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Investigation of farms linked to human patients with cryptosporidiosis in England and Wales

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ABSTRACT

The study investigates farms suspected of being sources of zoonotic human cryptosporidiosis. A variety of implicated farm animal species were sampled and tested to detect *Cryptosporidium* oocysts and investigate genetic linkage with human patients. Risk factor information was collected from each farm and analysed by multivariable logistic regression to detect significant associations between factors and *Cryptosporidium* in animals. The results showed that average sample prevalence of *Cryptosporidium* infection was highest in cattle, sheep and pigs (~40–50%), in the mid-range in goats and horses (20–25%) and lowest in rabbits/guinea pigs, chickens and other birds (~4–7%). A single sample from a farm dog was also positive. *Cryptosporidium parvum*, which has zoonotic potential, was the commonest species and was most likely to be present in cattle and, to a lesser extent, in sheep. In particular, young calves and lambs shed *C. parvum* and this finding was corroborated in a statistical model which demonstrated that samples from groups of preweaned animals were 11 times, and immature animal groups six times, more likely to be positive than groups of adult animals, and that samples from a farm with a cattle enterprise were twice as likely to be positive than farms without a cattle enterprise. On seven out of eight farms, at least one *C. parvum* isolate from an animal sample was indistinguishable at the gp60 locus from those found in the human patients, indicating that farm animals are a likely source of infection for humans.

1. Introduction

Human *Cryptosporidium* infection can result in the clinical disease cryptosporidiosis, which is mainly diarrhoeal, ranging in severity from mild to severe. In immuno-compromised patients, illness is often severe and can be fatal. The mean annual number of cases in England and Wales reported to the Health Protection Agency's Centre for Infection (CfI) over a 15 year period (1990–2005) was 4,559, an annual incidence of 8.9 cases per 100,000 population, with 10% cases linked to recognised outbreaks (Nichols et al., 2006). Patients are usually infected with one of two predominant human pathogenic species: *Cryptosporidium hominis*, acquired from other people or human sewage, and *Cryptosporidium parvum*, for which the main risk factor is contact with farmed animals (Hunter et al., 2004; Chalmers et al., 2009). However, animal- and human-specific *C. parvum* strains have been identified, which suggests that not all *C. parvum* isolates are equally infectious to the human population (Mallon et al., 2003; Alves et al., 2006; Hunter et al., 2007).

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C. parvum is a common cause of diarrhoea in young farmed animals, such as calves or lambs, and has been detected in all of the main livestock species, including in healthy animals. Large numbers of oocysts can be shed by asymptomatic, as well as symptomatic, lambs (Pritchard et al., 2007) and calves (Castro-Hermida et al., 2002), and the infectious dose for both animals and humans is low, thus it can be difficult to identify which animals should be isolated to restrict the chance of onward transmission (Defea, 2005). Animal infections with *C. hominis* are very rare and so far reported in a few farmed animals only (Ryan et al., 2005; Smith et al., 2005; Giles et al., 2009). The significance of the presence of this anthroponotic species in animals in relation to human infection is not known. *Cryptosporidium* infection has also been reported in a number of companion animals (pets) and wildlife in the UK and worldwide (Olson et al., 1997; Shukla et al., 2006; Ziegler et al., 2007; Smith et al., 2009).

Few practical, effective control measures have been identified for farmed animals, but these include the maintenance of closed herds or flocks, measures to limit spread between infected and uninfected groups of animals and minimising contamination of feed, housing, bedding and the wider environment (Tacal et al., 1987; Harp and Goff, 1998; Miller et al., 2008). Measures for the control of spread from animals to humans include hand hygiene following contact with animals (HSE, 2008) and improvements in source water catchment protection and water treatment (Sopwith et al., 2005).

The aim of this study was to investigate the range of farmed animal hosts potentially responsible for *Cryptosporidium* infection of humans, by estimating prevalence and comparing animal and human isolates, to indicate the importance of zoonotic transmission to human infection. An epidemiological analysis of farm questionnaire data was used to identify factors that were associated with *Cryptosporidium* infection in animals.

