**DETECTION AND MOLECULAR CHARACTERISATION OF *CRYPTOSPORIDIUM* *PARVUM* IN BRITISH EUROPEAN HEDGEHOGS (*ERINACEUS EUROPAEUS*)**

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Abstract

Surveillance was conducted for the occurrence of protozoan parasites of the genus *Cryptosporidium* in European hedgehogs (*Erinaceus europaeus*) in Great Britain. In total, 108 voided faecal samples were collected from hedgehogs newly admitted to eight wildlife casualty treatment and rehabilitation centres. Terminal large intestinal (LI) contents from three hedgehog carcasses were also analysed. Information on host and location variables, including faecal appearance, body weight, and apparent health status, was compiled. Polymerase Chain Reaction (PCR) targeting the 18S ribosomal RNA gene, confirmed by sequencing, revealed an 8% (9/111) occurrence of *Cryptosporidium parvum* in faeces or LI contents, with no significant association between the host or location variables and infection. Archived small intestinal (SI) tissue from a hedgehog with histological evidence of cryptosporidiosis was also positive for *C. parvum* by PCR and sequence analysis of the 18S rRNA gene. No other *Cryptosporidium* species were detected. PCR and sequencing of the glycoprotein 60 gene identified three known zoonotic *C. parvum* subtypes not previously found in hedgehogs: IIdA17G1 (n=4), IIdA19G1 (n=1) and IIdA24G1 (n=1). These subtypes are also known to infect livestock. Another faecal sample contained *C. parvum* IIcA5G3j which has been found previously in hedgehogs, and for which there is one published report in a human, but is not known to affect livestock. The presence of zoonotic subtypes of *C. parvum* in British hedgehogs highlights a potential public health concern. Further research is needed to better understand the epidemiology and potential impacts of *Cryptosporidium* infection in hedgehogs.

Keywords: *Cryptosporidium parvum*, *Erinaceus europaeus*, European hedgehog, 18S rRNA, *gp60*.

1. Introduction

The European hedgehog (*Erinaceus europaeus*) is a small, nocturnal mammals that inhabits a variety of urban and rural habitats, often resulting in frequent direct and indirect contact with humans, domestic animals, and wildlife (Amori et al., 2008). There are a small number of reports of *Cryptosporidium* infection in this species in continental Europe and Great Britain (Sturdee et al., 1999; Enemark et al., 2002; Meredith and Milne, 2009; Dyachenko et al., 2010; Barlow, *pers. comm.* 2014), and in captive African hedgehogs (*Ateletrix albiventris*) in the U.S.A. (Graczyk et al., 1998) and Japan (Abe and Matsubara, 2015).

Infections with *Cryptosporidium* spp. protozoan parasitesare ubiquitous in wildlife, domestic animals and humans (Gosling, 2005; OIE, 2008), but infection does not always cause disease (Xiao et al., 2004). A lack of morphological differences between different *Cryptosporidium* species and genotypes has often resulted in wildlife being incorrectly incriminated as a reservoir for human infection (Appelbee et al., 2005). Application of molecular techniques has revealed that species naturally infecting wild mammals are frequently host-adapted, so are of little concern to human health (Appelbee et al., 2005; Dyachenko et al., 2010). However, wild mammals can harbour both host-adapted and zoonotic species (Appelbee et al., 2005), including *Cryptosporidium parvum* (OIE, 2008; Xiao, 2010).

Sequence analysis of the small sub-unit ribosomal RNA (18S rRNA) gene (Enemark et al., 2002), the 60 kDa glycoprotein (*gp60*), *actin* and 70 kDa heat shock protein (*hsp70*) gene fragments has been used to detect and characterise *Cryptosporidium* infection in European hedgehogs in Germany (Dyachenko et al., 2010), Denmark (Enemark et al. 2002) and the Netherlands (Krawczyk et al. 2015). Dyachenko and colleagues (2010) identified a *gp60* genotype from captive *E. erinacei*, which they considered to be hedgehog-specific. This was subsequently classified as the new species, *Cryptosporidium erinacei* by Kváč et al. (2014a), who found it to infect *A. albiventris* through experimental exposure. *Cryptosporidium erinacei* infection has subsequently been identified in clinically healthy horses in Algeria (Laatamna et al., 2013) and in a human in the Czech Republic (Kvác et al., 2014b). These data suggest that *C.erinacei* has a broader host range than initially suspected.

