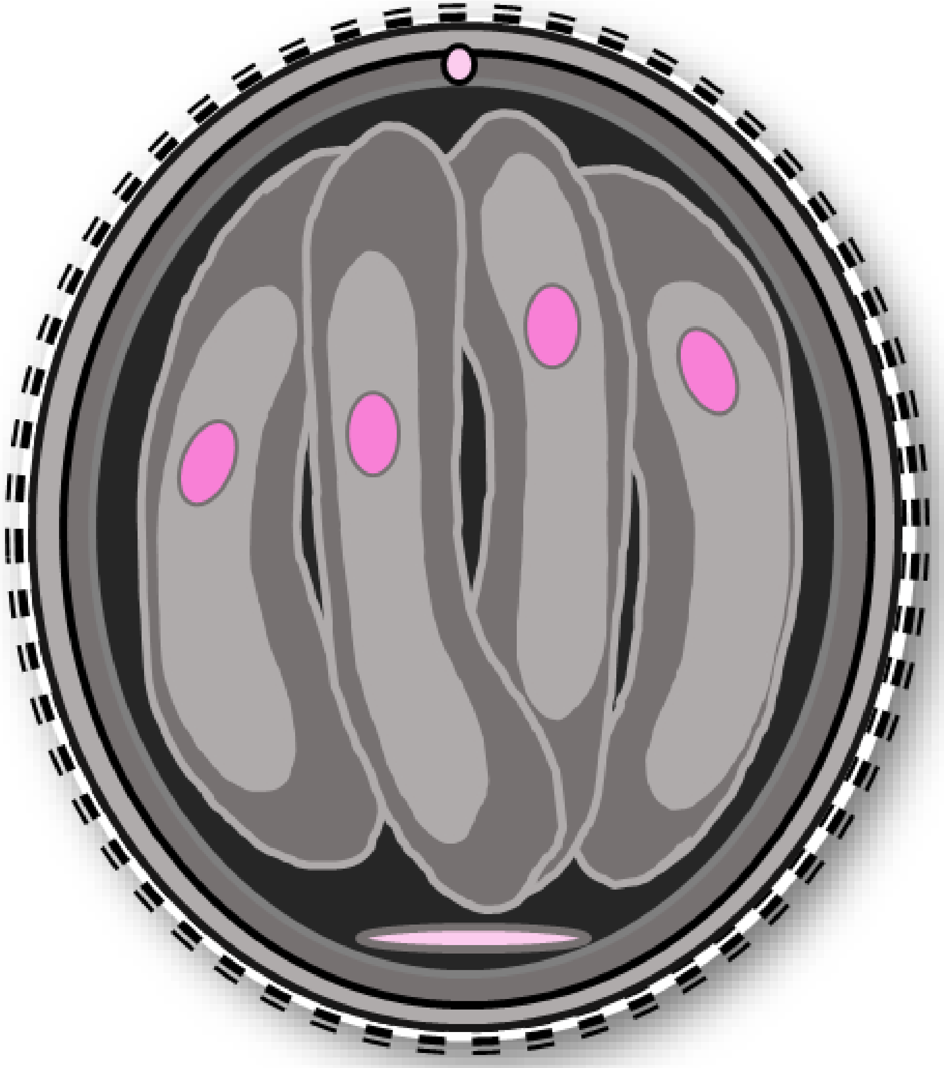
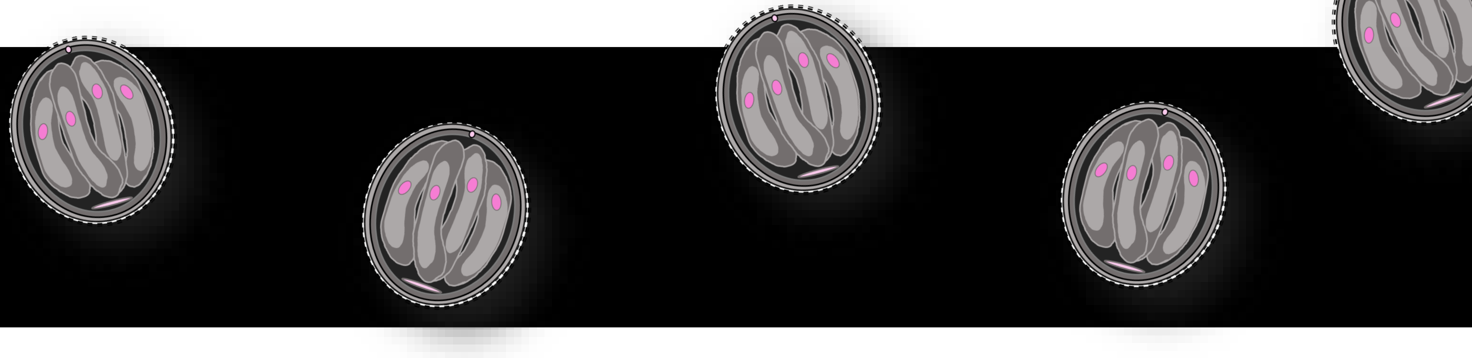
Investigation of the intestinal parasite *Cryptosporidium parvum*

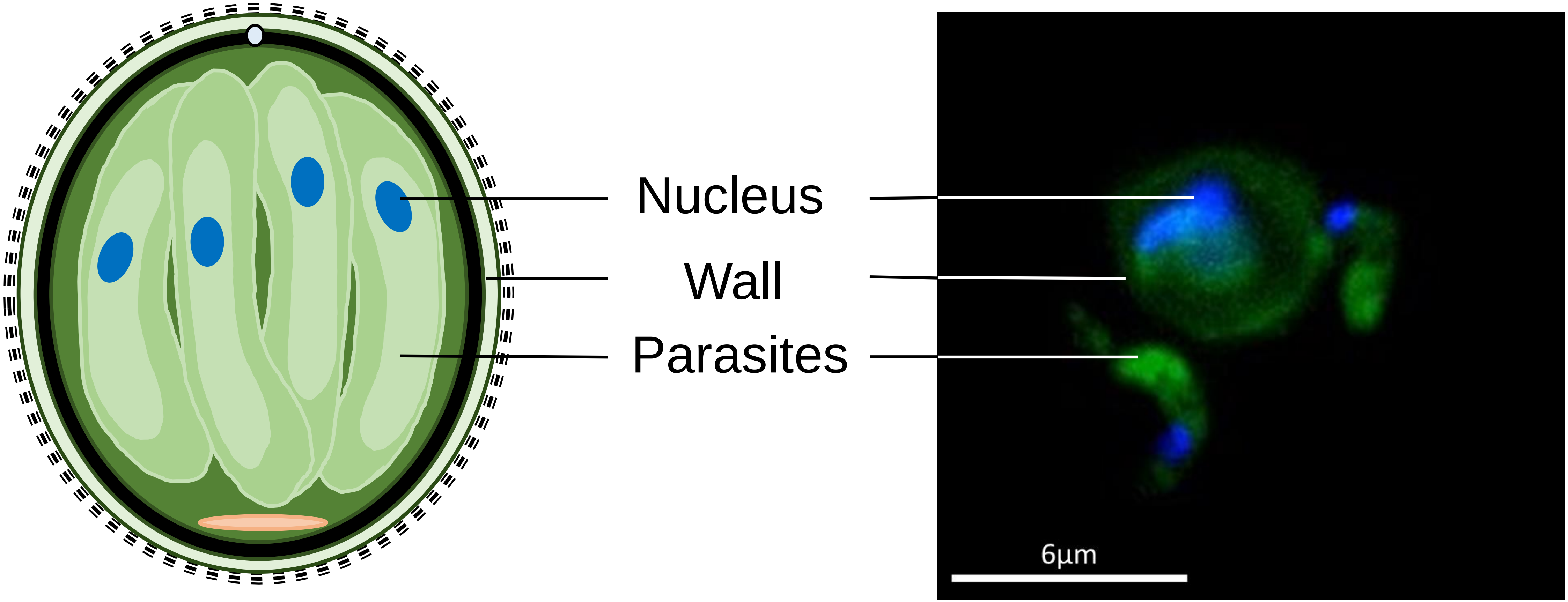


Bridget Lamont – Parasitology Lab

University of Otago

The Oocyst

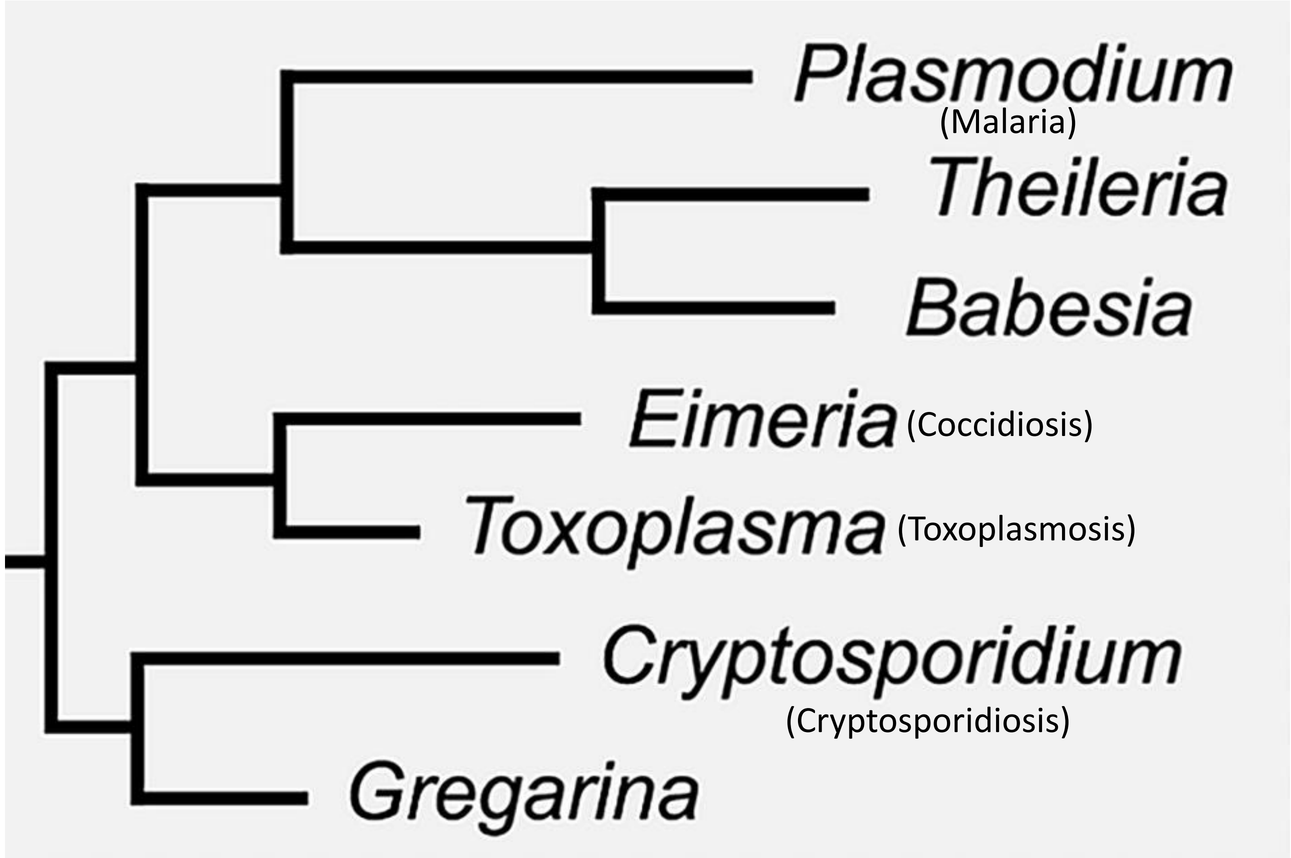




(Lab Data)

