NDP
National Development Plan 2007 - 2013

epa
Environmental Protection Agency

Cryptosporidiosis: Human, animal and environmental interface in the Liffey and Lough Gill catchments

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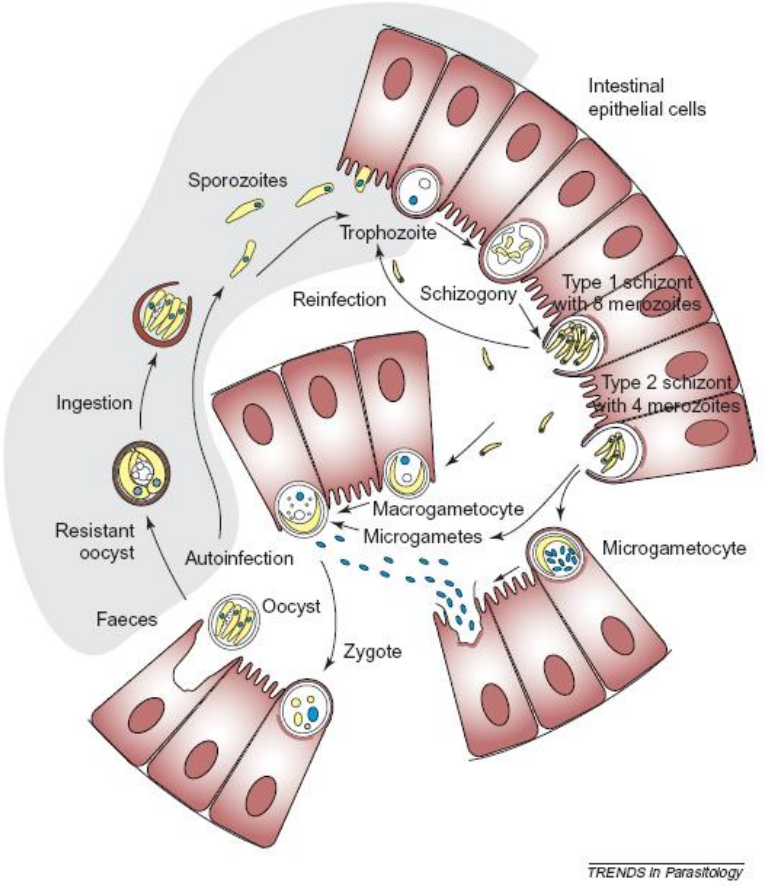
Outline

- Introduction & Background
- *Cryptosporidium* in humans
- *Cryptosporidium* in animals
- *Cryptosporidium* in surface water
- Conclusion

Cryptosporidium life cycle

- Direct life cycle
- Sporulated oocyst → environment
- Transmission: faecal-oral route
  - Waterborne
  - Foodborne
- Infect microvillus border of GIT – vertebrates
  - 3 spp: Gastric mucosa
  - 1 sp: Respiratory system



Cryptosporidium oocyst survival

- Very resistant!
  - Oocysts can remain viable in environment & animal liquid waste ~ 1 year
  - Resistant to environmental stressors
  - Resistant to most chemical disinfectants

http://www.bio-uv.com/fr/site/Piscines-spas-collectifs/Prevention-Cryptosporidium/Prevention-contre-les-pathogenes-parasitaires_129_.html

Cryptosporidium spp: Human

- 17 known *Cryptosporidium* species
  - 39 Cryptosporidium genotypes
- 9 *Cryptosporidium* species reported from human cases in England & Wales[1]
  - *C. hominis* (50.29%)
  - *C. parvum* (45.6%)
  - *C. meleagridis* (0.8%)
- Ireland[2,3]
  - *C. hominis* (20%)
  - *C. parvum* (80%)
  - *C. meleagridis*
  - *C.* deer genotype

1. Nichols, G., 2008. *Cryptosporidium* and Cryptosporidiosis 2nd Edition Fayer, R. & Xiao, L. (eds), CRC Press, USA.
2. Zintl, A., Proctor, A.F., Read, C., De Waal, T., Shanaghy, N., Fanning, S., Mulcahy, G., 2009, The prevalence of Cryptosporidium species and subtypes in human faecal samples in Ireland. Epidemiol. Infect. *137, 270-277.*
3. Graczyk, T.K., Lucy, F.E., Tamang, L. and Miraflor, A., 2007. Human enteropathogen load in activated sewage sludge and corresponding sewage sludge end products. Applied and Environmental Microbiology 73 (6):2013-2015.

Cryptosporidium in Ireland: Human

	2004	2005	2006	2007	2008 up to Sept
Cryptosporidiosis[1]	431	568	367	609	360

Crude incidence rate:

- 8.7 – 13.4/100,000 annually
- Rural areas reported more cases
- Regional as high as 31.4/100,000 per year

