Survey on diagnostic tests for ruminant helminth infections and anthelmintic resistance across COMBAR members' labs, and their Technology Readiness Level

A questionnaire on diagnostic tests used in the COMBAR members' labs was developed and made available online (from 26th September 2019 to 22nd April 2021) at the link https://survey.zohopublic.eu/zs/D9B86u.

The survey consisted of 11 questions listed on page 5.

A total of 37 researchers (out of 98 contacted) from 33 institutions (23 universities and 10 research centres) of 24 countries (Austria, Belgium, Czech Republic, Denmark, France, Germany, Greece, Hungary, Ireland, Italy, Lithuania, Netherlands, North Macedonia, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Spain, Switzerland, Tunisia, Turkey, United Kingdom) participated in the survey. In three institutions more than one researcher responded to the survey.

Most of the interviewed researchers focussed their studies on helminths and AR in sheep (89.2%), followed by goats (59.5%) and cattle (48.7%). In 43.2% of cases, researchers studied two/three ruminant species contemporaneously.

Moreover, most researchers (75.7%) were interested in both gastrointestinal nematodes (GIN) and liver flukes (*Fasciola hepatica*).

Coprological techniques and serological methods were the approaches most widely used for the diagnosis of GIN and *F. hepatica* infection in all ruminant species. In particular, McMaster (71.4%) and Mini-FLOTAC (40%) were the FEC methods most used to detect GIN infections, whereas sedimentation (56.7%) was the most used technique to detect *F. hepatica* infections.

As for the immunological techniques, the commercial ELISA kits (60%) were more used than the home-made ELISAs (40%) to detect GIN infections (e.g. bulk milk ELISA for *Ostertagia*), as well as for the diagnosis of *F. hepatica* (71.4%).

As for the DNA based techniques, the Real-time PCR (50%) and end-point PCR and/or nested-PCR (50%) were the most used approaches for GIN detection, while the PCR-end point and/or the nested-PCR (77.8%) and the Loop-mediated isothermal Amplification (LAMP) (44.4%) were the molecular techniques most used for the diagnosis of *F. hepatica*.

All the data collected in this survey are reported in the Annex 1. Links to dynamic maps of institutions that use the different diagnostic techniques are available under the static maps reported in the Annex 1.

A complete list of the diagnostics (tools, distributors, producers, prices, and targets) used in the COMBAR members' labs is reported on page 25.

The Technology Readiness Levels (TRLs) of the most used techniques for the diagnosis of GIN and *F. hepatica*, as well as for the detection of anthelmintic resistance are reported in Annex 2.

Noteworthy, also through the activities of this COST project techniques such as the Mini-FLOTAC method have been developed to TRL 9 level.

It appears remarkable that according to this survey still copromicroscopic techniques are by far the most often used methods for the direct detection of infections with helminth parasites in ruminants. This is certainly a major difference to other fields of infectious diseases such as bacteriology or virology, where molecular and proteomic approaches are being used in routine diagnostics to a much greater level. In addition to economics/costs also the comparatively high practicability and precision of copromicroscopic techniques might be regarded as reasons for their still high popularity. Concerning the molecular techniques for the identification of intestinal helminths in ruminants novel next-generation-sequencing approaches that have recently been described, are currently being established in several COMBAR labs. These as well as the already established molecular tools require further increase concerning TRL. However, proteomic tools such as Matrix-Assisteted-Laser-Desorption-Ionisation/Time-Of-Flight assays have thus far not been employed for the identification and differentiation of helminth infections in ruminants.

Annex 1- Outcome of the survey

WG1
Survey on Diagnostic Tools used in COMBAR members' labs

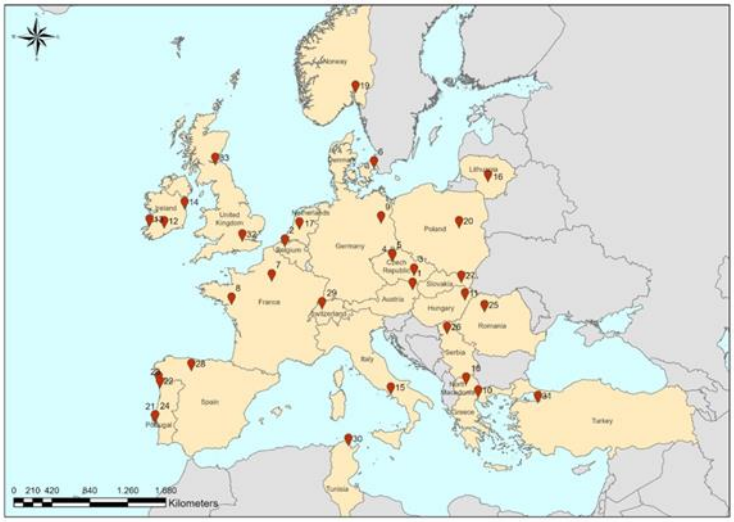
26th September 2019- 22nd April 2021

Aim: To create a list of harmonized, validated and newly introduced diagnostic tests across European COMBAR labs

Survey questionnaire

11 Questions:

1. Main ruminant species studied
2. Type of samples (faeces, blood, serum, milk, etc.) used to detect gastrointestinal nematode (GIN) infection
3. Type of samples (faeces, blood, serum, milk, etc.) used to detect *Fasciola hepatica* infection
4. Technique used to detect GIN infection
5. Technique used to detect *Fasciola hepatica* infection
6. Indicate the FEC technique used to detect GIN eggs
7. Indicate the FEC technique used to detect *Fasciola hepatica* eggs
8. Indicate the immunological technique used to detect GIN infection, if it is a home made or a commercial kit
9. Indicate the immunological technique used to detect *Fasciola hepatica* infection, if it is a home made or a commercial kit
10. Specify the DNA-based technique to detect GIN infection
11. Specify the DNA-based technique to detect *Fasciola hepatica* infection



33 Labs – 24 Countries

Link to dynamic map: https://arcg.is/190LWW

ID	Institution	Country	Survey responders
1	Institut für Parasitologie und Zoologie	Austria	1
2	Ghent University	Belgium	1
3	Masaryk University	Czech Republic	1
4	Czech University of Life Sciences Prague	Czech Republic	2
5	Charles University, Faculty of Pharmacy	Czech Republic	1
6	University of Copenhagen	Denmark	1
7	Agence Nationale de Sécurité Sanitaire Alimentaire	France	1
8	Institute National de la Recherche Agronomique	France	1
9	Institute of Parasitology and Tropical Veterinary Medicine, FU Berlin	Germany	1
10	Veterinary Research Institute Hellenic Agricultural Organization ELGO-DIMITRA	Greece	1
11	University of Debrecen	Hungary	1
12	Agriculture and Food Development Authority, Teagasc	Ireland	1
13	Department of Agriculture, Food and the Marine	Ireland	1
14	University College of Dublin	Ireland	1
15	University of Naples Federico II	Italy	1
16	Lithuanian University of Health Sciences, Veterinary Academy	Lithuania	1
17	Utrecht University	Netherlands	1
18	Local Action Group Agro Lider	North Macedonia	1
19	Norwegian University of Life Sciences	Norway	3
20	Warsaw University of Life Sciences	Poland	1
21	Instituto de Higiene e Medicina Tropical	Portugal	1
22	Escola Superior Agrária, Instituto Politécnico de Viana do Castelo	Portugal	1
23	Instituto Nacional de Investigação Agrária e Veterinária	Portugal	1
24	Lusofona University of Lisbon	Portugal	1
25	University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca	Romania	1
26	University of Novi Sad, Faculty of Agriculture	Serbia	1
27	Institute of Parasitology	Slovakia	1
28	Universidad de León, Institute of Mountain Livestock	Spain	1
29	Institute of Parasitology	Switzerland	1
30	Ecole Nationale de Médecine Vétérinaire	Tunisia	1
31	Bursa Uludag University	Turkey	1
32	Animal and Plant Health Agency	United Kingdom	1
33	Moredun Research Institute	United Kingdom	2

Figure 1. Map and list of COMBAR labs that participated at the questionnaire survey on diagnostic tests.

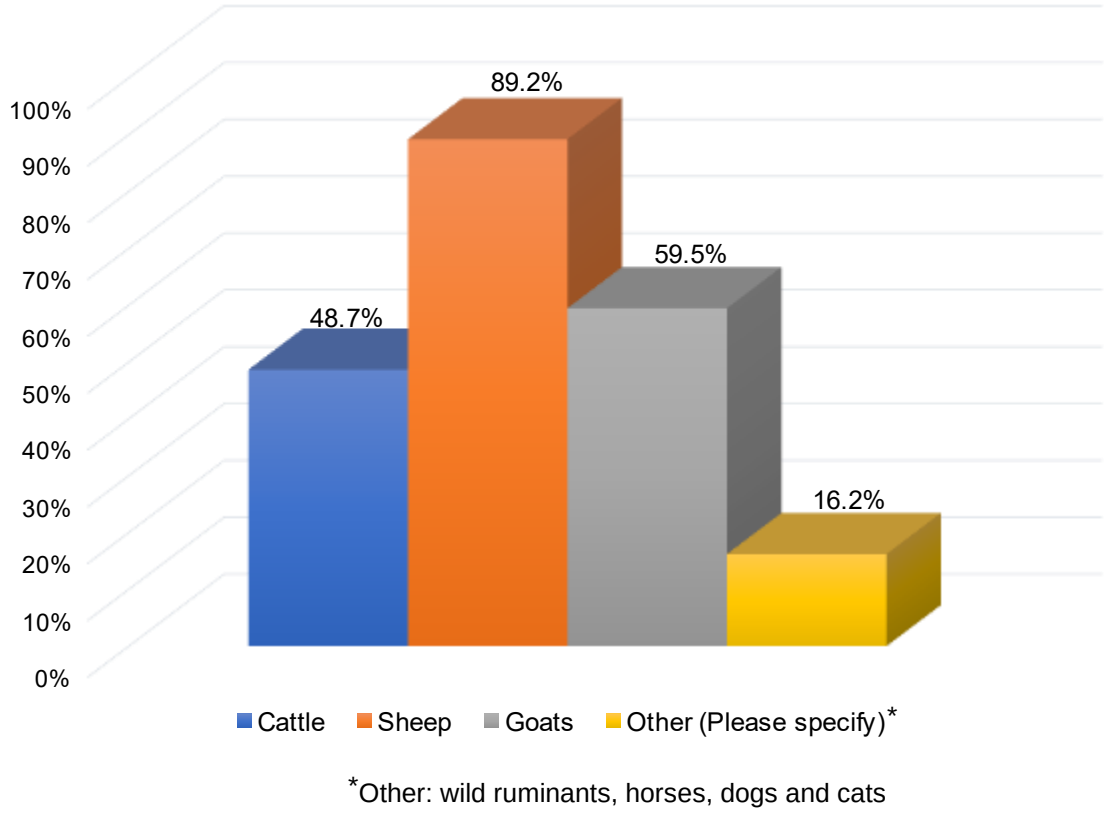


Figure 2. Main ruminant species studied in the COMBAR labs.

