A Method for the Continuous Culture of Cryptosporidium

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Culture Methods for Cryptosporidium

- Comparative development of *Cryptosporidium parvum* (Apicomplexa) in 11 continuous host cell lines. Upton et al., FEMS Microbiol. Lett. **118**:233–236 (1994).
- In vitro cultivation of *Cryptosporidium* species. Arrowood, MJ, Clin Microbiol Rev **15**:390-400 (2002)
- Detection of epithelial cell injury and quantification of infection in the HCT8 organoid model of cryptosporidosis. Warren et al., J. Infect Dis **198**:143-149 (2008).
- Human primary intestinal epithelial cells as an improved in vitro model for *Cryptosporidium parvum* infection. Castellanos-Gonzalez et al., Infect Immun **81**:1996-2001 (2013)
- A new in vitro model using small intestinal epithelial cells to enhance infection of *Cryptosporidium parvum*. Varughese, et al., J. Microbiol Met **106**:47-54 (2014)

Practical Issues with 2D Culture

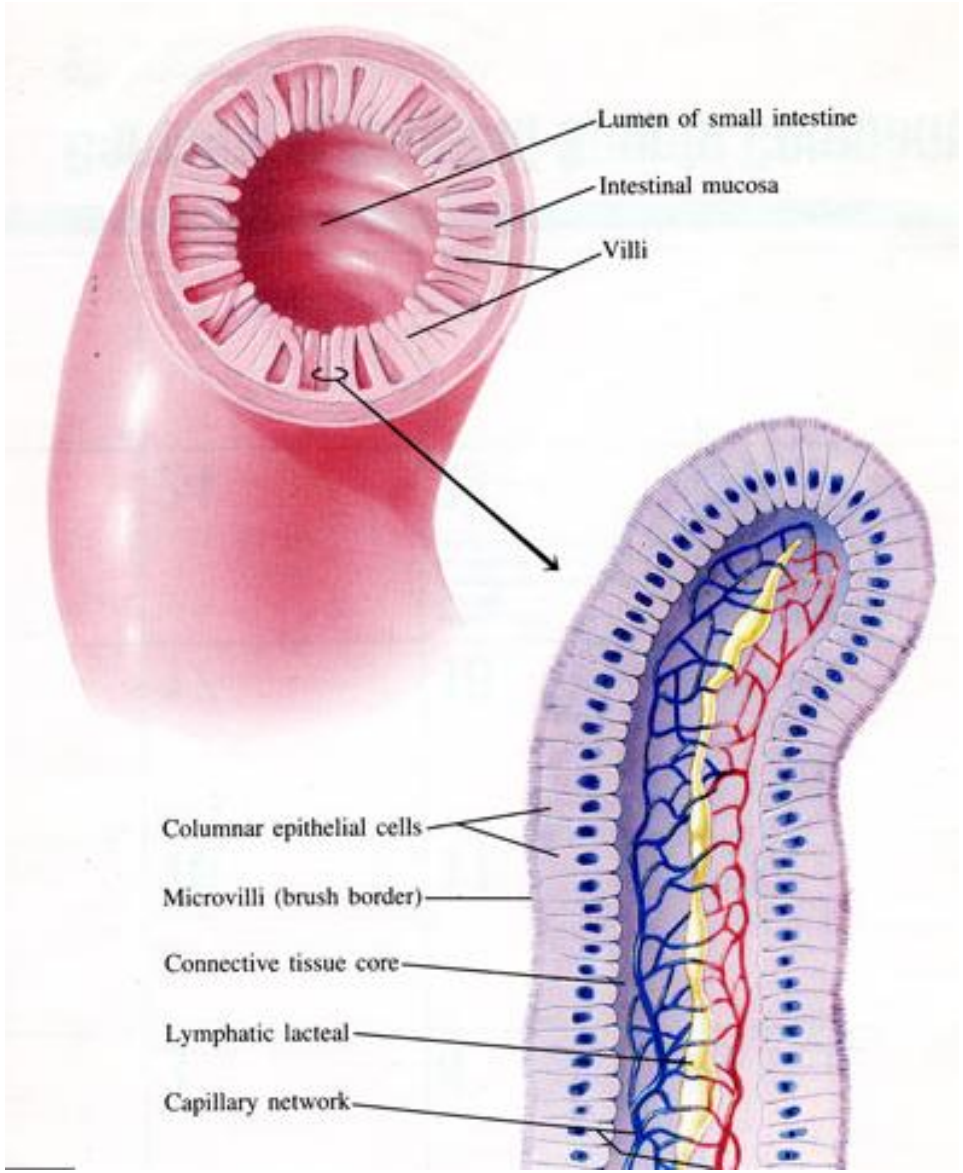
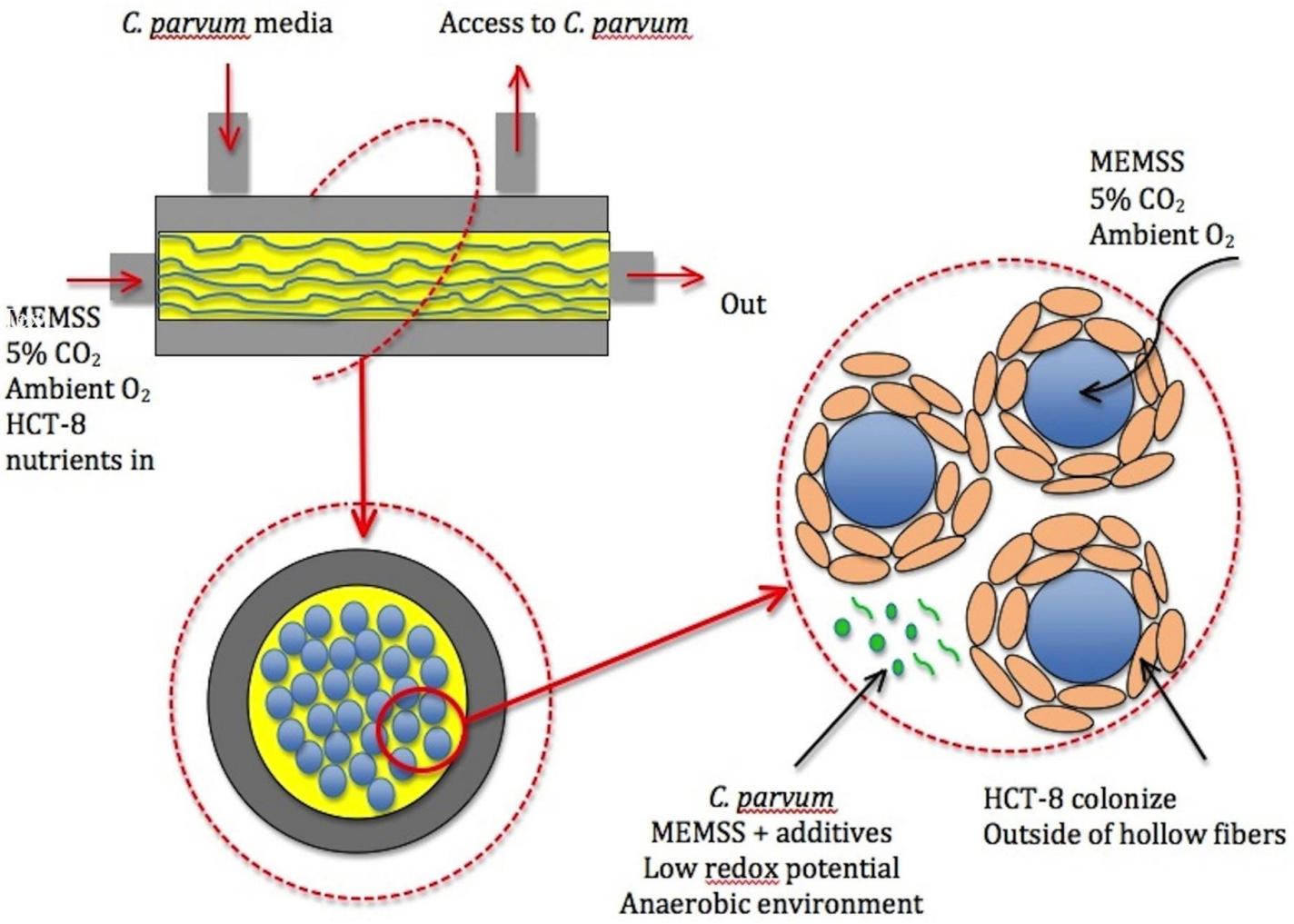


