Cryptosporidium in the Cairngorms – a whole catchment approach

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“To prevent and control infectious diseases of livestock”

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Powerful water - purity and safety tested

"Protecting the health of Europeans against contaminated drinking water through increased understanding and accurate testing for waterborne pathogens"

- Developing new technologies to prevent and control the spread of waterborne disease
- €8.9 million EU project involving over 40 European partners
- Aquavalens connects a multidisciplinary team of scientists, engineers, public health practitioners and policy makers from 13 countries across Europe
- The role of Moredun in Aquavalens is to provide molecular tools for the detection and quantification of the protozoan parasites *Cryptosporidium* and *Toxoplasma* and to develop a parasite viability assay
- The scientific resources generated here are critical to the future of the project

COMMENT

ANDREW BIRAJ/REUTERS/CORBIS

In young children, the parasitic infection cryptosporidiosis is one of four leading causes of severe diarrhoea.

Time to tackle cryptosporidiosis

The little-studied parasite *Cryptosporidium* is a major threat to infants. **Boris Striepen** calls on microbiologists and funders to give it more attention.

Earlier this year, a massive clinical and epidemiological study[1] involving 22,500 children from Africa and Asia revealed — unexpectedly — that the protozoan parasite *Cryptosporidium* is one of four pathogens responsible for the lion's share of severe diarrhoea in infants and toddlers. According to the World Health Organization, diarrhoea accounts for 10.5% of the nearly 8 million yearly deaths of children under five years of age[2]. (For comparison, malaria causes 7% of such deaths, and HIV/AIDS, just 2%.)

Vaccines and treatments are already available or fast being developed for three of the four pathogens identified: rotavirus, *Shigella* bacteria and enterotoxigenic *Escherichia coli* (see 'Child killer'). But for 'crypto', there is no fully effective drug treatment or vaccine, and the basic research tools and infrastructure needed to discover, evaluate and develop such interventions are mostly lacking.

The technical challenges of working on crypto in the laboratory have led to the perception that the pathogen is an intriguing yet intractable problem. Crypto lab cultures last a few days at most, for instance, and some of the species that infect humans cannot be easily studied in standard model organisms such as mice. As a result, funders and biologists have tended to shy away from the parasite: a search on the biomedical research database PubMed suggests that in the past five years, about 20 times more articles have been published on malaria than on crypto.

I believe, however, that with the right tools, research approach and financial backing, the prevention of deaths caused ▶

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an parasite

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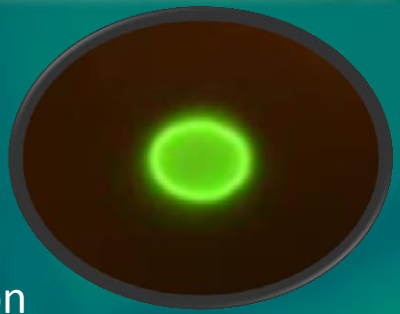