[1]Human cryptosporidiosis became a notifiable disease on January 1st 2004

Ireland: Seasonal distribution in humans

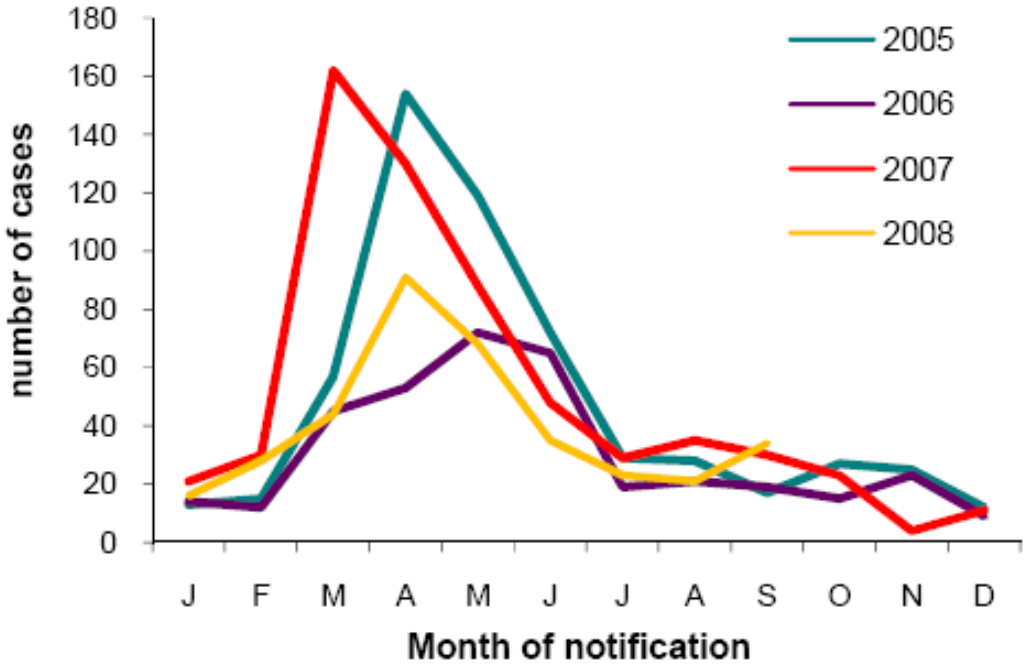


Figure 4. Seasonal distribution of cryptosporidiosis notifications 2005 to end quarter 3 2008

Cryptosporidium in Ireland: Animals

- Major cause of enteritis in neonatal animals
- Ireland - very little known
  - Calves
    - 2006: 25.9%[1]
  - Pigs
    - 2005: 15%[2]
  - Sheep/goats
    - ?

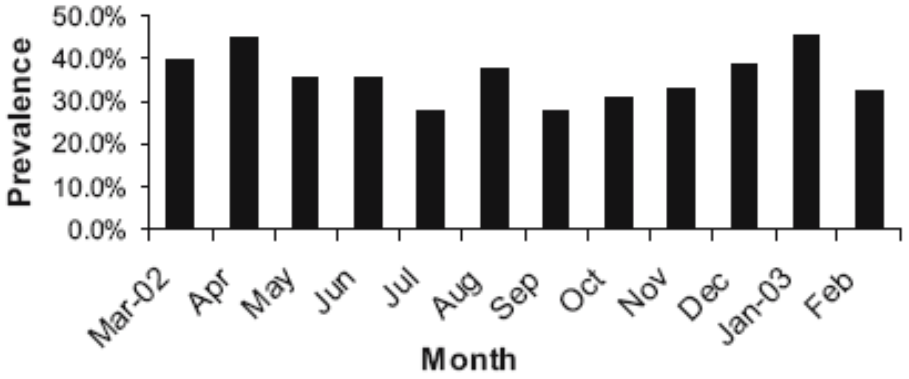


Fig. 1 Monthly prevalence of *Cryptosporidium* spp. in calves less than 30 days old in Northern Ireland March 2002 to February 2003

1 Thompson, H., Dooley, J., Kenny, J., McCoy, M., Lowery, C., Moore, J., Xiao, L., 2007, Genotypes and subtypes of *Cryptosporidium* spp. in neonatal calves in Northern Ireland. Parasitol. Res. *100, 619-624.*

2 Zintl, A., Neville, D., Maguire, D., Fanning, S., Mulcahy, G., Smith, H.V., De Waal, T., 2007, Prevalence of *Cryptosporidium* species in intensively farmed pigs in Ireland. Parasitology *134, 1575-1582.*

Cryptosporidium in Ireland: Animals

- Major cause of enteritis in neonatal animals
- Ireland - very little known
  - Horses
    - 1991: 29% of diarrhoeic foals[3]
  - Commercial deer herd
    - 2001: Common & asymptomatic[4]

Table 2
Mean oocyst counts for faecal samples taken from adult hinds from May 1996 to May 1997

Month	*n*	No. of positive samples	Mean ± S.D. (opg)	Range (opg)
May	30	0	0.0 ± 0.0	0
June	30	19	321.4 ± 1030.3	0–4704
July	30	16	2.8 ± 3.3	0–12
August	30	14	2.8 ± 3.5	0–12
October	30	8	2.1 ± 4.0	0–15
November	30	17	3.8 ± 4.4	0–15
January	30	6	6.1 ± 27.0	0–148
February	20	9	3.9 ± 10.1	0–44
April	30	12	1.7 ± 3.0	0–12
May	30	13	3796.8 ± 13503.4	0–67590

3 Browning, G.F., Chalmers, R.M., Snodgrass, D.R., Batt, R.M.,Hart, C.A., Ormarod, S.E., Leadon, D., Stoneham, S.J. and Rossdale, P.D. (1991). The prevalence of enteric pathogens in diarrhoeic Thoroughbred foals in Britain and Ireland. *Equine Veterinary Journal 23: 405-409.*

4 Skerrett, H.E. and Holland, C.V. (2001). Asymptomatic shedding of *Cryptosporidium oocysts by red deer hinds and calves. VeterinaryParasitology 94: 239-246.*

Cryptosporidium in Ireland: Environment

- Contamination of catchments used for drinking water abstraction has resulted in outbreaks of cryptosporidiosis worldwide
- Several Irish studies have detected *Cryptosporidium* species in Irish river basins[1,2]
- 2005 EPA risk assessment - Irish public water supply
  - 8% high risk
  - 13% very high risk
- Recent outbreaks in Ireland
  - Galway – 2007
    - ~240 confirmed cases

