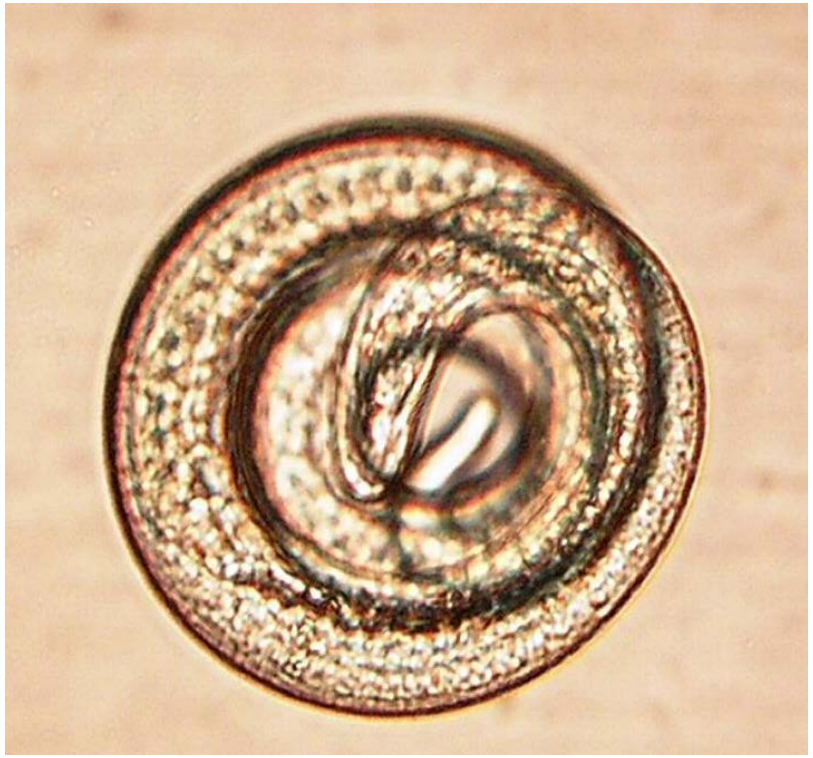
Identification of stage-specific *Trichinella spp.* antigens to develop a high sensitivity serological tool for diagnosis



By courtesy of Gianluca Marucci

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European Union Reference Laboratory for Parasites