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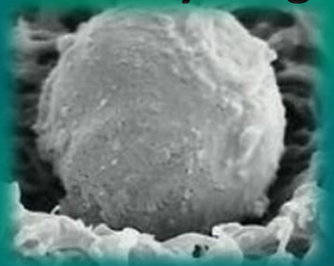
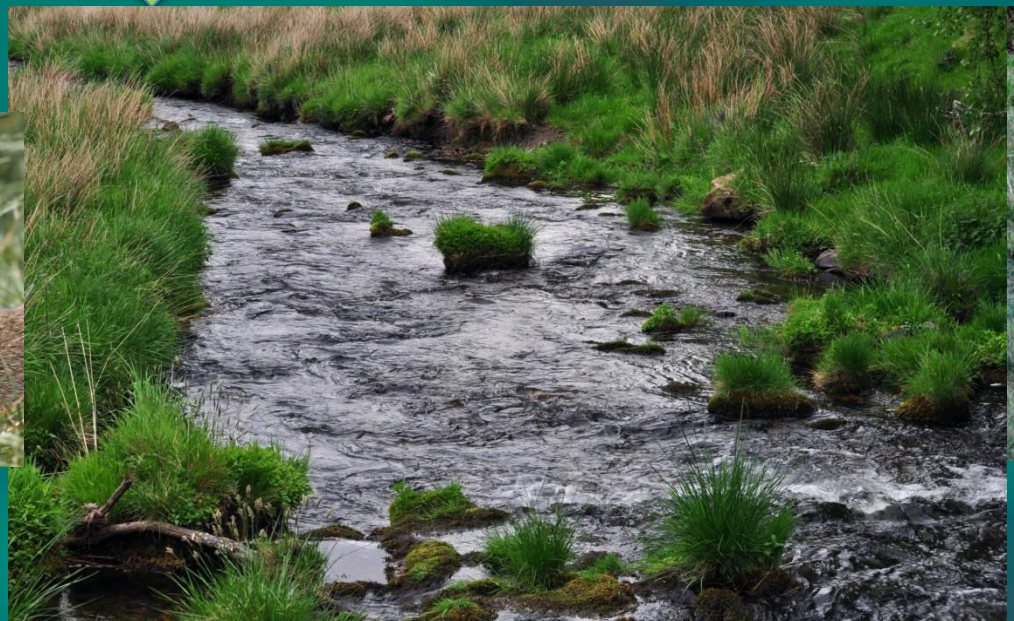


Transmission of *Cryptosporidium* oocysts in catchments



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The problem.....

Water is considered an important mechanism in the transmission of *Cryptosporidium*

This is particularly important where livestock have access to water courses

The *Cryptosporidium* (Scottish Water) Directive 2003: risk assessments for all public water supplies for *Cryptosporidium* was carried out and testing regimes implemented to monitor each supply according to the risk level

Is *Cryptosporidium* contamination in water a problem in the UK?

BBC NEWS

Sickness bug found in tap water

About 250,000 people in Northamptonshire are being told to boil tap water for drinking after a bug was found to have contaminated supplies.

Routine tests by Anglian Water found ***Cryptosporidium*** in a sample from supplies to Northampton, Daventry and surrounding villages. The parasite causes stomach upsets and the most common symptom is diarrhoea.

Anglian Water is now conducting further tests to track down the source of the bug and then they will flush it out.

Twenty schools in the area that rely on tap water for drinking fountains have now shut.

About 108,000 homes across 85 communities in Northamptonshire are affected by the contamination.

BBC Scotland

Water supply problems persist

About 140,000 people in Glasgow are still being advised to boil their tap water after the parasite cryptosporidium was found in supplies from Mugdock Reservoir in Milngavie, East Dunbartonshire

- Yes it is a problem! However most reported drinking water contamination issues do not result in cases of disease in humans due to effective testing regimes

- More than half of the human cases of cryptosporidiosis reported in the UK every year are caused by *C. parvum* – zoonotic transmission

A collaborative project to assess *Cryptosporidium* prevalence in a catchment in the Cairngorms National Park and determine the source of the parasite burden

Collaborators: Moredun Research Institute, Scottish Water, The Crown Estate and Cairngorm National Park

Pilot project where the results are being used to implement practical solutions in terms of livestock and wildlife management to reduce parasite contamination of the environment

Where on earth is Scotland???


THE GLENLIVET

Engagement was key:

- Water sampling and analysis – collaboration with Scottish Water
- Livestock – working with farmers and landowners
- Wildlife – engagement with gamekeepers, farmers and landowners

Project aims and methods

Aims:

To collect and analyse samples from water, sheep, lambs, cattle, calves and deer for *Cryptosporidium* - including speciation and genotyping

Apply innovative molecular diagnostic tools to enable source tracking – providing evidence for sound policy

Methods:

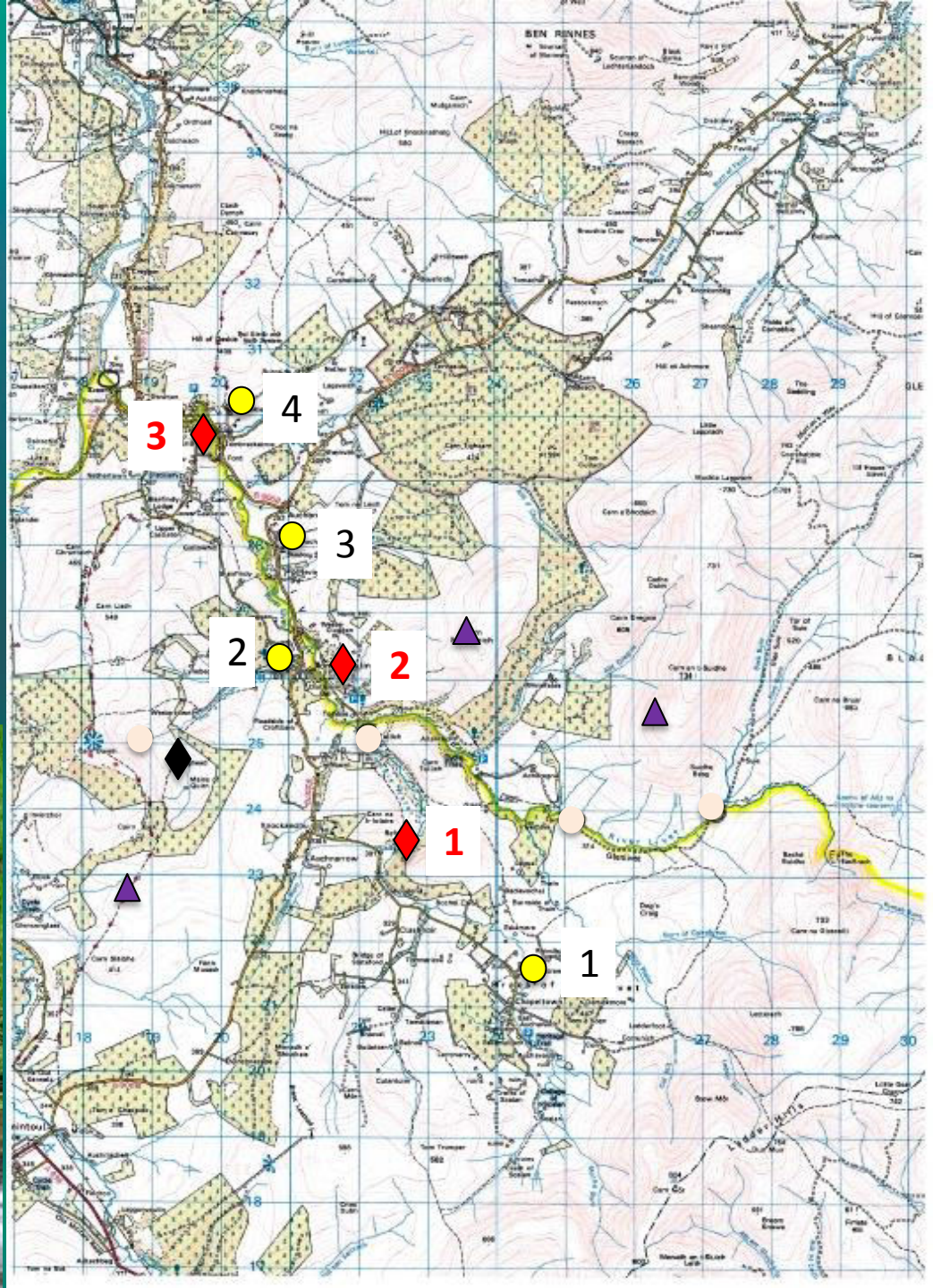
1. Three sample sets were collected – late March, early May and June 2014.