1. Graczyk, T.K., Conn, D.B., Lucy, F., Minchin, D., Tamang, L., Moura, L.N.S. and DaSilva, A.J., 2004. Human waterborne parasites in zebra mussels (Dreissena polymorpha) from the Shannon River drainage area, Ireland. Parasitology Research 93(5): 385-391.
2. Lucy, F.E., Graczyk T.K., Minchin, D., Tamang, L. and Miraflor, A. 2008..Biomonitoring of surface and coastal water for Cryptosporidium, Giardia and human virulent microsporidia using molluscan shellfish. Parasitology Research 103:1369-1375

Research need

- Research should be undertaken to: Elucidate the prevalence, epidemiology and mode of transmission of *Cryptosporidium in the* Irish context*

*Report of Waterborne Cryptosporidiosis Subcommittee of the Scientific Advisory Committee (2004)

Project objectives

- to identify the chief source(s) of *Cryptosporidium* oocysts in the environment during the spring peak
- to compile a database of *Cryptosporidium* species and subtypes that occur in livestock, wildlife, and the environment in 2 model water reservoir systems in the east and the west of the country.
- to identify species and subspecies that occur in the human population

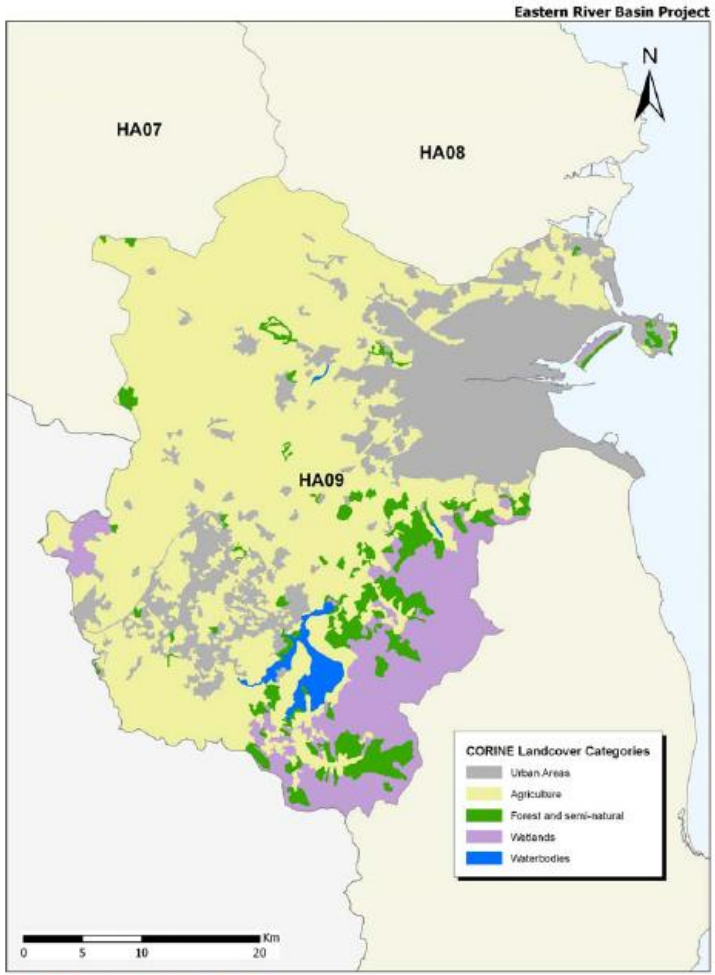
Study area 1: Hydrometric Area 09

- Eastern River Basin District
- HA09 (The “Liffey Catchment Area”) - most densely populated hydrometric areas in Ireland

Study area 1: Hydrometric Area 09

- Land use
  - Urban = 21%
  - Agricultural land = 61%
    - Pastures = 46%;
    - Arable land and crop cultivation = 12%
    - Managed forests = 3%
  - cattle in the middle catchment
  - sheep/forestry in the upper catchment

Figure 7.2: Diffuse Sources (HA09)



Study area 1: Hydrometric Area 09

- The Liffey Catchment area (HA09)
  - 676 km^2
  - 503.7 km of river channels
- 6 significant abstractions from surface waters
  - Poulaphouca Reservoir
    - 252,000 m^3/day
  - Leixlip Reservoir
    - 148,000 m^3/day

Study area 2: Lough Gill

- Western River Basin District
- HA35 (The Lough Gill catchment)

- Relatively sparsely populated
- < 0.5 mill people
- Urban infrastructure
  - ~ 0.03% of the basin area

Figure 2 Western RBD

Study area 2: Lough Gill

- Lough Gill 2 km east of Sligo town
- 10th largest lake in RI
  - 8 km long x 3.5 km wide
    - steep limestone shores and underwater cliffs
  - over 20m deep in places
  - surface area of 14km^2
- Catchment area 400km^2
  - Cattle & sheep farming & deer population
- Main water supply for Sligo town
  - Two water treatment plants - Cairn's Hill and Foxes Den
- Water supply for North Co. Leitrim

