

# Veterinary Vaccinology Network Conference

## *Fasciola hepatica* vaccines: case study

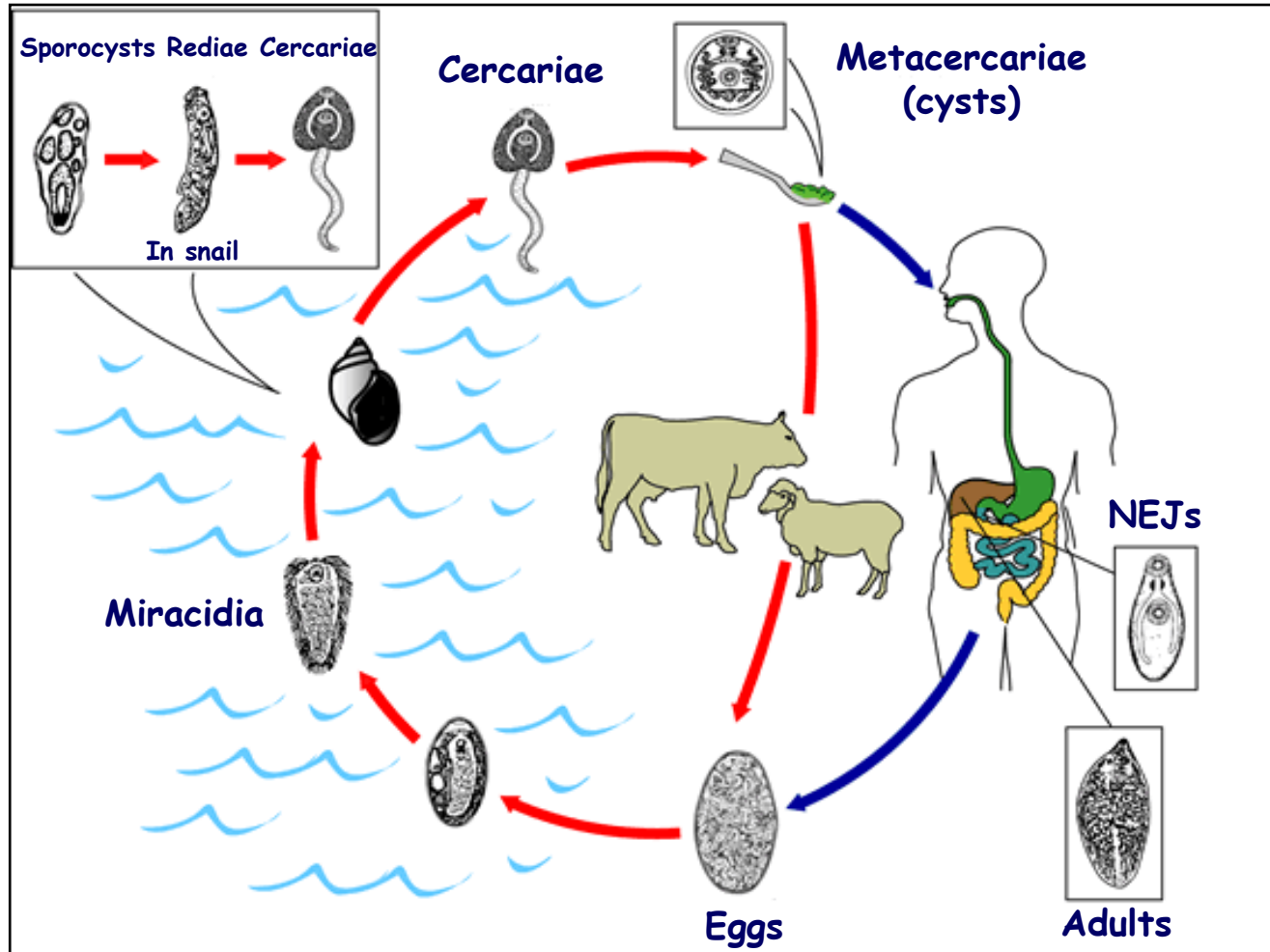


**John P. Dalton**

**FP7 PARAVAC CONSORTIUM / HORIZON 20:20**

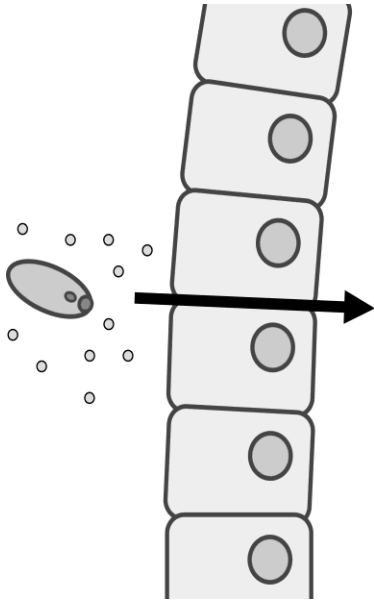
**ERC ADVANCED GRANT**

# *Fascioliasis: major food (grass)-borne disease*

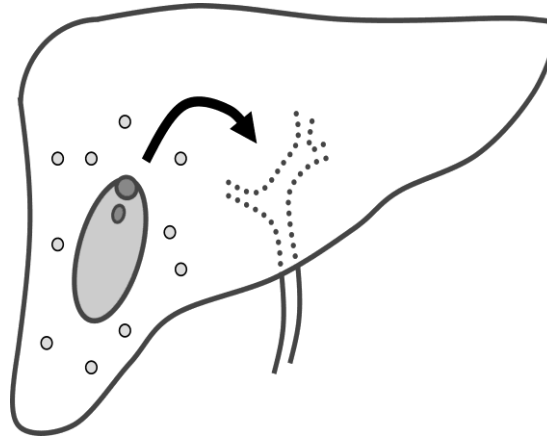


Adapted from [www.dpd.cdc.gov/dpdx](http://www.dpd.cdc.gov/dpdx)

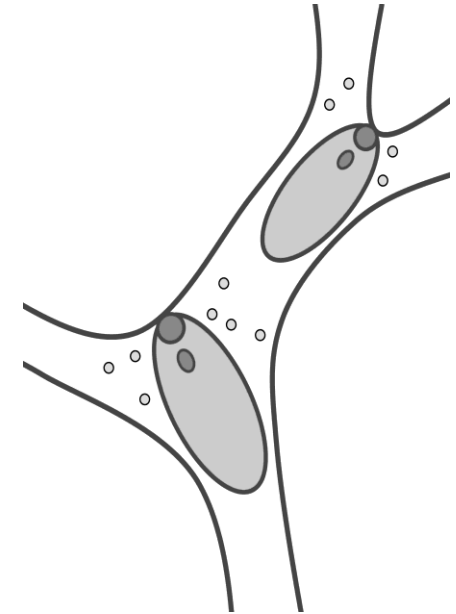
# Development of *Fasciola* in the mammalian host



1. Infective larvae  
**Penetration**



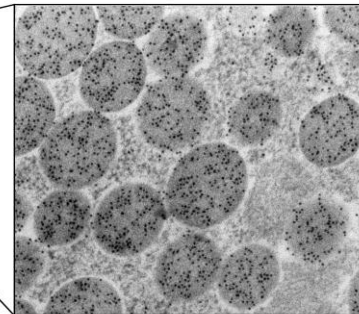
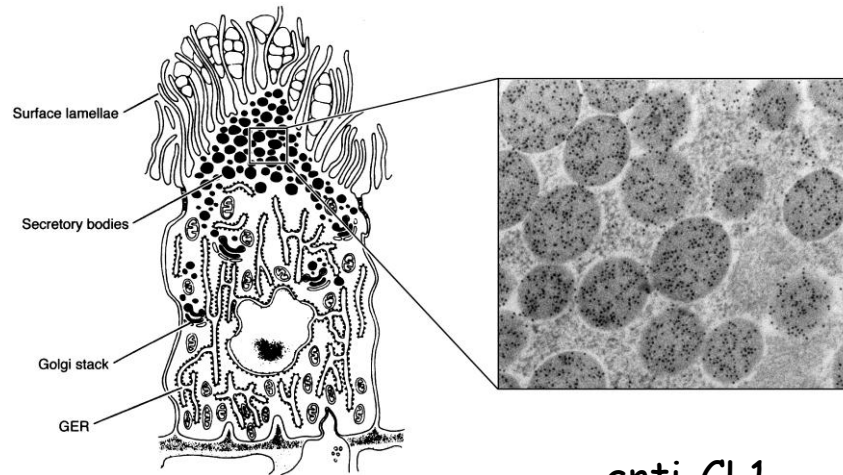
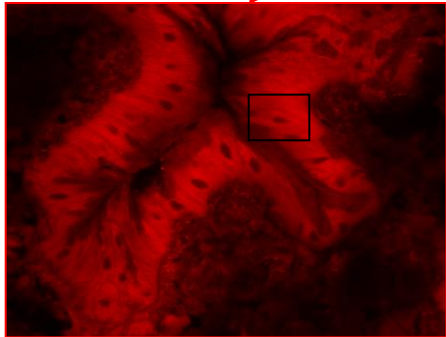
2. Immature stage  
**Migration**