2. Concentration and extraction of *Cryptosporidium* oocysts from adult cattle, sheep and deer faecal samples using a more sensitive method recently developed at Moredun

3. 18S nested species specific multiplex PCR for highly sensitive and specific detection of the common *Cryptosporidium* species infecting cattle, sheep and deer

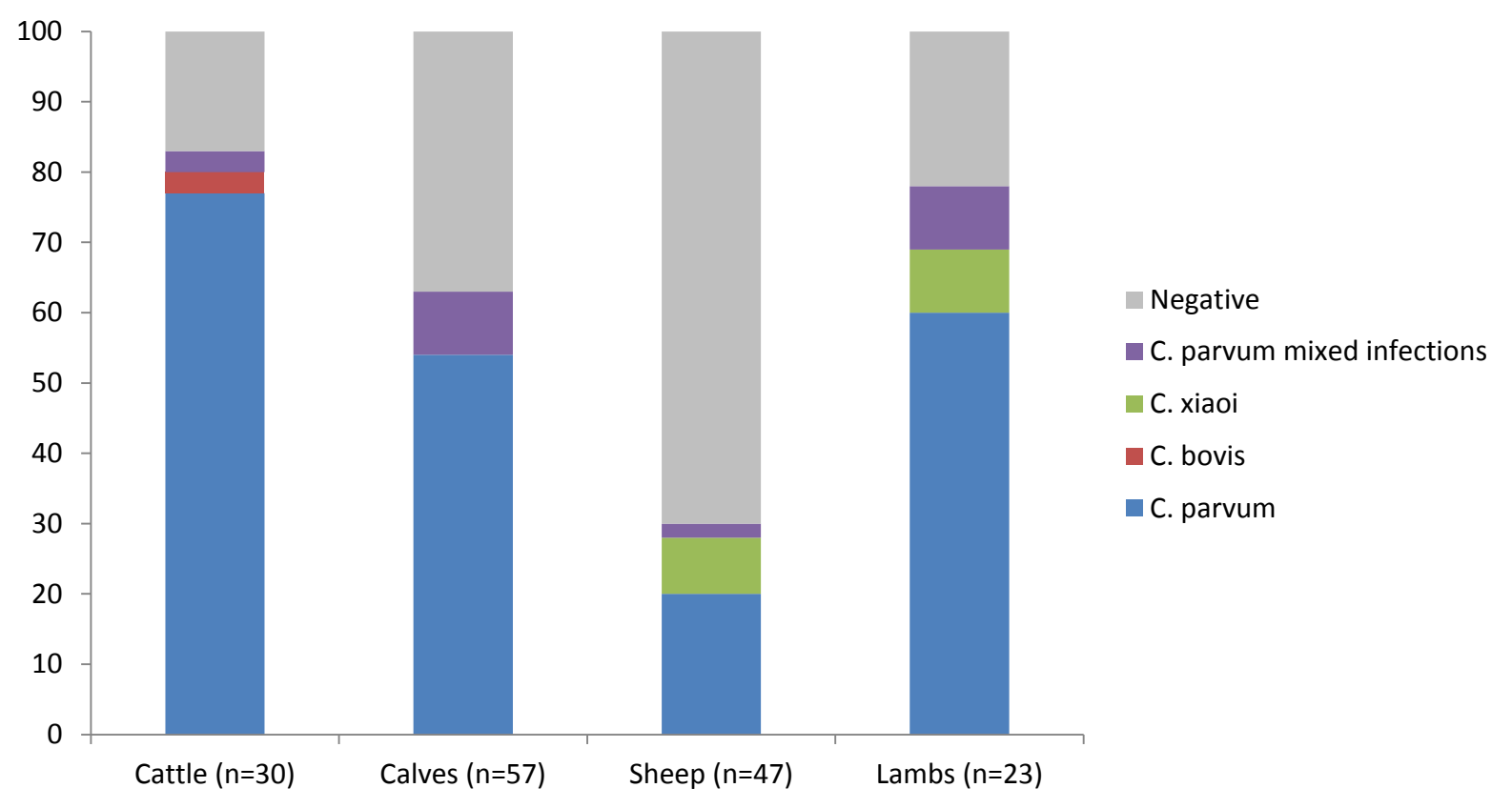
4. GP60 and other satellite marker analysis to establish *C. parvum* genotype (Emily Hotchkiss)