The *Cryptosporidium* genus

- Apicomplexan parasite
- Infects a broad range of hosts
- Spread via the fecal-oral route
- Cause the diarrheal disease, cryptosporidiosis

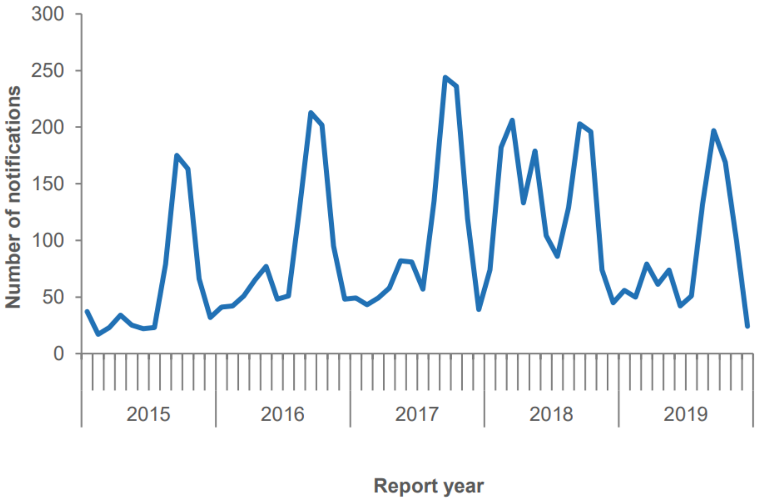


(Figure from Templeton and colleagues, 2016)

Cryptosporidiosis in Aotearoa

- Aotearoa has comparatively higher rates of cryptosporidiosis than other developed countries
- The livestock industry in Aotearoa is significantly impacted by cryptosporidiosis (mostly *C. parvum*)
- One of the top five notifiable human diseases in Aotearoa
  - *C. parvum* infection coincides with lambing/calving season in Spring
  - *C. hominis* infection is most common in Autumn

Figure 6. Cryptosporidiosis notifications by month, January 2015–December 2019



(ESR Report, 2019)

Parasitology Research (2020) 119:2317–2326
https://doi.org/10.1007/s00436-020-06729-w

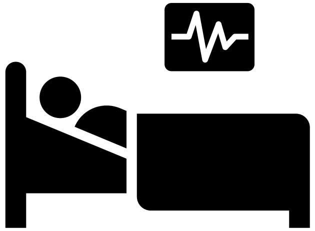
PROTOZOOLOGY - ORIGINAL PAPER

Species and genotypes causing human cryptosporidiosis in New Zealand

Juan C. Garcia-R[1] · Anthony B. Pita[1] · Niluka Velathanthiri[1] · Nigel P. French[1] · David T. S. Hayman[1]

Received: 2 April 2020 / Accepted: 25 May 2020 / Published online: 3 June 2020

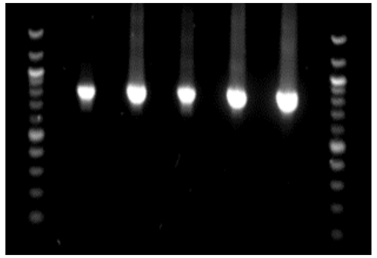
Creating a multi-locus phylogenetic tree



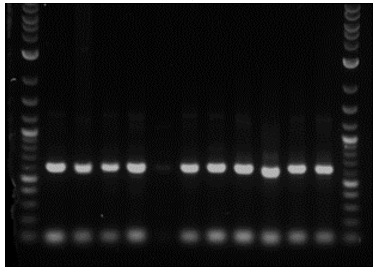
Patients with diarrhoeal symptoms

Southern Community Laboratories

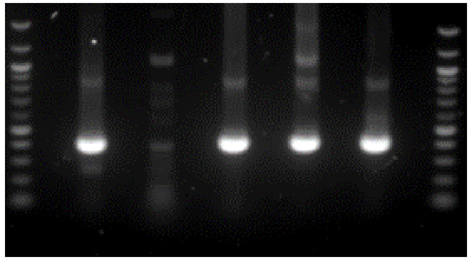
Stool sample sent for DNA extraction and analysis. DNA samples collected by us.



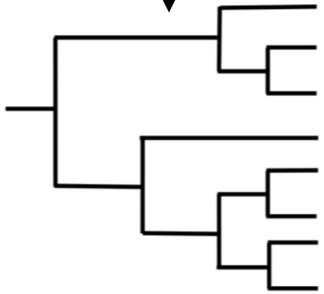
PCR amplification of the 18S rRNA target = *Cryptosporidium* confirmation



PCR-RFLP; secondary PCR product digested with AseI and SspI enzymes = *Cryptosporidium* species indication



PCR amplification of other targets including GP60, HSP70, CP47 and MSC6-7



Creation of multilocus phylogenetic tree based on SNPs

Southern Community Laboratories

Associate Professor James Ussher

Dr Jenny Grant

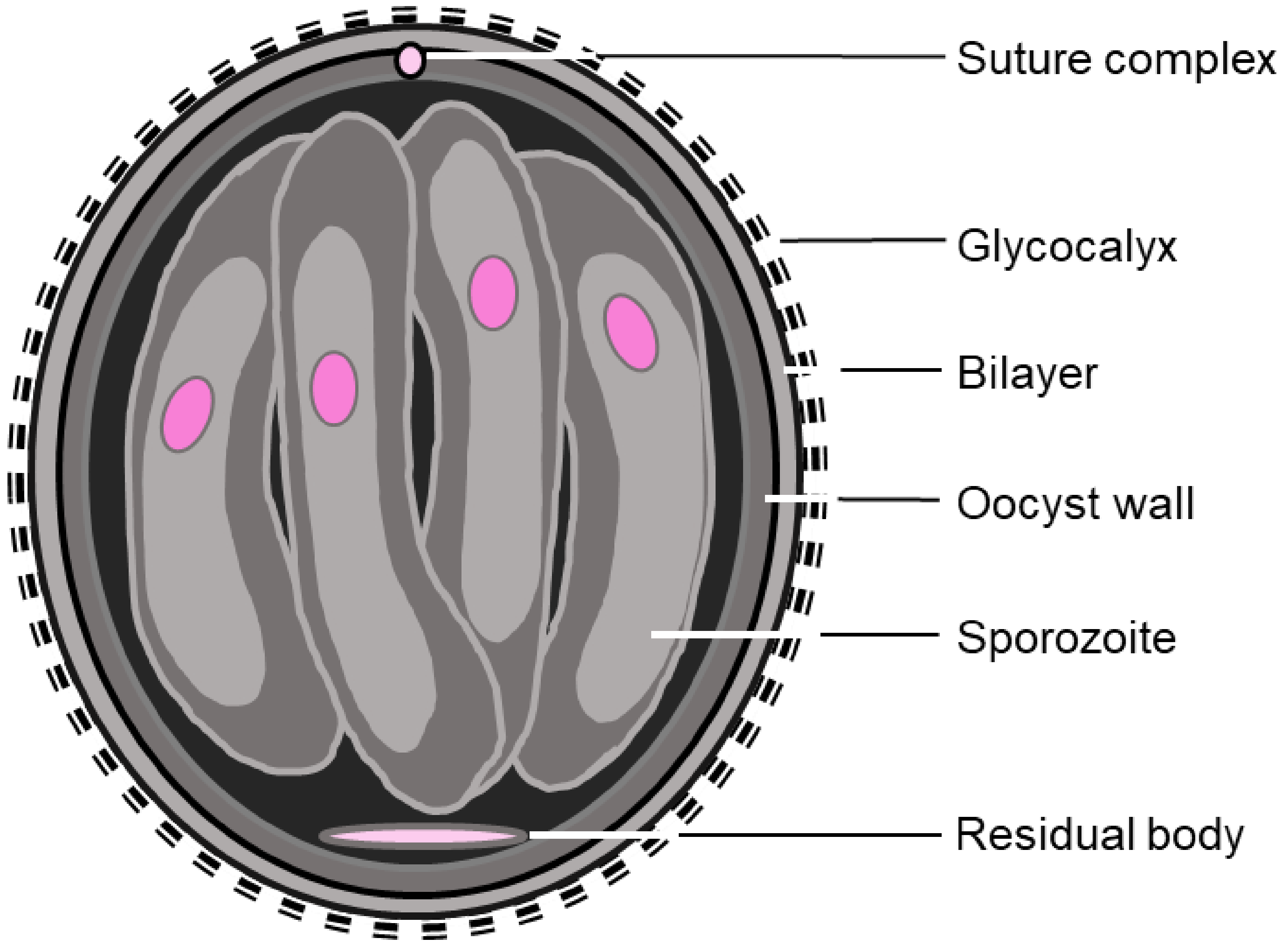
Cryptosporidiosis in the developing world

- The Ideal Niche:
  - Poor Sanitation
  - Malnourishment
  - High prevalence of HIV/AIDS

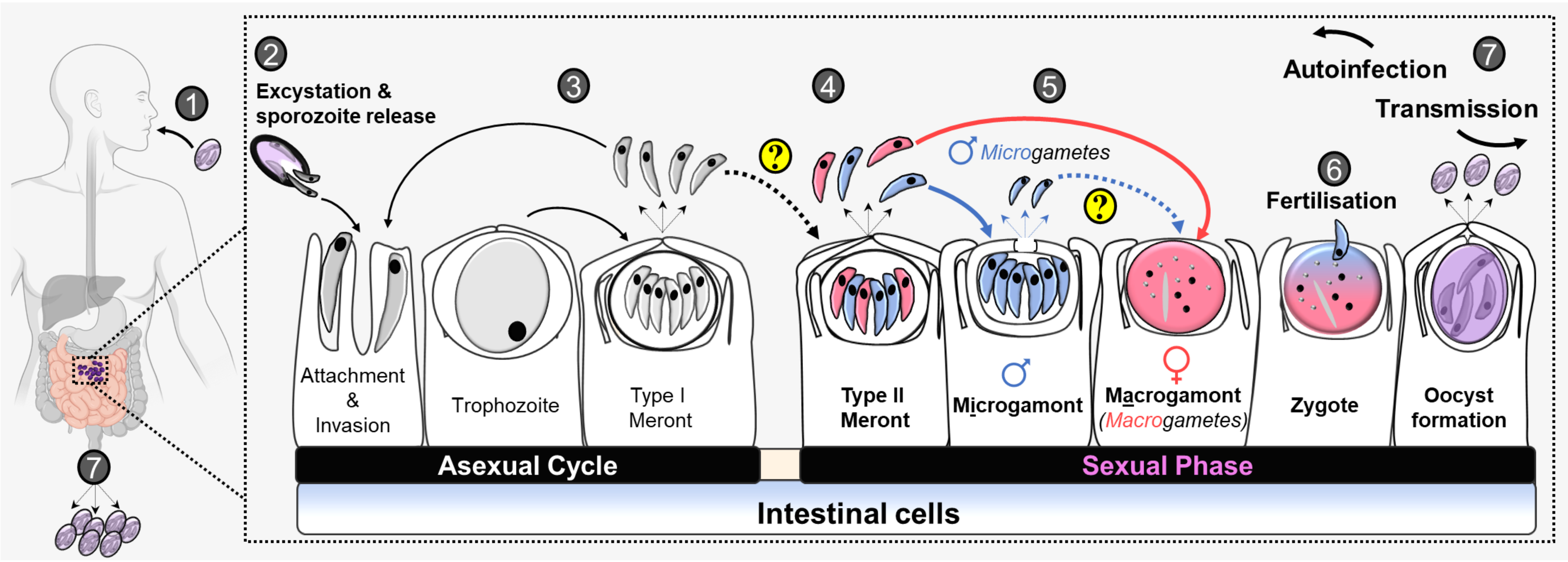
- Disease outcomes:
  - Prolonged symptoms
  - Growth developmental issues
  - Poor cognitive performance

