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Update on knowledge of transmission of *Echinococcus* to humans

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Revealing the transmission of *Echinococcus* eggs to humans

Cristian Alvarez Rojas

Institute of Parasitology, University of Zurich

1. Molecular diagnosis of echinococcosis in the definitive host

2. *Echinococcus* as a foodborne parasite?

1. Molecular diagnosis of echinococcosis in the definitive host

CHAPTER 3.1.6.

ECHINOCOCCOSIS (INFECTION WITH *ECHINOCOCCUS GRANULOSUS* AND WITH *E. MULTILOCULARIS*)

OIE *Terrestrial Manual* 2019

CoproDNA detection tests OIE manual, 2019

Table 3. PCR primers used for coproDNA detection (modified from Craig et al., 2015). Tissue indicates that the technique is also compatible with DNA extraction from metacestode tissues

Gene	Species	Copro-sample	Tissue	Reference
cox1 Forward primer: 5'-TCA-TAT-TTG-TTT-GAG-KAT-YAG-TKC-3', reverse primer: 5'-GTA-AAT-AAM-ACT-ATA-AAA-GAA-AYM-AC-3'	*E. granulosus*	Eggs	Yes	Cabrera *et al.*, 2002
EgG1Haelll Eg1121a 5'-GAA-TGC-AAG-CAG-CAG-ATG-3' (upstream) Eg1122a 5'-GAG-ATG-AGT-GAG-AAG-GAG-TG-3' (downstream)	*E. granulosus* s.l	Faeces	Yes	Abbasi *et al.*, 2003
12sRNA Eg1f, 5'-CAT-TAA-TGT-ATT-TTG-TAA-AGT-TG-3'; Eg1r, 5'-CAC-ATC-ATC-TTA-CAA-TAA-CAC-C-3'	*E. granulosus* G1	Eggs/ Faeces	Yes	Stefanic *et al.*, 2004
12S rRNA G1: E.g.ss1for. 5'-GTA-TTT-TGT-AAA-GTT-GTT-CTA-3' E.g.ss1rev. 5' CTA-AAT-CAC-ATC-ATC-TTA-CAA-T-3' G5, G6, G7: E.g.cs1for. (5'-ATT-TTT-AAA-ATG-TTC-GTC-CTG-3'), E.g.cs1rev. (5'-CTA-AAT-AAT-ATC-ATA-TTA-CAA-C-3' To discriminate between *E. ortleppi* and *E. granulosus* G5/7, semi-nested PCRs specific for G5/7 *(g5/7 PCR*, primer pair E.g.camel.for. 5'-ATG-GTC-CAC-CTA-TTA-TTT-CA-3' and E.g.cs1rev.) and for *E. ortleppi (g5 PCR*, primer pair E.g.cattle.for. 5'-ATG-GTC-CAC-CTA-TTA-TTT-TG-3' and E.g.cs1rev.)	*E. granulosus* G1, G5, G6/7	No	Yes	Dinkel *et al.*, 2004
Cox1, NAD, rrnS Multiple sequences referred to	*E. granulosus*, *Taenia* spp.	Eggs	Yes	Trachsel *et al.*, 2007
Real-time multiplex-nested PCR Primer/probe sequence (5'–3') P60.short.for TGG-TAC-AGG-ATT-AGA-TAC-CC P375.short.rev TGA-CGG-GCG-GTG-TGT-ACC CVF.for TTA-ATG-ACC-AAC-ATT-CGA-AA CVF.rev AGG/T-ACA/G-TAG/C-CCC-ATA/G-AAA/T-GC Pnest.for ACA-ATA-CCA-TAT-TAC-AAC-AAT-ATT-CCT-ATC Pnest.rev ATA-TTT-TGT-AAG-GTT-GTT-CTA CVF.light for TCA/T-GCC/T-TGA-TGA/G-AAC-TTC-GGA/G-TCC CVF.light rev AC/TA/G-ATT-CCA-ATA/G-TTT-CAT-GTC/T-TCT emult-6	*E. multiocularis*, *E. granulosus* (G1), *E. ortleppi*, *E. canadensis* (G6, G7), other taeniids	Faeces	Yes	Dinkel *et al.*, 2011

Gene	Species	Copro-sample	Tissue	Reference
CTA-AAA-CTA-CAC-AAA-CTT-ACA-TTA-CTA--FL emult-705 LC705-ACA-ATA-ATA-TCA-AAC-CAG-ACA-TAC-ACC-A--PH CaVulFe1-fl ATA-CAC-TAT-ACA-TCT-GAC-AC--FL CaVulFe2-640 LC640-GCT-ACT-GCT-TTC-TCA-TCT-G--PH				
LAMP method Eg1121gGAA-TGC-AAG-CAG-CAG-ATGEg1122aGAG-ATG-AGT-GAG-AAG-GAG-TGFIP1echCTT-TTC-CGG-ATG-GGT-AGG-CAT-CTT-TTG-ATC-ACT-CCT-ATT-CTA-GCA-TGTBIP1echCGT-GCT-GTG-GAG-GTA-GTT-TCG-TTT-TCA-GTG-AGA-TGA-GTG-AGA-AGG	*E. granulosus* G1	Eggs	Yes	Satanl *et al.*, 2012
ND1 Eg1F81, 5'-GTT-TTT-GGC-TGC-CGC-CAG-AAC-3' and Eg1R83, 5'-AAT-TAA-TGG-AAA-TAA-TAA-CAA-ACT-TAA-TCA-ACA-AT-3' EmF19/3, 5'-TAG-TTG-TTG-ATG-AAG-CTT-GTT-G-3' and EmR6/1, 5'-ATC-AAC-CAT-GAA-AAC-ACA-TAT-ACA-AC-3'	*E. granulosus* G1; *E. multiocularis*; *E. shiquicus*	Faeces	Yes	Boufana *et al.*, 2013
Many mitochondrial and nuclear primers	*E. granulosus* complex G1–G10	(Eggs)	Yes	Boubaker *et al.*, 2013
Nad5 gene primers inc. (LAMP method) Primer name Sequence (5' → 3') FIP TTA-ACC-AAC-CAA-TAA-CAA-CCC-AGT-gaattc-GTG-GTG-TTA-GTT-ATT-TGG-TTA-GG BIP ATG-TGA-CGT-TTG-GTG-TGG-TAG-TTA-gaattc-AAG-AAC-CAC-CAA-AAT-AAT-GTC-T F3 GTG-TGT-TGC-TAT-ATT-GCT-TGT B3 AAC-TTT-AAC-AAC-ATA-CAC-CTA-GT	*E. granulosus* s.s (G1)	Faeces	Yes	Ni *et al.*, 2014
Magnetic capture – PCR mt 12S rRNA gene EMRC01F' (5'-TGG-TAT-AAA-GGT-GTT-TAC-TTG-G-3'), EMRC01Rew' (5'-ACC-TAA-ACA-ACA-CTA-TAA-AAC-A-3'), and Zen probe: 5'-56-FAM-TCT-AGT-GTA/Zen-AAT-AAG-AGT-GAT-CCT-ATT-TTG-TGG-TGG-GT/3IABkFQ/-3'	*E. multiocularis*	Faeces	Yes	Isaksson *et al.*, 2014
Real-time PCR large ribosomal subunit gene (rrnL) Em-rrn forward primer: 5'-CTG-TGA-TCT-TGG-TGT-AGT-AGT-TGA-GAT-TT-3' Em-rrn reverse primer: 5'-GGC-TTA-CGC-CGG-TCT-TAA-CTC-3' Em-probe with reporter 6-carboxyfluorescein (FAM) and quencher tetramethylrhodamine (TAMRA): 5'-TGG-TCT-GTT-CGA-CCT-TTT-TAG-CCT-CCA-T-3'	*E. multiocularis*	Faeces	Yes	Knapp *et al.*, 2014

