Inter-laboratory comparison of tetracycline detection and age determination in red fox teeth

Fifth session (2022)

anses

EURL for Rabies Workshop, 22 June 2023, Ljubljana, Slovenia

EUROPEAN UNION REFERENCE LABORATORY RABIES

1 — Introduction

ORGANISATION OF ILT FOR TTC AND AGE DETERMINATION

- Tetracycline (TTC) is an antibiotic widely used as a biomarker for monitoring oral vaccine bait consumption.
- When administrated orally, TTC can be detected as early as 2 days post consumption on bones section under UV lights (Frost, et al. 1959).
- In EU, after ORV campaign, red foxes from vaccinated areas are sampled to assess the bait uptake proportion in the target population.
- ILT to compare laboratory performances on TTC detection and age determination on red fox teeth is assessed regularly by Anses since EURL designation (2008).
- 2022 was the fifth session (one ILT every ≈ 3 years).

PARTICIPATING LABORATORIES

- Eligible participants (Expenses covered by EURL funds):

- NRLs from EU member states and 9 bordering third countries (ME, MK, AL, RS, XK, BA, TR, MD, UA)

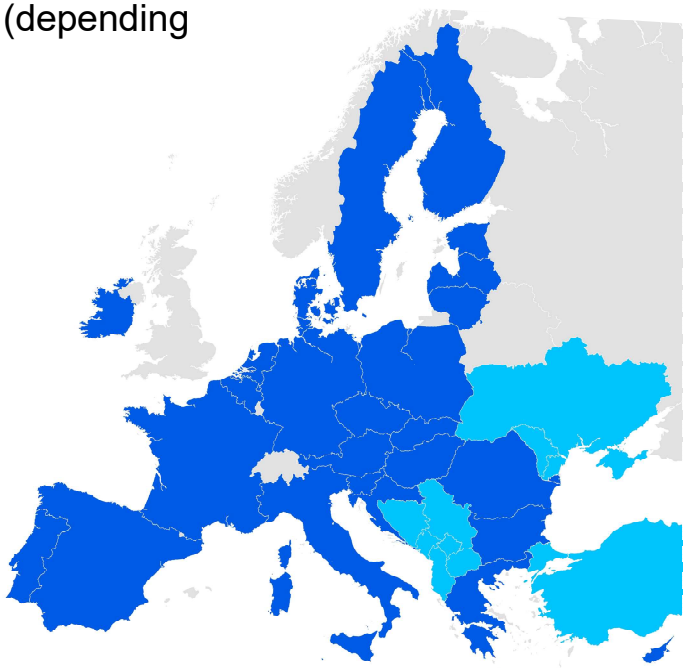
- Priority given to countries involved in at least one ORV campaigns (depending of the number of panels available)

- Participants in the 2022 session:

- 11 NRLs from EU member states

- 5 NRL from bordering third countries

- All except one involved in at least ORV campaign during the last two years.



2 — Material and Methods

PANEL COMPOSITION

- Jaws collected from red foxes sampled in the field in Romania following ORV campaign performed in Autumn 2021 (S1, S2, S3, S5, S6), and from Croatia, Italy, and Poland for S4.

- Assigned status of the jaw

Jaw divided in two parts:

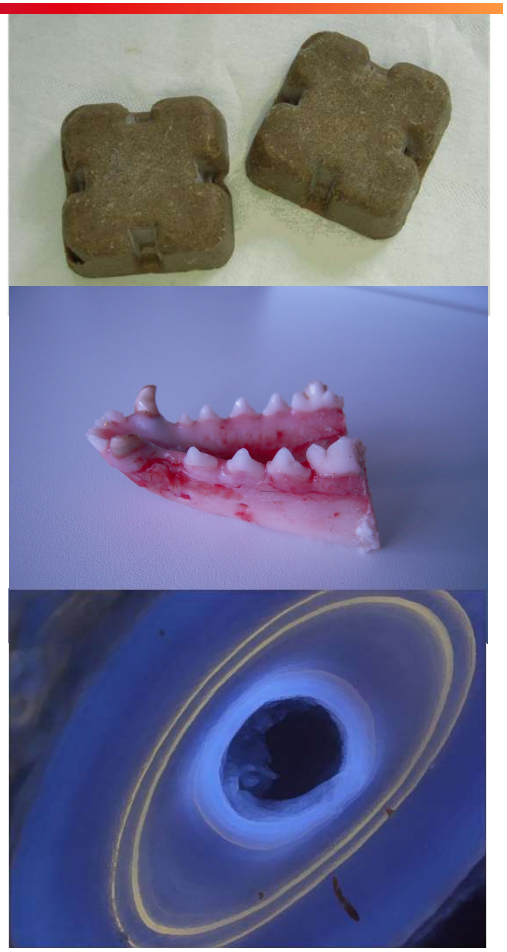
- One to determine the status of the sample in Anses (2 readers).
- The other one to be sent to participating laboratory.