- Water sampling sites (**1-3**)
- Scottish Water public supply
- Farms for livestock sampling (**1-4**)
- Deer sampling areas



Results – farm livestock

Cryptosporidium species detected (%) in livestock across all time points and farms



High *Cryptosporidium* prevalence in cattle, calves and lambs

Most prevalent species *C. parvum* – important in terms of animal health, water quality and public health

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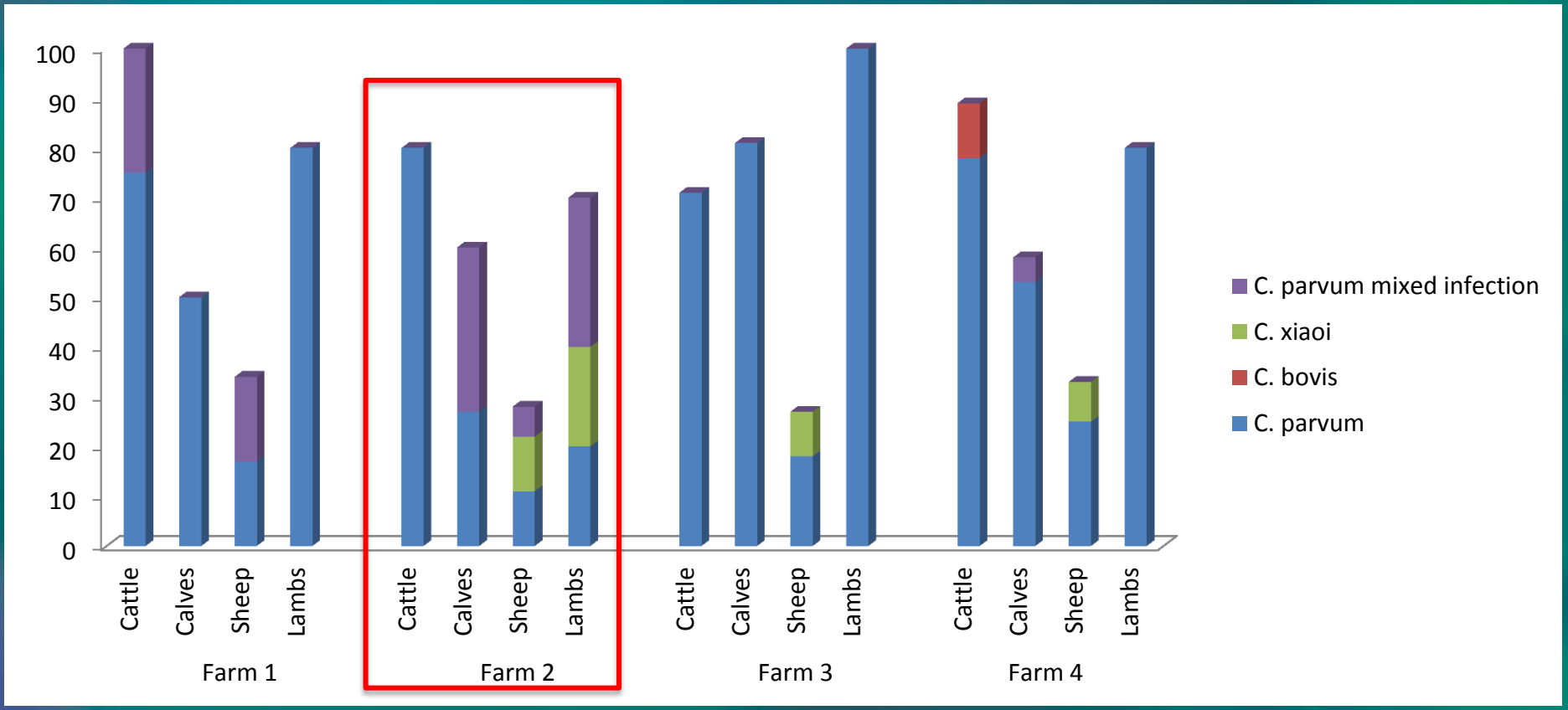
Results – farm livestock