LAMP: loop-mediated isothermal amplification

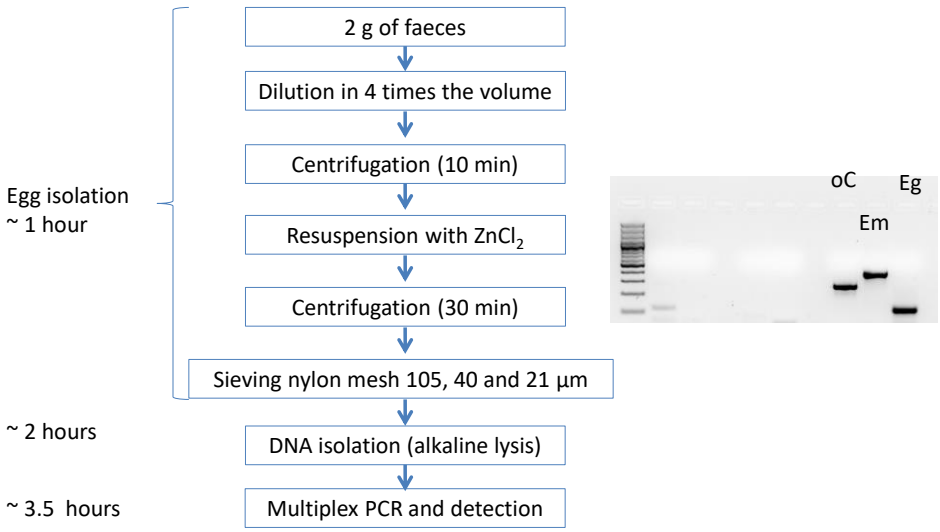
Initial material

	Total faeces	Eggs isolated from faeces
Starting material	Usually up to 0.5 g	2-20 g
Pre patency detection	Yes	No
Free DNA	Can also detect free DNA	Tests can detect 1 egg
Microscopic confirmation	No	Yes
Inhibition PCR	Possible	Less likely

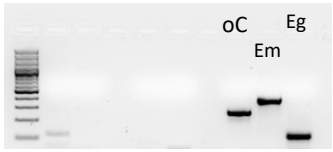
DNA isolation method from eggs

	Method	Reference
Not mentioned	50 µL of lysozyme (10 mg/ml) ON Freezing thaw liqN2 Proteinase K/SDS 10% lysis buffer (100 µL of 5 M ClNa and 100 µL of CTAB/ClNa solution chloroform: isoamyl alcohol (24:1) extraction Isopropanol precipitation	Cabrera et al, 2002
2 g	Alkaline lysis KOH + DTT Tris-HCl+ HCl AL buffer (Qiagen) + proteinase K Chelex Qiamp DNA mini kit	Stefanic et al 2004
2 g	Same as above	Trachsel et al, 2007

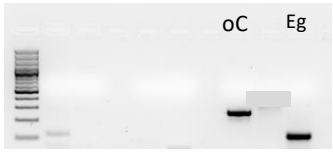
Multiplex PCR for the diagnosis of *E. granulosus*, *E. multilocularis* and other cestodes (Trachsel et al, 2007)



Multiplex PCR for the diagnosis of *E*. *granulosus*, *E*. *multilocularis* and other cestodes (Trachsel et al, 2007)

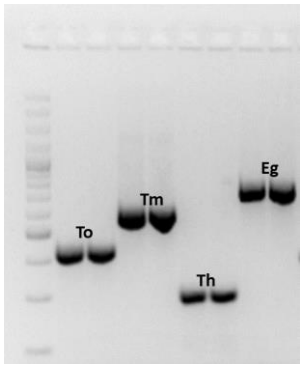


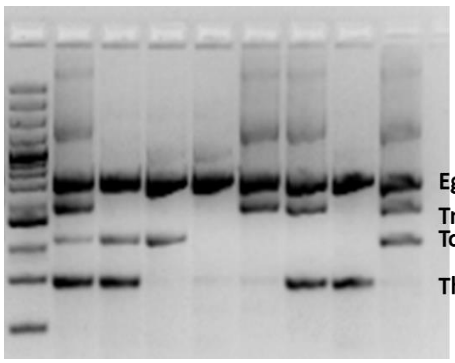
Europe, China, Kzrgyzstan



Europe, South America, Africa and Asia

Multiplex PCR for identification of dog-sheep taeniid

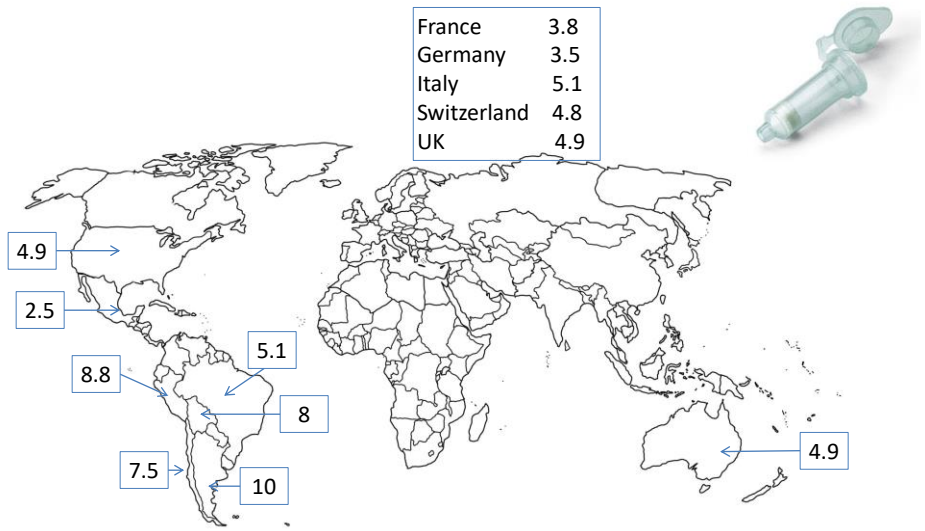




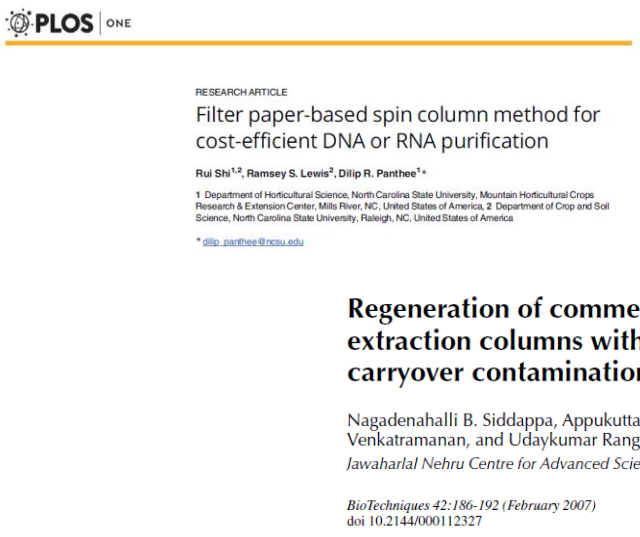
DNA isolation method from faeces

Starting material	Method	Reference
	QIAmp DNA Stool Mini Kit Invisorb Spin Stool DNA Kit	Abbasi et al, 2003
0.5g	Alkaline lysis KOH + DTT Tris-HCl+ HCl phenol-chloroform-isoamyl alcohol (25:24:1) Prep-A-Gene purification kit	Dinkel et al, 1998
	Alkaline lysis KOH + DTT Tris-HCl+ HCl phenol-chloroform-isoamyl alcohol (25:24:1) Prep-A-Gene purification kit	Dinkel et al, 2011
1-2 g	QIAamp DNA Mini Stool Kit	Boufana et al, 2013
0.5 g	QIAamp DNA Stool kit InhibitEX tablet	Knapp et al, 2014
3 g	zirconia beads to get the target DNA streptavidin sepharose a biotinylated DNA hybridization probe streptavidin-coated paramagnetic beads	Isaksson et al, 2014

Cost (USD) DNA isolation/sample (QIAamp DNA Mini Kit)



Feeling creative? Recycling?



PLOS ONE

RESEARCH ARTICLE

Filter paper-based spin column method for cost-efficient DNA or RNA purification

Rui Shi[1,2], Ramsey S. Lewis[2], Dilip R. Panthee[1]*

1 Department of Horticultural Science, North Carolina State University, Mountain Horticultural Crops Research & Extension Center, Mills River, NC, United States of America, 2 Department of Crop and Soil Science, North Carolina State University, Raleigh, NC, United States of America

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Regeneration of commercial nucleic acid extraction columns without the risk of carryover contamination

Nagadenahalli B. Siddappa, Appukuttan Avinash, Mohanram Venkatramanan, and Udaykumar Ranga

Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore, India

BioTechniques 42:186-192 (February 2007)
doi 10.2144/000112327

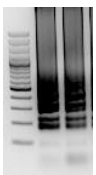
Alternatives for DNA "isolation"

- Frozen hydatid fluid (HF)
  - 1 ml was heated to 100°C for 30 min (positive PCR)
- Germinal layers and protoscoleces (frozen and fresh)
  - Alkaline lysis (NaOH and DTT)/HCl and Tris-HCl (positive PCR with 1:8 or 1:10 dilutions)

Boubaker et al, 2013

Alternatives to PCR

- Isothermal amplification of DNA
  - Loop-mediated isothermal amplification (LAMP)
  - Strand displacement amplification (SDA)
  - Helicase-dependent amplification (HDA)
  - Nicking enzyme amplification reaction (NEAR)



LAMP

Compared with PCR:

- Cheaper, faster YES
- More sensitive in theory YES
- Easier to perform NO
  - need of trained staff
  - High chance of contamination
- Is a test for the field YES/NO
  - It requires separate rooms as a PCR lab

Detection of *Echinococcus* using LAMP

Species	Faeces	Egg isolation	DNA isolation	Detection limit (DNA)	Detection limit (eggs)	Detection method
E. multilocularis (*)	2 g	NaCl flotation	QIAamp DNA Stool		4 eggs/g faeces	agarose gel with and SYBR Green I
E. granulosus (*)	2 g	NaCl flotation	QIAamp DNA Stool	10 pg	5 eggs/g faeces	agarose gel with and SYBR Green I
Taeniids (**)	NA	NA	QIAamp DNA mini	1 to 10 pg	3 eggs	Real-time turbidimeter
E. granulosus s.s. (***) *E. equinus* *E. ortleppi* *E. canadensis* *E. felidis*	NA	NA	Alkaline lysis		1/50 of an egg	agarose gel and UV light
E. granulosus (****)	0.2 g	NA	Spin Stool DNA Plus extraction	100 fg 1 pg/200 mg feces	1 egg/ 0.2 g (3/6)	agarose gel with and SYBR Green I

(*)Ni et al, 2014, (**) Feng et al, 2017, (***) Wassermann et al, 2014, (****) Salant et al, 2012

Isolated eggs: LAMP/lateral flow assay

- Detection of *E. granulosus s.s.* and *E. multilocularis*

Barbara Bucher

LAMP detection of *E. granulosus* and *E. multilocularis*

Egg isolation
~ 1 hour

- 2 g of faeces
- ↓ Dilution in 4 times the volume
- ↓ Centrifugation (10 min)
- ↓ Resuspension with $ZnCl_2$
- ↓ Centrifugation (30 min)
- ↓ Sieving nylon mesh 105, 40 and 21 µm

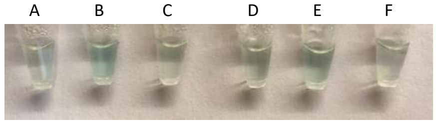
~ 15 min — DNA “isolation” NAOH | DNA “isolation” NAOH

1 hour 45 min — LAMP reaction and detection | LAMP reaction and detection

Preliminary results LAMP

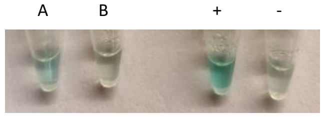
1 egg

A B C D E F



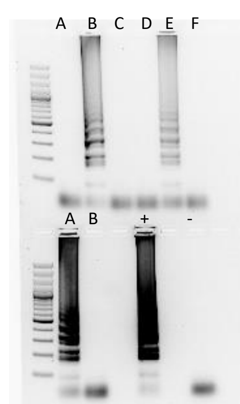
2 eggs

A B + -



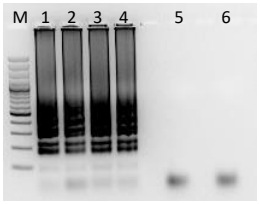
M Marker
1 1 egg
2 2 eggs

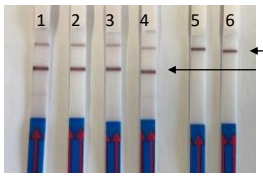
\+ Positive control
\- Negative control



Preliminary results LAMP

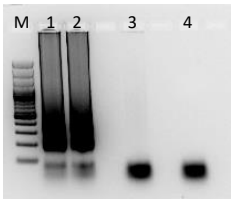
E. multilocularis:

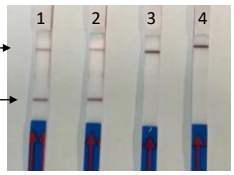




1-4: Em; 5: Eg; 6: H_2O

E. granulosus:





1-2: Eg; 3: Em; 4: H_2O

Echinococcus as a foodborne parasite

Current Clinical Microbiology Reports (2018) 5:154–163
https://doi.org/10.1007/s40588-018-0091-0

FOODBORNE PATHOGENS (S JOHLER, SECTION EDITOR)

Assessing the Contamination of Food and the Environment With Taenia and Echinococcus Eggs and Their Zoonotic Transmission

Cristian A. Alvarez Rojas[1] · Alexander Mathis[1] · Peter Deplazes[1]

1. The isolation and molecular identification of taeniid eggs is technically challenging and little standardized
2. Assessment of the environmental contamination is required
3. The detection of taeniid DNA ≠ viability
4. Need of viability tests for eggs

Transmission of *Echinococcus* eggs to humans

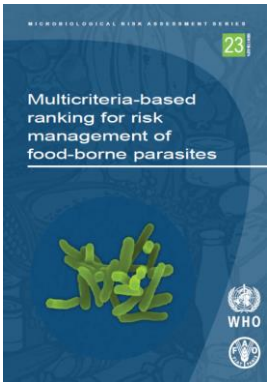
Direct contact with infected dogs

Ingestion through food and water

Direct ingestion of soil

Echinococcus as a foodborne parasite

- *Taenia solium* – Pork
- *Echinococcus granulosus* – Fresh produce
- *Echinococcus multilocularis* – Fresh produce
- *Toxoplasma gondii* – Meat from small ruminants, pork, beef, game (red meat and organs)
- *Cryptosporidium* spp. – Fresh produce, fruit juice, milk
- *Entamoeba histolytica* – Fresh produce
- *Trichinella spiralis* – Pork
- Opisthorchiidae – Freshwater fish
- *Ascaris* spp. – Fresh produce
- *Trypanosoma cruzi* – Fruit juices
- *Giardia duodenalis* – Fresh produce
- *Fasciola* spp. – Fresh produce (aquatic plants)
- *Cyclospora cayetanensis* – Berries, fresh produce
- *Paragonimus* spp. – Freshwater crustaceans
- *Trichuris trichiura* – Fresh produce
- *Trichinella* spp. – Game meat (wild boar, crocodile, bear, walrus, etc.)
- *Anisakidae* – Salt water fish, crustaceans, and cephalopods
- *Balantidium coli* – Fresh produce
- *Taenia saginata* – Beef
- *Toxocara* spp. – Fresh produce
- *Sarcocystis* spp. – Beef and pork
- Heterophyidae – Fresh and brackish water fish
- Diphyllobothriidae – Fresh and salt water fish
- *Spirometra* spp. – Fish, reptiles and amphibians



Evidence of Taeniid eggs in vegetables

Country	Number of samples	Weight of sample	Taeniid eggs
Iran	772	200-300 g	0.9-1.8%
	1,205	200-250 g	1.8-9.2% (unwashed) 0 (washed)
Jordan	133	250 g	6%
Lybia	126	100 g	22%
Nigeria	2,289	200-250 g	0.5-18.3%
Turkey	111	100 g	2.7%
	609	200 g	3.5% (unwashed) 0 (unwashed)

Varmia-Masuria Province, Poland

Matrix	**Egg isolation**	**PCR**	**Detection limit**	**Field samples**
Soil (*)	flotation with ZnCl2	Nested PCR 12S ribosomal RNA	1 egg	11.3% (7/62 samples)
Fruits, vegetable and mushrooms (**)	flotation with ZnCl2	Nested PCR 12S ribosomal RNA	100 eggs	23.3 %
Fruits, vegetable and mushrooms (***)		Nested PCR 12S ribosomal RNA		
Water (****)	Filtration	nested PCR and real-time (rrnL gene)	10 eggs/10 L	1.9% (2/105)

(*) Szostakowska et al, 2015; (**)(***) Lass et al, 2015; (****)Lass et al, 2019

Detection of DNA ≠ detection of eggs ≠ detection of viable eggs

Viability of *E. multilocularis* eggs

- Subcutaneous infection of the mice
- Dissection after 8 weeks

Federer et al., 2015

Hand-to-mouth contact

- Average frequency in children (Xue et al, 2007)
  - 6.7 to 28.0 contacts/h in indoor situations
  - 2.9 to 14.5 contacts/h outdoors

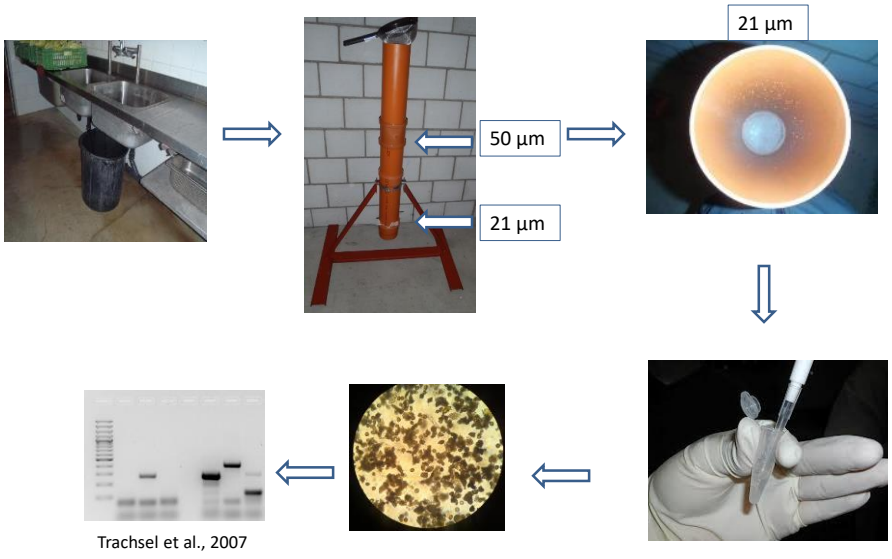
Soil and sediment ingestion

- 26 mg soil/day for children (1–8 years) faecal tracer studies (Stanek et al, 2012)
- 80 mg soil/day for children (7 months-4 years) (Health Canada, 2010)
- 18 to 72 mg sediment/h in aquatic areas (Wilson et al, 2014)

Detection of taeniid (*Taenia* spp., *Echinococcus* spp.) eggs contaminating vegetables and fruits sold in European markets

- 141 samples
- 60 kg per sample
- Each sample:
  - 40 heads of lettuce
  - carrots, bell pepper, leek, beetroot, fennel
- Rinsing water from the vegetables/fruits
  - 240 L per sample

Federer et al, 2016



Trachsel et al., 2007

Detection of taeniid (Taenia spp., Echinococcus spp.) eggs contaminating vegetables and fruits sold in European markets

Location	Number of samples	Taeniids	Number of positives
Basel	95	*T. hydatigena* *T. polyacantha* *T. ovis* *T. taeniformis* Other cestodes	4 2 3 5 3
		Total	17
Various European countries and Switzerland	46	*T. hydatigena* *T. saginata* *T. crassiceps* *T. taeniformis* *T. multiceps/T. serialis* *E. granulosus* Other cestodes	2 1 1 5 2 2 2
		Total	13

Detection of taeniid eggs in vegetables sold in Zurich 2019

- Standardization of the method at a lower scale
- Detection of other parasites

Annina Guggisberg

https://www.travelistas.info/home/ein-tag-zurich-oerlikon/attachment/markt-in-zurich-oerlikon/

https://www.friday-magazine.ch/articles/darum-lohnt-sich-einkaufen-auf-dem-markt

https://www.zuercher-maerkte.ch/oerlikon-1.html

Material and methods

Processing the salad

Sample size: 9 salad heads, 900-1500g from the outer/dirty leaves

Washing with 500 ml 0.02% Tween water per 300g

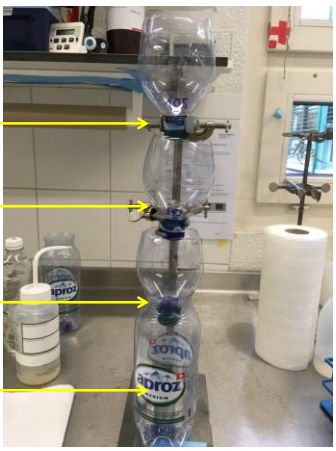
Material and methods

105 µm: debris

40 µm: *Toxocara* spp.

21 µm: Taeniids

Flow through: Protozoa

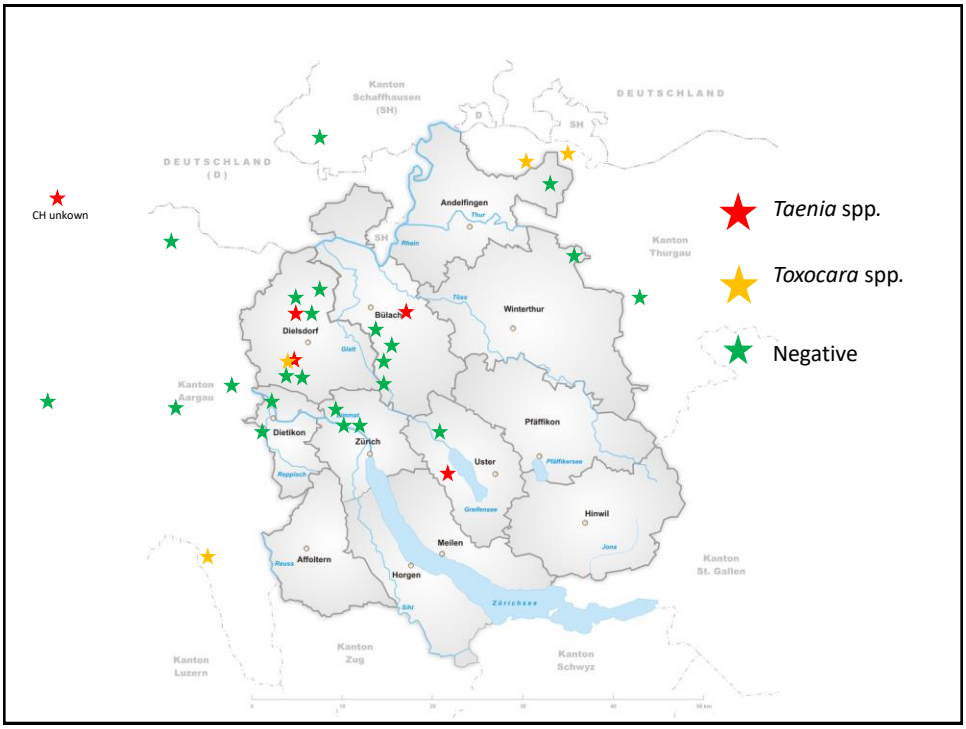


Recovery experiments

Substrate	No. of taeniid eggs	PCR *E. multilocularis* No. positives	Inverted Microscopy No. of positives	Metacestode growth
Water	40	5/5	4/5	0/5
Water	8	5/5	4/5	0/5
Salad	40	5/5	5/5	0/5
Salad	8	5/5	1/5	1/5

Preliminary results from 119 samples= >1,000 lettuce heads = >100 kg

Parasite species	Number of positives
Taenia taeniformis	2
Taenia martis	1
Taenia polyacantha	2
Toxocara cati	4
	9



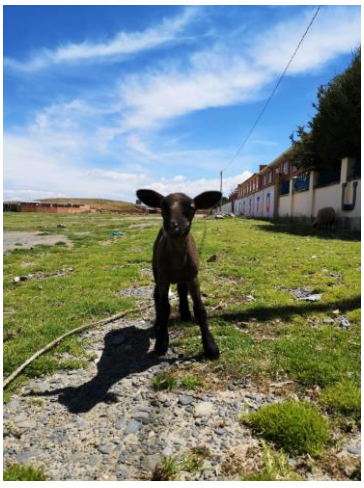
Conclusions

- A standardized method for the detection of taeniid and other parasites in salad had been developed.
- We have not found *E. multilocularis* in vegetables sold in Swiss markets.

Conclusions

- The contribution of food sources for the transmission of *Echinococcus* species requires critical assessment.
- Similar studies could be performed in endemic areas for *E*. *granulosus.*
- Similar investigations using other substrates as soil are needed.

Gracias



Vigilancia de Equinococosis quística en PERROS
Surveillance of CE in DOGS

Dr. Edmundo Larrieu
Universidad Nacional de Rio Negro
Vicepresidente para América
Asociación Internacional Hidatidologia

DEFINICION DEL PROBLEMA

- **DIAGNOSTICO INDIVIDUAL DE EQ EN UN PERRO ?**
- **(INDIVIDUAL DIAGNOSES)**
- **DIAGNOSTICO POBLACIONAL / TENDENCIA ?**
- **(POBLATIONAL DIAGNOSES TENDENCY)**

Test de arecolina, unidad de analisis perro, tasa x 100 (dog analysis unit, rate x 100)

100% especificidad

80% sensibilidad

- **Bajo valor predictivo a prevalencias bajas (Low predictive value at low prevalences)**
- **Impacto educativo positivo (Positive educational impact)**
- **No aleatorizado (not randomized)**

COPROANTIGENOS / ELISA

Allan JC, Craig PS, et al (1992) Coproantigen detection for immunodiagnosis of echinococcosis and taeniasis in dogs and humans. Parasitology 1104: 347-56

Guarnera E, Santillan G, et al (2000) Canine echinococcosis: an alternative for surveillance epidemiology. Vet Parasitol 88: 131-134

Pierangeli ND, Soriano SV, et al (2010) Usefulness and validation of a coproantigen test for dog echinococcosis screening in the consolidation phase of hydatid control in Neuquén, Argentina. Parasitol Int 59: 394–399

Morel N, Lassabe G, et al (2013) A monoclonal antibody-based copro-ELISA kit for canine echinococcosis to support the PAHO effort for hydatid disease control in South America PLoS Negl Trop Dis 7: e1967

Van Kesteren F, Mastin A, et al (2017) Evaluation of the impact of 2 years of a dosing intervention on canine echinococcosis in the Alay Valley, Kyrgyzstan. Parasitology 144; 1328-1337.

Jara LM, Rodriguez M, et al (2019) Development and Validation of a Copro-Enzyme-Linked Immunosorbent Assay Sandwich for Detection of Echinococcus granulosus-Soluble Membrane Antigens in Dogs. Am J Trop Med Hyg 100(2): 330-335

Reacciones cruzadas con otras tenias.
FALSOS POSITIVOS (Cross reactions with other tapeworms)

FALSOS NEGATIVOS (si hay pocos parasitos)

sensibilidad (78%-100%) especificidad (85%)

Sensitvity and specificity for the copro ELISA, computed by laboratory processing the samples and the sample source.

Laboratory processing the samples	Laboratory #1		Laboratory #2		Laboratory #3		Laboratory #4	
	Se	Sp	Se	Sp	Se	Sp	Se	Sp
Laboratory #1	-	-	40	40	20	20	33.3	42.9
Laboratory #5	0	60	60	100	0	80	0	71.4

CoproELISA atencion con el “n”

* Si el número de muestras fecales es representativo, la clasificación de un establecimiento como positivo será útil para la vigilancia en un programa de control, para determinar los campos donde se deben aumentar las medidas de control

CoproELISA

* * Marcador de alimentacion del perro con visceras y No desparasitacion (Marker of food of the dog with viscera and not deworming)

Screening con Test de coproELISA, unidad de analisis establecimiento ganadero, tasa x 100 de campos con transmision presente (farms analysis unit, rate x 100 of fields with present transmission)

PCR PARA CONFIFRMACION

1. Bowles J, Blair D, McManus DP. Genetic variants within the genus Echinococcus identified by mitochondrial DNA sequencing. Mol. Biochem. Parasitol. 1992;54:165-173
2. Cabrera M, Canova S, et al Identification of *Echinococcus granulosus* eggs. Diagn Microbiol Infect Dis. 2002;44(1):29-34.
3. Mathis A, Deplazes P. Copro-DNA tests for diagnosis of animal taeniid cestodes. Parasitol Int. 2006;55 Suppl:S87-90.
4. Abbasi I, Branzburg A, et al. Copro-Diagnosis of *Echinococcus granulosus* infection in dogs by amplification of a newly identified repeated DNA sequence Am J Trop Med. 2003;69:324–330.

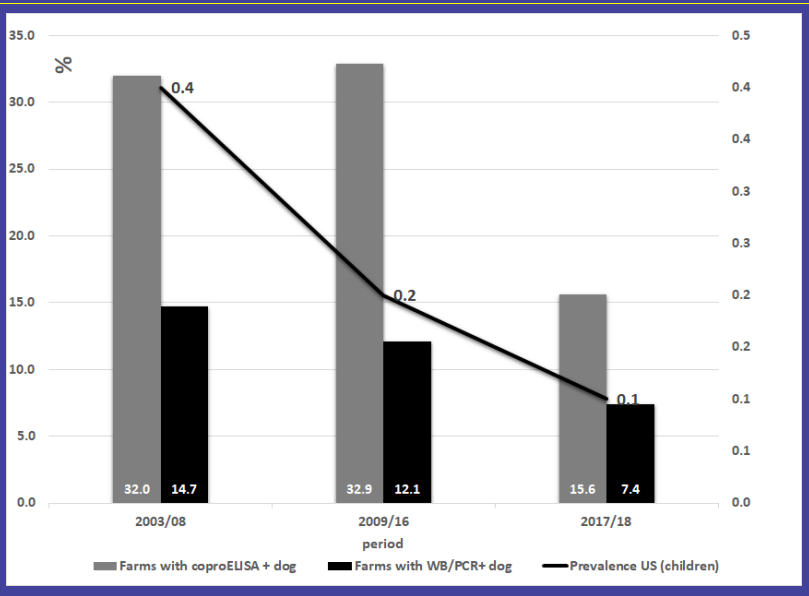
Sensitivity (Sn) and specificity (Sp) for the copro PCR, computed by laboratory processing the samples and sample source.

Laboratory processing the samples		Laboratory #1		Laboratory #2		Laboratory #3		Laboratory #4		Laboratory #5	
		Se	Sp	Se	Sp	Se	Sp	Se	Sp	Se	Sp
Laboratory #1	Estimate	-	-	40	60	20	40	33.3	42.9	20	66.7
	95% CI	-	-	5.3 - 85.3	14.7 - 94.7	0.5 - 71.6	5.3 - 85.3	0.8 - 90.6	9.9 - 81.6	0.5 - 71.6	9.4 - 99.2
Laboratory #2	Estimate	0	100	-	-	60	80	33.3	57.1	50	60
	95% CI	0 - 52.2	47.8 - 100	-	-	14.7 - 94.7	28.4 - 99.5	0.8 - 90.6	18.4 - 90.1	6.8 - 93.2	14.7 - 94.7
Laboratory #3	Estimate	20	80	80	80	-	-	33.3	42.9	20	80
	95% CI	0.5 - 71.6	28.4 - 99.5	28.4 - 99.5	28.4 - 99.5	-	-	0.8 - 90.6	9.9 - 81.6	0.5 - 71.6	28.4 - 99.5
Laboratory #4	Estimate	0	60	60	100	100	100	-	-	20	80
	95% CI	0 - 52.2	14.7 - 94.7	14.7 - 94.7	47.8 - 100	47.8 – 100	47.8 - 100	-	-	0.5 - 71.6	28.4 - 99.5
Laboratory #5	Estimate	0	100	25	100	20	100	100	100	-	-
	95% CI	0 - 52.2	47.8 - 100	0.6 - 80.6	47.8 - 100	0.5 - 71.6	47.8 - 100	29.2 - 100	59.0 - 100	-	-

Kappa coefficient for the copro PCR results across laboratories

	Laboratory #1		Laboratory #2		Laboratory #3		Laboratory #4		Laboratory #5	
	Kappa	p-value	Kappa	p-value	Kappa	p-value	Kappa	p-value	Kappa	p-value
Laboratory #1	-	-	-0.25	0.96	-0.04	0.62	-0.16	0.88	-0.2	0.93
Laboratory #2			-	-	0.51	<0.001	0.26	0.03	0.21	0.06
Laboratory #3					-	-	0.38	<0.01	0.03	0.41
Laboratory #4							-	-	0.34	<0.01
Laboratory #5									-	-

Screening con coproELISA-Wb/PCR



MUCHAS GRACIAS

Departamento de Parasitología
INEI ANLIS “ Carlos G Malbran”

Cystic echinococcosis : Environmental Diagnosis

Dra Graciela Santillán

Lic. Graciela Cespedes, Farm. Gerardo Ricoy , Tec. Sonia Sosa, Bioq. Marta Cabrera , Tec. Ignacio Velazquez , Tec. Rocio Garcia , Bioq. Gustavo Diego, Ms Ariana Gutierrez ,Ms Ariel Naidich

28th World Congress of Echinococcosis
Tuesday, October 29 - Thursday, October 31, 2019
(Lima, Peru)

Epidemiological unit -

the micro environment of rural housing

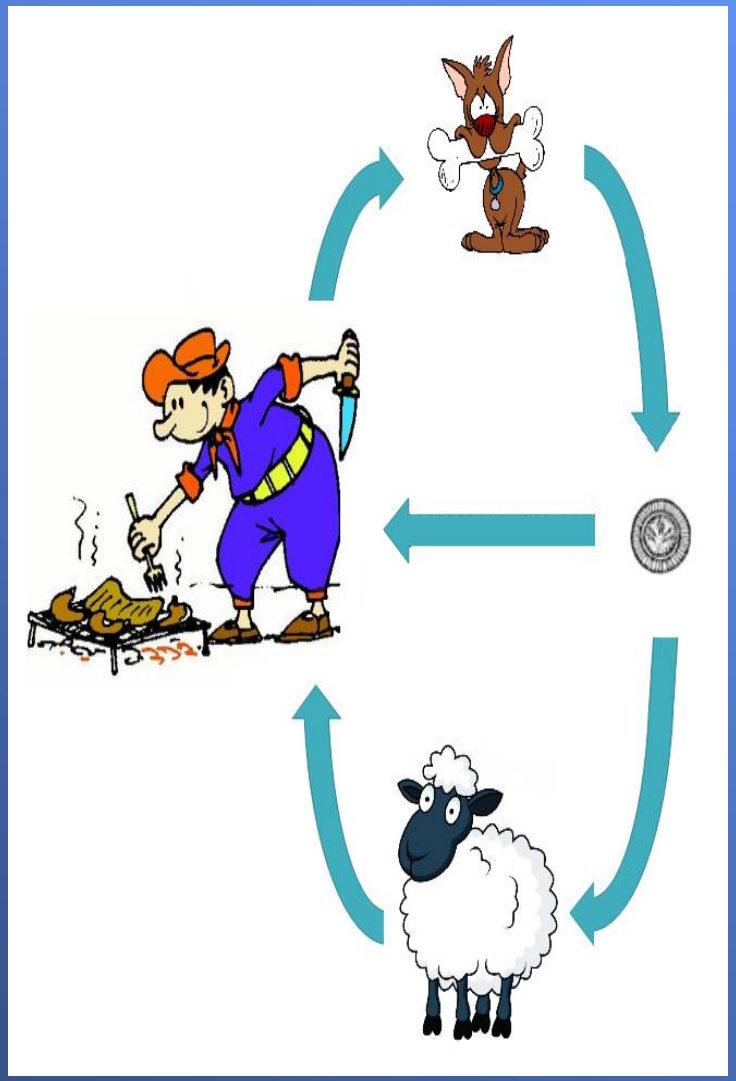
Most polluted sites, where dogs spend an amount of time

PERI-DOMICILE

Definite host

Zoonitic cycle

Intermediate slaughtered host



Definite host

Environmental cycle

Environment

COPROANTIGENS

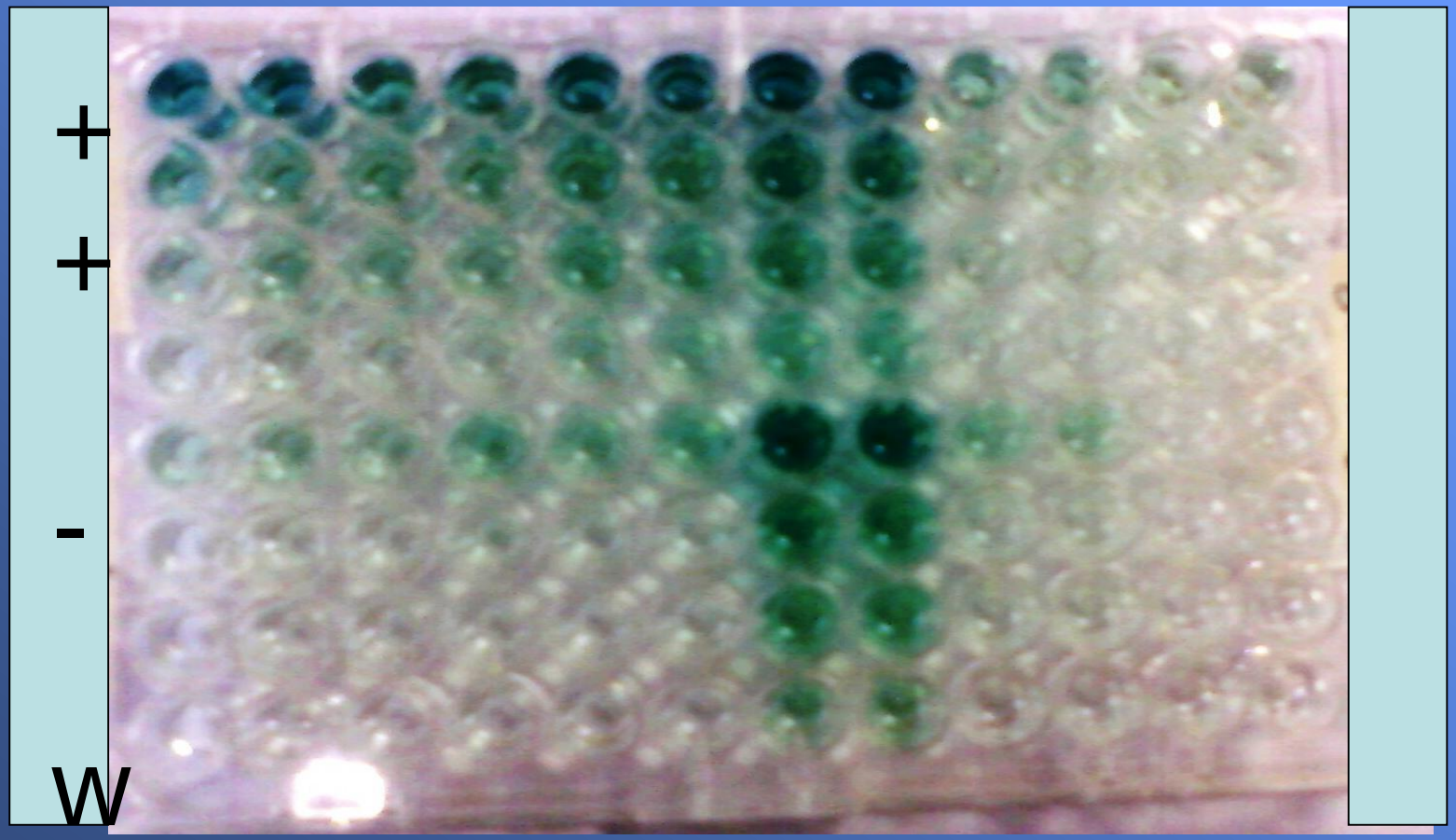
- Feces of dogs newly emitted or previously emitted and collected from the environment.

- Survillance.

- Control programmes.

- It is not recommended for the individual diagnosis of dogs.

ELISA



ANTIGENS

- Are detected between 6 and 13 days p.i.
- Levels decrease seventy-two hours after administration of Albendazole/Praziquantel.
- Administration of Arecholine Bromide does not eliminate all parasites.
- Coproantigens are detected in the pre-patent period of infection before the proglotids are eliminated into the environment.