In 2016, 80% of deaths from *Cryptosporidium* were in children under 5 years old

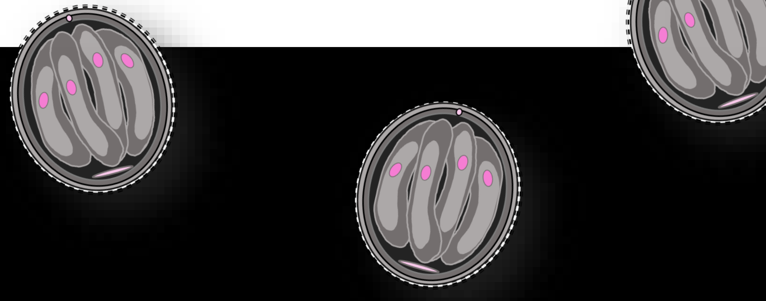
The Culprit – The *Cryptosporidium* Oocyst

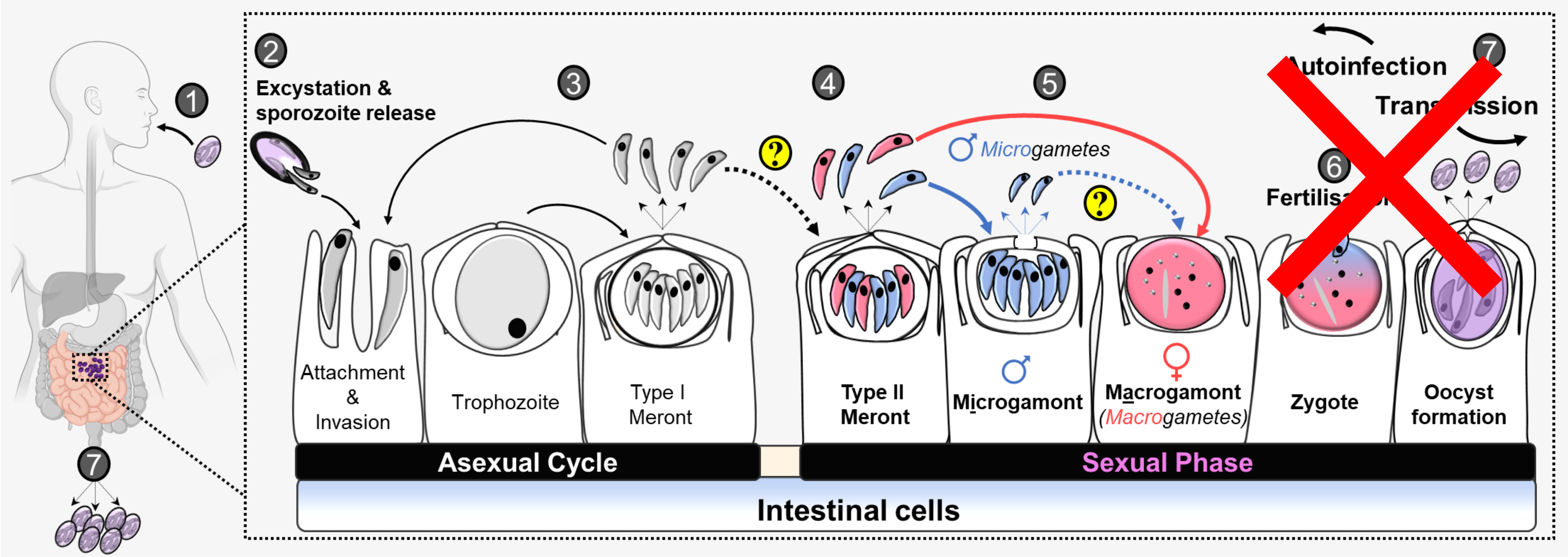


One Single Host

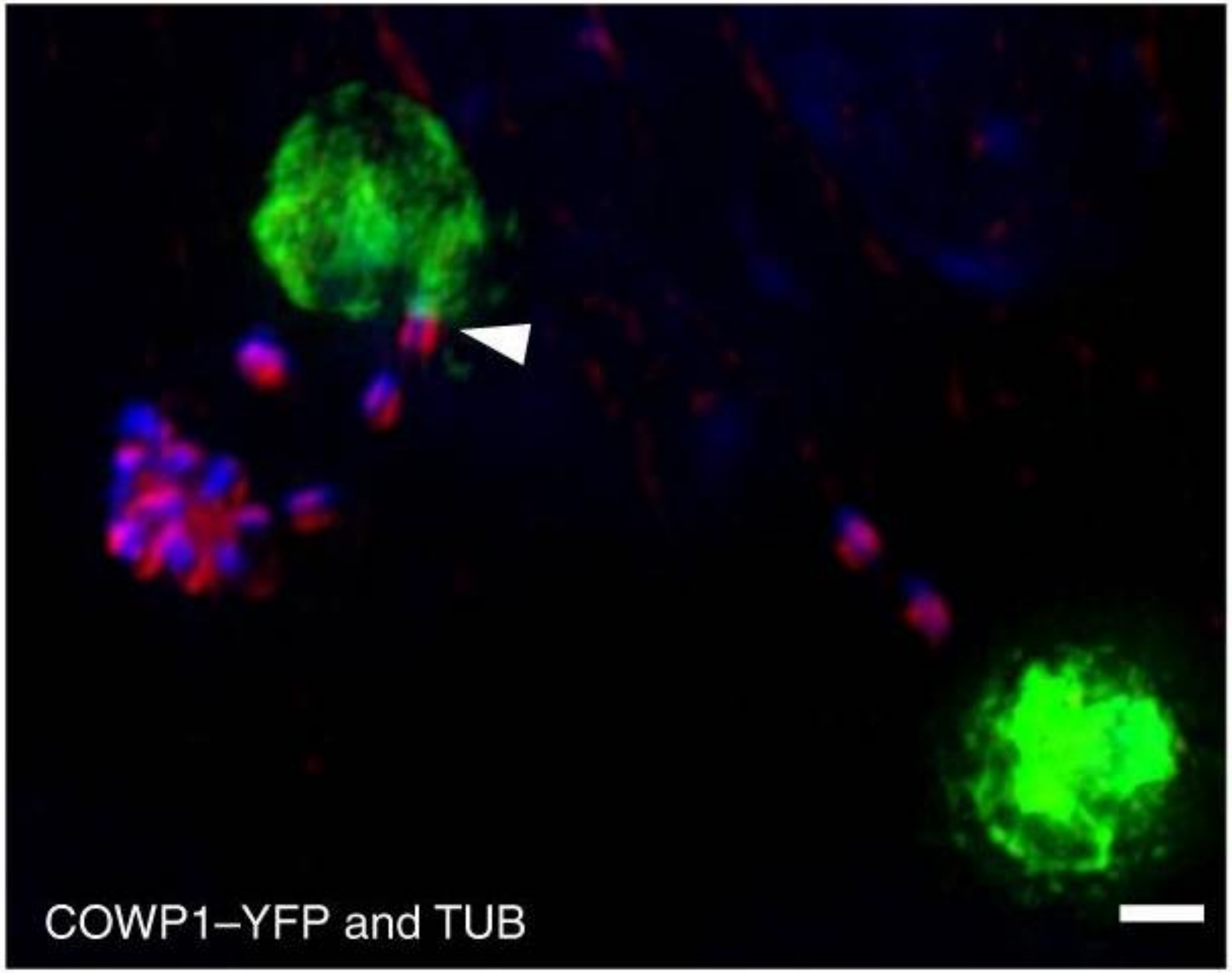


Not in cell culture





Male and female gametes find each other



Female (Macrogamont)

Male (Microgamete)

ARTICLES
https://doi.org/10.1038/s41564-019-0539-x
nature microbiology
OPEN

Life cycle progression and sexual development of the apicomplexan parasite *Cryptosporidium parvum*

Jayesh Tandel[1], Elizabeth D. English[1], Adam Sateriale[1], Jodi A. Gullicksrud[1], Daniel P. Beiting[1], Megan C. Sullivan[1], Brittain Pinkston[1,2] and Boris Striepen[1*]

Continuously trialling continuous cultures

Animal Models:

- Mice
- Neonatal calf

In vitro continuous culture models:

