

Veterinary vaccines for parasites

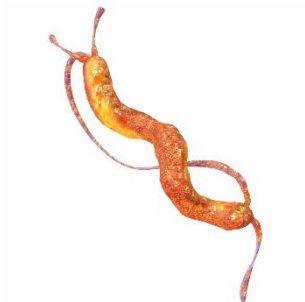
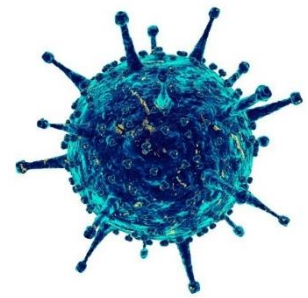


Damer Blake

Parasites are everywhere...

- 'Parasitism'

"Intimate relationship between two organisms in which one (the parasite) lives at the expense of the other (the host)"



...parasite vaccines are not

- Still no vaccines to protect against malaria, trypanosomiasis, hookworm, ascarids...
- Parasites continue to be common causes of morbidity and mortality

Table 7.3 Twenty top diseases/pathogens ranked according to their impact on the poor, by region (listed alphabetically within each rank group)

	West Africa (WA)	Eastern, Central and Southern Africa (ECSA)	South Asia (SA)	South-East Asia (SEA)
	Anthrax	→ East Coast fever (ECF)	<i>Brucella abortus</i>	Duck virus enteritis (DVE)
	Black-leg	→ Ectoparasites	→ Coccidiosis	
	Contagious bovine pleuro-pneumonia (CBPP)	→ GI parasitism	FMD	→ Ectoparasites
		→ Haemonchosis	HS	FMD
T		Infectious coryza	→ Liver fluke	Fowl cholera
o	Dermatophilosis	ND	Neonatal mortality	Fowl pox
P →	Ectoparasites	Neonatal mortality	Nutritional/	→ GI parasitism
→	Gastro-intestinal (GI) parasitism	Nutritional/	micronutrient	HS
10		micronutrient	deficiencies	Hog cholera
→	Heartwater	deficiencies	PPR	ND
→	Liver fluke (fascioliasis)	Respiratory complexes	Reproductive disorders	→ <i>Toxocara vitulorum</i>
	Respiratory complexes	RVF	→ <i>Toxocara vitulorum</i>	
→	Trypanosomosis			

...parasite vaccines are not

- Why?
 - Complex lifecycles
 - Complex antigenic profiles
 - Immune escape mechanisms
 - Rapid evolution – genetic diversity
- But...
 - Economic, welfare & social imperatives



Parasite vaccines of today... and the future

- Today
 - Whole parasite vaccines
 - Subunit (native) antigens
 - Recombinant antigens
- The future
 - More...
 - DNA vaccines
 - Dietary vaccines
 - **Vectored vaccines**

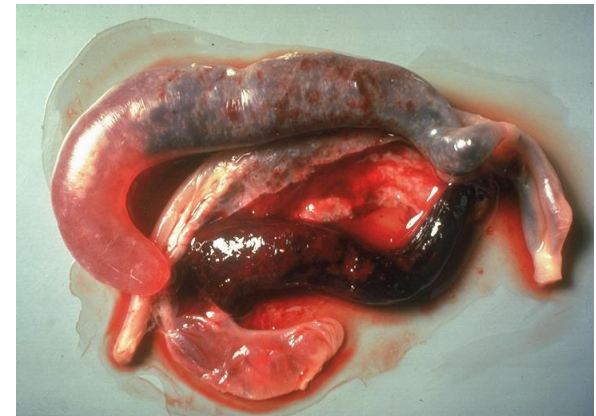
Parasite vaccines of today... and the future

- Today
 - Whole (live) parasites, 'natural' immunity

Eimeria spp.



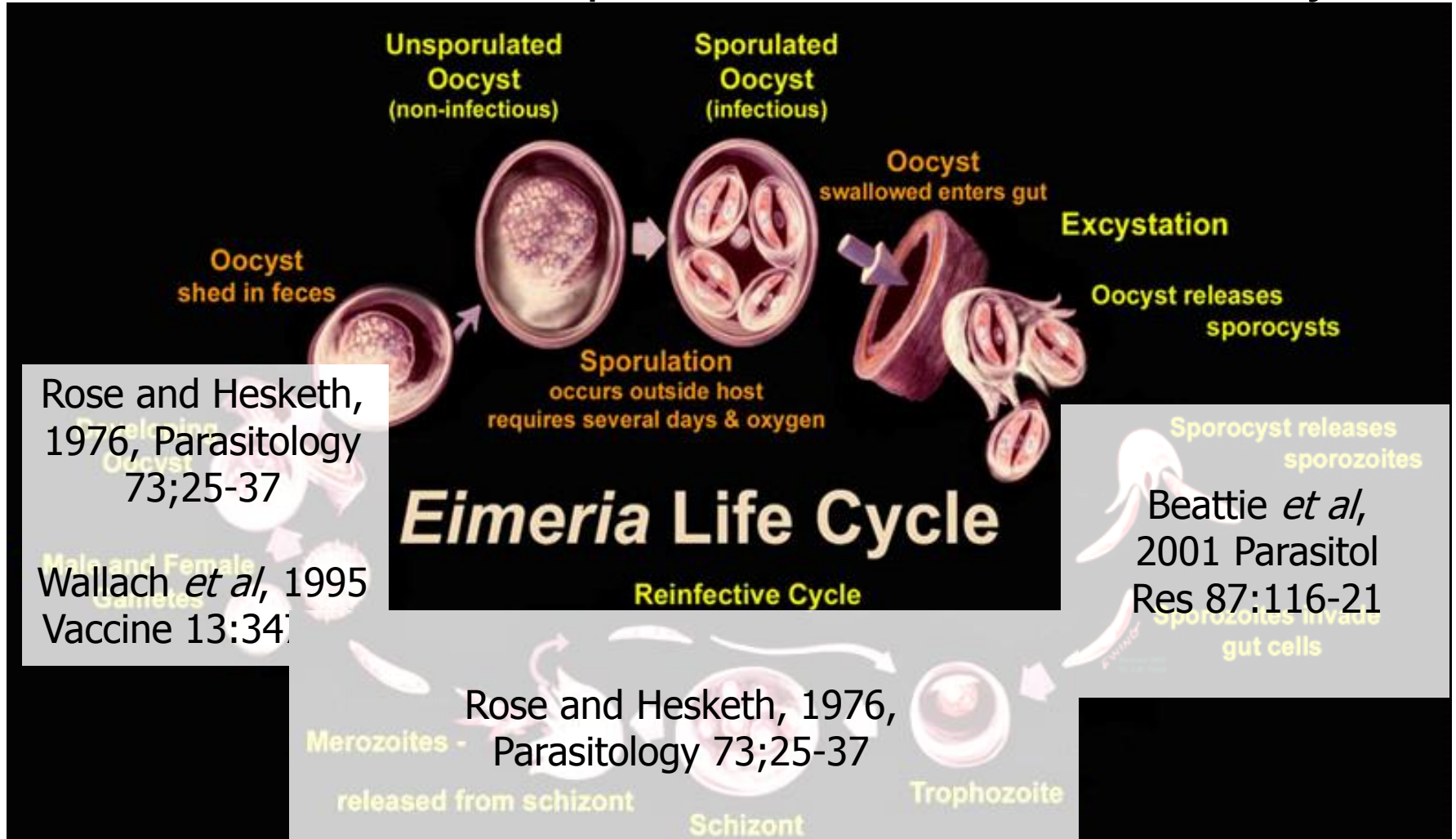
Eimeria maxima



Eimeria tenella

Parasite vaccines of today... and the future

- Today
 - Whole (live) parasites, 'natural' immunity



Rose and Hesketh, 1976, Parasitology 73;25-37

Wallach *et al*, 1995 Vaccine 13:34

Beattie *et al*, 2001 Parasitol Res 87:116-21

Rose and Hesketh, 1976, Parasitology 73;25-37

Parasite vaccines of today... and the future

- Today
 - Anti-*Eimeria* vaccines



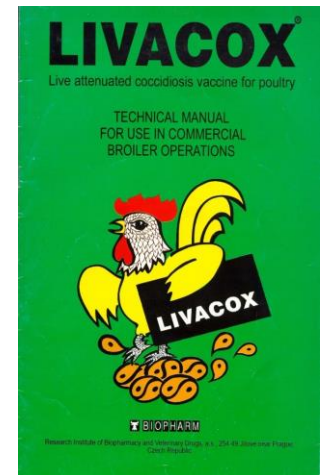
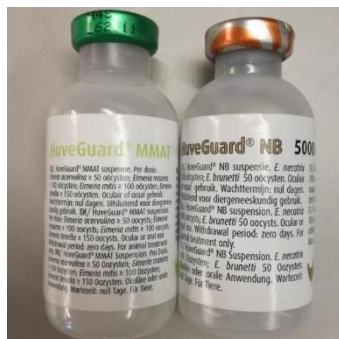
- Early form introduced in 1952 (USA)
- Very low oocyst doses per chicken
- Relies on parasite recycling
- Limited production capacity
- 1° breeder and layer stock

Parasite vaccines of today... and the future

- Today
 - Anti-*Eimeria* vaccines in Europe



- No licensed wild-type vaccines
- Attenuated alternatives
- Selected for precocious development
- Significantly limited production capacity



Parasite vaccines of today... and the future

- Today

- Whole (live) parasites, 'natural' immunity

- Dose and treat



- Attenuated



- Irradiated



Image for illustration only.
Supplied as single dose

Parasite vaccines of today... and the future

- Today

- Whole (live) parasites, 'natural' immunity
 - Robust immune protection
 - Require parasite production

- Subunit (native) proteins

- Leishmune (Fort Dodge/Pfizer)

- *L. donovani* fucose-mannose ligand
- First available in Brazil



- CaniLeish (Virbac)

- *L. infantum* excreted/secreted (ES) proteins
- Available in Portugal, UK (2012), ...



Parasite vaccines of today... and the future

- Today

- Whole (live) parasites, 'natural' immunity
 - Robust immune protection
 - Require parasite production
- Subunit (native) proteins
 - Barbervax (*Haemonchus contortus*)
 - Protein from *H. contortus* intestinal walls
 - Circulating antibodies stimulated by vaccination
 - Blood meal antibodies bind to the parasite gut
 - Interfere with digestion/uptake
 - Starvation, reduced reproduction
 - 'Hidden gut' antigen



Parasite vaccines of today... and the future

- Today

- Whole (live) parasites, 'natural' immunity
 - Robust immune protection
 - Require parasite production
- Subunit (native) proteins
- Subunit (recombinant) proteins

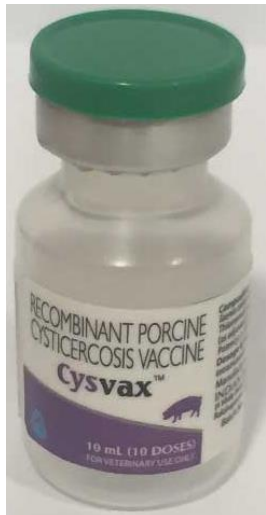
- TickGARD Plus
- *Rhipicephalus microplus*
- Recombinant Bm86
- Multiple immunisations required



Parasite vaccines of today... and the future

- Today

- Whole (live) parasites, 'natural' immunity
 - Robust immune protection
 - Require parasite production
- Subunit (native) proteins
- Subunit (recombinant) proteins



- *Cysvax* – *Taenia solium*
- Recombinant oncosphere antigen (TSOL18)
- Vaccinate pigs, reduce human infection

And what of the future?



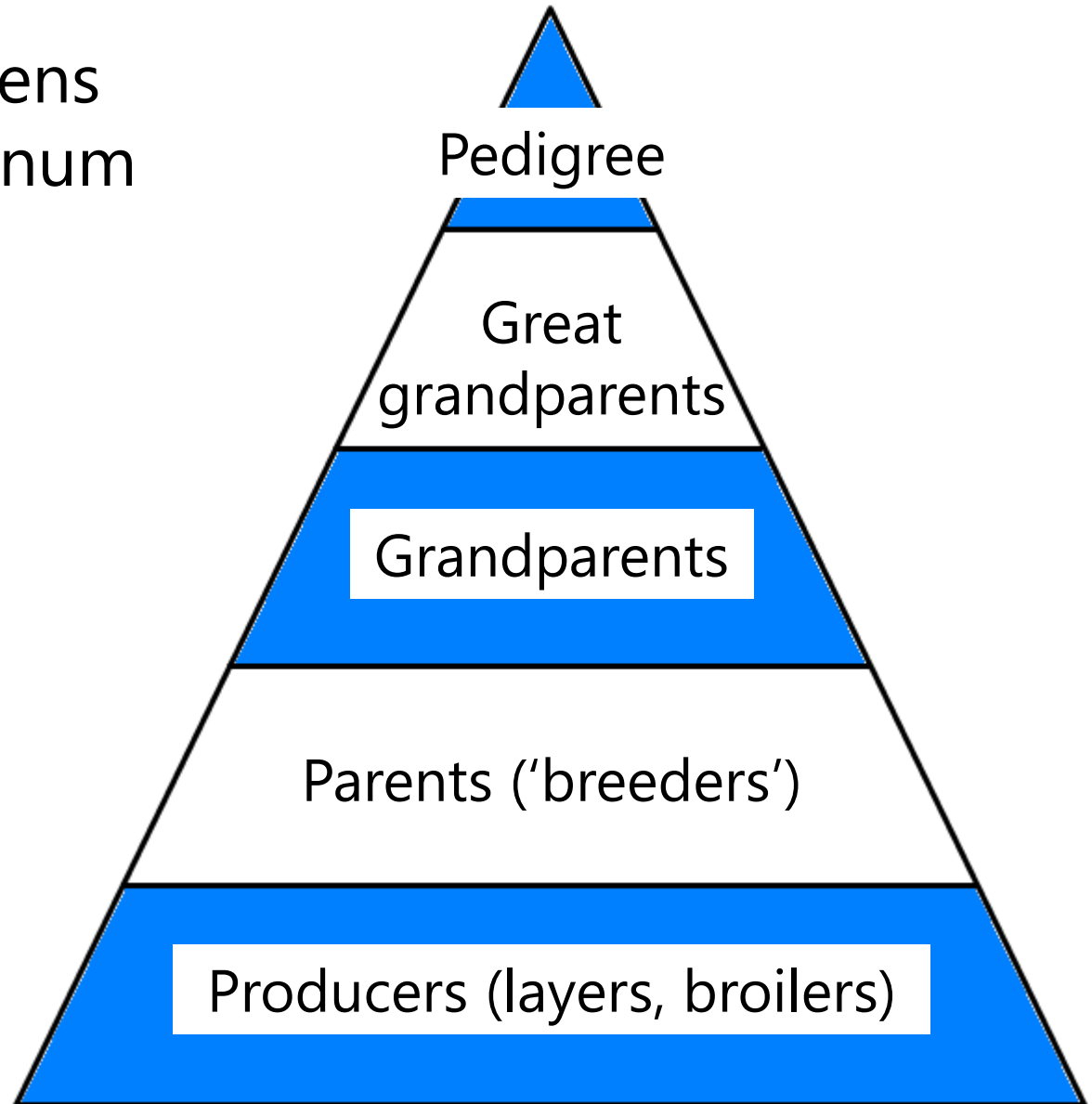
Background: poultry production



Background: poultry production

- >62 billion chickens produced per annum
 - Broilers
 - Layers
 - Breeders
 - Others

Pathogen control



Vaccination

- Broiler breeders:
11 pathogens (+)
- Layers:
9 pathogens (+)
- Broilers:
4 pathogens (+)
- Additional pathogens?
- Zoonoses?

Age	Disease	Vaccine Strain	Route
day one	Marek's Coccidiosis	HVT + SB ₁ , Rispens	Inject / <i>in-ovo</i> Spray
1-2 weeks	Reovirus	1133	Water Spray
2 weeks	ND IB IBD	B ₁ , B ₁ Mass +/-Conn Intermediate	Water or Spray Water
4 weeks	ND IB	B ₁ , B ₁ M-9	Spray

Improve current vaccines

Develop new vaccines

Replace antibiotics
(*Eimeria...*)

	CAV (opt) IBD & Reo	inactivated	injection
18 weeks	Fowl Cholera IBD, IB, Reo & ND	PM-1 or M-9 inactivated	Wing Web injection

Legislation and commerce

- 2008 - EU report on the use of anticoccidial substances as feed additives
- Designed to inform the phasing out of anticoccidials by December 2012 (Regulation [EC] No. 1831/2003, Article 11)
– no ban
- Similarly, the US FDA introduced its first ban of an agricultural drug in 2005
- Now, 'No antibiotics, ever' - calls to reduce ionophore use
- 2016 - EU consultation to revise guidelines for anticoccidials



Vaccine considerations

- ??? for poultry and all livestock

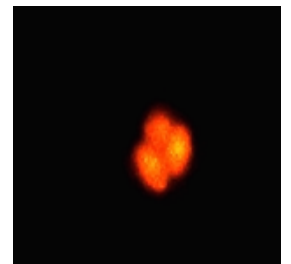
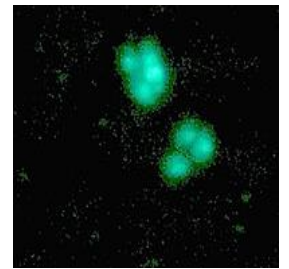
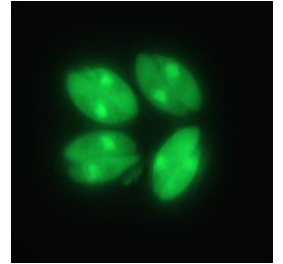
Vaccine considerations

- Effective
- Simple delivery mechanism **Vectored**
- Host specificity
- Immunogenic – adjuvanting properties
- Non-pathogenic
- Self-limiting **Bacterial**
- Capacity for multi-valence **Viral**

Transgenic live *Eimeria* vaccines?

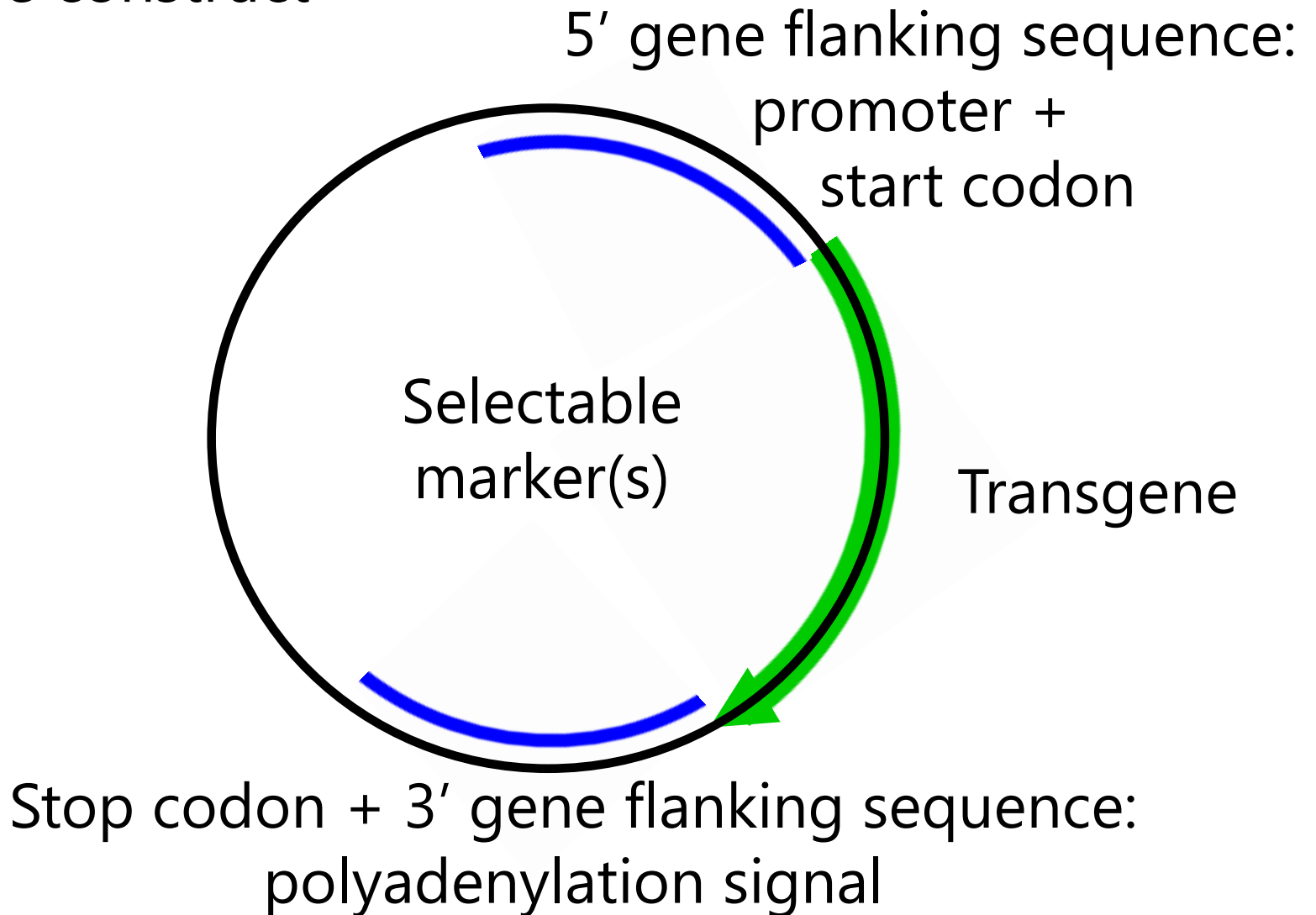
GM coccidia – the state of play

- Well established for *Toxoplasma gondii*
- Valuable laboratory tool
- *Eimeria* species: obligate *in vivo* phase
- Stable transfection established
 - Random integration
 - Targeted insertion (knock in/out)...
 - Stable transgene expression




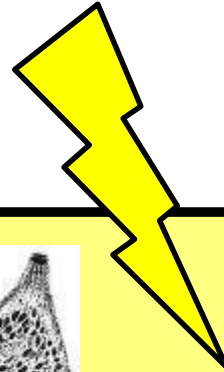
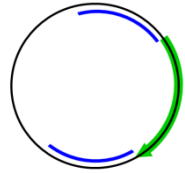
Eimeria transfection

- The construct



Eimeria transfection

- The construct
- The cell



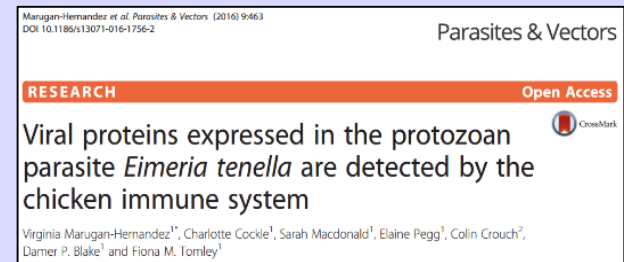
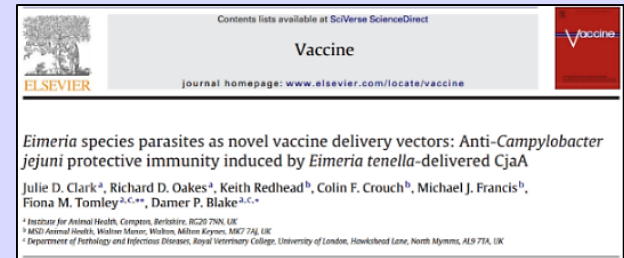
• Transient
(Kelleher and Tomley, 1998)

• Stable
(Clark et al, 2008)

The complex block contains a large yellow rounded rectangle. On the left side of this rectangle, there is a circular inset showing a petri dish with several pipettes. In the center of the rectangle is a black and white micrograph of an Eimeria cell, showing its internal organelles and a large nucleus. To the right of the micrograph, there are two bullet points: 'Transient (Kelleher and Tomley, 1998)' and 'Stable (Clark et al, 2008)'. A yellow lightning bolt symbol points from the top right towards the cell image.

Eimeria as a vaccine vector

- *E. tenella* parasites expressing the **CjaA** protein induced immune protection against *Campylobacter jejuni* challenge (Clark et al., 2012)
- *E. tenella* parasites expressing the **vvVP2** (IBDV) and the **gI** (ILTV) proteins induced specific antibodies (Marugán-Hernández et al., 2016)



**Inconclusive immune
recognition or low antibody
titers**

**Low-expression
promoters**

Anticoccidial antigens?

Timing of secondary immune response

- Primary infection: 100 *E. maxima* W oocysts
- Secondary infection (homologous): +3 weeks

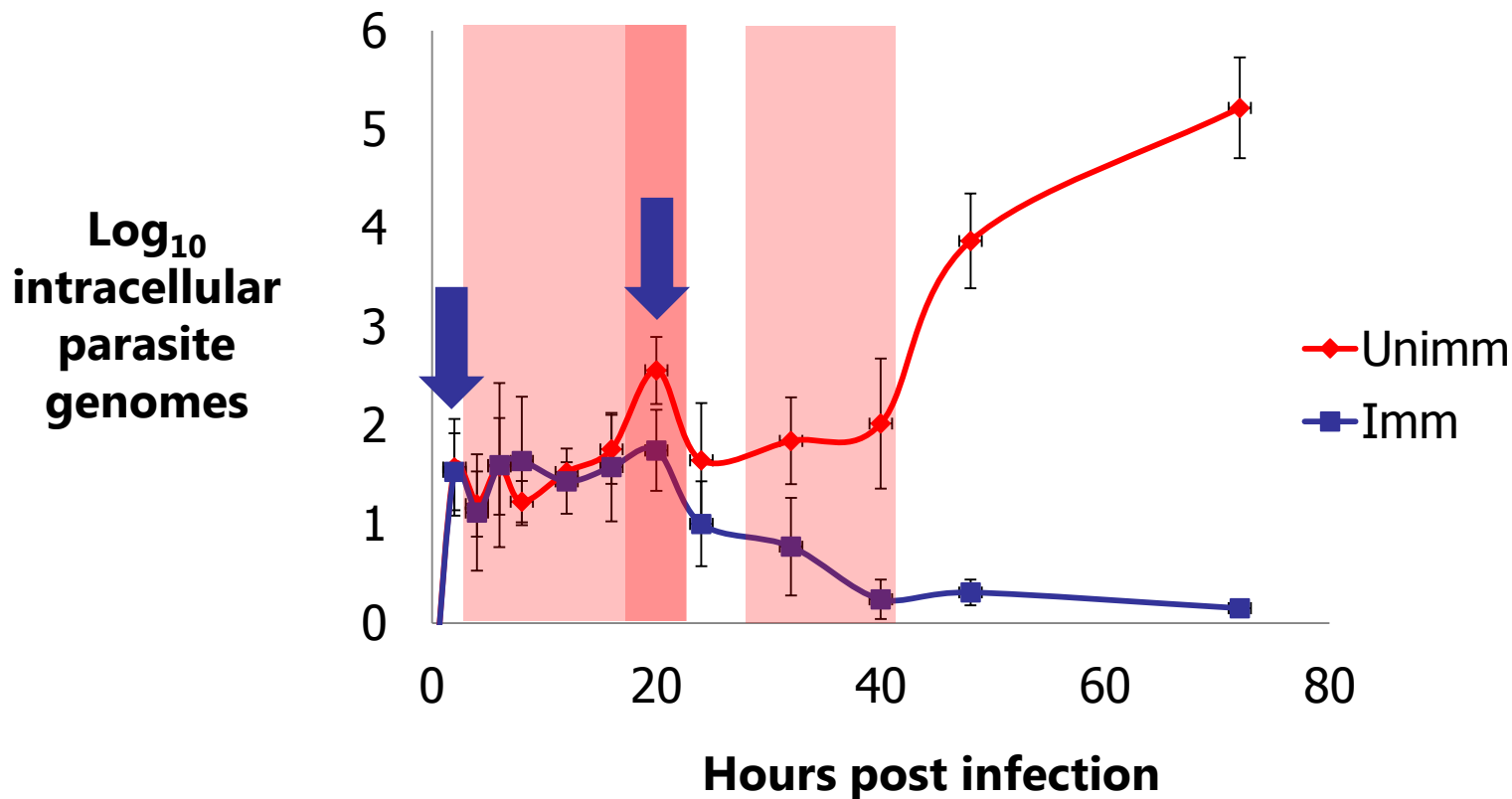
Quantified parasite replication
in vivo by TaqMan qPCR

— Unimmunised
— Immunised

Anticoccidial antigens?

Timing of secondary immune response

- Primary infection: 100 *E. maxima* W oocysts
- Secondary infection (homologous): +3 weeks



Anticoccidial antigens

Recombinant protein vaccination:

- Apical membrane antigen 1 (AMA1)

- *E. maxima*: 42%*
- *E. tenella*: 66%*

OPEN ACCESS Freely available online

PLoS PATHOGENS

Genetic Mapping Identifies Novel Highly Protective Antigens for an Apicomplexan Parasite

Damer P. Blake^{1,2*}, Karen J. Billington¹, Susan L. Copestake¹, Richard D. Oakes¹, Michael A. Quail³, Kiew-Lian Wan^{4,5}, Martin W. Shirley¹, Adrian L. Smith^{1,6*}

- Immune mapped protein 1 (IMP1)

- *E. maxima*: 45%*
- *E. tenella*: 67%*



Research paper

Humoral and cytokine response elicited during immunisation with recombinant Immune Mapped protein-1 (EtIMP-1) and oocysts of *Eimeria tenella*



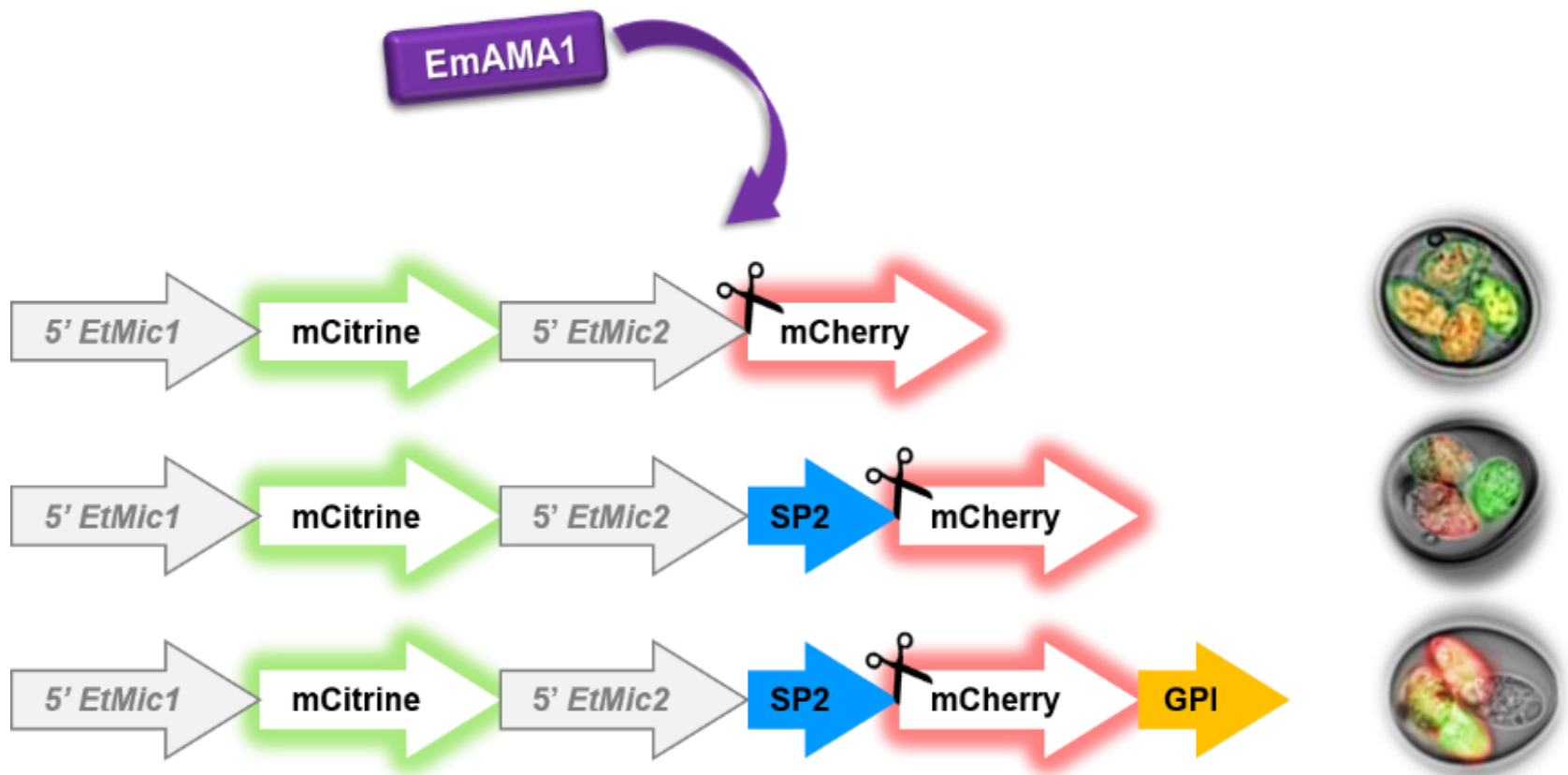
Krishnendu Kundu^{a,1}, Rajat Garg^a, Saroj Kumar^a, Mrityunjay Mandal^{b,2}, Fiona M. Tomley^b, Damer P. Blake^b, Partha Sarathi Banerjee^{a,*}

- ...

*Reduction in parasite replication immunised : mock immunised

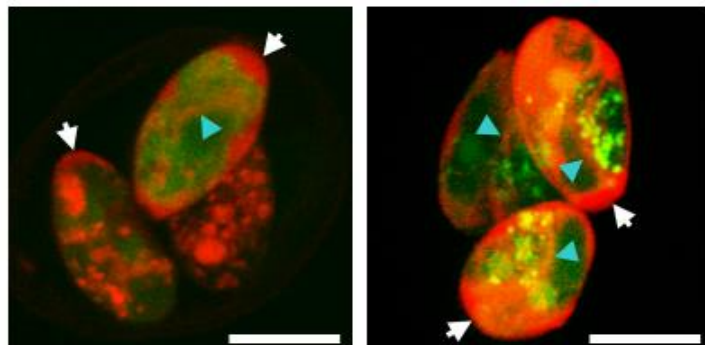
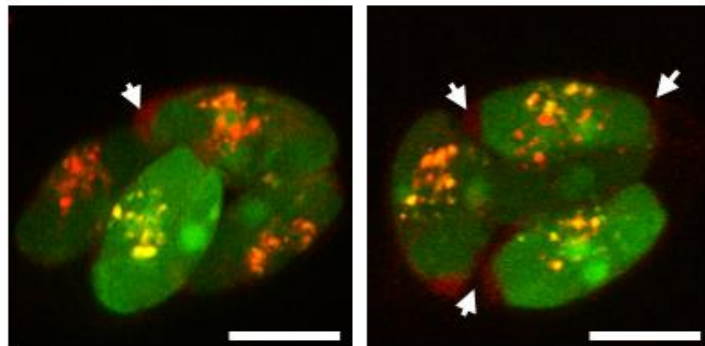
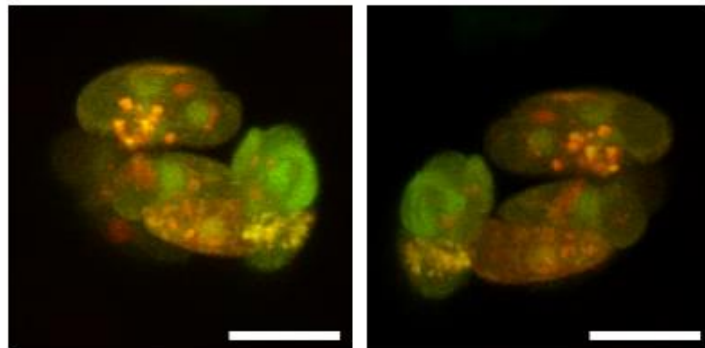
E. tenella-based vaccines against *E. maxima*

- Construct optimisation
- Additional of AMA1 ectodomain



Et-[EmAMA1] parasites: characterization

- EmAMA1 expressed by *E. tenella*, trafficking differs:



Et-[EmAMA1] parasites: vaccine trial



- Experimental design:





White Leghorn 15I

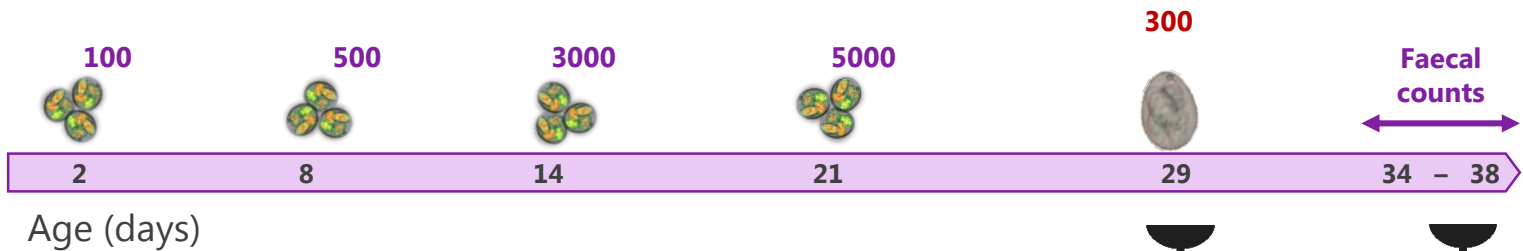


Susceptible to *E. maxima*

Groups (5-7 birds/treatment)

	EmAMA1-Ch		Vector (SP2-Ch-GPI)
	SP2-EmAMA1-Ch		PBS - NCh
	SP2-EmAMA1-Ch-GPI		PBS - Ch

Serial immunisations to mimic oocyst recycling
(oocysts/bird)

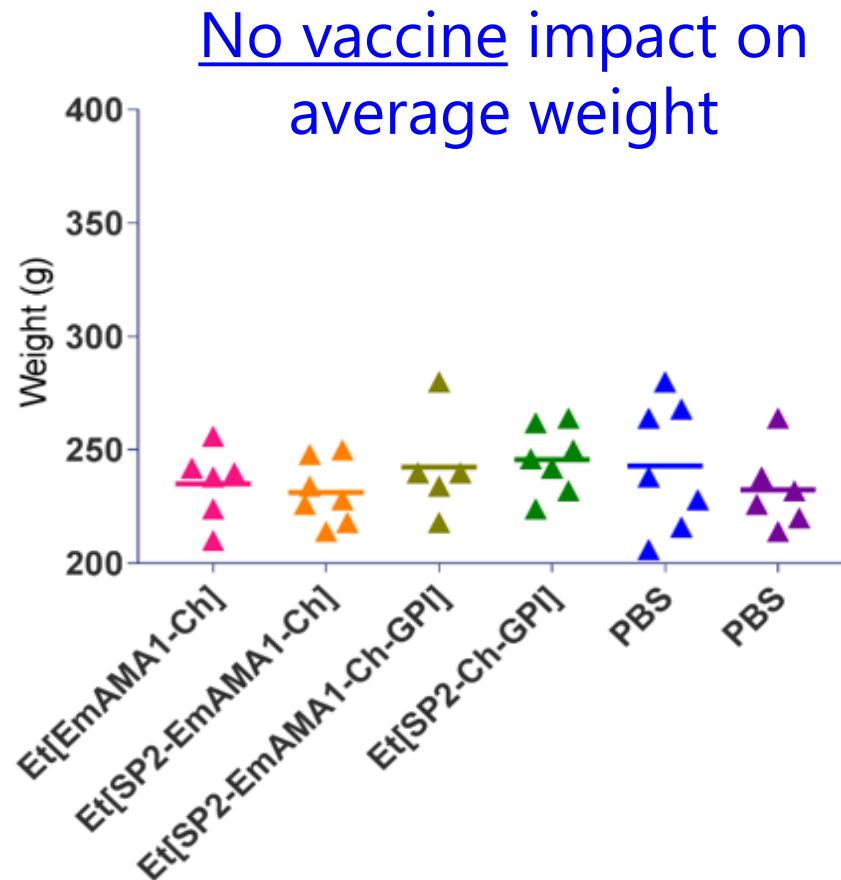


Age (days)

Et-[EmAMA1] parasites: vaccine trial



- *Et*-[EmAMA1] parasites are **safe** when used as vaccines

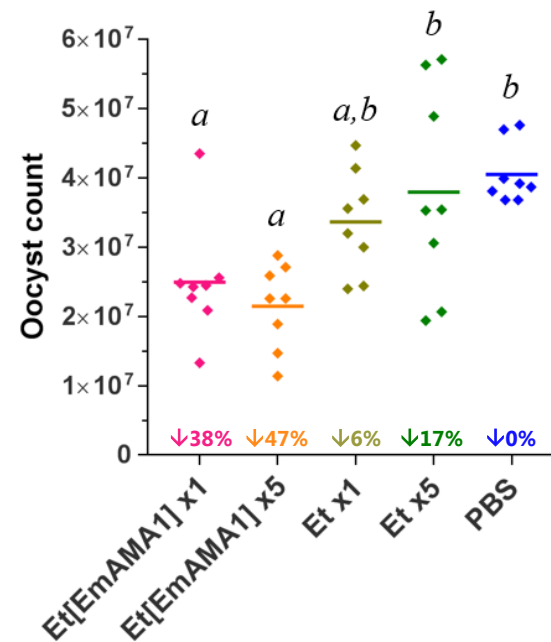
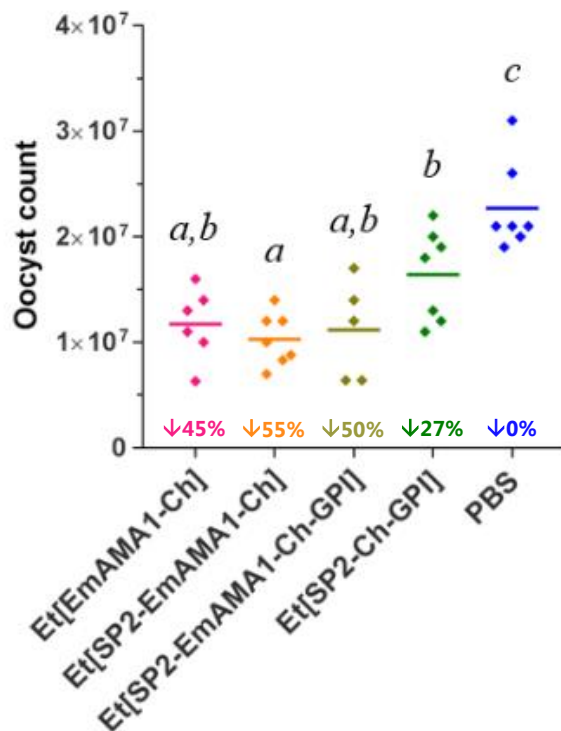


Et-[EmAMA1] parasites: vaccine trial



- Et*-[EmAMA1] parasites induce **significant levels of cross protection** against *E. maxima* challenge

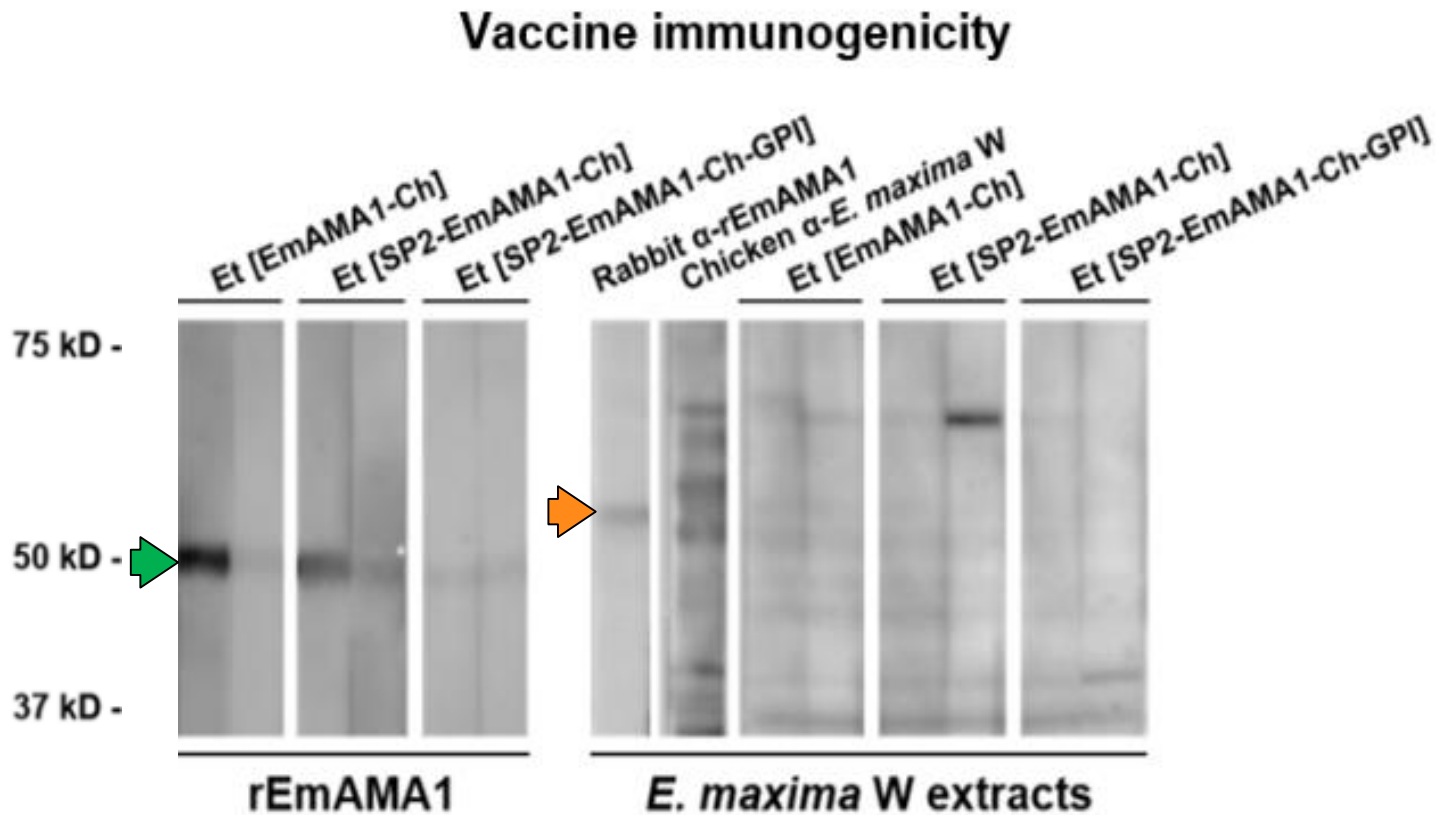
Total oocyst output (after challenge)



Et-[EmAMA1] parasites: vaccine trial



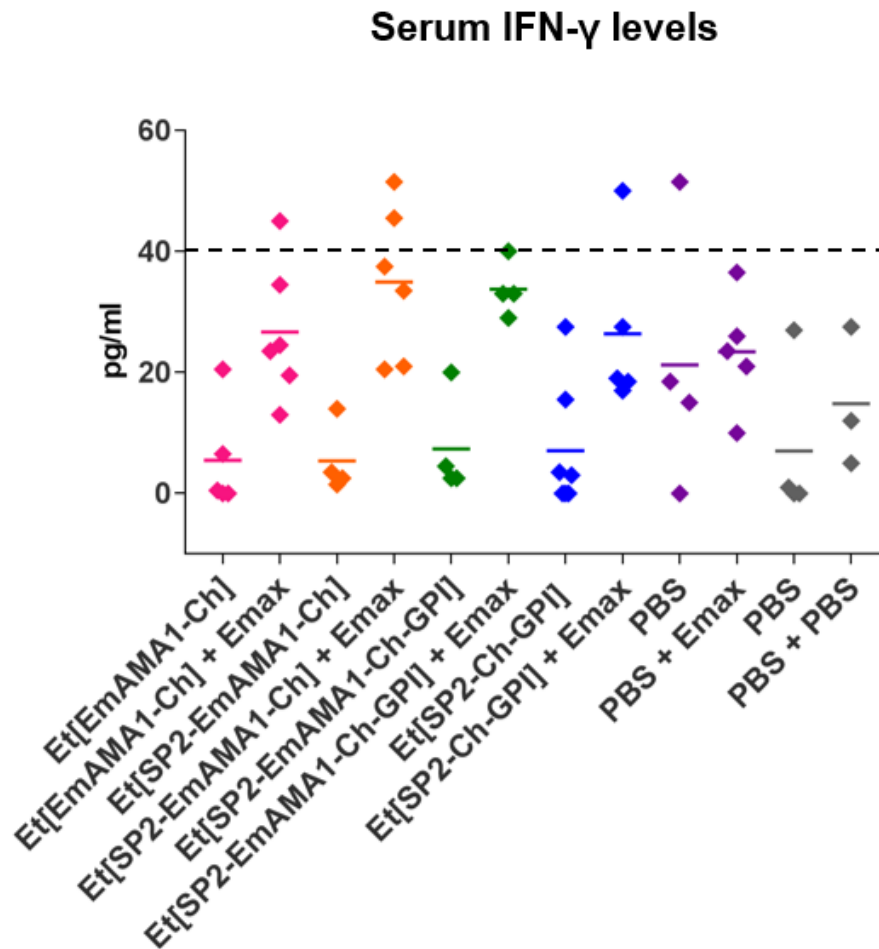
- Reduction in parasite replication was not mediated by increased levels of α -EmAMA1 antibodies after vaccination