To investigate the occurrence of *Cryptosporidium* spp. in the free-living British hedgehog population, a survey was undertaken through testing voided faeces from hedgehogs recently admitted to wildlife centres and terminal large (LI) intestinal contents from hedgehogs found dead or euthanased. Isolates were characterised and associations assessed between *Cryptosporidium* infection and host and location variables.

2. Materials and methods

*2.1. Collection of samples*

From April-June 2014, a single voided faecal sample was collected from each of 108 individually housed hedgehogs newly admitted (most within 48 hours of admission) to eight geographically dispersed wildlife casualty treatment and rehabilitation centres in Great Britain (Fig. 1). Maternally-dependent juveniles were excluded from the study. A standardised submission form provided basic information about the individual, including body weight, sex, approximate location found and reason for casualty presentation. Faecal consistency, colour and the presence of blood were recorded. Terminal LI contents were collected from three carcasses of free-living hedgehogs during post mortem examination as part of the Garden Wildlife Health project (GWH, 2014).

Samples (minimum 1 g) were stored for up to one week at 4 oC, after which they were stored in 2.5% (w/v) potassium dichromate at 4oC until processing.

Additionally, a sample of small intestine was examined from a juvenile female hedgehog found in Worcestershire, England, in October 2012 and admitted to wildlife centre G (Fig. 1). During a period of circa three months in care, the hedgehog received antimicrobial and anthelminthic treatment and fluid therapy. It gained body weight from its admission at 173 g to a maximum recorded value of 411 g whilst in captivity. It had variable appetite throughout its time in care, but deteriorated to become inappetent and latterly anorexic, when it developed abnormal green faeces sometimes described as of ‘runny’ consistency. The hedgehog progressed to a recumbent state and was euthanased and submitted for post-mortem examination in February 2013. Histopathological examination showed evidence of intestinal cryptosporidiosis. An archived intestinal tract tissue sample (stored at -20 oC) was examined to characterise the *Cryptosporidium* species involved.

*2.2. Faecal processing and DNA purification*

To recover and enrich oocysts, faecal samples and terminal LI contents were centrifuged at 200 g for five minutes, the potassium dichromate decanted and one gram of faeces subjected to saturated salt flotation (Kuczynska and Shelton, 1999). Four surface aliquots per sample were pooled, washed in phosphate buffered saline (PBS) by centrifugation at 4,000 g for one minute, the sediment re-suspended in 200 µl of PBS, boiled for five minutes and centrifuged at 10,000 g for one minute (process adapted from Abe et al. 2002). DNA was extracted from the resulting supernatant using a Qiagen DNeasy Blood and Tissue Kit (Qiagen©, Germany) following the manufacturer’s protocol. Samples were stored at -20 oC prior to ethanol precipitation (0.1 volumes sodium acetate, 3 M pH 5.2; 2.5 volumes ice cold 100% ethanol and 1 µl glycogen as a carrier; centrifugation at 10,000 g for 15 minutes; washed in one volume 70% ethanol and re-suspended in 20 µl molecular grade water).

Additionally, DNA was extracted from four 1 cm2 sections of archived SI tract collected from the hedgehog with histological evidence of cryptosporidiosis using a Qiagen DNeasy Blood and Tissue Kit (Qiagen©, Germany) following the manufacturer’s instructions for tissue samples.

