

Control programs against Cystic Echinococcosis in southern Italy and innovative tools including microRNAs

Elena Ciccone and Laura Rinaldi

Department of Veterinary Medicine and Animal Production, University of Naples Federico II, Naples, Italy

ECHINOCOCCUS GRANULOSUS → Cystic echinococcosis (CE)

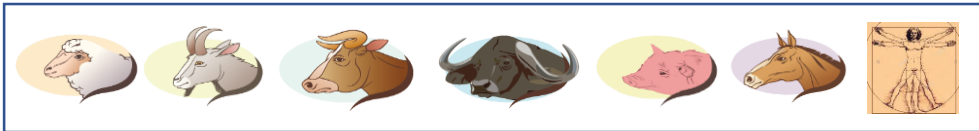


Final hosts = canids

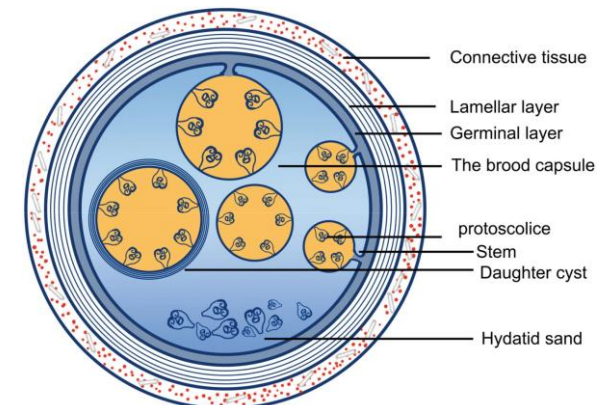
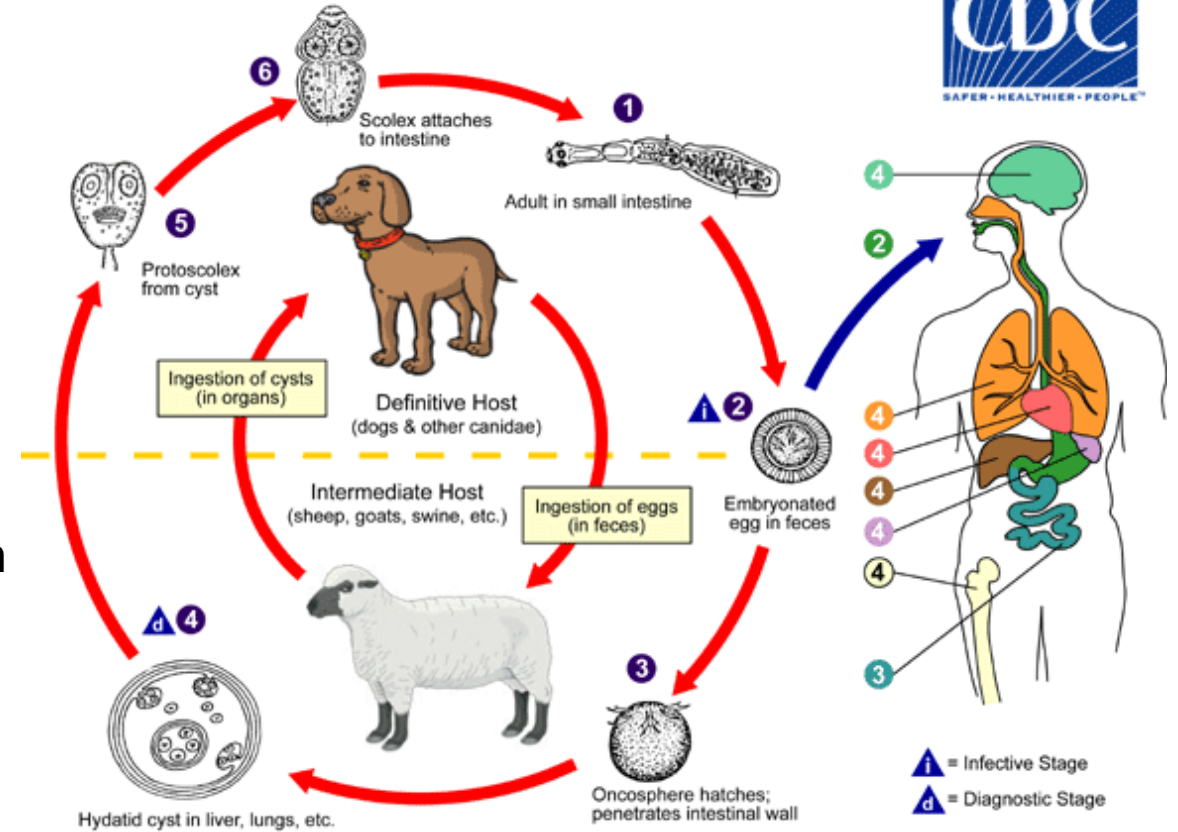


Adult and egg of *E. granulosus*

Intermediate hosts = ruminants, pigs, equids, human

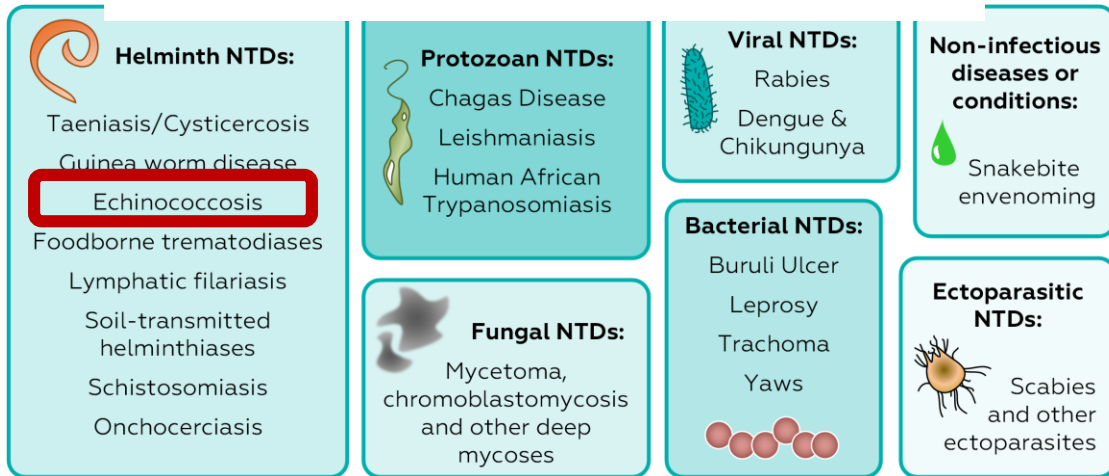


Hydatid cysts



ECHINOCOCCUS GRANULOSUS → Cystic echinococcosis (CE)

NEGLECTED TROPICAL DISEASES



WHO 2030 roadmap to end the neglect



Targeted for control

Disease	Critical action 1	Critical action 2	Critical action 3
Buruli ulcer	Build capacity of health workers to clinically diagnose and treat the disease and community health workers to detect and refer cases for early treatment, furthering integration among skin NTDs.	Develop rapid diagnostic tools for use in public health and community centres to ensure early diagnosis, reduce morbidity and confirm cases.	Create comprehensive surveillance systems in all endemic countries, including micro-mapping, to improve targeting and integrating interventions with those for other NTDs in co-endemic areas to improve case detection.
Dengue and chikungunya	Continue developing preventive vaccines for all at-risk populations.	Further develop the evidence base on effectiveness of vector control strategies.	Continue collaborating with environmental sector and engineers to reduce mosquito habitats.
Echinococcosis	Map disease prevalence to establish baseline data, and strengthen integrated national surveillance.	Develop guidelines for effective prevention and control strategies, and implement them in the field.	Strengthen implementation of ultrasound diagnosis and effective interventions, and ensure access to albendazole.
Foodborne trematodiasis	Develop accurate surveillance and mapping tools and methods, with	Estimate number of tablets required for control and secure donations of	Promote application and awareness of preventive chemotherapy, WASH and

Impact of CE on human health

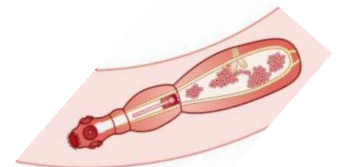
- Annual global burden at **19,300 deaths**
- Approximately **871,000 disability-adjusted life years (DALYs)**

Impact of CE on the livestock industry

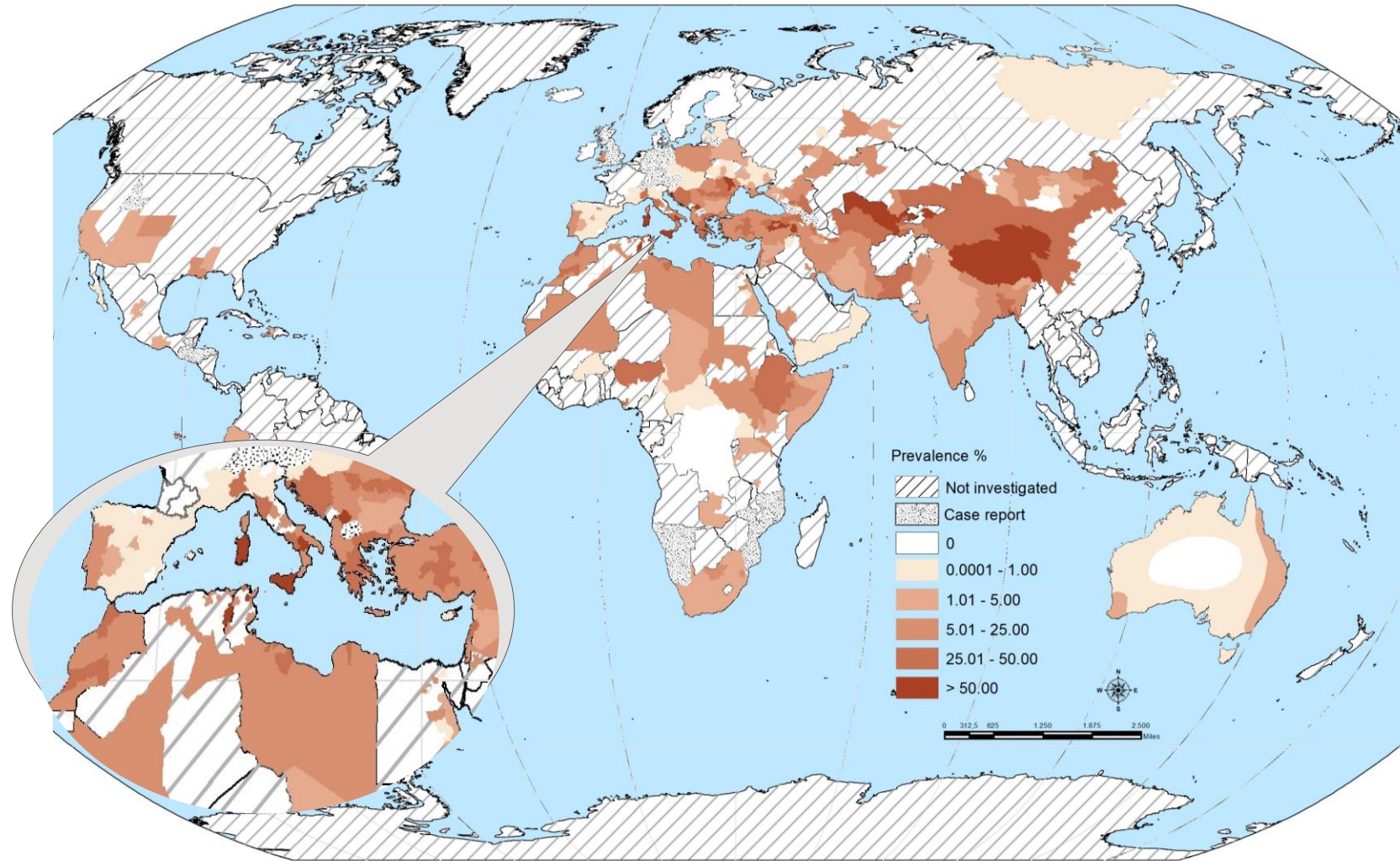
- quality of **meat, milk** and **wool** affected
- reduced **birth rate**
- delayed **performance** and **growth**
- post-mortem rejections of **infected organs** at slaughtering

Impact of CE on global economy

- The total **annual cost** of CE is estimated at **3 billion US dollars**, including costs to humans and livestock



Distribution of *E. granulosus*



Cystic echinococcosis (CE) has a worldwide distribution with **high prevalence** in communities where pastoral activities predominate, as **the Mediterranean areas**

Cystic echinococcosis in Campania region (southern Italy)

1999-2009



Prevalence = 10.5%
Cyst fertility = 20.6%
Genotypes = G1-G3

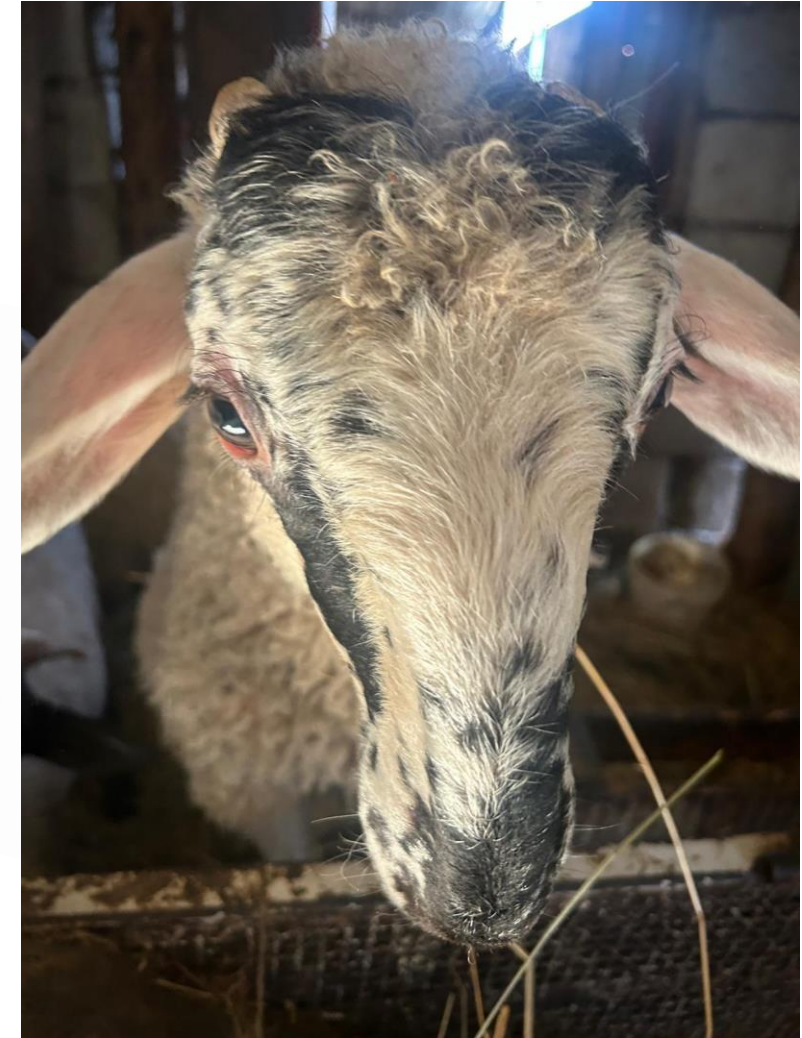
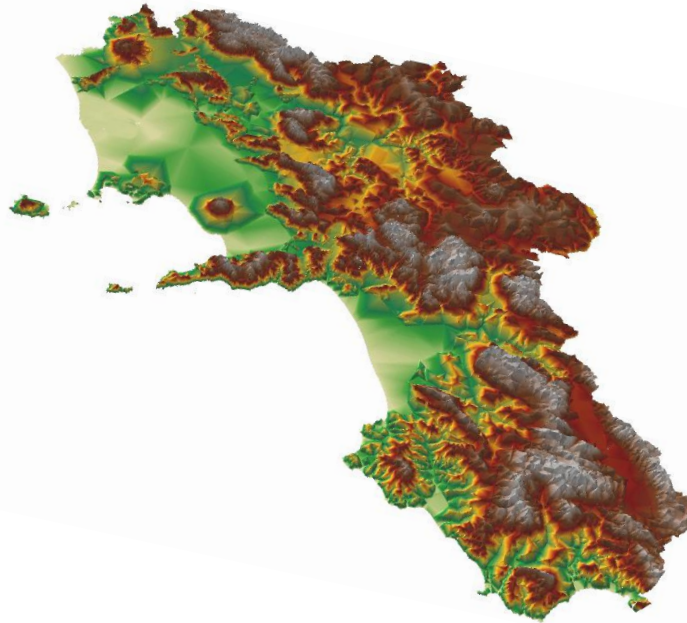