Cryptosporidium species detected (% of positive samples) in livestock on individual farms



- Consistent results across the 4 farms

- High *Cryptosporidium* prevalence shown in adult cattle is unusual and is likely to be important for transmission through the catchment

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Water

Historical records show an ongoing *Cryptosporidium* problem in the public water supply in this catchment

These outbreaks have caused sickness and in at least one case hospitalisation in infected humans

The problems generally follow high intensity rainfall events

Date of sampling	Sampling site	Crypto Oocyst Count/10L	18S nssm PCR result	Species present
27.03.14	1	2	Pos	*C. parvum*
	2	<0.2	Neg	
	3	0.8	Neg	
05.05.14	1	1.67	Pos	*C. parvum*
	2	<0.2	Neg	
	3	2	Pos	*C. parvum*
03.06.14	1	0	Neg	
	2	0.3	Pos	*C. xiaoi*
	3	0	Neg	

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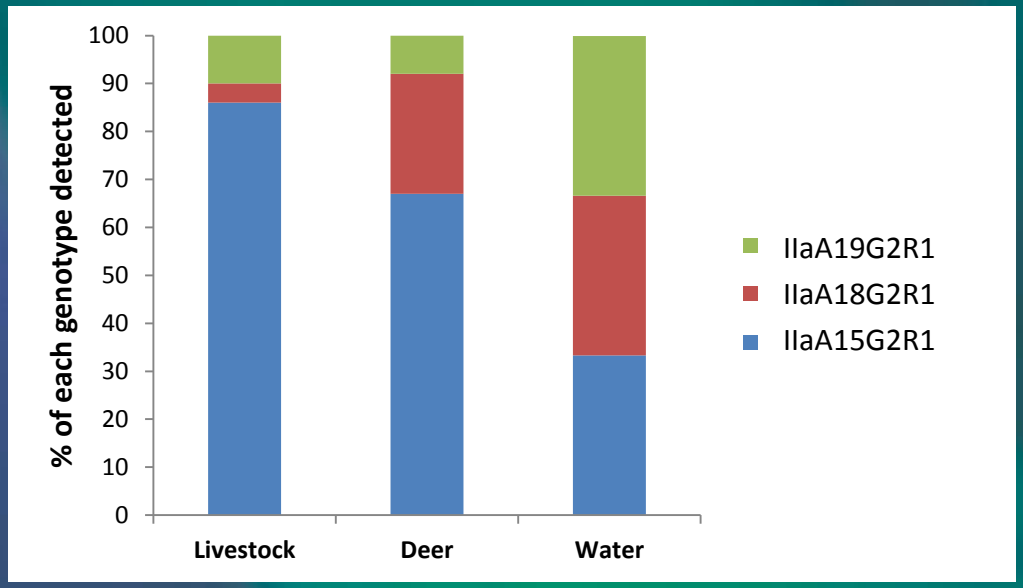
C. parvum GP60 genotyping – parasite transmission routes

- All farms had the same genotypes present – one very common one (IIaA15G2R1) and 2 less common (IIaA19G2R1 and IIaA18G2R1)

- All calves and most other livestock had mainly the IIaA15G2R1 genotype (found on all 4 farms)

- All 3 genotypes were isolated from red deer and from water samples

- Genotyping suggested sheep had a lesser role to play in water contamination in this area compared to cattle and deer



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In summary :

- High prevalence of *Cryptosporidium* in the Livet catchment particularly in the cattle, calves , lambs and red deer sampled

- Previous studies have found significantly lower prevalence in adult cattle and deer, indicating the higher sensitivity of the newly developed oocyst concentration technique

- The most prevalent species was *C. parvum* which has consequences for animal health, water quality and public health

- Sequencing has confirmed the PCR results

- GP60 marker analysis has established cattle, calves, lambs and deer as potential parasite sources for transmission into water