Project Team

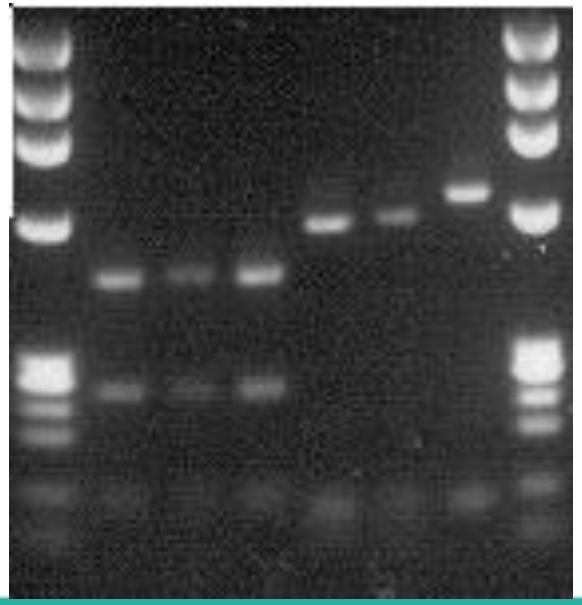
- **University College Dublin**
  - Dr Theo de Waal
  - Dr Annetta Zintl, Ms Carolyn Read, Prof Grace Mulcahy
  - **PhD student: Ms Marzieh Mirhashemi**
- **Institute of Technology, Sligo**
  - Dr Frances Lucy
  - **Technician: Mr Declan Feeney**
- **USA: Johns Hopkins Bloomberg School of Public Health**
  - **Prof Thaddeus Graczyk**
  - **Technician: Ms Leena Tamang**
- Teagasc: Animal Production Research Centre
  - Dr Barbara Good
- UK : Cryptosporidium Reference Unit
  - Dr Rachel Chalmers
- Fingal County Council
    - Mr George Sharpson

Analysis of human Cryptosporidium isolates

- *Cryptosporidium* species and subspecies present in human cryptosporidiosis cases
  - 186 *Cryptosporidium*-positive human stool samples collected from Irish patients between 2000 and 2007
  - 95 stool samples submitted to the UK *Cryptosporidium* reference lab in 2008

Typing to species …

- Oocyst concentration
- DNA extraction
- PCR-RFLP analysis of the SSU rRNA (Xiao et al 2001) and/or COWP loci (Spano et al 1997; Pedraza-Diaz et al 2001)



Xiao, *et al*., 2001, *Appl. Environ. Microbiol*. 67, 1097-1101.
Spano, *et al*., 1997, *FEMS Microbiol. Lett*. *150, 209-217.*
Pedraza-Diaz, *et al*., 2001, *Emerg. Infect. Dis*. *7, 49-56.*

.. and subspecies level

- sequence analysis of the gp60 region (Peng et al 2001; Alves et al 2003)

>DQ192508.gp60-IIa allele

…CAGCCGTTCCACTCAGAGGAACTTTAAAGGATGTTCCTGTTGAGGGC

TCA TCA TCG TCA TCG TCA TCG TCA TCA TCA TCA TCA TCA TCA
TCA TCA TCA TCA TCA TCA TCA

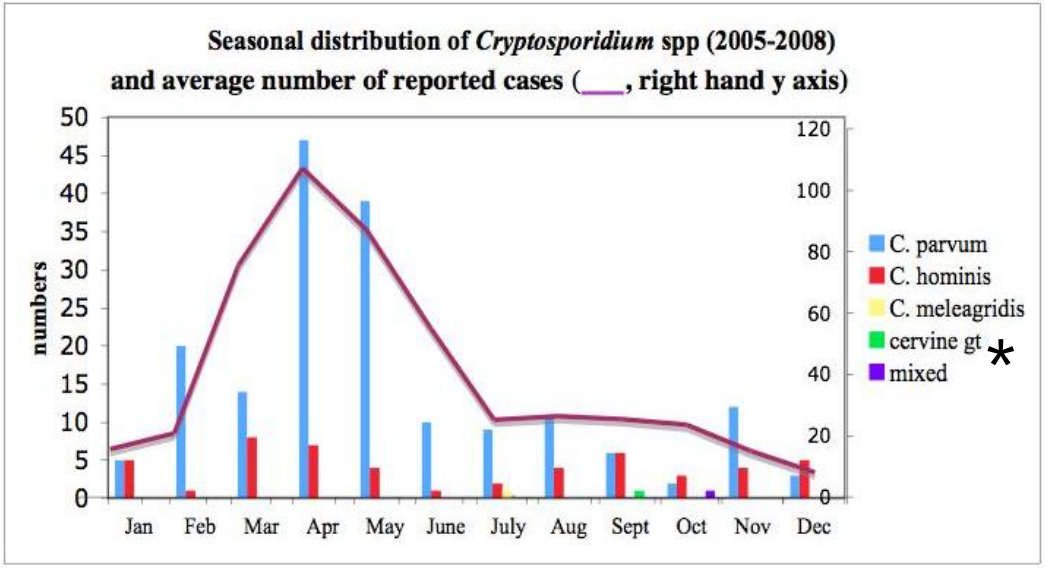
ACATCAACCGTCGCACCAGCAAATAAGGCAAGAACTGGAGAA…

'IIaA18G3R1'

Peng, *et al.*, 2001, *J. Eukaryot. Microbiol. Suppl*, 28S-31S.
Alves, *et al.*, , 2003, *J. Eukaryot. Microbiol. 50 Suppl, 529-530.*

