EUROPEAN UNION REFERENCE LABORATORY RABIES

anses

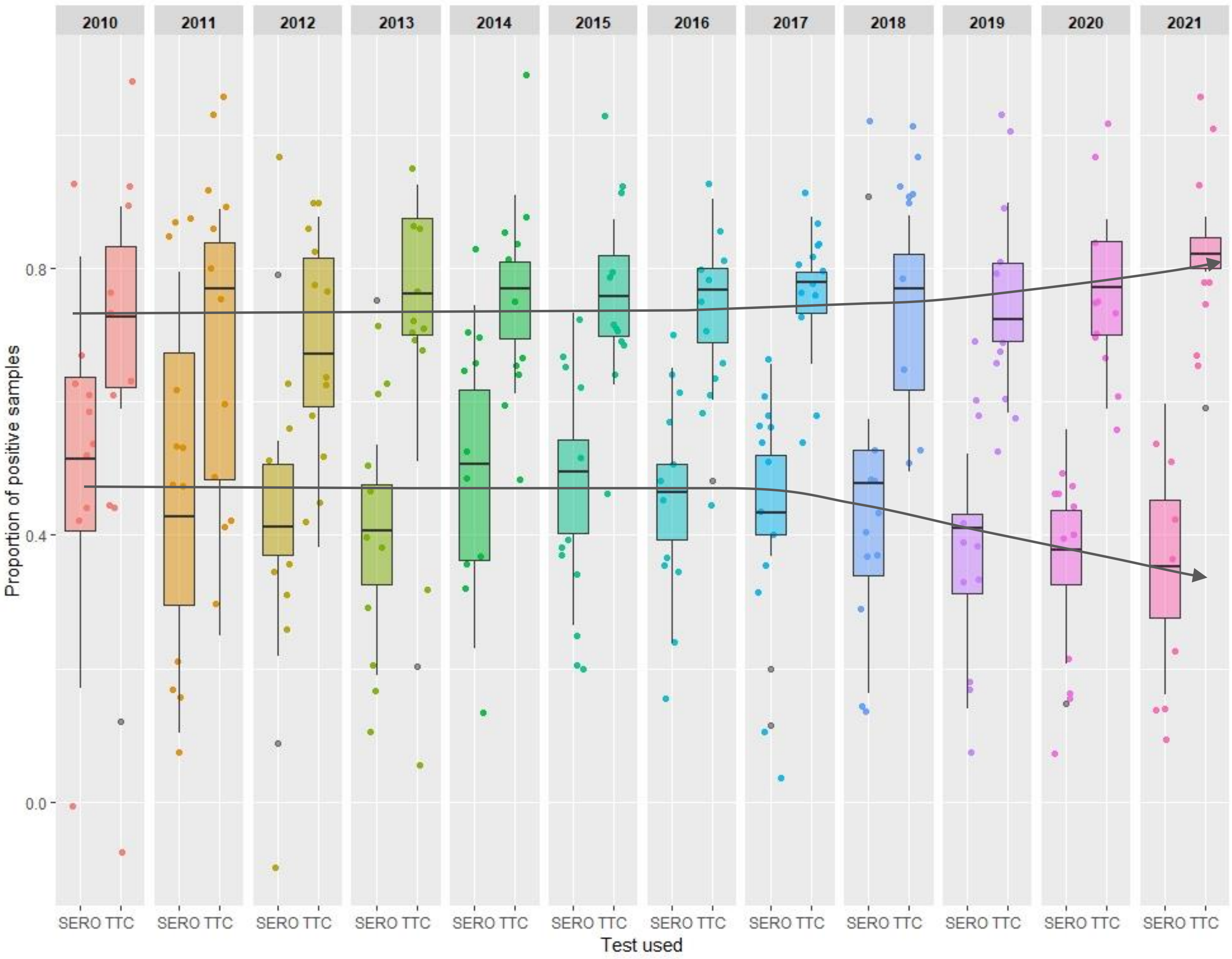
RETROSPECTIVE ANALYSES OF BAIT UPTAKE AND SEROCONVERSION LEVELS IN FOXES

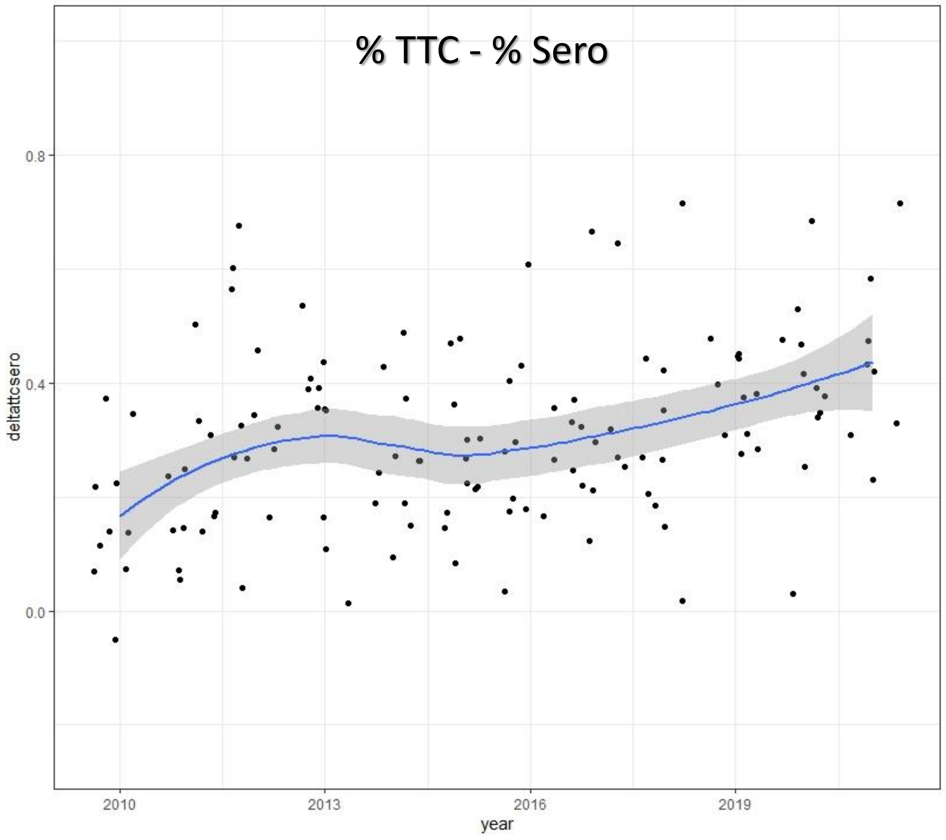
Context of the study

- PAFF Committee meeting of 23-24 September 2020: Member States (MS) presented the results of their 2019 rabies control programmes, including the oral rabies vaccination (ORV) of wildlife and results of its monitoring (biomarker and serological testing of indicator animals) to determine levels of vaccine uptake and immune response.
- One of the issues discussed was a new trend observed in some MS after a few years of continuous implementation of ORV with appropriate