Prevalence = 10.4%
Cyst fertility = 0%
Genotypes = G1-G3



Prevalence = 51.2%
Cyst fertility = 24.5%
Genotypes = G1-G3

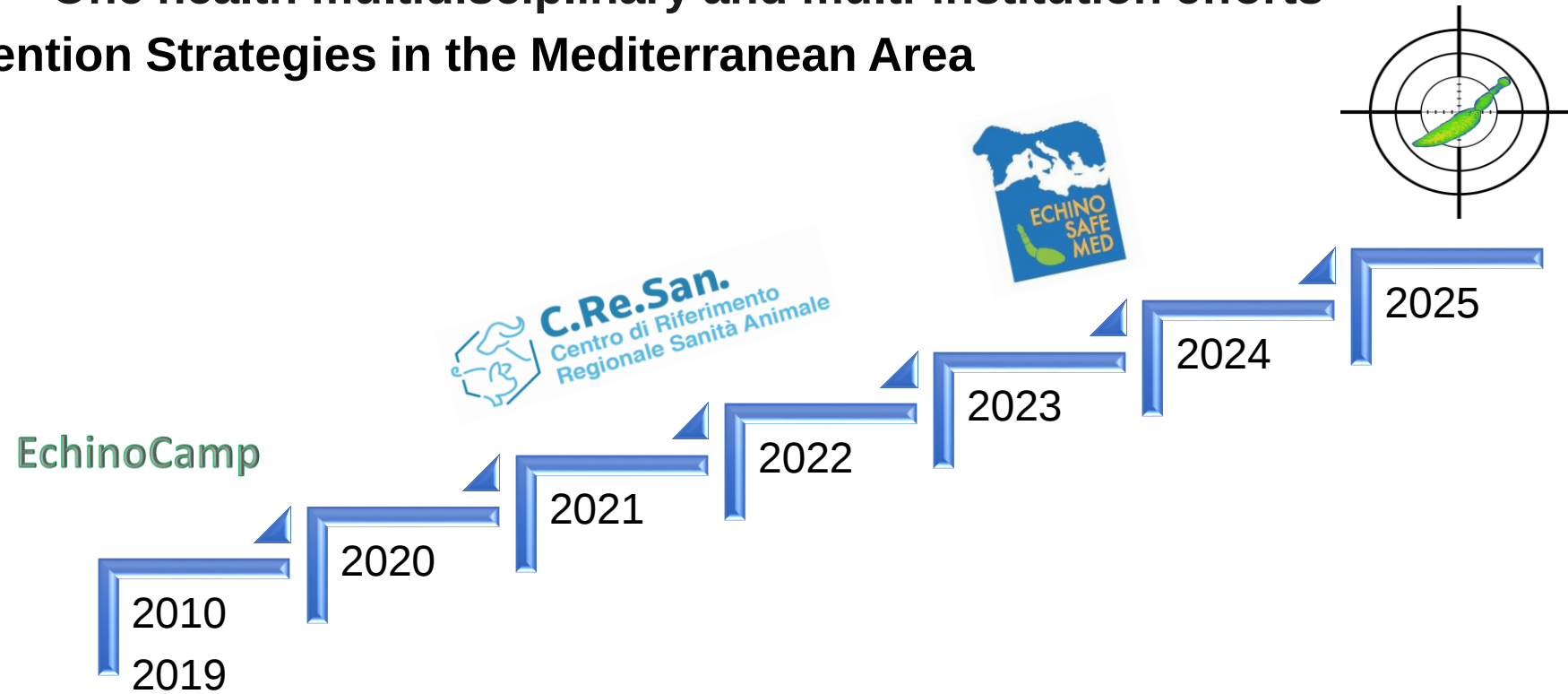
Echinococcus granulosus sensu stricto



Capuano et al., Vet Parasitol, 2006; Cringoli et al., Geospat Health, 2007; Rinaldi et al., Parasitol Res, 2008; Maurelli et al., Parasitol Res, 2009.

ECHINOCOCCOSIS CONTROL

Integrated Approach – One health multidisciplinary and multi-institution efforts Intervention Strategies in the Mediterranean Area



Since 2010 the Campania regional government have boosted and financed “**EchinoCamp**”, a control programme aimed at developing an integrated approach to control CE in animals and in humans.

- Characterized by different tasks based on livestock surveillance at slaughterhouses, early diagnosis in dogs and sheep, targeted deworming of dogs and outreach activities.

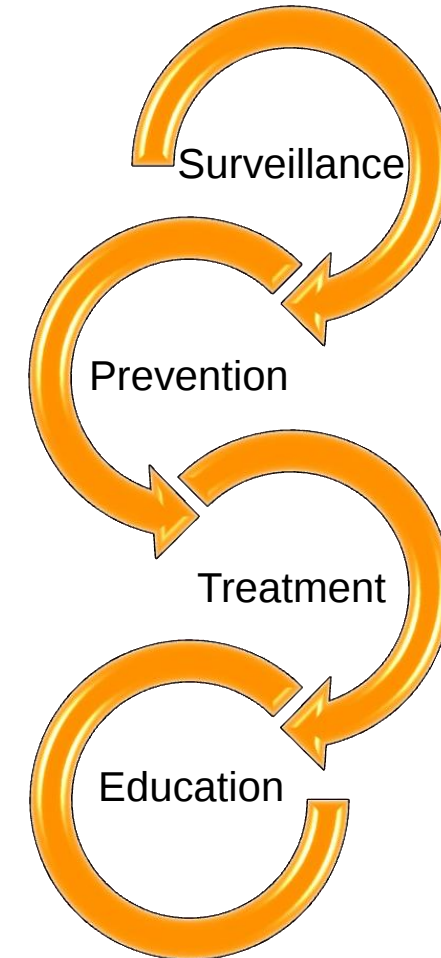
Integrated Approach – One health multidisciplinary and multi-institution efforts



Deworming of owned dogs



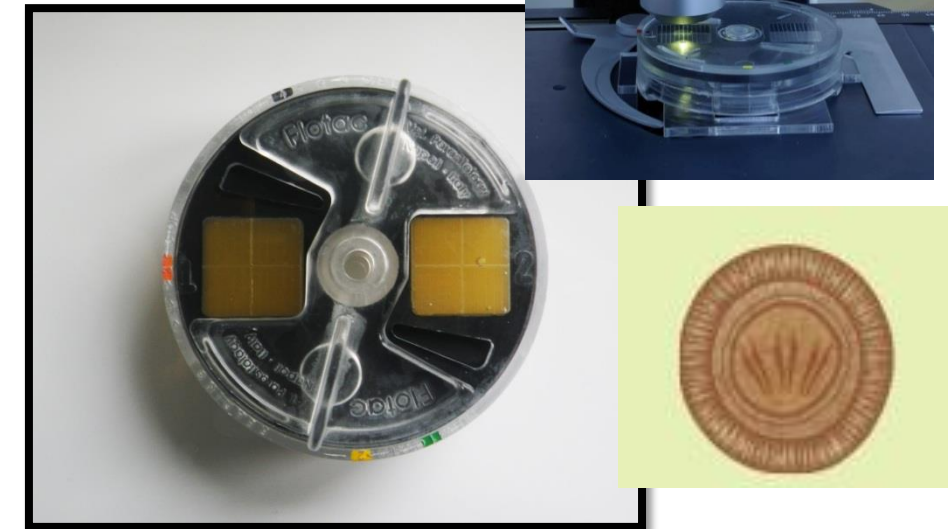
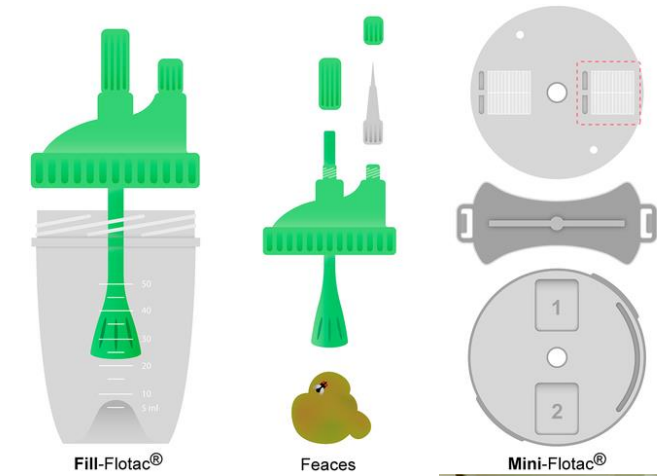
Treatment of not-owned dogs and vaccination of lambs were not included



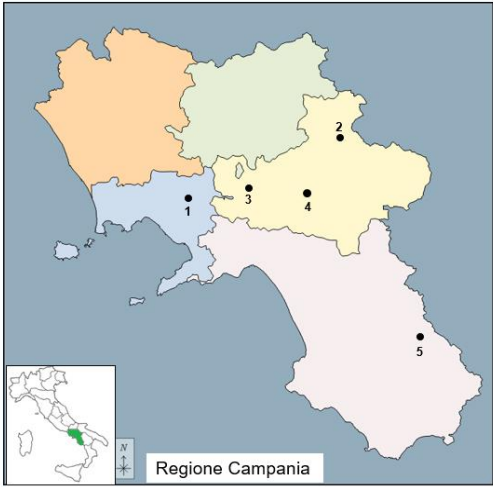
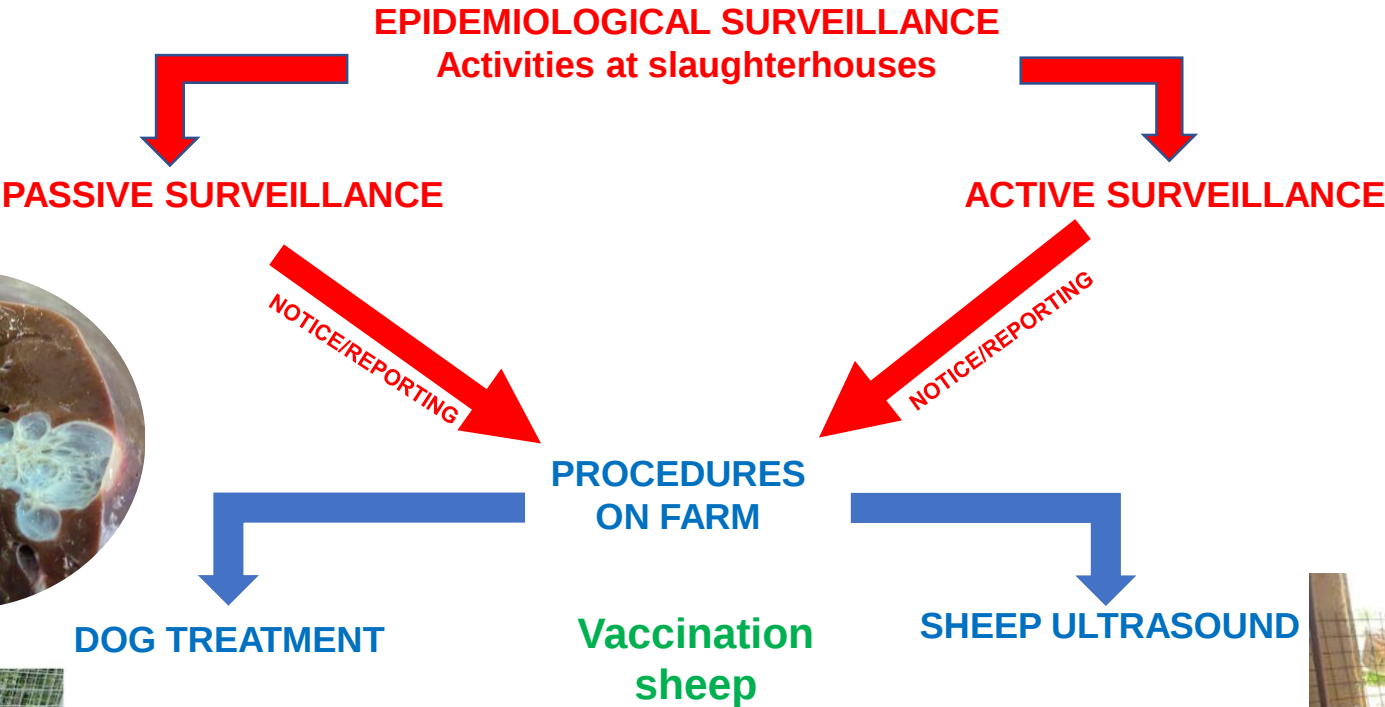
EchinoCages



FLOTAC - Techniques



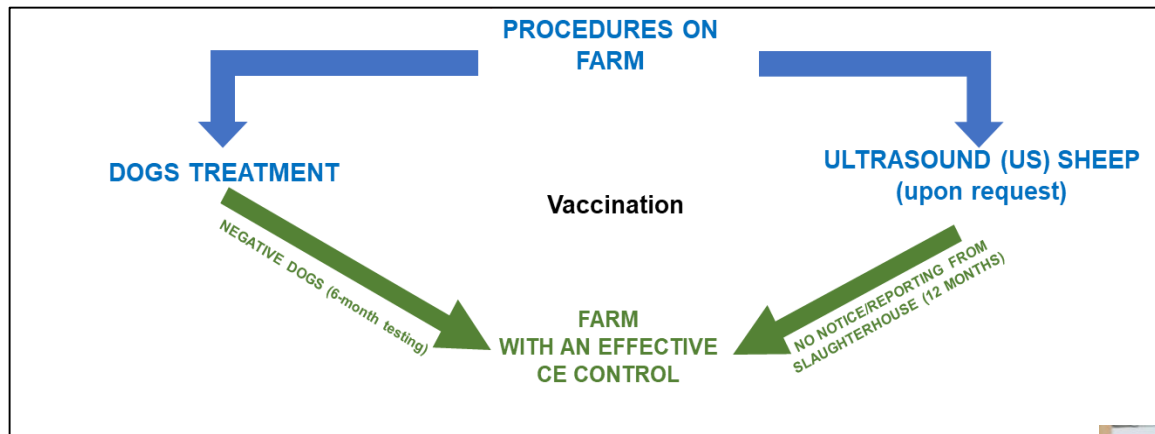
Since 2021, the control of CE in ruminants in Campania has been officially included as a mandatory task in the annual regional planning document (DPAT) of the veterinary services (AASSLL)