Prevalence and seasonal distribution of *Cryptosporidium* spp

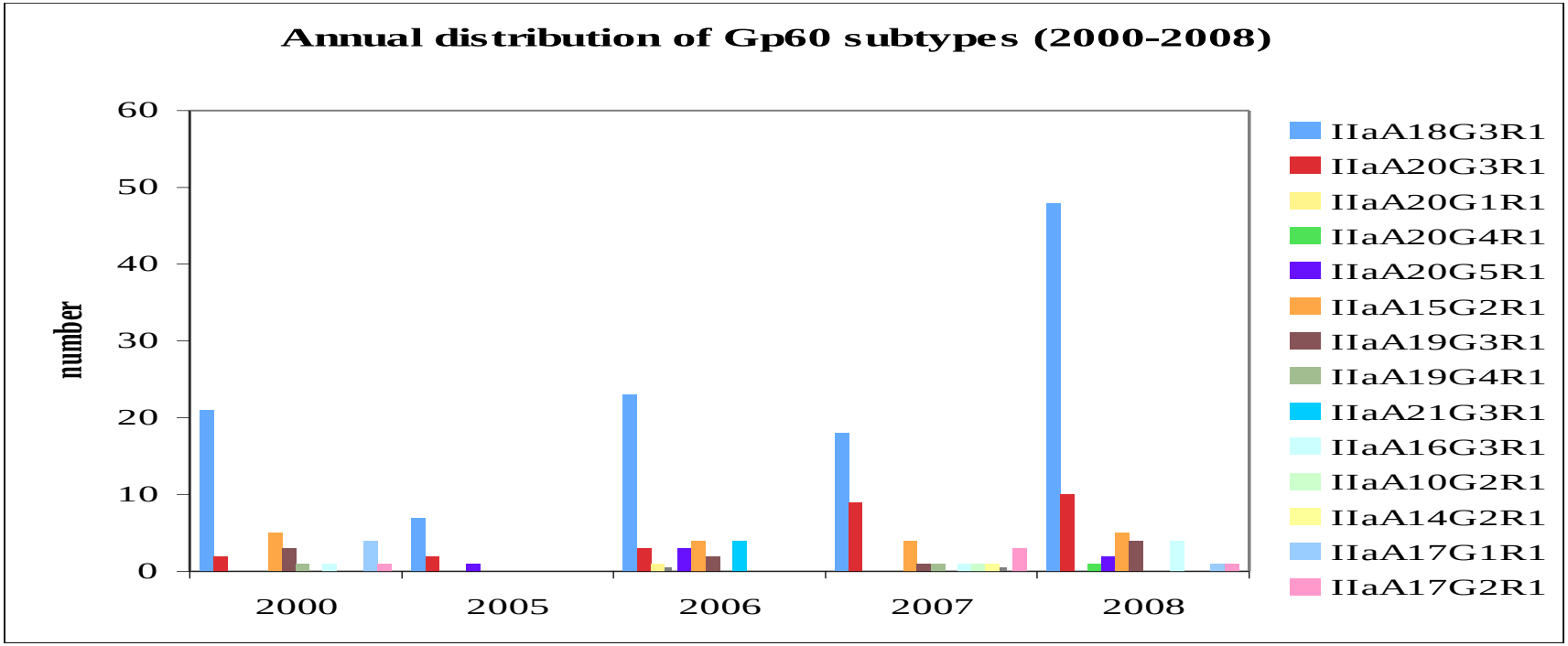
	2000	2005	2006	2007	2008
C. parvum	48	12	46	40	80
C. hominis	2	13	6	16	15
C. meleagridis					1
cervine gt *					1
mixed					1



**C. ryanae*

Annual distribution of *C. parvum* gp60 subtypes

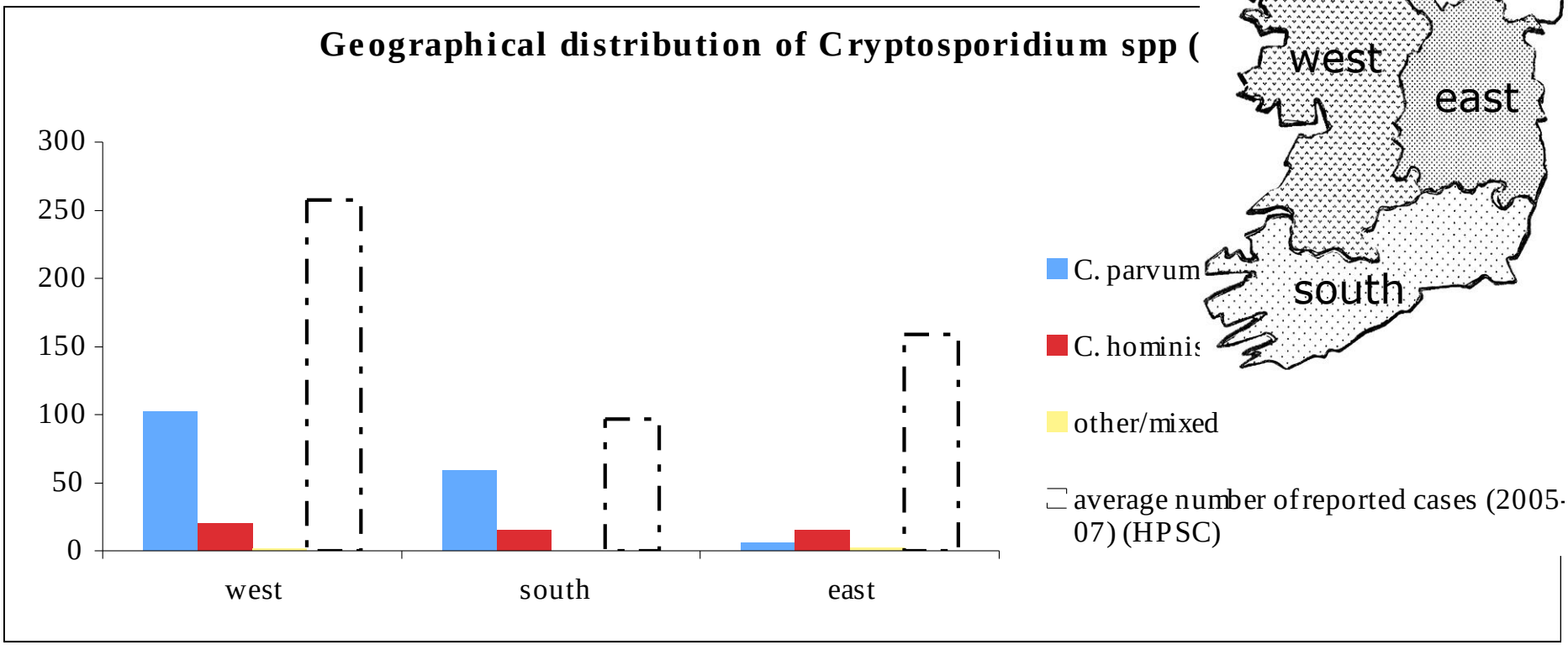
- 14 different gp60 subtypes identified
- IIaA18G3R1 type most prevalent