- COLO-680N
- Axenic Culture
- Hollow Fibre Technology

Continuously trialling continuous cultures

- **COLO-680N**
- Axenic Culture
- Hollow Fibre Technology


International Journal for Parasitology 48 (2018) 197–201

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

ELSEVIER

IJP

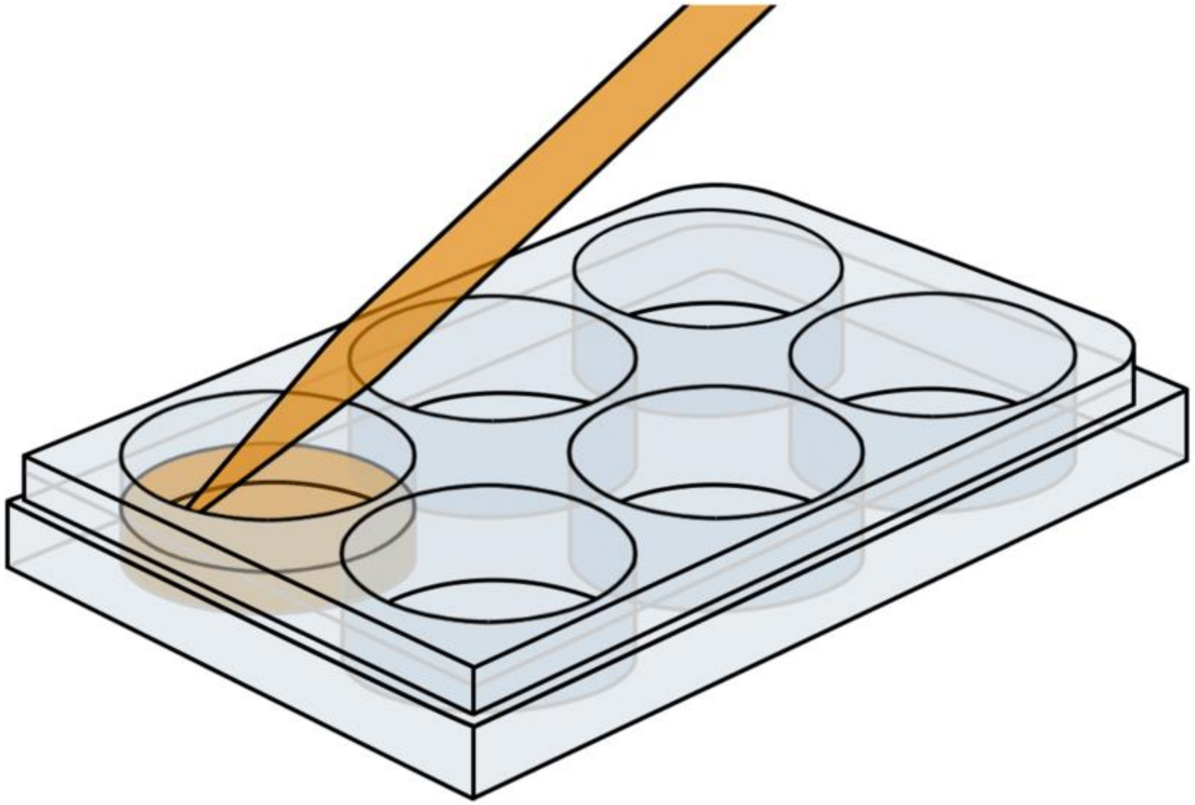
Succinctus

A cell culture platform for *Cryptosporidium* that enables long-term cultivation and new tools for the systematic investigation of its biology

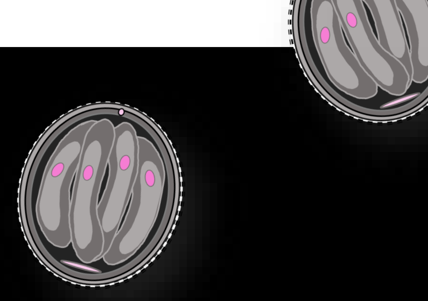
Check for updates

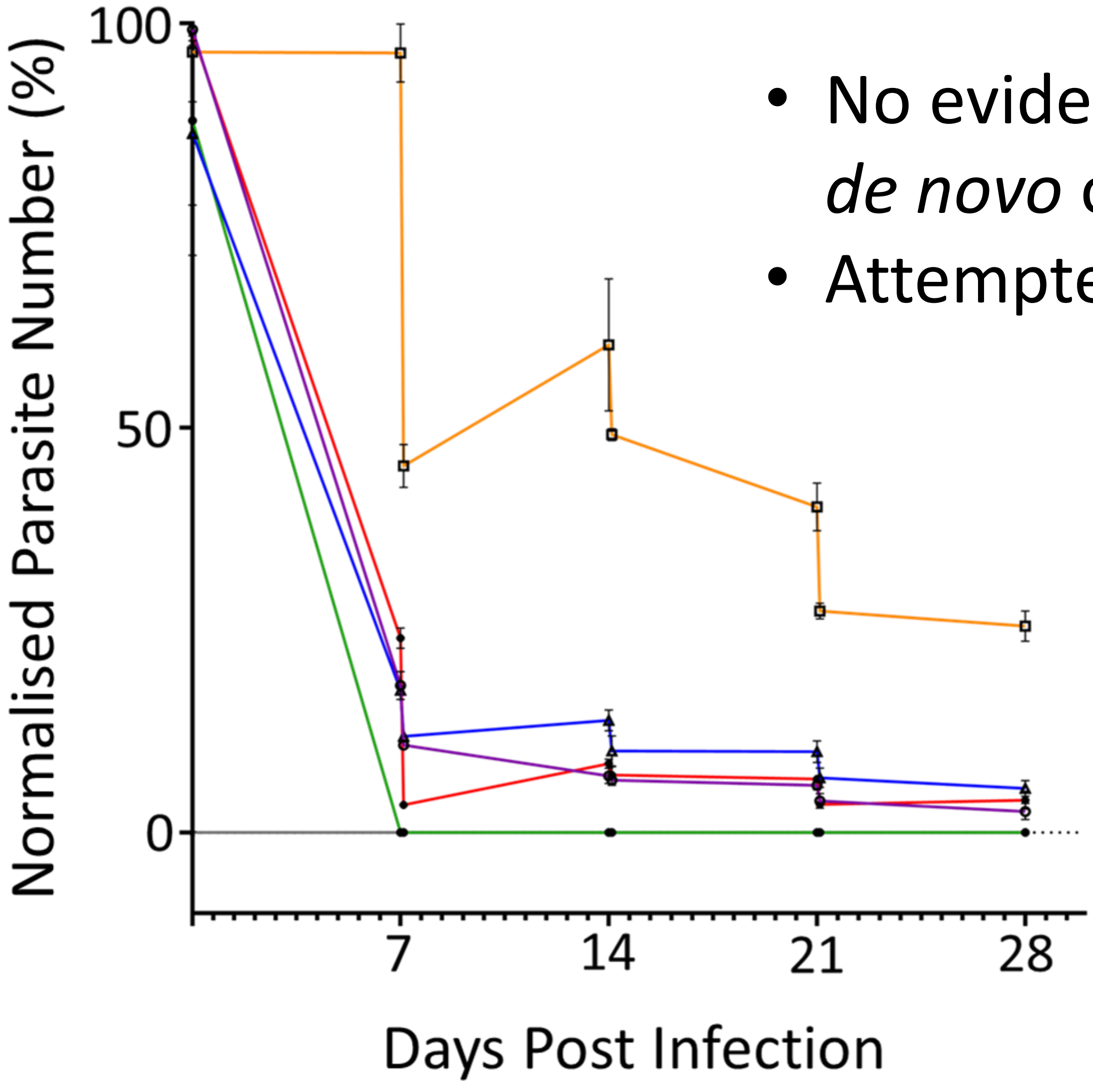
Christopher N. Miller[a,b], Lyne Jossé[a,b,c], Ian Brown[b], Ben Blakeman[b], Jane Povey[c], Lyto Yiangou[a,b,c], Mark Price[d], Jindrich Cinatl Jr.[e], Wei-Feng Xue[b], Martin Michaelis[b,c,*], Anastasios D. Tsaousis[a,b,*]

[a] Laboratory of Molecular & Evolutionary Parasitology, RAPID Group, School of Biosciences, University of Kent, Canterbury, UK
[b] School of Biosciences, University of Kent, Canterbury, UK
[c] Industrial Biotechnology Centre, School of Biosciences, University of Kent, Canterbury, UK
[d] School of Physical Sciences, University of Kent, Canterbury, UK
[e] Institut für Medizinische Virologie, Klinikum der Goethe-Universität, Frankfurt am Main, Germany



COLO-680N Cell Monolayers



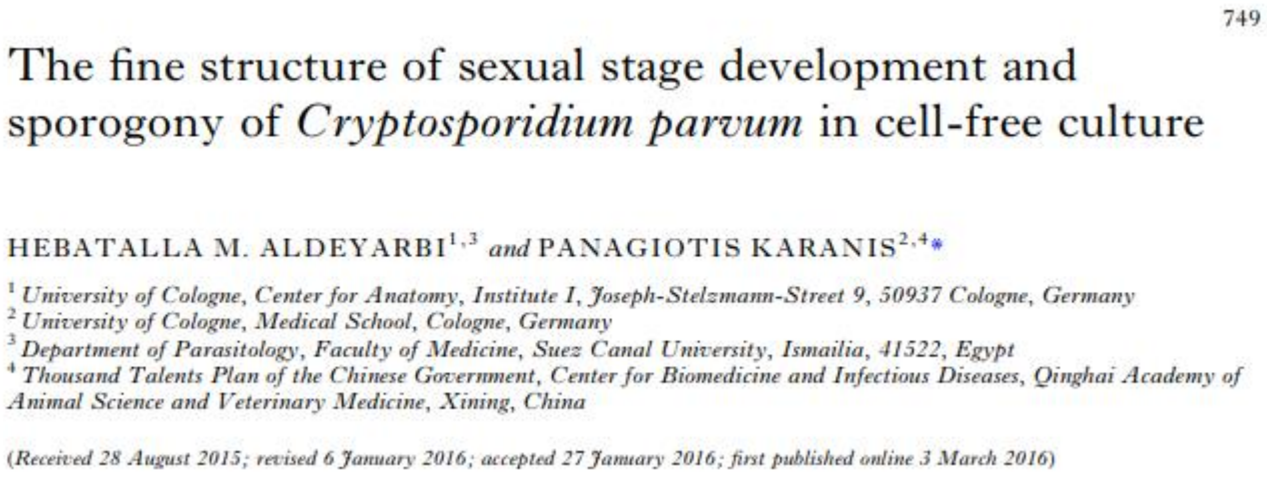


- No evidence of significant parasitic growth or *de novo* oocyst production
- Attempted to optimise with different media:

RPMI+ 10% FBS
RPMI + 10% Horse Serum
RPMI + Glucose
RPMI + Reducing agents
R10 + No parasites
R10 + Anti-crypto drug