monitoring, i.e. without obvious or serious shortcomings and in accordance with the guidelines currently in place.
- This new trend is that the percentage of vaccine uptake (% of sampled indicator animals that are tetracycline-positive) keeps increasing, or at least remains stable, in consecutive years while the percentage of seroconversion (% of sampled indicator animals that are seropositive) decreases every year.

➡ **EURL sollicitated to analyse on a large dataset factors affecting seroprevalences and proportions of bait uptake**

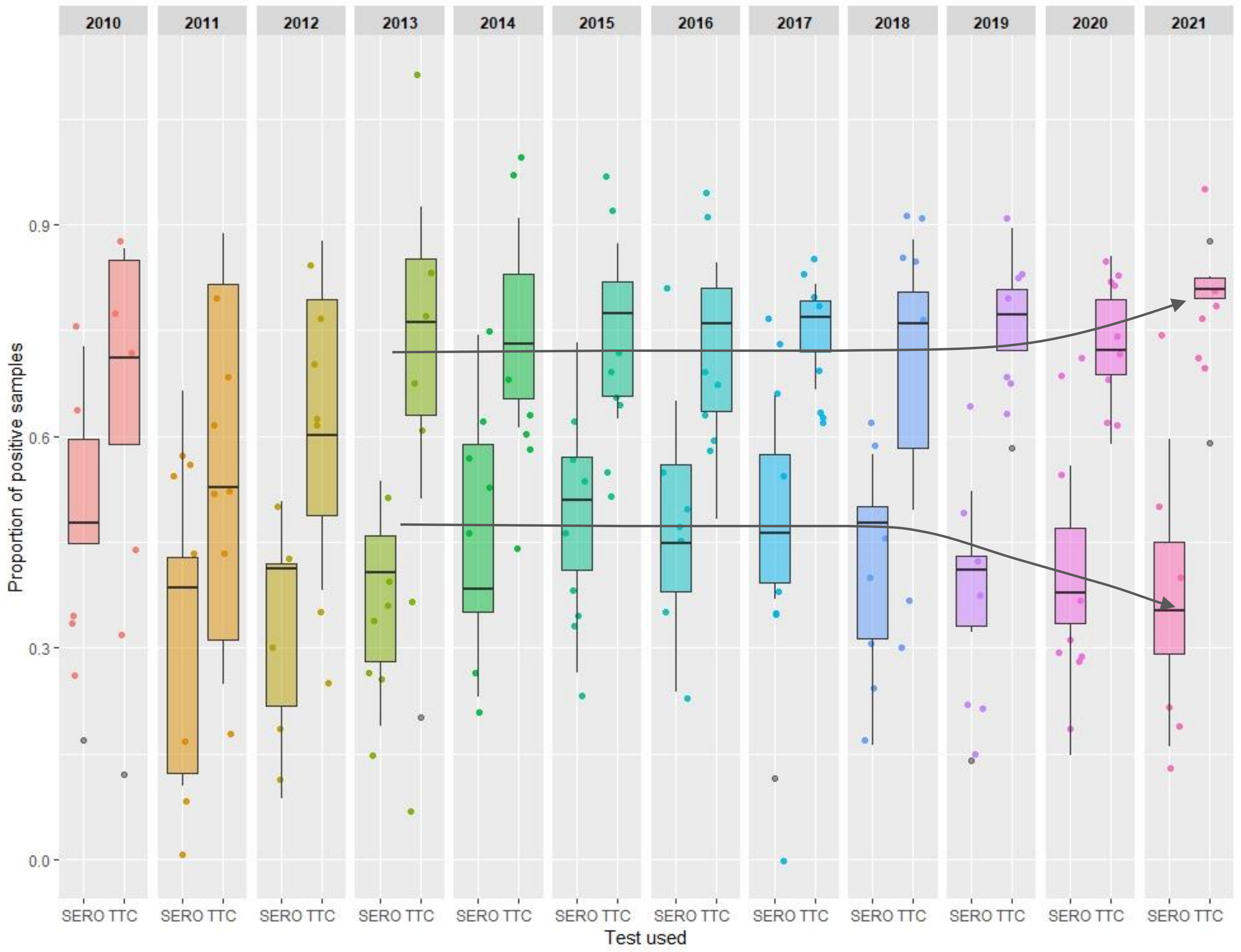
Evolution of annual seroprevalence and bait uptake estimation within EU countries (data source: annual NRL review by the EURL)