C. hominis gp60 subtypes

- 2000, 2005, 2006 and 2007: only IbA10G2R1 (n=25)
- 2008 (n=15): Detection of ‘new’ subtypes IbA9G3R1 (n=1) and IdA2 (n=2)

Geographical distribution of *Cryptosporidium* spp (2005-2008)

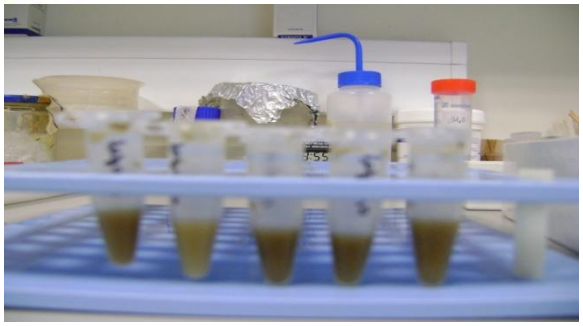


Cryptosporidium species and subspecies in livestock and wildlife spp.

- Lough Gill catchment
  - 4 Cattle, 3 Sheep and 1 Cattle/ Sheep farms
- Liffey catchments
  - 3 Cattle, 3 Sheep, 3 Horse farms

Month	Liffey catchments			Lough Gill catchment	
	Cattle	**Sheep**	**Horse**	**Cattle**	**Sheep**
March	13	48	25	37	20
April	13	48	29	36	29
May	16	34	26	50	55
June	22	30	17	60	75