PANEL TEST COMPOSITION: 6 red fox half-jaws

Sample 1: 1 juvenile (<1 year) negative TTC jaw,
Sample 2: 1 adult (1-2 years) positive TTC jaws,
Sample 3: 1 adult (2-3 and >3 years) positive TTC jaw,
Sample 4: 1 mix of age and positive TTC jaw,
Sample 5: 1 juvenile (<1 year) negative TTC jaw,
Sample 6: 1 juvenile (<1 year) negative TTC jaw.

- Panel sent in dry ice under UN3373 conditions.

- Testing:
  - Determination of tetracycline (results expressed as positive or negative).
  - Determination of animal age (juvenile/ adult).



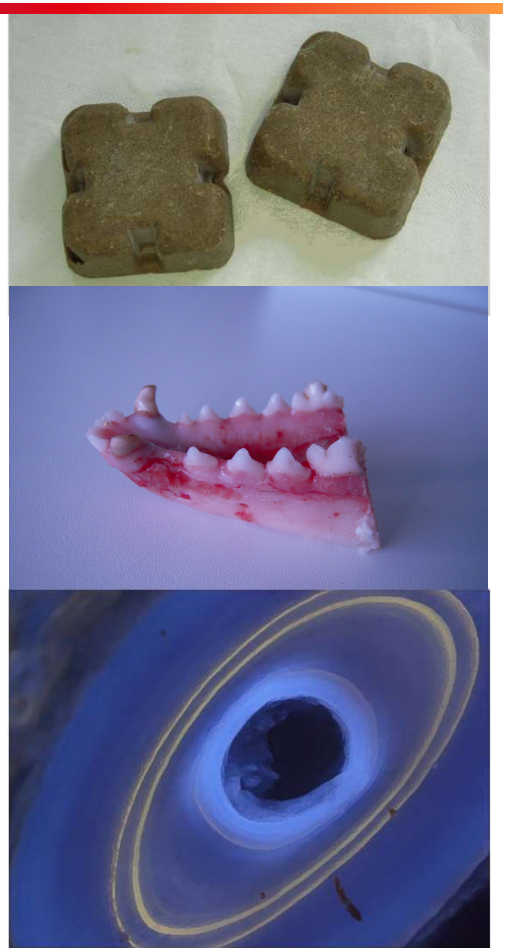
HOMOGENEITY AND STABILITY

- **Stability:**

Microstructures of increments of tooth cement used for age determination and tetracycline marking being permanent, there is no stability issue on teeth samples stored at -20°C.

- **Homogeneity:**

As no significant difference in the presence of tetracycline in the right and left canines and premolars of marked animals has been demonstrated, there is no homogeneity issue between the participant and the organiser results on the samples (Algeo, Norhenberg et al. 2013).



3 — Results

Laboratory results on TTC detection – Raw Data

	JUVENILE and TTC NEGATIVE			ADULT and TTC POSITIVE			ADULT and TTC POSITIVE			MIX and TTC POSITIVE			JUVENILE and TTC NEGATIVE			JUVENILE and TTC NEGATIVE		
code_lab	code_1	status	observed	code_2	status	observed	code_3	status	observed	code_4	status	observed	code_5	status	observed	code_6	status	observed
L01	22090814	NEGATIVE	NEGATIVE	22090488	POSITIVE	POSITIVE	22090882	POSITIVE	POSITIVE	22090191	POSITIVE	POSITIVE	22090029	NEGATIVE	NEGATIVE	22090962	NEGATIVE	NEGATIVE
L02	22090809	NEGATIVE	NEGATIVE	22090643	POSITIVE	POSITIVE	22090249	POSITIVE	POSITIVE	22090192	POSITIVE	POSITIVE	22090636	NEGATIVE	NEGATIVE	22090753	NEGATIVE	NEGATIVE
L03	22090539	NEGATIVE	NEGATIVE	22090948	POSITIVE	POSITIVE	22090961	POSITIVE	POSITIVE	22090102	POSITIVE	POSITIVE	22090744	NEGATIVE	NEGATIVE	22090756	NEGATIVE	NEGATIVE
L04	22090947	NEGATIVE	NEGATIVE	22090851	POSITIVE	POSITIVE	22090173	POSITIVE	POSITIVE	22090151	POSITIVE	POSITIVE	22090447	NEGATIVE	NEGATIVE	22090696	NEGATIVE	NEGATIVE
L05	22090520	NEGATIVE	NEGATIVE	22090937	POSITIVE	POSITIVE	22090475	POSITIVE	POSITIVE	22090379	POSITIVE	POSITIVE	22090434	NEGATIVE	NEGATIVE	22090031	NEGATIVE	NEGATIVE
L06	22090830	NEGATIVE	NEGATIVE	22090004	POSITIVE	POSITIVE	22090791	POSITIVE	POSITIVE	22090691	POSITIVE	POSITIVE	22090739	NEGATIVE	NEGATIVE	22090290	NEGATIVE	NEGATIVE
L07	22090654	NEGATIVE	NEGATIVE	22090527	POSITIVE	POSITIVE	22090258	POSITIVE	POSITIVE	22090111	POSITIVE	POSITIVE	22090168	NEGATIVE	NEGATIVE	22090578	NEGATIVE	NEGATIVE
L08	22090038	NEGATIVE	POSITIVE	22090085	POSITIVE	POSITIVE	22090215	POSITIVE	POSITIVE	22090611	POSITIVE	POSITIVE	22090936	NEGATIVE	NEGATIVE	22090465	NEGATIVE	NEGATIVE
L09	22090124	NEGATIVE	NEGATIVE	22090427	POSITIVE	POSITIVE	22090236	POSITIVE	POSITIVE	22090063	POSITIVE	POSITIVE	22090485	NEGATIVE	NEGATIVE	22090112	NEGATIVE	NEGATIVE
L11	22090223	NEGATIVE	NEGATIVE	22090990	POSITIVE	POSITIVE	22090324	POSITIVE	POSITIVE	22090316	POSITIVE	POSITIVE	22090449	NEGATIVE	NEGATIVE	22090451	NEGATIVE	NEGATIVE
L12	22090525	NEGATIVE	NEGATIVE	22090532	POSITIVE	POSITIVE	22090406	POSITIVE	POSITIVE	22090041	POSITIVE	POSITIVE	22090695	NEGATIVE	NEGATIVE	22090863	NEGATIVE	NEGATIVE
L13	22090879	NEGATIVE	POSITIVE	22090203	POSITIVE	POSITIVE	22090326	POSITIVE	POSITIVE	22090057	POSITIVE	POSITIVE	22090400	NEGATIVE	NEGATIVE	22090305	NEGATIVE	POSITIVE
L14	22090517	NEGATIVE	NEGATIVE	22090931	POSITIVE	POSITIVE	22090154	POSITIVE	POSITIVE	22090610	POSITIVE	POSITIVE	22090946	NEGATIVE	NEGATIVE	22090142	NEGATIVE	NEGATIVE
L15	22090918	NEGATIVE	NEGATIVE	22090758	POSITIVE	POSITIVE	22090055	POSITIVE	POSITIVE	22090701	POSITIVE	POSITIVE	22090056	NEGATIVE	NEGATIVE	22090404	NEGATIVE	NEGATIVE
L17	22090216	NEGATIVE	POSITIVE	22090769	POSITIVE	POSITIVE	22090926	POSITIVE	POSITIVE	22090663	POSITIVE	POSITIVE	22090876	NEGATIVE	NEGATIVE	22090762	NEGATIVE	POSITIVE
L18	22090059	NEGATIVE	NEGATIVE	22090944	POSITIVE	POSITIVE	22090058	POSITIVE	POSITIVE	22090189	POSITIVE	POSITIVE	22090624	NEGATIVE	NEGATIVE	22090296	NEGATIVE	NEGATIVE

Laboratory results on TTC detection - Summary

Tested samples	N participating laboratories	% laboratories with satisfactory results	Binomial proportion confidence interval	N samples analysed	% discordant results	Binomial proportion confidence interval
TTC Positive Adult	16	**100** (n=16)	[79.4 – 100]	32	0 (n=0)	[0.0 – 10.9]
TTC Positive Mix	16	**100** (n=16)	[79.4 – 100]	16	0 (n=0)	[0.0 – 20.6]
Total TTC Positive	16	**100** (n=16)	[79.4 – 100]	48	0 (n=0)	[0.0 – 7.4]
TTC Negative Juvenile	16	**81** (n=13)	[54.4 – 95.6]	48	10 (n=5)	[3.5 – 22.7]
Total TTC Negative	16	**81** (n=13)	[54.4 – 95.6]	48	10 (n=5)	[3.5 – 22.7]
Total	16	**81** (n=13)	[54.4 – 95.6]	96	5 (n=5)	[1.7 – 11.7]

In conclusion, the overall success rate of the tetracycline detection test of this session is satisfactory (81% of laboratories succeeded in all tests). The success rate of laboratories on positive and negative samples was 100% and 81% respectively.

Laboratory results on age determination – Raw Data

	JUVENILE and TTC NEGATIVE			ADULT and TTC POSITIVE			ADULT and TTC POSITIVE			Mix and TTC POSITIVE			JUVENILE and TTC NEGATIVE			JUVENILE and TTC NEGATIVE		
code_lab	code_1	status	observed	code_2	status	observed	code_3	status	observed	code_4	status	observed	code_5	status	observed	code_6	status	observed
L01	22090814	Juvenile	Juvenile	22090488	Adult	Adult	22090882	Adult	Adult	22090191	Mix	Juvenile	22090029	Juvenile	Juvenile	22090962	Juvenile	Juvenile
L02	22090809	Juvenile	Juvenile	22090643	Adult	Adult	22090249	Adult	Adult	22090192	Mix	Juvenile	22090636	Juvenile	Juvenile	22090753	Juvenile	Juvenile
L03	22090539	Juvenile	Juvenile	22090948	Adult	Adult	22090961	Adult	Adult	22090102	Mix	Adult	22090744	Juvenile	Juvenile	22090756	Juvenile	Juvenile
L04	22090947	Juvenile	Juvenile	22090851	Adult	Adult	22090173	Adult	Adult	22090151	Mix	Juvenile	22090447	Juvenile	Juvenile	22090696	Juvenile	Juvenile
L05	22090520	Juvenile	Juvenile	22090937	Adult	Adult	22090475	Adult	Adult	22090379	Mix	Juvenile	22090434	Juvenile	Adult	22090031	Juvenile	Juvenile
L06	22090830	Juvenile	Juvenile	22090004	Adult	Adult	22090791	Adult	Adult	22090691	Mix	Adult	22090739	Juvenile	Juvenile	22090290	Juvenile	Adult
L07	22090654	Juvenile	Juvenile	22090527	Adult	Adult	22090258	Adult	Adult	22090111	Mix	Adult	22090168	Juvenile	Juvenile	22090578	Juvenile	Juvenile
L08	22090038	Juvenile	Juvenile	22090085	Adult	Adult	22090215	Adult	Adult	22090611	Mix	Juvenile	22090936	Juvenile	Juvenile	22090465	Juvenile	Juvenile
L09	22090124	Juvenile	Juvenile	22090427	Adult	Adult	22090236	Adult	Adult	22090063	Mix	Adult	22090485	Juvenile	Juvenile	22090112	Juvenile	Juvenile
L11	22090223	Juvenile	Juvenile	22090990	Adult	Adult	22090324	Adult	Adult	22090316	Mix	Adult	22090449	Juvenile	Juvenile	22090451	Juvenile	Juvenile
L12	22090525	Juvenile	Juvenile	22090532	Adult	Adult	22090406	Adult	Adult	22090041	Mix	Adult	22090695	Juvenile	Juvenile	22090863	Juvenile	Juvenile
L13	22090879	Juvenile	Juvenile	22090203	Adult	Adult	22090326	Adult	Adult	22090057	Mix	Adult	22090400	Juvenile	Juvenile	22090305	Juvenile	Adult
L14	22090517	Juvenile	Juvenile	22090931	Adult	Adult	22090154	Adult	Adult	22090610	Mix	Juvenile	22090946	Juvenile	Juvenile	22090142	Juvenile	Juvenile
L15	22090918	Juvenile	Juvenile	22090758	Adult	Adult	22090055	Adult	Adult	22090701	Mix	Adult	22090056	Juvenile	Juvenile	22090404	Juvenile	Juvenile
L17	22090216	Juvenile	Adult	22090769	Adult	Adult	22090926	Adult	Adult	22090663	Mix	Adult	22090876	Juvenile	Juvenile	22090762	Juvenile	Adult
L18	22090059	Juvenile	Juvenile	22090944	Adult	Adult	22090058	Adult	Adult	22090189	Mix	Adult	22090624	Juvenile	Juvenile	22090296	Juvenile	Juvenile