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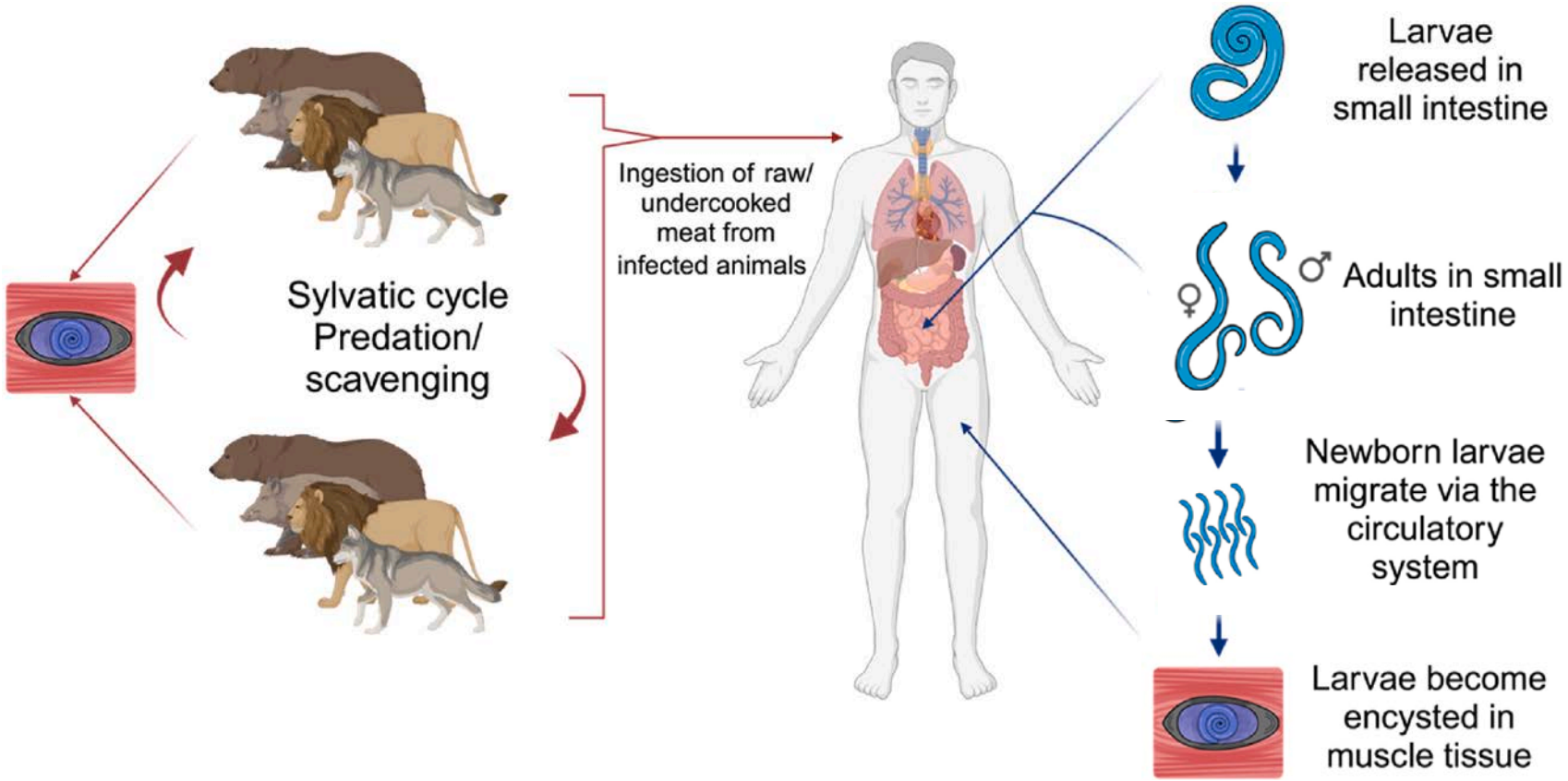
Istituto Superiore di Sanità, Rome

- *Trichinella spp* are foodborne parasites widely distributed all around the world

- the genus is currently comprised of 13 taxa: 10 named species [*T. spiralis* (T1), *T. nativa* (T2), *T. britovi* (T3), *T. pseudospiralis* (T4), *T. murrelli* (T5), *T. nelsoni* (T7), *T. papuae* (T10), *T. zimbabwensis* (T11), *T. patagoniensis* (T12), *T. chanchalensis* (T13) and three unnamed genotypes (*Trichinella* T6, T8, and T9) (Pozio, 2021; Sharma et al., 2020).

- encapsulated species vs non-encapsulated species (*T. pseudospiralis*) (Garkavi 1972)