*2.3 Polymerase chain reaction* Cryptosporidium *detection and genotyping*

Purified DNA was tested for *Cryptosporidium* spp. using conventional PCR targeting a ~300 bp region of the *Cryptosporidium* 18S gene as described elsewhere (Morgan et al., 1997). *Cryptosporidium parvum* 18S rDNA derived from an infection in a domestic dog and cloned into pGEM-T easy (Promega, Southampton, UK) was used as a positive control and molecular grade water as a negative control. PCR amplification was performed in a volume of 25µl. PCR amplicons were resolved by electrophoretic separation through 2% (w/v) agarose gel (Ultrapure™ agarose powder in 0.5x Tris-Borate-EDTA buffer) stained with 0.01% (v/v) SafeView nucleic acid stain (NBS Biologicals, U.K.) and visualised under ultraviolet light using an U:Genius Image Capture gel documentation system (Syngene, U.K). Samples positive by 18S PCR were subtyped using a nested PCR targeting the *Cryptosporidium* *gp60* gene (Alves et al., 2003; Dyachenko et al., 2010). PCR products of the anticipated size were purified using a Qiagen MinElute Purification Kit (18S) or QIAquick Gel Extraction Kit (*gp60*) as recommended by the manufacturer (Qiagen©, Germany). Purified PCR products were sequenced in each direction by GATC Biotech (Cologne, Germany) using the same primers as used for the original PCR.

Sequences were analysed in CLC Main Workbench Version 5.7.1 using BLASTn against the National Centre for Biotechnology Information (NCBI) non-redundant nucleotide collection. The *gp60* genotypes were identified according to sequence and serine repeat characteristics (Sulaiman et al., 2005).

*2.4. Phylogenetic analysis*

To assess the relationship between *gp60* subtypes identified from the hedgehogs in this study and those published in GenBank (reference accession numbers as shown in Supplementary Figure 1), a series of phylogenetic trees were constructed, incorporating the reference sequences used by Kváč et al. (2014a). All sequences were aligned using ClustalW and trimmed in CLC Main Workbench using default parameters. The assembled sequences were analysed using the maximum likelihood (ML), neighbour joining (NJ) and maximum parsimony (MP) methods. ML analysis used the Kimura 2 + Gamma distribution model in MEGA 5.10, identified using the Bayesian Information Criterion with 1000 bootstrap replicates. NJ and MP analyses used the Kimura 2 model in MEGA 5.10 with 1000 bootstrap replicates.

*2.5 Statistical analyses*

For analysis of infection status and host and location variables, individuals were classed, based on their reason for admittance, as ‘apparently healthy’ (victims of road traffic accidents and other physical reasons for presentation, e.g. entanglement) or ‘apparently unhealthy’ (those classified as ‘underweight’ by centre staff or that were found ‘out during the day' since these observations are known to be common in hedgehogs with disease when submitted as wildlife casualties (Robinson and Routh, 1999)). Hedgehogs that had been predated were assessed on an individual basis for classification using body weight and any other supporting information that was available.

Faeces were classified as ‘normal’ (solid, brown and free of blood) or ‘abnormal’.

Statistical analyses were performed using ‘R’ (version 3.1.0) and significance was assigned when P < 0.05. Possible associations between the presence/absence of *Cryptosporidium* by 18S PCR and the host and location variables sex, centre from which the sample originated, apparent health status and faecal appearance, were tested by Fisher’s exact test and mean body weight by Welch’s t-test.

The distribution of the sampling centres was mapped (QGIS 2.4; www.qgis.org) to demonstrate the sample and *Cryptosporidium* spp. and genotype distribution.

3. Results

3.1. *Post-mortem examination*

Post-mortem examinations were performed on three hedgehogs from which LI contents were collected for testing; two had been euthanased with traumatic injuries apparently caused by predation while the third had a disseminated bacterial infection which was considered to be the cause of death. No other significant abnormalities were detected.

The juvenile female hedgehog that was euthanased in a wildlife centre was in good body condition with ample fat deposits. Macroscopic examination revealed subcutaneous oedema and visceral congestion. Light microscopic examination of a saline-mount direct preparation of SI contents was negative for metazoan parasites. Microbiological examination of the liver and SI contents yielded no significant isolates. Histological examination revealed non-suppurative meningo-encephalitis and localised jejunal cryptosporidiosis. Numerous round bodies (2-3.5 µm diameter and morphology characteristic of *Cryptosporidium* sp. parasites) were adherent to the intestinal epithelium and free within the lumen. These were associated with blunting and shortening of the villi in which there was interstitial oedema and a plasmalymphocytic inflammatory cell infiltrate (Fig. 2). Sparse parasitic infection was observed in a second SI section, whilst no evidence of infection was observed in three other sections of different regions of the small intestine.