Milbemax® tab

0.5 mg/kg milbemycin oxime
5 mg/kg praziquantel

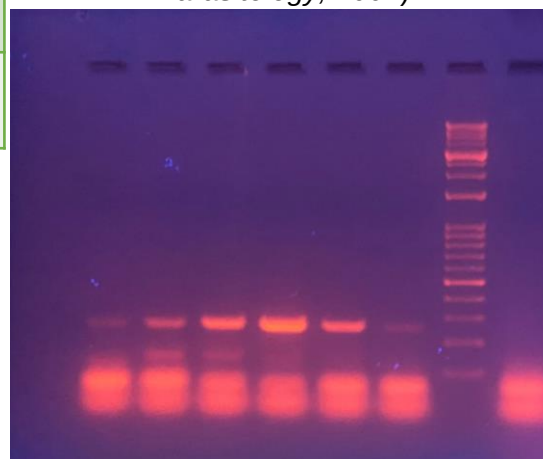


Providean Hidatec EG 95®

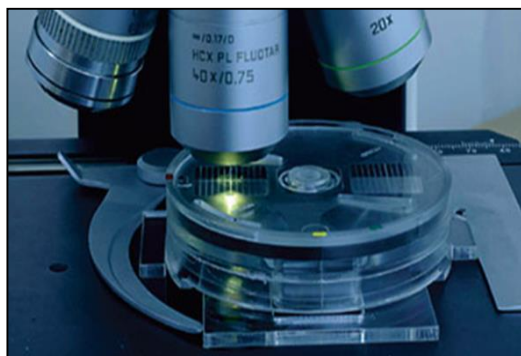
	No. Dogs analyzed	No. Dogs positive to Taeniidae
2022	160	8
2023	219	9
2024	557	33
2025 Ongoing...	511	33

19 positive to *Taenia* spp.
25 positive to *E. granulosus*
39 co-infections

Multiplex PCR (Trachsel et al.,
Parasitology, 2007)

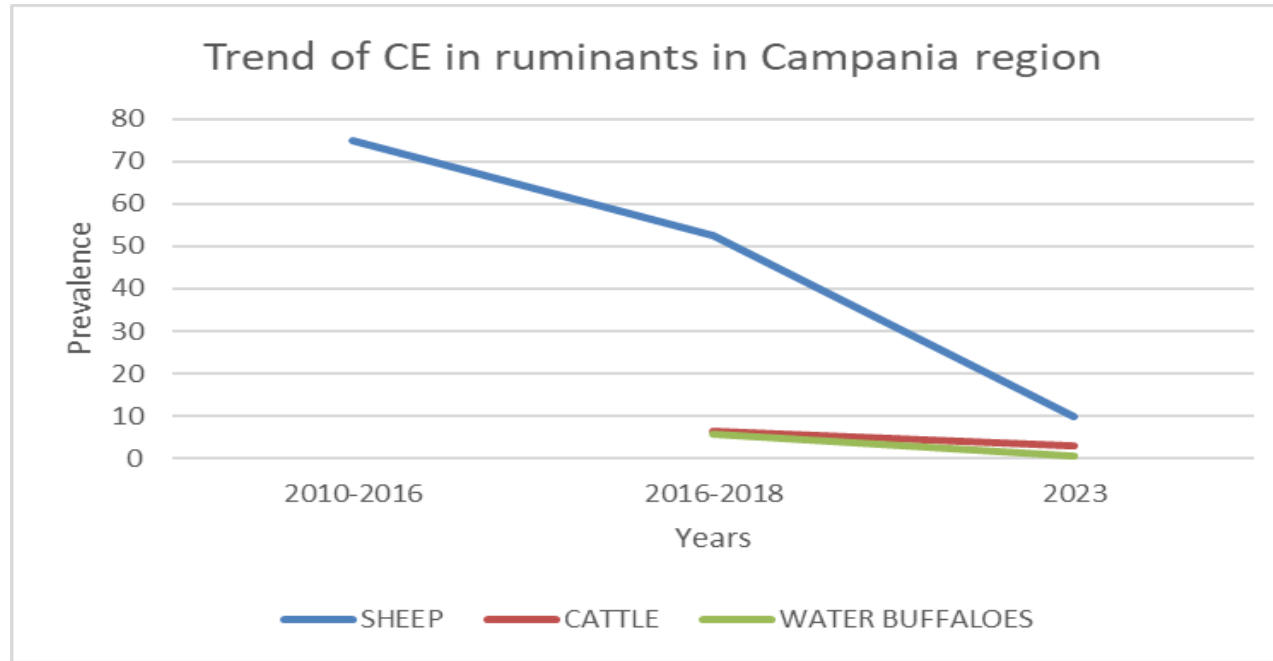


→ *Taenia* spp. 267 bp
→ *Echinococcus granulosus* 117 bp

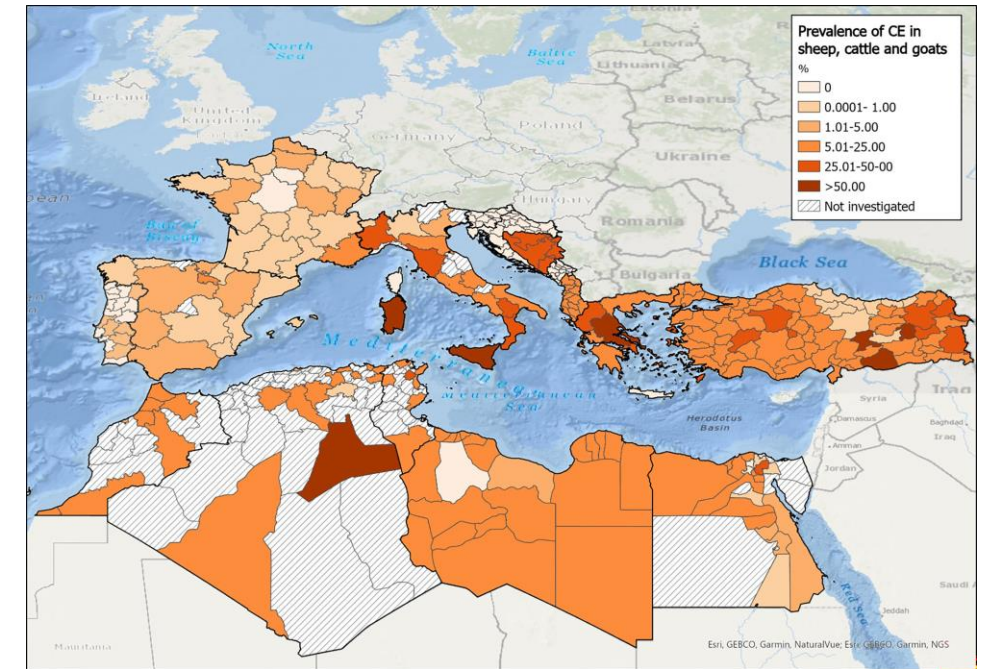


	No. Lambs Vaccinated (2 doses) (Procedura)	No. Lambs vaccinated (2 doses) (Open Day)
2022	395	0
2023	447	0
2024	979	334
2025 Ongoing...	1252	216

Epidemiological updates



The number of cysts per animal decreased and most cysts were small and calcified.



Region	before 2000	2000-2014	2015-2020	2021-2023
Campania (Italy)		63.1%	52.2%	9.7%

Rinaldi et al. 2008; Cringoli et al. 2021; Rinaldi et al. 2023 personal communication

Epidemiological update of cystic echinococcosis in livestock and assessment of practices related to its control in the Mediterranean area

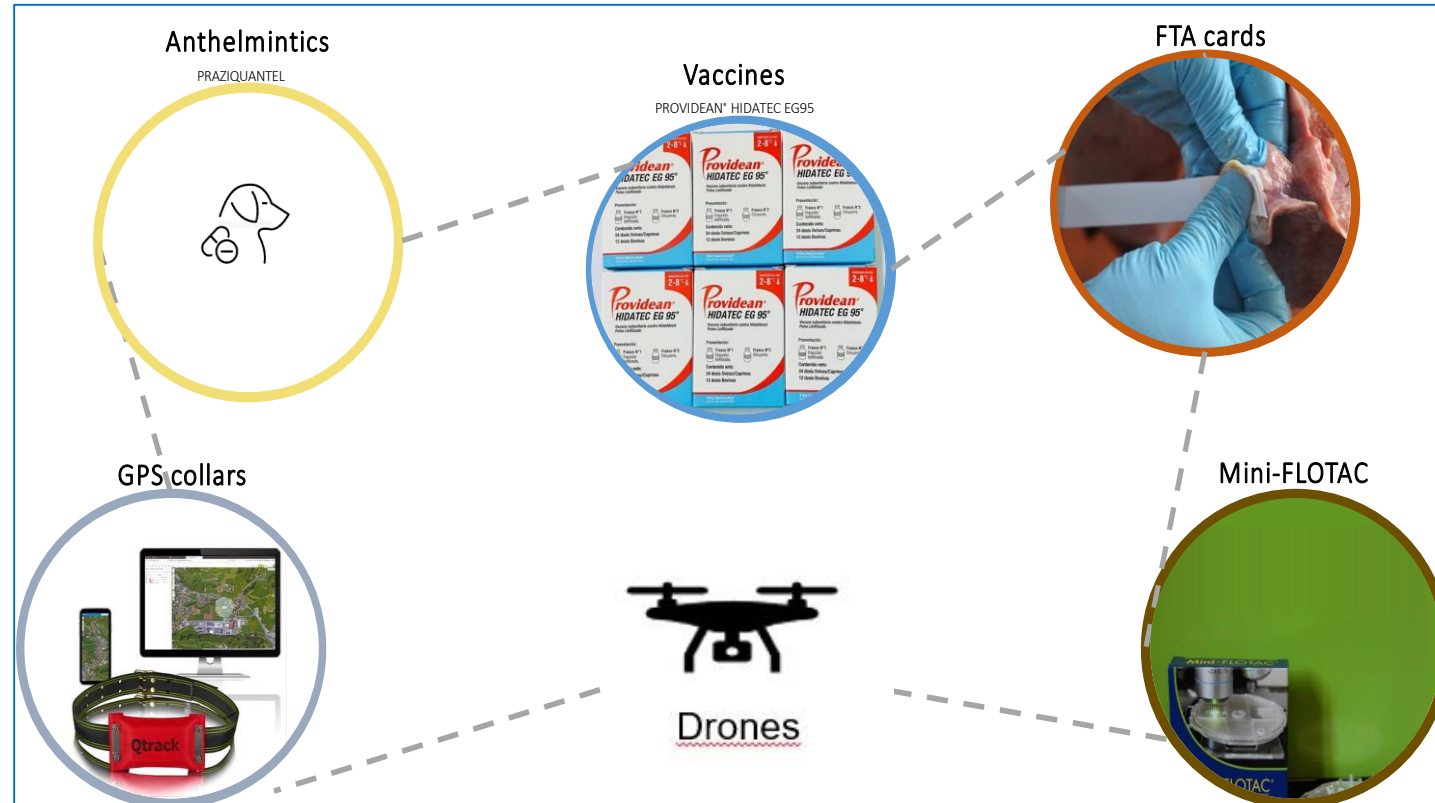
Martina Nocerino ^a, Paola Pepe ^a ✉, Elena Ciccone ^{a b}, Maria Paola Maurelli ^{a b}, Antonio Bosco ^{a b}, Franck Boué ^c, Gérald Umhang ^c, Samia Lahmar ^d, Yousra Said ^d, Smaragda Sotiraki ^e, Panagiota Ligda ^e, AbdElkarim Laatamna ^f, Nassiba Reghaissia ^g, Giorgio Saralli ^h, Vincenzo Musella ⁱ, Maria Chiara Alterisio ^a, Giuseppe Piegari ^a, Laura Rinaldi ^{a b}

Acronym: ECHINO-SAFE-MED

Project Title: New sustainable tools and innovative actions to control cystic **ECHINO**coccosis in sheep farms in the **MED**iterranean area: improvement of diagnosis and **SAF**ety in response to climatic changes

Coordinator: University of Naples Federico II (UNINA)

No. of years: 4 years (May 2021 - May 2025)



*PRIMA Projects are financed through a combination of **funding from PRIMA Participating States** and contribution from the EU through **Horizon 2020***

Partners

Italy: University of Naples Federico II (**UNINA**); Istituto Zooprofilattico Sperimentale del Lazio e della Toscana (**IZSLT**)

Algeria: Ziane Achour University of Djelfa (**UZAD**)

Greece: Vet Res Institute Hellenic Agricultural Organisation (**VRI-HAO**)

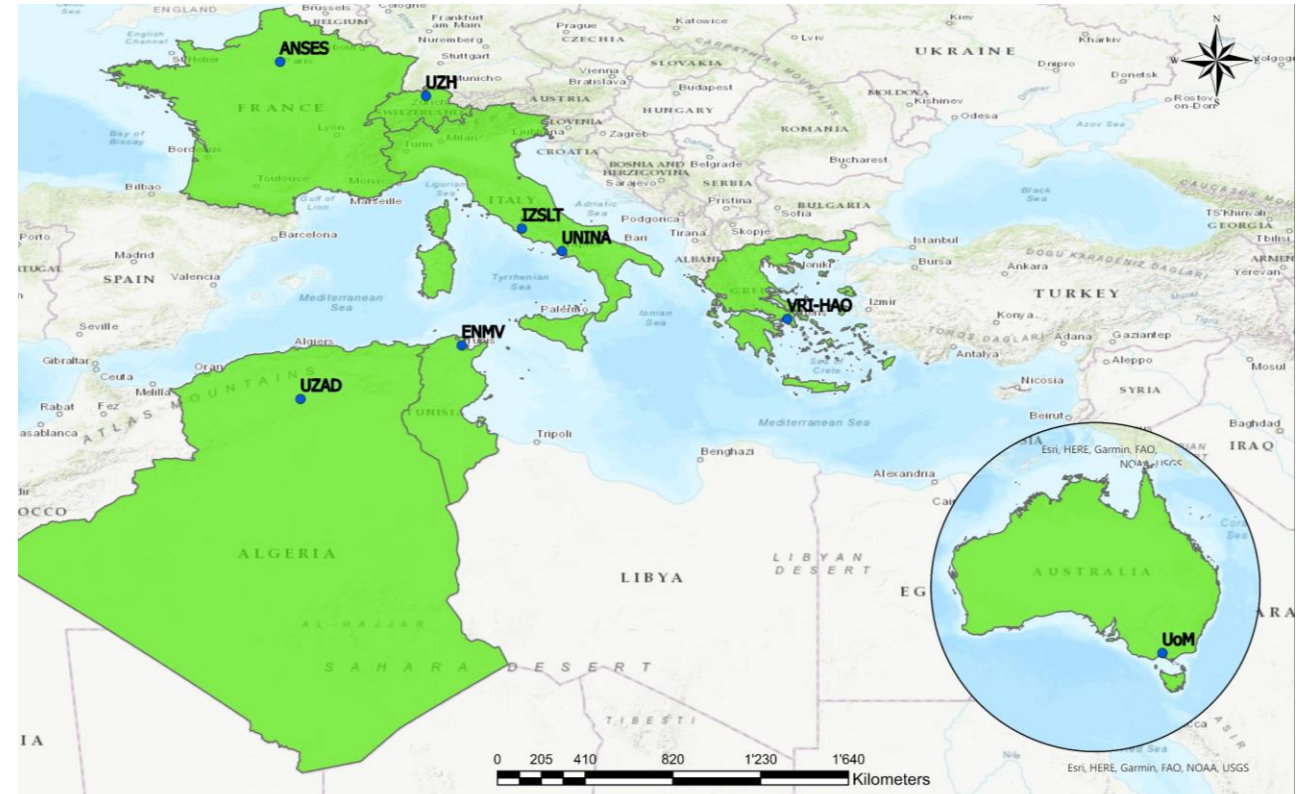
Tunisia: National School of Veterinary Medicine, Sidi Thabet (**ENMV**)