Diagram of Culture System



Hollow Fiber Culture System

Culture conditionS

Extra capillary space

1L MEM + 4 g dry mix + 10% horse serum + 4.5 g glucose

Lipid mix:
oleic acid (67 mg/10 mL)
cholesterol (180 mg/10 mL)

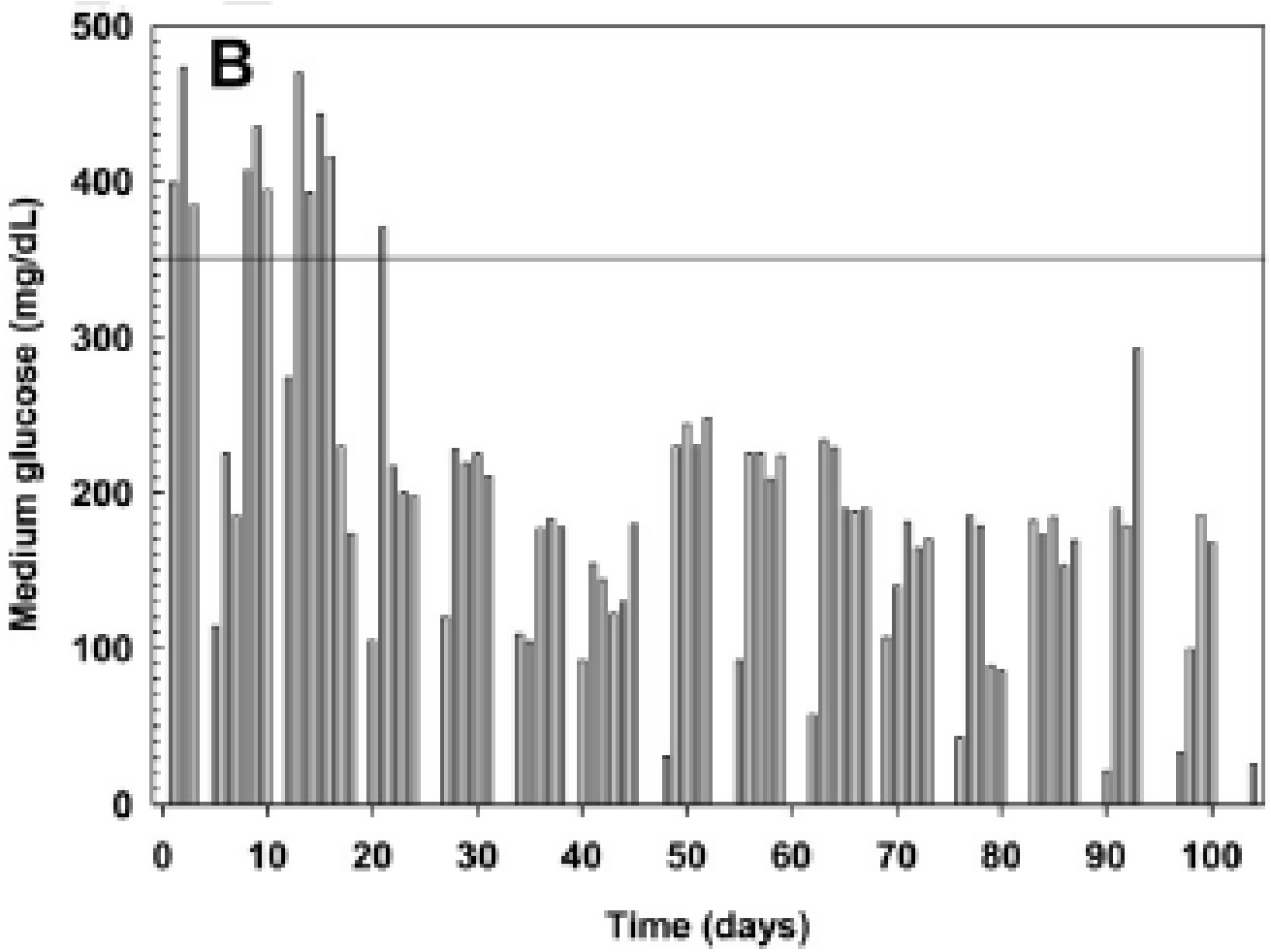
Redox buffer:
glutathione 200 mg/10 mL
taurine 200 mg/10 mL
betaine 200 mg/10 mL
cysteine 200 mg/ 10 mL

Reservoir hollow fibers

1L MEM + 10% horse serum

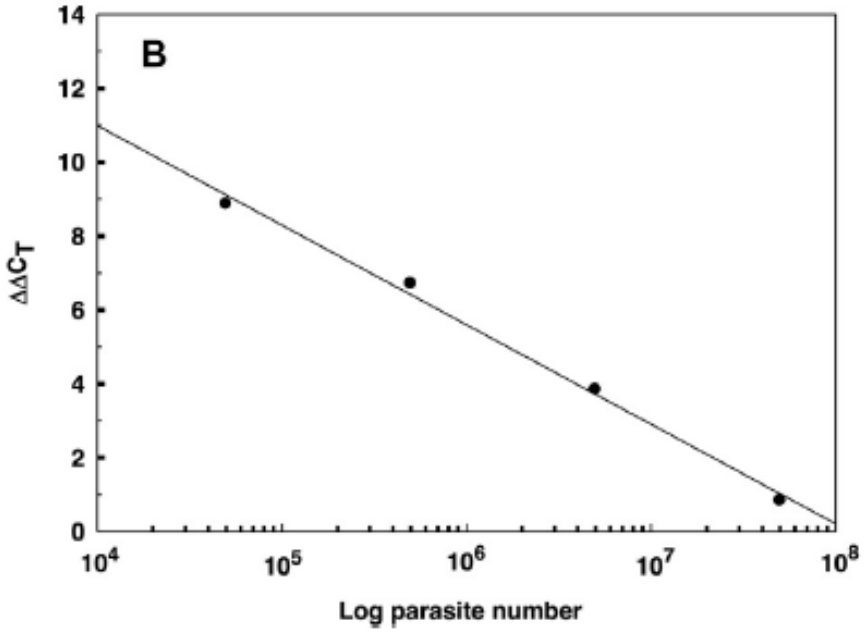
Dry mix (4 g):
0.29 g L-glutamine
3.57 g HEPES
4.5 g glucose
0.035 g ascorbate
0.04 g PABA
0.02 g Ca pantothenate
0.001 g folic acid