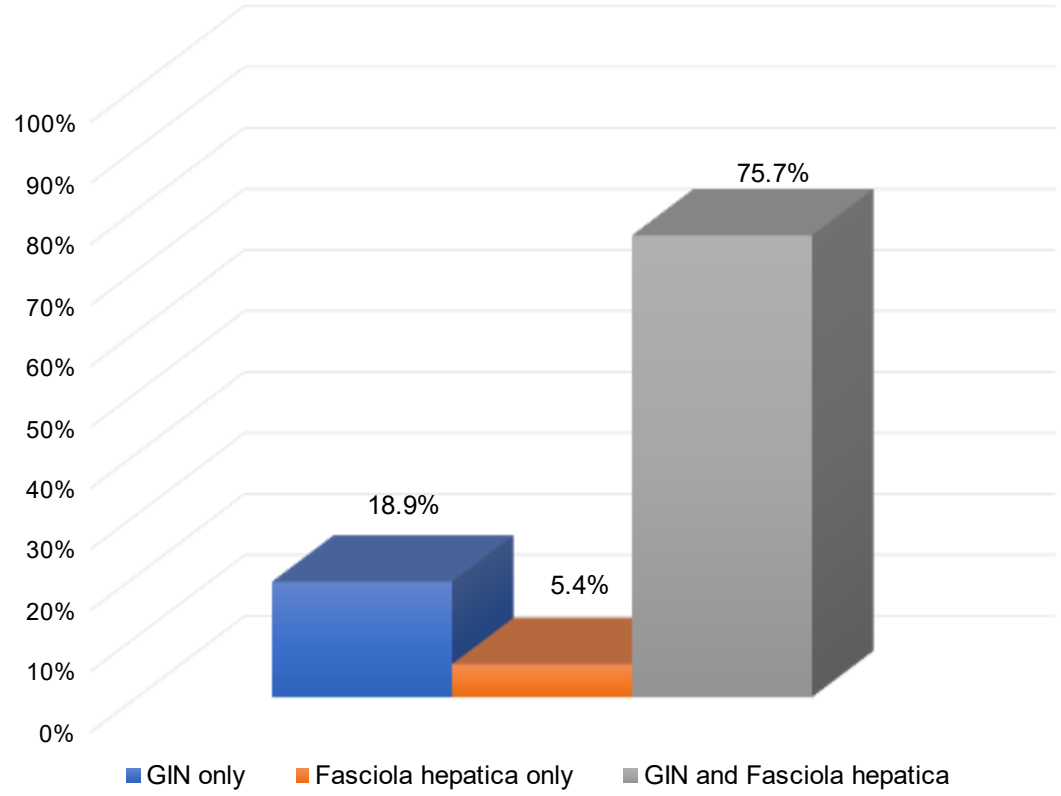
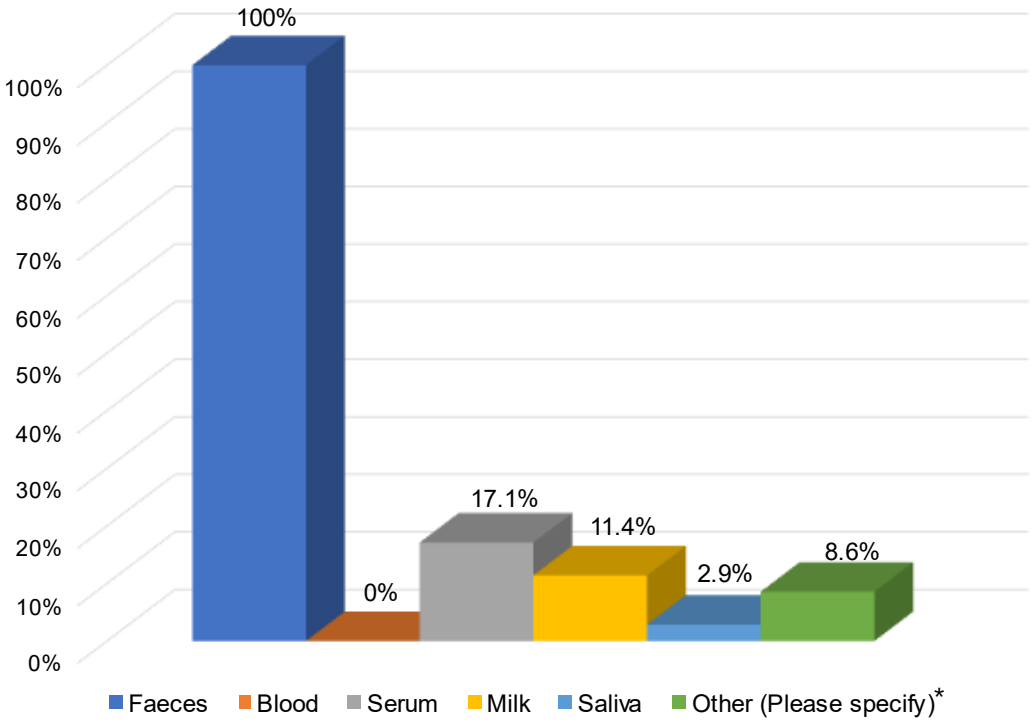


Figure 3. Main parasites diagnosed in the COMBAR labs.



*Other: necropsy - nasal secretions

Figure 4. Type of matrices (faeces, blood, serum, milk etc.) used for the diagnosis of GIN in the COMBAR labs.

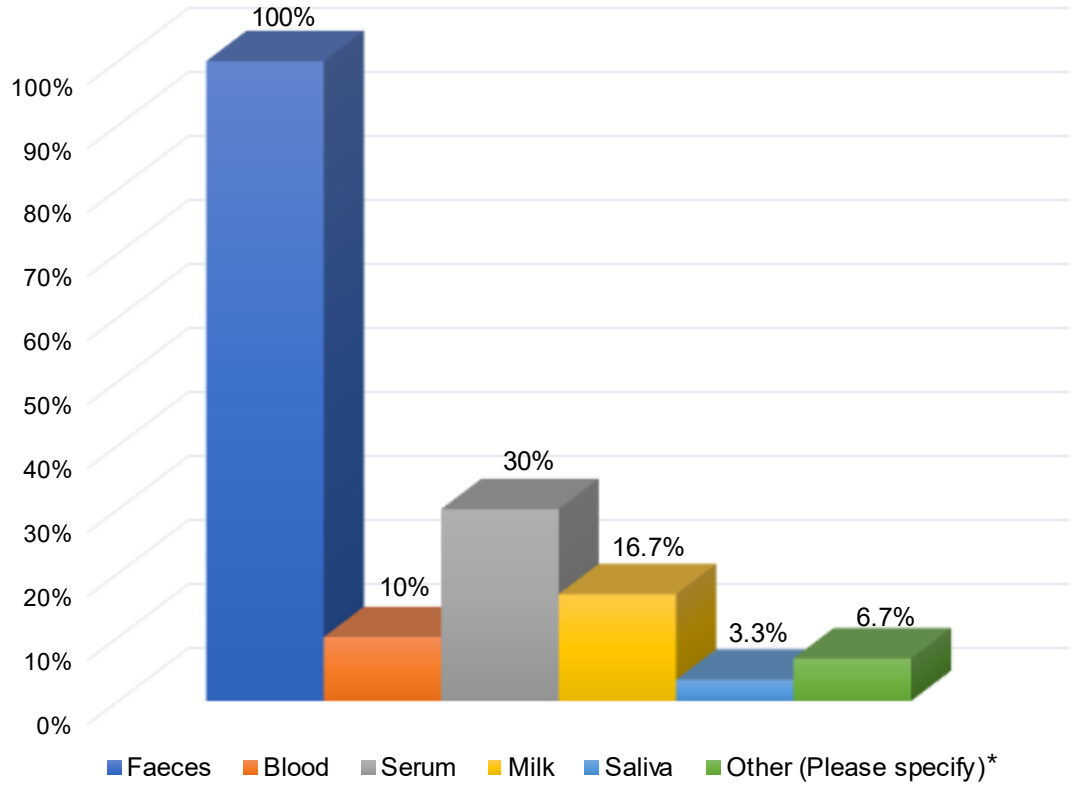


Figure 5. Type of matrices (faeces, blood, serum, milk etc.) used for the diagnosis of *Fasciola hepatica* in the COMBAR labs.

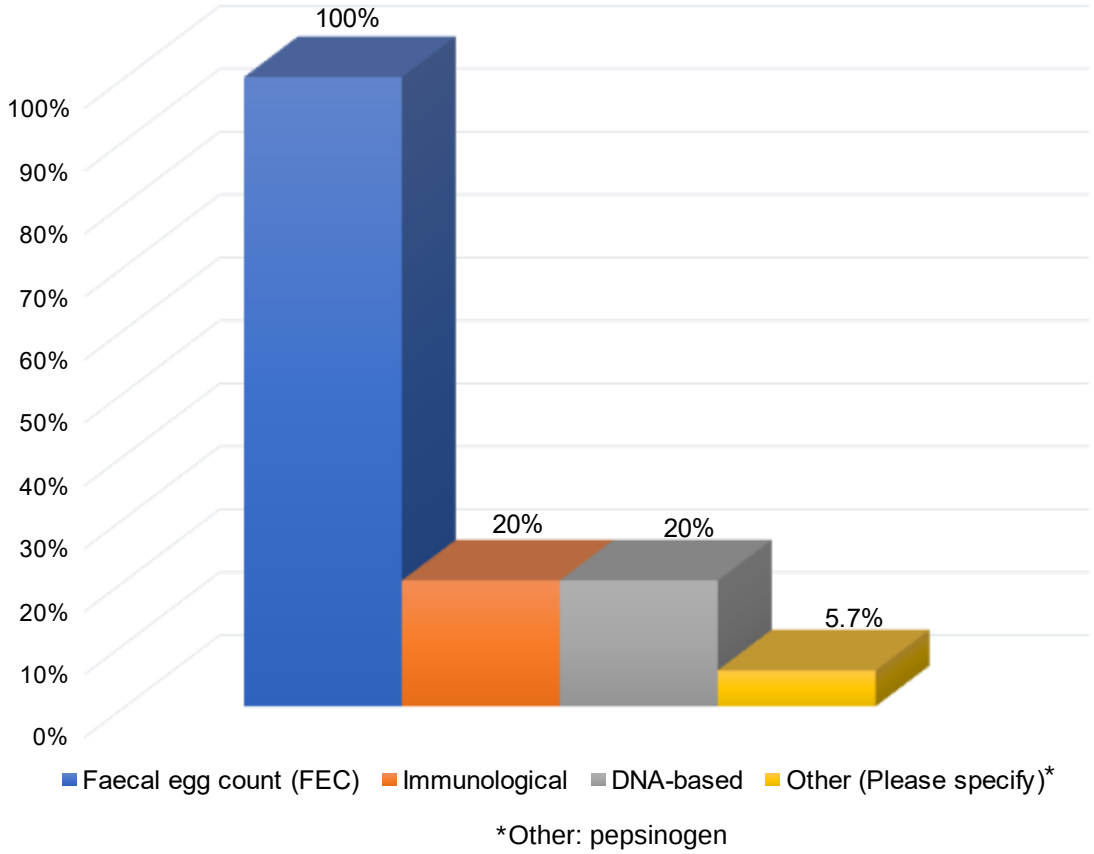


Figure 6. Techniques used to detect GIN infection in the COMBAR labs.