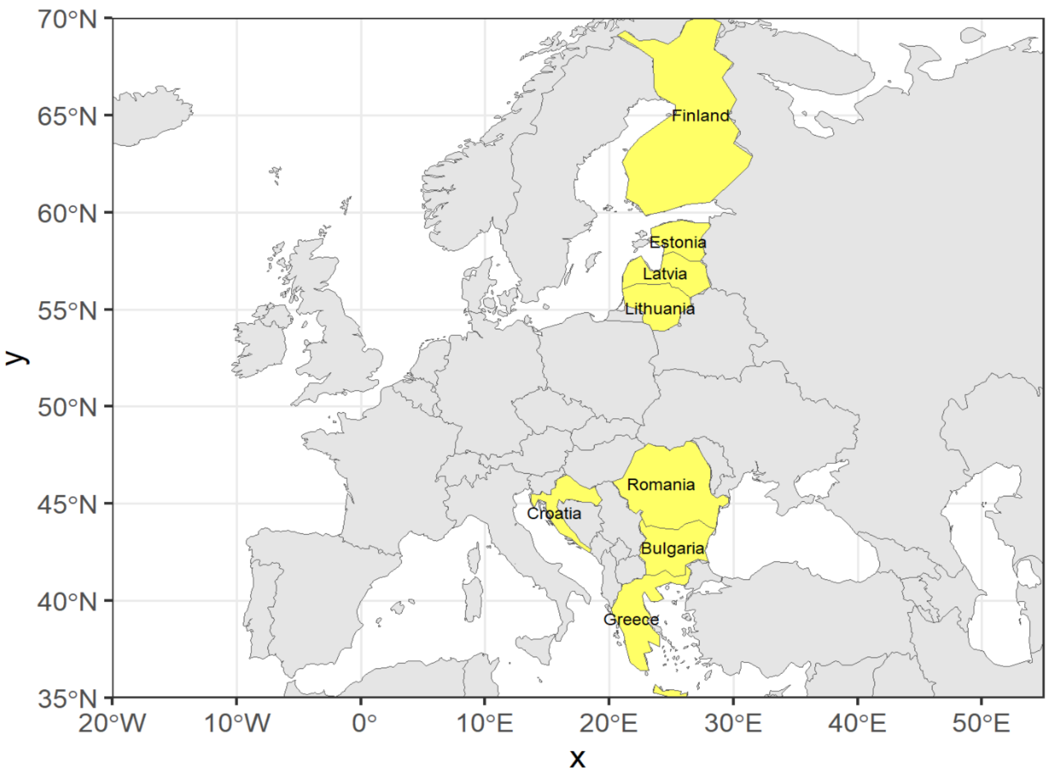


Differences between %TTC and %Sero per year and countries are significant ($F = 8.76$; $p = 0.003$)

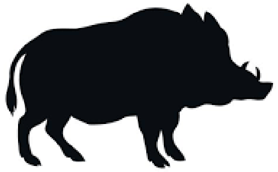
Evolution of annual seroprevalence and bait uptake estimation in participating countries (data source: annual NRL review by the EURL)



8 participating countries



Setting up factors to consider and (harmonised) data to collect



Sampling; Type of test used

- High Ratio perimeter/vaccinated area, (probability to collect unvaccinated animals↑)
- Blood sample (availability, quality?)
- Collection one month after ORV? (timeframe of sampling)
- Test used (Type of test, cut-off used)

Environmental conditions

Bait accessibility

- Competitors (Wild board: hunting bags?)
- Melting (Temperature max in spring and min in autumn)
- Deteriorated vaccine strain by heat/ frozen/defrozen (T°C max min

BAIT-UPTAKE (BAIT ACCESSIBILITY)

IMMUNE RESPONSE (SEROCONVERSION)

Vaccine

- Non appetent (vaccine type)
- Unadequat vaccine titre at start of ORV (titer check)
- Vaccine degraded

Distribution

- Inadequate distribution (bait density checked based on GPS data)
- Inadequate period of distribution
- Inadequate density (Eval fox population? hunting bags?)

Statistical analysis used

Response variables analysed:

- Number of positive and negative serological samples.
- Number of positive and negative bait uptake samples.

Explanatory variables assessed:

- Type of oral **vaccine** used (Fuchsoral; Lysvulpen; Rabigen; Rabitec).
- Size of the **vaccinated area** (continuous variable).
- Relative density of the **red fox population** (n hunting bags/area of the country x10). One country provided snow-tracking indexes for red fox population relative densities. These data were removed from the analysis as snow tracking and hunting bags are not comparable indicators.
- Relative density of the **wild boar population** (n hunting bags/area of the country x10).
- Tmax: **maximum temperature** observed during ORV campaigns, from Day 1 of the ORV to one month after the start (continuous variable).

and

- Type of **serological test** used (for the analysis of serological samples only) (ELISA Bio-Rad with cut-off 0.3, ELISA Bio-Rad with cut-off 0.5, ELISA BioPro 40% BP, FAVN, RFFIT).

Logistic regression (GLM)
with ad hoc Williams correction
for over-dispersed data (minimize type I errors, FP concl.)