Continuously trialling continuous cultures

- COLO-680N
- **Axenic Culture**
- Hollow Fibre Technology


749

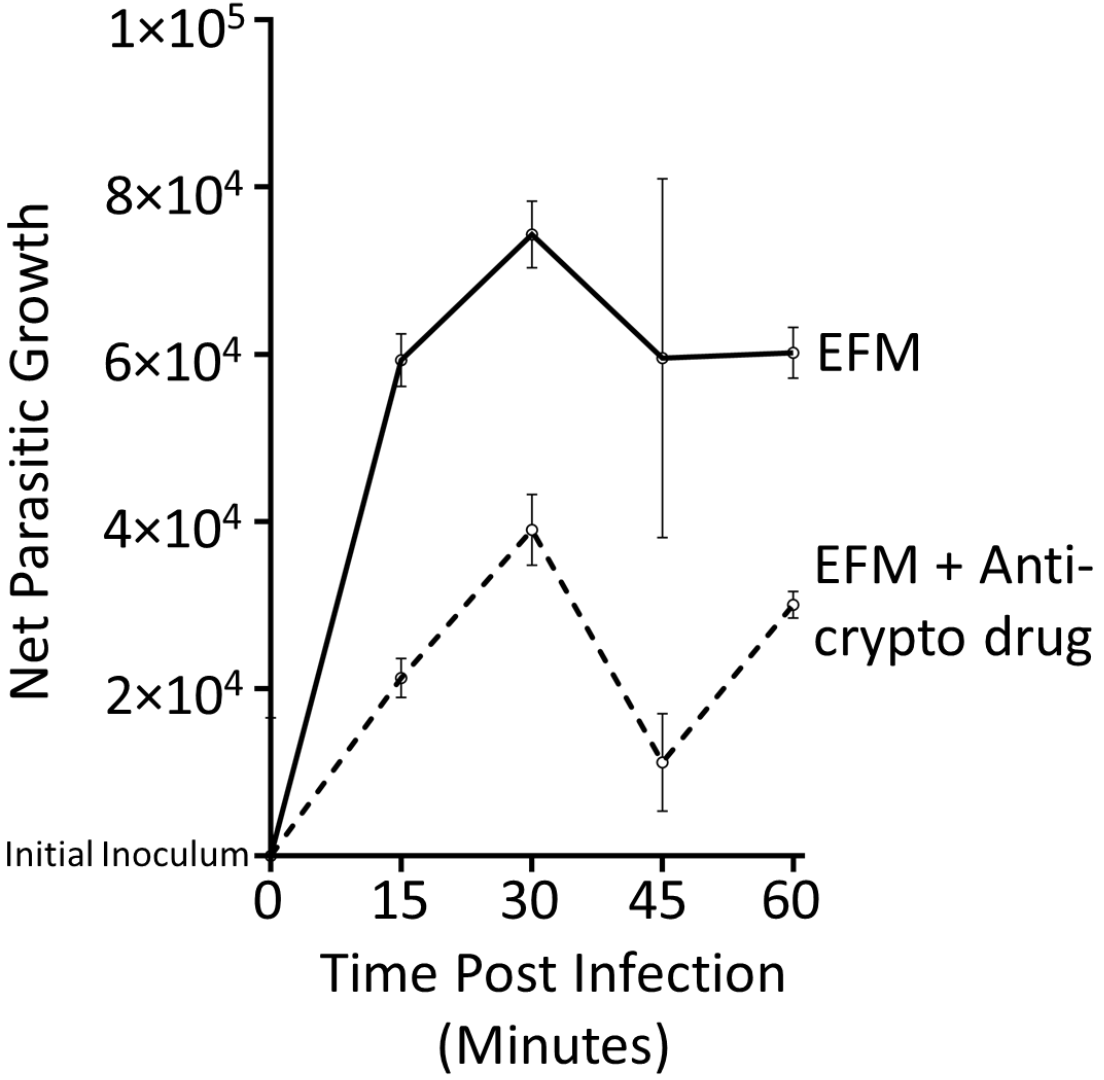
The fine structure of sexual stage development and sporogony of *Cryptosporidium parvum* in cell-free culture

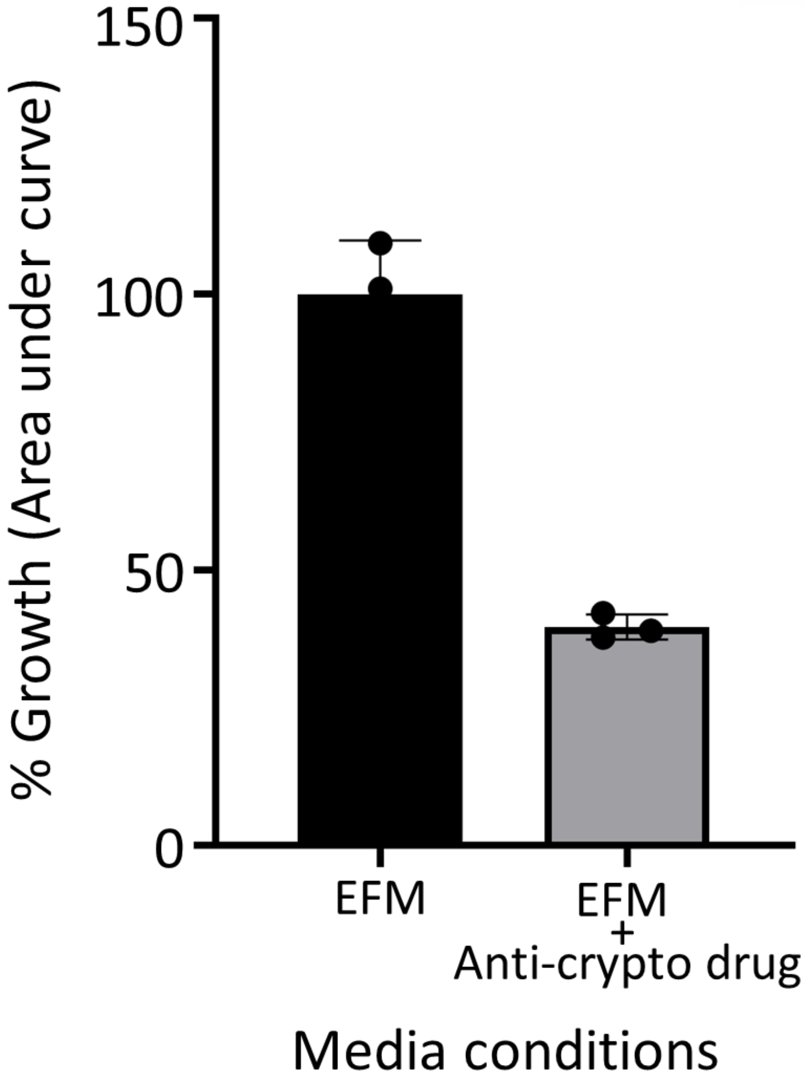
HEBATALLA M. ALDEYARBI[1,3] *and* PANAGIOTIS KARANIS[2,4]*

[1] *University of Cologne, Center for Anatomy, Institute I, Joseph-Stelzmann-Street 9, 50937 Cologne, Germany*
[2] *University of Cologne, Medical School, Cologne, Germany*
[3] *Department of Parasitology, Faculty of Medicine, Suez Canal University, Ismailia, 41522, Egypt*
[4] *Thousand Talents Plan of the Chinese Government, Center for Biomedicine and Infectious Diseases, Qinghai Academy of Animal Science and Veterinary Medicine, Xining, China*

(*Received 28 August 2015; revised 6 January 2016; accepted 27 January 2016; first published online 3 March 2016*)

Axenic Culture





Continuously trialling continuous cultures

- COLO-680N
- Axenic Culture
- **Hollow Fibre Technology**

International Journal for Parasitology 46 (2016) 21–29

Contents lists available at ScienceDirect

International Journal for Parasitology

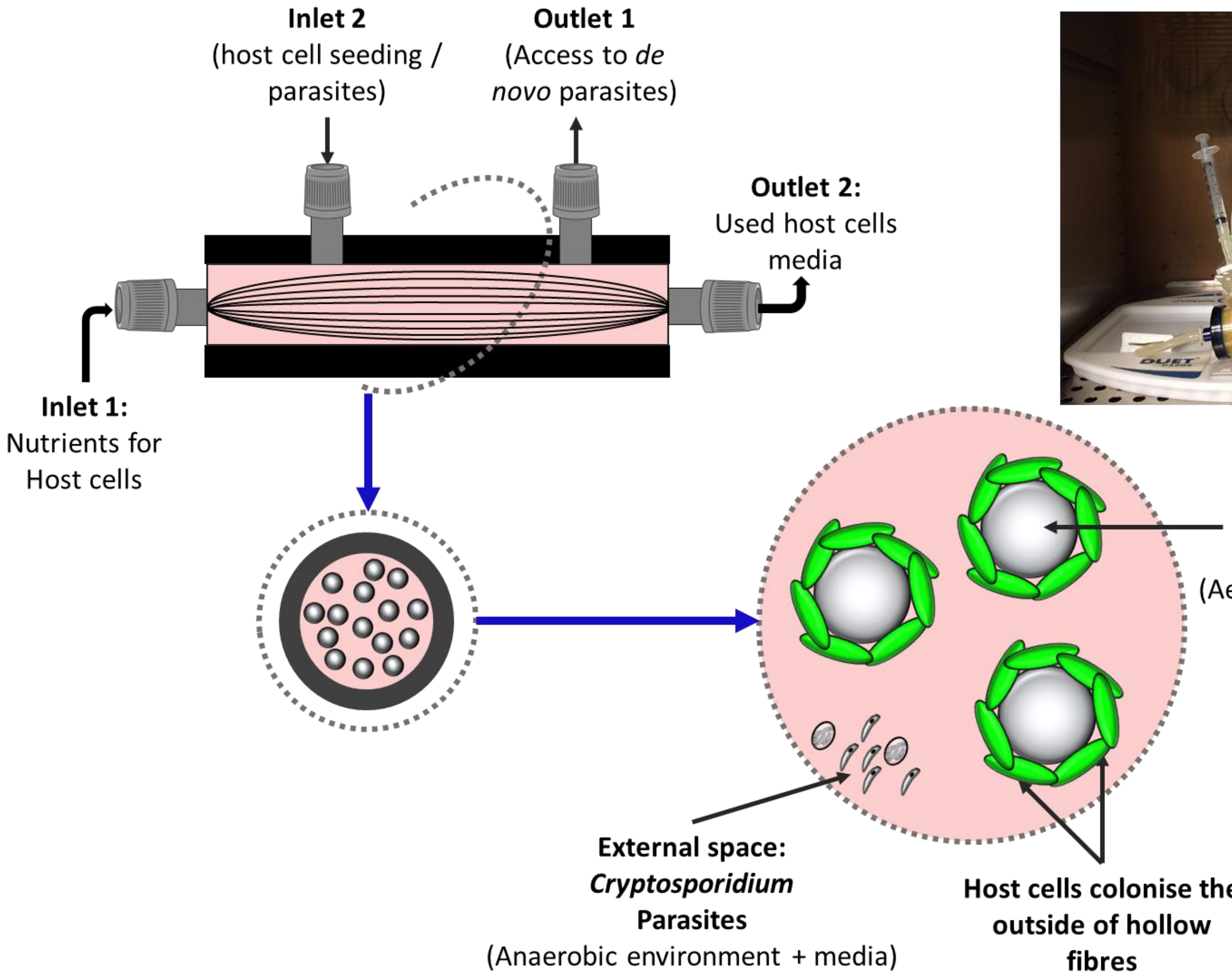
journal homepage: www.elsevier.com/locate/ijpara

Continuous culture of *Cryptosporidium parvum* using hollow fiber technology

Mary Morada [a], Sangun Lee [b], Leslie Gunther-Cummins [c], Louis M. Weiss [d,e], Giovanni Widmer [b], Saul Tzipori [b], Nigel Yarlett [a,*]