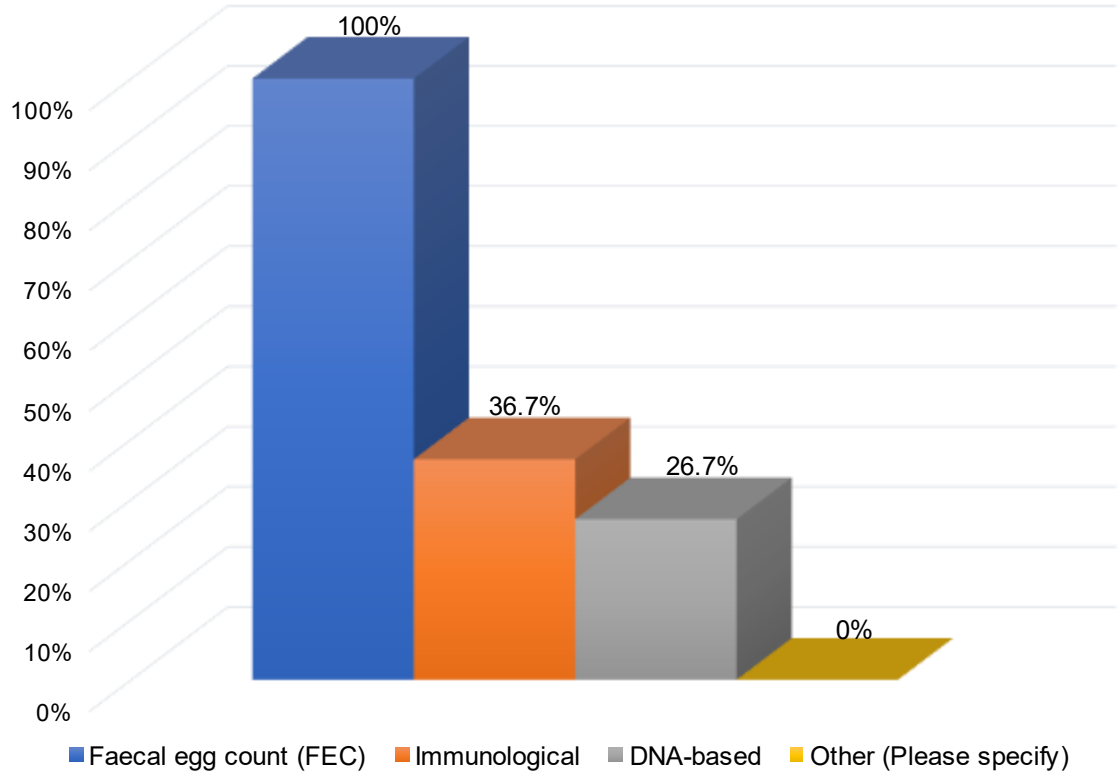
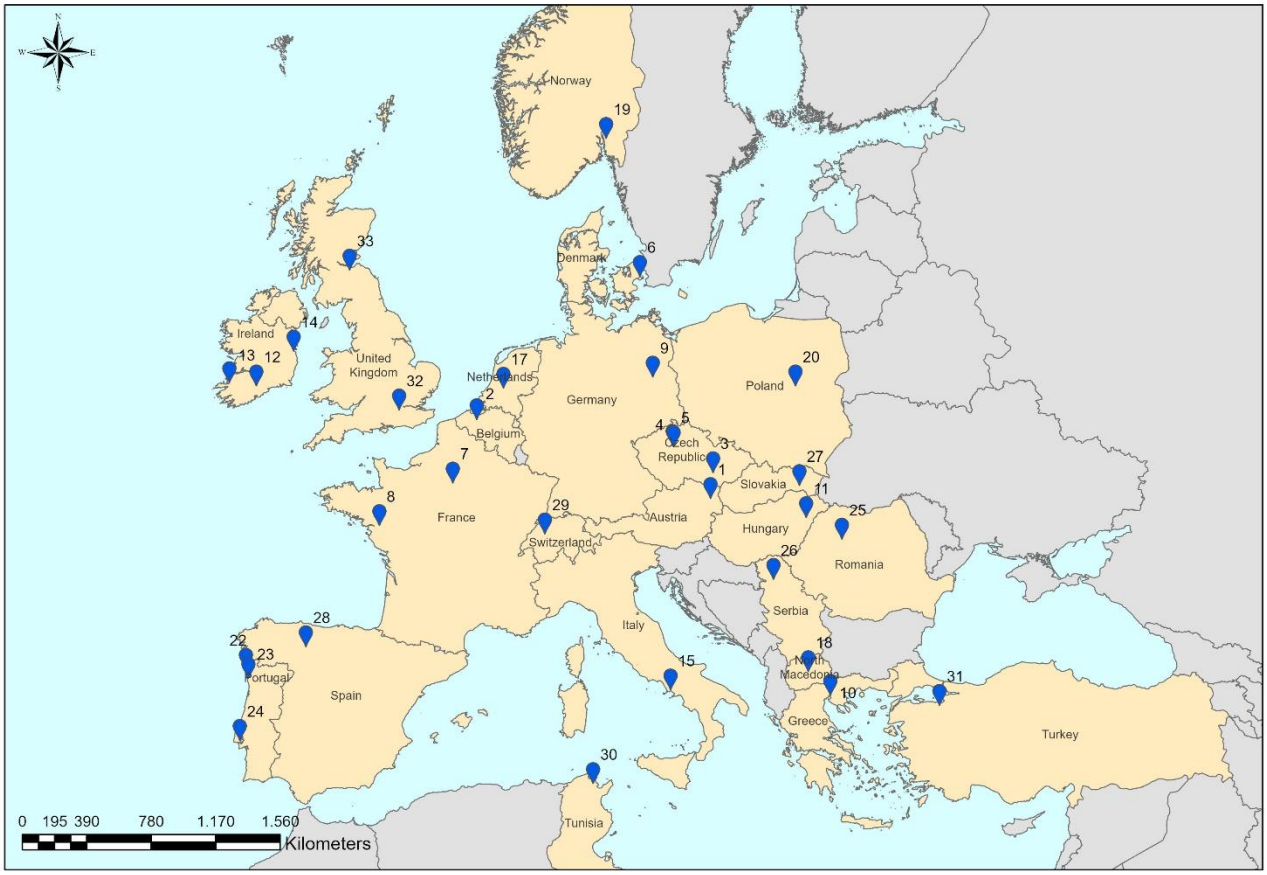


Figure 7. Techniques used to detect *Fasciola hepatica* infection in the COMBAR labs.



31 Labs – 23 Countries

Link to dynamic map: https://arcg.is/0SiGDP

Figure 8. COMBAR labs where FEC techniques are used for diagnosis of GIN.

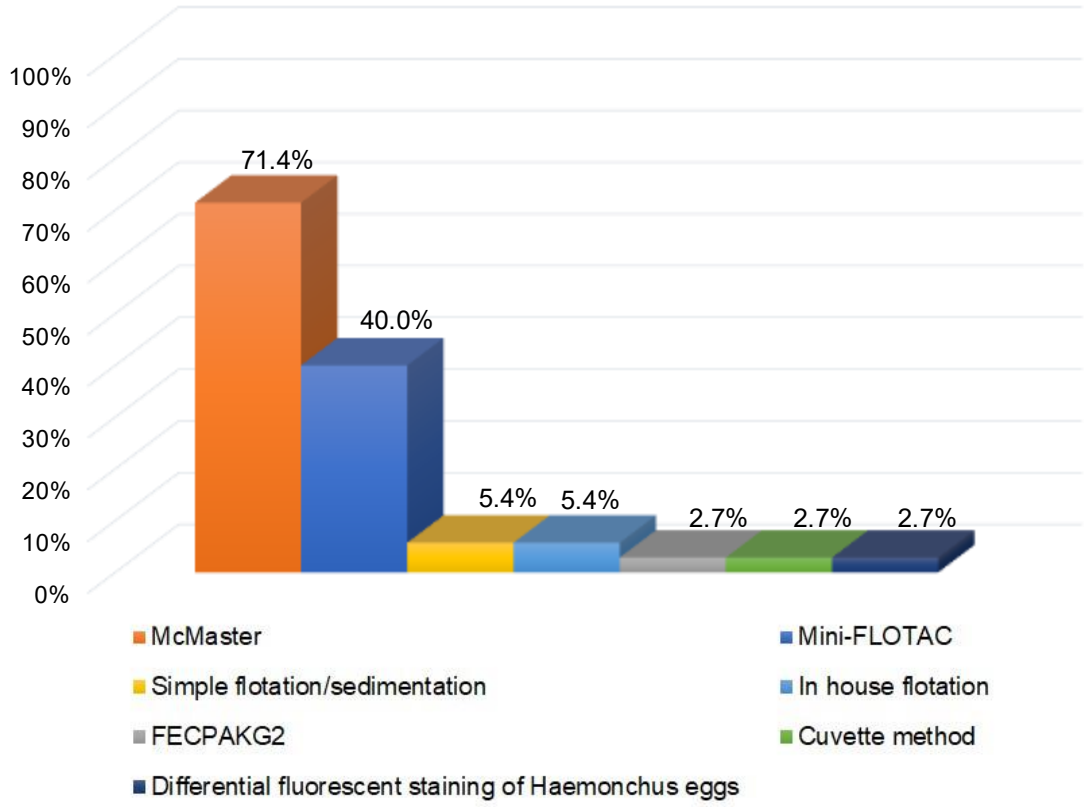
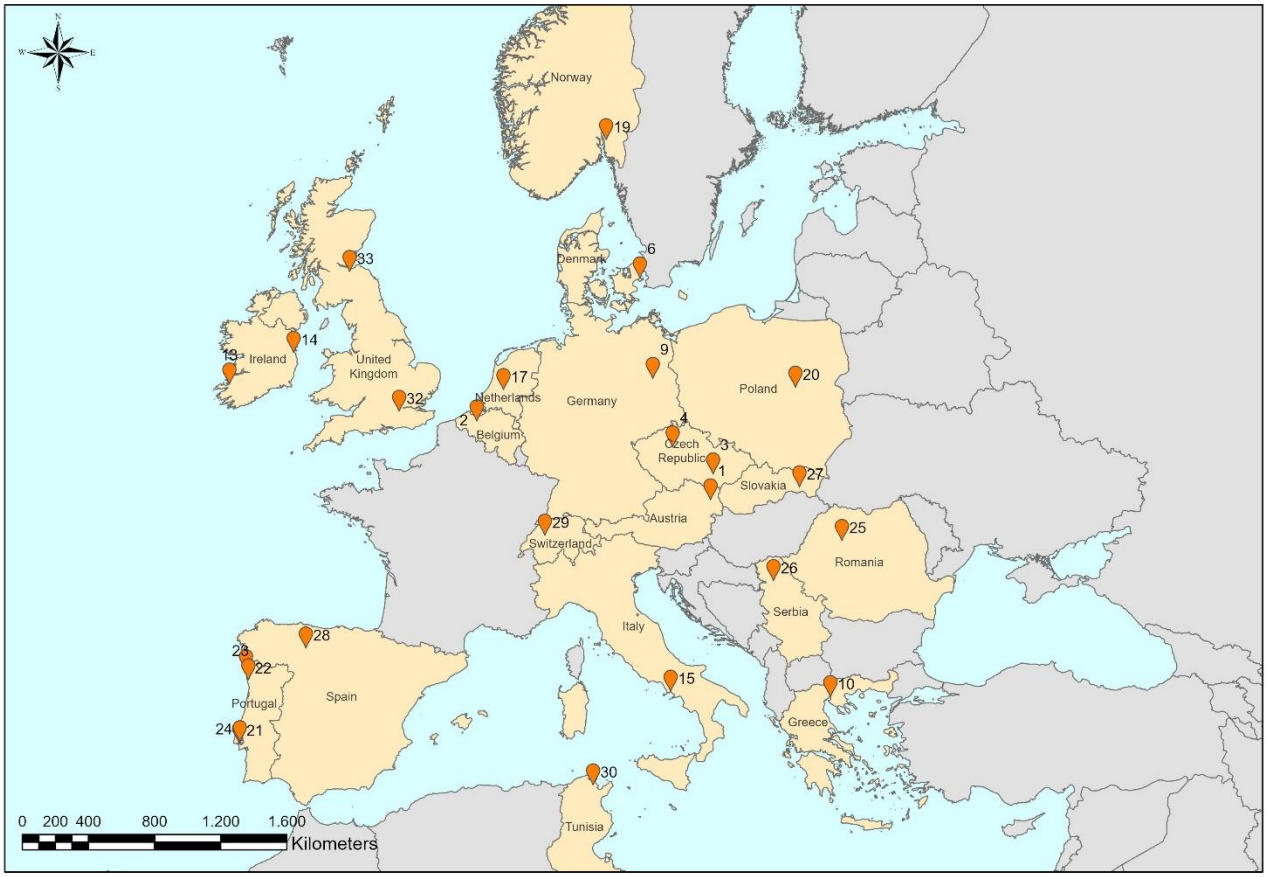


Figure 9. FEC techniques used for diagnosis of GIN eggs in the COMBAR labs.



25 Labs – 19 Countries

Link to dynamic map: https://arcg.is/0SiGDP

Figure 10. COMBAR labs where FEC techniques are used for diagnosis of *Fasciola hepatica*.

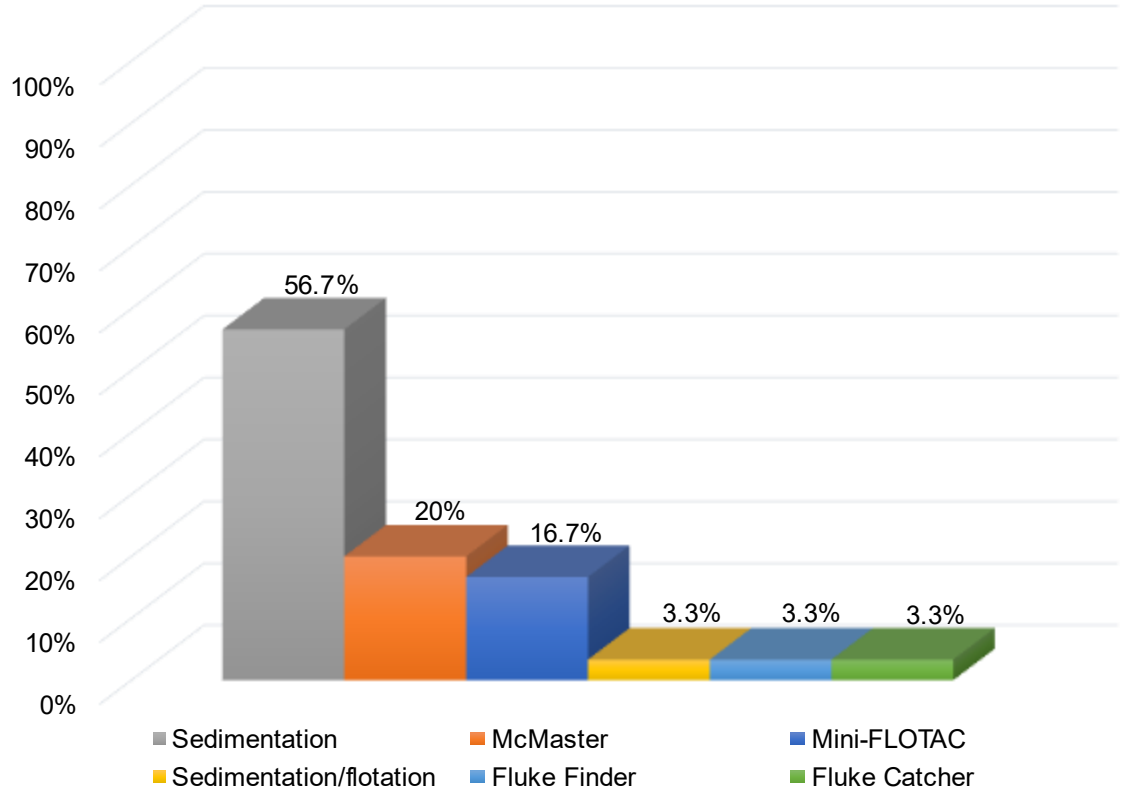
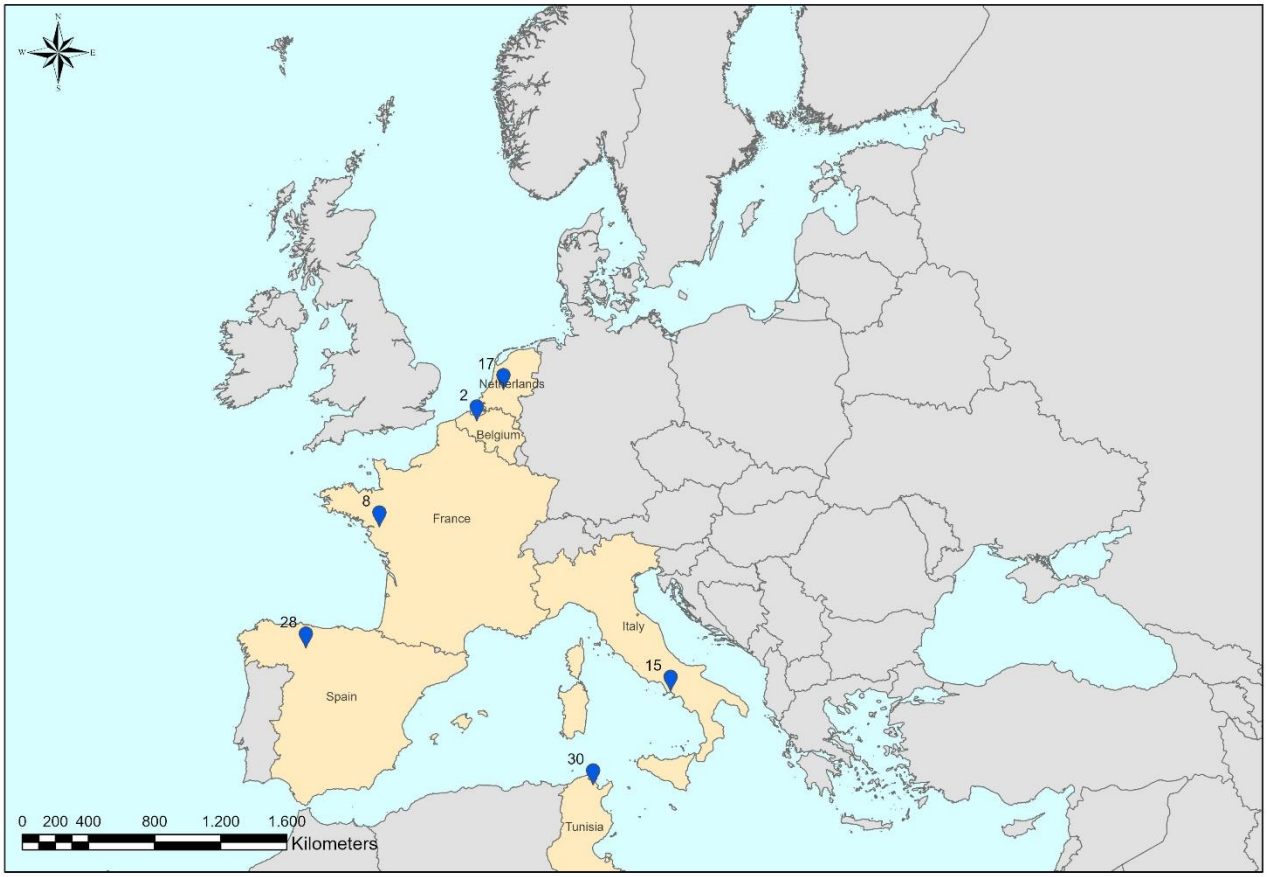


Figure 11. FEC techniques used for diagnosis of *Fasciola hepatica* eggs in the COMBAR labs.



6 Labs – 6 Countries

Link to dynamic map: https://arcg.is/SvKWa1

Figure 12. COMBAR labs where immunological techniques are used for diagnosis of GIN infection.

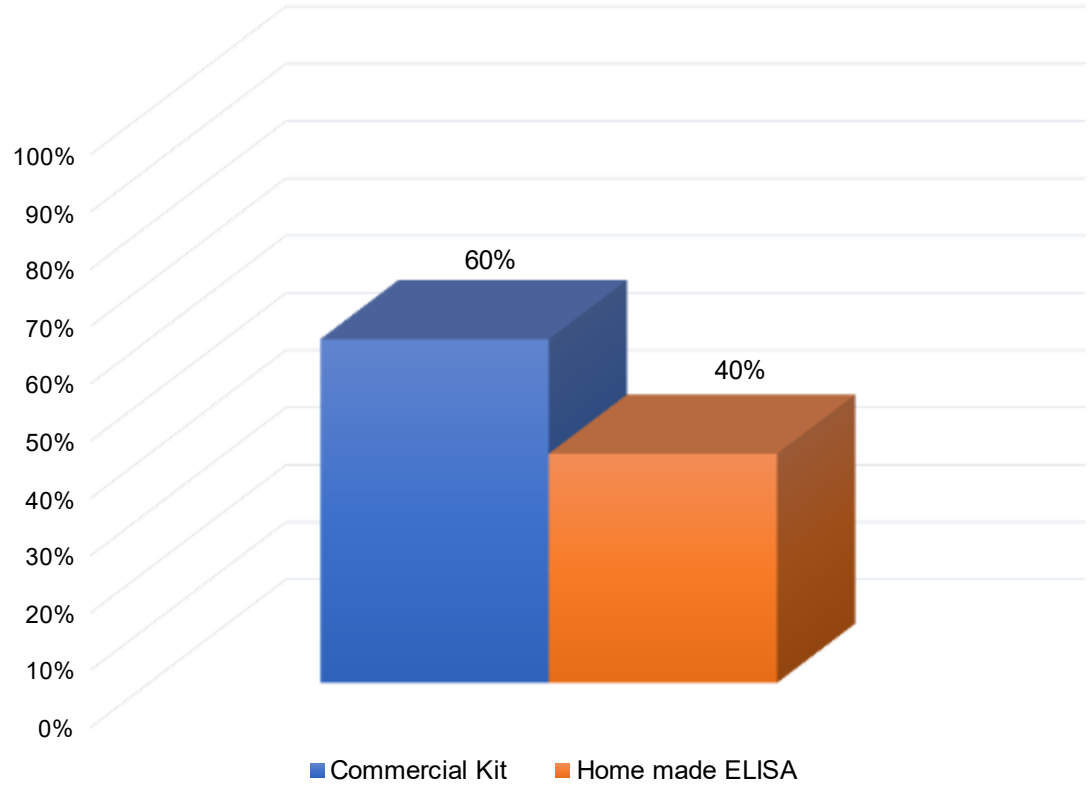
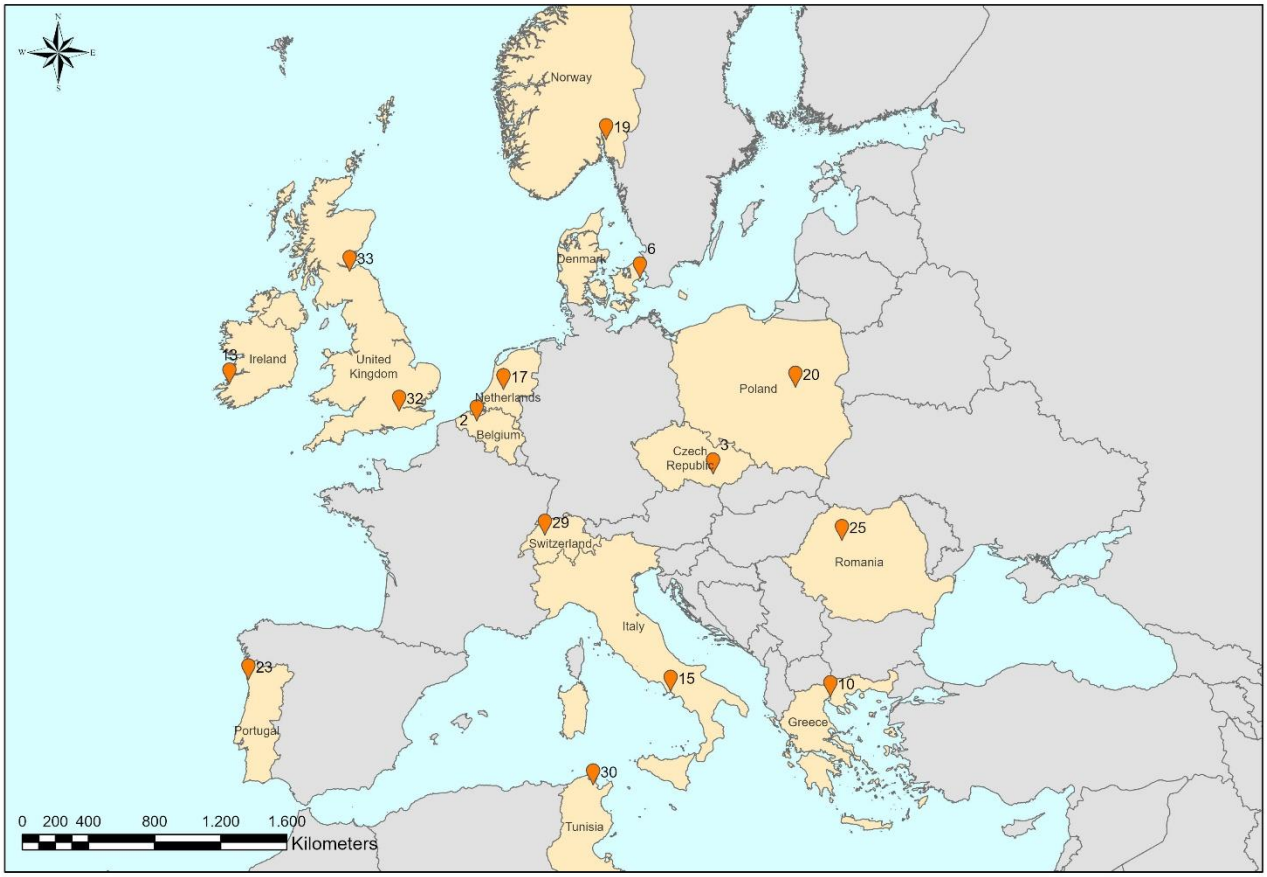


Figure 13. Immunological techniques used to detect GIN infection in the COMBAR labs.



15 Labs – 14 Countries

Link to dynamic map: https://arcg.is/SvKWa1

Figure 14. COMBAR labs where immunological techniques are used for diagnosis of *Fasciola hepatica* infection.

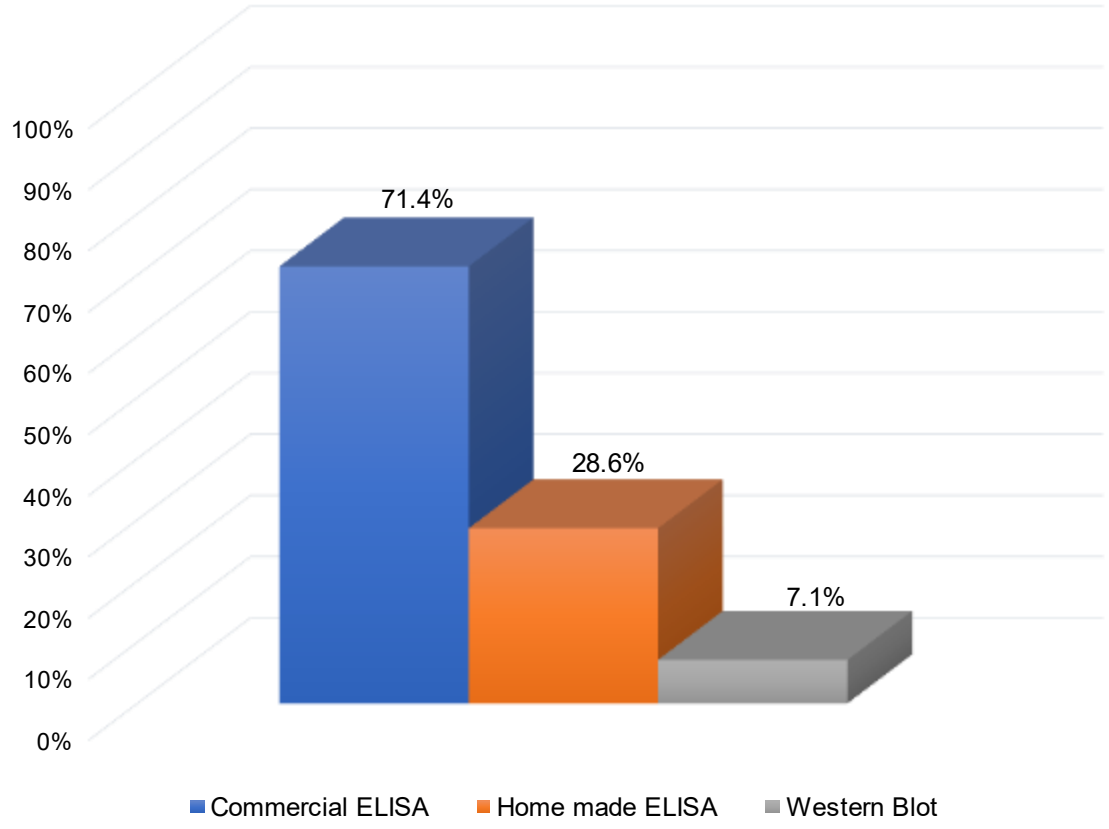
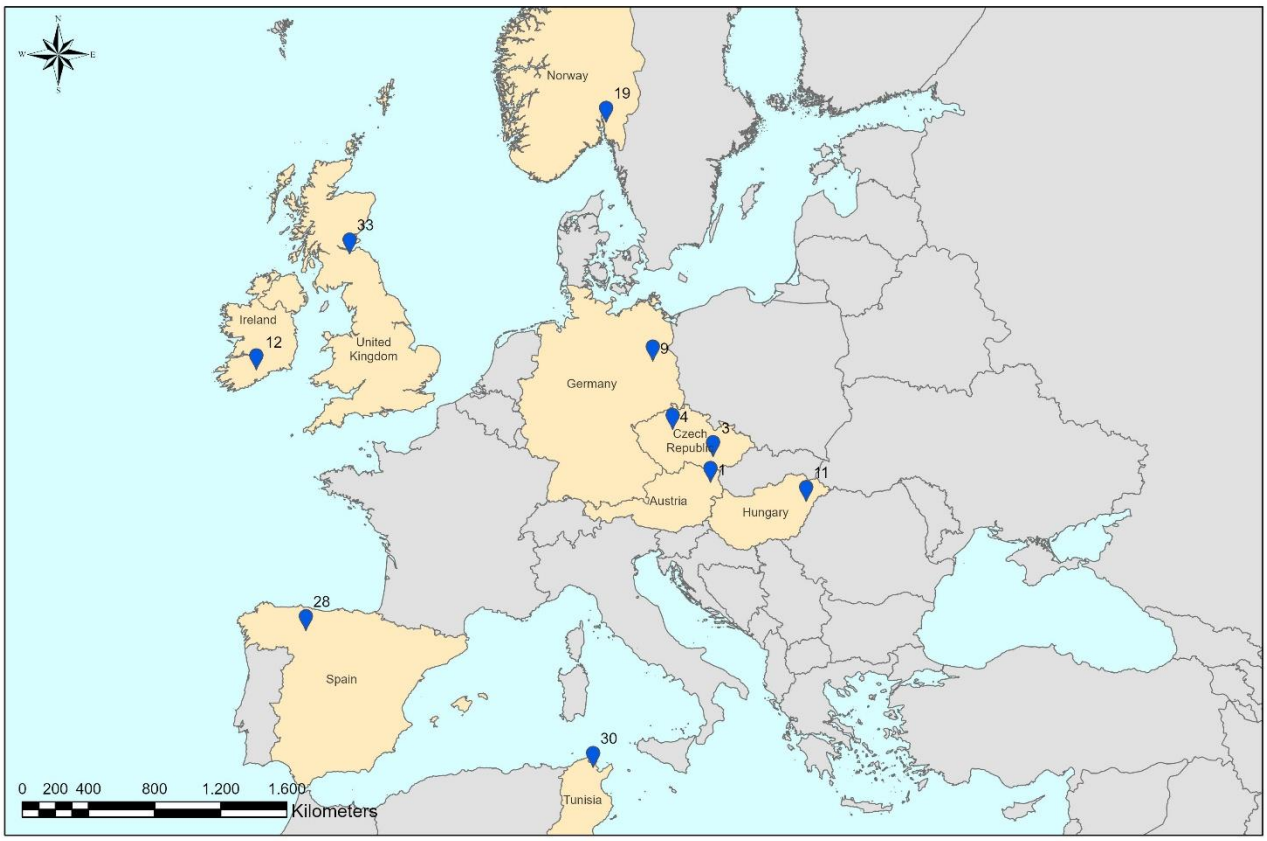


Figure 15. Immunological techniques used to detect *Fasciola hepatica* infection in the COMBAR labs.



10 Labs – 9 Countries

Link to dynamic map: https://arcg.is/ieOz90

Figure 16. COMBAR labs where DNA-based techniques are used for diagnosis of GIN infection.

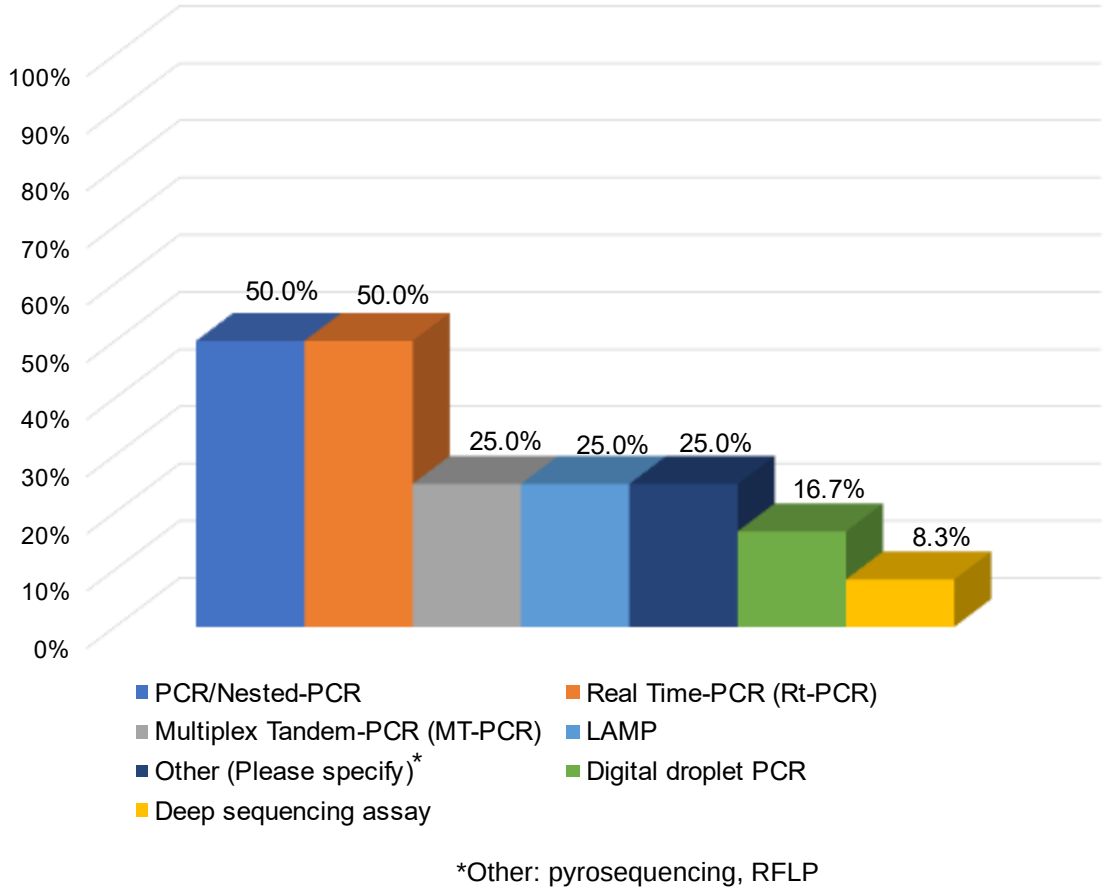
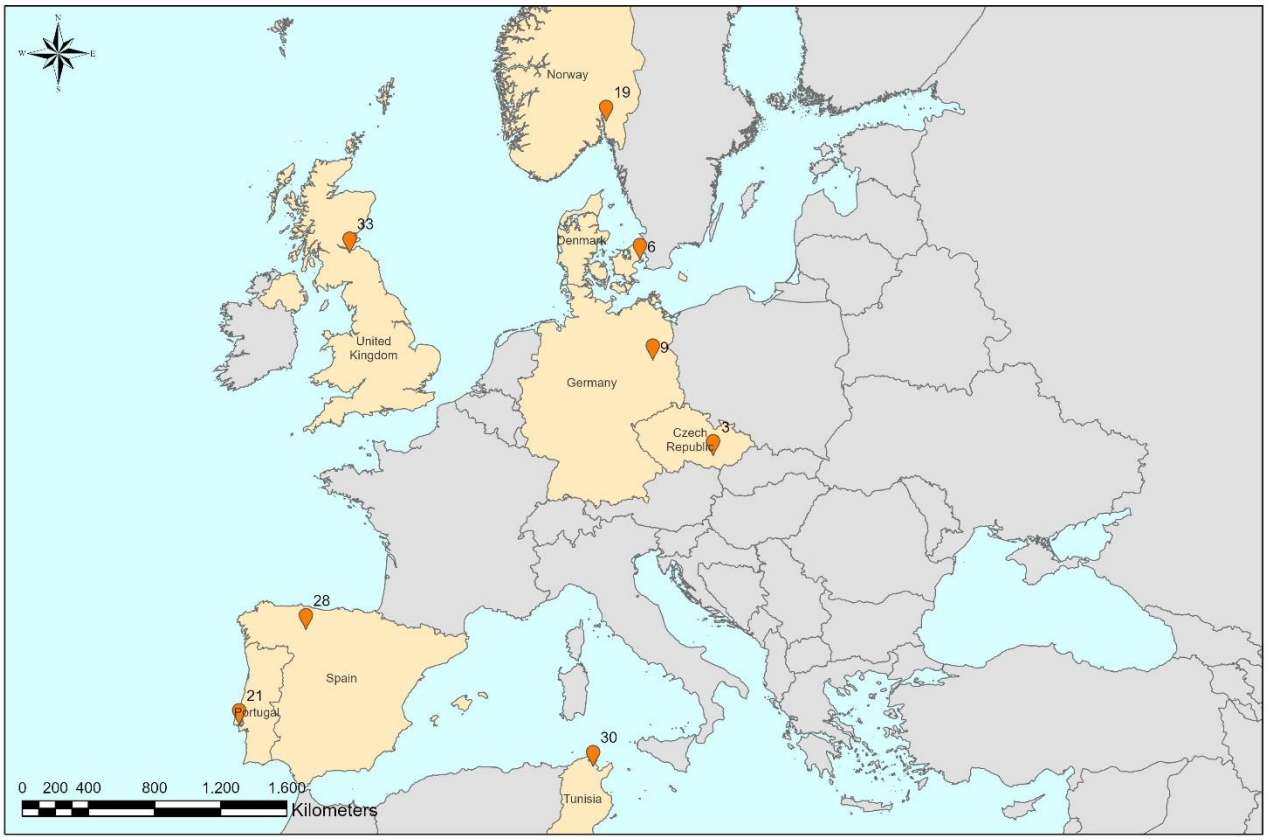