3.2. *Detection of* Cryptosporidium *in hedgehogs by PCR*

Based on 18S PCR amplicon sequencing, nine of the 111 (8%; 95% CI: 3-13%) faecal and LI contents samples were positive for *C. parvum*; all were from hedgehogs < 48 h in captivity. The SI tissue sample from the hedgehog examined post mortem which had been in captivity for circa three months was also positive.

No significant association was found between being positive for C*ryptosporidium* and any of the host and location variables examined (see Supplementary Table 1).

Of the 10 samples found to be positive using 18S PCR, seven (six faecal, one SI tissue) were also positive using *gp60* PCR. Six *gp60* PCR products were assigned to subtype family IId by BLASTn. Three different IId subtypes were identified (IIdA17G1, IIdA19G1, IIdA24G1). The seventh *gp60* PCR product, from a faecal sample, was assigned to subtype family IIc, identified as subtype IIcA5G3j. The locations at which these different subtypes occurred are shown in Fig. 1. The sequences generated in this study have been submitted to GenBank with the accession numbers LN714778-87 (18S) and LN714788-94 (*gp60*).

3.3. *Phylogenetic analysis*

The ML tree for the *gp60* gene (see Supplementary Figure 1) showed that six of the seven sequences in this study form a clade with the annotated *C. parvum* IId GenBank sequence to which they are most similar on BLASTn analysis (JF727809), as well as AY738194, identified from human hosts in Kuwait (Sulaiman et al. 2005). This clade is separate to those sequences published for *C. erinacei* from theCzech Republic(KF612329) (Kváč et al., 2014b)and Germany (e.g. GQ214081 and GQ259140) (Dyachenko et al., 2010). The remaining *gp60* sequence annotated as IIc was identical to sequence GQ259136, isolated previously from a German hedgehog (Dyachenko et al., 2010). The NJ and MP methods produced phylogenies with comparable topologies (data not shown).

3.4. *Spatial distribution*

There was a wide spatial distribution of *Cryptosporidium* infection in the hedgehogs examined (Fig 1). Subtype IIdA17G1 was found in hedgehogs at multiple locations (centres B, C, F, G) and multiple subtypes (IIdA17G1, IIdA19G1 and IIdA24G1) were identified from hedgehogs admitted to centre B. Subtype IIcA5G3j was identified from a hedgehog from centre G.

4. Discussion

We found *C. parvum* infection in ten European hedgehogs, of which six samples were identified as *gp60* subtype family IId. To our knowledge, this is the first report of these potentially zoonotic *C. parvum* subtypes in hedgehogs (IIdA17G1, IIdA19G1 and IIdA24G1). Our findings indicate that subtype IIdA17G1 appears widespread in British hedgehogs, but the sample size is too small to make conclusions on the distribution of the other subtypes found.

A seventh sample was confirmed as *gp60* subtype IIcA5G3j, previously identified in the United Kingdom from humans (Chalmers et al., 2011a) and European hedgehogs in Germany (Dyachenko et al., 2010). Subtype IIcA5G3 was identified in a study of hedgehogs in the Netherlands (Krawczyk et al., 2015), but without the suffix or sequences deposited on GenBank it is not possible to compare this with that found in our study.

Our results are consistent with previous published (Meredith & Milne 2009) and unpublished (Barlow, *pers. comm.* 2014) reports describing *Cryptosporidium* infection in hedgehogs in Great Britain. *Cryptosporidium* infection has previously been identified in European hedgehogs admitted to wildlife centres in Germany (Dyachenko et al., 2010), Denmark (Enemark et al., 2002) and the Netherlands (Krawczyk et al., 2015). The 8% occurrence in this study is similar to published values from the Netherlands (9%; 8/90) (Krawczyk et al., 2015), but considerably lower than in Germany (Dyachenko et al., 2010), where prevalence was estimated at up to 39.4% (45/114) in animals commencing treatment and rehabilitation (based on an immunoassay for coproantigen detection and microscopy of faeces). However, the study by Dyachenko and colleagues (2010) used a non-randomised population as individuals with diarrhoea were preferentially selected and the sample included juveniles. In other species, such as cattle, cryptosporidiosis is usually more common in juveniles than in adults (Constable, 2010). In the current study, sample collection was from maternally-independent hedgehogs newly admitted to the participating wildlife centres without further selection criteria.