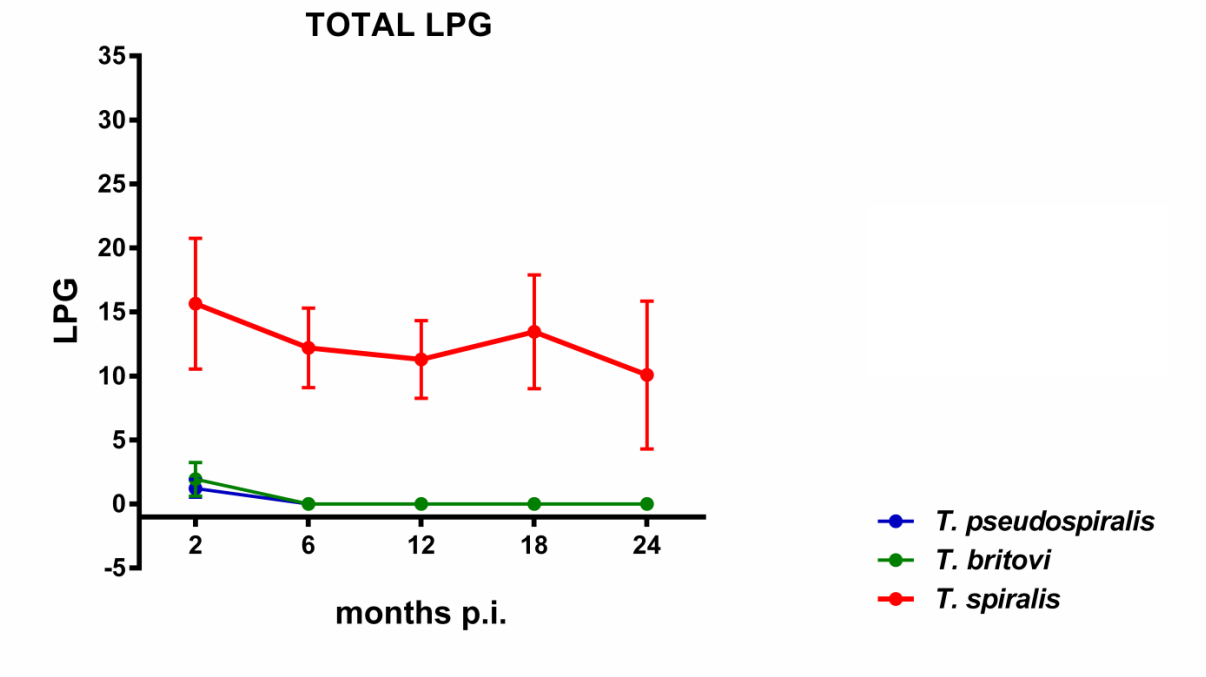
Malone et al, 2024

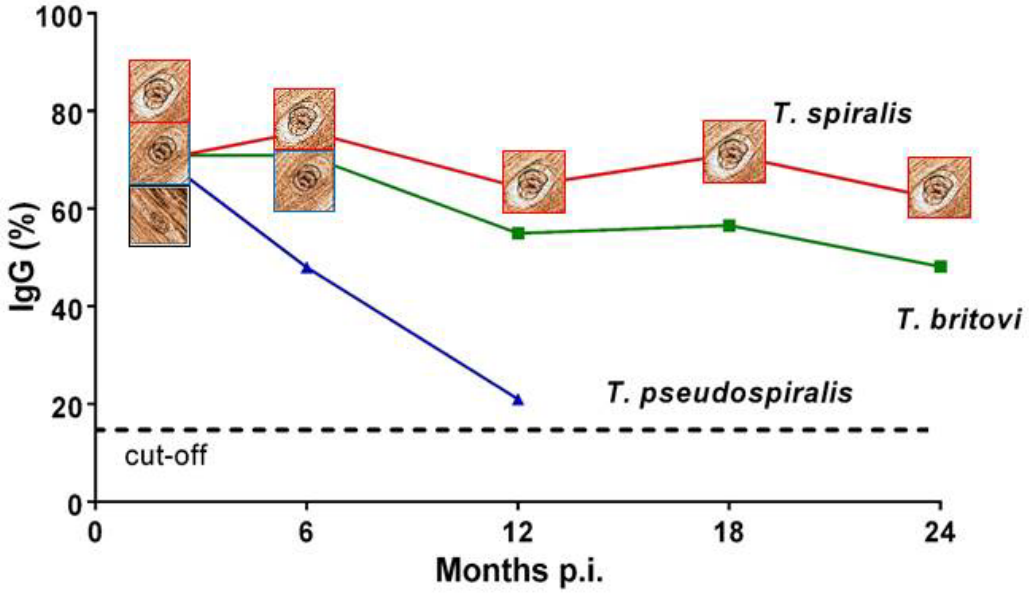
- All *Trichinella*-susceptible animals intended for human consumption are required to be tested for the presence of *Trichinella* larvae in the muscles (Commission Implementing Regulation (EU) 2015/1375).

- No stage-specific antigens are available for diagnosis so usually *Trichinella* is detected in late stage, when muscle larvae are formed

Actually, an antigene from excretory/secretory (ES) apparatus from muscle larvae is used for diagnosis

Serological diagnosis of *Trichinella spp.* in pigs





Pozio et al, 2020; adapted

Trichinella spiralis: highest infectivity and immunogenicity in pigs. IgG level significantly increased at 30 days p.i. and reached a peak at about 60 days p.i.

T. britovi-infected pigs, LPG was about 70 times lower than for *T. spiralis* at 2 months p.i. The IgG pattern showed by *T. britovi*-infected pigs was similar to that of *T. spiralis*-infected pigs, although seroconversion occurred some days later.

The larval burden of *T. pseudospiralis* was slightly greater than for *T. britovi* at 2 months p.i., but no larvae were detected at 6 and 12 months p.i. The IgG level showed a significant drop at 6 months p.i. and declining to the cut-off value at 12 months p.i.

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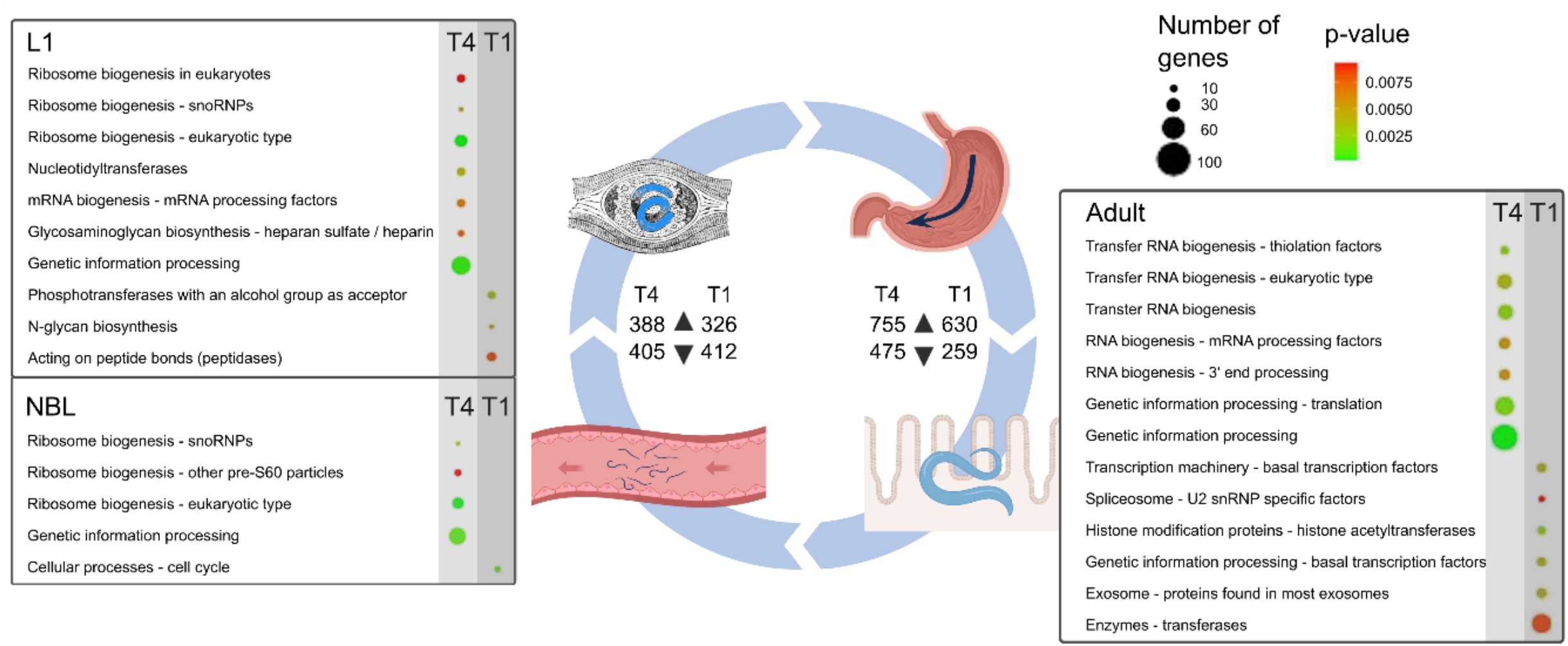
Article