Figure 17. DNA-based techniques used to detect GIN infection in the COMBAR labs.



8 Labs – 8 Countries

Link to dynamic map: https://arcg.is/ieOz90

Figure 18. COMBAR labs where DNA-based techniques are used for diagnosis of *Fasciola hepatica* infection.

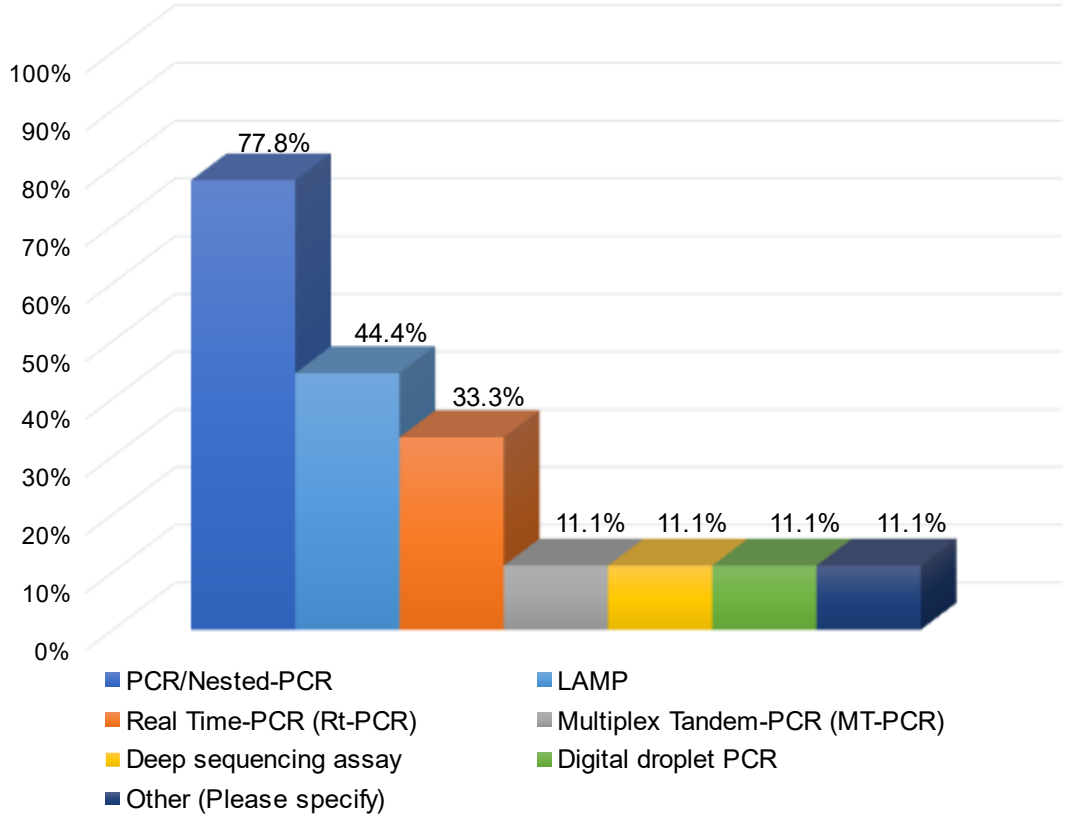


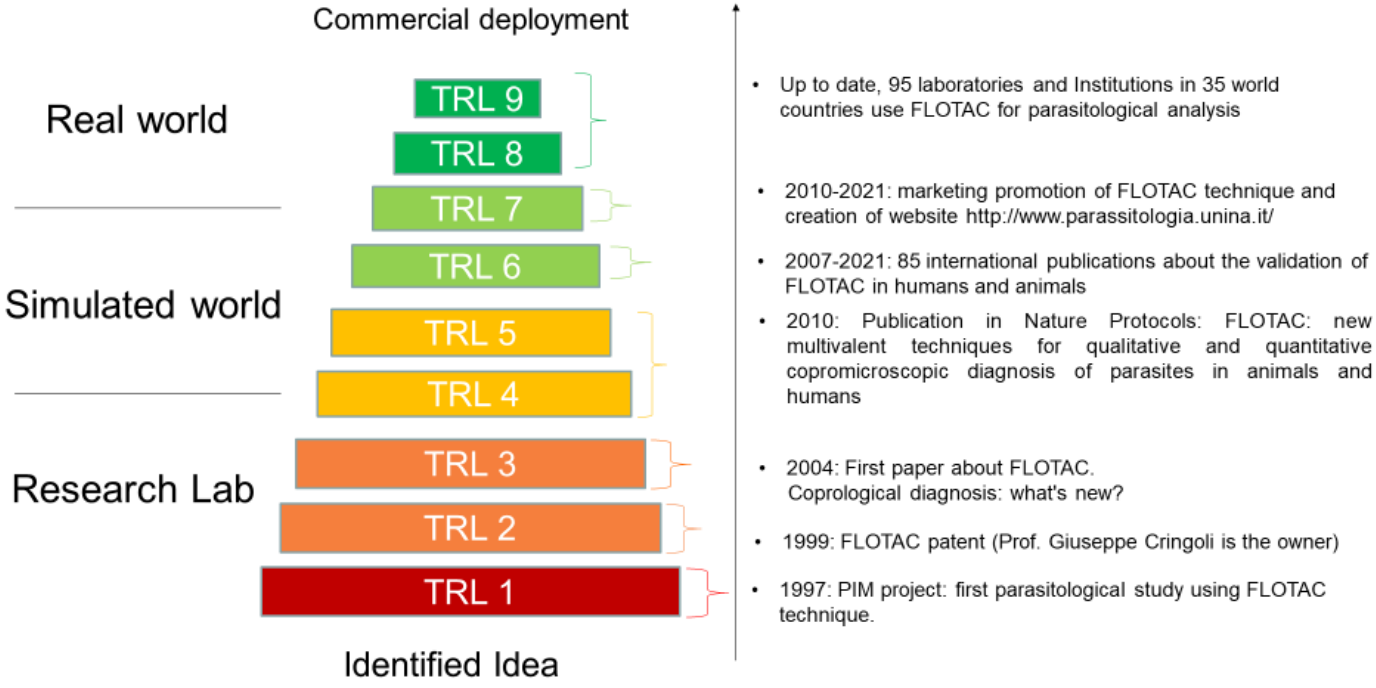
Figure 19. DNA-based techniques used to detect *Fasciola hepatica* infection in the COMBAR labs.

Tool	Distributor	Producer	Price	TARGET
Simple flotation				All
Sedimentation				All
McMaster (acrylic)	Vetlab supplies	Vetlab supplies	£39.50 (ex VAT)	All
	Focal Point (www.mcmaster.co.za)	Focal Point (www.mcmaster.co.za)	30 $	All
	Eggzamin	Eggzamin	17.95$	All
	Chalex	Chalex	15$ (opaque lines)-20$ (green lines)	All
McMaster (glass)	Marienfeld Superior	Marienfeld Superior	£119.00 (ex VAT)	All
FECPAK	Techion Group LTD	Techion Group LTD	600 €	All
FLOTAC Kit	University of Naples Federico II	Ideal Plastik Sud srl	250 €	All
Mini-FLOTAC	University of Naples Federico II	Ideal Plastik Sud srl	15 €	All
Fill-FLOTAC	University of Naples Federico II	Ideal Plastik Sud srl	10 €	All
Fluorescent stains - GIN eggs			/	Livestock
Cuvette method (Jackson, F., 1974)				All
Flukefinder	Flukefinder	Flukefinder	$149.00	Livestock
FlukeCatcher	Provinos	Provinos	95 €	Livestock
ELISA-Fasciola hepatica	IDEXX	IDEXX	550 €	Cattle and sheep
ELISA- SVANOVIR Ostertagia Ostertagi	SVANOVA	SVANOVA	583,20 €	Cattle
ELISA - SVANOVIR Fasciola hepatica	SVANOVA	SVANOVA	583,20 €	Cattle
BioX C-Elisa Fasciola	Listarfish	BioX Diagnostic S.A.	486,00 €	Livestock
ISOLATE Fecal DNA Kit	Bioline	Bioline	/	All
QIAamp DNA Stool Mini Kit	Qiagen	Qiagen	240 €	All
Quick-DNA Fecal/Soil Microbe Kits	Zymo Research	Zymo Research	/	All
Stool DNA Isolation Kit	Biochain	Biochain	/	All
Genomic DNA Extraction Kit - Stool	Cepham Life Sciences, Inc.	Cepham Life Sciences, Inc.	/	All
PCR end-point/nested/multiplex			/	All
Rt-PCR			/	All
MT-PCR	AusDiagnostics	AusDiagnostics	1000€ x 96 samples	All
Droplet-PCR	Biorad	Biorad	/	All
LAMP			/	All

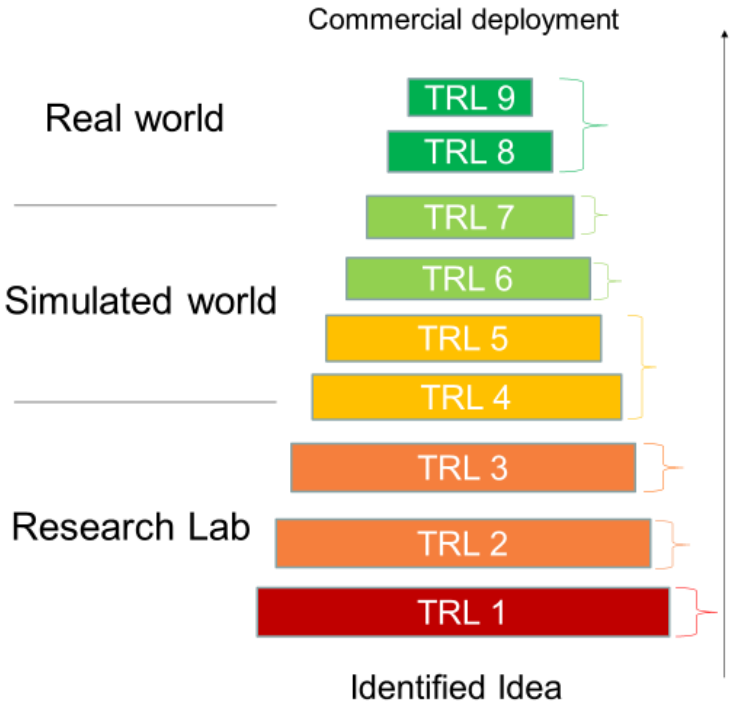
Figure 20. List of diagnostics used in the COMBAR labs.

