

Development of a National Reference Facility for *Cryptosporidium*

National Zoonoses Conference
June 9th 2011

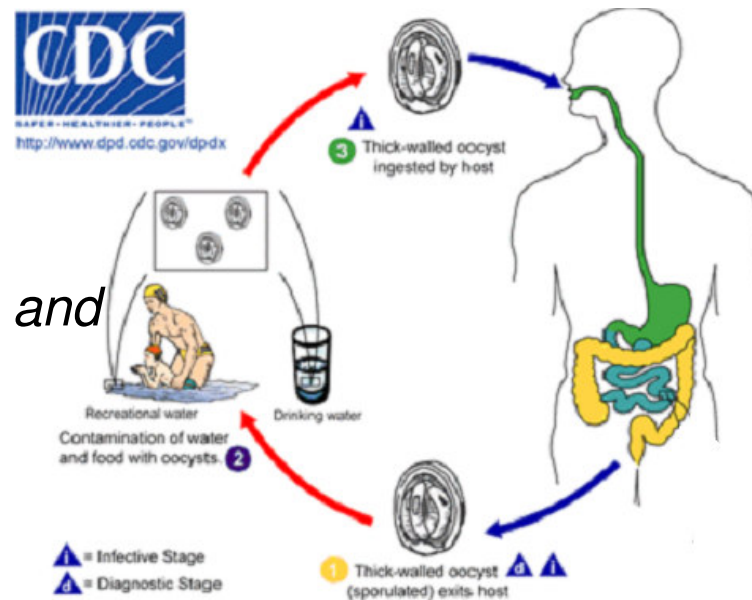
Carolyn Read



Cryptosporidium



- 20 valid species
- Many genotypes
- Host specific: *C. hominis*
- Broad host range: *C. parvum* and *C. ubiquitum*



Waterborne Outbreak 1993!

Vol. 331 No. 3

CRYPTOSPORIDIUM INFECTION FROM MILWAUKEE'S PUBLIC WATER SUPPLY

161

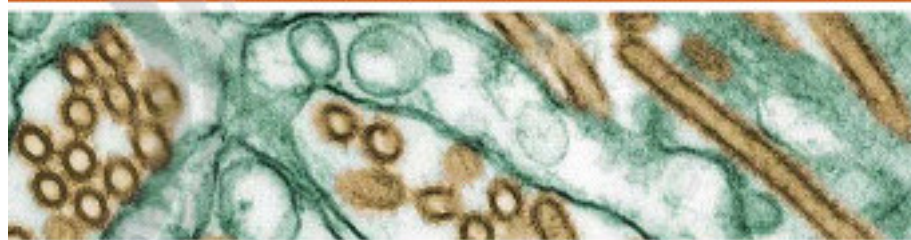
A MASSIVE OUTBREAK IN MILWAUKEE OF CRYPTOSPORIDIUM INFECTION TRANSMITTED THROUGH THE PUBLIC WATER SUPPLY

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Number and notification rate of reported cases of cryptosporidiosis in the EU and EEA/EFTA, 2006–08