- The results are to be used in a Bayesian Belief Network (Ron Smith, Centre of Ecology and Hydrology) to highlight the real environmental costs of different management options and their effects on water quality

Project outputs

1. Improved land management of the catchment above the water supply intake

• Fencing, riparian woodland creation and grazing management

• Provision of water troughs – benefits livestock health by reducing livestock infection through the water source

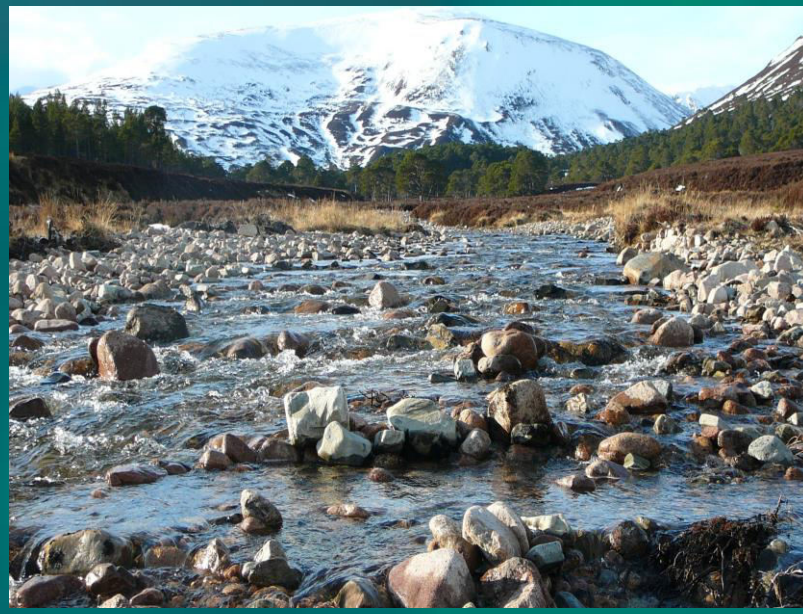
2. Management advice to farmers and vets – reduction of *Cryptosporidium* prevalence on farm

3. Meetings with Scottish Water Catchment Officers – improving understanding and dialogue between farmers and water providers

Outcomes for the catchment: everyone's a winner!

Potential Payment for Ecosystems Services (PES) schemes will:

- reduce water treatment costs
- improve water quality
- improve fish habitat
- enhance biodiversity
- landscape benefits



Reduction in oocyst burden in the catchment will lead to:

- healthier livestock and improved production
- improved food security
- less risk to the human population

Acknowledgements

Moredun
Lee Innes
Frank Katzer
Emily Hotchkiss
Hannah Shaw
Sarah Thomson
Stefano Guido
Alison Burrells
Clare Hamilton
Paul Bartley

Funders
Scottish Government
Aquavalens (EU)
Devolved Levy Boards
Genomia
Scottish Water

Collaborators
Scottish Water
The Crown Estate
Cairngorm National Park
Cryptosporidium Reference Unit
Centre of Ecology and Hydrology

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Thank you for your attention!

"The existence of *Cryptosporidium* in the water supply affects the management strategy of the water company and potentially the surrounding land managers. An ecosystem services approach highlights the real environmental costs of different management options, which can be explored within a decision framework that includes ecological, social and economic models to investigate potential benefits and trade-offs. Genotyping the *Cryptosporidium* species to allow source tracking allows better assessment of the strength of the possible pathways from ecology through management to delivery of clean water for the customer. The ecosystem approach with its transparency of the decision process fosters better understanding and agreement between the different stakeholders." **Ron Smith, Centre of Ecology and Hydrology**