Annex 2- Technology Readiness Levels (TRLs)

FLOTAC

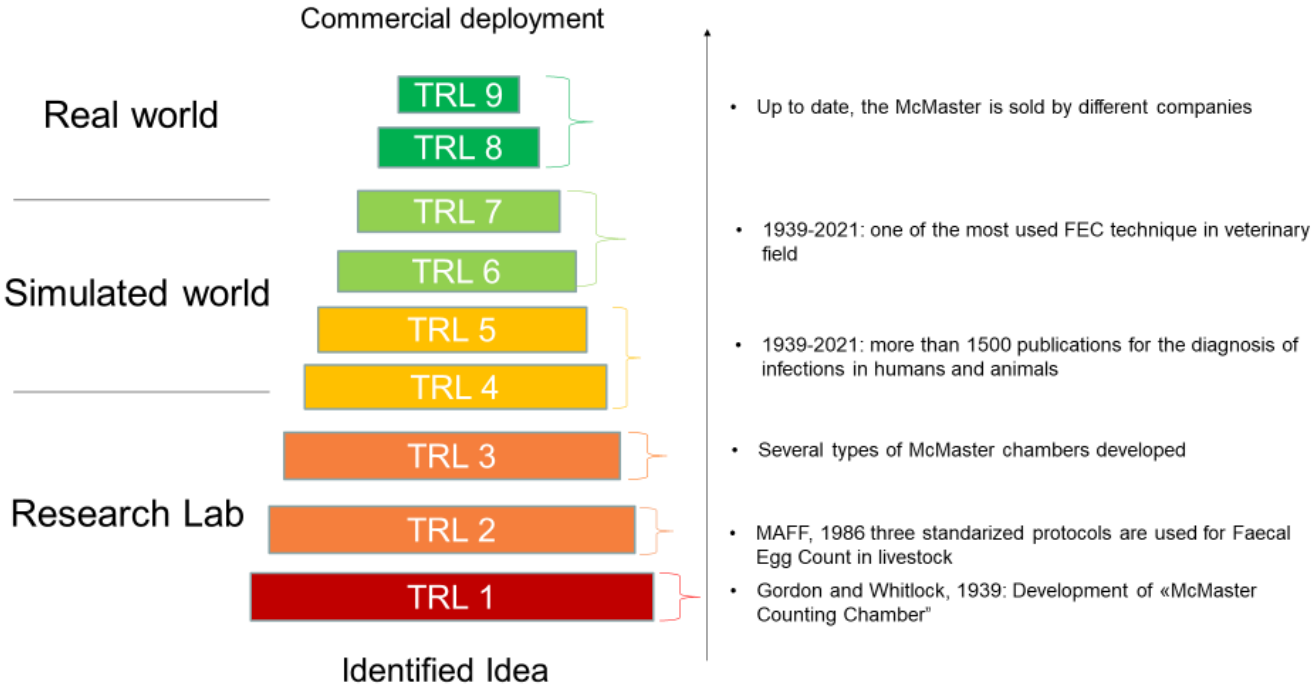


Mini-FLOTAC

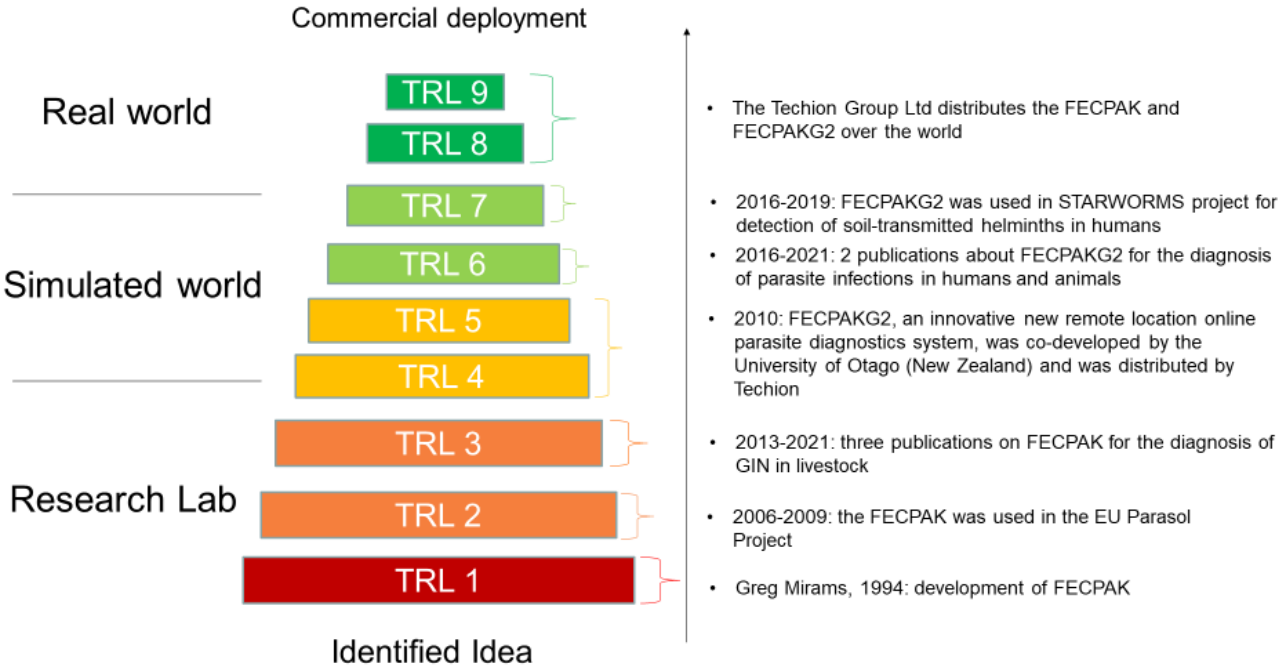


- Up to date, 488 laboratories and Institutions in 61 world countries use Mini-FLOTAC and Fill-FLOTAC for parasitological analysis
- SWOT and PESTEL analysis published by Maurelli et al., 2020.in Front Vet Sci, 7: 580649
- SOPs for SGI diagnosis published in Charlier et al., 2021 in Adv. Parasitol.
- 2013-2021: marketing of Mini-FLOTAC technique and creation of website http://www.parassitologia.unina.it.
- 2017: publication in Nature Protocols «The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals».
- 2013-2021: 64 international publications about the validation of Mini-FLOTAC in humans and animals.
- 2013: First full paper about Mini-FLOTAC technique: «Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of Lake Victioria»
- 2013: Mini-FLOTAC patent (Prof. Giuseppe Cringoli is the owner)
- 2012: GLOWORM project (first study using Mini-FLOTAC technique) : «Innovative and sustainable strategies to mitigate the impact of global change on helminth infections in ruminants»

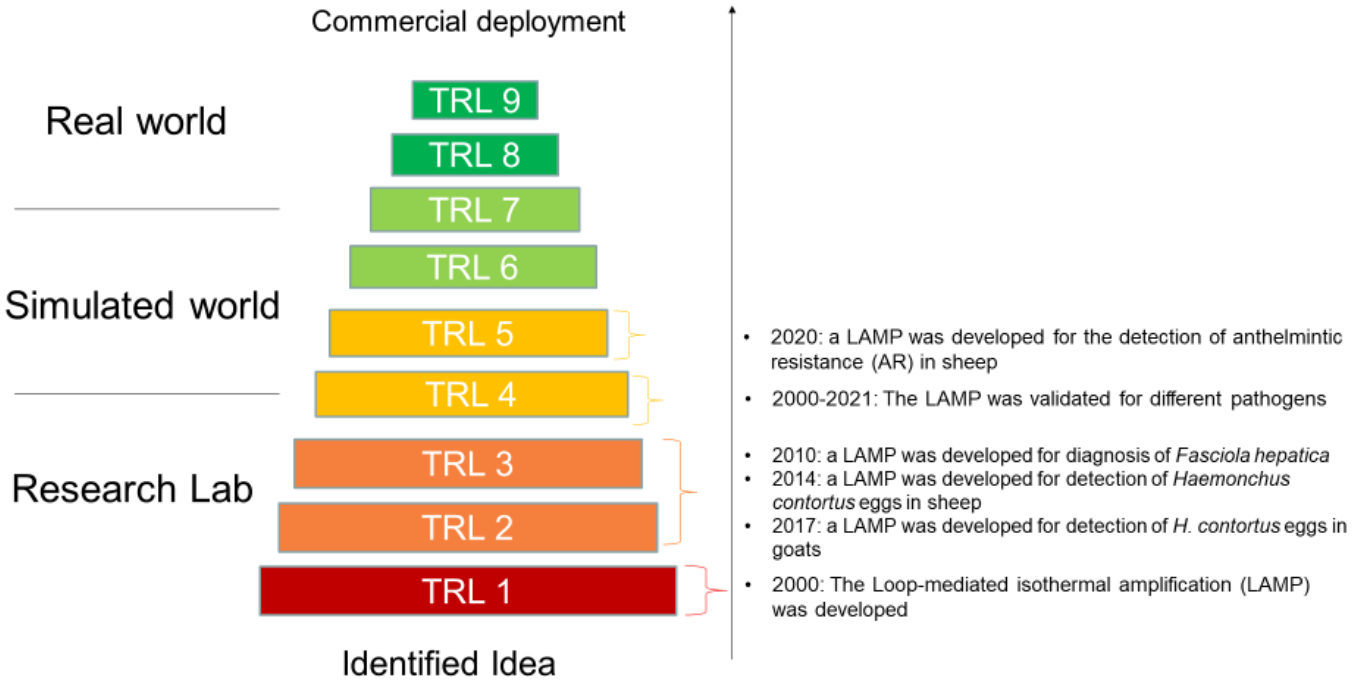
McMaster


Commercial deployment
Real world
TRL 9
TRL 8
TRL 7
Simulated world
TRL 6
TRL 5
TRL 4
TRL 3
Research Lab
TRL 2
TRL 1
Identified Idea
• Up to date, the McMaster is sold by different companies
• 1939-2021: one of the most used FEC technique in veterinary field
• 1939-2021: more than 1500 publications for the diagnosis of infections in humans and animals
• Several types of McMaster chambers developed
• MAFF, 1986 three standarized protocols are used for Faecal Egg Count in livestock
• Gordon and Whitlock, 1939: Development of «McMaster Counting Chamber"

