INVESTIGATION OF THE ZOONOTIC TRANSMISSION OF *CRYPTOSPORIDIUM*

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Aim:
This study investigated the relationship between *Cryptosporidium* isolates of human origin and isolates from animal contacts in the two weeks prior to the onset of symptoms. By analysing the *Cryptosporidium* species and subtypes present in the human cases and their animal contacts we addressed the question of whether the human infection could have been caused by zoonotic transmission.

Introduction:
Cryptosporidium is an important protozoan parasite which can cause diarrhoeal disease in humans. In healthy individuals it is usually self-limiting, but can be fatal in immunocompromised patients. There are two principal species which cause human disease in the UK:*Cryptosporidium parvum* and *Cryptosporidium hominis*, which between them account for 96% of cases in approximately equal proportions. *C. parvum* is considered to be a zoonotically acquired infection, while *C. hominis* is generally considered to be primarily spread within human populations. Both species can be transmitted directly or indirectly e.g. waterborne. The relative risk of human infection through direct or indirect contact with animals or animal faeces during occupational or recreational activities is largely unknown.

Method:
Between Nov 2004 and Nov 2006, 28 diagnostic laboratories, in 41 local authorities within the study areas of South West and East England and Wales, submitted 1030 human *Cryptosporidium*-positive faecal samples to the UK Cryptosporidium Reference Unit for species/subtype identification by PCR-RFLP and sequencing. Human cases were asked by the Local Authority Environmental Health Department to complete questionnaires, to identify cases reporting farmed or companion animal contact.

Eligible cases were identified which were not from an outbreak, not associated with foreign travel and had no other household members with diarrhoea. Permission was sought to follow up exposure with sampling of the companion and farm animals contacted by cases. The samples were analysed for the presence of *Cryptosporidium* oocysts by immunomagnetic separation (IMS) and immunofluorescence microscopy (IFM). PCR-RFLP at the 18s rRNA locus, PCR at the dhfr locus and analysis of part of the GP60 gene, were completed on a representative amount of samples from each animal species linked to each human case. Sequencing was also used to confirm speciation results and for GP60 subtyping.

Cryptosporidium stained with monoclonal IF antibodies

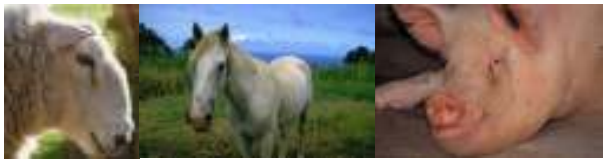
Results:
Of the 358 eligible cases, 206 had contact with companion animals and 80 with farm animal contact (63 had both companion and farm contact). 27 farm visits and 55 samplings of pet households were completed. A total of 669 samples were collected and *Cryptosporidium* was detected by IMS/IFM and/or PCR analysis in 33% of the animal samples (Table 1). Of those tested and positive by PCR, 77% were *C. parvum,* 8% were *C. andersoni*, 6% were *C. bovis,* 1% environmental genotype and 8% unclear patterns (Table 2). The results indicate that cattle had the highest percentage of positives in farmed animals and dogs had the highest in the companion animals. *C. parvum* was detected in cattle, dogs, horses, pigs and sheep.

Table 1: *Cryptosporidium* presence in farmed and companion animals.
*Muck heap samples from cattle sources

Sample source	No. positive samples	No. samples tested	% positive
Caged birds	0	4	0.0%
Cattle	62	116	53.4%
Cat	3	23	13.0%
Dog	19	59	32.2%
Goats	3	15	20.0%
Horses	6	20	30.0%
Llama	0	1	0.0%
Muck Heap*	2	4	50.0%
Other birds	2	30	6.7%
Pigs	17	43	39.5%
Poultry & game	3	58	5.2%
Rabbits & rodents	1	36	2.8%
Sheep	103	260	39.6%
Total	221	669	33.0%

Table 2: *Cryptosporidium* species detected by PCR from animal samples

Species	*C. andersoni*	*C. bovis*	*C. parvum*	Environmental genotype	Unclear pattern	Number of samples
Cattle	7 (16%)	2 (5%)	33 (77%)		1 (2%)	43
Cat				1 (100%)		1
Dog			4 (100%)			4
Horse			1 (100%)			1
Muck heap			1 (100%)			1
Pig			3 (60%)		2 (40%)	5
Sheep		3 (9%)	26 (79%)		4 (12%)	33



A positive animal sample was detected from investigations to 24/77 (31%) human cases.

For 13/16 of the human cases, the human *Cryptosporidium* species matched that of a animal sample (all *C. parvum*). For the other 8 either the human or animal sample did not provide a positive PCR result.

The matched results came from 3 dogs, 3 pigs, 1 horse, 22 cattle and 23 sheep.

The matched subtypes were:- IIaA17G1R1 (originating from 1 dog, 10 cattle, 3 pigs and 8 sheep); IIdA22G1 (1 dog); and IIaA15G2R1 (1 dog and 1 sheep).

***Cryptosporidium* Species Comparison**

For 9/10 of the human cases, the human sample subtype matched the animal subtypes

Conclusion:
Our data provides molecular evidence of matched species and subtypes in humans and animals who had recent contact, to support the evidence of a zoonotic transmission pathway of *C. parvum* through direct or indirect contact with farm and companion animals. Further analysis of the companion animal data is presented in R.P. Smith, R.M. Chalmers, et al (2009) Investigation of the role of companion animals in the zoonotic transmission of cryptosporidiosis. Zoonoses & Public Health, 56: 24-33.

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