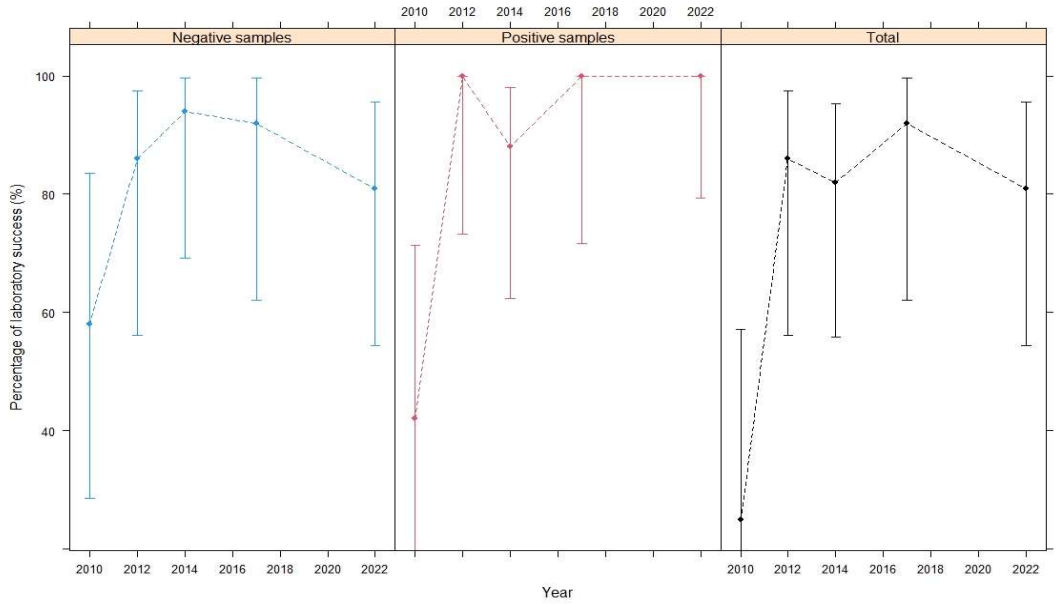
Laboratory results on age determination – Summary

Tested samples	N participating laboratories	% laboratories with satisfactory results	Binomial proportion confidence interval	N samples analysed	% discordant results	Binomial proportion confidence interval
Total adult samples	16	**100** (n=16)	[79.4 – 100]	32	0 (n=0)	[0.0 – 10.9]
Juvenile samples	16	**75** (n=12)	[47.6 –92.7]	48	10 (n=5)	[3.5 – 22.7]
Total	16	**75** (n=12)	[47.6 – 92.7]	80	6 (n=5)	[2.1 – 14.0]

Rq: « Mix positive » not evaluated for age determination as this group included several age categories

Seventy five percent of laboratories estimated the correct age class on all samples. Five discordant results (6%) were detected on the total of 80 samples analysed for age estimation. All discordant results were detected in juvenile samples (<1 year) identified as adult samples.