France: Agence Nationale de Sécurité San. Alim., Envir. et du Travail (**ANSES**)

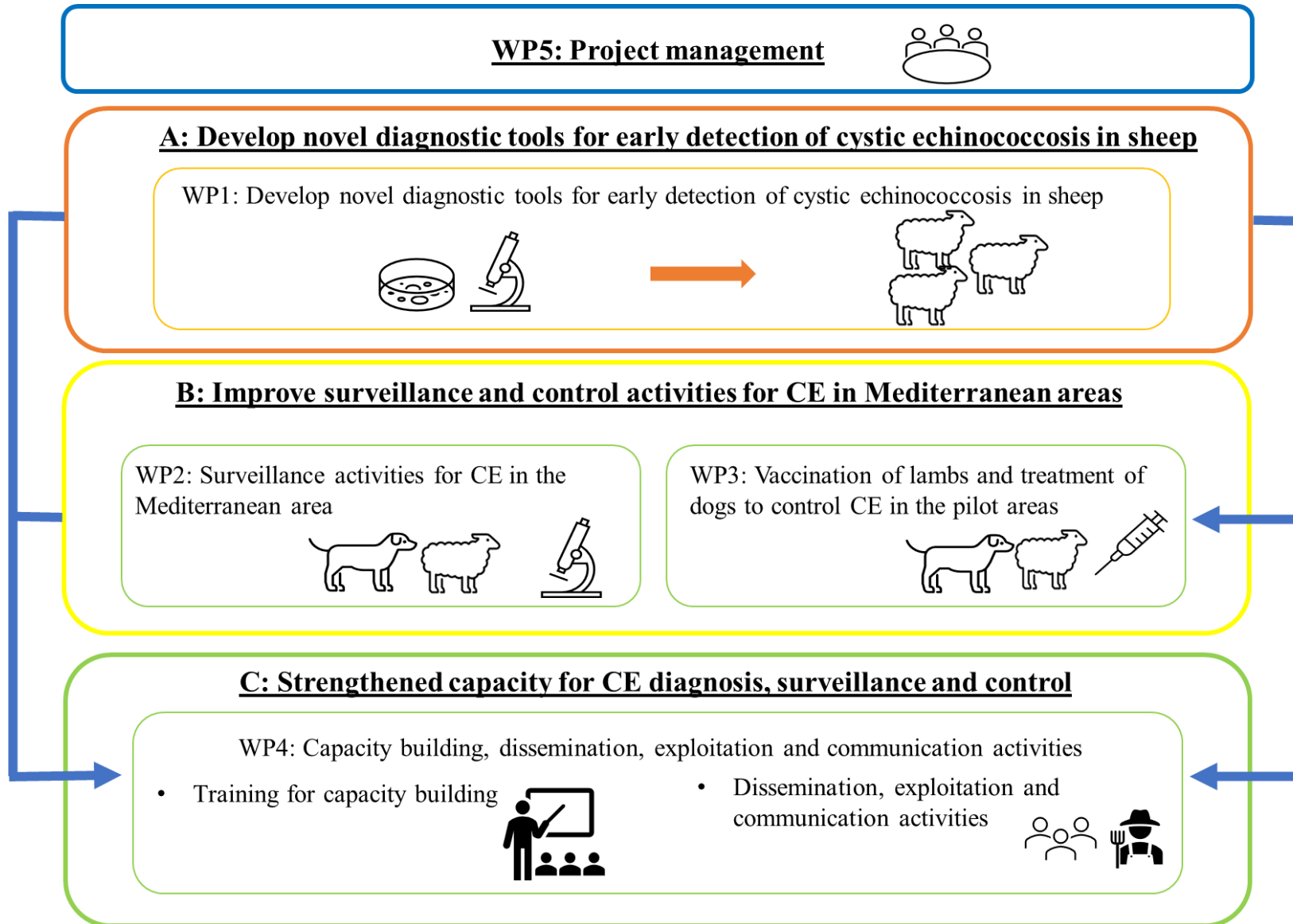
External Advisors

Switzerland: University of Zurich (**UZH**)

Australia: University of Melbourne (**UoM**)



ECHINO-SAFE-MED: PERT diagram



Diagnostic tools for early detection of CE in sheep



ECOGRAPHY (ULTRASOUND)

(Borriello et al., Animals, 2021; Mercaldo et al., Vet. Parasitol., 2025)

US Sensitivity = 92.4%; Specificity = 85.6%

Reducing the effects of **logistic difficulties** to improve the use of this technique **for CE diagnosis in field conditions**



ELISA TEST

(Kronenberg et al., Pathogens, 2022)

- *E. granulosus* s.s. vesicle crude antigens (EgVC), obtained from fertile cysts of infected sheep liver, were used in ELISA to evaluate their diagnostic performance.
- **Very low specificity due to cross-reactivity** with other parasites (e.g. *T. hydatigena*, *F. hepatica*) compared to that obtained using human sera



SKIN TEST

(Piegari et al., 2025, In press)

Sheep inoculated with different *E. granulosus* antigens at different dilutions:

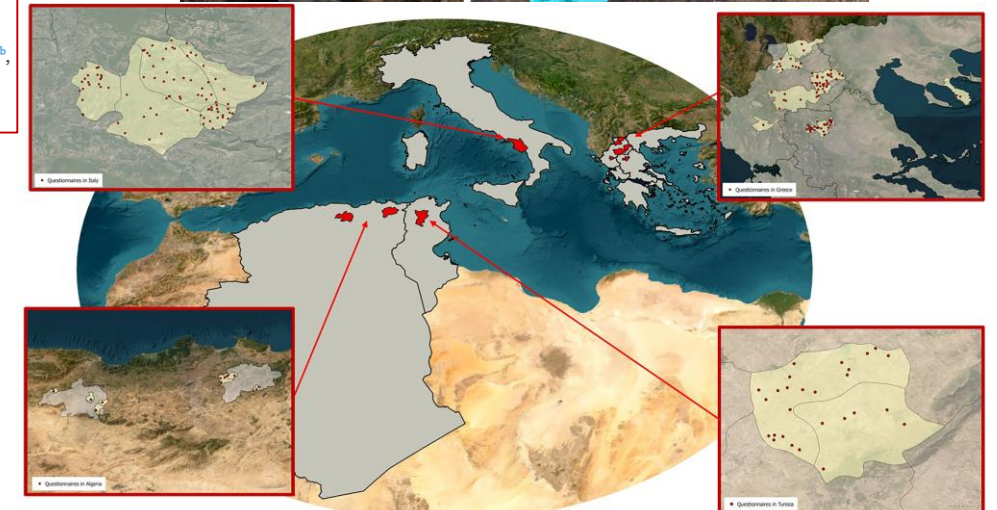
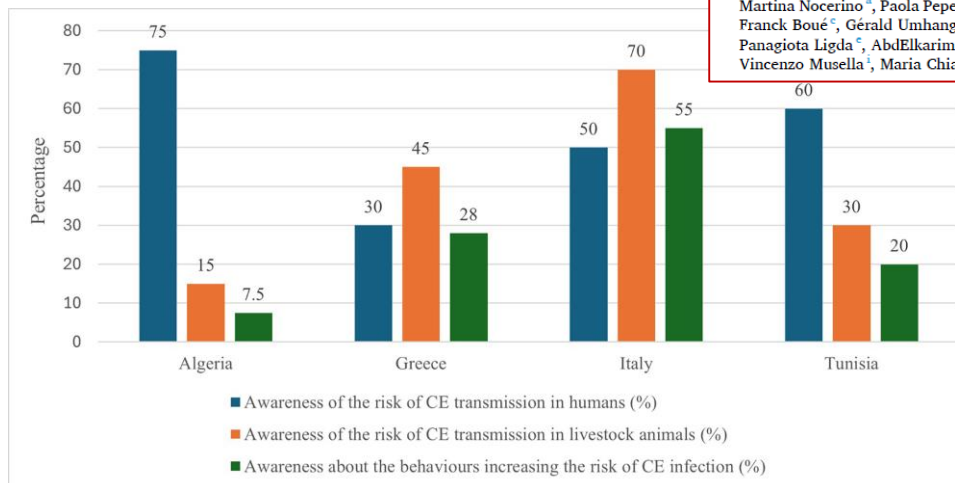
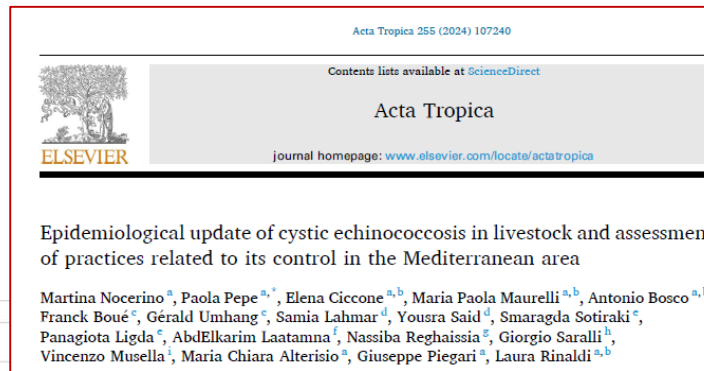
- Crude antigen of *in vitro* cultivated vesicles, **EgVC antigen**
- Vesicle fluid of *in vitro* cultivated vesicles, **EgVF antigen**
- Antigen from protoscolices, **EgP antigen**
- Antigen from cyst fluid, **EgCF antigen**





Surveillance activities for CE in the Mediterranean area

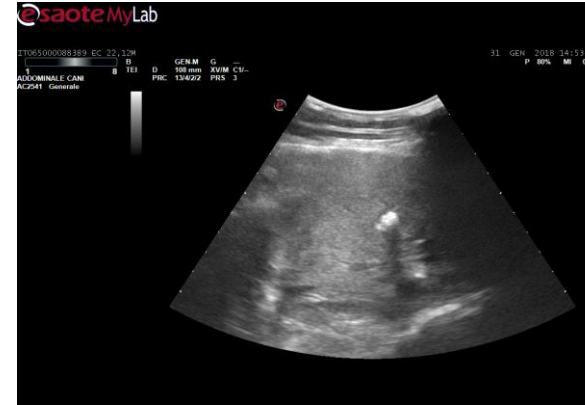
- Deepening the **epidemiological data** on CE in ruminant intermediate hosts and identification of the **high-risk endemic areas in Mediterranean basin**
- Identification of the **practices in use** that potentially allow the **spread of CE** in four countries of the Mediterranean basin (**Algeria, Greece, Italy and Tunisia**)
- Assessing the **knowledge attitudes** of shepherds towards CE



Surveillance of CE in sheep

Field Activities

>500 sheep



Ultrasound



Collection of FTA cards

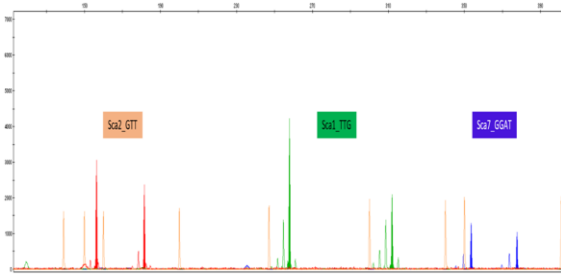
Necroscopy

Lot # 1
GE Healthcare companies. Covered under US Patents 5496562, 5756126, 5972386 and patents pending.
CAT No. 29277434 Lot No. 17027908
Rev. AD

Surveillance of CE in sheep and in dogs



Microsatellite analyses were performed on FTA Cards collected, to describe genetic diversity of *E. granulosus sensu stricto*



Examples of the electrophoretic profiles obtained for one *E. granulosus* s.s. sample for three of the selected loci.

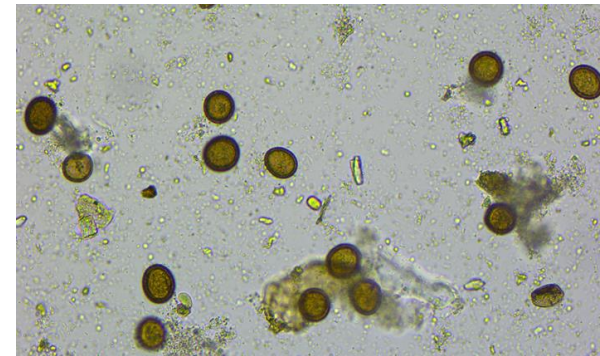
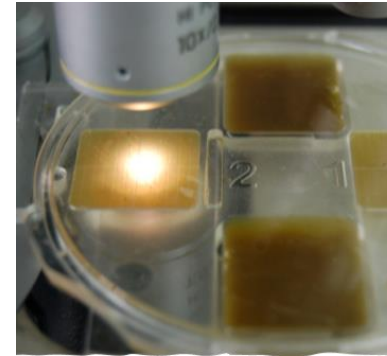
Country	No. FTA Cards collected	No. FTA Cards analyzed
Italy	263	263
Greece	207	207
Tunisia	50	50
Algeria	50	35



Faecal samples collection and copromicroscopic analysis, using Mini-FLOTAC technique, for detecting and counting Taeniidae eggs

Aliquots to ANSES (either pos or neg)

Country	No. Dog faeces collected	No. Dog faeces analyses
Italy	145	145
Greece	240	240
Tunisia	87	87
Algeria	200	200



ITALY

- No. faecal samples analysed = **145**
- No. faecal samples positive to Taeniidae eggs = **8**
- **Prevalence = 5.5%**

Control activities: Vaccination of lambs

Tecnovax Sanidad Animal

Buenos Aires, Argentina

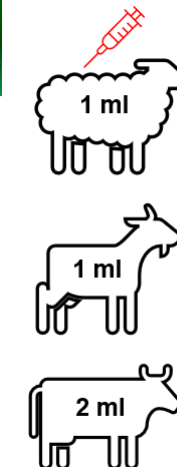
TECNOVAX
Sanidad Animal

PROVIDEAN® HDATEC EG95

10,000 doses of **Providean Hidatec EG95** for **ITALY**
received in **December 2021**



Prof. Marshall Lightowlers
(University of Melbourne – Australia)



1st dose	→	2-12 months
2nd dose	→	After 1 month
Annual booster	→	Every year



Ministero della Salute

DIREZIONE GENERALE DELLA SANITA' ANIMALE E DEI FARMACI VETERINARI
Uff. 3-4-8 DGSF

Registro – Classif:

Allegati: 0

Giunta Regionale della Campania
Direzione Generale per la Tutela della Salute
ed il Coordinamento del Sistema Sanitario
Regionale

Oggetto: autorizzazione per l'importazione in Italia di organismi patogeni: vaccino ad uso veterinario non registrato in Italia denominato PROVIDEAN HDATEC EG 95.

