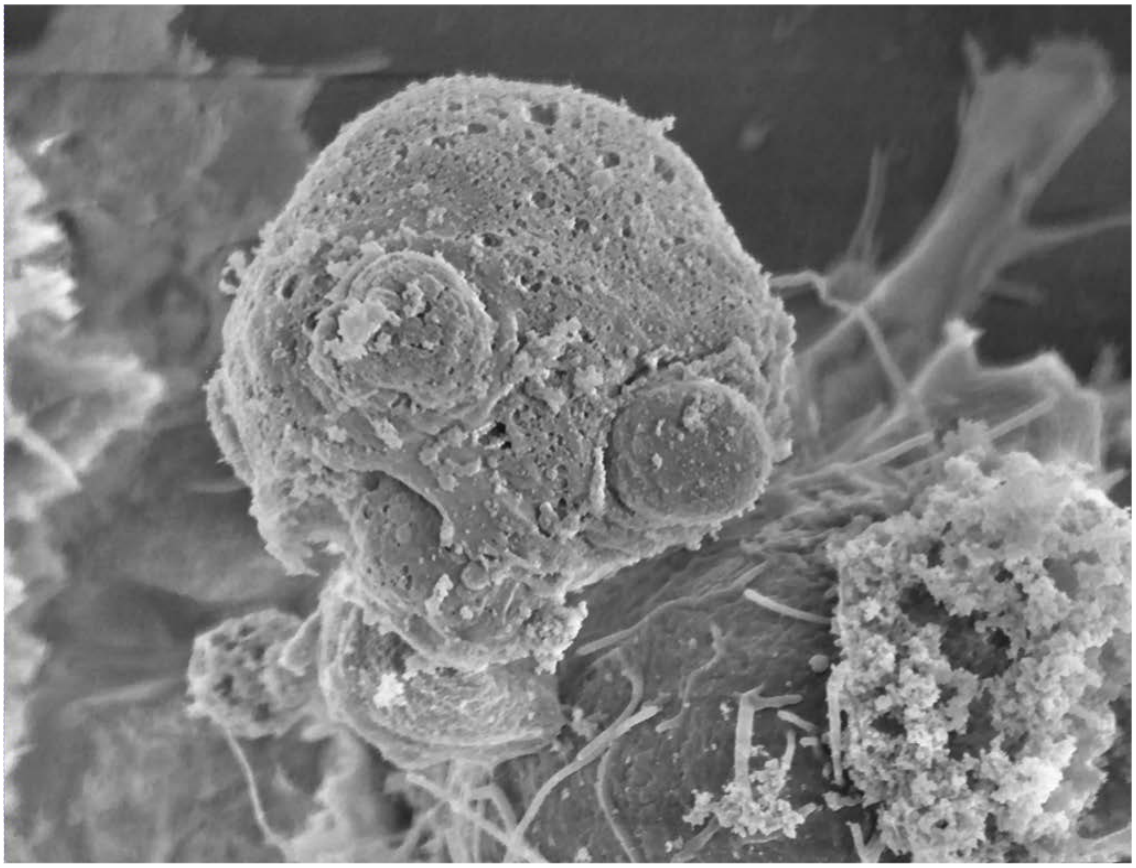
The 100 Faces of *Cryptosporidium parvum*



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Declaration

I declare that this thesis is a true account of my own research and contains work, which has not been submitted for a degree at any other educational institution.

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Abstract

Cryptosporidium parvum is a protozoan enteric parasite of humans and livestock. *C. parvum* infection mainly affects the ileum, where it has the potential to cause severe enteric disease. Drugs for the treatment of cryptosporidiosis are still not available and the biology and life cycle of *C. parvum* remain incompletely understood. The present study gives new insight into the parasite's morphology, life cycle and host cell relationship.