2. Method

2.1. Enrolment and sampling

Farms were identified through the investigation of laboratory confirmed human cryptosporidiosis cases reported to routine surveillance. Between November 2004 and November 2006, the 41 participating Local Authorities in Wales and parts of the South West and East of England were asked to complete an enhanced questionnaire for each patient. From this, patients with farm animal contact up to two weeks before onset of illness were identified for follow-up. Patients who may have acquired the infection through anthroponotic or foreign transmission routes were excluded, i.e. those linked to outbreaks were excluded; also patients who had recently travelled abroad or who had a household member with diarrhoea in the two weeks before illness (Smith et al., 2009).

If the patient and the farmer gave consent, the farm was visited to collect samples of fresh farm animal faeces and to complete a standardised farm visit questionnaire. The animal group or groups with which the human patient had reported contact were targeted for sampling. If the "population" of such animals was small in number (e.g. farm dogs; bottle fed lambs; hand reared calves), the aim was to obtain samples from every single animal. If the population size was large (e.g. a herd of dairy cows), a minimum number was sampled according to a standard table (Cannon and Roe, 1982), sufficient to estimate 10% prevalence (at 95% precision) in that group.

The interval between the recognition of human disease and animal sampling was kept as short as possible, to maximise oocyst detection and typing and thus the chance of successfully correlating human disease with animal excretion.

2.2. Sample testing

Full details of the human and animal testing are given in a previous publication (Smith et al., 2009). Briefly, for animal samples, two grams of faeces were suspended in water and centrifuged at $1{,}100 \times g$ for 15 min. The resultant pellet was purified by immuno-magnetic separation (IMS) (Isolate^{TM} TCS Biosciences Ltd UK) and then examined for recovered oocysts by immunofluorescence microscopy (IFM) (CryptoCel, TCS Biosciences Ltd) and DAPI staining. Oocyst shaped objects showing between two to four sporozoite nuclei were recorded as *Cryptosporidium* oocysts. Objects resembling oocysts but without DAPI stained sporozoites were also counted and recorded as oocyst-like bodies, but not included in the *Cryptosporidium* count. Enumeration results that were 'too numerous to count' were recorded as 1×10^6 oocysts per gram for comparison and analysis.

The *Cryptosporidium*-positive animal samples were prepared for DNA extraction using a freeze/thaw technique in a cardice/methanol bath followed by using the Qiagen stool extraction kit (Qiagen, Crawley, UK) (Robinson, 2005; Mueller-Doblies et al., 2008). Analysis was performed by Multiplex Allele Specific PCR (MAS-PCR) for *C. parvum* and *C. hominis* (Giles et al., 2002) and a nested PCR-RFLP based upon the SSU rRNA gene (Xiao et al., 2001) for differentiation of all *Cryptosporidium* species. Confirmatory double-stranded sequencing of purified SSU PCR products (Qiaquick PCR purification kit, Qiagen, Crawley, UK) was applied where equivocal RFLP patterns were obtained or as confirmation of the RFLP pattern. The results were compared with published sequences using the BlastN search programme (Altschul et al., 1997) on the Genbank and EMBL databases.

Cryptosporidium was identified in faecal samples from patients by routine microscopy in local diagnostic laboratories (HPA BSOP31) and positive samples submitted for typing to the UK *Cryptosporidium* Reference Unit (CRU; Chalmers et al., 2009). Briefly, DNA extracted from oocyst suspensions (Elwin et al., 2001) was initially tested by PCR-RFLP of the *Cryptosporidium* oocyst wall protein gene (Spano et al., 1997). Isolates where no amplicons were obtained or equivocal results generated using the COWP PCR were further tested by PCR-RFLP using nested primer sets for the SSU rRNA gene (Xiao et al., 2001).

A representational subset of all *C. parvum* isolates was investigated for variation within the gp60 gene by DNA sequence analysis using a nested PCR protocol (Alves et al.,