SURVEILLANCE REPORT

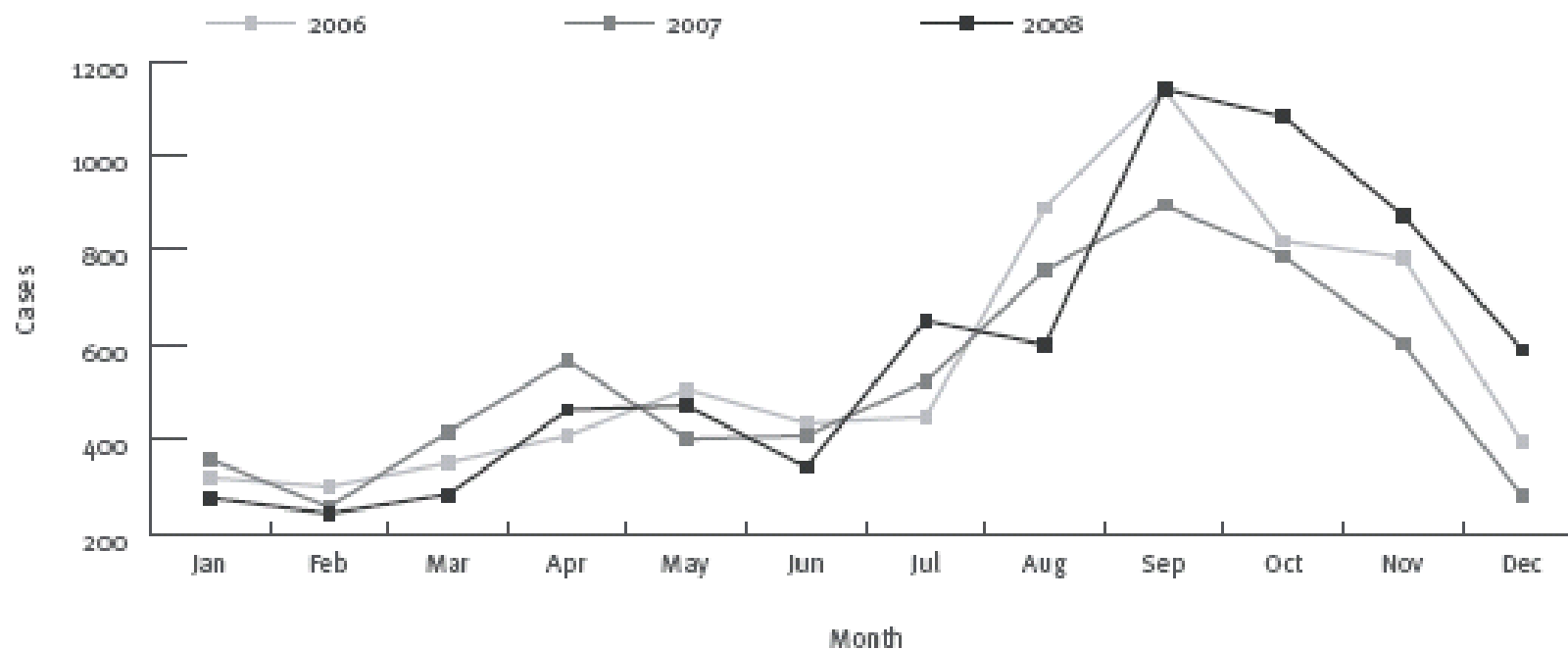


Annual epidemiological report
on communicable diseases in Europe

2010

Country	Report type*	2008		
		Total cases	Confirmed cases	Notification rate per 100 000 population
Austria ^(a)	C	16	13	0.2
Belgium	C	396	396	3.7
Bulgaria	C	0	0	0.00
Cyprus	C	0	0	0.00
Czech Republic	C	0	0	0.00
Denmark	—	—	—	—
Estonia	C	0	0	0.00
Finland	C	11	11	0.21
France	—	—	—	—
Germany	C	1 014	1 014	1.2
Greece	—	—	—	—
Hungary	C	10	10	0.10
Ireland	C	414	412	9.4
Italy	—	—	—	—
Latvia	C	0	0	0.00
Lithuania	C	0	0	0.00
Luxembourg	C	0	0	0.00
Malta	C	0	0	0.00
Netherlands	—	—	—	—
Poland	A	1	1	0.00
Portugal	—	—	—	—
Romania	C	0	0	0.00
Slovakia	C	0	0	0.00
Slovenia	C	6	6	0.30
Spain ^(b)	C	75	75	—
Sweden	C	148	148	1.6
United Kingdom	C	4 941	4 941	8.1
EU total		7 032	7 027	2.44