- X Adequat batch titer (rarely inadequate)
- X Bait density (evaluted by GPS analysis)
- X Blood sample quality (no specific trend indicated by respondants)

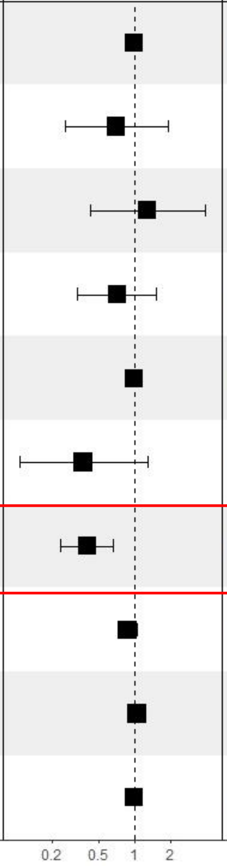
Life span of foxes ≈ 2/3 years
Annual data were used (and not per campaign) to minimise non independance of data.
Juvenile and Adult together
(missing data if considered per age category and per campaign)

Parameter estimates of the best model selected to explain seroprevalences

glmfinal<-glm.binomial.disp (cbind(sero_pos,sero_neg)~vaccine_used+serotype+ fox_dens+ wildboar_dens + orv_area, family=binomial)

Models compared by AiC and
« T max » not retained as explanatory variable

Variable		N	Odds ratio		p
vaccine_used	Rabigen	8		Reference	
	Lysvulpen	35		0.70 (0.26, 1.93)	0.49
	Fuchsoral	3		1.29 (0.42, 4.00)	0.65
	Rabitec	3		0.72 (0.33, 1.54)	0.40
sero_type	ELISA BioPro	14		Reference	
	ELISA BR03	10		0.37 (0.11, 1.29)	0.12
	ELISA BR05	25		0.40 (0.24, 0.66)	<0.001
fox_dens		49		0.87 (0.72, 1.06)	0.17
wildboar_dens		49		1.05 (0.95, 1.17)	0.34
areascaled		49		0.99 (0.97, 1.00)	0.09



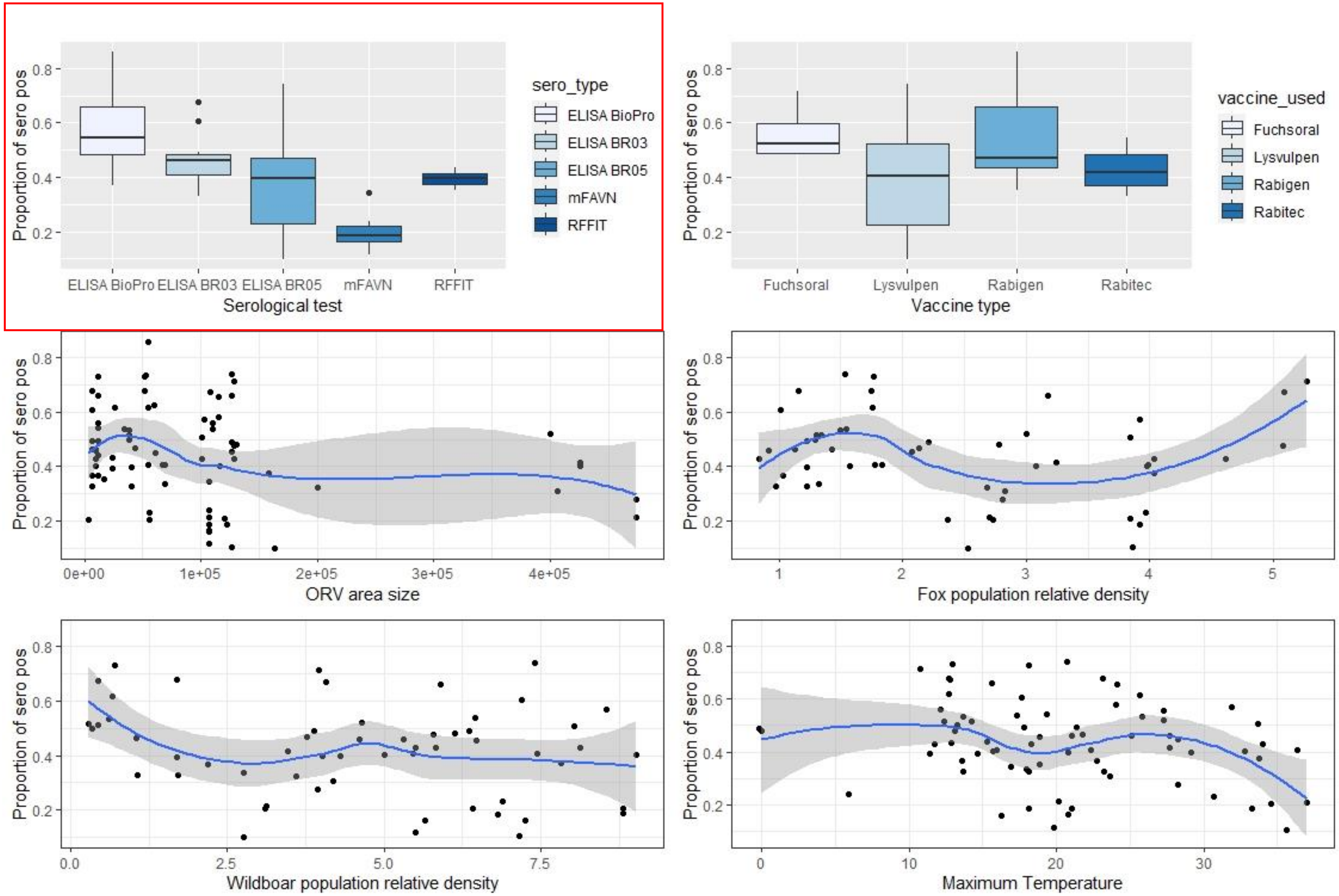
Odds ratio >1: positive impact on bait uptake
Odds ratio <1: negative impact on bait uptake