Evolution of ILT results on tetracycline detection

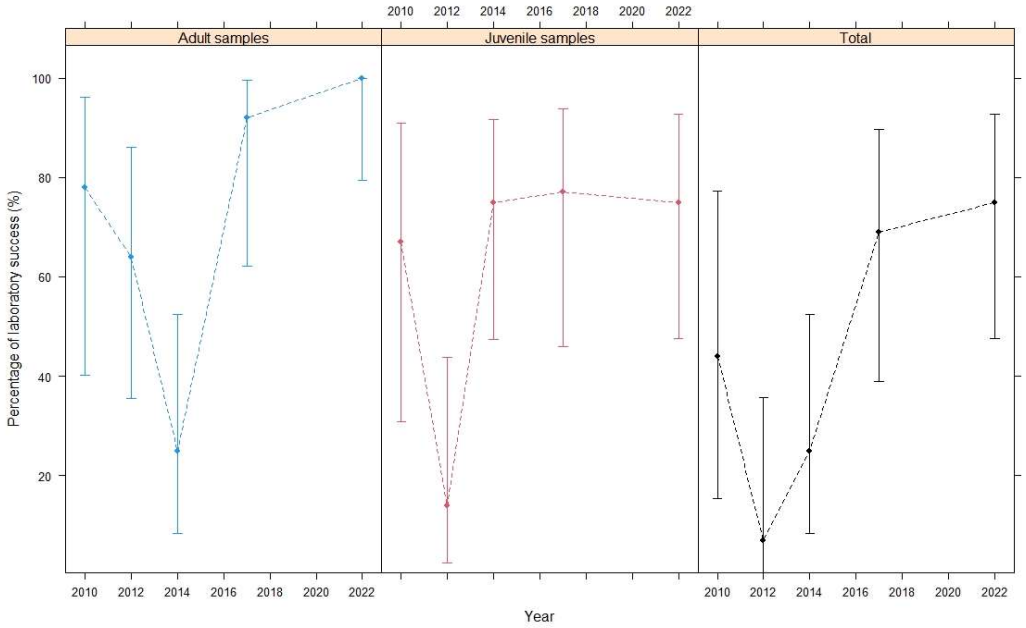


2022: 13/16 participating laboratories (81%) presented 100% concordant results in TTC test (100% on pos, 81% on neg).

Comparable proportion compared to previous sessions (2017, 2014, and 2012)

but with a higher performance compare to the first session of 2010.

Evolution of ILT results on age determination



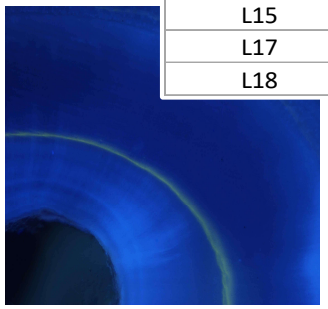
2022: 12/16 laboratories (75%) estimated a correct age class on the whole panel.

This is a comparable result with the 2017 session, with a higher performance compare to 2012 session.

Evolution of results in participating laboratories

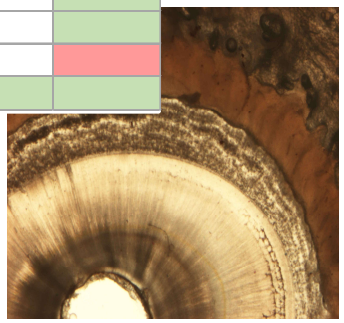
TTC detection

Laboratory code	2010	2012	2014	2017	2022
L01					
L02					
L03					
L04					
L05					
L06					
L07					
L08					
L09					
L11					
L12					
L13					
L14					
L15					
L17					
L18					



Age determination

Laboratory code	2010	2012	2014	2017	2022
L01					
L02					
L03					
L04					
L05					
L06					
L07					
L08					
L09					
L11					
L12					
L13					
L14					
L15					
L17					
L18					



Conclusion (1/2)

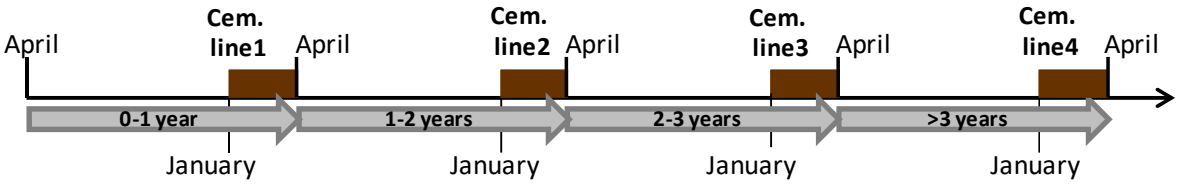
Constant satisfactory level of performance of the laboratories in both detection of tetracycline and age determination since the 2017 session. They are encouraging and demonstrate the laboratories capacity and the satisfactory results comparability for bait uptake estimations performed at EU level in the frame of ORV campaigns.

But

Conclusion (2/2)

Frequently, misinterpretations of age estimation are observed between juvenile samples (0-1 year) and adult samples of 1-2 years.

Taking into account the birth period of cubs in Europe in March-April, animals sampled in October-November, after the summer season, harbouring one pale line should have been aged >1-2 years and not 1-2 years old. This observation highlights the importance of taking into account the age of death of the animal for proper age estimation. **Animals sampled in autumn are easy to assess, while spring's samples are not because cementum line just appeared.**



Acknowledgments

- Laboratories who participated in this assessment
- NRL from Romania, specifically Vlad Vuta, for having provided positive jaws with TTC
- The Anses Staff, and principally Christophe Caillot and Léo Damoiseaux for the involvement in this work
- The EC for fundings this activity

anses

Thank you for your attention!

Questions?