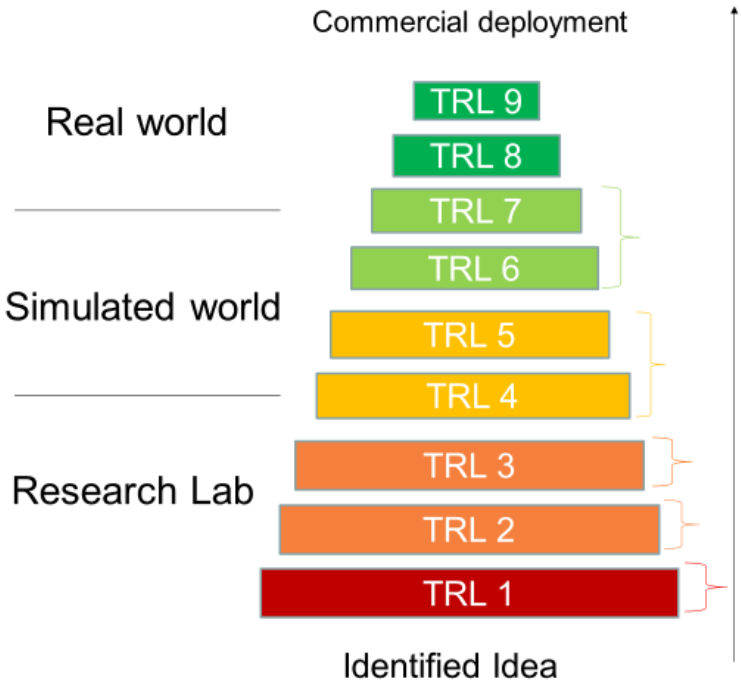
FECPAK/FECPAKG2



Loop-mediated isothermal Amplification (LAMP)

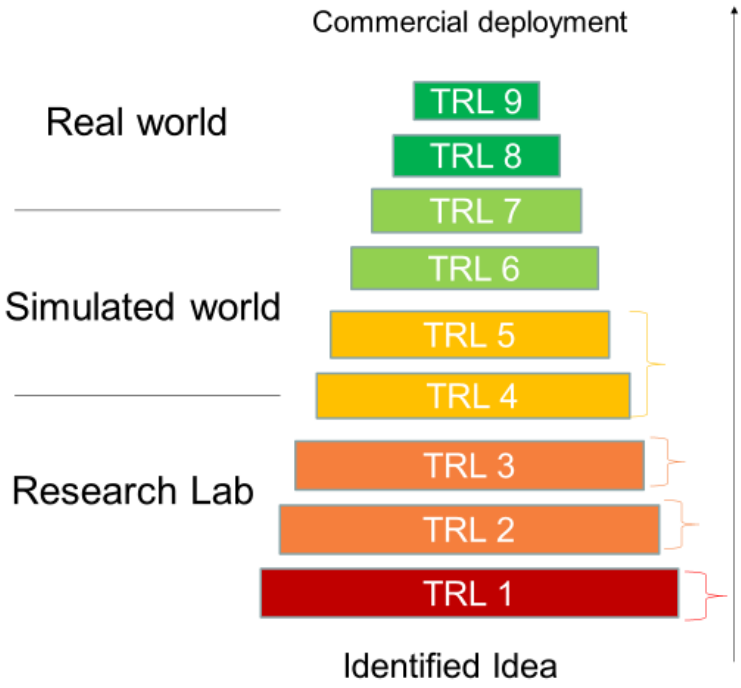


Multiplex Tandem-PCR (MT-PCR)

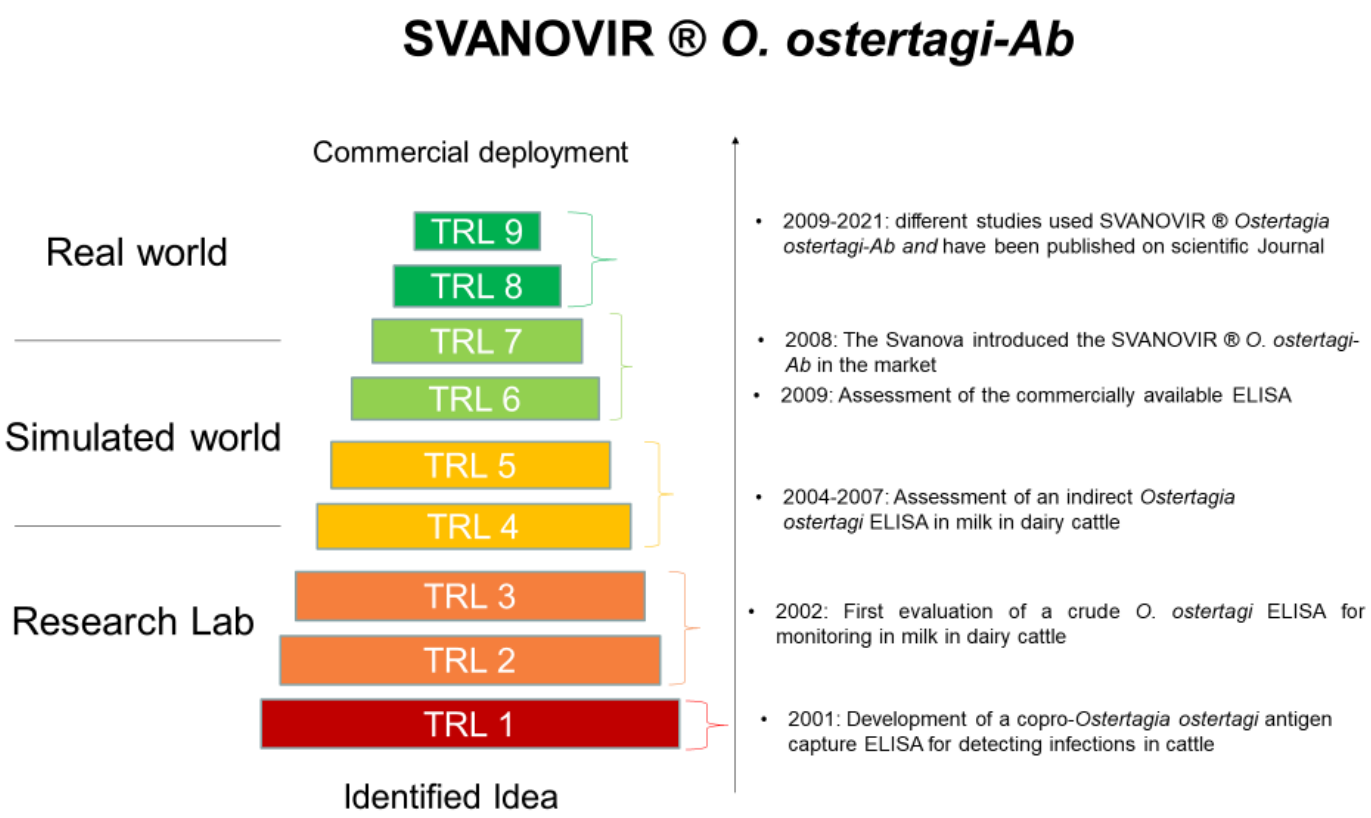


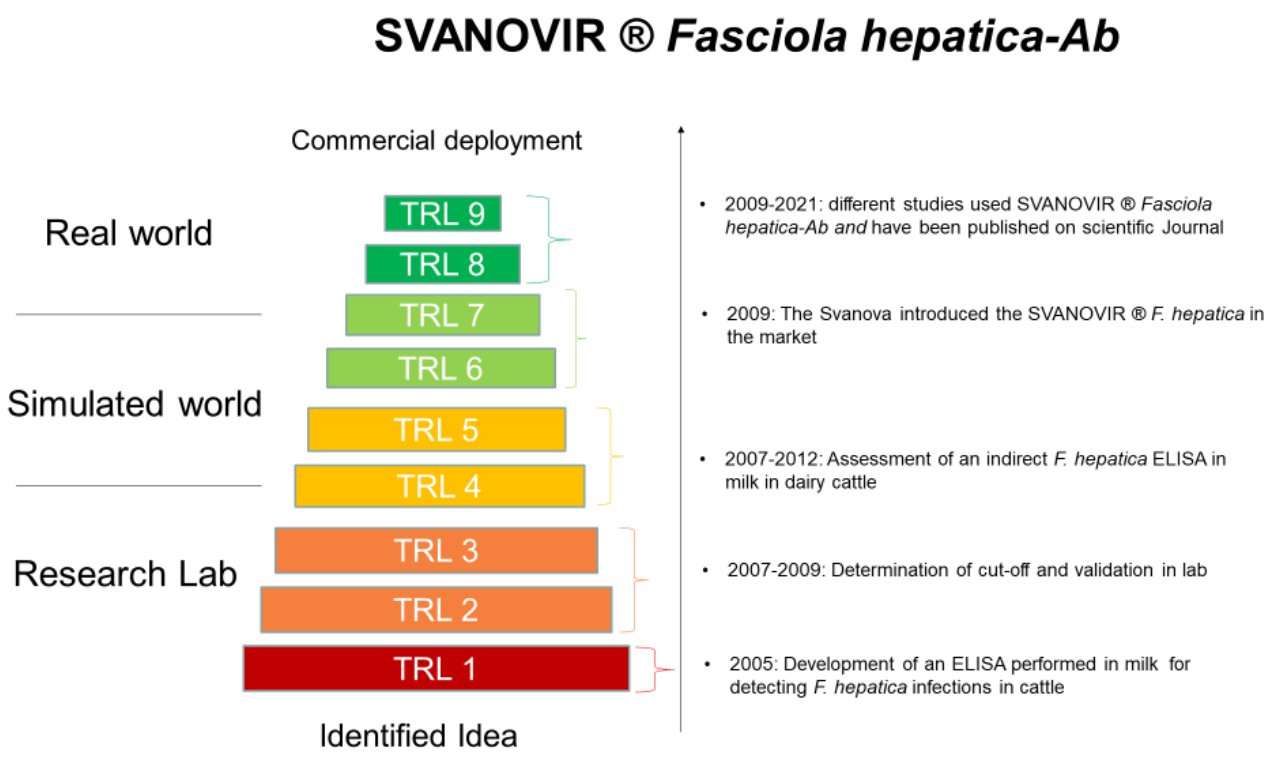
- Patented MT-PCR platform distributed by AusDiagnostics. In Europe is present in two labs
- 2012-2021: 9 studies have been performed using MT-PCR to detect GINs or AR
- 2018-2019: Identification of alpacas GINs and anthelmintic resistance evaluation
- 2017: First Australian-European validation of the MT-PCR for the diagnosis of cattle and sheep GINs
- 2012: University of Melbourne (Australia) evaluated a semi-automated high throughput MT-PCR platform for the diagnosis of sheep Gastrointestinal nematodes (GINs)
- 2005: MT-PCR was described for the first time

DROPLET DIGITAL PCR (ddPCR)



- 2020: Studies published on anthelmintic resistance diagnosis with ddPCR
- 2018-2021: 9 papers on use of the ddPCR for identification of Gastrointestinal nematodes (GIN) and anthelmintic resistancein livestock
- 2014-2021: Validation for different pathogens
- 2007-2008: An emulsification water-oil technique for the digital PCR was developed
- 1999: A digital PCR was developed


SVANOVIR ® O. ostertagi-Ab
Commercial deployment
Real world
TRL 9
TRL 8
TRL 7
TRL 6
Simulated world
TRL 5
TRL 4
Research Lab
TRL 3
TRL 2
TRL 1
Identified Idea
• 2009-2021: different studies used SVANOVIR ® Ostertagia ostertagi-Ab and have been published on scientific Journal
• 2008: The Svanova introduced the SVANOVIR ® O. ostertagi-Ab in the market
• 2009: Assessment of the commercially available ELISA
• 2004-2007: Assessment of an indirect Ostertagia ostertagi ELISA in milk in dairy cattle
• 2002: First evaluation of a crude O. ostertagi ELISA for monitoring in milk in dairy cattle
• 2001: Development of a copro-Ostertagia ostertagi antigen capture ELISA for detecting infections in cattle


SVANOVIR ® Fasciola hepatica-Ab
Commercial deployment
Real world
TRL 9
TRL 8
TRL 7
TRL 6
Simulated world
TRL 5
TRL 4
Research Lab
TRL 3
TRL 2
TRL 1
Identified Idea
• 2009-2021: different studies used SVANOVIR ® Fasciola hepatica-Ab and have been published on scientific Journal
• 2009: The Svanova introduced the SVANOVIR ® F. hepatica in the market
• 2007-2012: Assessment of an indirect F. hepatica ELISA in milk in dairy cattle
• 2007-2009: Determination of cut-off and validation in lab
• 2005: Development of an ELISA performed in milk for detecting F. hepatica infections in cattle