Seasonal distribution of cryptosporidiosis cases in the EU and EEA/EFTA, 2006-08



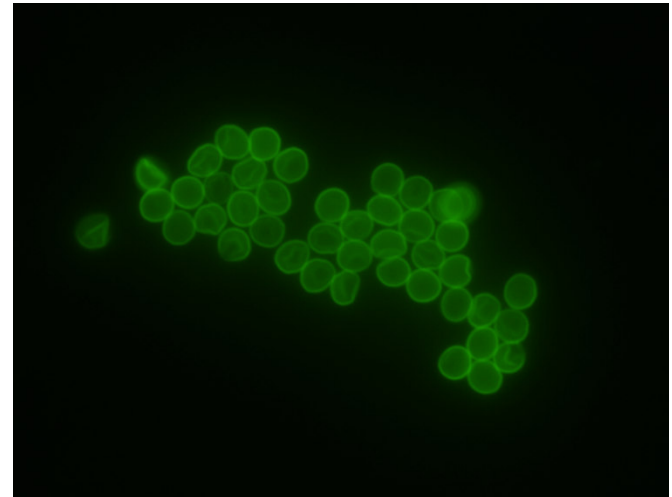
Cryptosporidium in Ireland

- East/ West incidence difference
- Role of Direct transmission?
- Surface water (82% of drinking water)
- Number of septic tanks
- High rainfall
- Livestock density
- Inadequate treatment



Why do we need a reference laboratory?

- No genotyping facilities in Ireland
- Sources of contamination ?
- Public health risk ?
- Significance of direct versus indirect transmission?



FITC stained *Cryptosporidium* oocysts

STRIVE Call: Towards Developing a National *Cryptosporidium* Monitoring Protocol

- Jointly funded by EPA and DAFF
- UCD awarded project - Started September 2010
- Project team:
 - Dr. Theo de Waal – UCD
 - Dr. Annetta Zintl - UCD
 - Dr. Tom Murphy - DAFF
 - Carolyn Read – DAFF/UCD
- Collaborators:
 - Grace Mulcahy – UCD
 - Rachel Chalmers – UK CRU
 - George Sharpson – Fingal Co. Co.
 - Frances Lucy- Institute Technology Sligo
 - Kevin Callanan – Dublin City Council

Research Focus:

- *Cryptosporidium* subtyping in humans - Annetta Zintl
- Epidemiology and on-farm transmission of *C. parvum* in neonatal calves.
Funded: Research Stimulus Fund (Dept. Agric.)
Principal investigator: Dr Tom Murphy (Parasitology Section, CVRL)
Research Student: Ms Valerie de Waele
- Development of risk assessment models for contamination of river catchments with *Cryptosporidium* spp. oocysts.
Funded: EPA; EU FP6 (Marie Curie, TOK).
Principal investigators: Prof. Nicholas Holden (Sch. Agric. Vet. Med., UCD) and Dr Tom Murphy
Research student: Stephen McDonald
Postdoctoral Research Fellows: Drs M. Berzano, X. Peng, S. Sammader, J. Tang and P. Zeigler
- Cryptosporidiosis: Human, animal and environmental interface
Funded: EPA STRIVE Programme
Principal investigator: Dr. Theo de Waal , Postdoctoral Research: Annetta Zintl
Research Student: Marzieh Mirhashemi

Project objectives

- Survey of public water supplies
- Establish national reference facility for *Cryptosporidium* in Water
- Emerging contaminants
- Strategies for service delivery beyond project

Survey- Annetta Zintl

Survey of Water Service Authorities about Cryptosporidium testing in drinking water - Microsoft Internet Explorer

File Edit View Favorites Tools Help

Back Forward Stop Reload Home Search Favorites Print Mail

Address http://www.surveymonkey.com/s/9KS53CH Go Links

UCD DUBLIN Department of Agriculture, Fisheries and Food An Roinn Talmhaíochta, Iascaigh agus Bia EPA Environmental Protection Agency Exit this survey

Survey of Water Service Authorities about Cryptosporidium testing in drinking water

1. Cryptosporidium monitoring in specific water supplies

1. Cryptosporidium risk

	What is the Cryptosporidium risk score?	Is this supply being tested for Cryptosporidium oocysts?
supply 1	<input type="text"/>	<input type="text"/>
supply 2	<input type="text"/>	<input type="text"/>
supply 3	<input type="text"/>	<input type="text"/>
supply 4	<input type="text"/>	<input type="text"/>
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supply 7	<input type="text"/>	<input type="text"/>
supply 8	<input type="text"/>	<input type="text"/>
supply 9	<input type="text"/>	<input type="text"/>
supply 10	<input type="text"/>	<input type="text"/>

Internet

Routine *Cryptosporidium* testing in Ireland: reasons for monitoring

- 227 Public water supplies currently being monitored (PWS register =926)
- Supplies on remedial action list due to lack of effective barrier
- Population served
- Surface water
- Supplies that are high to very high risk

Cryptosporidium monitoring

- Considerable proportion of drinking water treatment plants monitor for *Cryptosporidium*
- Monitoring frequencies generally low (<10/year)
- Reasons why supplies not monitored:
 - Lack of resources
 - Low risk scores

Report Recommends

- Risk assessment- short term high frequency monitoring
- Catchment management - raw water monitoring
- Efficacy of treatment - both raw and treated water monitoring
- Subsequent monitoring in response to triggers such as increase in turbidity and if cases are reported in community

Cryptosporidium Reference Laboratory



Laboratory Resources

- Equipment for filter processing
- Molecular biology
- Unidirectional workflow



REAGENT
PREP

EXTRACTION

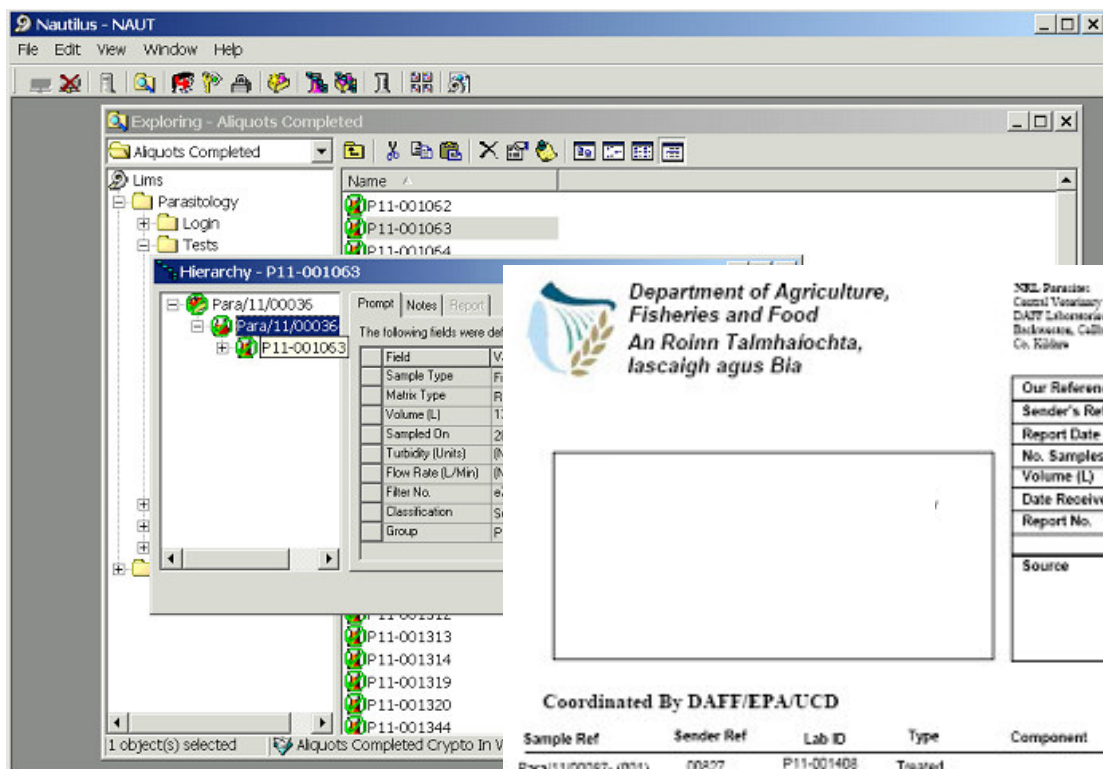
AMPLIFICATION

POST PCR



UNIDIRECTIONAL WORKFLOW

Laboratory Information Management System



Nautilus - NAUT

File Edit View Window Help

Exploring - Aliquots Completed

Aliases Completed

Aliases

Parasitology

Login

Tests

Hierarchy - P11-001063

Para/11/00036

P11-001063

The following fields were displayed:

Field	Value
Sample Type	Fi
Matrix Type	R
Volume (L)	1
Sampled On	2
Turbidity (Units)	0
Flow Rate (L/Min)	0
Filter No.	0
Classification	Si
Group	P

Department of Agriculture, Fisheries and Food
An Roinn Talmhaíochta, Iascaigh agus Bia

NGL Parasitology
Central Veterinary Research Laboratory
DAFF Laboratory
Barnmore, Galway
Co. Galway

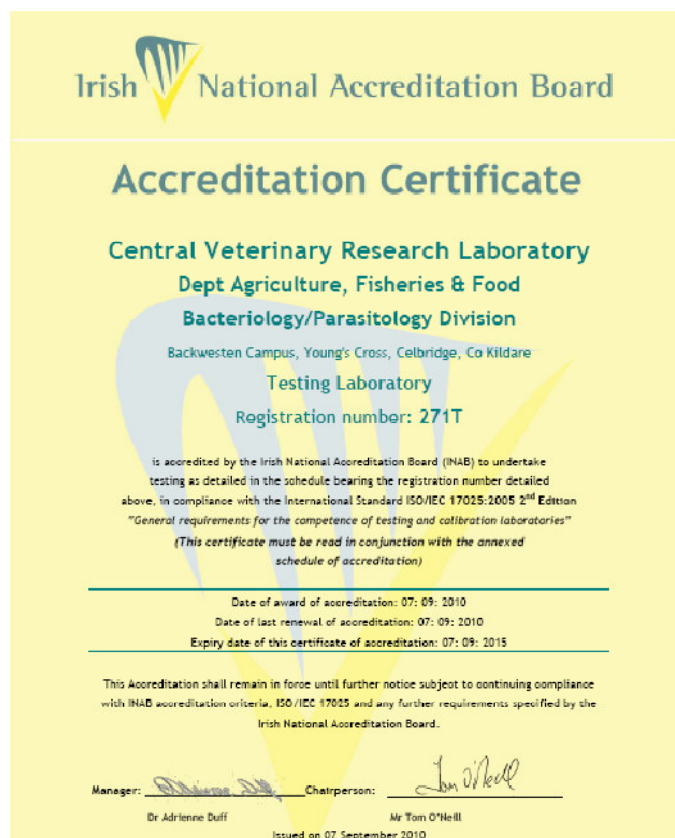
Phone: 01-4157141
Fax: 01-415 7116
Email:

Our Reference	Para/11/00087
Sender's Reference	00827
Report Date	24/05/2011
No. Samples	1
Volume (L)	1016
Date Received	15/05/2011
Report No.	1
Source	Castlerea Urban

Coordinated By DAFF/EPA/UCD

Sample Ref	Sender Ref	Lab ID	Type	Component	Result	Units
Para/11/00087- (001)	00827	P11-001408	Treated	Volume Analysed	1016	Litre
				Cryptosporidium in Water	<0.01	Cryptosporidium/10L

Backweston Laboratory



Scope of Accreditation



CVRL Bacteriology/Parasitology Division

Permanent Laboratory:
Category A

Biological Testing Laboratory

INAB Classification number (P9) Materials/products tested	Type of test/properties measured Range of measurement	Standard specifications Equipment/techniques used
819 .31 Microbiological tests on other materials Veterinary clinical specimens	Detection of <i>Salmonella</i> spp	SOP.001 based on ISO 6579:2002
819 .99 Microbiological tests on other materials Other Materials: - Dust - environmental swabs from feedmills and animal poultry houses - faecal material - fluff, chick box liners, digestion residues - Compost samples from compost plants.	Detection of <i>Salmonella</i> spp	SOP.001 based on ISO 6579:2002
811 .03 Microbiological tests on foods Meat and Meat products	Detection of <i>E. coli</i> O157 using immunomagnetic separation	SOP.011 based on ISO 16654:2001

Detection of *Cryptosporidium* in Water



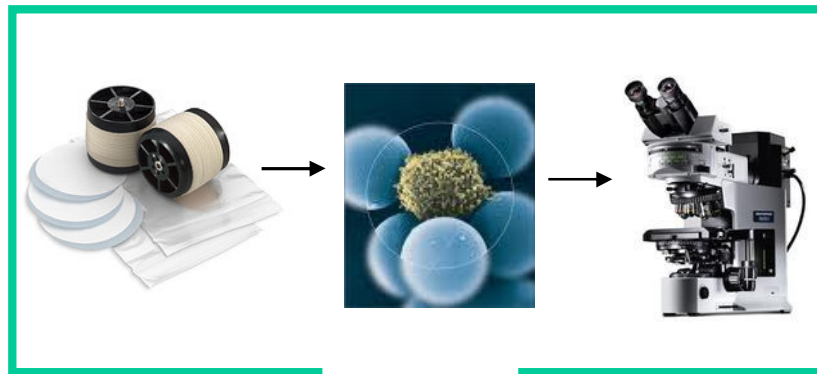
Method 1622: *Cryptosporidium* in Water
by Filtration/IMS/FA

December 2005

- 1996 Draft Method
- 1998 Validated
- 1999- 2005 Revised

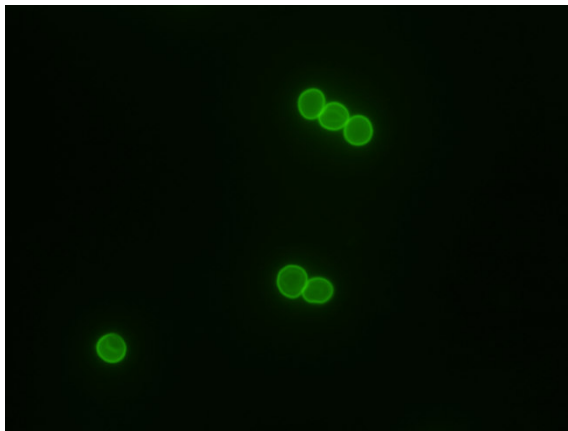


Method:1622



Enumeration

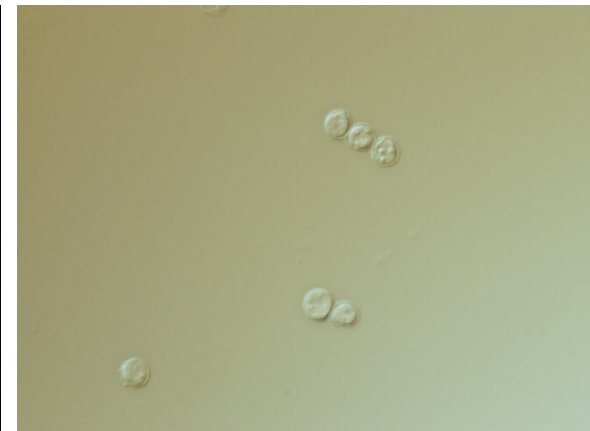
Oocyst detection and Enumeration



FITC



DAPI



DIC

Setting up the Laboratory- USEPA 1622

VALIDATION



→ Initial Precision and Recovery

→ Ongoing Precision and Recovery

→ External Proficiency Test-
CRYPTS Scheme

• Method Performance

• Quality Control
Acceptance Criteria:

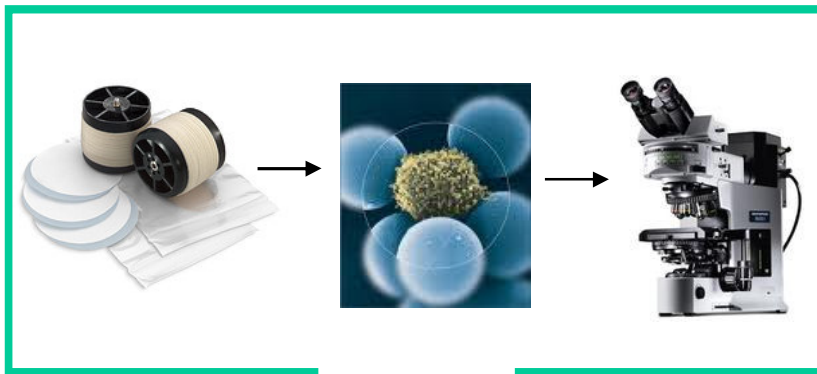
ACCREDITATION

→ INAB

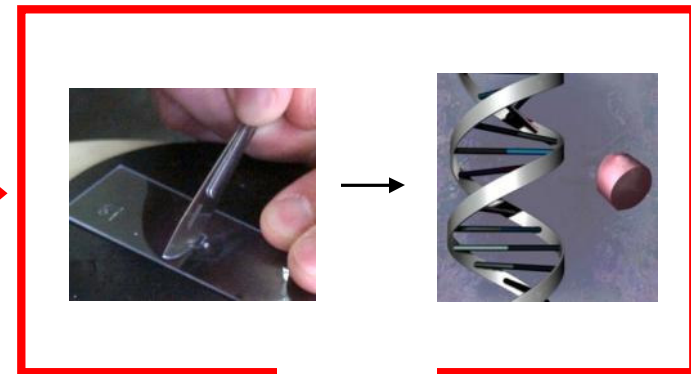
→ 12-18 Months



Method: Genotyping oocysts from water samples

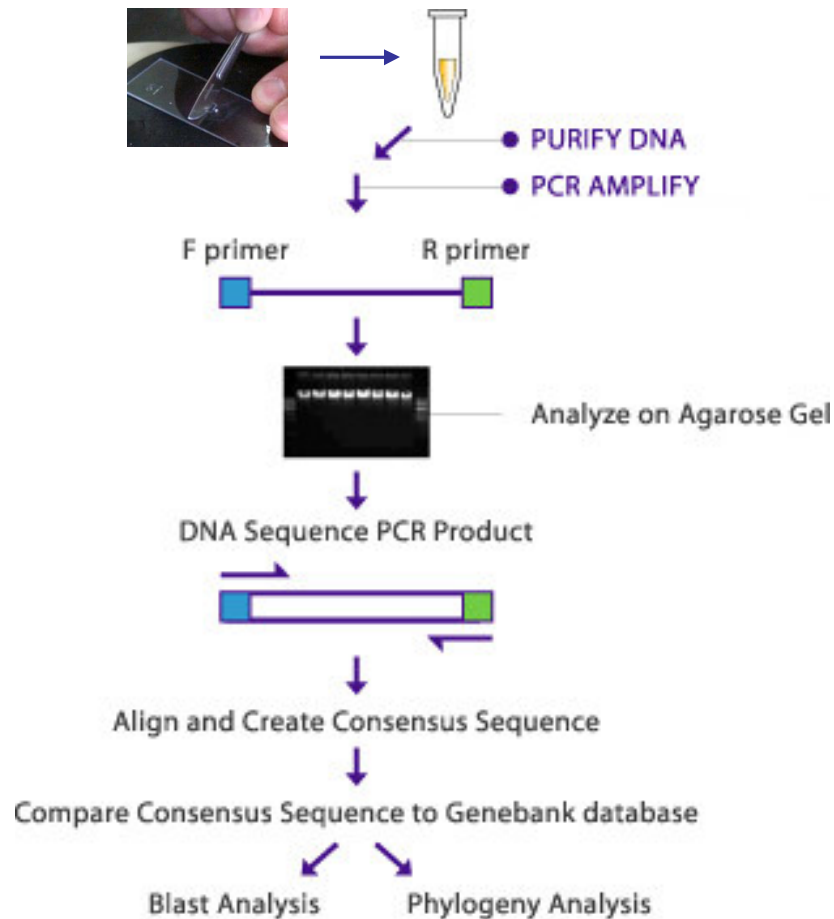


Enumeration



Genotyping

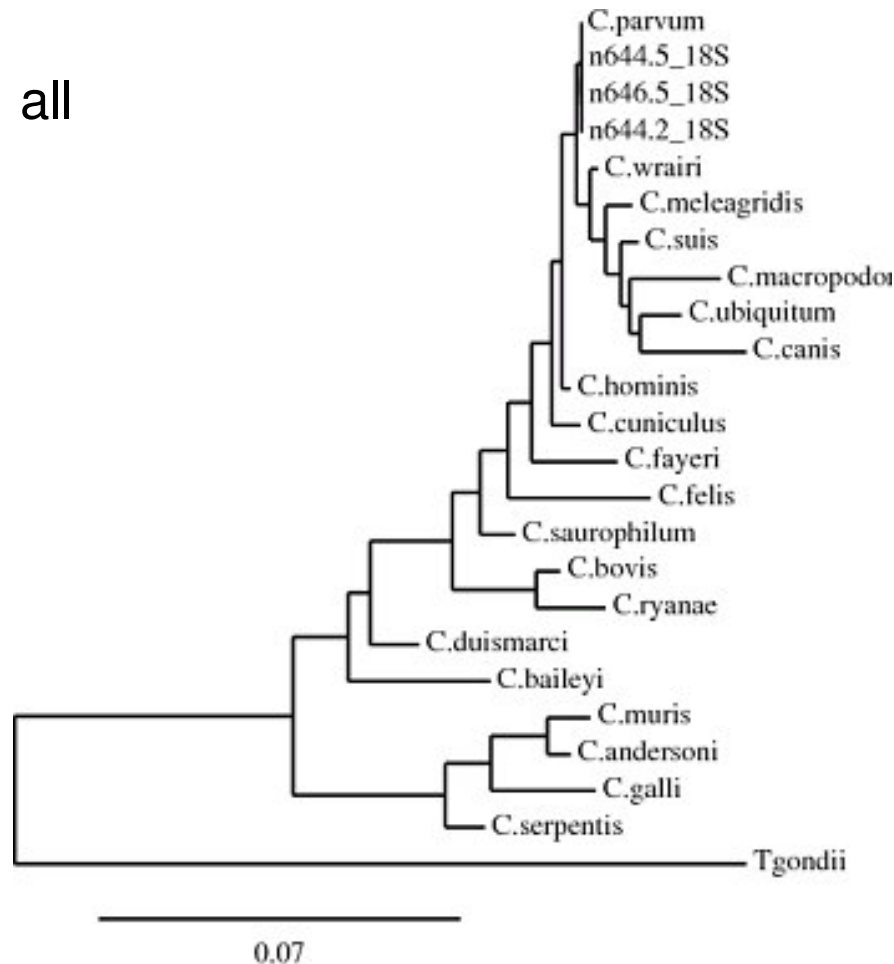
Genotyping : PCR-Sequencing SSU-rRNA



- Multicopy locus
- Variable region
- Multiple replicates

Genotyping Result: Phylogenetic analysis

- Distinguish between all known species and genotypes
- Novel genotypes
- Mixed genotypes



Collaboration: UK *Cryptosporidium* Reference Unit

- Method Development
- Optimisation of real time PCR
- Interlab proficiency test for genotyping

Water Research Foundation: Genotyping of *Cryptosporidium* oocysts from USEPA and UKDWI Regulatory Slides

- Selecting and standardising the most appropriate tool for regulatory genotyping
 - Lihua Xiao – Centers for Disease Control, Atlanta
- Development of a method for the detection of human pathogenic *Cryptosporidium* oocysts
 - George Digiovanni – Texas A and M University

WRF – Project outcomes

- Method sensitive to genotype from 1 oocyst on slide
- Simplified SOP for genotyping in routine monitoring
- Method development- Validation studies, internal controls, round robin and field test between international laboratories

Laboratory Services

- Currently running pilot monitoring scheme with number of local authorities:

Detection and genotyping of *Cryptosporidium* oocysts in 'model' catchments of supplies on RAL

- Genotyping of slides referred from other laboratories
- Support for outbreak investigation

Outcomes.....

- Accredited water testing laboratory
- Genotyping facility
- Laboratory support during outbreak investigations
- Towards better understanding of *Cryptosporidium* contamination in associated supplies on RAL