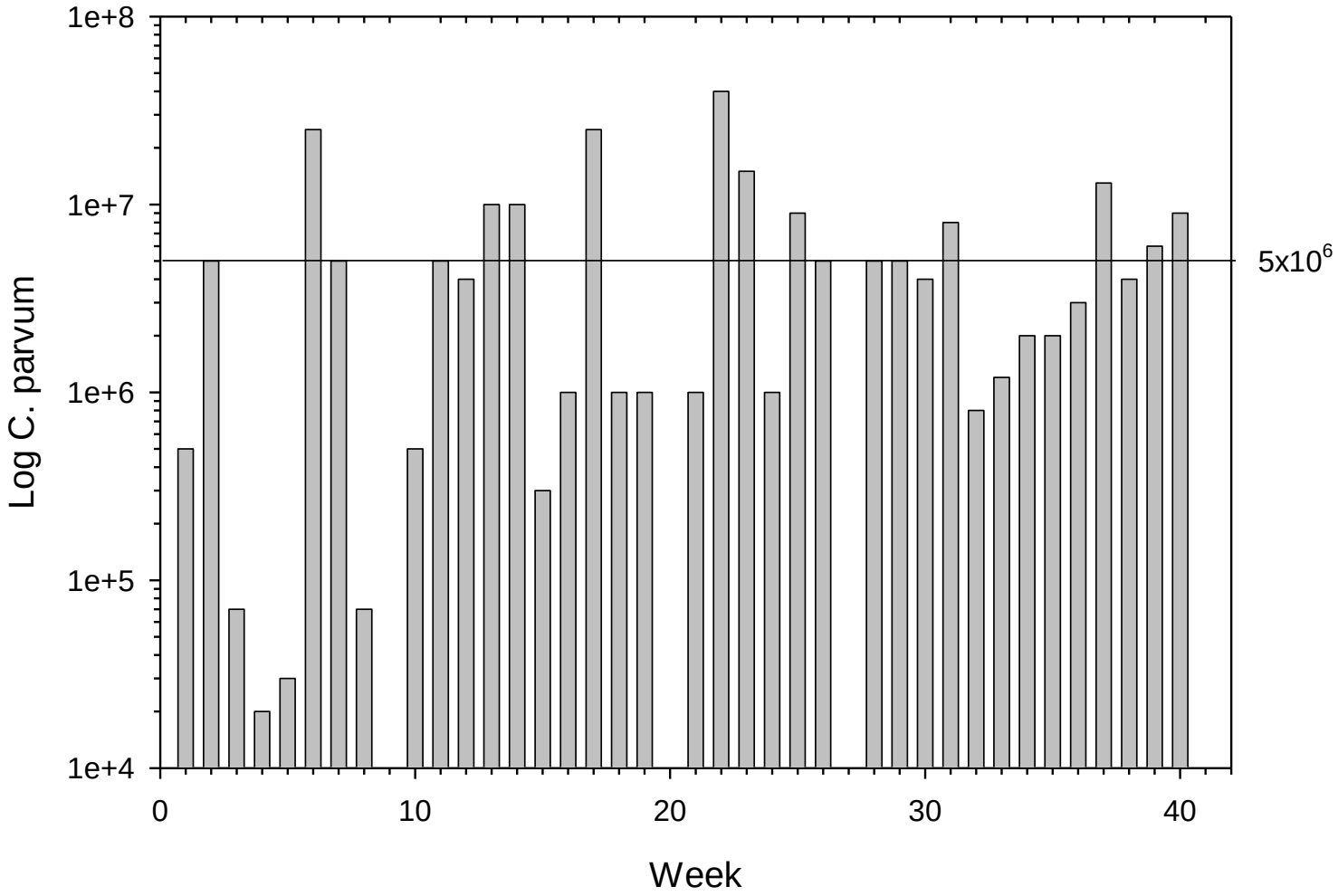
Medium glucose levels over 100 days. Glucose levels cycled for the first 20 days, exhibiting a dampened oscillation. The line at 350 mg/dL signifies the glucose concentration of the growth medium.



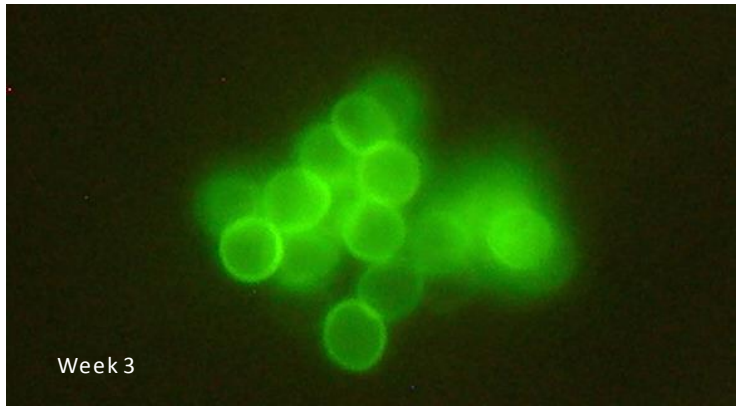
C. Parvum growth determined by qRT-PCR analysis of Cp18S-rRNA.

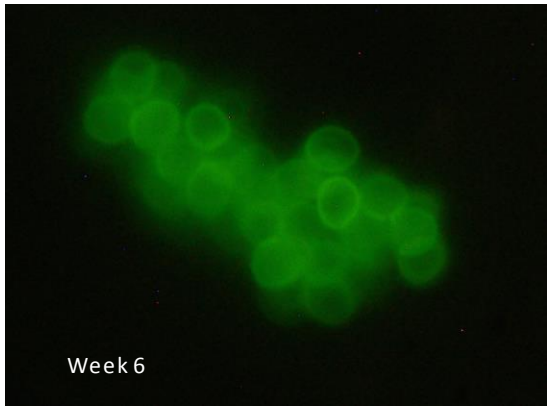
The line at $5x10^6$ is the mean number of parasites/mL over 40 weeks