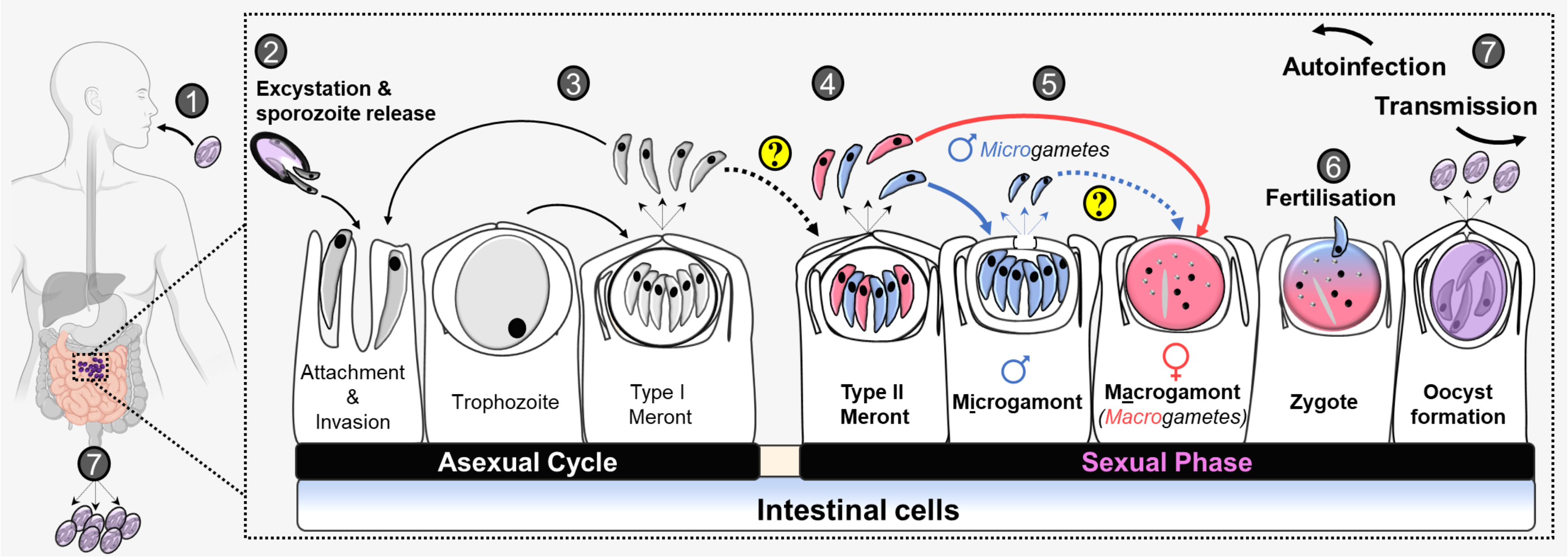
[a] Haskins Laboratories, and Department of Chemistry and Physical Sciences, Pace University, New York, USA
[b] Cummings School of Veterinary Medicine, Tufts University, N. Grafton, MA, USA
[c] Analytical Imaging Facility, Albert Einstein College of Medicine, Bronx, NY, USA
[d] Department of Pathology, Albert Einstein College of Medicine, Bronx, NY, USA
[e] Department of Medicine, Albert Einstein College of Medicine, Bronx, NY, USA

Hollow Fibre Technology



A reproducible *in vitro* continuous culture

- Life cycle progression and production of *de novo* oocysts
- Maintain transgenic lines of *C. parvum* parasites
- Testing novel compounds against the important life cycle stages of fertilisation and oocyst production



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Life cycle progression and sexual development of the apicomplexan parasite *Cryptosporidium parvum*

Jayesh Tandel[1], Elizabeth D. English[1], Adam Sateriale[1], Jodi A. Gullicksrud[1], Daniel P. Beiting[1], Megan C. Sullivan[1], Brittain Pinkston[1,2] and Boris Striepen[1*]

Table 1: cpAP2 genes' expression in *C. parvum* during the sexual phase.[3]

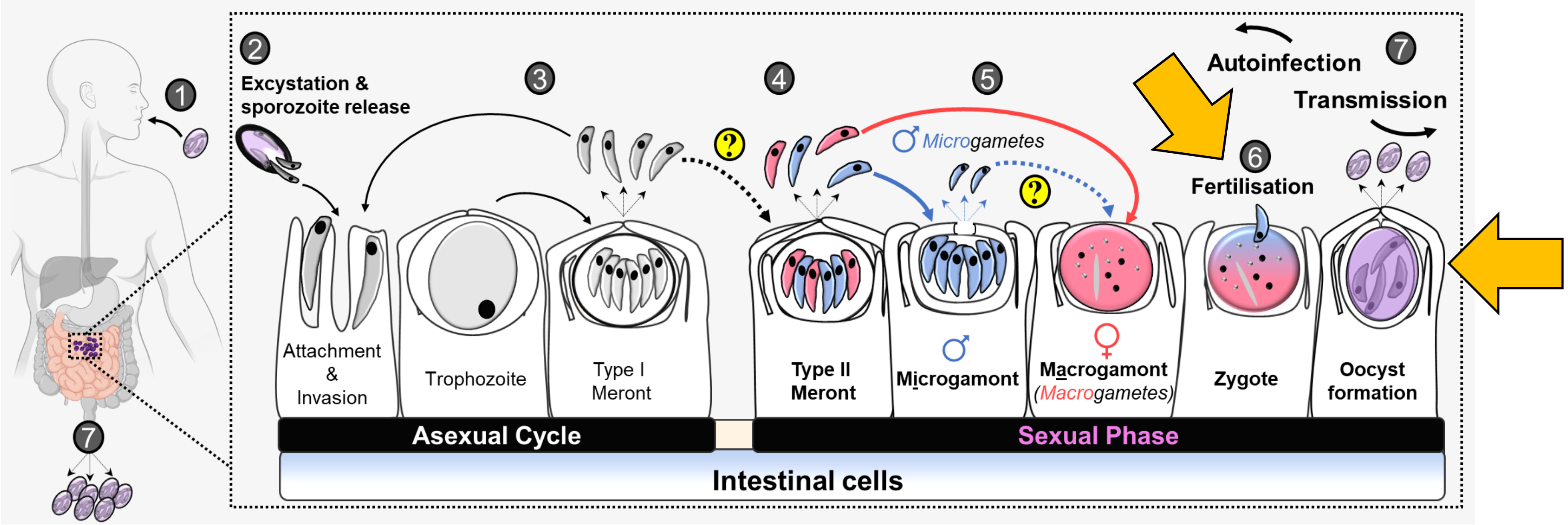
Gene code[$]	Name	Level*
cgd6_1140	AP2/DNA-binding domain containing protein	↑
cgd5_4250	Uncharacterised protein	↑
cgd6_2600	Uncharacterised protein	↑
cgd4_2950	AP2/DNA-binding domain containing protein	↑
cgd8_3130	AP2/DNA-binding domain containing protein	↑
cgd4_600	AP2/DNA-binding domain containing protein	↑
cgd4_3820	Uncharacterised protein	↑
cgd5_2570	Uncharacterised protein	↑
cgd4_1110	AP2/DNA-binding domain containing protein	↑
cgd2_3490	AP2/DNA-binding domain containing protein	↑
cgd8_810	AP2/DNA-binding domain containing protein	↑
cgd1_3520	AP2/DNA-binding domain containing protein	↑
cgd6_2670	Uncharacterised protein	↑
cgd8_3230	AP2/DNA-binding domain containing protein	↓
cgd3_1980	Uncharacterised protein	↓
cgd6_5320	AP2/DNA-binding domain containing protein	↓
cgd3_2970	Uncharacterised protein	↓

*Expression levels of *C. parvum* in vivo compared to in vitro

[$]Main database cryptodb.com

A reproducible *in vitro* continuous culture

- Life cycle progression and production of *de novo* oocysts
- Maintain transgenic lines of *C. parvum* parasites
- **Testing novel compounds against the important life cycle stages of fertilisation and oocyst production**

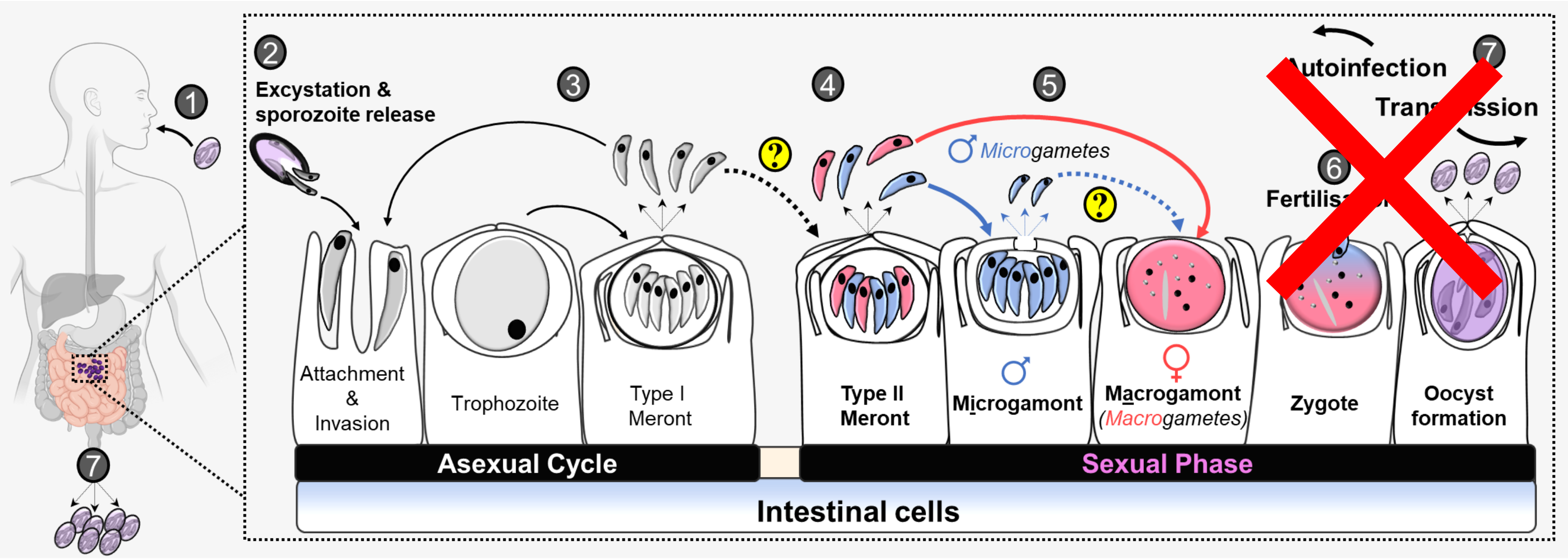


Lack of new cryptosporidiosis treatments

- Nitazoxanide / paromomycin for humans
- Halofuginone lactate (marketed as Halocur) for livestock