BioPro: BioPro used with 40%PB

BR03: BioRad used with 0.5 EU/ml cut off

BR03: BioRad used with 0.3 EU/ml cut off

Link of the studied factors with serological results

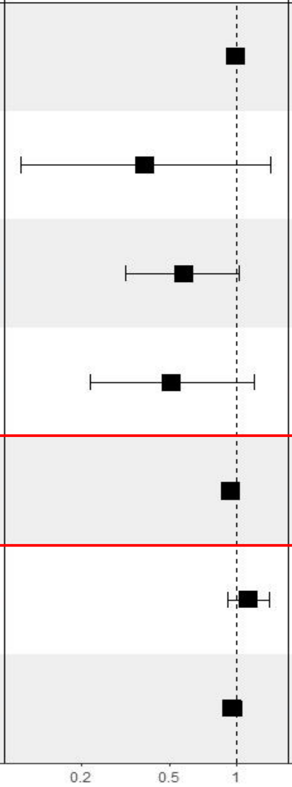


Parameter estimates of the best model selected to explain bait uptake variations

glmfinal<-glm.binomial.disp (cbind(ttc_pos,ttc_neg)~vaccine_used+Tmax+fox_dens+wildboar_dens, family=binomial)

Models compared by AiC and
« Size of ORV » not retained as explanatory variable

Variable		N	Odds ratio		p
vaccine_used	Rabigen	8		Reference	
	Fuchsoral	3		0.39 (0.11, 1.43)	0.15
	Lysvulpen	35		0.58 (0.32, 1.02)	0.07
	Rabitec	3		0.51 (0.22, 1.21)	0.12
Tmax		49		0.94 (0.91, 0.97)	<0.001
fox_dens		49		1.13 (0.92, 1.41)	0.27
wildboar_dens		49		0.95 (0.87, 1.05)	0.35



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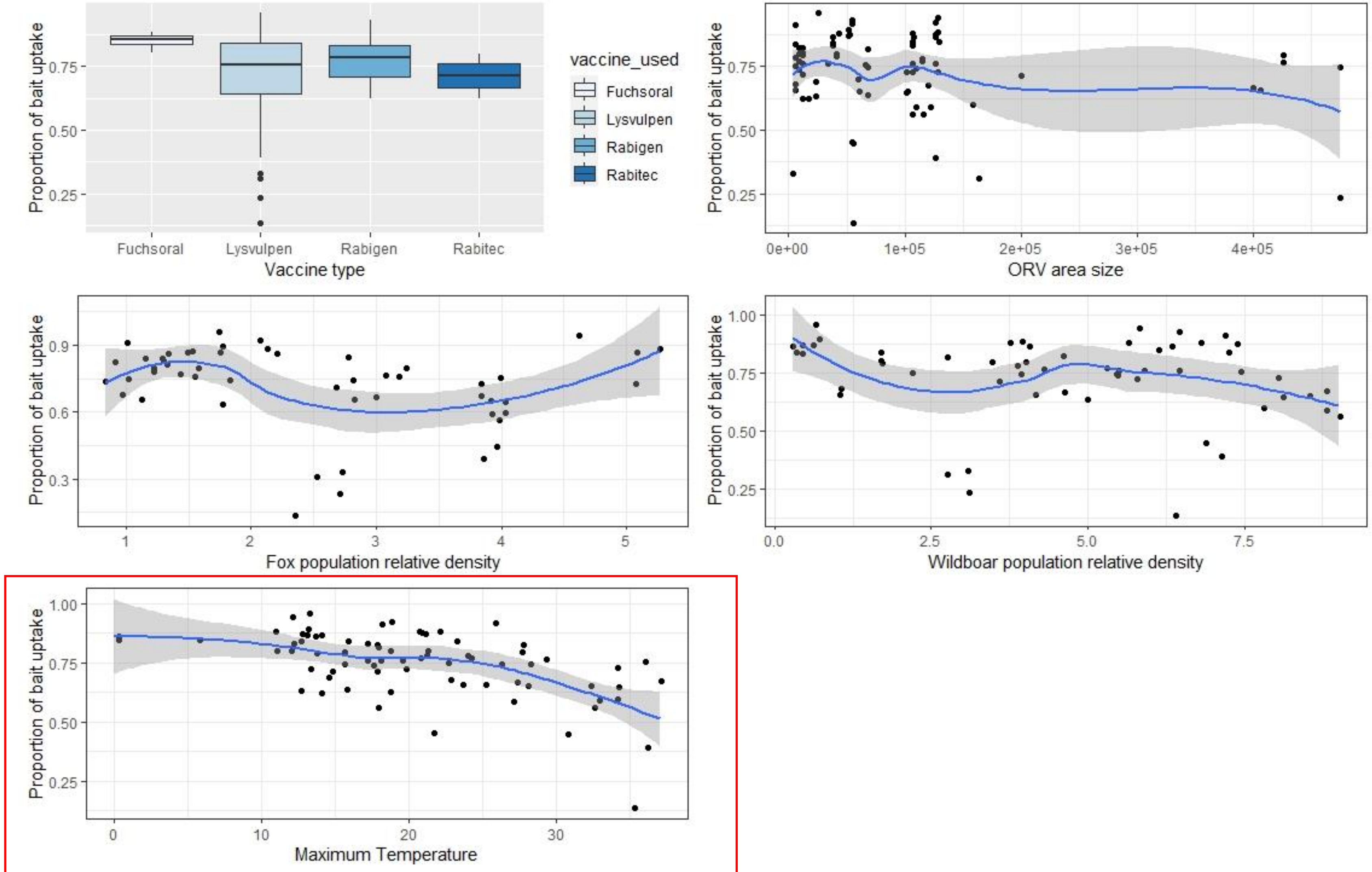


Serological tests used for seroconversion evaluation

- At least four different types of test were used, including the Bio-Rad ELISA kit used 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 levels than levels from BioPro kits. The variety of tests used and their different threshold values for serological evaluation undoubtedly makes the comparison of oral vaccination results between countries difficult to assess.
- **To allow merged and easy use of oral vaccination efficacy surveillance data at community level, a single serological test with a common threshold value is recommended to be used for the evaluation of seroconversion level within the community**.
- **The significant effect of the factor 'Type of test used' could indeed hide other effects, with less variability but just as important to consider for the successful completion of oral vaccination programmes**.