Sample processing



Faecal sample

ELISA

Sugar Flotation

Smear preparation

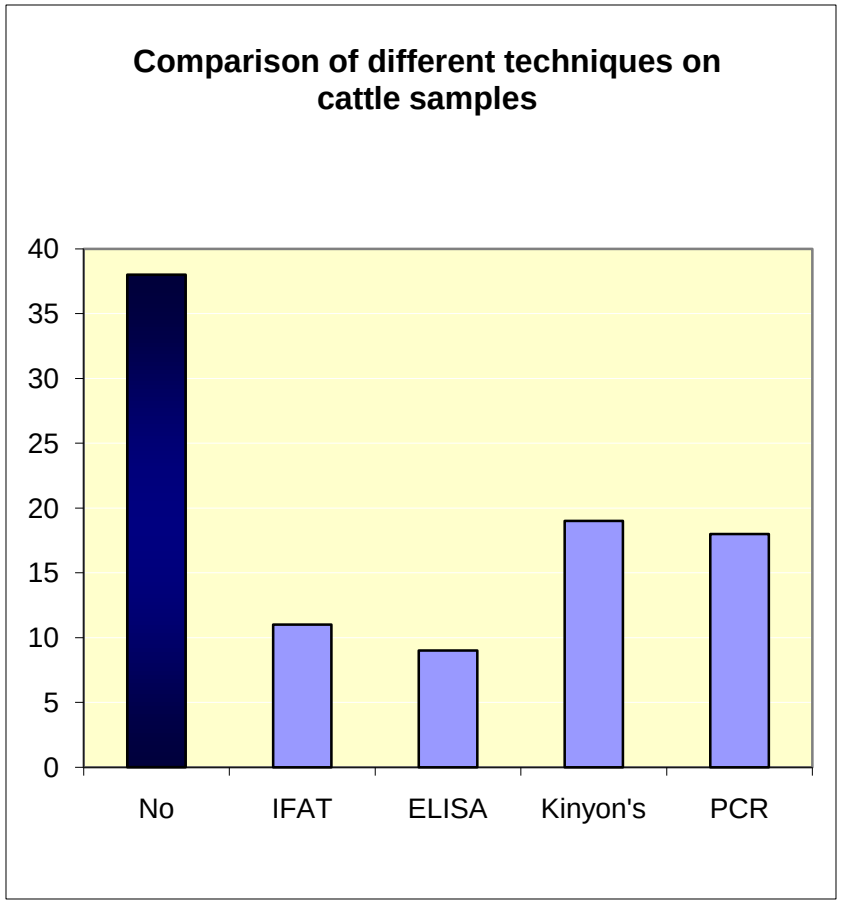
DNA extraction

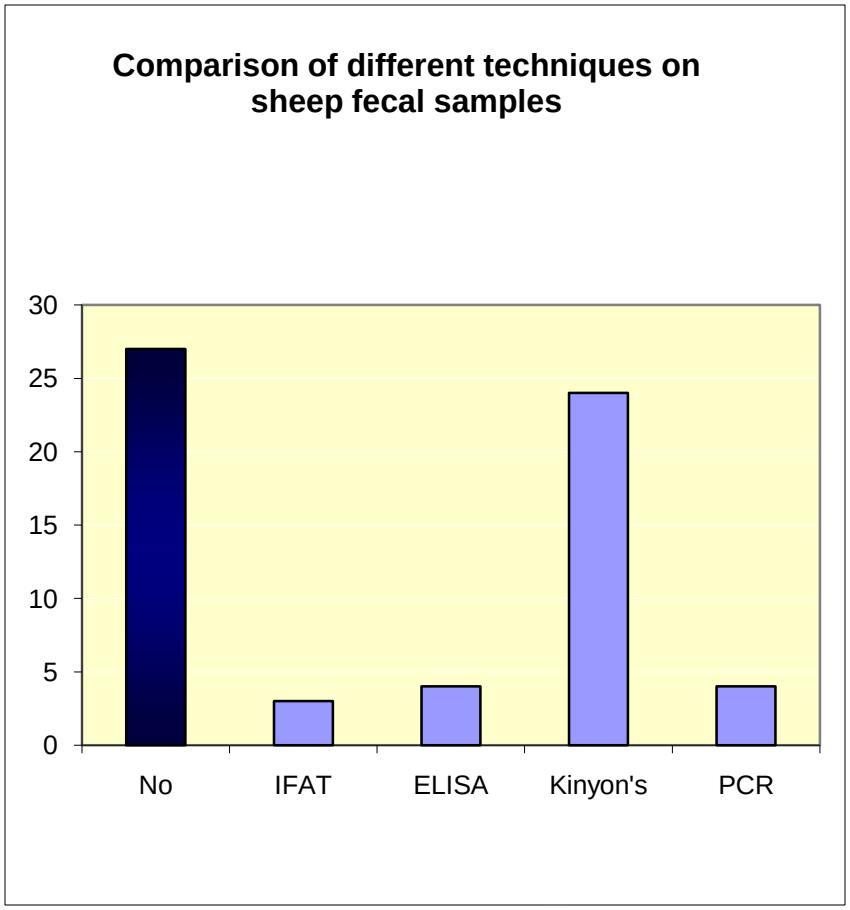
18S rRNA - Nested PCR

IFAT

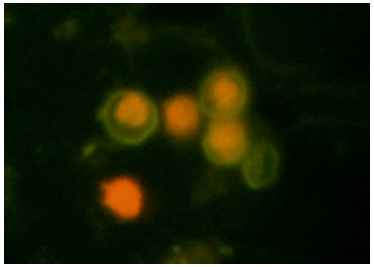
Kinyon's stain

Results


Comparison of different techniques on cattle samples
40
35
30
25
20
15
10
5
0
No
IFAT
ELISA
Kinyon's
PCR


Comparison of different techniques on sheep fecal samples
30
25
20
15
10
5
0
No
IFAT
ELISA
Kinyon's
PCR

Environmental sampling 2009



- Sampling
  - Lough Gill & Liffey Catchment
    - Winter - January
    - Spring/Summer – March, April, May, June, July
    - Autumn – October
- Biomonitors

Biomonitors

Asellus aquaticus - water hoglouse

- 30 minute sample
- Four sites on the River Liffey
  - Sites downstream of storm-water sewage overflows

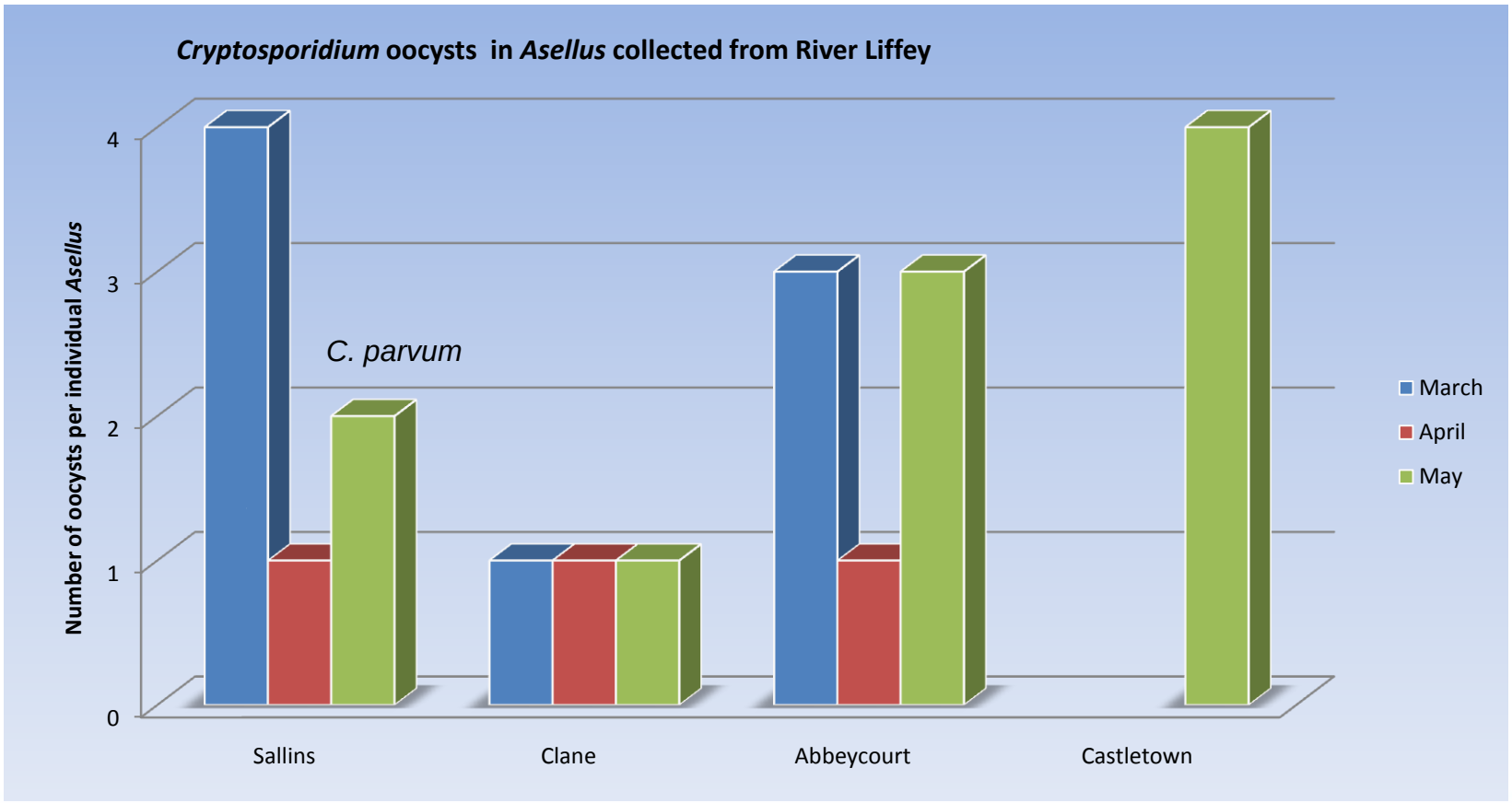
Dreissena polymorpha, zebra mussels

- 300g
- Four sites at Lough Gill

- Analysis by IFA, FISH[1] and nested PCR[2]