Et-[EmAMA1] parasites: vaccine trial

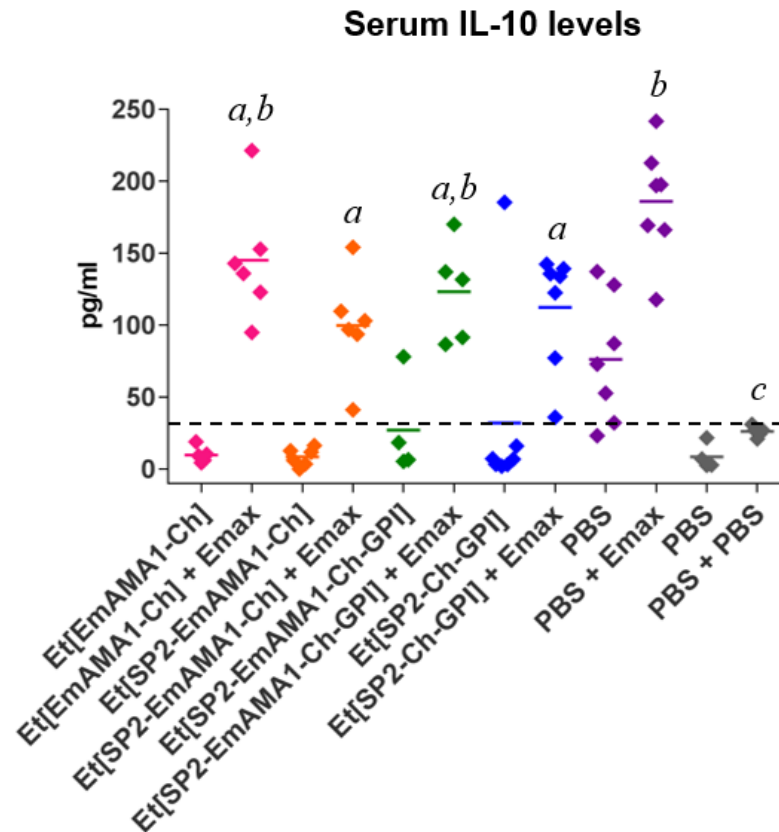


- Serum IFN- γ levels were not enhanced after final vaccination or after challenge in any group



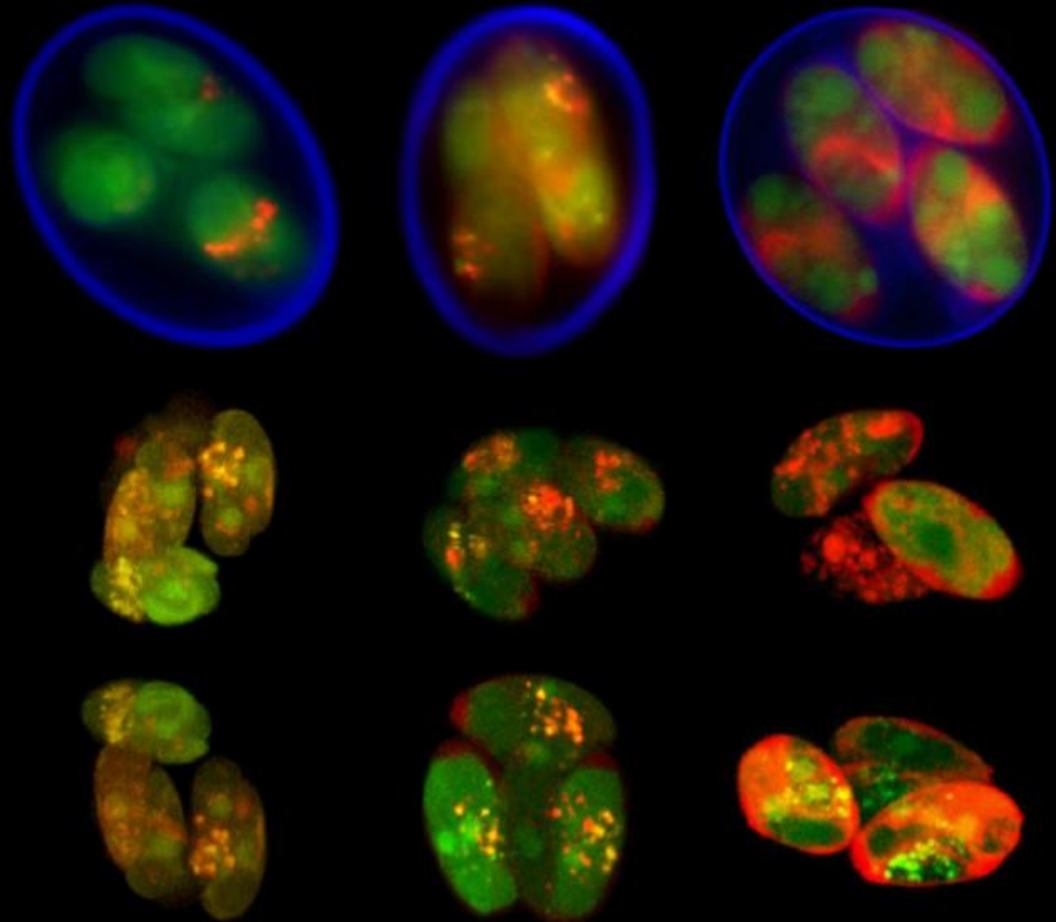
Et-[EmAMA1] parasites: vaccine trial

- Serum IL-10 levels were low after final vaccination, and lower in all groups immunised with transgenic parasites:
 - ✓ Not mediated by EmAMA1 expression: *Eimeria* vector acts as an adjuvant



I J P

INTERNATIONAL JOURNAL FOR PARASITOLOGY



Summary

- Development of anti-parasite vaccines is immensely challenging
- Many parasite targets, few commercial vaccines
- Range of vaccine formulations available
 - Must be effective, safe, cheap & easy
- *Eimeria* can function as a vaccine vector
- Specific humoral responses may not be appropriate



How many antigens?

- 7 *Eimeria* species
- AMA1: 42%-66% reduction in parasite replication
- Monensin (ionophore): 53%-98%

- Selection for vaccine escape?
- Increased antigen numbers reduces the risk...
- Incomplete selection an advantage



Contents lists available at [ScienceDirect](#)

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid



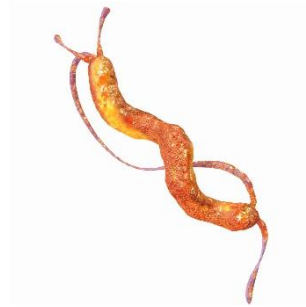
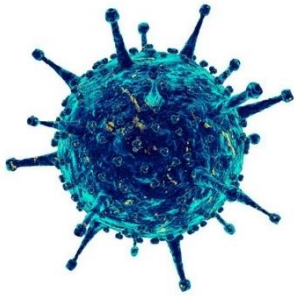
Review

Recombinant anticoccidial vaccines - a cup half full?

Damer P. Blake*, Iván Pastor-Fernández, Matthew J. Nolan, Fiona M. Tomley



What else could be a target?





<https://www.h2020-saphir.eu/>



<https://www.paragoneh2020.eu/>

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BSU: Animal services

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development and
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The logo for the EU Horizon 2020 program, featuring a globe in the center with the text 'HORIZON 2020' on either side, set against a blue background with light rays.

HORIZON 2020