Examination of a larger number of samples is required to robustly document the prevalence of *Cryptosporidium* infection in British hedgehogs and to explore whether seasonal or spatial variation occurs. Future application of a nested PCR approach targeting the multi-copy 18S rRNA gene would be expected to improve sensitivity of detection (Jiang et al., 2005).

The specific *gp60* IId subtypes found in the current study have been previously identified in a range of species, including humans (see Supplementary Table 2). Subtype IIdA17G1 has been identified in Spanish lambs and goat kids, (Quílez et al., 2008), cattle in Portugal (Alves et al., 2006) and Sweden (Silverlås et al., 2013), and in immunocompetent humans in England and Wales (Chalmers et al., 2011b). Subtype IIdA24G1 was identified in lambs in Spain (Quílez et al. 2008) and has also been found in humans in Australia (Waldron et al., 2009) and Sweden (Gherasim et al., 2012). Subtype IIdA19G1 has previously been found in calves in China (Wang et al., 2011) and in Spanish lambs and goat kids (Quílez et al., 2008), as well as cattle in Hungary and Sweden (Plutzer and Karanis, 2007; Silverlås et al., 2013), and humans in Sweden (Insulander et al., 2013). Subtype IIdA19G1 has also been identified in HIV-positive humans in China (Wang et al., 2013) and Portugal (Alves et al., 2006) and in urban wastewater in China (Li et al., 2012). Infection (and associated disease) in humans with *gp60* family IId has been seen in several countries but less frequently than *gp60* family IIa (Nichols et al., 2014).

*Cryptosporidium parvum* IIc subtypes are considered another important causative agent of cryptosporidiosis in humans (Xiao, 2010) and, until recently, this subtype family was considered to be human-specific. Dyachenko and colleagues (2010) identified IIc subtypes in *E. europaeus* in Germany, including the subtype IIcA5G3j (GenBank Accession GQ259136), also identified in the current study. In the United Kingdom, this subtype has previously only been reported from humans (Chalmers et al., 2011b).

The current study found no evidence of *C. erinacei* in British hedgehogs. This could relate to the small sample size, and further examination may reveal the presence of this parasite in the future. Alternatively, this could be due to parasite evolutionary divergence between Great Britain and mainland Europe.

We found no evidence of an association between *Cryptosporidium* infection and any of the host or location variables tested. Histological evidence of localised cryptosporidiosis in a hedgehog infected with *C. parvum* subtype IIdA17G1 indicates that this subtype, at least, can cause disease in some animals. Since this hedgehog had been kept in captivity for a period of circa 3 months and had concurrent disease (meningoencephalitis), it was considered likely to have been in an immunocompromised state. Previous reports of cryptosporidiosis, some fatal, have been described in European hedgehogs held in captivity long term (Meredith and Milne, 2009; Barlow *pers. comm*. 2014). The extent to which cryptosporidiosis occurs as a primary disease of free-living hedgehogs is unknown. Future studies could combine molecular subtyping with microscopic examination of faecal smears or quantitative PCR in order to quantify infection intensities and identify active intestinal infection as opposed to parasite oocyst transport alone that could have resulted from ingestion of contaminated foodstuffs.

The occurrence of *C*. *parvum* infection in the European hedgehog in the absence of clinical signs is noteworthy. To safeguard people against occupational zoonotic infection and prevent the spread of this parasite to uninfected animals, staff in wildlife centres should understand that infected hedgehogs may appear clinically healthy with normal faeces and should employ routine hygiene precautions when handling these animals or potentially-contaminated materials. The hedgehog also could be a reservoir or vector of *C*. *parvum* infection for livestock, the importance of which requires further investigation.

Further work is needed to investigate the extent to which *Cryptosporidium* is a health threat to hedgehogs and what importance, if any, this parasite may have at a population-level.