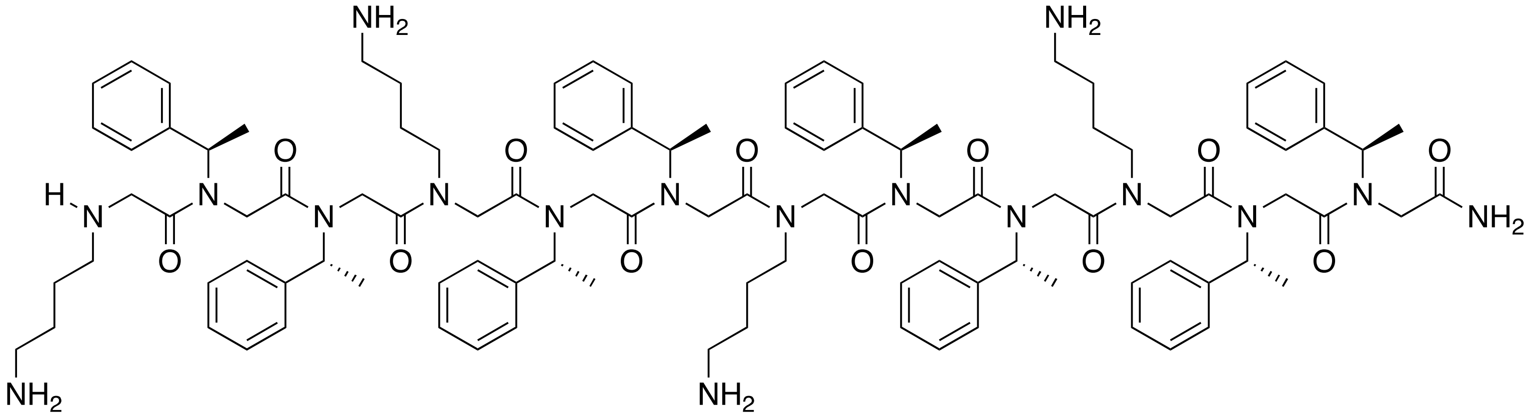
The ideal treatment for cryptosporidiosis

- Easy to administer
- Non-toxic
- Fast acting
- Prevents shedding of infectious **oocysts** (disrupts life cycle)



Peptoids as therapeutics

- Class of peptidomimetics which could be used as a therapeutic
- Side chain branches from amide nitrogen group
- Unique chemical structure has several advantages over peptides
- Peptoids could be used to treat cryptosporidiosis



Peptoid 1

Chemical Formula: $C_{104}H_{139}N_{17}O_{12}$
Exact Mass: 1818,08
Molecular Weight: 1819,36

Peptoids as therapeutics for cryptosporidiosis

1. Initial screening → 2. Staging → 3. Toxicity

1. Initial screening

- 18 Peptoids
- Different doses
- 2-3 Peptoids chosen for further analysis

Dr Daniel Pletzer
University of Otago

2. Staging

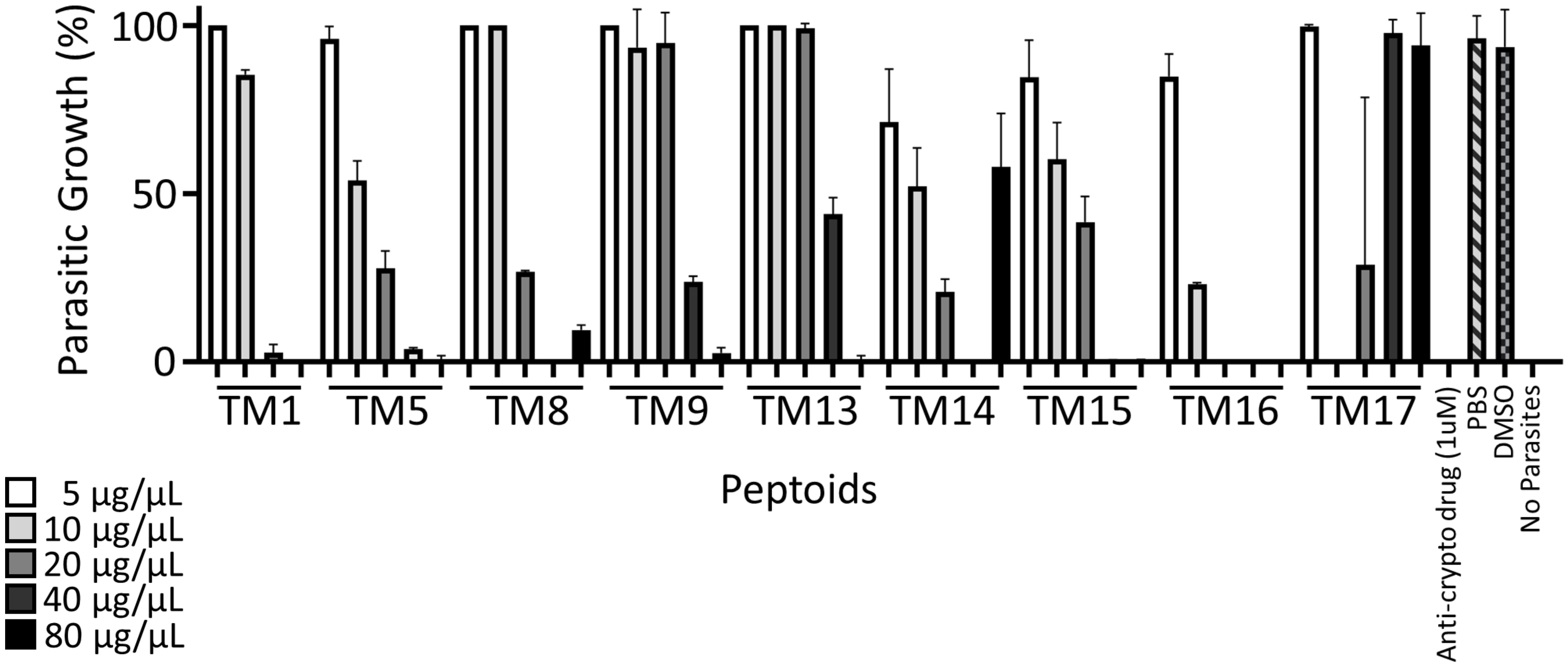
Effects on:

- Invasion
- Merozoite egress (popping)
- Sexual gene expression
- Parasitic DNA replication

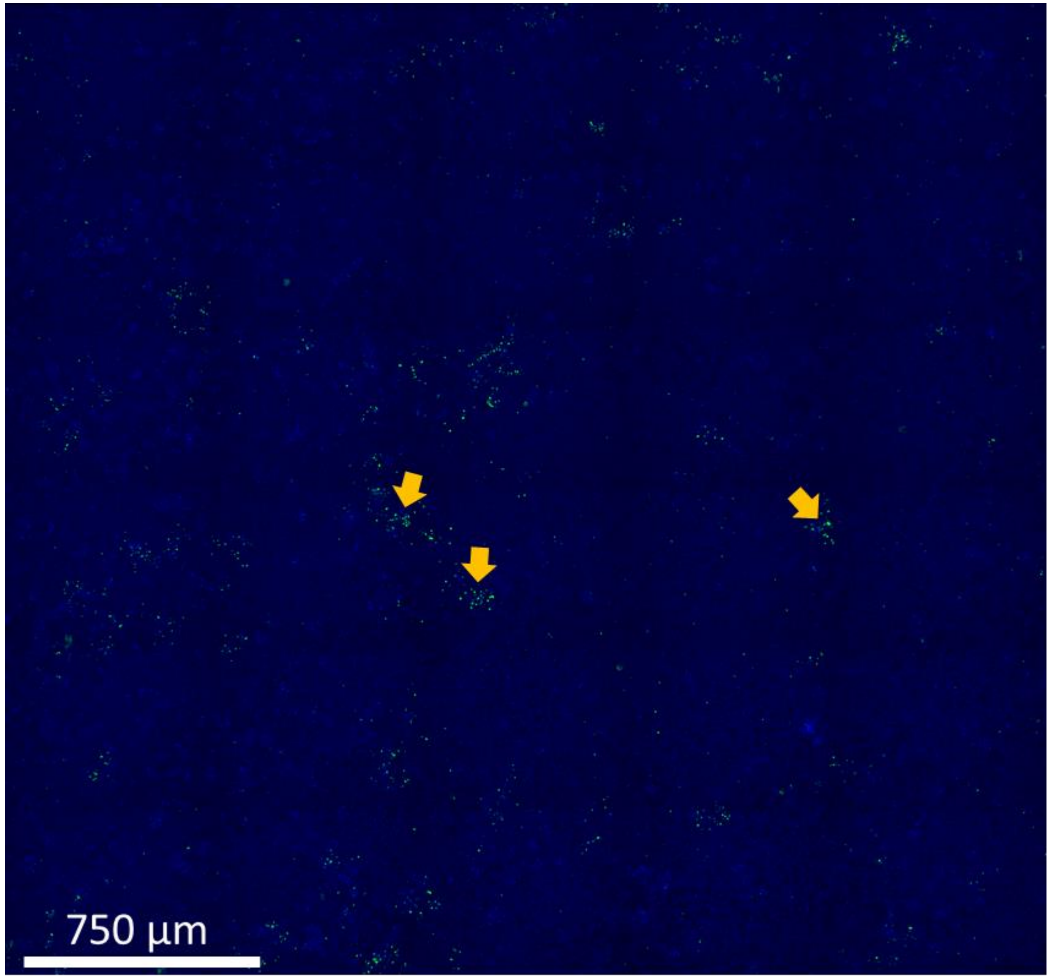
3. Toxicity

- Mitochondrial health
- Cell cycle analysis
- EM for phenotype of Peptoid-parasite interactions