3. Mature stage  
**Feeding**



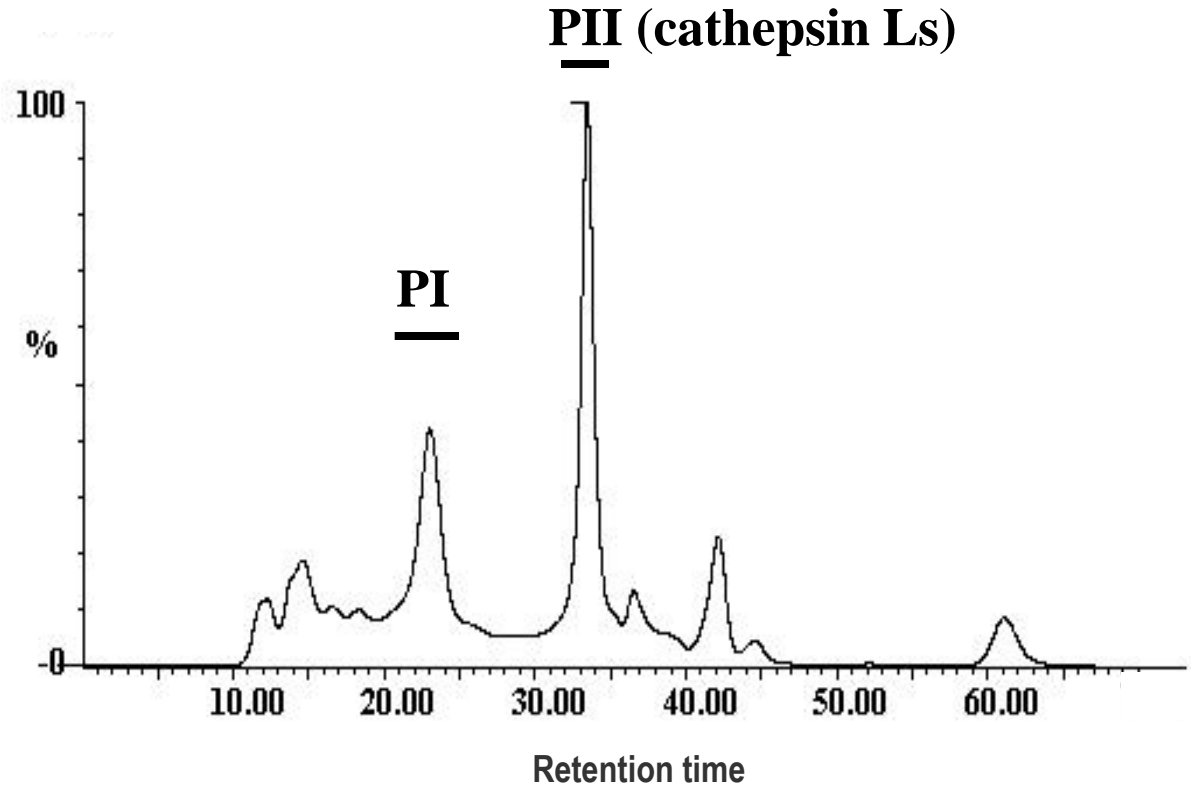
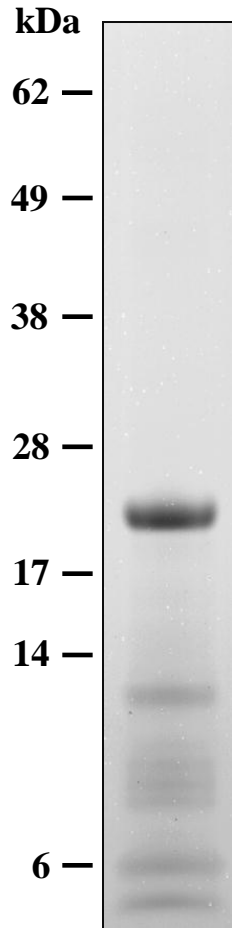
# Cathepsin L cysteine proteases are secreted from specialised epithelial cells lining the parasite gut lumen



anti-CL1



# Separation of *F. hepatica* excreted/secreted proteins by gel filtration



HPLC size exclusion chromatography

## Vaccination with cathepsin L1 and L2 & HMW fraction

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<b>TRIAL NO.</b>	<b>HOST</b>	<b>VACCINE</b>	<b>PROTECTION (%)</b>	<b>ANTI-VIABILITY EFFECT (%)</b>
<b>2</b>	<b>Cattle</b>	<b>Cathepsin L1</b>	<b>42.5</b>	<b>61</b>
<b>2</b>	<b>Cattle</b>	<b>Cathepsin L2 + HMW fraction</b>	<b>73</b>	<b>98</b>
<b>2</b>	<b>Cattle</b>	<b>Cathepsin L1 + HMW fraction</b>	<b>64</b>	<b>82</b>
<b>2</b>	<b>Cattle</b>	<b>HMW fraction</b>	<b>43</b>	<b>72</b>

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# Vaccination with cathepsin L1

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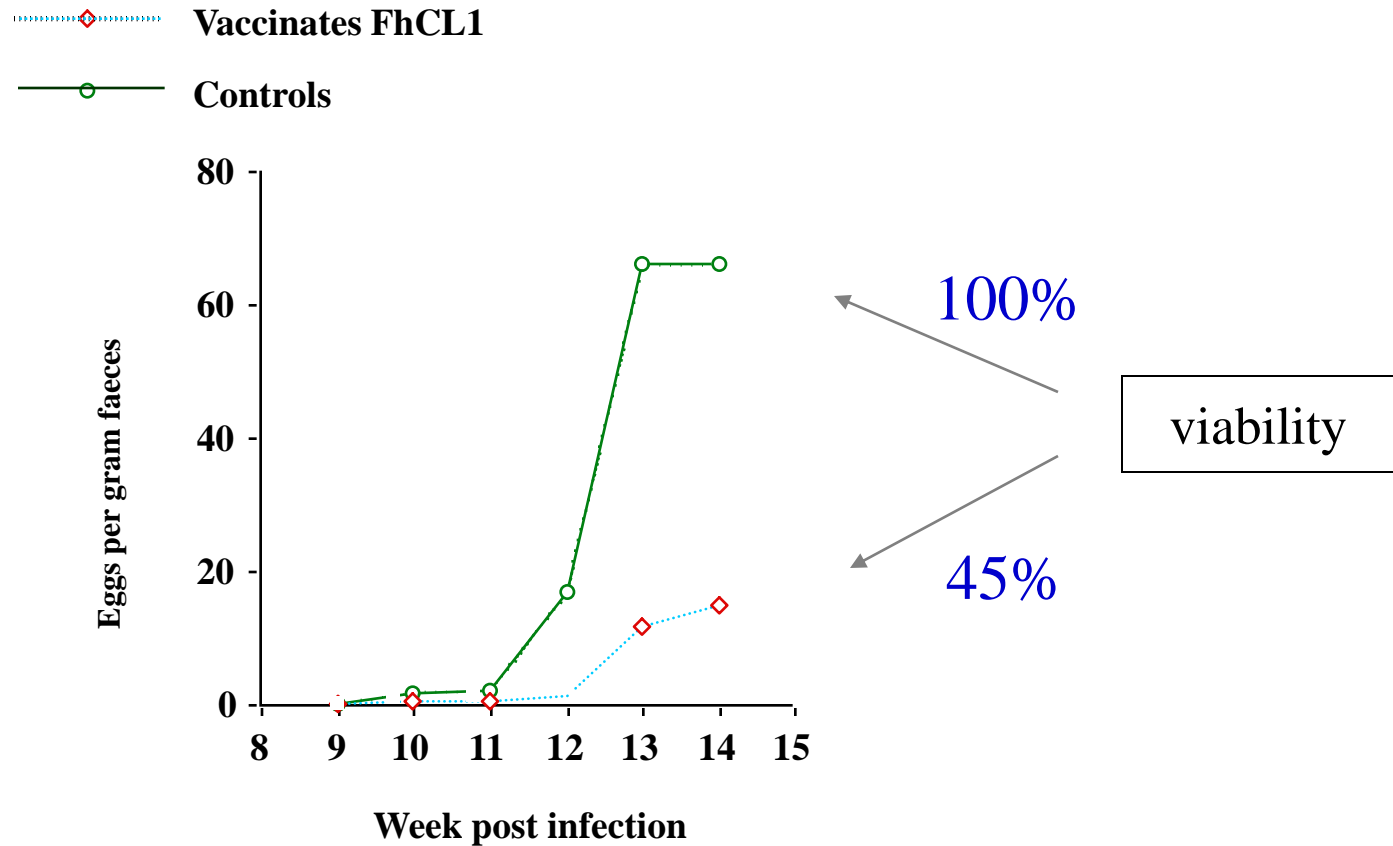
TRIAL NO.	HOST	ADJUVANT	PROTECTION (%)
6	Cattle	FCA/FIA	55%
6	Cattle	FIA	11% (NS)
6	Cattle	Alum	0 (NS)
7	Cattle	Quil A	0 (NS)

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Fasciola hepatica cathepsin L-like proteases: biology, function, and potential in the development of first generation liver fluke vaccines. **Dalton** JP, Neill SO, Stack C, Collins P, Walshe A, Sekiya M, Doyle S, **Mulcahy** G, Hoyle D, Khaznadji E, Moiré N, Brennan G, Mousley A, Kreshchenko N, Maule AG, Donnelly SM. Int J Parasitol. 2003 Sep 30;33(11):1173-81. Review.

Parasite vaccines--a reality? **Dalton** JP, **Mulcahy** G. Vet Parasitol. 2001 Jul 12;98(1-3):149-67. Review.