Enhanced Genomic and Transcriptomic Resources for *Trichinella pseudospiralis* and *T. spiralis* to Underpin the Discovery of Molecular Differences between Stages and Species

Pasi K. Korhonen [1], Giuseppe La Rosa [2], Sunita B. Sumanam [1], Maria Angeles Gomez Morales [2], Alessandra Ludovisi [2], Edoardo Pozio [2], Daniele Tonanzi [2], Bill C. H. Chang [1], Neil D. Young [1] and Robin B. Gasser [1,*]

- enhance genomic and transcriptomic resources for selected representatives - ***T. pseudospiralis*** **(non-encapsulated**) and ***T. spiralis*** **(encapsulated**);
- to characterise and compare the composition of the transcriptomes of these two representatives;
- transcriptional variation between or among selected **developmental stages** of each of these species, and link these differences to respective stage-specific biological pathways or processes;
- to explore transcriptional differences at key points of the life cycle between these representative non-encapsulated and encapsulated species.



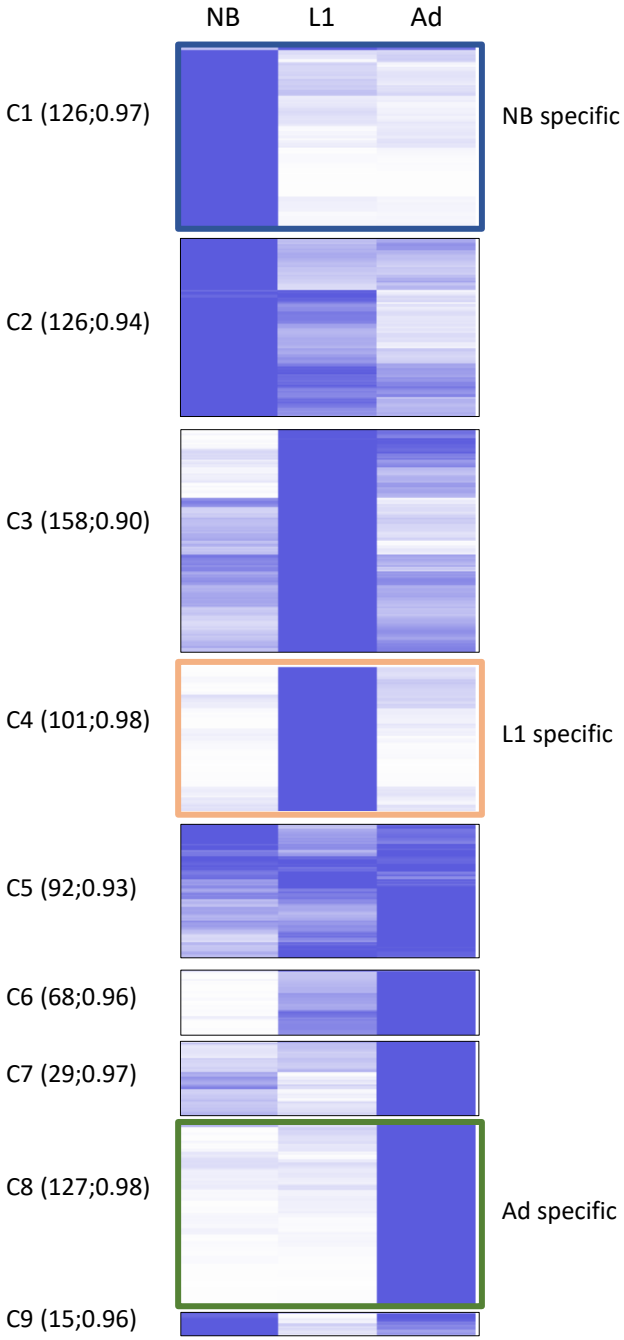
Korhonen et al. 2024

Looking for new candidates as potential markers for stage-specific serological diagnosis!