IL DIRETTORE GENERALE

AUTORIZZA

la Regione Campania - Direzione Generale per la Tutela della Salute ed il Coordinamento del Sistema Sanitario Regionale - Unità Operativa Dirigenziale Prevenzione e Sanità Pubblica Veterinaria, con sede legale in Via S. Lucia, 81 - 80132 Napoli e sede operativa in Centro Direzionale Isola C 3 - 80143 Napoli, ad importare, in più consegne, dall'**Argentina** (Ditta di provenienza e di produzione: *Tecnovax Sanidad Animal - Luis Viale 2853 - Buenos Aires - Argentina*) **n. 17.000 (diciassettemila)** dosi del vaccino ad uso veterinario denominato **PROVIDEAN HDATEC EG 95** (*principio attivo: proteina ricombinante EG 95*), regolarmente autorizzato nel Paese terzo di provenienza, da impiegare, con le modalità previste dalla normativa vigente, esclusivamente per la **vaccinazione contro l'echinococcosi**, presso allevamenti di **ovini e caprini**, ubicati nelle **Province di Avellino e Salerno**, al fine di dare attuazione al **"Piano vaccinale per il risanamento dell'Echinococcosi Idatidosi in allevamenti ovini e caprini in aree endemiche della Regione Campania. Supporto Tecnico-scientifico al personale AA.SS.LL. per le attività territoriali"**, predisposto dall'Università di Napoli Federico II - Dipartimento di Medicina Veterinaria e Produzioni Animali Zootecniche - Unità di Parassitologia e Malattie Parassitarie.

Vaccination of lambs



DEVELOPMENT OF A STRATEGY FOR DEWORMING NOT OWNED DOGS

Baiting not-owned dogs against *Echinococcus granulosus*: innovative tools for integrated control

Elena Ciccone^{1,2} , Antonio Bosco^{1,2} , Paola Pepe¹, Martina Nocerino¹, Nicola Lattero^{1,2}, Gerald Umhang³ , Laatamna AbdElkarim⁴ , Samia Lahmar⁵ , Yousra Said⁵ , Giorgio Saralli⁶, Giuseppe Piegari¹, Maria Chiara Alterisio¹, Rania Baka⁷, Smaragda Sotiraki⁷, Franck Boué³ and Laura Rinaldi^{1,2} 

The potential role of not-owned dogs in contaminating the environment by shedding worms or eggs is a **critical issue** in the control of CE and other zoonoses (FAO, 2014).

PRELIMINARY TRIAL

Test suitable baits for the treatment of stray dogs

Evaluate the composition of baits

1

Evaluate the durability and palatability over time of different type of baits

2

Evaluate the integrity resistance from different heights

3

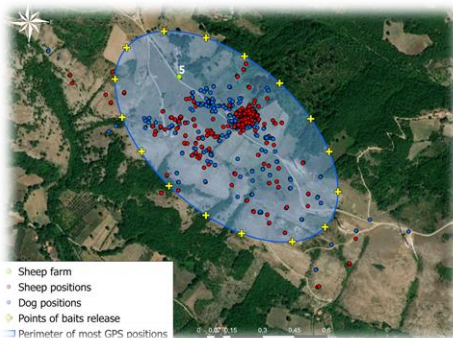


4

Evaluate the acceptance of the baits from stray dogs

5

Identification of bait consumers (stray dogs and wildlife)




Stray dogs

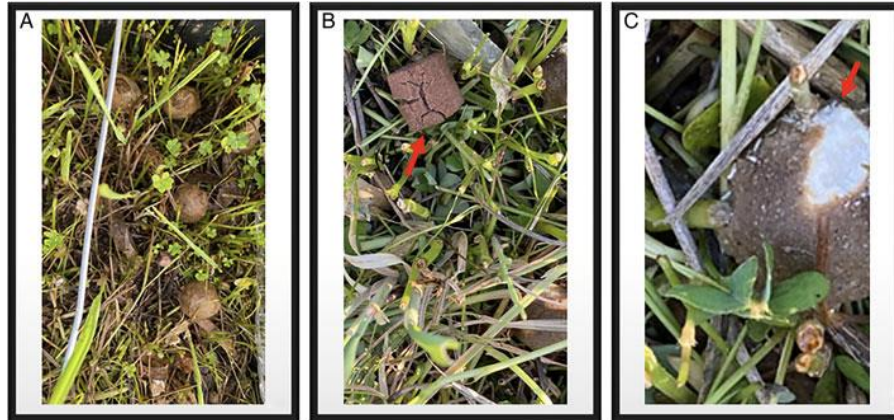


BAITING ASSESSMENT TRIAL ON FIELD

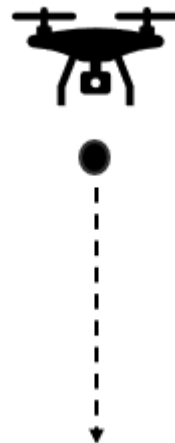
Validation of the use of the baits tested in extra-urban area (near the CE positive sheep farms)

PRELIMINARY TRIAL

Integrity, attractiveness, palatability (%) of the baits over time, and mechanical resistance

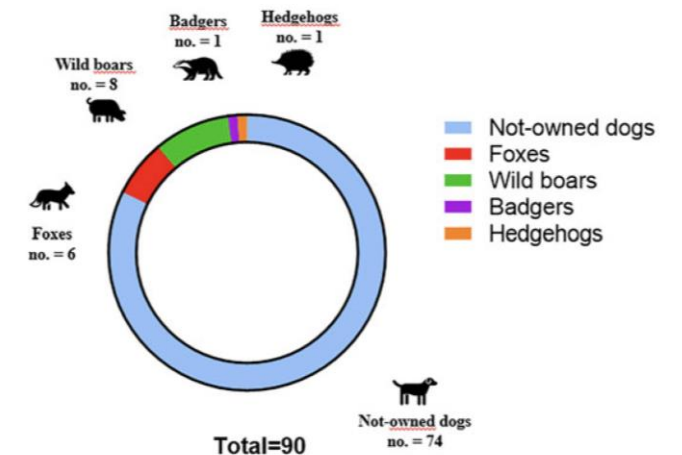
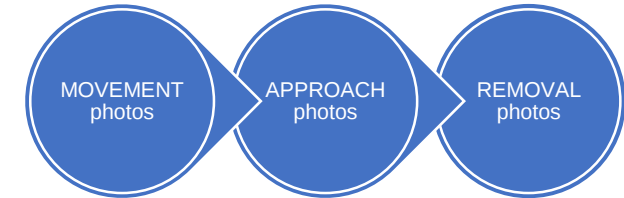


Type of baits	Attractiveness over time (No. Dogs (%) that found and consumed the baits)		Palatability (%)
	Day 5	Day 10	
Single PZQ-laced	4 (33.3%)	2 (16.7%)	25.0
Double PZQ-laced	7 (58.3%)	10 (83.3%)	70.8
PZQ chewable	1 (8.4%)	0 (0%)	4.2



FIELD TRIAL

Baits' acceptability by not owned dogs



Usefulness of geospatial technology in supporting **parasite control strategies** to reduce disease transmission

Nocerino et al. *Parasites & Vectors* (2024) 17:86
<https://doi.org/10.1186/s13071-024-06184-x>

Parasites & Vectors

RESEARCH

Open Access

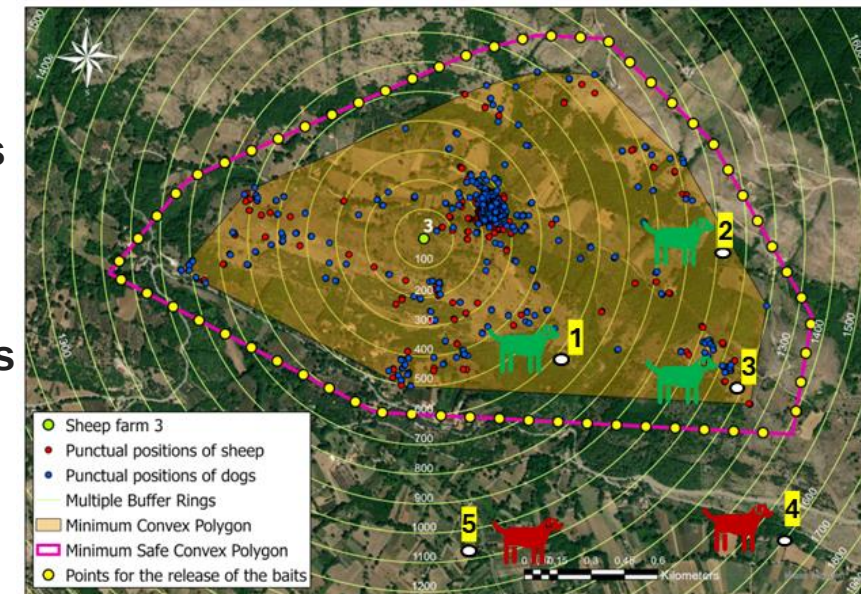


An innovative strategy for deworming dogs in Mediterranean areas highly endemic for cystic echinococcosis

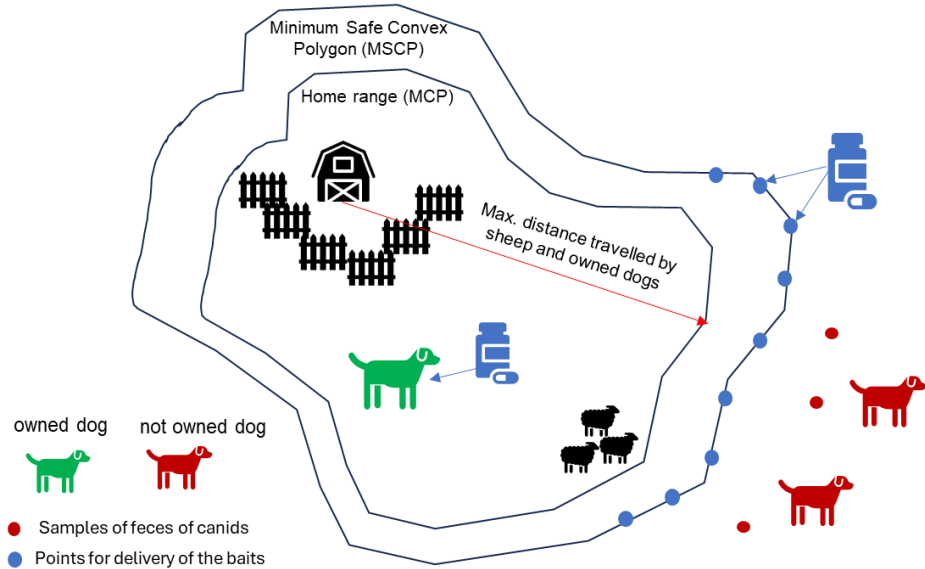
Martina Nocerino¹, Paola Pepe^{1*}, Antonio Bosco^{1,2}, Elena Ciccone^{1,2}, Maria Paola Maurelli^{1,2}, Franck Boué³, Gérald Umhang³, Justine Pellegrini³, Samia Lahmar⁴, Yousra Said⁴, Smaragda Sotiraki⁵, Panagiota Ligda⁵, AbdElkarim Laatamna⁶, Giorgio Saralli⁷, Orlando Paciello¹, Maria Chiara Alterisio¹ and Laura Rinaldi^{1,2}



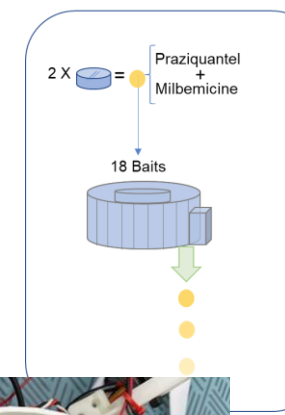
- Collecting data on animal movements and behavior using geospatial technologies and **GPS dataloggers** applied to sheep and dogs
- Based on the home range of the animals a potential scheme for the **delivery of praziquantel-laced baits for the treatment of not owned dogs** gravitating around the grazing area was designed



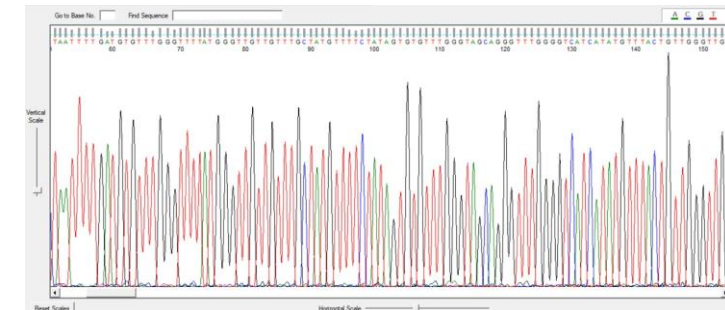
Delivery of anthelmintic PZQ baits



Medicated baits release



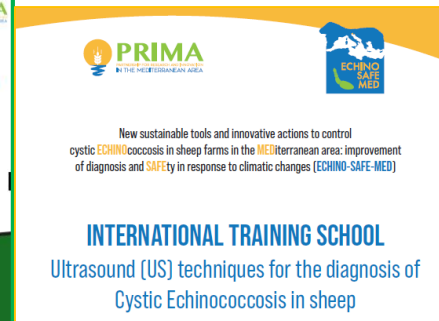
- The **faecal samples of canids** falling **outside the Minimum Safe Convex Polygon (MSCP)** of six farms tested positive for Taeniidae eggs at T0 (start of the intervention)
- The molecular analysis showed that all faecal samples were positive to *E. granulosus*. Since the second treatment, none of the fecal samples, analyzed every 2 months, tested positive for Taeniidae eggs