This study utilised light microscopy, scanning electron microscopy, transmission electron microscopy and labeling of *C. parvum* surface receptors to examine infected cell cultures, cell-free cultures and oocyst stocks of *C. parvum*. Hence, this study compared different culture and different microscopic examination methods to determine the most suitable way to examine *C. parvum'*s morphology. Cell-free culture did not provide additional information to this study. However, it served as a valuable comparison for life cycle stages detected in the supernatant above cells which are expected to occur in the intestinal lumen of infected hosts. Scanning electron microscopy was the most suitable tool for obtaining information on the parasite's morphology, whereas transmission electron microscopy enabled a view into the interior of stages. Employing light microscopy in this study was essential to progressively monitor live samples and visualise stages in the supernatant above cells, which were not attached to host tissue. In the course of this study a protocol was developed, which enabled the visualisation of *Cryptosporidium* receptors on the surface of parasites and/or host cell material via immunogold labeling with scanning electron microscopy.

For the first time, the entire range of *C. parvum's* life cycle stages has been morphologically characterised (including their interactions with host cells) and presented in one study. A better understanding of the parasite's biology, proliferation in host tissue and interactions with host cells will aid the drug development process.

Recent electron micrographs acquired in the course of this study revealed new life cycle stages, provided new information about the parasite's morphology and its relationship with host cells. New insight into the host cell invasion process of *C. parvum* sporozoites as well as merozoites I and II was obtained. Features of gliding motility of the invasive stages were visualised and explained. Phenomena including binary fission - commonly employed by bacteria for the production of two identical daughter stages from one parent stage - and syzygy - the pairing of gamonts to exchange genetic material, described in gregarines - , was observed and described. Extracellular gamonts and gamont-like stages were also characterised; developing from intracellular trophozoites to finally break host cell contact and take their place in the life cycle of *C. parvum,* travelling free in the intestinal lumen. The morphology of the two different types of oocysts has been described and findings on receptor expression in their outer membranes are presented. Furthermore, *C. parvum* receptors were identified in the apical membranes surrounding parasite stages. *C. parvum* surface receptors were also found on host cell microvilli in proximity to invading and/or gliding parasites.

Additionally, the present study observed the effect that a *C. parvum* infection exerts on host tissue with respect to necrosis and apoptosis. This study also poses new ideas regarding the parasite's host-dependent feeding behaviour.

Publications

Part of the work presented in this thesis has been accepted for publication or presented in scientific conferences as described below:

Publications in Journals

BOROWSKI, H., CLODE, P. L. & THOMPSON, R. C. (2008) Active invasion and/or encapsulation? A reappraisal of host-cell parasitism by Cryptosporidium. *Trends in Parasitology*.

BOROWSKI, H., THOMPSON, R. C., ARMSTRONG, T. & CLODE, P. L. (2009) Morphological characterization of Cryptosporidium parvum life-cycle stages in an in vitro model system. *Parasitology***,** 1-14.

EDWARDS, H., THOMPSON, R. C., KOH, W. H. & CLODE, P. L. (2011) Labeling surface epitopes to identify Cryptosporidium life stages using scanning electron microscopy-based immunogold approach. *Molecular and Cellular Probes*, 1-8.

Presentations at Conferences

BOROWSKI, H., CLODE, P. L. & THOMPSON, R. C. (2007) News about Cryptosporidium parvum: New insights into the invasion process, developmental stages and effects on host cells in vitro. ASP Conference 2007, Canberra, Australian Capital Territory, Australia.

CLODE, P. L., BOROWSKI, H., ARMSTRONG, T., KOH, W. H. & THOMPSON, R. C. (2009) SEM imaging of Cryptosporidium parvum life cycle stages. ICOPA Conference 2009, Ovieto, Italy.