Initial BBN for *Cryptosporidium* issue at Tomnavoulin

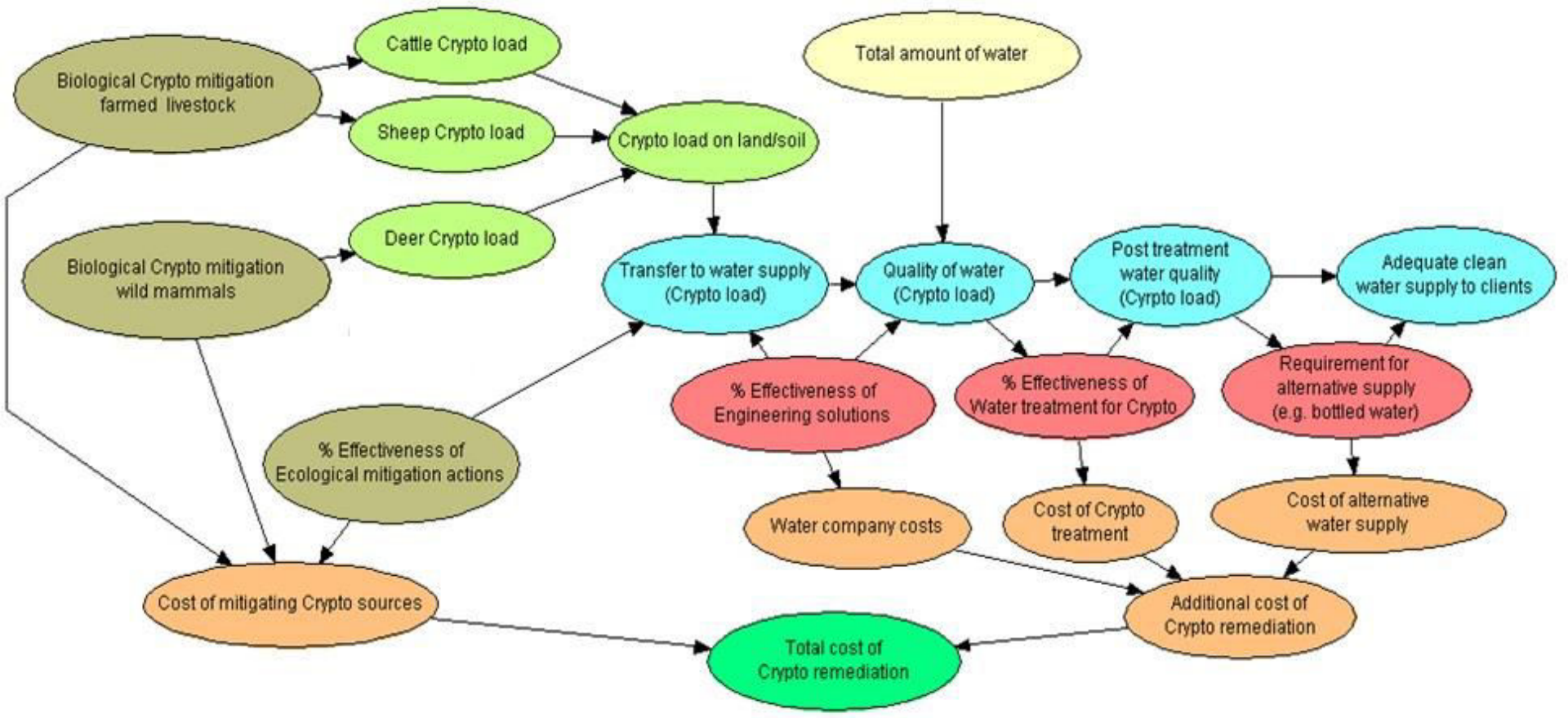

Table 3: Total percentages of livestock samples positive for *Cryptosporidium* in all the farms sampled

Livestock	Farm 1	Farm 2	Farm 3	Farm 4	Mean
Cattle	100 (n=4)	80 (n=10)	86 (n=7)	78 (n=9)	80 (n=30)
Calves	50 (n=8)	57 (n=14)	81 (n=16)	58 (n=19)	63 (n=57)
Sheep	33 (n=6)	28 (n=18)	27 (n=11)	42 (n=12)	30 (n=47)
Lambs	80 (n=5)	70 (n=10)	100 (n=3)	80 (n=5)	78 (n=23)