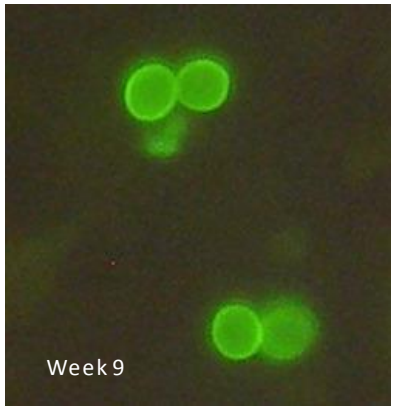


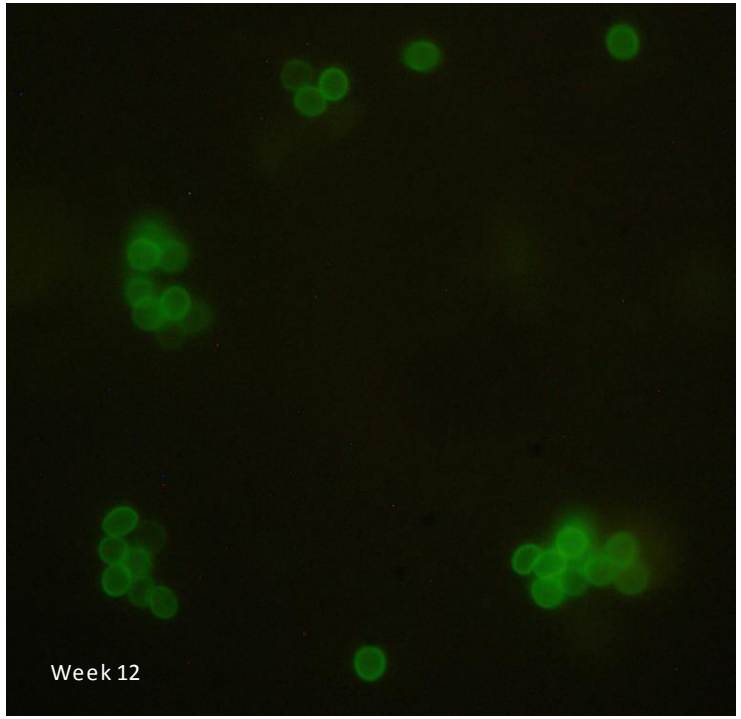


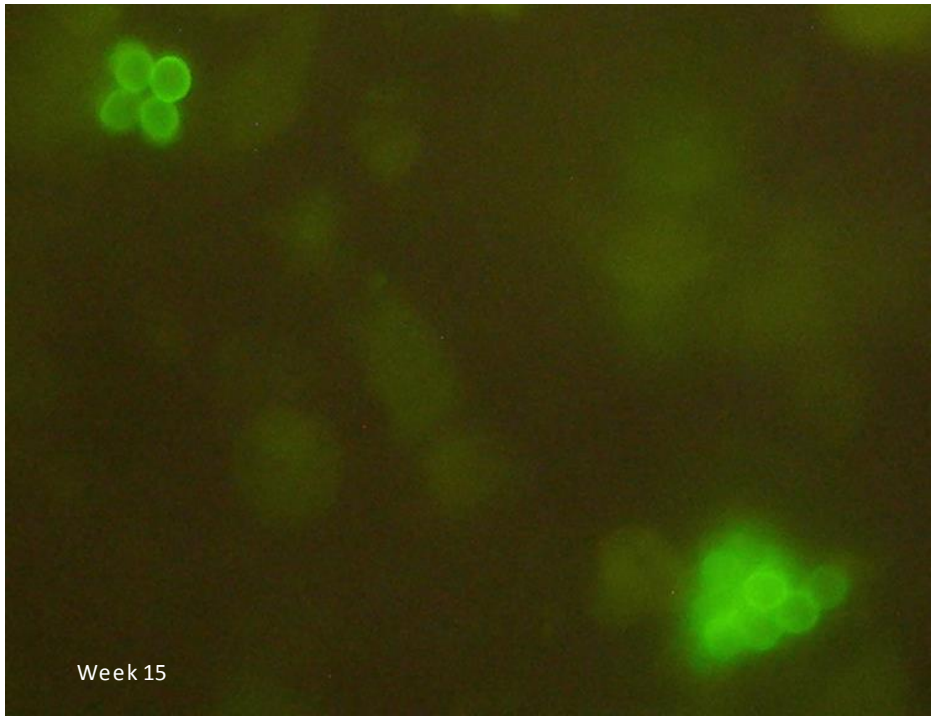
Crypt-a-Glo stained oocysts from culture system