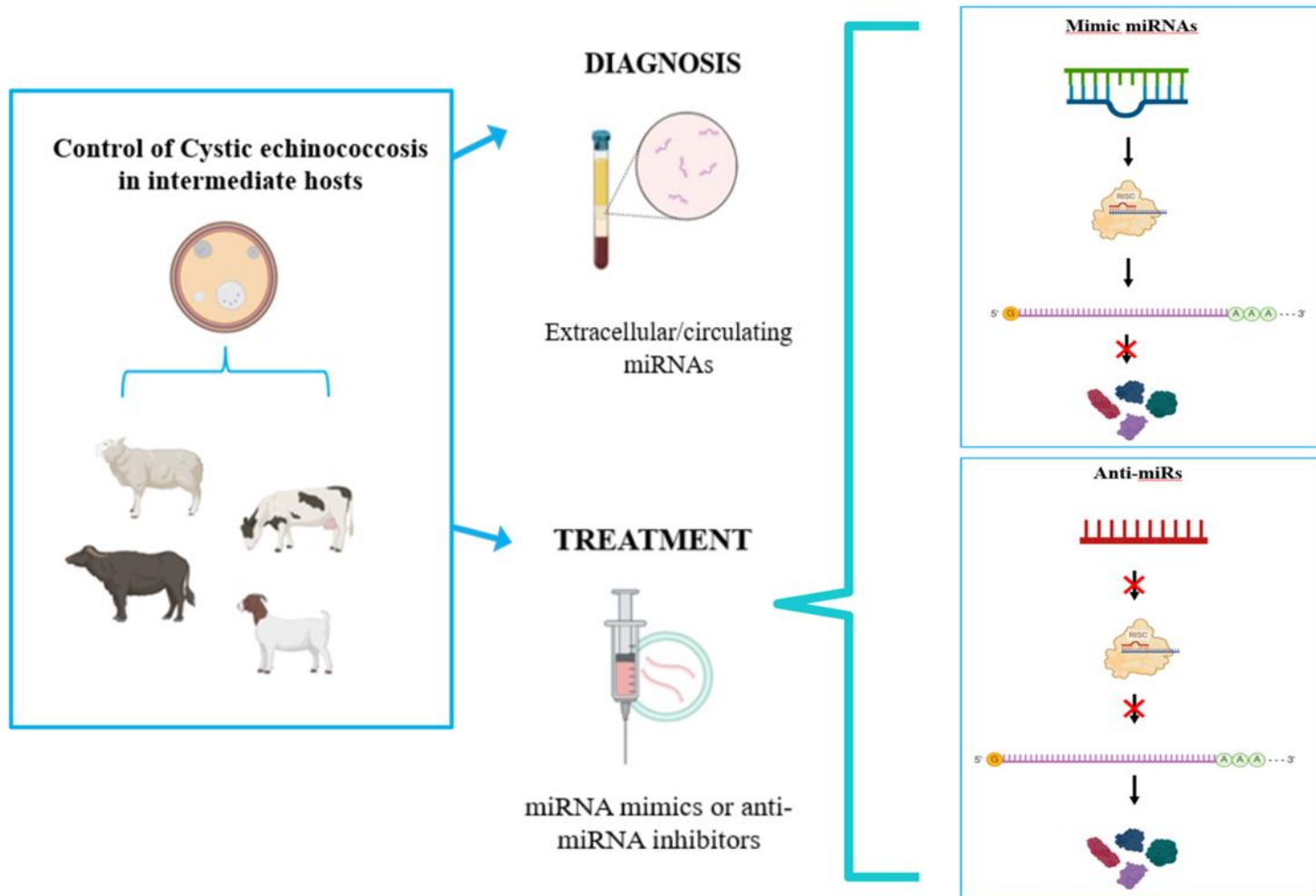
These results highlight the importance of including, in the CE control strategies, the treatment of not-owned dogs and other canids in the grazing areas

Capacity building, dissemination, exploitation and communication activities

- Standard Operating Procedures (SOPs), training videos and dissemination material shared among the partners and uploaded on Youtube and website
- Exchange of Staff Members (i.e. 15 among Professors, Post-Doc, PhD students and master students)
- Training School
- Posters/brochures translated into five languages (English, French, Greek Arabic and Italian)
- Dissemination meetings with veterinarians, farmers and school-aged children
- Presentations at national/international congresses



microRNAs (miRNAs) could be used as diagnostic and therapeutic targets to enhance control programs for Cystic Echinococcosis



- Exploding area of research that is generating considerable scientific interest and funding opportunities



PNRR CN3 - Spoke 5 - Task 4.2 – Task Leader: UNINA

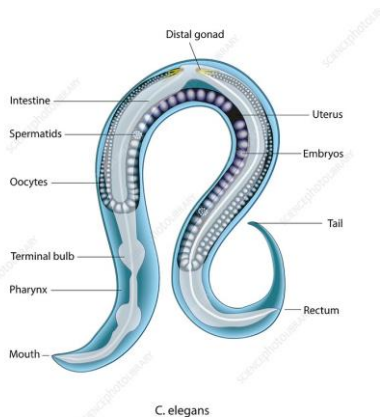
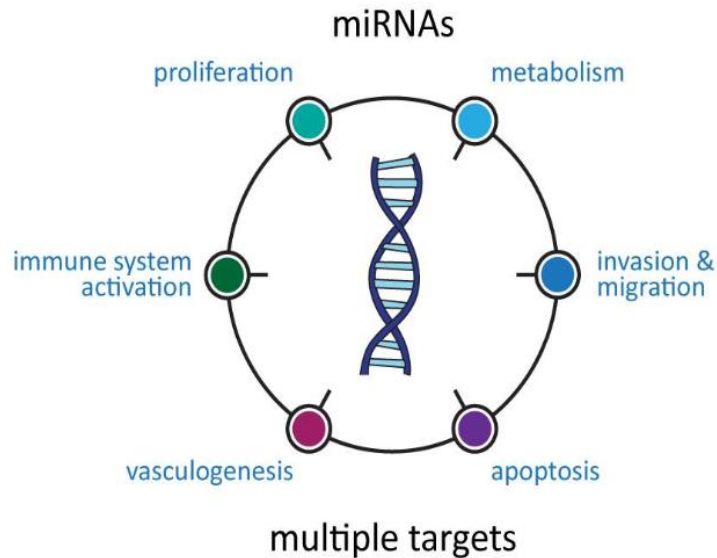
Spoke 5 Inflammatory and Infectious

Task 4.2 Identification and characterization of non-coding RNA [small interfering RNAs (siRNAs), **microRNAs** (miRNAs) and long non-coding RNA (lncRNA)] implicated in different parasitic stages of *Echinococcus granulosus*.

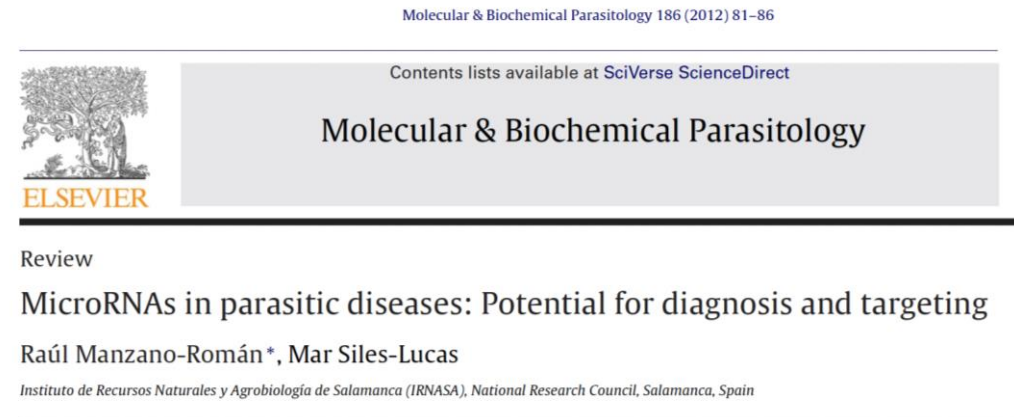
The final aim is to identify **new potential targets** for the early **diagnosis** and **treatment** of cystic echinococcosis.

microRNA (miRNA, miR)

miRNAs have been explored as diagnostic biomarkers and therapeutic targets in various infectious diseases

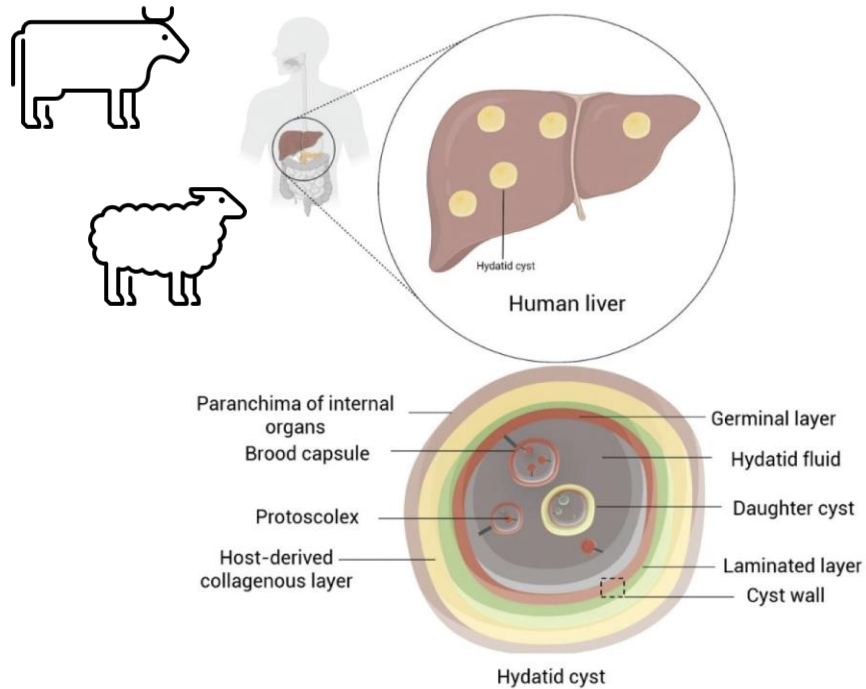


➤ First miRNAs discovered in *Caenorhabditis elegans*: **lin-4** and **let-7** (Lee et al., *Cell*, 1993)



- The miRNAs functions and characteristics make them **very attractive for target-specific treatment of diseases, as well as for the diagnosis**
- Alteration of intracellular miRNAs expression has been associated with many pathological conditions, e.g. oncological pathologies, Alzheimer's etc.

Expressed miRNAs in different components of hydatid cyst



Expressed miRNAs in different components of hydatid cyst

Hydatid fluid	Protoscolex	Cyst wall
egr-miR-4989-3p egr-miR-125-5p egr-let-7-5p egr-miR-71-5p egr-miR-61-3p egr-miR-277a-3p egr-miR-10a-5p egr-bantam-3p egr-miR-2162-3p egr-miR-2a 3p egr-let-7-3p egr-miR-96-5p	miR-2 miR-71 let-7 miR-10 miR-9 miR-277 miR-125 miR-2d	miR-2 miR-71 let-7 miR-10 miR-9 miR-277

Reviewed in Habibi et al., 2023.
Expert Rev Mol Diagn, 23: 885-94.

A total of 167 mature miRNAs in *E. granulosus* (different stages) are documented in the miRBase database (www.mirbase.org)

Table 2. Common miRNAs in *Echinococcus* spp.

Author/year	Parasite	miRNAs	Target genes	Biological function
Juvvuna et al. 2012 Guo et al. 2017 Cucher et al. 2015	<i>E. granulosus</i>	MiR-71 MiR-36	Nemo-like kinase Unknown	Involved in protoscolex development and regulates host macrophage functions in <i>Echinococcus</i> May correspond with the increased regenerative capacity of <i>E. multilocularis</i> with respect to that of <i>E. granulosus</i> s.l.
BaiY et al. 2014 Mortezaei et al. 2019		Let-7	Unknown	May be associated with the capability of <i>E. granulosus</i> for bi-directional development, can be significantly affected in the microcyst stage of <i>E. granulosus</i> and can exhibit different changes in expression in response to albendazole sulfoxide
Macchiaroli et al. 2017		MiR-277 MiR-10	ECANG7_01278; ECANG7_02522 MAPKs; ECANG7_04447; ECANG7_01705; ECANG7_09658	May be involved in the regulation of Wnt signaling pathways regulating the pluripotency of stem cells in <i>Echinococcus</i> May be involved in regulating the MAPK and Wnt signaling pathways in <i>Echinococcus</i>
		MiR-125 MiR-7	ECANG7_01292; ECANG7_01524 ECANG7_04919 and ECANG7_00818; ECANG7_03238	May regulate developmental genes in <i>Echinococcus</i> Potentially involved in the developmental morphogenesis of <i>Echinococcus</i>
		miR-195-5p ↑ miR-16-5p miR-19 ↓	BCL2 COL1A1 and COL3A	Promotion of apoptosis Suppression of cell proliferation by blocking signal transmission in the TGF-β pathway
Mortezaei et al. 2019		MiR-61	Unknown	Significantly affected in the microcyst stage of <i>E. granulosus</i> and exhibits different changes in expression in response to albendazole sulfoxide
Alizadeh et al. 2020		egr-miR-71* egr-let-7*	Unknown	Unknown
Fasihi et al. 2022		egr-miR-125-5p and egr-miR-2a-3p	Unknown	Unknown
Fasihi et al. 2023		egr-miR-125,5p, egr-let-7,5p, egr-miR-2, and egr-miR-71	Unknown	Unknown

Note: *Parasite derived miRNAs ↑ Indicates upregulated ↓ Indicates downregulated.

Diagnostic and Therapeutic Targets

> Expert Rev Mol Diagn. 2023 Feb;23(2):133-142. doi: 10.1080/14737159.2023.2178903.
Epub 2023 Feb 18.

Diagnosis of echinococcosis by detecting circulating cell-free DNA and miRNA

Mahboubeh Hadipour¹, Majid Fasihi Harandi², Hossein Mirhendi¹, Hossein Yousofi Darani¹



During *E. granulosus* infection, **parasite-derived miRNAs** are released into circulation from mammalian cells and tissues and **can be stably detected in the plasma or and serum of infected hosts**, even without the presence of parasites in the blood circulating system

Not only cellular but also circulating miRNA
= early **diagnosis** of CE



International Journal for Parasitology

Available online 10 September 2023

In Press, Corrected Proof ? What's this? ↗



microRNA silencing in a whole worm cestode model provides insight into miR-71 function

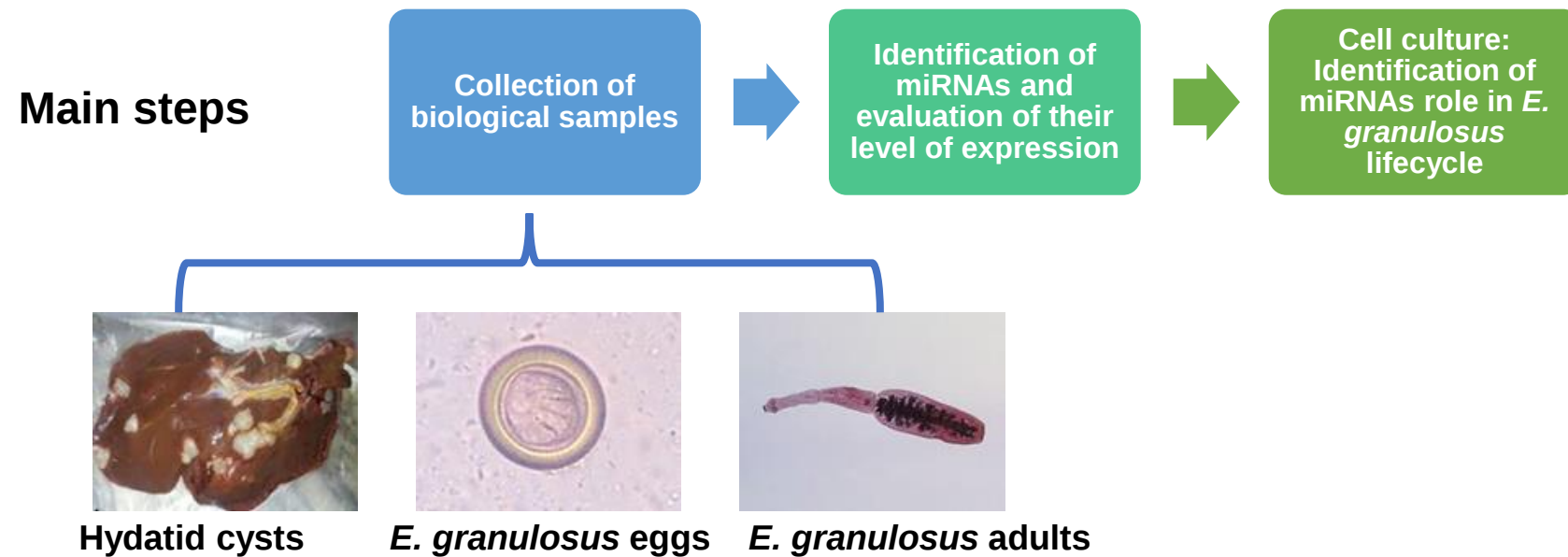
Andrés Grecco^{a b}, Natalia Macchiaroli^c, Matías Gastón Pérez^{a b}, Adriano Casulli^{d e},
Marcela Alejandra Cucher^{a b}, Mara Cecilia Rosenzvit^{a b}  