Testing immune sera from animals with different timing of infection

Development of a sentitive diagnostic tool, able to identify stage-specific infection

Here I joined the group…



Clustering (hierarchical) of genes differentially expressed in the 3 development stages in *T. spiralis*

Genes encoding excretory/secretory (ES) proteins, secreted or with a predicted extracellular localization in Newborn Larvae (NBLs), first-stage larvae (L1) and adult stages of *Trichinella spiralis* and *Trichinella pseudospiralis*

Identification of 3 genes NBL specific, 3 genes L1 specific and 4 genes Adult specific with respective orthologue in *T. pseudospiralis* for a total of 10 candidates

(Dr. Elisabetta Pizzi, ISS)

A	B	C	D	E	F	G
Code	**Sequence (cloning region)**	**PTM/processing**	**TMHMM**	**Alpha Fold**	**Cloning region**	**Cysteines**
Tsp_05827	MAAIAQLLLQLYAAVTLTNAVHPAFEANPALVSPGGSQCCILISTSSCCPHMYGDRPTCI GSGGIAPYPLGPTAPGGVQFPSIFQPTAPQPQPRPGGVFPGPIQTPPVGGGQVNIMWPGG VQLPITEQNQRCQDILREICKYCQAAGIQPIPQPYWPRPWPTATQQTFAPFNPSAGQMQV PSPFASNQDQTQMTSLDINLPIWSPTSGQQPLPPTGVDSPWAVTWEGNPIDWSKVQIPPL SPNPETNMPPNFNQQIQTDENPSVVNYPVQATTNNINRPYQ	signal peptide (1-20): MAAIAQLLLQLYAAVTLTNA	none	partial model of low confidence	VHPAFEANPALVSPGGSQCCILISTSSCCPHMYGDRPTCIGSGGIAPYPLGPTAPGGVQFPSIFQPTAPQPQPRPGGVFPGPIQTPPVGGGQVNIMWPGGVQLPITEQNQRCQDILREICKYCQAAGIQPIPQPYWPRPWPTATQQTFAPFNPSAGQMQVPSPFASNQDQTQMTSLDINLPIWSPTSGQQPLPPTGVDSPWAVTWEGNPIDWSKVQIPPLSPNPETNMPPNFNQQIQTDENPSVVNYPVQATTNNINRPYQ	8
Tsp_06566	MATTCIISQIFHFHKIWAVHCTNCPLAIGQFEVQPSSTSETGLKQAFKAARRLRNFASNIVVPNTANDPCENLIQVGTVLIERERRRIIKKLLKSLVKSAERNKRRGNRGMRTCNRMLRIVQG		none	high confidence for C-terminus but disordered N-terminus	SSTSETGLKQAFKAARRLRNFASNIVVPNTANDPCENLIQVGTVLIERERRRIIKKLLKSLVKSAERNKRRGNRGMRTCNRMLRIVQG	5
Tsp_00273	MQTQSVNISFIKTDPRESSCISTLELTIEGKSYTEQNVKNFSYCLSCVTVHSSENSNLSE DNLKNYLTSKNMRVPVSDDHCRETLPTELPGRHLVPIYYCSSGACVKIKGTFQDEIYVVR ECWDRLWDRPIKHGWQGCSVVDFMYDSMAHICVCLSNDLCNSALKIGSGQIYLLLMLFMP LTGLCFFNQILF	SigPep says none but homolog in Uniprot has one. In alpha fold model it's disoredered + helix	res 169-191	confident globular domain	VKNFSYCLSCVTVHSSENSNLSEDNLKNYLTSKNMRVPVSDDHCRETLPTELPGRHLVPIYYCSSGACVKIKGTFQDEIYVVRECWDRLWDRPIKHGWQGCSVVDFMYDSMAHICVCLSNDLCNSALKI	10
Tsp_00292	MKKYKYPLEKDVCMYYSKMVRFCSLVILLLLVPLIIDTEAKKKAKSRSPAVRNKNLESDA ENSSELVNNEEDEEGILKLAKKPVKQPPEEGSEMQPPKQPPEEPRGEPMEPPAEMPAGPP IDEPTEIKPEDQFEPPPPPPPEMEAGDADDVENAVEE	none	res-20-37	extremely low and unuseful. Predicted to be disordered with a few helices. In fact it is proline rich and lys rich.	TEAKKKAKSRSPAVRNKNLESDAENSSELVNNEEDEEGILKLAKKPVKQPPEEGSEMQPPKQPPEEPRGEPMEPPAEMPAGPPIDEPTEIKPEDQFEPPPPPPEMEAGDADDVENAVEE	0
Tsp_09226	MSYYASYEQILRWDIRSKYGTKHALPIHSLFSFLLFEYRILHSRGFTRRHKRNNWFQKDK LKRCTEKQTEICAQTECKAEDAAMTDLLLEGESDIFDHSDFTSYATCMHRCCARLNGAAV PPLKEGEKRRGPSKLPFQSIFEVADQKTVERCDETMCKSHRQKYENLVARTSSYKKLRSS QELKDYKECIERCDAKLNG	none	none	extremely low and unuseful	MSYYASYEQILRWDIRSKYGTKHALPIHSLFSFLLFEYRILHSRGFTRRHKRNNWFQKDKLKRCTEKQTEICAQTECKAEDAAMTDLLLEGESDIFDHSDFTSYATCMHRCCARLNGAAVPPLKEGEKRRGPSKLPFQSIFEVADQKTVERCDETMCKSHRQKYENLVARTSSYKKLRSSQELKDYKECIERCDAKLNG	10
TSP_03319	MSSKCLALMLFGSLVLMMVMGFDNKPALSTSSHAYDCGNENRIIRLYSFRLGPMPLVFPDVLQMHIDLEIMDKIPSQVDAHVRVEKQVTKTIWVRVPCMLQFGSCDYYRIDACLLTEEMFGCPIEIGRHNHTETYLLDSPETFNPNSLRGNYRTLVEINNSDTGEPLACFNFIYSINGSRW	signal peptide (1-20): MSSKCLALMLFGSLVLMMVM	none	globular. High confidence A fold model	GFDNKPALSTSSHAYDCGNENRIIRLYSFRLGPMPLVFPDVLQMHIDLEIMDKIPSQVDAHVRVEKQVTKTIWVRVPCMLQFGSCDYYRIDACLLTEEMFGCPIEIGRHNHTETYLLDSPETFNPNSLRGNYRTLVEINNSDTGEPLACFNFIYSINGSRW	6
Tsp_09073	MALKFCNAITVGLITFCCLFFAASADATTNCAEDVANFYKCIDRHYDQELGKIFTYHTAE AFSEKAIECLTKNGCEKPAFHKFKPTEESDEDDDEEGFIGKQLDAFVFRKILNP	signal peptide (1-25): MALKFCNAITVGLITFCCLFFAASAD	none	globular. High confidence A fold model	ATTNCAEDVANFYKCIDRHYDQELGKIFTYHTAEAFSEKAIECLTKNGCEKPAFHKFKPTEESDEDDDEEGFIGKQLDAFVFRKILNPEWFPNA	

Methods

A. Construct design and cloning

- **pET14b** – N-terminal His-tag
- **His-SUMO-pET21b** – N-terminal His-SUMO tag. The SUMO tag enhances expression and is readily removed using the SUMO-protease with limited non-specific proteolysis activities.
- **pET27b** – N-terminal signal peptidase (SP) for pelB for periplasmic expression and C-terminal His tag. Selected for secreted and extracellular proteins may have disulphide bonds.