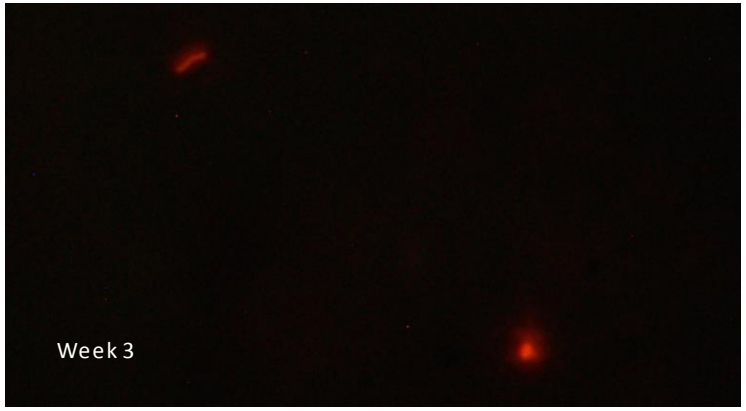


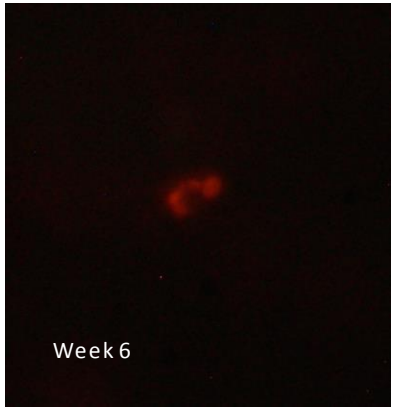


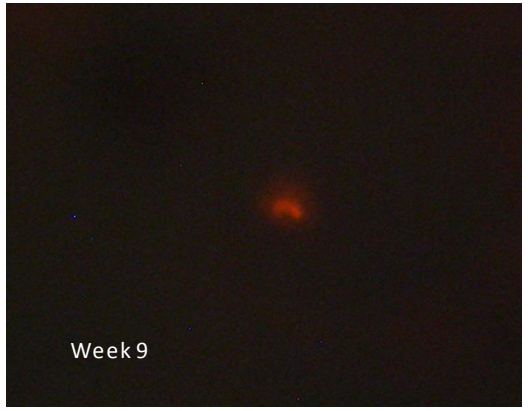




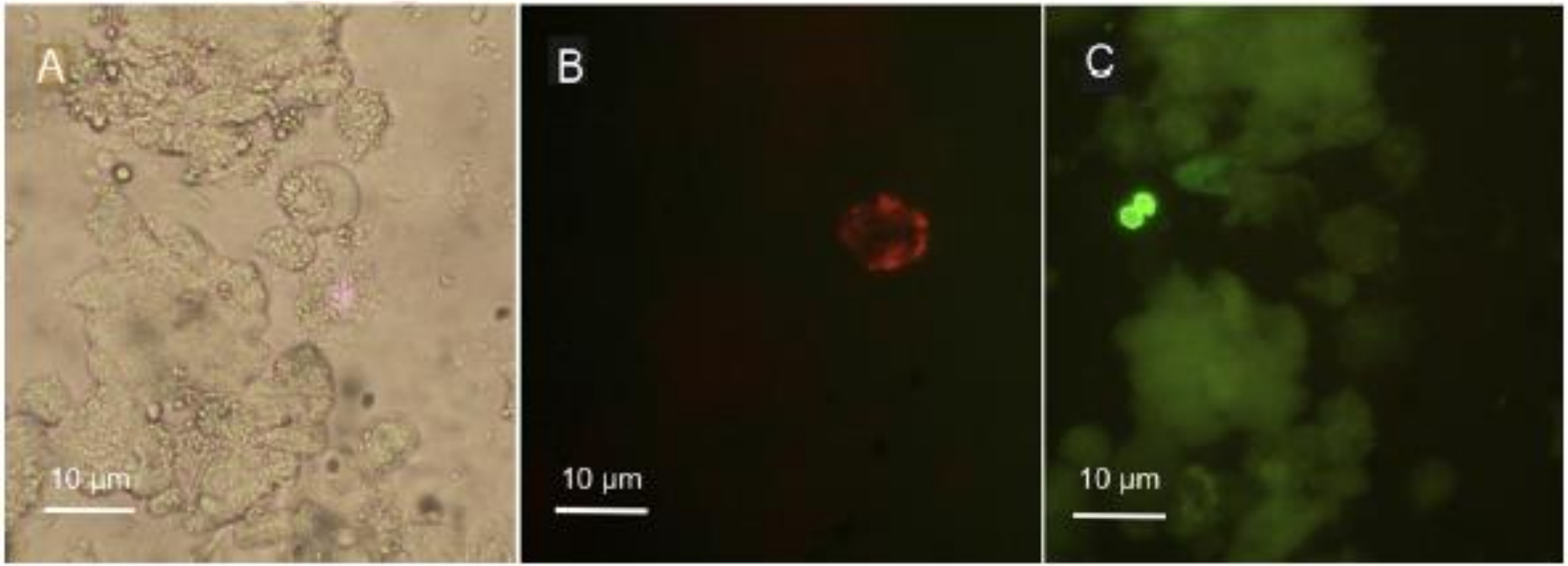
Spor-a-Glo stained motile stages from culture system







Overlay of sample from the culture system stained to show internal stages and oocysts.
(A) Light microscopy; (B) SporoGlo stained showing infected host cell; (C) Cryptoglo stained showing oocysts



C. parvum propagation in mouse model - S. Tzipori laboratory

C. parvum oocysts prepared from 6 weeks culture
C. parvum Iowa oocysts
15 CD1 Female 3-5 weeks old

Group	Mouse	Description
101-A (No treated)	5	Dex only; No oocysts
101-B (Culture)	5	Dex + C. parvum oocysts (Culture) 10^6 per an individual
101-C (Iowa)	5	Dex + C. parvum Iowa oocysts 10^6 per an individual

Dexamethasone treatment

Dexamethasone was administered in the drinking water (16 ug/ml) ad libitum from 4 days before inoculation to the end of study.