Analysed at the organism level in a larval-stage **cestode model**, demonstrating its central role in the establishment of successful infection in the experimental host

Involved in parasite development and host
parasite interaction
= Potential **therapeutic** target

Task 4.2 - Study Design



Research steps



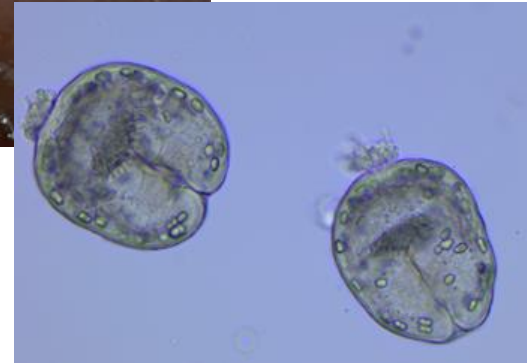
- 1) Hydatid cyst collection (water buffaloes, cattle, sheep, goats, humans). Different typology (fertile/sterile etc)
- 2) Collection of *E. granulosus* eggs and adults
- 3) Total RNA extraction
- 4) Bioinformatics analysis and identification of miRNA most present in the various stages of development
- 5) Real-Time PCR to quantify miRNA identified
- 6) Cell cultures overexpression/silencing miRNA to identify the role in the biological cycle of *E. granulosus*
 - ✓ Diagnostic marker (especially for sheep)
 - ✓ Therapeutic target

Collection of biological samples

Hydatid cysts



- Sheep, goats, cattle and water buffaloes were screened for the presence of hydatid cysts during the **ordinary slaughterhouse activities**
- Germinative membranes and hydatid liquid (containing vital protoscoleces) were sampled and stored for the RNA extraction



E. granulosus eggs



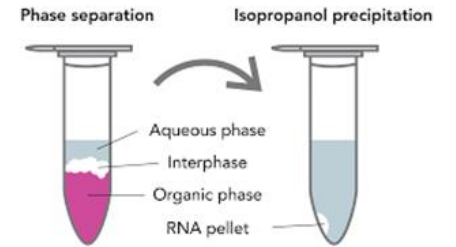
- Screening of faecal samples for the detection of Taeniidae eggs
- Multiplex PCR to identify *E. granulosus* (Bowles and McManus, *International Journal for Parasitology*, 1993)
- Aliquots of *E. granulosus* eggs for RNA extraction



RNA extraction, identification and evaluation of miRNA level expression in each development stage of *E. granulosus*

Total RNA extraction is carried out for all biological samples collected

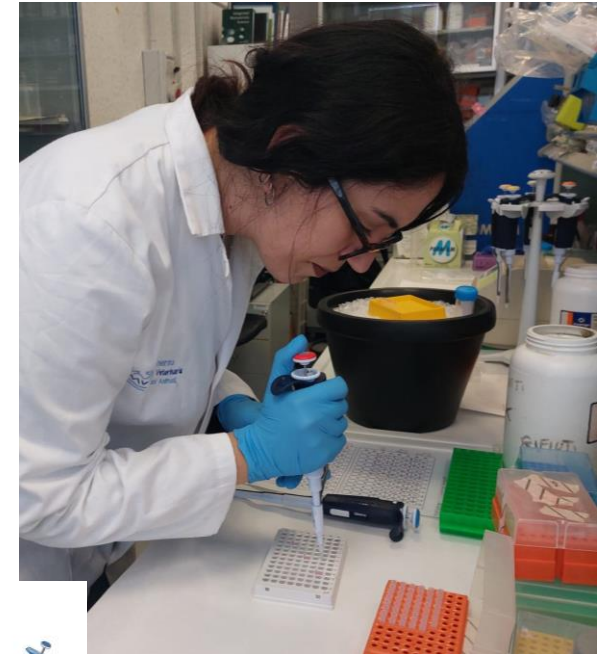
Only samples with a **good quality of RNA extracted** (e.g. concentration, 260/280 and 260/230 ratios and no nucleic acid degradation) are used for further analysis



RNA SAMPLES

Bioinformatics analysis and identification of miRNAs most present in each development stage of *E. granulosus* (Germinative membranes, protoscoleces, eggs)

Evaluation through Real-Time PCR of the levels expression of the most important miRNAs identify in literature (**miR-71, miR-125, miR-10 and let-7**) in our samples



Preliminary results – Sample collection

INTERMEDIATE HOSTS – Hydatid cysts



To date:

> **3000** animals screened

123 positive to CE

Animal Species*	Germinative membranes (Fertile cysts)	Protoscoleces	Germinative membranes (Sterile cysts)
Sheep	66	66	17
Cattle	0	0	27
Water buffalo	4	4	9
Total	70	70	53

Only Sterile cysts!

*No goats resulted positive to CE

DEFINITIVE HOSTS - Eggs



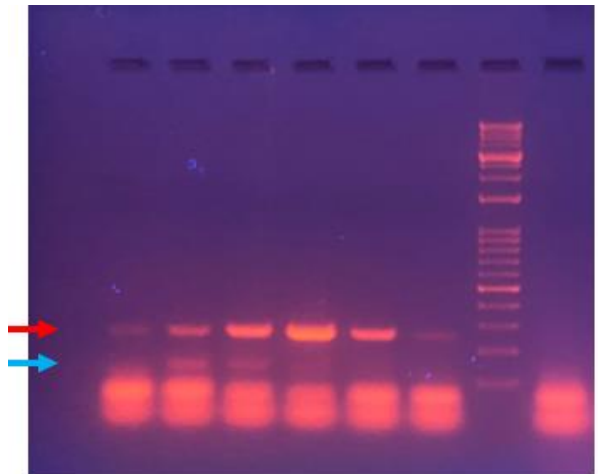
To date:

937 faecal samples analyzes

53 positive to Taeniidae

20 positive only to *E. granulosus*

(Trachsel et al., *Parasitology*, 2007)



- *Taenia* spp 267 bp
- *Echinococcus granulosus* 117 bp
- *Echinococcus multilocularis* 395 bp

45 samples (germinative membranes, protoscoleces, eggs) were selected for bioinformatic analysis

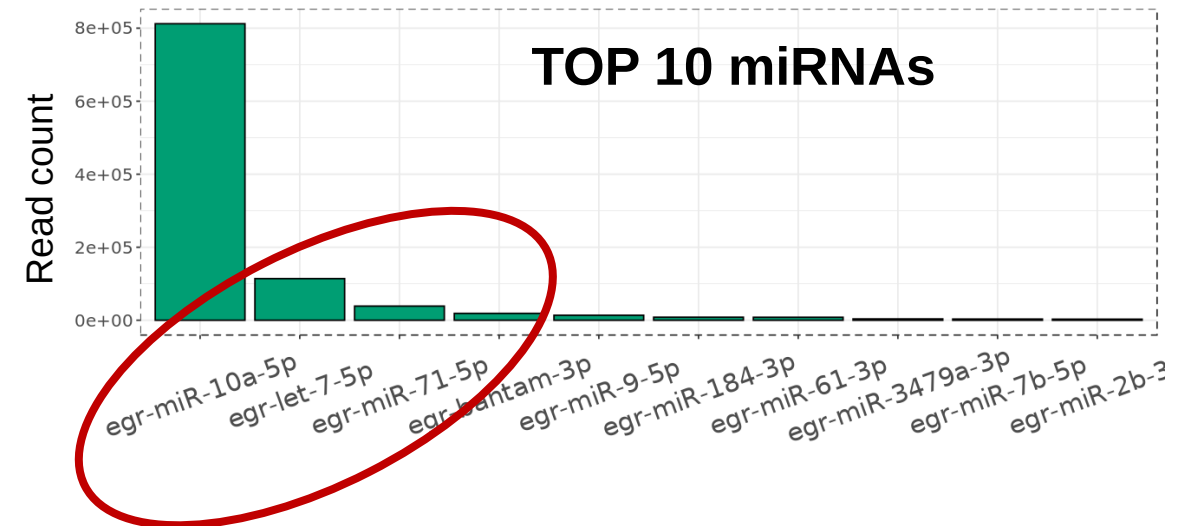
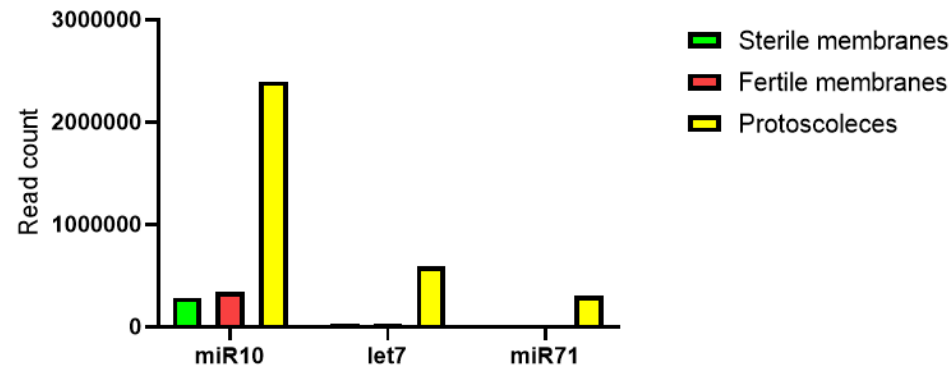
Preliminary results – Bioinformatic analysis

! Bioinformatic analysis on *E. granulosus* eggs is still ongoing

The results on hydatid cysts showed the presence of **169 mature miRNAs**

The **3** most abundant miRNAs are:

- egr-miR-10a-5p
- egr-let-7-5p
- egr-miR-71-5p



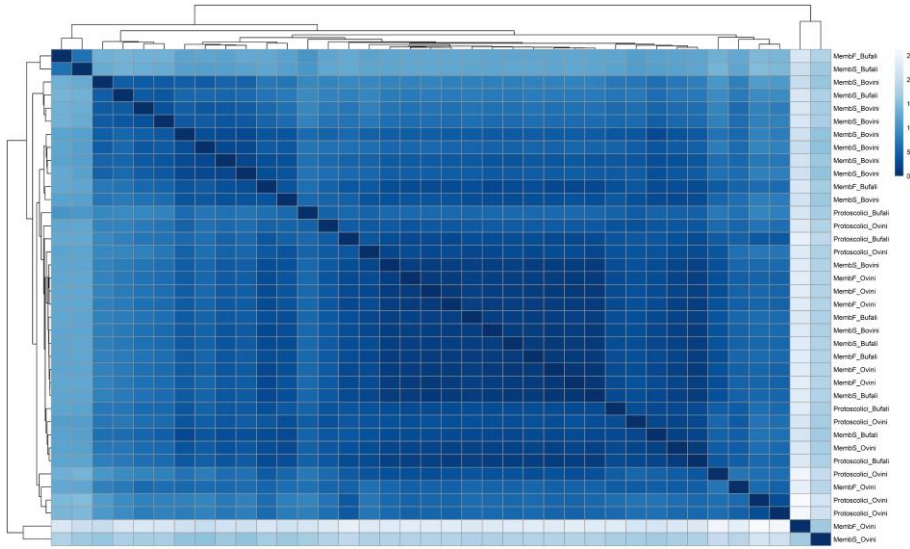
- **miRNA 10a-5p** resulted the most abundant with over 2,000,000 and 300,000 reads expressed respectively in Small RNA libraries from protoscoleces and cysts of *E. granulosus*.

- No differences of miRNAs presence were found between animal species (sheep, cattle and water buffalo).

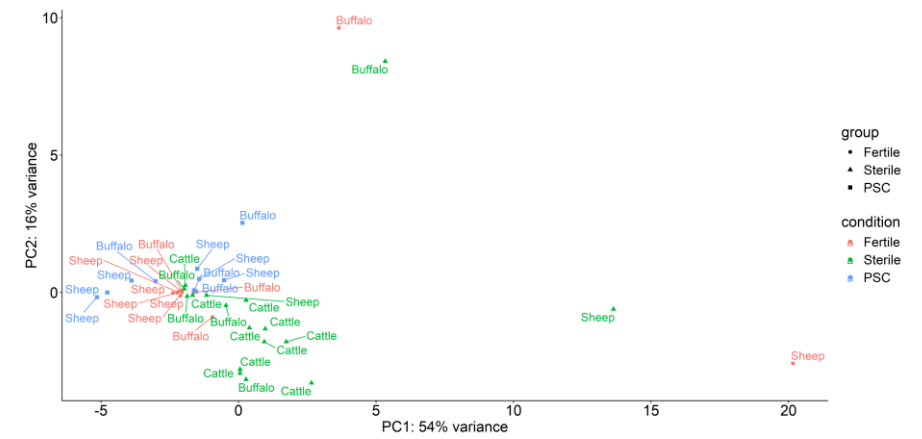
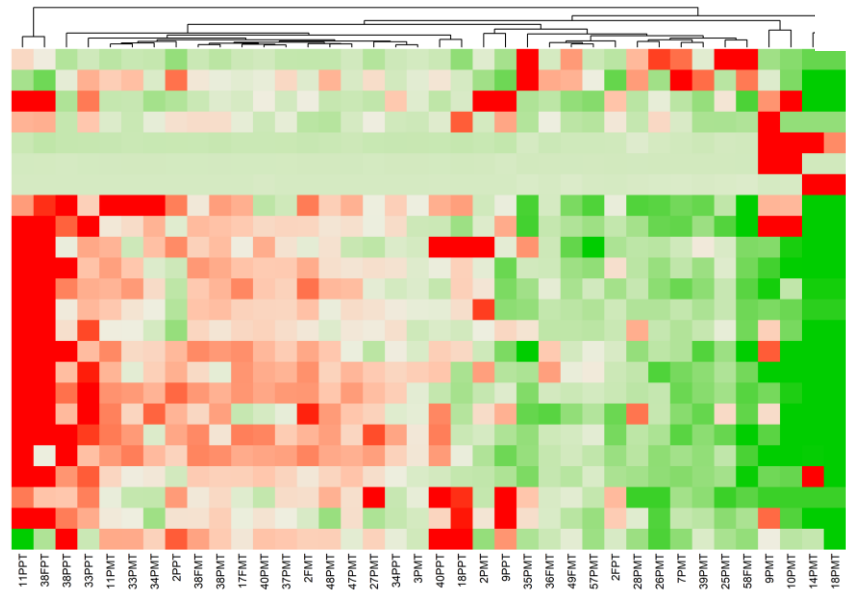
Preliminary results – Bioinformatic analysis

Differential Expression Analysis

- 38 Samples
- 3 conditions: Fertile, Sterile, Protoscoleces
- 3 ruminant species: sheep, cattle, water buffaloes



Heatmap Expression



PCA (Principal component analysis)

