

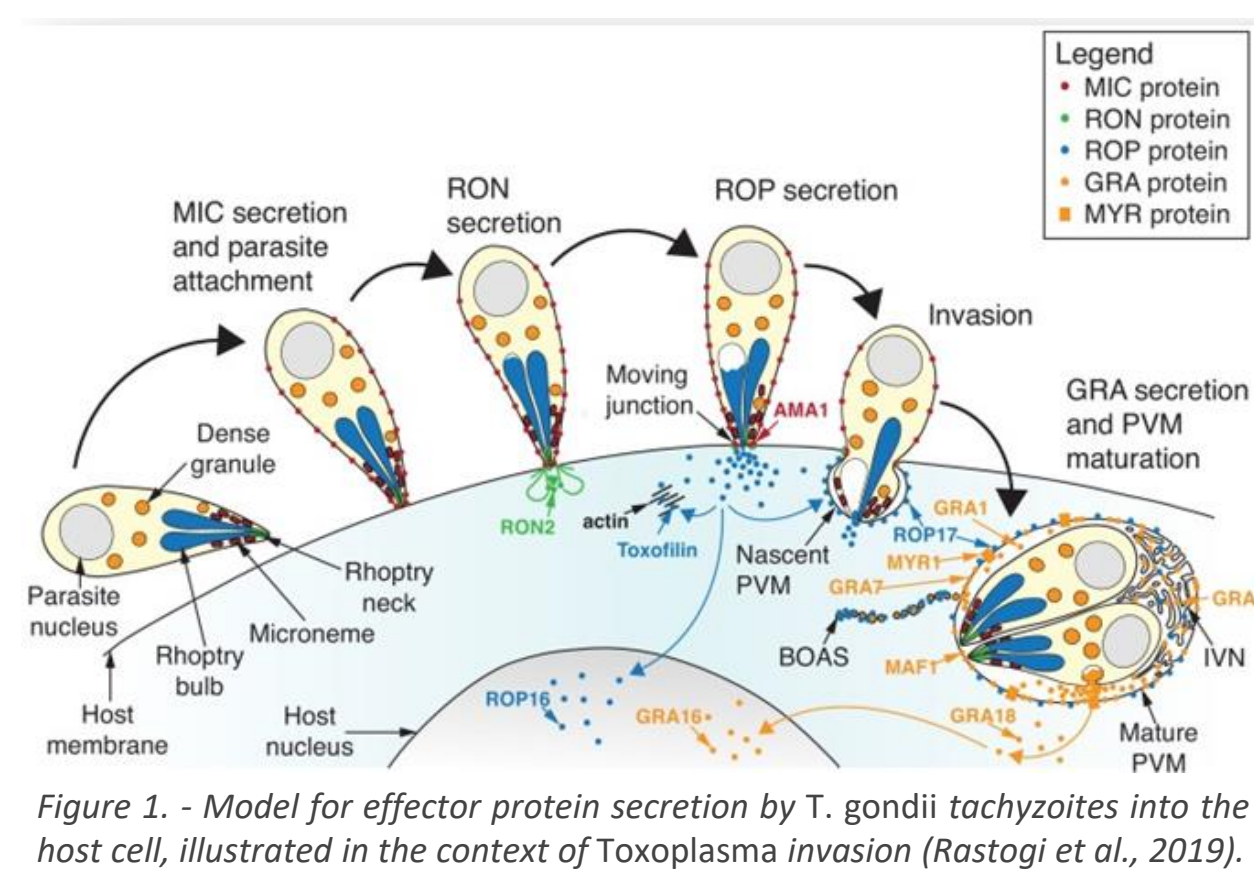


# Novel vaccine candidates related to the endogenous development in *Eimeria*

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## Introduction

- **Chicken coccidiosis** - enteritis caused by intracellular parasites of the genus *Eimeria*. Economic losses to the poultry industry estimated in **£10.4 billion** worldwide annually (1).
- **Control**: live vaccines (high cost - limited use) & chemoprophylaxis (drug resistance, new regulations in AMR) (2,3).
- **Proteins of dense granules (GRAs)**: important role in interaction with the host cell in *Toxoplasma* (closely related to *Eimeria* (Fig.1)). The presence of dense granules has not been proved in *Eimeria* and limited information is available regarding dense granules orthologues.



## Methodology

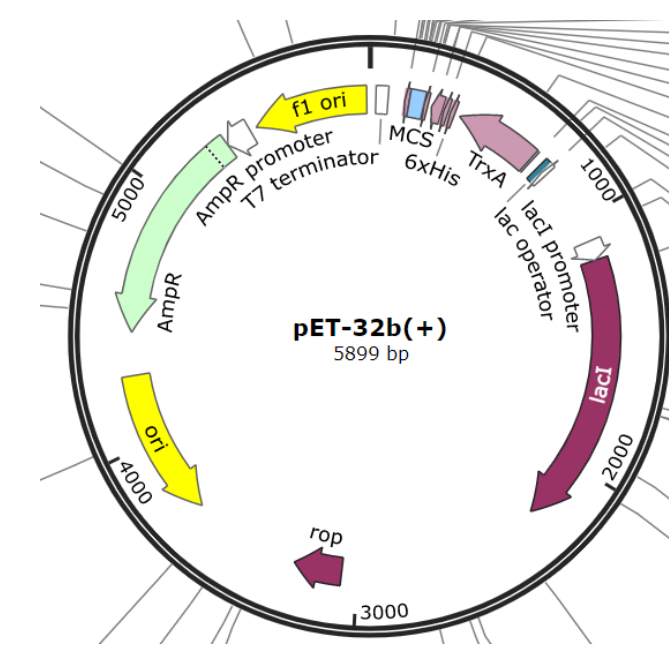
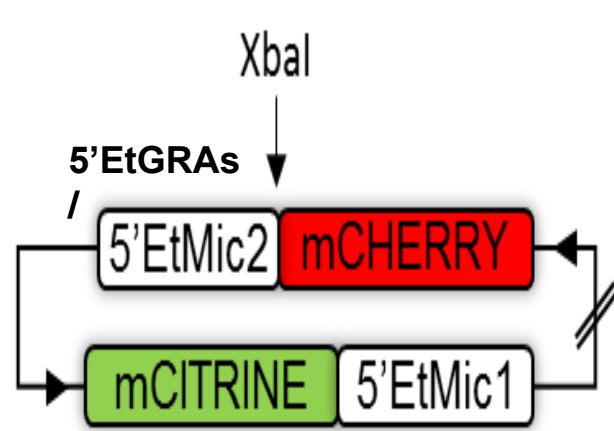
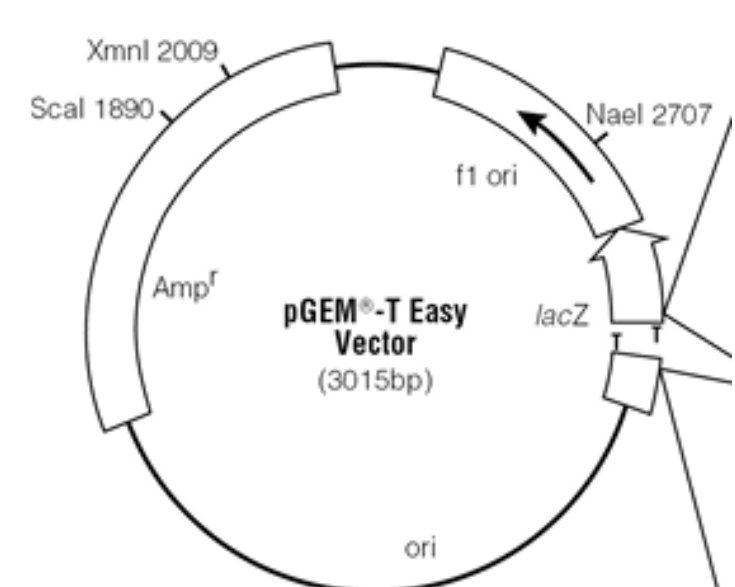
### In silico analysis



- Antigenic regions
- Signal peptides
- Transmembrane Domains
- GPI anchors
- Sequence analysis & verification
- Clone selection
- SNPs analysis



### DNA manipulation and cloning



Candidate genes subcloning and sequencing

Expression of candidate proteins fused to fluorescent reporter

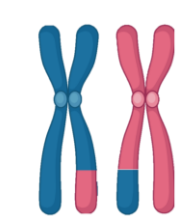
Expression of polyhistidine tagged recombinant proteins in *E. coli*

## Hypothesis

1. GRA proteins in *Eimeria* are stored in **equivalent structures** to classical dense granules (DG- like), however with a relatively different composition and regulation of secretion into the parasitophorous vacuole.



2. GRAs orthologues identified in *Eimeria* conserve **immunoprotective** properties, similar to what was found in *Toxoplasma* (4).



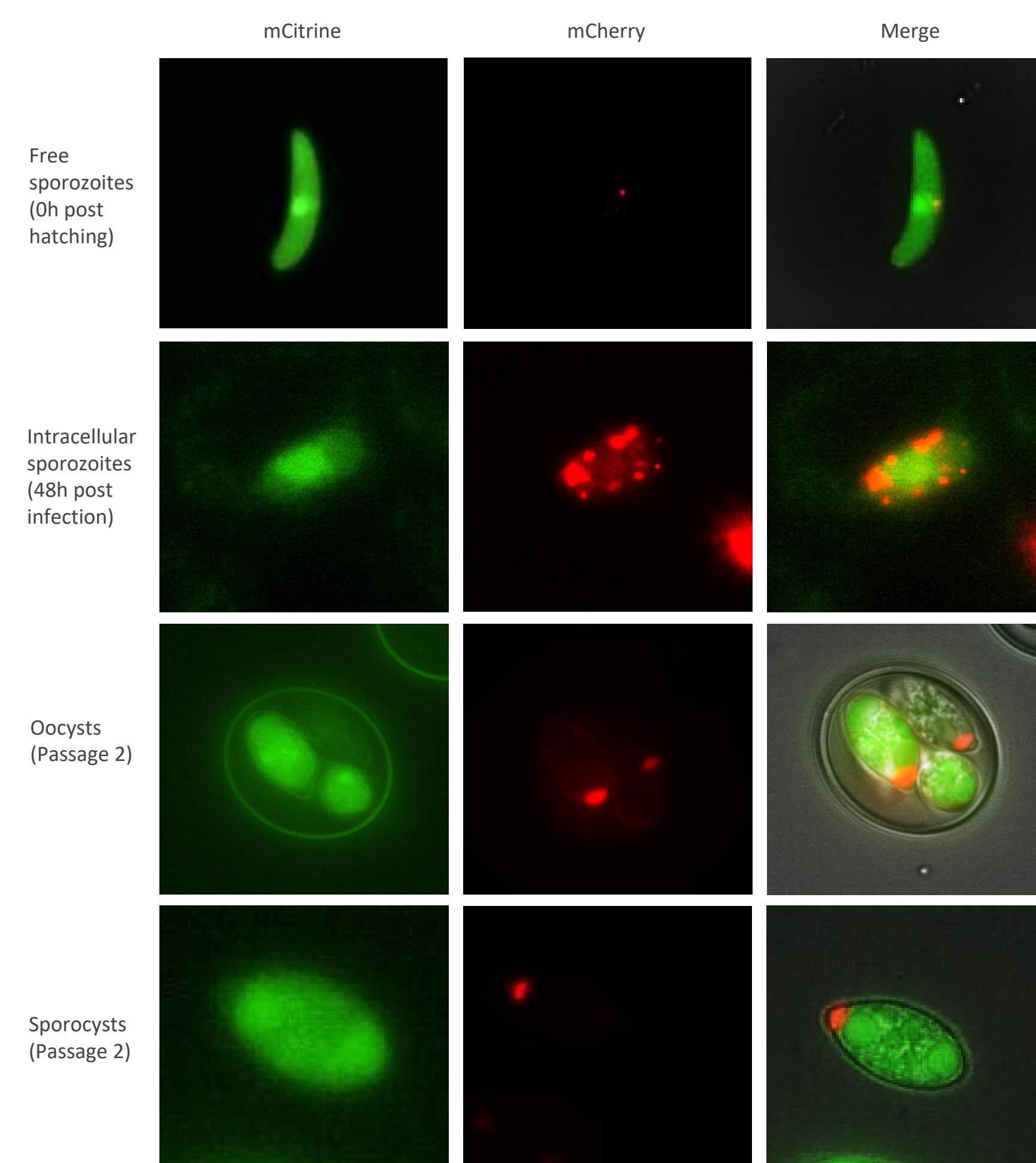
## Objectives

- Generate **new molecular and structural information** regarding *Eimeria* GRA proteins by in silico procedures.
- Tag candidate proteins to identify **location** through fluorescence microscopy and elucidate the presence of vesicles equivalent to dense granules.
- Assess the existence of pre-existing **genetic diversity** between geographically distinct *Eimeria* GRAs.
- Evaluate natural **recognition** of recGRAs by serum of infected chickens with *E. tenella*.