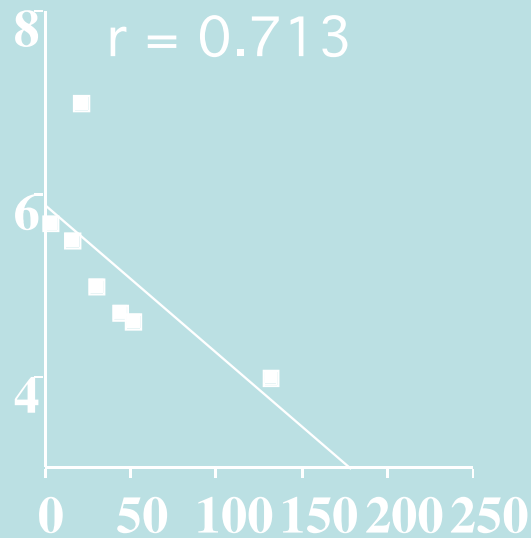
# Vaccination with cathepsin L1: FECs



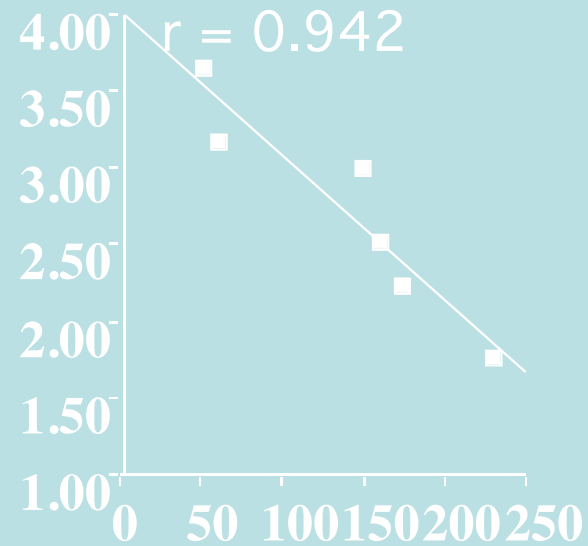


IgG2 (Th1 response) production correlates with protection

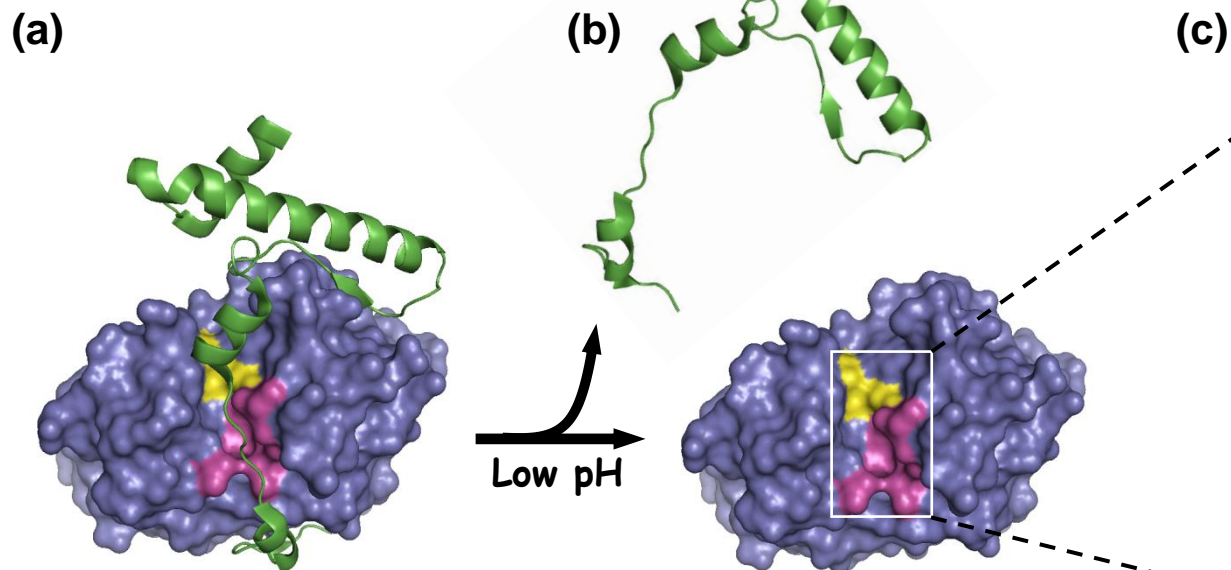
IgG2 Avidity



IgG2 titre

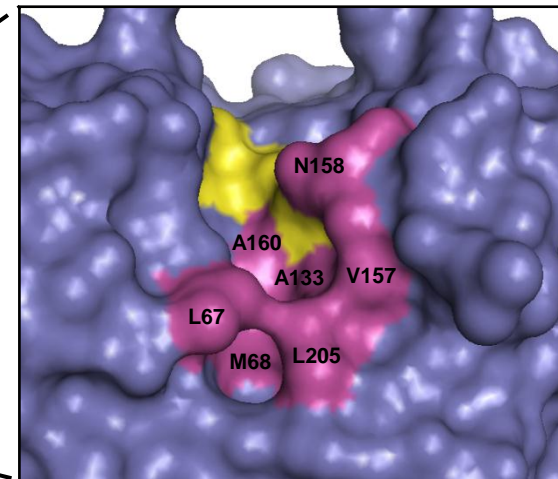


No. of Flukes

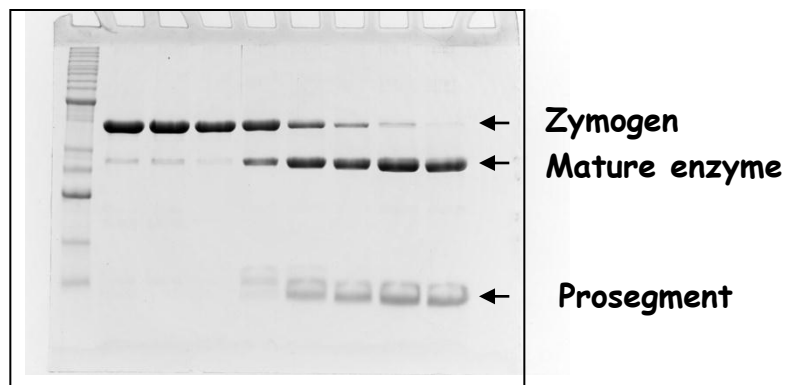


**FheCL1**

PDB ID: 206X

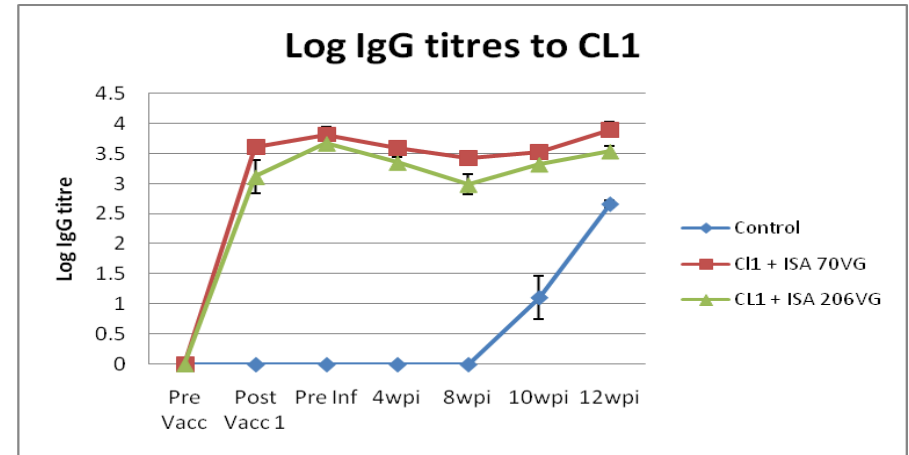


**pH 4.5-induced autocatalytic activation**

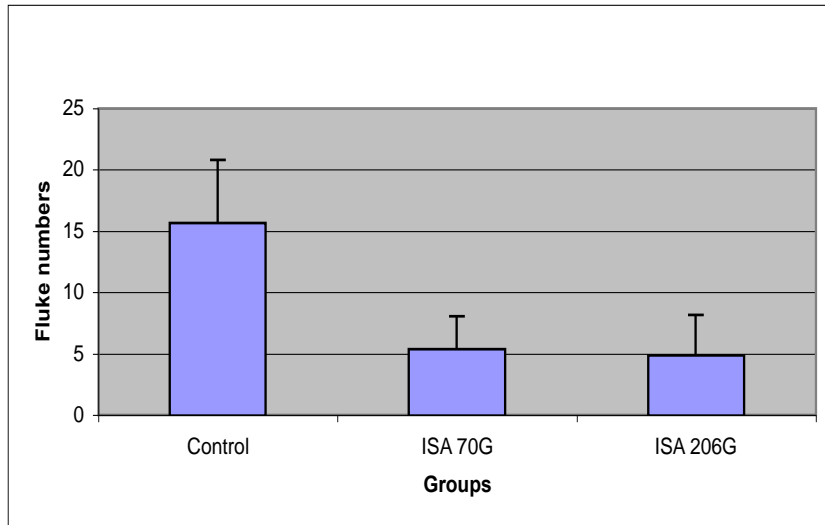


# Vaccine field trial in cattle using Montanide adjuvants

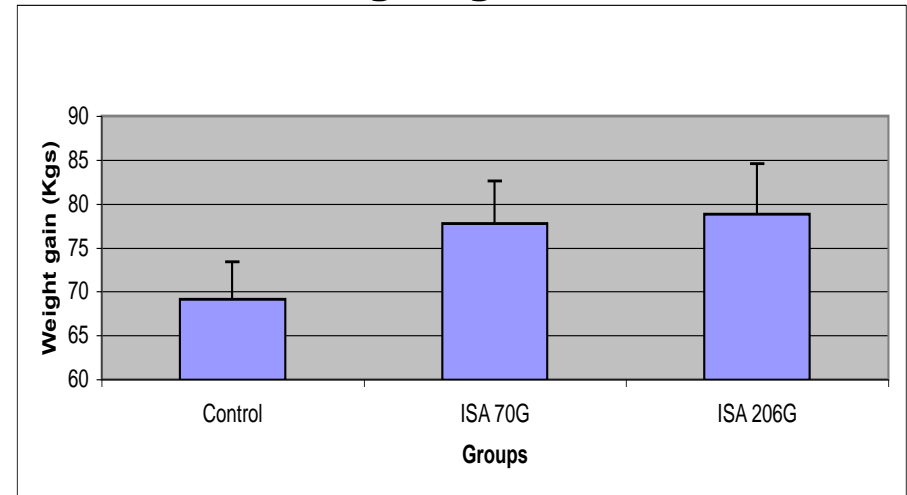
## Antibody responses



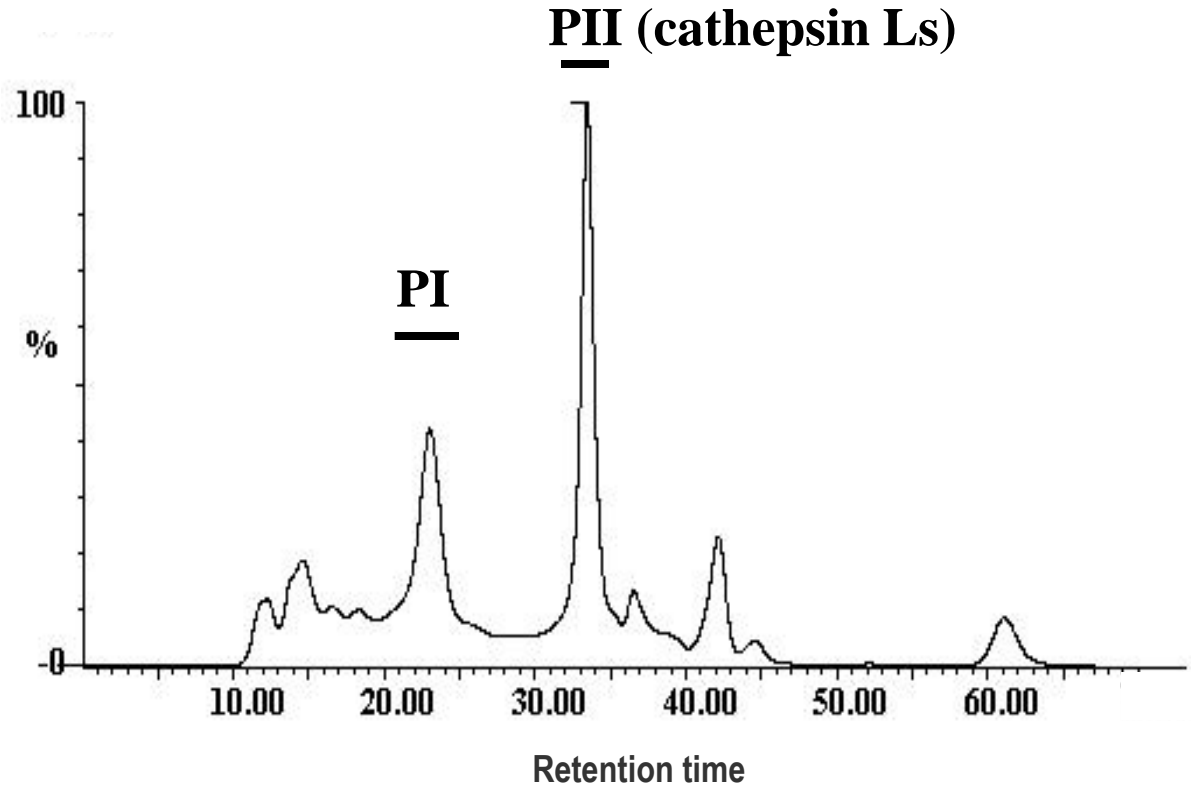
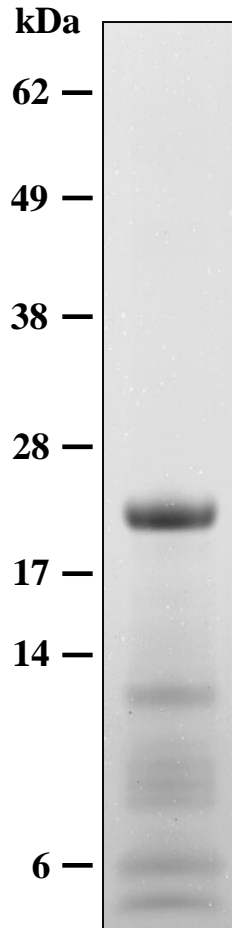
## Parasite burdens



## Weight gains

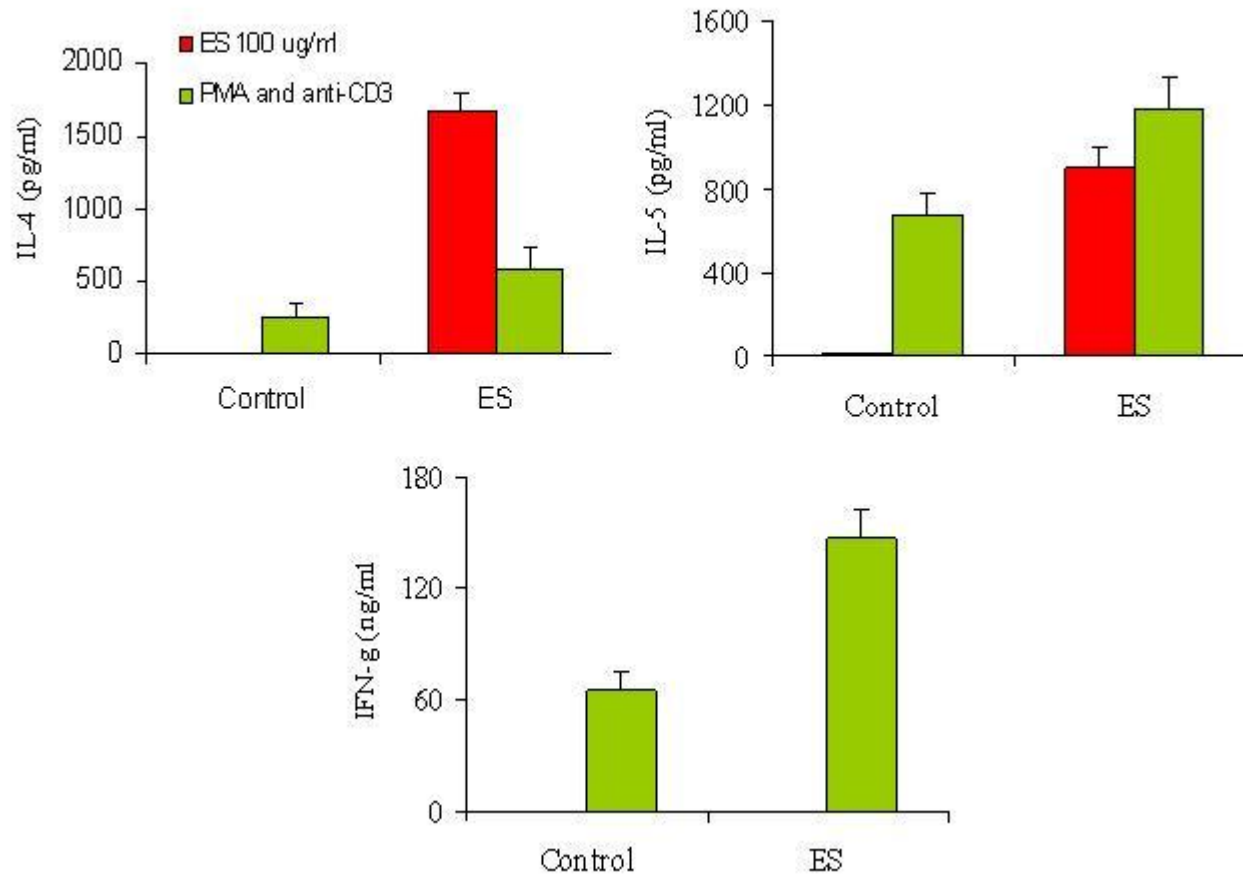


# Separation of *F. hepatica* excreted/secreted proteins by gel filtration

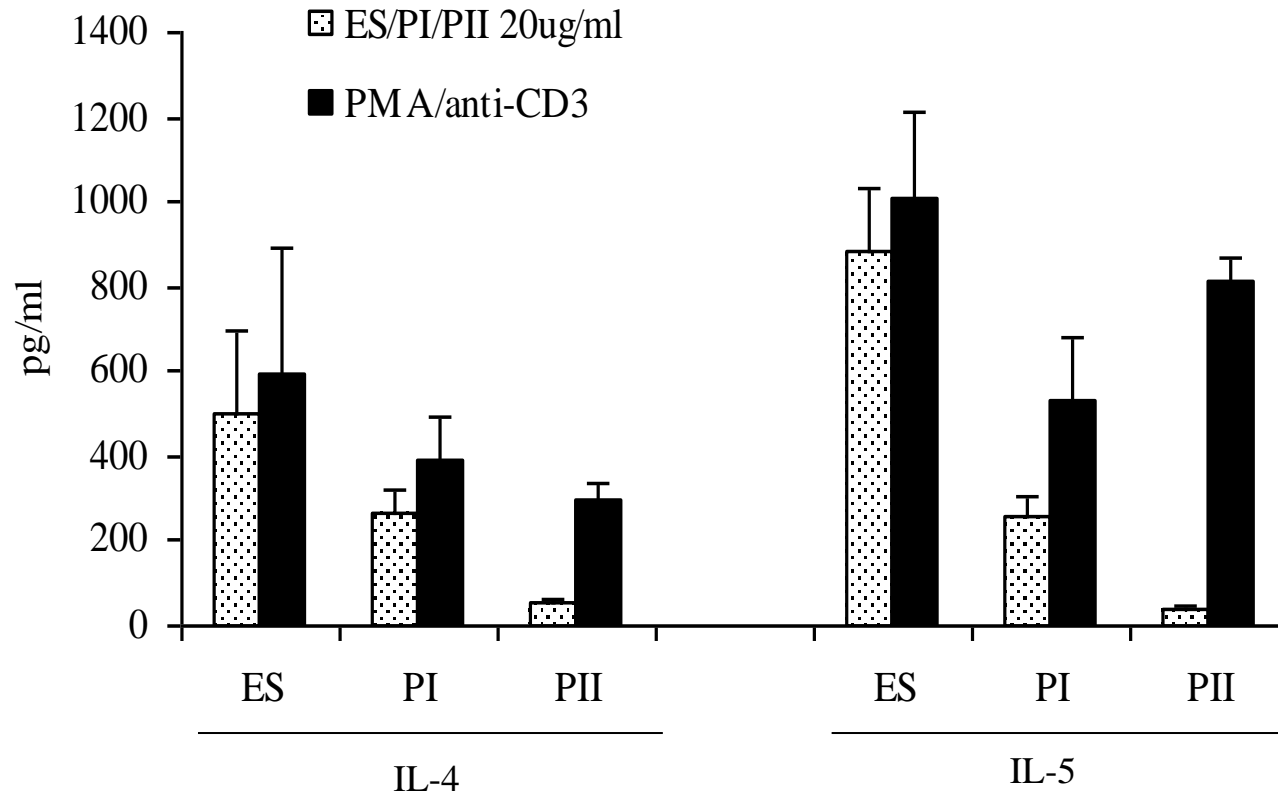


HPLC size exclusion chromatography

## *F. hepatica* excretory/secretory products stimulate a potent Th2 immune response *in vivo*

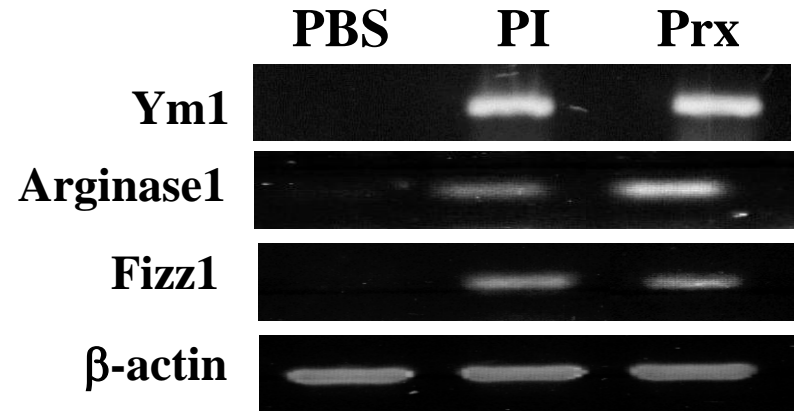
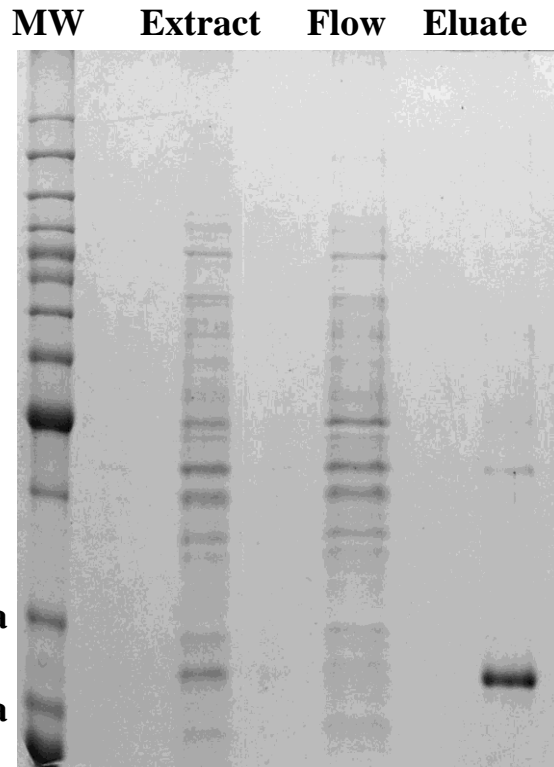


# Peak I, but not Peak II, of *F. hepatica* ES products induces a Th2 response



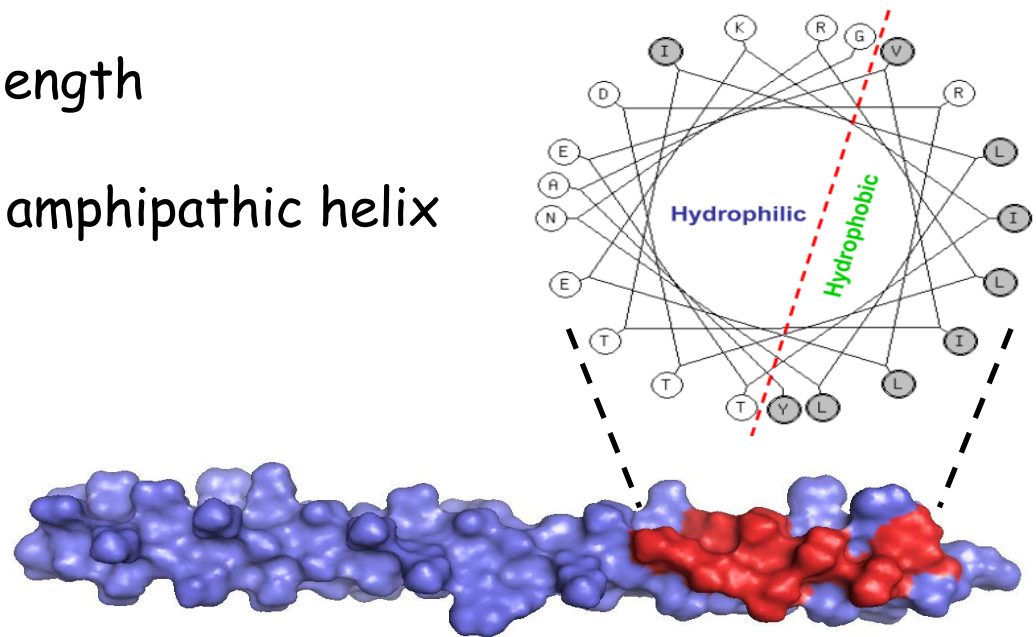
# *F. hepatica* Peroxiredoxin: Recruits M2 macrophages when injected into mice

## SDS-PAGE



# Helminth defense molecule - FhHDM

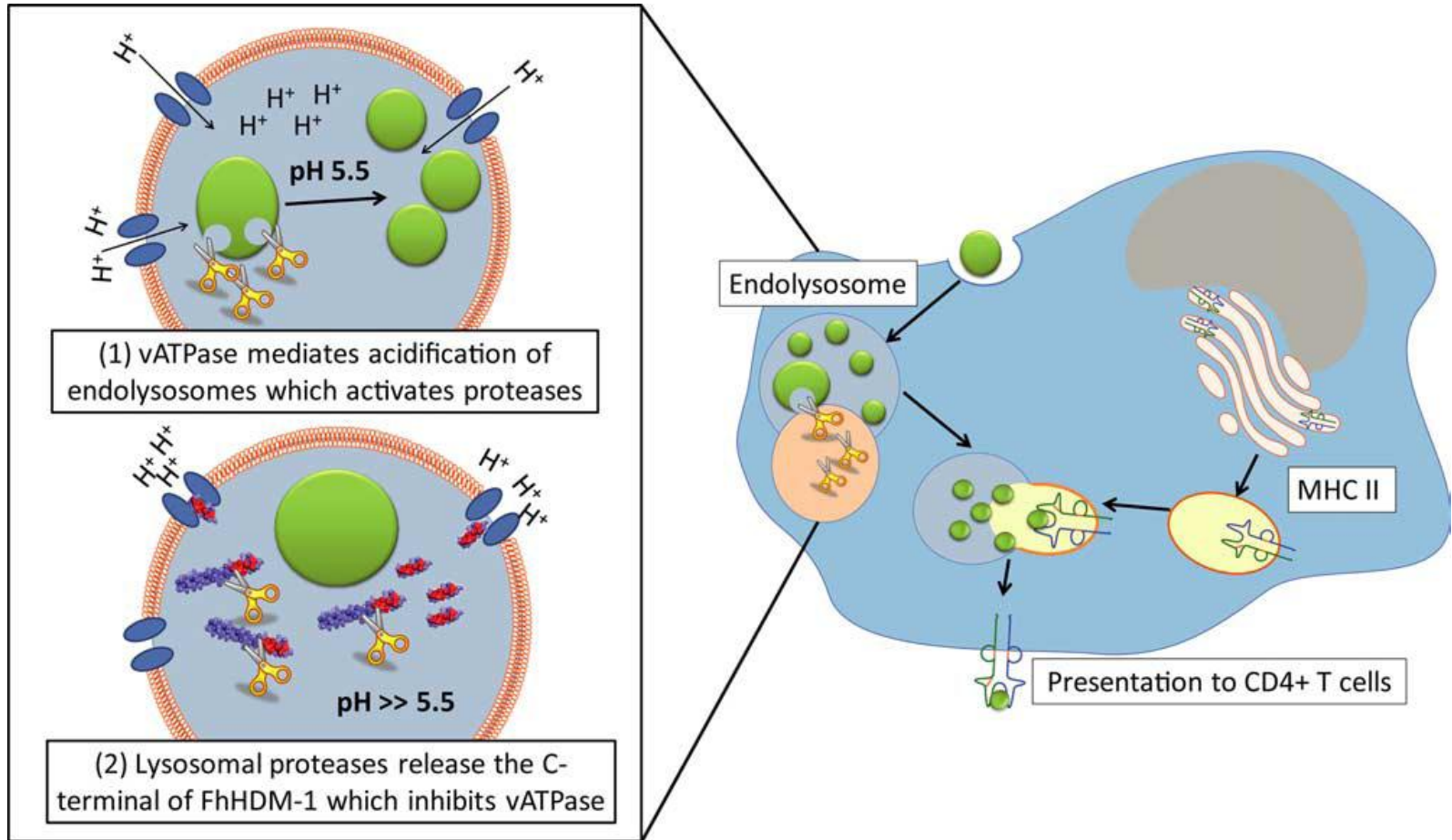
- FhHDM is a novel protein
- Homologs of FhHDM are found in the secretions of all medically relevant trematode worms
- FhHDM 70 amino acid in length
- The C-terminus forms an amphipathic helix





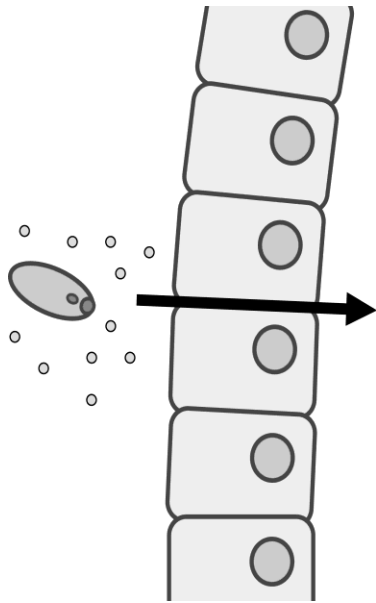
# Proposed mechanism of action of FhHDM

## Inhibition of vATPase



 : FhHDM-1

# Developmental regulation of *Fasciola* cathepsin proteases

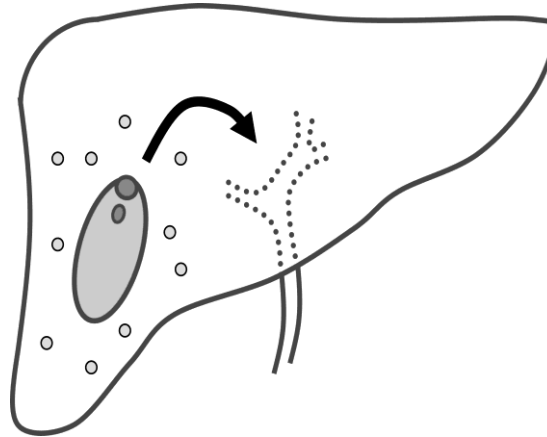


## 1. Infective larvae

### Penetration

Cathepsin B1, B2, B3

Cathepsin L3



## 2. Immature stage

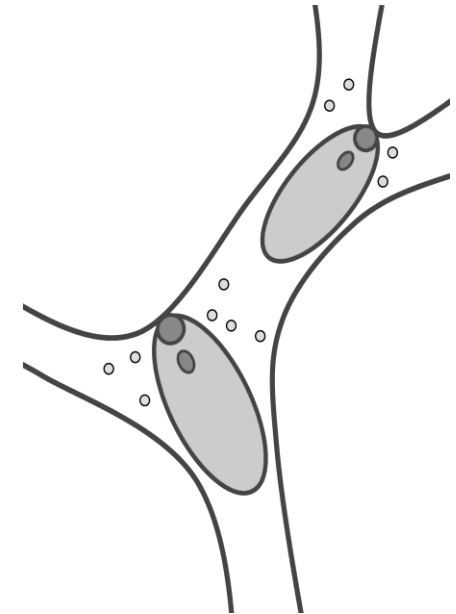
### Migration

Cathepsin B1, B2, B3 ↓

Cathepsin L3 ↓

Cathepsin L1 ↑

Cathepsin L2 ↑



## 3. Mature stage

### Feeding

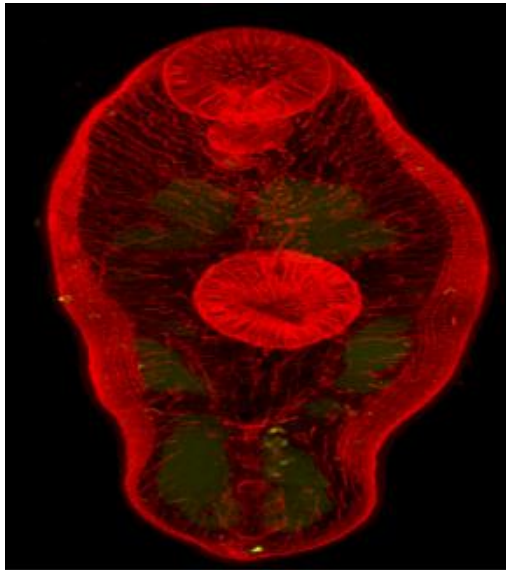
Cathepsin L1

Cathepsin L2

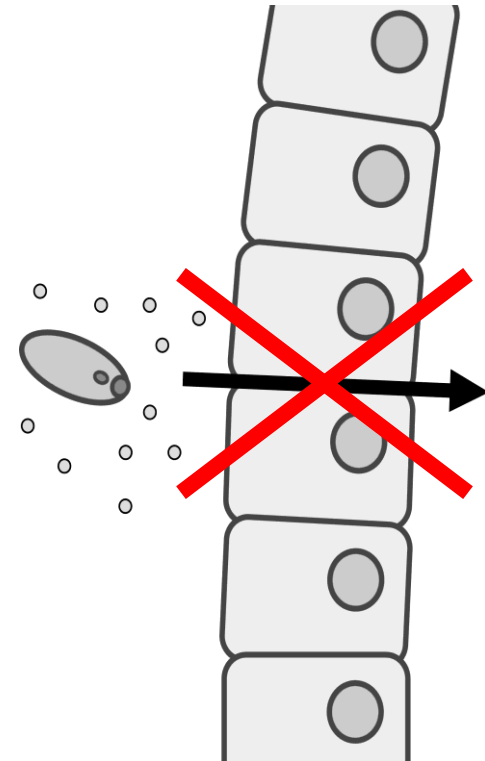
Cathepsin L5

# RNAi of *Fasciola* cathepsin L and cathepsin B

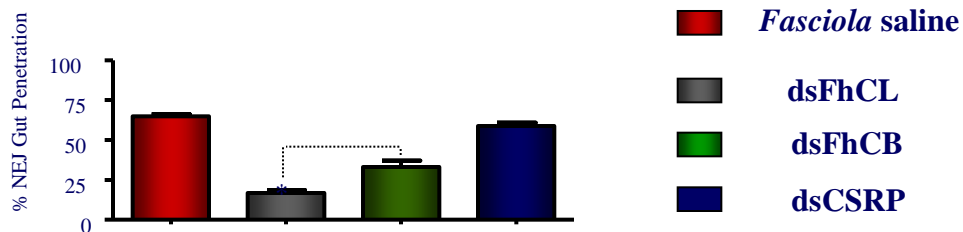
dsFhCB



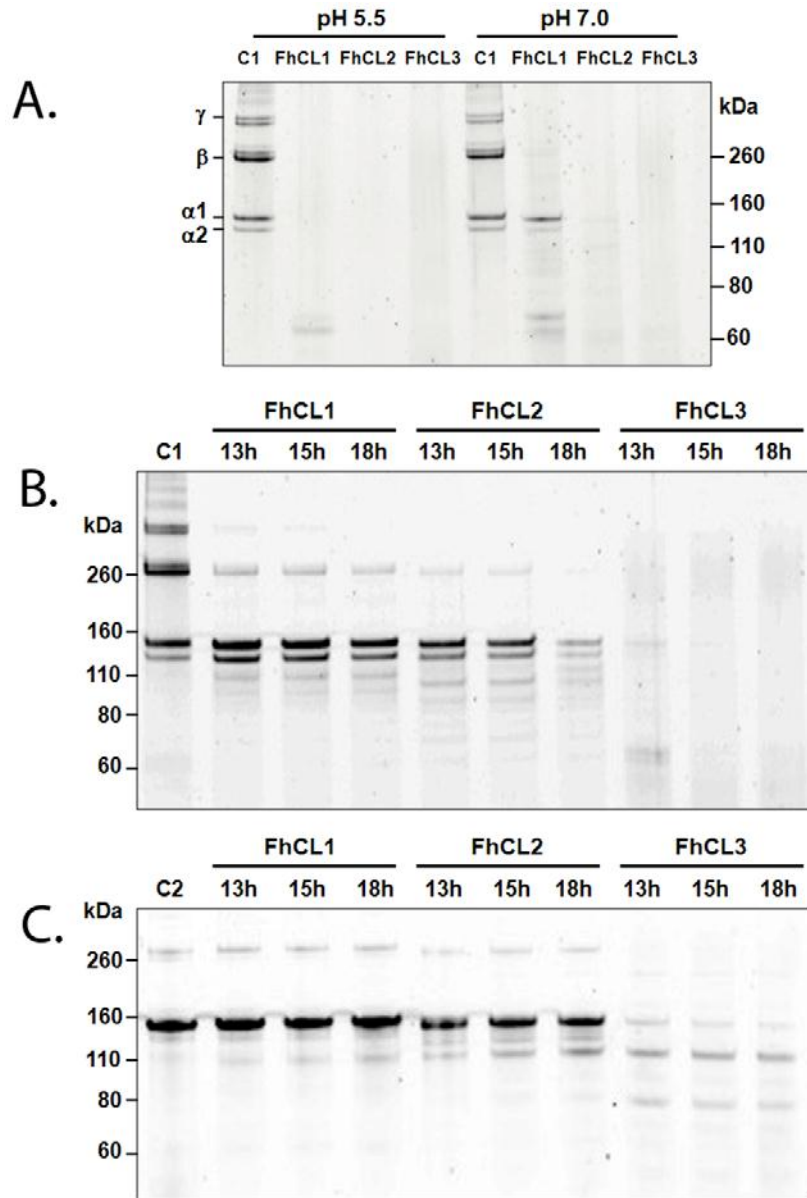
control



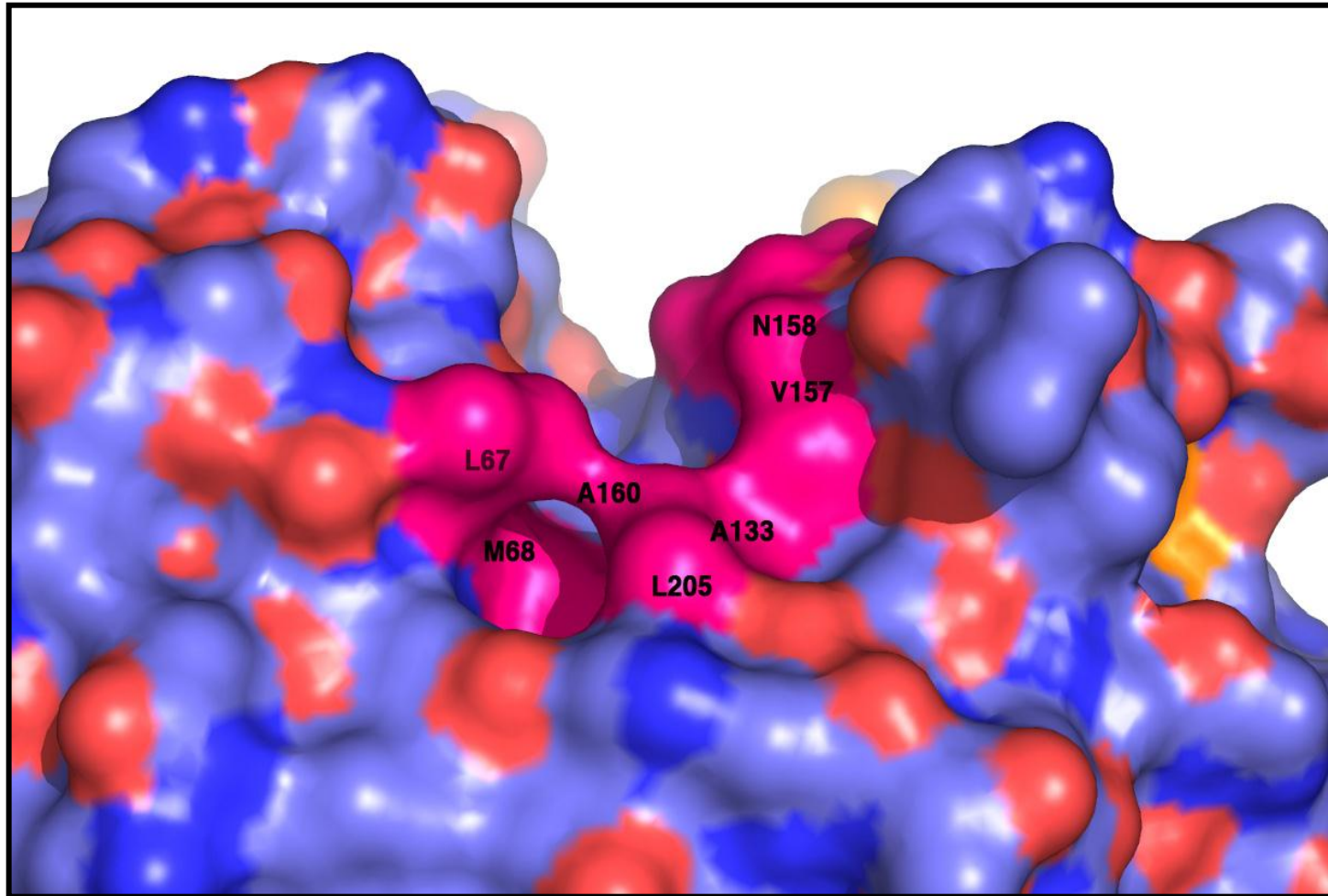
## Blocking of tissue invasion



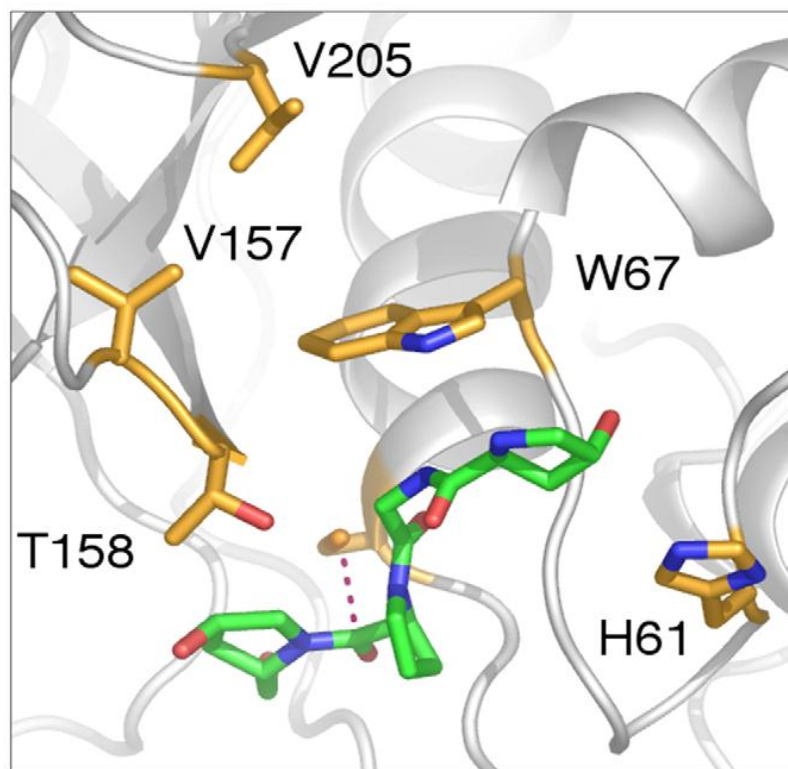
# Digestion of collagen *Fasciola cathepsin Ls*



# S2 site residues that determine substrate specificity of *F. hepatica* cathepsin L proteases

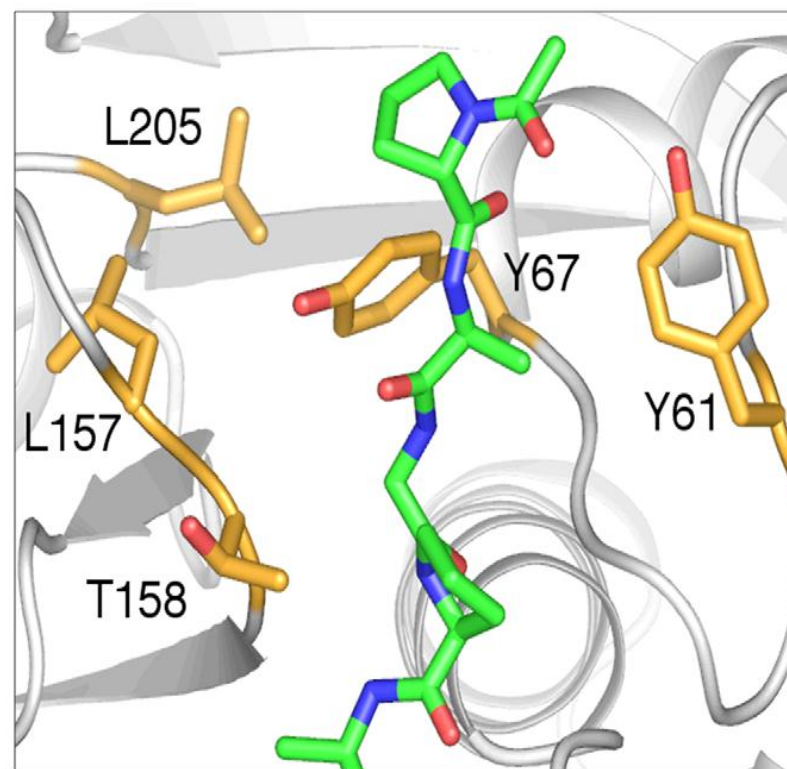


**A.**

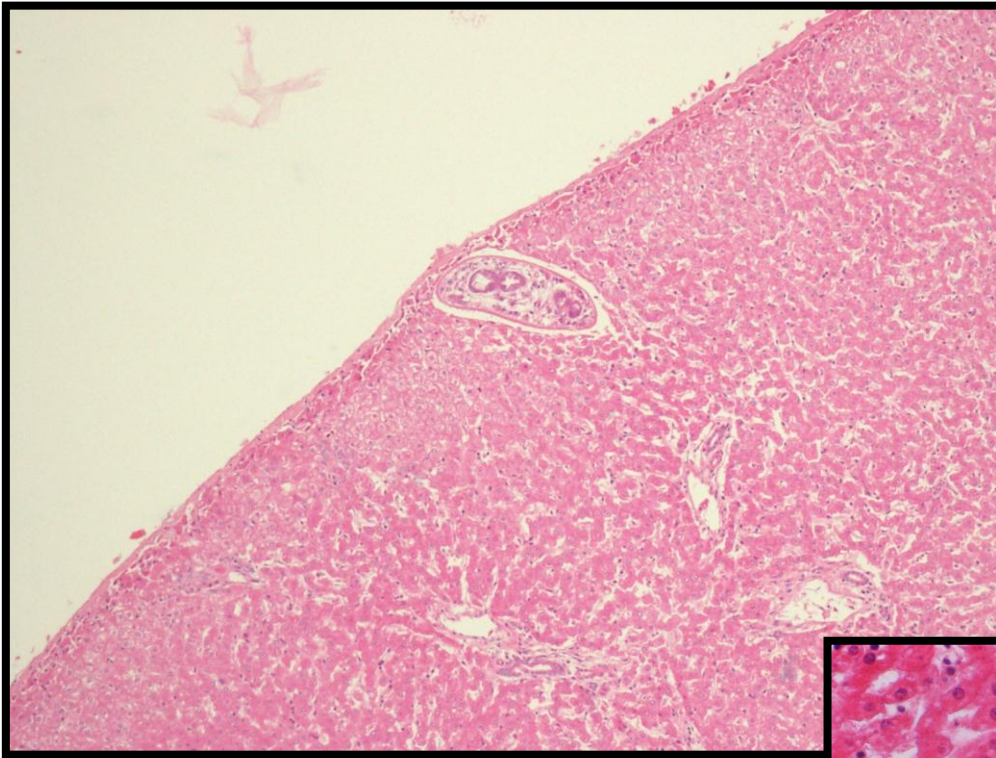


**FhCL3 – Ligand F**

**B.**

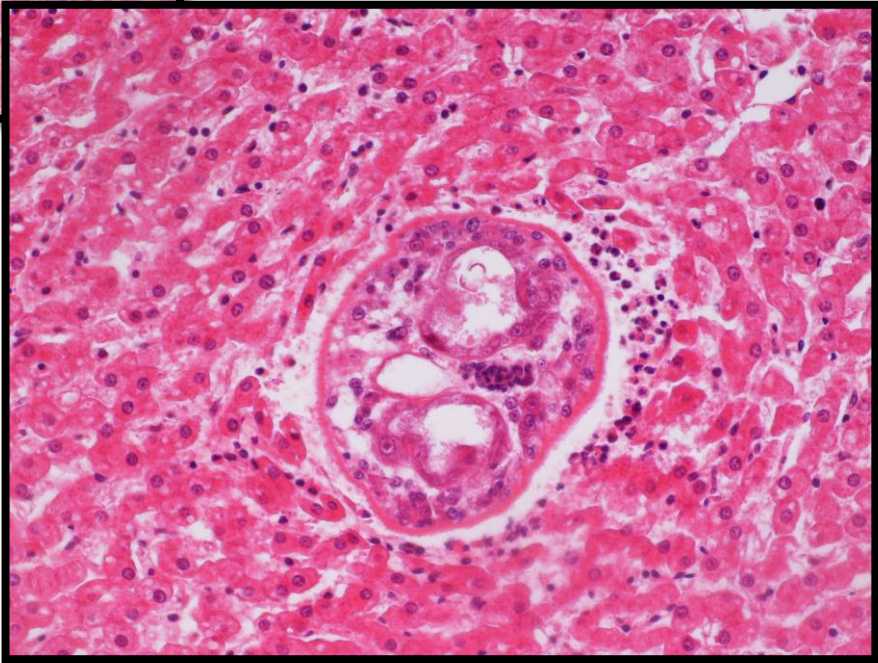


**FhCL2 – Ligand D**

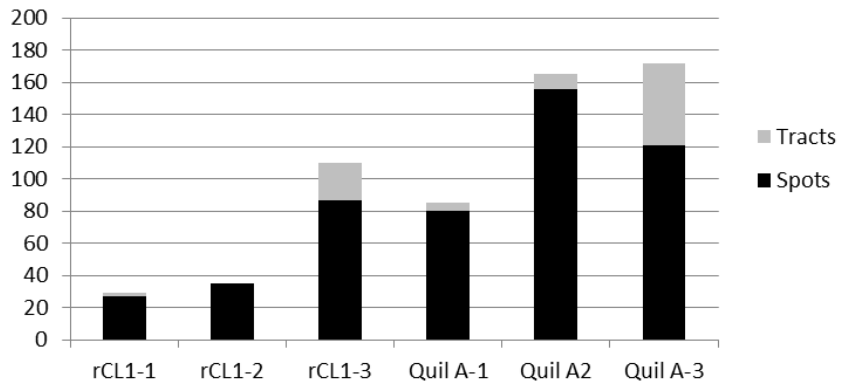


Penetration of liver capsule

Invasion of inflammatory cells

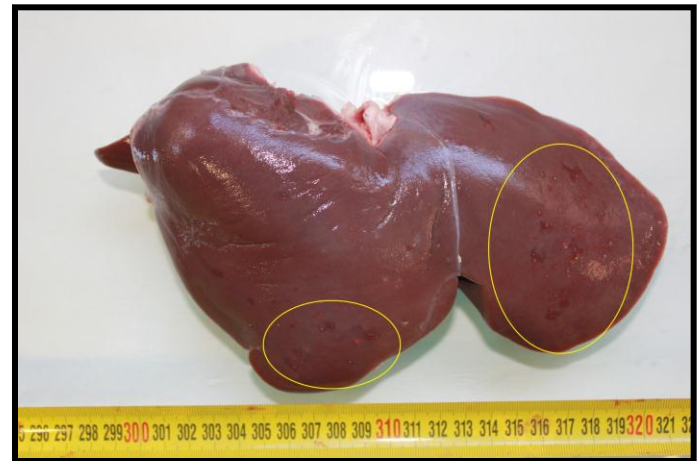
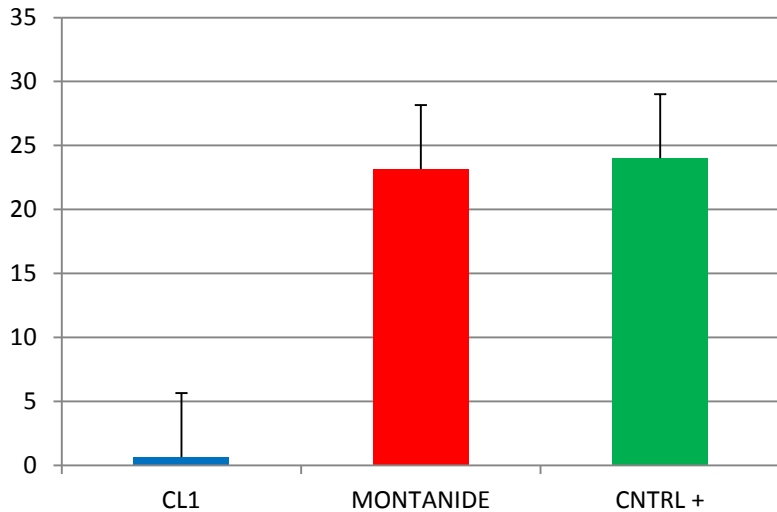


### Number of gross hepatic lesions



Zafra et al. J. Com. Pathol. 2013, 148(4):373-84.

### Lesions

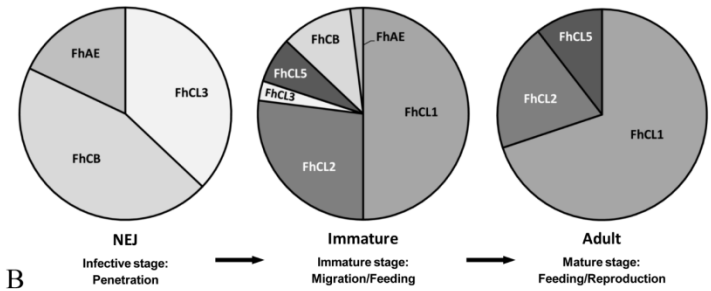






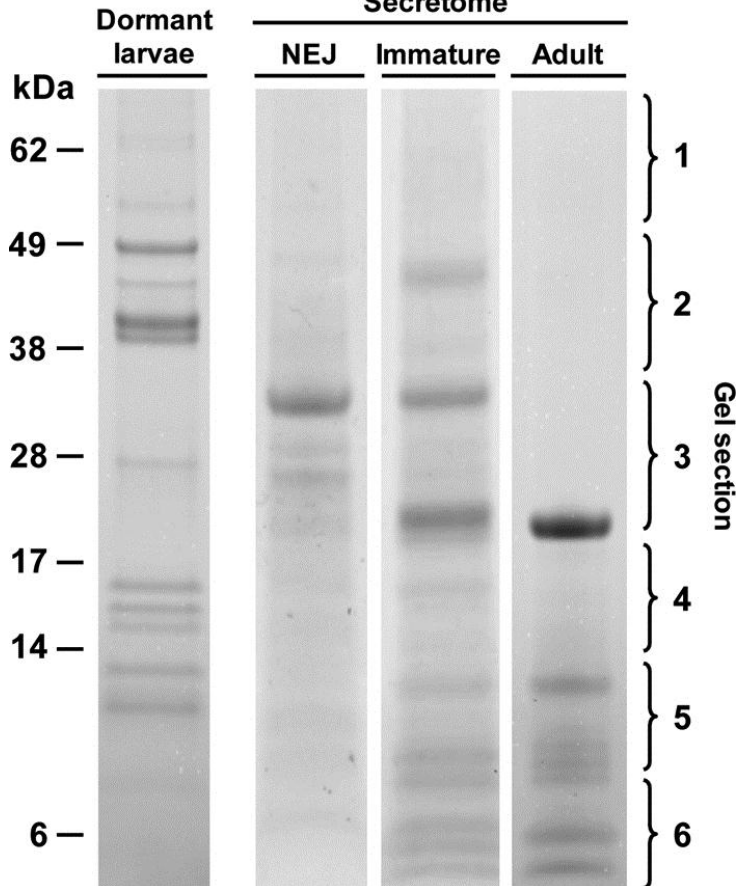
A

## F. hepatica protease expression



B

Accession	Fasciola protein	Score <sup>1</sup>	Unique peptides <sup>2</sup>	% cover	Mr	emPAI <sup>1</sup>	Section <sup>1</sup>
<b>Dormant larvae somatic</b>							
A7UNB2	Cathepsin B	294	5	24	37.6	0.29	2
Q817B2	Procathepsin B2 precursor	111	3	9	38.0	0.09	2
A5X494	Cathepsin B3	223	1	22	31.0	0.36	1-3
A8W7J0	Procathepsin L	235	7	21	35.6	0.30	2
Q9GRW6	Procathepsin L3	194	7	21	35.3	0.31	2
Q711M2	Legumain-like precursor 1	266	7	21	47.8	0.30	1-3
Fhep29h09.q1k	Putative legumain 2	76 <sup>3</sup>	5	12	-	-	1
<b>NEJ secretome</b>							
P80527	Hemoglobinase-like protein 1	117	2	85	2.2	2.17	2,3
Fhep29h09.q1k	Putative legumain 2	99	12	12	-	-	1-5
Q9GRW4	Partial procathepsin L3	99 <sup>3</sup>	11	23	35.1	-	2-5
Q9GRW6	Partial procathepsin L3	99 <sup>3</sup>	8	23	35.4	-	2-5
Q95VA7	Cathepsin L	99 <sup>3</sup>	5	27	37.4	-	2-5
B3TM67	Cathepsin L3	99 <sup>3</sup>	9	26	35.1	-	2-5
B3TM68	Cathepsin L3	99 <sup>3</sup>	9	39	35.3	-	2-5
A8W638	Metacercarial cathepsin L	99 <sup>3</sup>	12	34	37.4	-	2-5
A8W7J0	Metacercarial procathepsin L	99 <sup>3</sup>	13	38	35.7	-	2-5
HAN4015b05.q1kT3	Putative cathepsin B4	88 <sup>3</sup>	3	11	-	-	3
<b>Immature fluke secretome</b>							
A7UNB2	Cathepsin B	113	2	7	37.6	0.09	2
Q817B2	Pro-cathepsin B2 precursor	99	2	6	38.0	0.18	5
Fhep45b05.q1k	Putative cathepsin B5	97 <sup>3</sup>	6	13	-	-	2
HAN4006g01.q1kT3	Putative cathepsin B6	99 <sup>3</sup>	6	9	-	-	2
Q711M2	Legumain-like precursor	412	31	16	47.8	0.70	2
A6Y9U8	Legumain-1	328	15	20	47.9	0.59	2
A6Y9U9	Legumain-2	176	3	10	48.3	0.22	2
Q8T5Z9	Cathepsin L	334	12	32	35.1	1.06	3
Q7JNQ9	Secreted cathepsin L1	447	16	37	36.7	1.82	3
Q6R018	Cathepsin L protein	292	13	24	36.6	1.00	3
Q24940	Cathepsin L-like proteinase	356	15	32	36.8	1.36	3
Q9GRW5	Cathepsin L1	282	12	27	35.1	0.88	3
Q7JNQ8	Secreted cathepsin L2	363	12	37	37.0	0.67	3
A5Z1V3	Secreted cathepsin L2	470	14	39	37.0	0.98	3
A5X483	Cathepsin L2	367	14	39	24.5	1.78	3
A3FMG6	Cathepsin L	228	9	30	36.9	0.19	3
Q95VA7	Cathepsin L	235	7	15	37.4	0.18	3
A8W638	Cathepsin L	274	6	25	37.3	0.29	3
Q9NGW3	Cathepsin L	204	7	11	36.9	0.54	3
Q9NB30	Cathepsin L	257	5	15	37.1	0.41	3
Q2HPD3	Cathepsin L1 proteinase	220	12	19	36.5	0.63	6
HAN4005b02.q1kT3	Putative prolylcarboxypeptidase	99 <sup>3</sup>	10	33	-	-	1
<b>Adult secretome</b>							
Q7JNQ9	Secreted cathepsin L1	552	20	39	36.7	1.82	3
A5Z1V3	Secreted cathepsin L2	444	12	34	37.0	1.16	3
Q9NB30	Cathepsin L	372	9	18	37.1	0.41	3
Q8T5Z9	Cathepsin L	481	17	40	35.1	1.25	3
Q9GRW5	Cathepsin L1	476	21	32	35.1	1.06	3
Q6R018	Cathepsin L protein	451	22	38	36.6	1.37	3
Q24940	Cathepsin L-like proteinase	444	19	33	36.8	1.58	3
A5X483	Cathepsin L2	401	16	45	24.5	2.16	3
Q24945	Cathepsin L-like protease	348	7	35	18.5	0.95	3
P81222	Cathepsin L-like proteinase	297	12	57	12.3	4.58	3
Q9NGW3	Cathepsin L	254	7	12	36.9	0.41	3
A5X484	Cathepsin L1	114	4	20	24.3	0.14	3
A81598	Cathepsin L	77	2	7	28.1	-	3
Fhep30b01.q1k	Putative prolylcarboxypeptidase	92 <sup>3</sup>	8	13	-	-	1



# What does the animal industry want?

- Efficacy against all parasitic stages (TBZ resistance)
- Reduction in clinical signs and lesions attributable to immature and adult parasites
- Reduction of egg shedding to reduce pasture contamination

# Vaccine profile (cattle)

- Two immunisations 4 weeks apart followed by yearly boost
- 10 - 200  $\mu\text{g}$  / dose
- 10 dose vials (stable and safe)
- Injectable administered subcutaneously
- (One vaccine per ml of fermentation)

# Veterinary Vaccinology Network Conference

## *Fasciola hepatica* vaccines: case study

John P. Dalton

FP7 PARAVAC CONSORTIUM / HORIZON 20:20  
ERC ADVANCED GRANT

- **Dr. Krystyna Cwiklinki (QUB, Northern Ireland)**
- **Dr. Mark Robinson (QUB, Northern Ireland)**
- **Prof. Aaron Maule (QUB, Northern Ireland)**
- **Dr. Sheila Donnelly (UTS, Australia)**
- **Prof. Grace Mulcahy (UCD, Republic of Ireland)**
- **Steve Paterson, Jane Hodgkinson, Diane Williams (Liverpool, UK)**
- **Jose Perez & Alvaro Moreno, University of Cordoba, Spain.**