Preliminary results – Bioinformatic analysis

Differential Expression Analysis: Comparison Protoscoleces vs Sterile membranes

miRNAs	Base mean	Fold Change	PADJ
egr-miR-10240-5p	173,2627	-20,9174	2,76E-07
egr-miR-4989-3p	1049,854	-2,37378	0,002815
egr-miR-9-5p	5613,743	-2,22079	0,006513
egr-miR-2c-5p	140,979	1,927532	1,18E-06
egr-miR-3479a-3p	1196,39	1,887539	0,002815
egr-miR-124b-3p	174,4296	1,886974	0,002815
egr-miR-4989-5p	145,4578	1,647932	0,002815
egr-miR-8-3p	25,09782	1,72649	0,002815
egr-miR-2a-3p	1120,663	2,149147	0,002994
egr-let-7-3p	293,6827	1,578332	0,006628
egr-miR-87-5p	225,3361	1,8356	0,00732
egr-miR-7b-3p	38,24587	1,801538	0,00732
egr-miR-124a-3p	48,67281	1,816394	0,008065
egr-miR-71-5p	15144,87	1,343511	0,008699
egr-bantam-3p	7226,981	1,441625	0,011317
egr-miR-125-5p	948,596	1,861841	0,011317
egr-miR-125-3p	71,66744	1,684876	0,014748
egr-miR-10232-3p	10,32128	2,138402	0,018616
egr-miR-133-3p	42,25157	1,891206	0,023525
egr-miR-10450-3p	28,10387	1,596814	0,023835
egr-miR-10230-5p	96,28447	1,39832	0,028217
egr-miR-61-5p	41,41247	1,578358	0,028217
egr-miR-133-5p	6,704414	2,224892	0,035773
egr-miR-2b-3p	1504,124	1,483393	0,047306

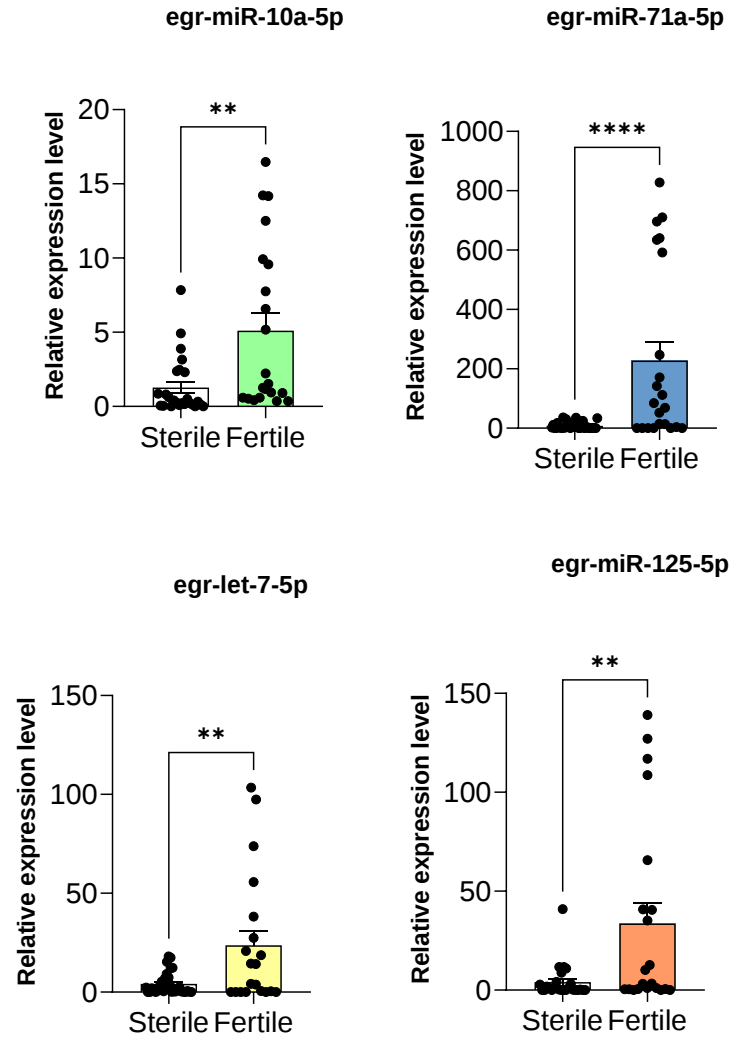


24 miRNAs differentially expressed

● 3 down- regulated

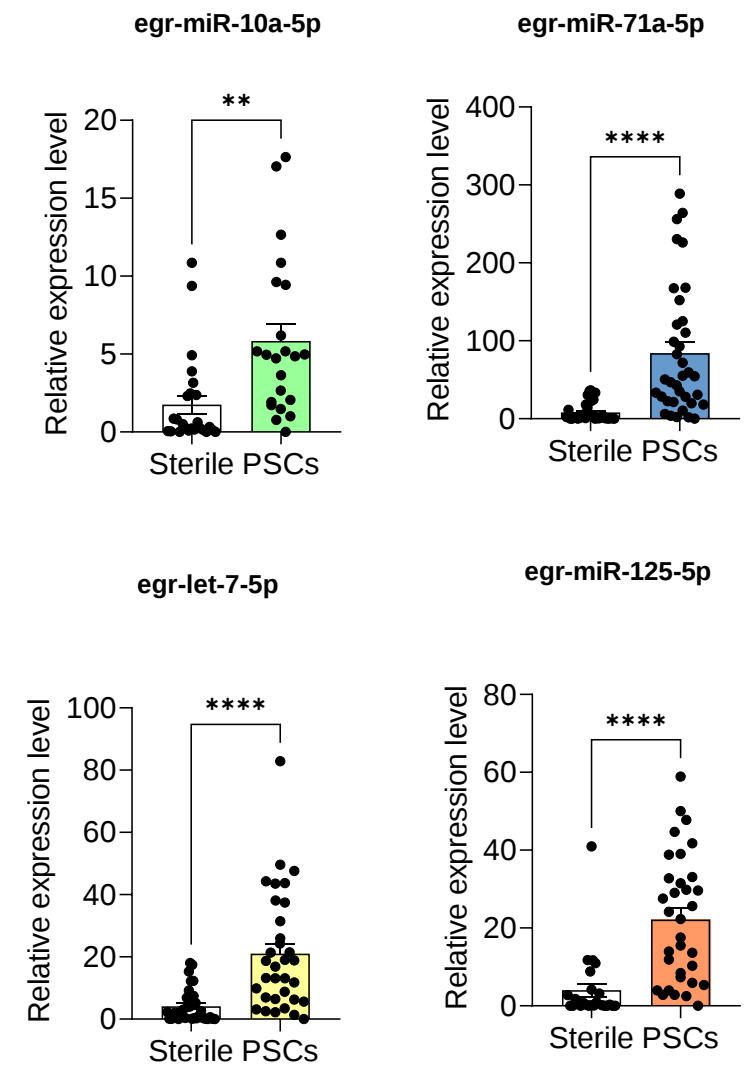
● 21 up-regulated

Preliminary results – Real-Time PCR



Sterile vs Fertile

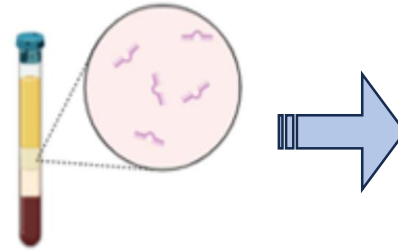
NO statistically significant differences of miRNAs level expression were found between the different species ($p > 0.05$)



Sterile vs Protoscoleces

No differences
Fertile vs Protoscoleces

BLOOD SAMPLING IN INTERMEDIATE HOSTS FOR THE EARLY DIAGNOSIS OF CE

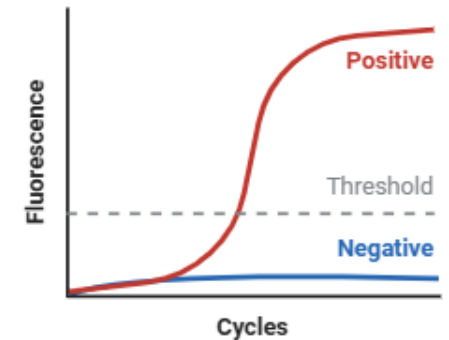


Evaluation through **RealTime and Digital PCR**

miR-2, miR-9, let-7 and miR-71

Orsten et al., *Journal of Helminthology*, 2021;
Orsten et al., *Parasitology research*, 2022;
Orsten et al., *Fabad Journal of Pharmaceutical Sciences*, 2023;
Karami et al., *Acta Parasitologica*, 2023;

Species	No. of serum samples	Type of cysts
Water buffaloes	4	Sterile
Cattle	9	Sterile
Goats	4	Sterile/Fertile
Sheep	14	Sterile/Fertile



Ongoing....

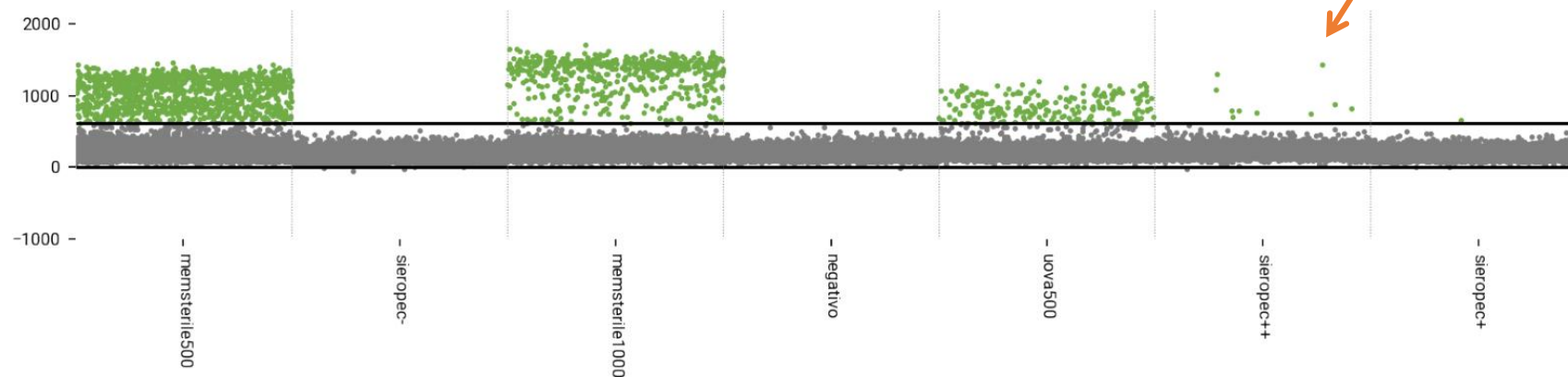
Preliminary Results – Circulating miRNAs

miRNAs investigated:
miRNA 71, Let-7
miRNA 2 and miRNA 9

Slight positivity to
miRNA 71 in a highly
positive sheep serum



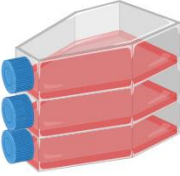
mir71
VIC: mir71



Ongoing....

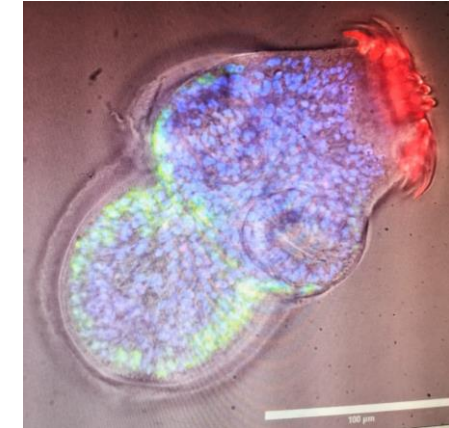
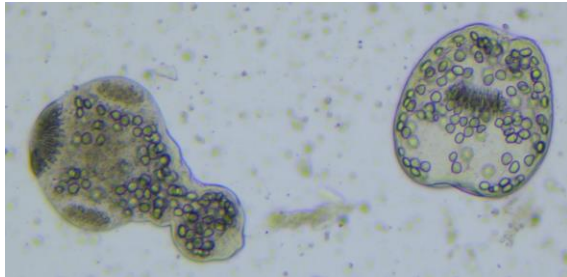
FUTURE STEPS AND PERSPECTIVES:

- Cell cultures overexpression/silencing miRNA to identify the role in the biological cycle of *E. granulosus*



Prof. Klaus Brehm

GENOMIC and TRANSCRIPTOMIC AND EXPRESSION ANALYSIS OF CODING GENES IN THE METACESTODE STAGE of *Echinococcus multilocularis*



Institute of Hygiene and Microbiology, University of Würzburg, Germany



Parasitology International
Volume 59, Issue 4, December 2010, Pages 647-652



Short communication

Gene silencing in *Echinococcus multilocularis* protoscoleces using RNA interference

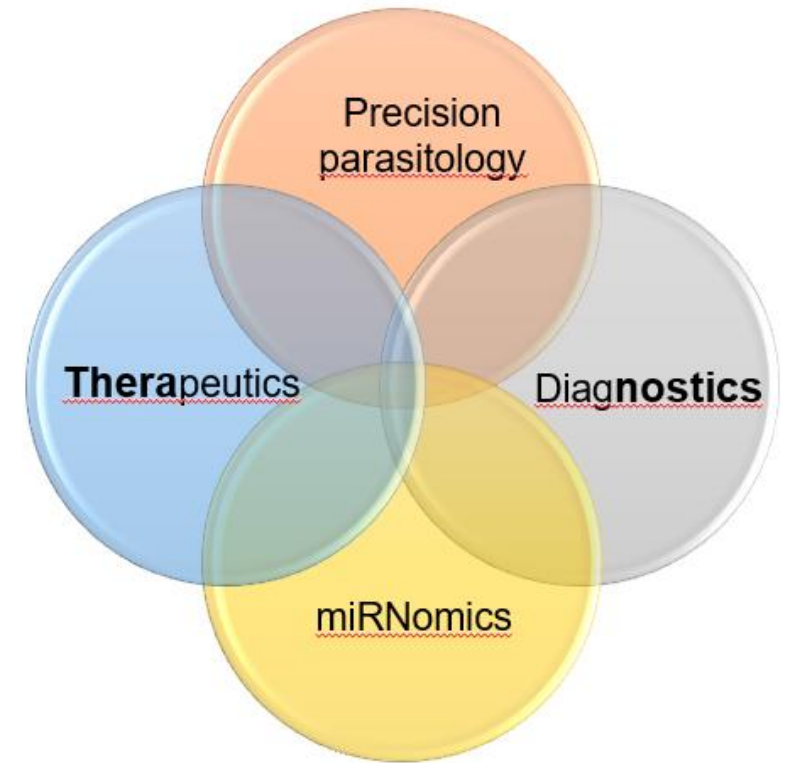


RNA interference (vs miRNA mimics)
to target genes involved in protoscoleces development

CONCLUSIONS

- **Integrated control programs** such as **EchinoCamp** have proven effective in reducing CE prevalence in southern Italy
- **ECHINO-SAFE-MED** has expanded these efforts to a **Mediterranean level**, building strong cooperation among countries
- **miRNA research** is opening new frontiers for **early diagnosis and RNA-based therapy**, represent promising steps towards reducing the burden of CE

Although much progress has been made, continuous surveillance, the involvement of all stakeholders, and the One Health perspective remain essential to achieve sustainable control of CE





Dr. Elena Ciccone
DVM, PhD student
elena.ciccone@unina.it



UNIVERSITÀ
DEGLI STUDI DI NAPOLI
FEDERICO II



Dipartimento
Medicina Veterinaria
Produzioni Animali



**THANKS FOR YOUR
ATTENTION**



Prof. Laura Rinaldi
PhD, Ass. EVPC
Full Professor of Parasitology
and Parasitic Diseases
Director WHO Collaborating
Centre for Diagnosis of Intestinal
Helminths and Protozoa
l.rinaldi@unina.it