C. parvum oocysts inoculation

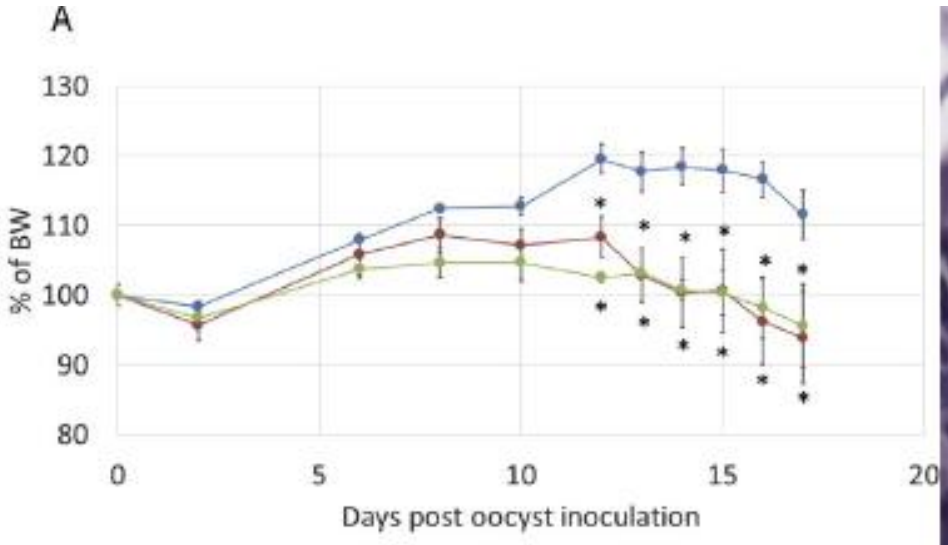
Mice were orally infected with 10^6 *C. parvum* oocysts in a 200 ul volume of sterile water via gavage.

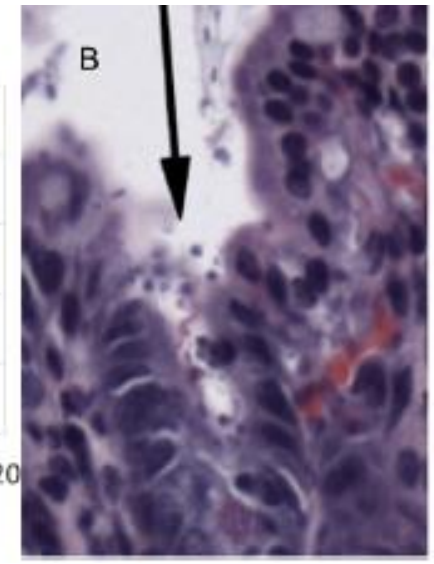
Animal monitoring and Sample collection

Mice were monitored twice daily following infection. Animals were weighed 3x each week. Following infection, fecal samples were collected daily and microscopically examined for the presence of oocysts.

At the end of study, Mice were euthanized and collect gastrointestinal tissues for histopathological examination.

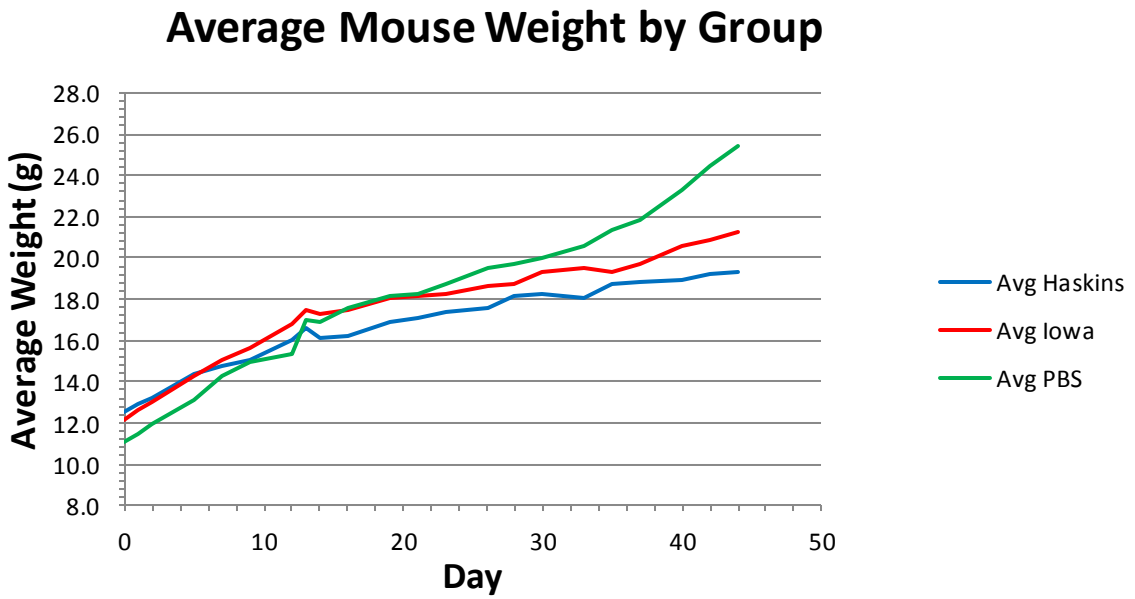
Propagation in immunosuppressed mouse model after 6 weeks – Tzipori Laboratory