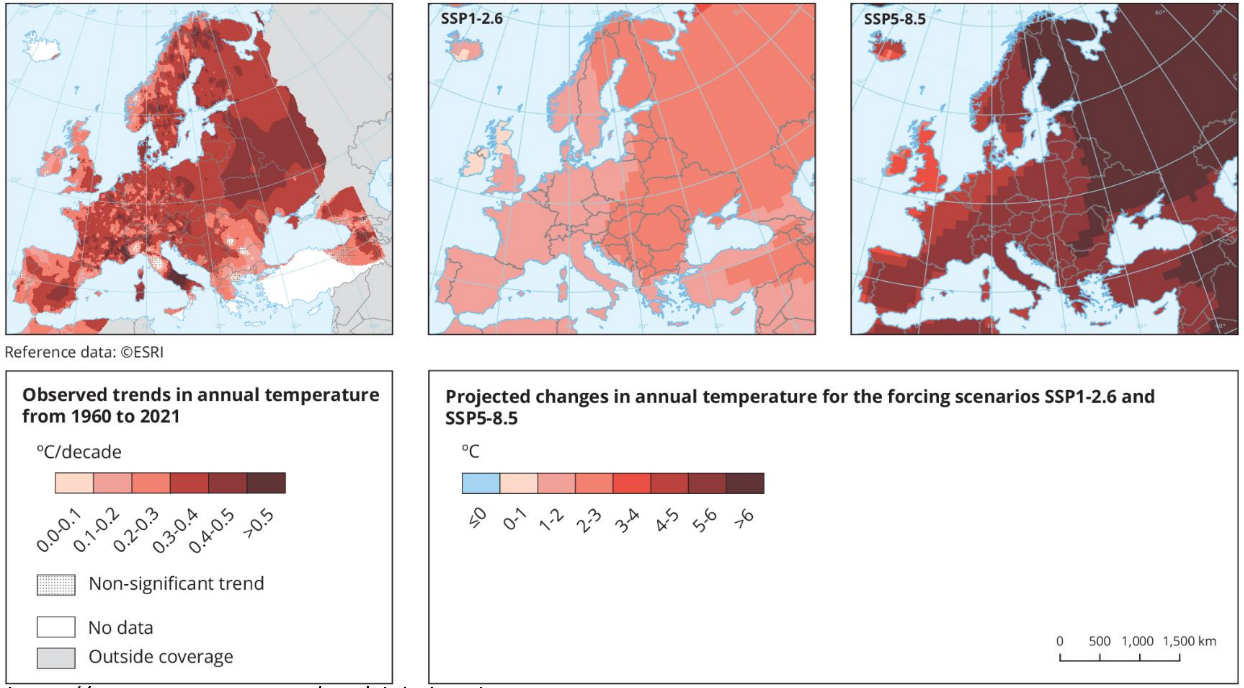
Temperature

- **Significant factor correlated with bait uptake data. When maximum temperature increases bait uptake decreases.**

- Impact of climate change and local extreme temperature on bait uptake?

In late spring 2022, for example, Europe experienced local temperatures over 30°C (measurements taken in the shade) which are the kind of temperatures that are expected in the summer, a season when ORV campaigns are not usually carried out, partly because of these high temperatures.

Figure 2. Observed annual mean temperature trend from 1960 to 2021 (left panel) and projected 21st century temperature change under different SSP scenarios (right panels) in Europe



https://www.eea.europa.eu/ims/global-and-european-temperatures

Temperature

- Oral vaccines currently used indicates:

  - maximum 7 days at 25°C for optimal use.

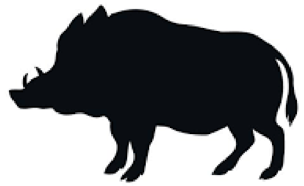
  - not to be used over 25°C or 30°C.

- Ph. Eur. monograph 0746 : Address vaccine titre stability and bait casing stability. Bait casing: Vaccines must be designed not to melt 1hour at +40°C (**“Heat the bait at 40°C for 1 hour. The bait casing complies with the test if it remains in its original shape and adheres to the vaccine container”**).

Exemple of melting tempertatures of various oils included in oral rabies vaccine baits composition

Oil	Melting point
Coconut oil	24 to 25°C
Palm oil	30 to 40°C
Beef tallow	43 to 49°C
Hard parafin	50 to 70°C