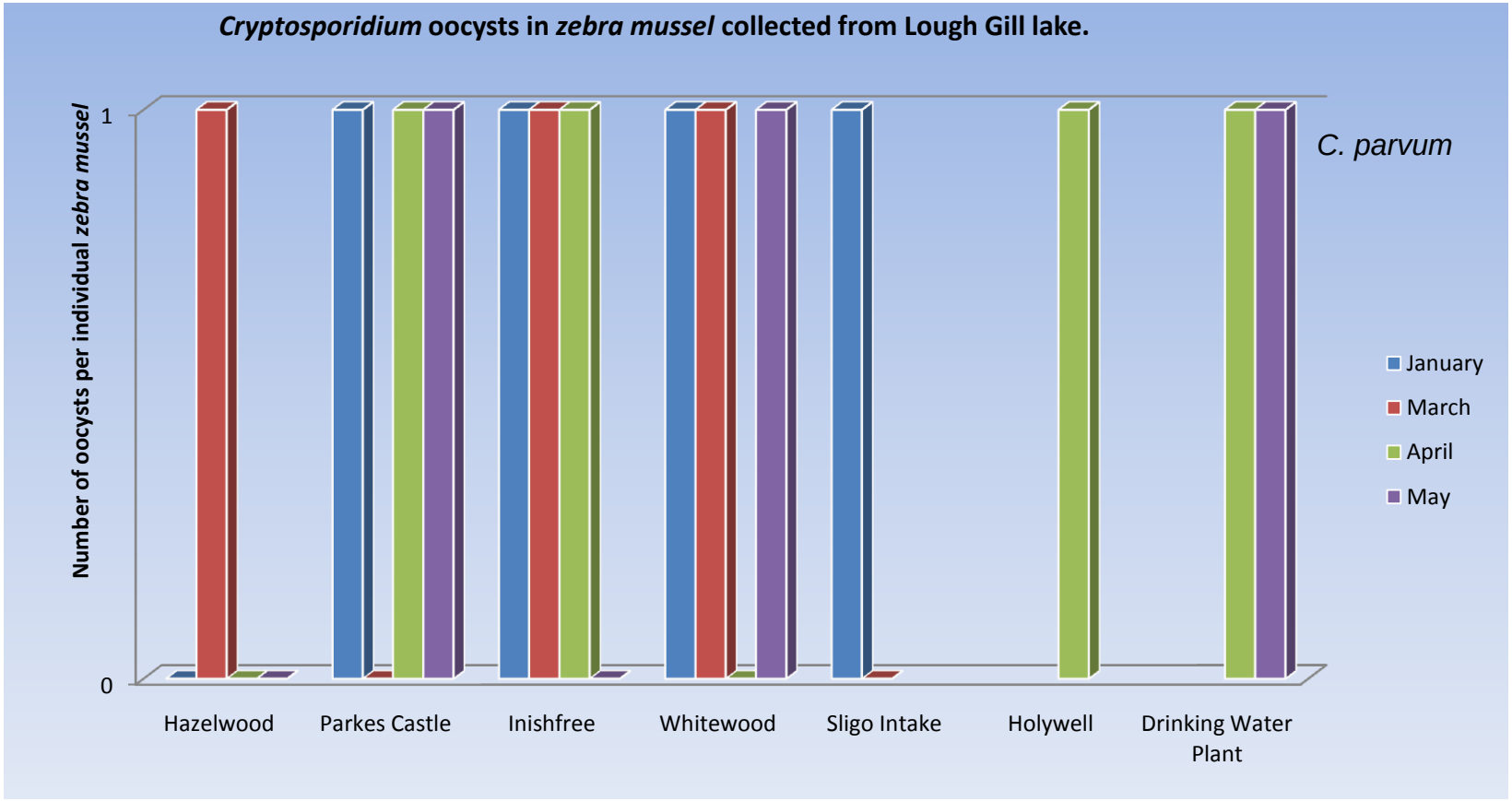
[1]Graczyk, *et al*., 2006. *Applied Environmental Microbiology*; 72: 3390-3395
[2]Downey, *et al.*, 2007. *Applied Environmental Microbiology*; 73: 6910-6915..

Biomonitoring Results – River Liffey



Lough Gill – Zebra Mussel Samples

Biomonitoring Results – Lough Gill



Summary & Conclusions

- Humans
  - *C. parvum* predominant, spring peak
  - *C. hominis*: weak bi-modal patter
  - *C. meleagridis*, *C. ryanae* first reported in 2008
  - gp60 subtypes
    - *C. parvum* - IIaA18G3R1 predominant in all parts of the country
    - *C. hominis* - IbA10G2R1 predominates

Summary & Conclusions

- Animals
  - IFAT useful screening techniques
  - ~28% samples PCR+
- Environment
  - Hoglouse & Zebra mussels good biomonitors
  - *Cryptosporidium* detected in all sites

Acknowledgements

- Human study
  - Annetta Zintl
- Animal study
  - Marzieh Mirhashemi
- Environmental study
  - Frances Lucy, Declan Feeney
  - Thaddeus Graczyk, Leena Tamang
- Funding
  - EPA – STRIVE programme

http://www.caricatures-ireland.com/blog/category/galway-water-crisis/