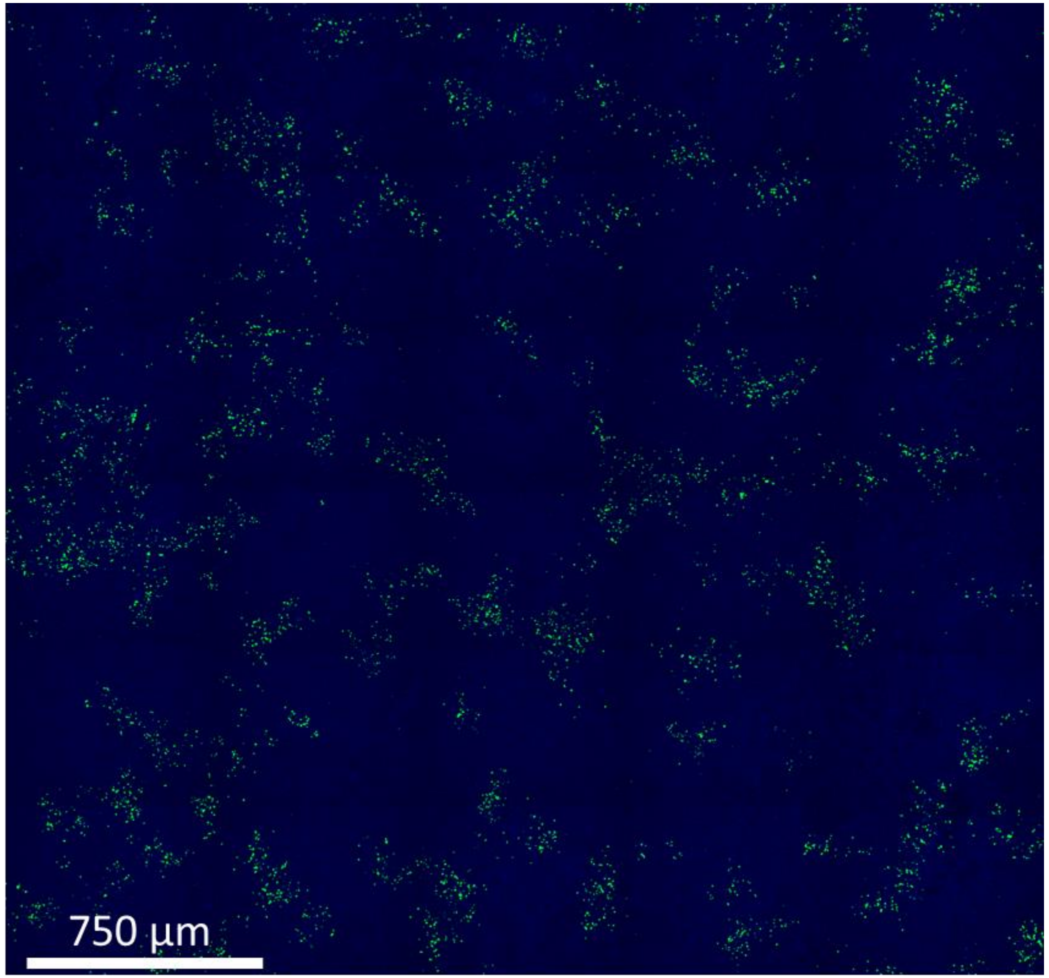
Peptoid Initial Screening



Peptoid Initial Screening

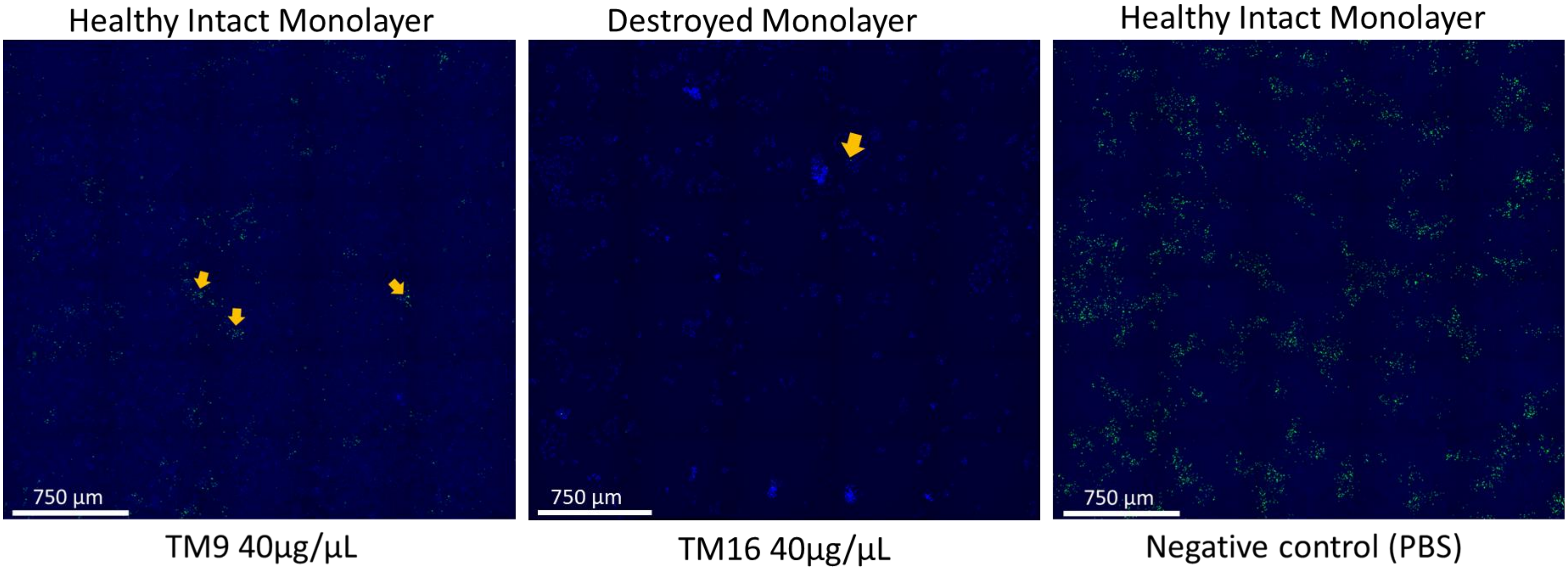


TM9 40µg/µL



Negative control (PBS)

Peptoid toxicity to host cells



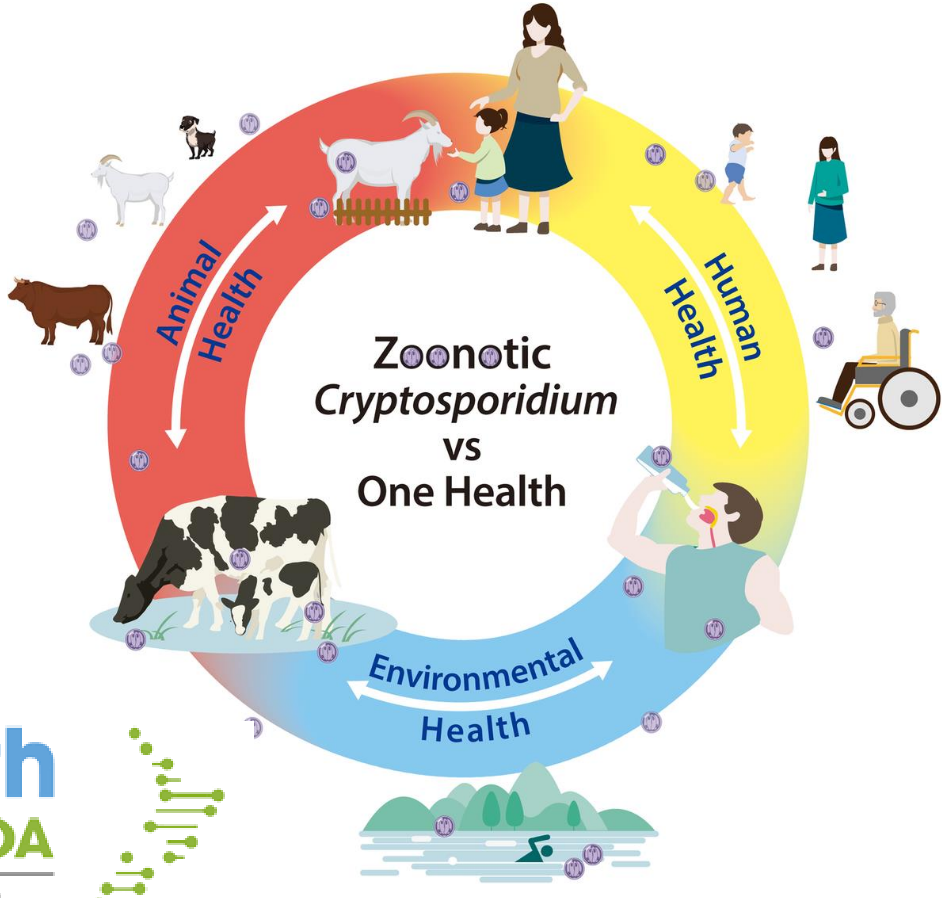
Next phase: *in vivo* mouse model

- *In vivo* mouse model
- Measure anti-cryptosporidial activity of candidate peptoids from cell culture studies
- Pathology and pharmacokinetic studies
- Discover whether Peptoids protect prophylactically or cure (therapeutically)

The One Health Approach

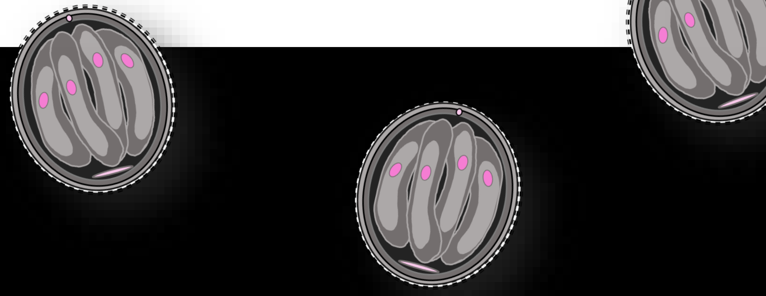
- Surveillance of circulating subtypes in humans in Aotearoa
- Continue to investigate how this important zoonotic pathogen functions
- Potential treatment of cryptosporidiosis in livestock
- Ultimately combat cryptosporidiosis in Aotearoa and worldwide

One Health
AOTEAROA
An integrative approach to understanding, preventing, and controlling infectious disease



(Figure from Zhu et al, 2021)

Acknowledgements



- **Dr Remy Muhsin (Supervisor)**
- **Professor Bruce Russell (Supervisor, Parasitology Lab PI)**
- Dr Noi Suwanarusk
- Parasitology lab members (Saffron, Nick, Jess, Natalie)
- Dr Daniel Pletzer and Deborah Yung (Peptoid work)
- Southern Community Laboratories (Subtyping samples)
- Associate Professor James Ussher (Subtyping samples)
- Tsauosis Lab (COLO-680N system correspondence)
- Professor Boris Striepen (Support, Plasmids)
- Dr Deborah Schaefer (Oocysts)
- Adeline Chua and Pablo Bifani
- Bryan Yeung
- Sibley Lab
- University of Otago Doctoral Scholarship
- Maurice and Phyllis Paykel Trust equipment grant (Hollow fibre)
- MBIE Science Whitinga Fellowship (Peptoids)

UNIVERSITY of OTAGO
Te Whare Wānanga o Otāgo
NEW ZEALAND
SAPERE AUDE

One Health AOTEAROA SYMPOSIUM

MAURICE & PHYLLIS PAYKEL TRUST

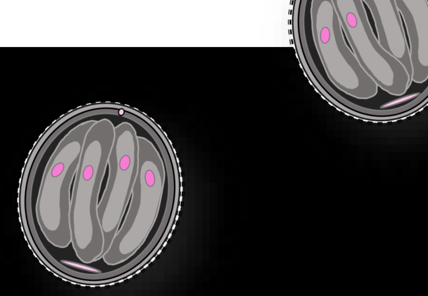
New Zealand Society for Parasitology

MICROBIOLOGY & IMMUNOLOGY
Te Tari Moromoroiti me te Ārai Mate

Contact email: bridget.lamont@postgrad.otago.ac.nz

Supplementary Data

Peptoid toxicity to host cells



	Toxicity Score: 0 = Monolayer intact 0.5 = Some damage to monolayer 1 = Monolayer completely destroyed							
Peptoid	**5µg/µL**	**10µg/µL**	**20µg/µL**	**40µg/µL**	**80µg/µL**	**Anti-crypto effect**	**Variance (High or Low)**	**Is the anti-crypto effect true?**
TM1	0.5	0.5	1	1	N/A	✓	Low	Not true
TM5	0	0	0	0.5	1	✓	Low	Likely true
TM8	0	0	0.5	1	1	✓	Low	Repeat
TM9	0	0	0	0.5	1	✓	Low	Repeat
TM13	0	0	0	0.5	1	✓	High	Repeat
TM14	0	0	0.5	1	0	✓	Low	Repeat
TM15	0	0	0	0.5	1	✓	Low	Likely true
TM16	0	0.5?	0.5	1	1	✓	Low	Not true
TM17	0	0	0	0	0	?	High	Repeat