Wild boar densities



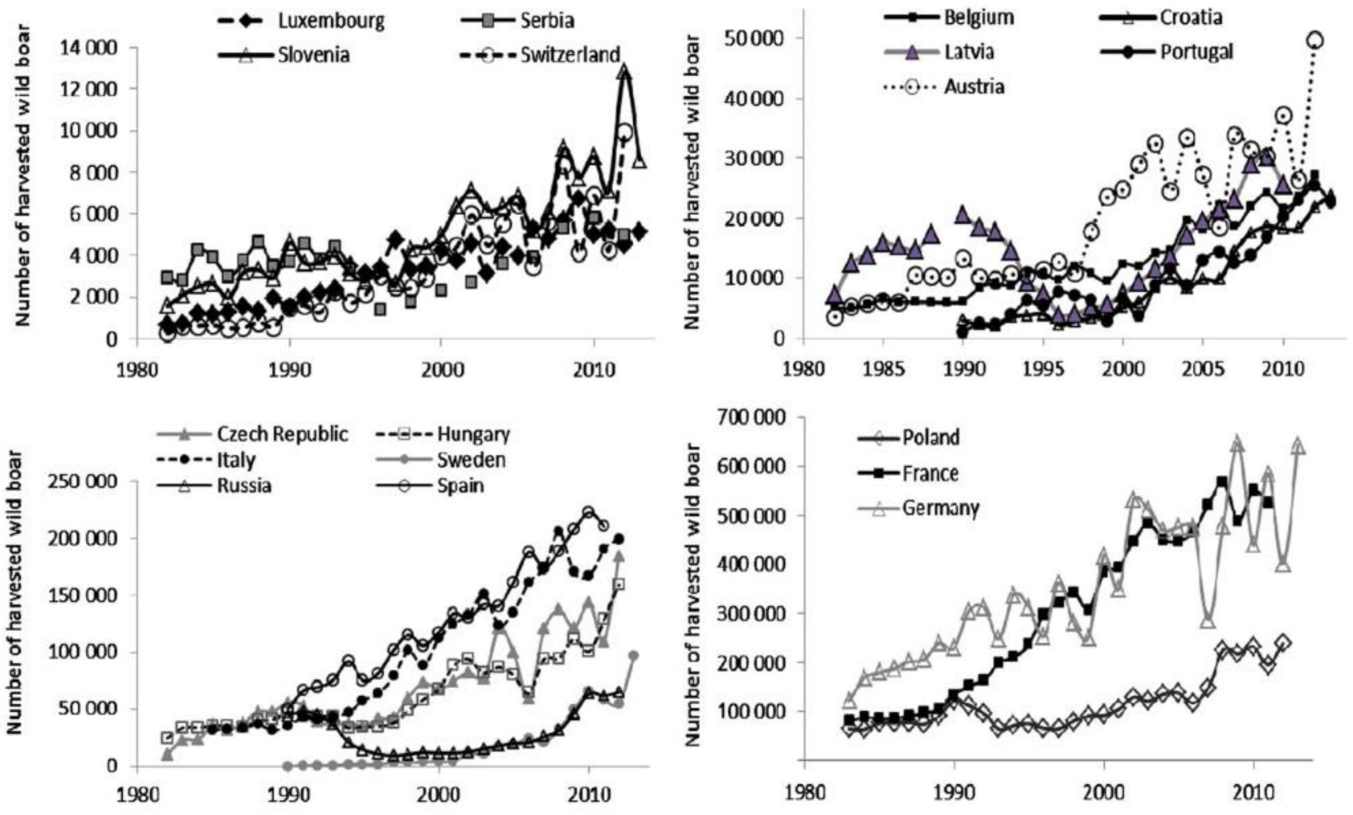
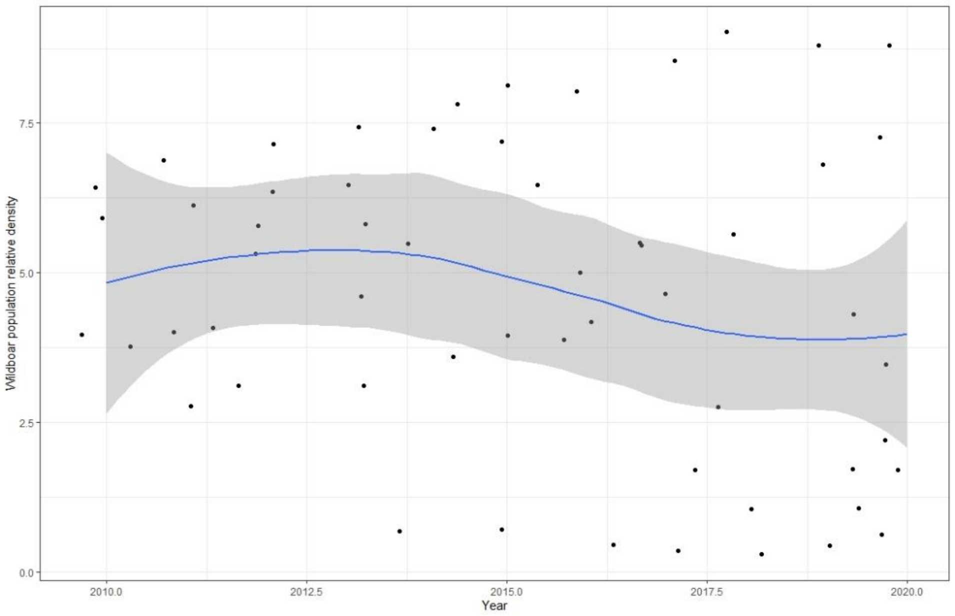


Figure 1. Wild boar hunting bags from selected European countries.

Massei, 2015



Wild boar population are increasing in Europe since 1980s (Massei, 2015), and are possible competitor for Oral Vaccines comsumption (Anti-rabies antibodies detected in wild boars in Slovenia, Romania, etc). **The evolution of wild boar relative densities in our dataset did not support the increase in this population and no link was found with the seroconversion level or bait uptake of foxes**.