B. Screening: Expression, purification and SDS-PAGE analysis

BL21(DE3)Star, Shuffle(DE3), C41(DE3): Autoinduction media, growing at 37°C, then transferring to 20°C overnight. Plate repeated for Superior broth and IPTG-based induction. Example plate:

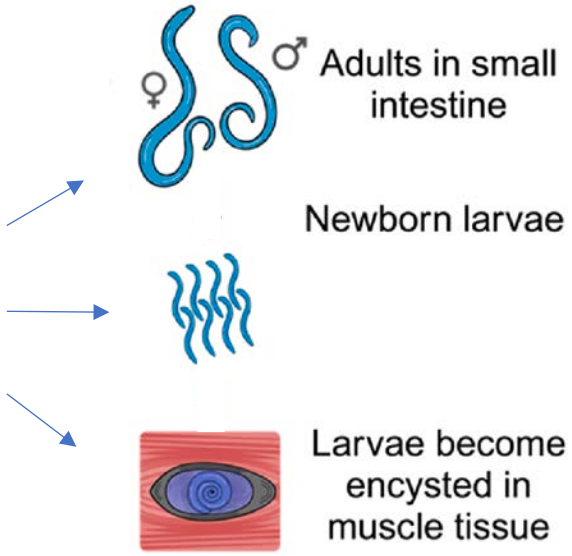
Vector	Ag1	Ag2	Ag3	Ag4	Ag5	Ag6	Ag7	Ag8	Ag9	Ag10	pelB	pelB
pET14b											Ag1	Ag10
pET21b											Ag2	Ag11
pET27b											Ag3	Ag12
pET14b											Ag4	
pET21b											Ag5	
PET27b											Ag6	
pET14b											Ag7	
pET21b											Ag8	

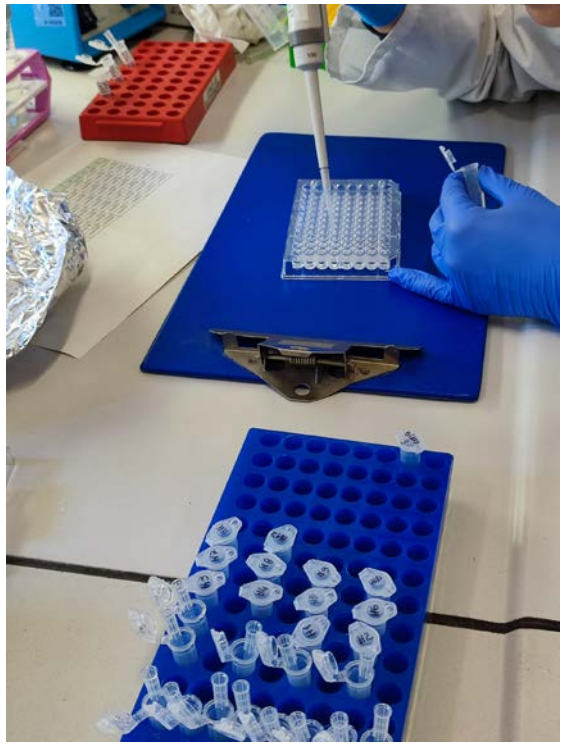
C. Large-scale chromatographic purification of 10 antigens

(Dr. Gourlay, University of Milano)

- Testing of **immune sera** collected at different time point of infection (human or animal) already available in our lab

(Dr. Alessandra Ludovisi and colleagues, ISS)





- Ability to discriminate the stage-specific infection

- VALIDATION of the diagnostic tool

Microchip device (easy to use, for slaughter, correct treatment, outbreaks, etc….)

Thank you for your attention!!!