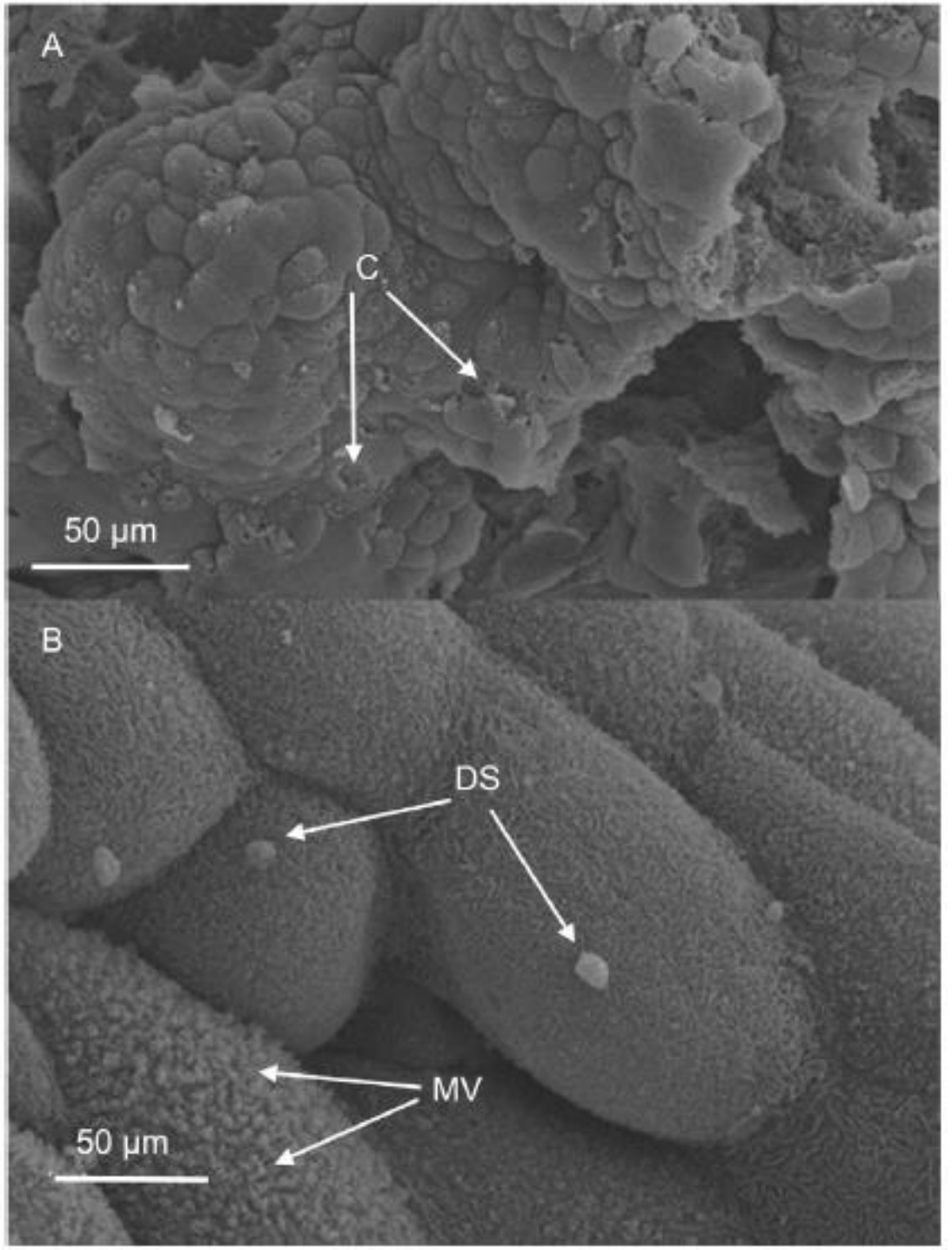
Group	Mouse #	Days post oocyst inoculation		
		13	14	17
No treated	m1	0	0	0
	m2	0	0	0
	m3	0	0	0
	m4	0	0	0
	m5	0	0	0
Culture	m1	0	1	8
	m2	**7**	**5**	**151**
	m3	3	0	0
	m4	**15**	**96***	NA
	m5	**3**	**3**	**7**
Iowa	m1	2	0	0
	m2	**71**	**19**	**58****
	m3	0	0	0
	m4	**2**	**5**	**4**
	m5	NA	NA	NA

Propagation in TCR-alpha mouse model after 6 months – Yarlett Laboratory



Day	control	culture	Iowa
14	0	43	32
16	0	58	29
18	0	65	47
21	0	111	93
23	0	161	181
25	0	130	208
28	0	210	190
30	0	189	211
32	0	235	190

SEM of 8 week culture on hollow fibers.
A. Proximal end of cartridge; B. Distal end of cartridge. Microvilli (MV), developmental stages (DS), craters (C).



Conclusion

- A technique allowing the continuous in vitro cultivation of *C. parvum*
- A valuable addition to the current techniques for studying the parasite
- Allows growth of *C. parvum* in the absence of other gut organisms
- Provides approximately 10^8 parasites per column volume
- Allows drug studies to be performed longer than 48 hours
- Allows pharmacokinetic studies to be performed

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