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List of Abbreviations

a:	apical
ACBP:	fatty acid acyl-CoA binding protein
AGS:	human stomach adenocarcinoma
AIDS:	autoimmune deficiency syndrom
AP:	arginine aminopeptidase
AQP1:	aquaporin 1
ARP2/3:	actin related proteins 2 and 3
ASM :	acidic-sphingomyelinase
ATCC:	American type cell culture collection
ATP:	adenosine triphosphate
b:	rudimentary body
BSD:	backscattered electron detector/detection
c:	cyst
Caco:	human Caucasian colon adenocarcinoma cell line
C. andersoni :	*Cryptosporidium andersoni*
C. baileyi:	*Cryptosporidium baileyi*
CD:	cluster of differentiation
Cdc42:	cell division cycle 42
C. muris:	*Cryptosporidium muris*
cm :	centimetre(s)
cn :	contact region
CoA :	Coenzyme A
CP:	Cryptosporidial protein

Cpa:	*Cryptosporidium parvum*
CpABC:	*C. parvum* ATP-binding cassette protein
C. parvum:	*Cryptosporidium parvum*
CpATPase:	*C. parvum* adenosine triphosphatase
CPS:	*C. parvum* sporozoite protein
CSL:	*C. parvum* sporozoite ligand
c-Src:	cellular Src
db :	dense band
DMSO :	dimethyl sulfoxide
DNA :	Desoxyribonucleic acid
EM:	electron microscopy
ER :	endoplasmatic reticulum
et. al. :	and others
f :	feederorganelle
F-actin:	filamentous actin
Fas :	apoptosis stimulating fragment
FasL :	Fas ligand
FCS:	foetal calf serum
Fig.:	figure
Figs.:	figures
g:	gram(s)
G:	gamont
Gal:	Galactose
Gal/GalNAc:	N-acetylgalactosamin
GDP:	guanosine diphosphate

GEFs:	guanine nucleotide exchange factors
GP:	glycoprotein
GTP:	guanosine triphosphate
GTPase:	guanosine triphosphatase
gz:	gametocyte
H:	hole
HCT-8:	human ileocecal tumor (adenocarcinoma) epithelial cell line
HEPES:	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV:	human immunodeficiency virus
hrs:	hours
Ig:	Immunoglobulin
INF:	interferron
j:	junction
k-Da:	kilo-Dalton
kV:	kilo-Volts
kx:	thousand times
l:	litre(s)
mAB:	monoclonal antibody
m:	membrane
M/Ma:	macrogamont
Mi:	microgamont
min:	minute(s)
MDCK:	Madin-Darby Canine kidney epithelial cell line
mf:	membrane folds
mg:	milligram(s)

ml:	milliliter(s)
mm:	millimetre(s)
mv:	microvillus, microvilli
mz:	merozoite
n:	nucleus
NK:	natural killer
nm:	nanometer
no:	nucleolus
O:	oocyst
p:	protein
P:	parasite
P_3:	phosphatidylinostitol 3,4,5-trisphosphate
p34-Arc:	subunit protein with 34 k-Da of the actin-related protein complex (Arp2/3)
PBS:	phosphate buffered saline
PCR:	polymerase chain reaction
pH:	power of hydrogen
Ph:	pleckstrin homology domains
PI-3K:	phosphoinositide 3-Kinase
PM:	parasite membrane
psi:	pounds per square inch
PTPase:	protein tyrosine phosphatase
PV:	parasitophorous vacuole
PVM:	parasitophorous vacuole membrane
r :	residual body
rhp :	rhoptries

rpm:	revolutions per minute
RPMI:	Rapid prototyping and Manufacturing Institute
RT:	room temperature
s:	stork
SE:	secondary electron
sec:	second(s)
SEM:	scanning electron microscopy
SEMs:	sphingolipid-enriched membrane microdomains
SGLT1:	sodium/glucose cotransporter
sp./spp.:	species
$sPLA_2$:	secretory phospholipase A_2
Src:	a group of non-receptor tyrosine kinases
T:	trophozoite
TEM:	transmission electron microscopy
TKGFR:	tyrosine kinase growth factor receptor
TNF:	tumor necrosis factor
TRAP:	thrombospondin-related adhesive protein
TRAP-C1:	thrombospondin-related adhesive protein 1 of *C. parvum*
TSP:	thrombospondin
VASP:	vasodilator-stimulated phosphoprotein
VPSEM:	variable pressure scanning electron microscope
µg:	microgram(s)
µl:	microlitre(s)
µm:	micrometer(s)
x:	times

z: zoite