5. Conclusion

We found infection with zoonotic subtypes of *C. parvum* to be geographically widespread in the European hedgehog in Britain. These results should be taken into account by those handling this species, such as wildlife rehabilitators, and appropriate hygiene measures should be taken to minimise occupational exposure risks. Similarly, the possibility of hedgehogs acting as a source of *C. parvum* infection of livestock should not be discounted. Surveillance of *Cryptosporidium* in free-ranging hedgehogs across age groups is warranted to further our knowledge of the epidemiology of this parasite in this species and our understanding of the individual and population impacts of infection on the hedgehog. The identification of novel subtypes in hedgehogs, and the difference between those found in Great Britain and mainland Europe, also warrant further investigation.

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References

Abe, N., Sawano, Y., Yamada, K., 2002. *Cryptosporidium* infection in dogs in Osaka, Japan. *Vet. Parasitol.* 108, 185–193.

Abe, N. Matsubara, K. 2015. Molecular identification of Cryptosporidium isolates from exotic pet animals in Japan. *Vet. Parasitol.* 209(3-4): 254-257

Alves, M. Xiao, L., Antunes, F., Matos, O., 2006. Distribution of *Cryptosporidium* subtypes in humans and domestic and wild ruminants in Portugal. *Parasitol. Res.* 99, 287–292.

Alves, M., Xiao, L., Sulaiman, I., Lal, A. A., Matos, O., Antunes, F., 2003. Subgenotype analysis of *Cryptosporidium* isolates from humans, cattle, and zoo ruminants in Portugal. *J. Clin. Microbiol.* 41(6), 2744–2747.

Amori, G., Hutterer, R., Kryštufek, B., Yigit, N., Mitsain, G., Palomo, L.J., 2008. *Erinaceus europaeus*. In: IUCN 2013. *IUCN Red List of Threatened Species. Version 2013.2.* Available at: <www.iucnredlist.org>. Accessed 23 January 2014.

Appelbee, A.J., Thompson, R.C.A., Olson, M.E., 2005. *Giardia* and *Cryptosporidium* in mammalian wildlife-current status and future needs. *Trends Parasitol.* 21(8), 370–376.

Chalmers R.M., Smith R., Elwin K., Clifton-Hadley F.A., G.M., 2011a. Epidemiology of anthroponotic and zoonotic human cryptosporidiosis in England and Wales, 2004–2006. *Epidemiol. Infect.* 139(5), 700–712.

Chalmers, R.M. Smith, R.P., Hadfield S.J., Elwin, K., Giles, M., 2011b. Zoonotic linkage and variation in *Cryptosporidium parvum* from patients in the United Kingdom. *Parasitol. Res.* 108(5), 1321–1325.

Constable, P.D., 2010. An Overview of Cryptosporidiosis. In C. Kahn, ed. *The Merck Veterinary Manual*. New Jersey: Merck & Co., Inc., p. 134.

Dyachenko, V. Kuhnert, Y., Schmaeschke, R., Etzold, M., Pantchev, N., Daugschies, A., 2010. Occurrence and molecular characterization of *Cryptosporidium* spp. genotypes in European hedgehogs (*Erinaceus europaeus* L.) in Germany. *Parasitol.* 137(2), 205–216.

Enemark, H.L., Ahrens, P., Juel, C. D., Petersen, E., Petersen, R. F., Andersen, J. S., Lind, P., Thamsborg, S. M., 2002. Molecular characterization of Danish *Cryptosporidium parvum* isolates. *Parasitol.* 125( 4), 331–341.

Gherasim, A. Lebbad, M., Insulander, M., Decraene, V., Kling, A., Hjertqvist, M., Wallensten, A., 2012. Two geographically separated food-borne outbreaks in Sweden linked by an unusual *Cryptosporidium parvum* subtype, October 2010. *Euro Surveillance* 17(46),1–8.

Gosling, P.J., 2005. *Dictionary of Parasitology*, Boca Raton, Florida, USA: CRC Press Taylor & Francis Group.

Graczyk, T.K., Dunning, T., Strandberg, J., 1998. Fatal cryptosporidiosis in a juvenile captive African hedgehog (*Ateletrix albiventris)*. *J. Parasitol.* 84(1), 178-180.

GWH, 2014. Garden Wildlife Health. Available at: http://www.gardenwildlifehealth.org/about-2/ [Accessed November 10, 2014].

Insulander, M. Silverlås, C., