284	+	-0.0848	8	-1366.05	2751.7	15.98	0
59		-0.0234		-0.0315	+	-0.0743	7	-1368.40	2753.5	17.80	0
48	-0.0024	-0.0270	0.0573	-0.0337		-0.0881	6	-1400.02	2814.0	78.32	0
47		-0.0264	0.0467	-0.0365		-0.0753	5	-1405.50	2822.4	86.67	0
44	-0.0015	-0.0258		-0.0330		-0.0760	5	-1411.26	2833.9	98.19	0
43		-0.0256		-0.0349		-0.0688	4	-1413.59	2836.1	100.37	0
32	0.0048	-0.0217	0.0267	-0.0467	+		8	-1434.58	2888.8	153.03	0
28	0.0047	-0.0211		-0.0446	+		7	-1436.72	2890.2	154.45	0

model id: model number; areascaled: size of ORV; density: bait density; fox_pop: relative index of fox densities; Tmax: maximum temperature that occurred during the ORV program; vaccine_used: type of vaccine used; wildboar_pop: relative index of wildboar densities. df: number of parameters in the model; logLik: Log-likelihood; AICc: bias corrected Akaike’s Information Criterion for fitted models; delta: (AIC c) i -(AIC c) min; weight = Akaike weights.

large wild boar population could therefore jeopardise the effectiveness of ORV campaigns by reducing the availability of baits for red foxes (Musto et al., 2022). Interestingly, the evolution of wild boar abundances in our dataset did not support the continuous increase in this population. Although considered to be good indicators of population size, hunting data may be biased, for example by the number of hunters (which is known to be decreasing in Europe), or by any particular management campaign which would bias the collection pressure on

populations (Husek et al., 2021). By example, African swine Fever is or has been present in some participating countries (all countries excepted Croatia and Greece) (Sauter-Louis et al., 2021). In this context, increased hunting efforts could have been set up as a mean to limit the spread of the disease, modifying substantially the figures of hunting bags that have been considered here as indicators for population abundances.

Over several decades, Western Europe has also experienced an increase in red fox populations followed by the emergence of urban fox

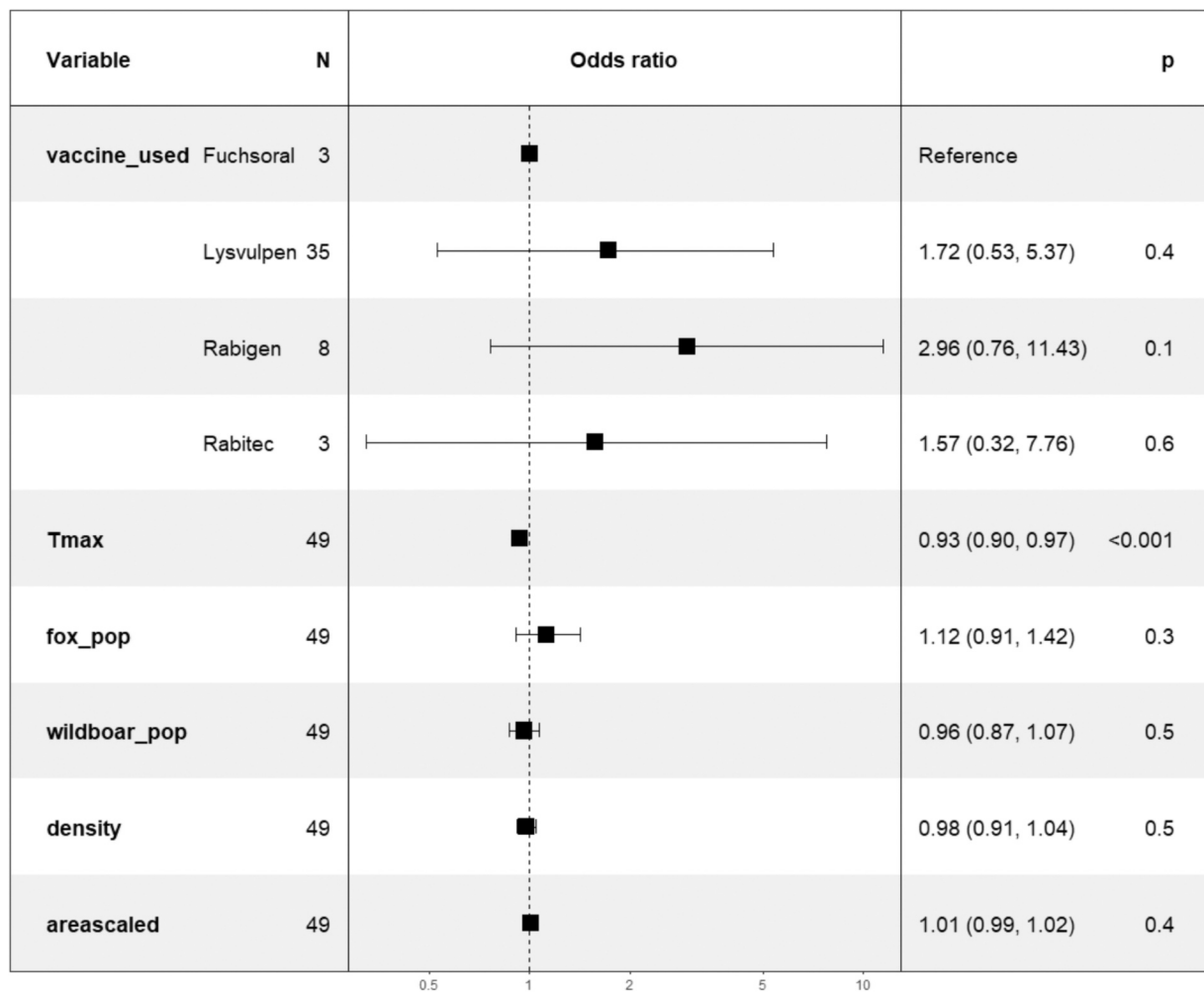


Fig. 6. Parameter estimates of the best model selected to explain oral rabies bait uptake variations in foxes based on analysis of foxes sampled after ORV programmes. All the data were collected per year and per participating country. “vaccine_used” is the type of vaccine used during the ORV programme. “Tmax” is the maximum temperature recorded during the ORV programme. “fox_pop” is the number of hunting fox bags during the ORV programme for 10 km² (used as indicator of red fox population abundances). “wildboar_pop” is the number of hunting wildboar bags observed during the ORV programme for 10 km² (used as indicator of wildboar population abundances). “density” is the bait density dropped during the programme. “areascaled” is indicator of the size of the vaccinated area. Odds ratio > 1 reflects a tendency to positively impact the bait uptake; Odds ratio < 1 reflects a tendency to negatively impact the bait uptake.

populations (Chautan et al., 2000). Rabies elimination thanks to ORV could not be the only reason for this increase in population as rabies-free areas have also experienced it (Chautan et al., 2000). Around the 2000 s, rural fox populations have appeared to reach densities close to carrying capacity, and populations have become more stable. However, from the mid-2000 s, a decrease in populations has been observed on both hunting data and road traffic accidents (Delcourt et al., 2022). As all indicators of fox population trends currently used are subject of bias, understanding the evolution dynamic of red fox populations at European level and their impact on the success of oral vaccination campaigns is still challenging. Also, if vaccination campaigns would become less effective and bait uptake reduced permanently, this may not be detected immediately as a single ORV induces permanent marking and replacement of the fox population would be needed before new tendencies become detectable.

5. Conclusion

This large-scale study revealed a relationship between the serological test used and the seroconversion rate detected in fox samples collected after ORV campaigns. In our study, performance variations of serological kits were too high to allow a common interpretation of the

serological values. The variability due to the tests themselves was indeed greater than any observed variability potentially due to biological or natural phenomena. To allow pooling and easier interpretation of ORV efficacy data at regional/EU level, a single serological test with a common threshold value should be recommended.

This study also highlighted a link between the maximum temperature reached during ORV campaigns and bait uptake rates. To our knowledge, this study is the first field study highlighting such a link. This finding could be due to a confounding variable or due to a real relationship of bait uptake decreasing with increasing temperature. As correlation does not confirm causality, further experimental studies and laboratory investigations should be conducted to assess whether a potential decrease in palatability of baits could be associated with increased temperatures.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Emmanuelle Robardet reports financial support was provided by European Commission.

Acknowledgements

We would like to thank the National Reference Laboratories, the competent authorities who kindly participated in this study and the European Commission for supporting this work in the framework of the support provided to the EU Reference Laboratory for Rabies.

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