Cross reaction:

Trichuris vulpis,

Uncinarias

***Taenia hydatigena*.**

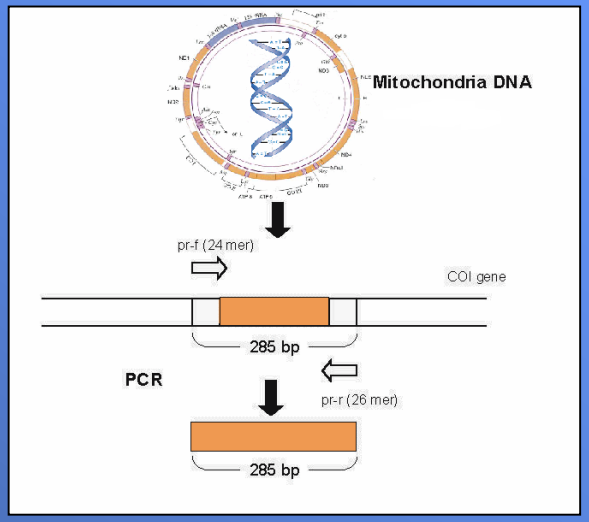
ADVANTAGES

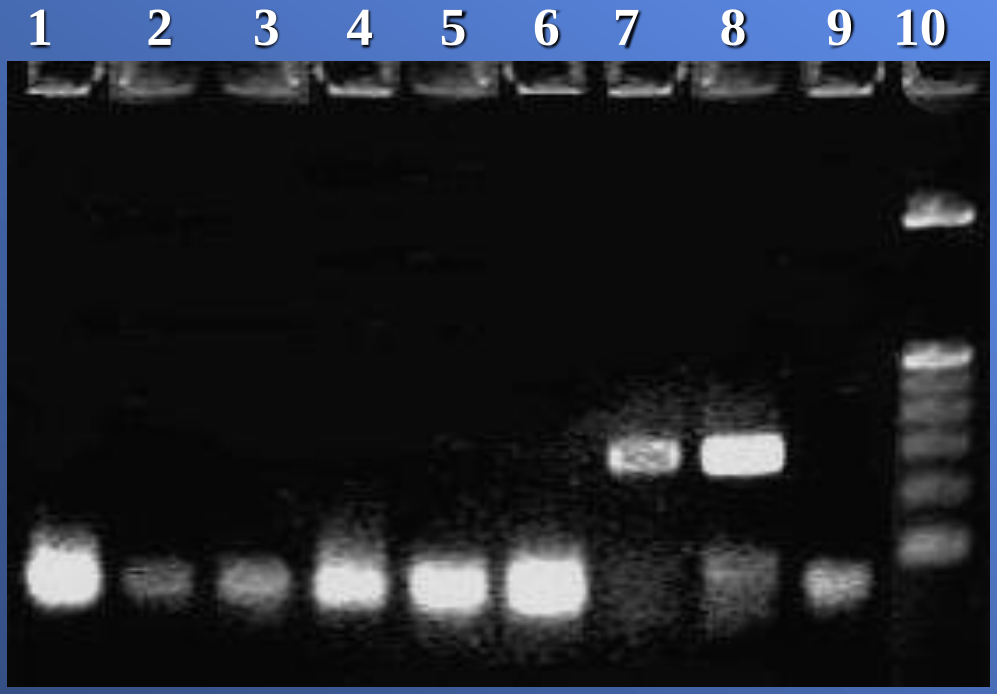
- Process at the same time a large number of samples.
- Detect antigens in the pre-patent period.

Relative Diagnostic Sensitivity : 90%

Relative Diagnostic Specificity: 88.37%

PCR


Mitochondria DNA
pr-f (24 mer)
COI gene
285 bp
PCR
pr-r (26 mer)
285 bp


1 2 3 4 5 6 7 8 9 10
MRx TS Hn Th Dc Em Eg Eg MRx 100bp

ENVIROMENTAL SAMPLES

- ✓dog coat swab
- ✓fresh feaces
- ✓ dry dog feaces
- ✓ soil
- ✓ vegetables
- ✓ water

DOG COAT SWAB

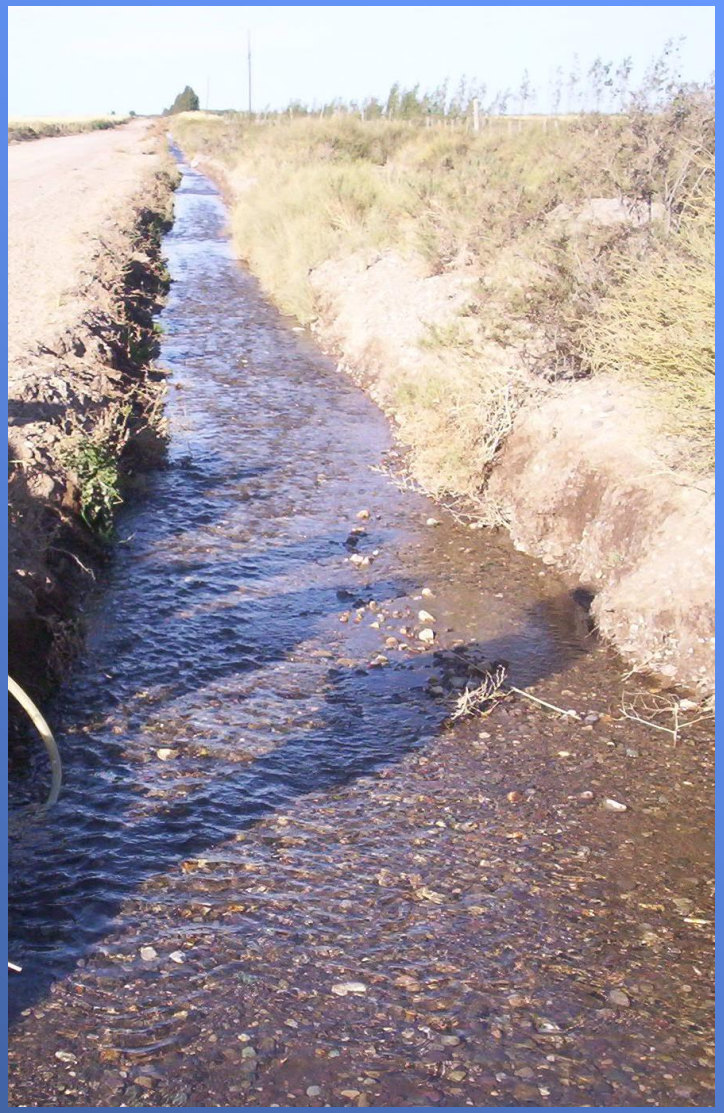
FRESH STOOL FEACES

SOIL

DRY FAECES

GRASS AND VEGETABLES

WATER



The positive reaction in samples taken from the environment surrounding the rural home, such as the soil , the grasses and pastures and a water course and the material extracted from the fur of dogs, suggest the magnitude of environmental pollution in a rural housing located in the endemic area.

The importance of diagnosis in housing is due to the fact that the two cycles of transmission, zoonotic and environmental, occur in the house and, from there, extend to the environment.

Muchas Gracias!!!

Thank you very much !!!!