Red fox densities

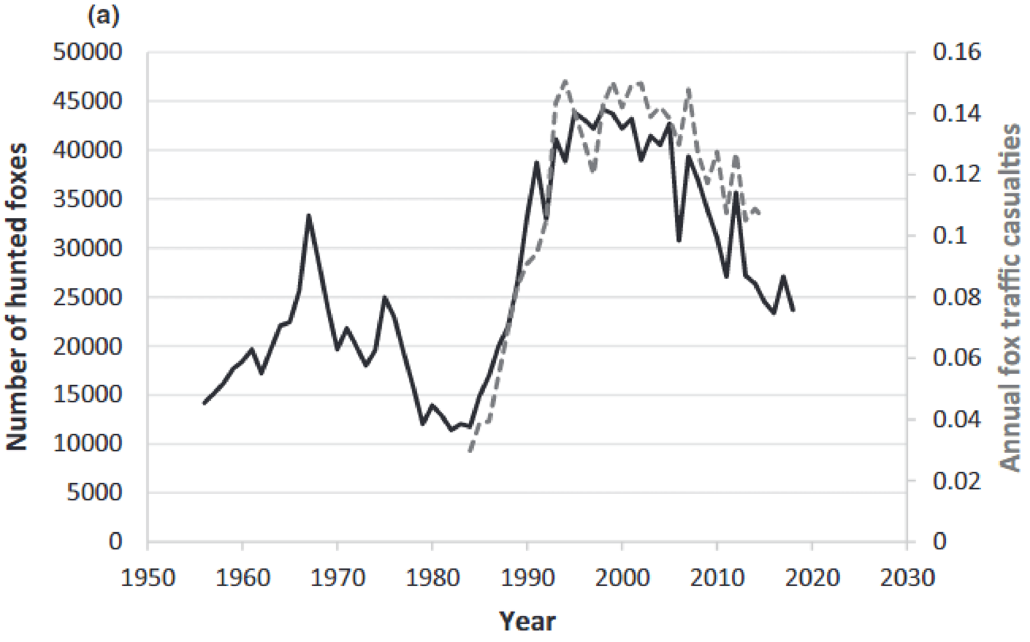


Fig. 2 Red fox *Vulpes vulpes* population trends in Switzerland. (a) Continuous line, y axis on left: annual number of foxes hunted (source: Bundesamt für Statistik, Federal Office for Environment, Switzerland). Dashed line, y axis on right: index of annual fox road traffic casualties from 1986 to 2015, with the y-scale (right) corrected for the sum of kilometres travelled per year in Switzerland (traffic casualties/million vehicles*km, source: Geiger et al. 2018).

Delcourt, 2022 (Switzerland)

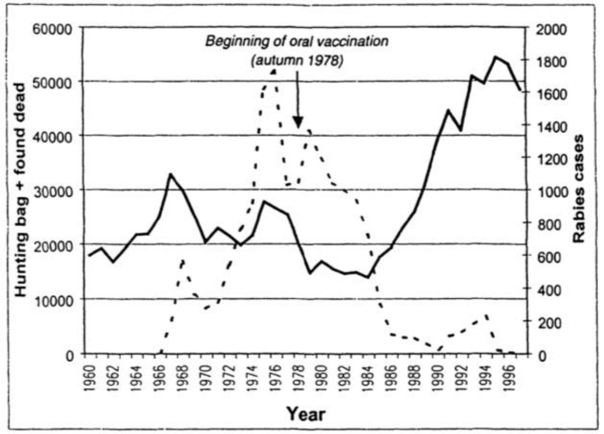
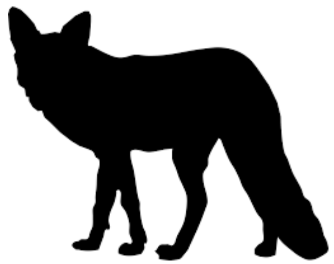
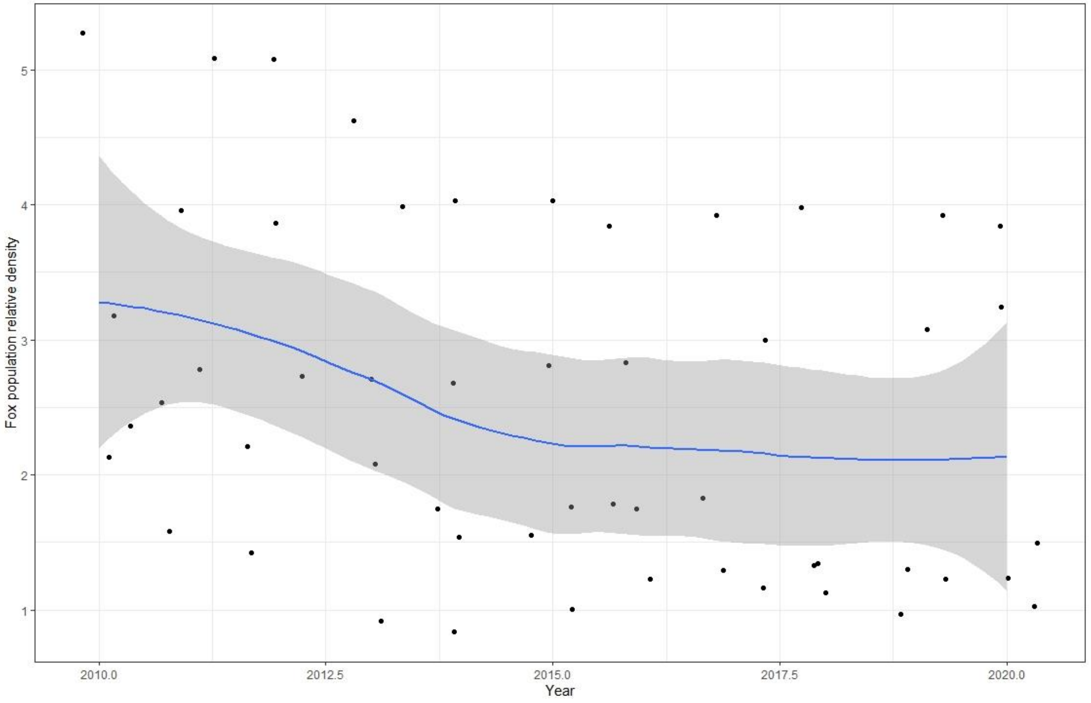


Fig. 2. – Annual hunting bag records (solid line) and annual number of rabies cases (dashed of foxes in Switzerland from 1960 to 1997. (After Breitenmoser and Mueller, in prep.).

Chautan, 1998 (Switzerland)





In our study, the relative densities of red foxes seems to decrease and did not appear to be linked with variation of seroconversion level or bait uptake. However, due to many missing red fox population data, further investigations should be needed to exclude their impact on ORV efficiency.

What is next?

- Can an unique ELISA test be used within EU?
- Correction of data to reduce the bias due to test used for serology testing?
- More laboratory investigations related to raised questions?

Thank You!