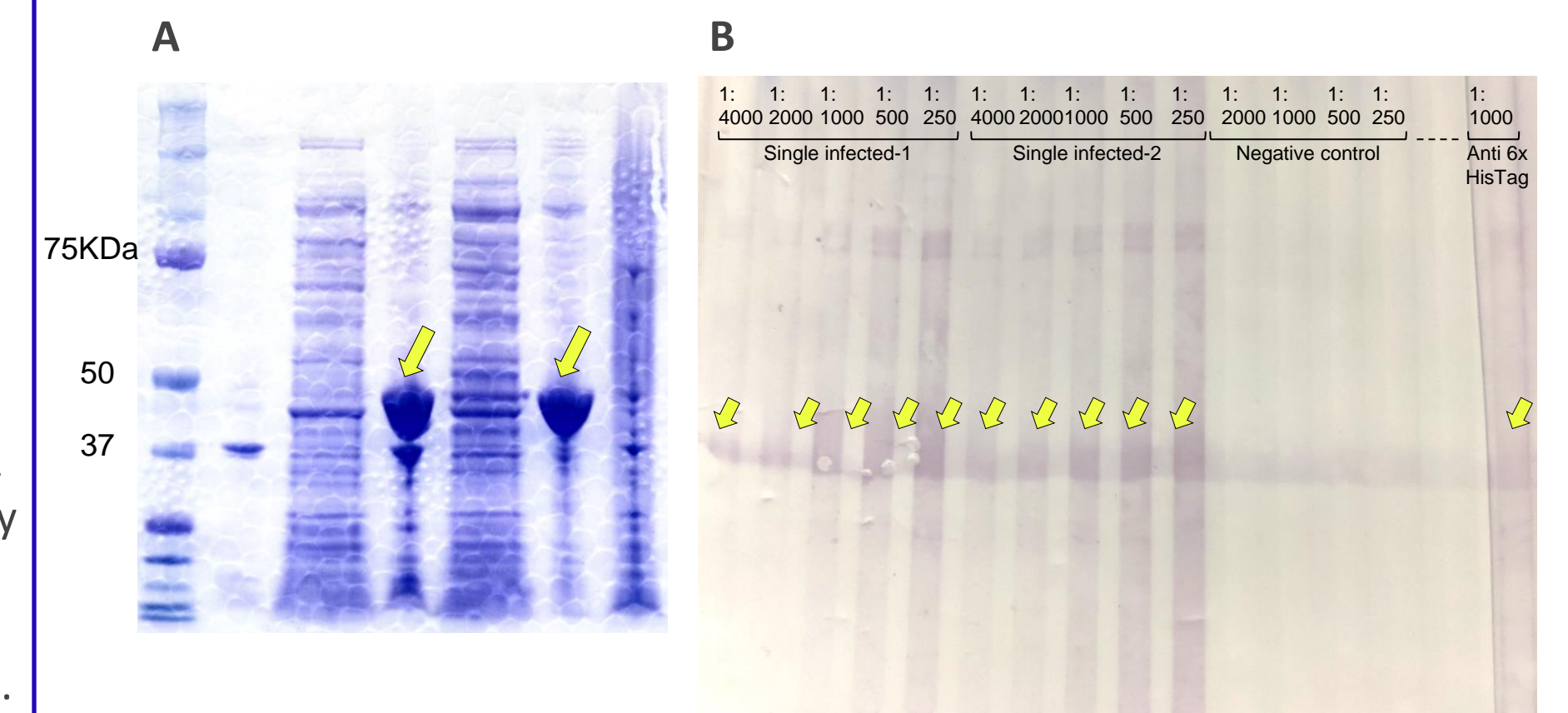
## Results

*Eimeria* GRA fused to **mCherry** was visualised scattered in the cytosol of transgenic sporozoites and secreted into the sporocyst cavity in oocysts.

**mCitrine** expressed without any regulatory element was always expressed in the cytosol of sporozoites.



**High expression** levels of an *Eimeria* GRA expressed as recombinant polyhistidine tagged fusion were obtained **(A)**. This recombinant GRA was **recognised** by sera from chickens infected with *E. tenella* **(B)**.



The **genetic diversity** based in analysis of SNPs in *Eimeria* spp. isolates from different geographic regions was **low** for *E. tenella* GRAs and moderate for *E. maxima* orthologues.

	EIGRA-A	EIGRA-B	EIGRA-C	EmGRA-A	EmGRA-B	EmGRA-C
NP sequences	15	16	14	11	8	10
NP sites	1005	1400	1305	1121	1311	1019
NP polymorphic sites	1	1	1	13	2	3
NP mutations	1	1	1	13	2	3
NP haplotypes	2	2	2	10	2	3
Haplotype diversity	0.133	0.233	0.440	0.982	0.429	0.511
VA of haplotype diversity	0.01261	0.01578	0.01254	0.00215	0.02846	0.02700
SD of haplotype diversity	0.112	0.126	0.112	0.046	0.169	0.164
Nucleotide diversity (Pi)	0.00013	0.00017	0.00034	0.00563	0.00069	0.00090

## Conclusions

- The localisation study could support the existence of **vesicles equivalent to dense granules** in *Eimeria*, but further work is needed to determine their composition, nature and function.
- Sera from chickens immunised with a single dose of *E. tenella* **recognised** the polyhistidine fused recombinant protein, evidencing the presence of **specific antibodies** against this GRA protein.
- Antigenic variation is a common reason for failure and a contributor to the complexity of vaccine design, as implies ability to escape from immune response. Antigenic variability is **low** in the selected genes from *E. tenella*, which makes studied genes good candidates for potential vaccine antigens; whereas in the case of *E. maxima*, which are known to accommodate greater genetic diversity than *E. tenella*, the results show a **higher number** of polymorphisms.

## References

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(3) Marugan-Hernandez, V., Long, E., Blake, D., Crouch, C., Tomley, F. (2017) 'Eimeria tenella protein trafficking: differential regulation of secretion versus surface tethering during the life cycle', *Scientific Reports*, 7(1), 1-13.

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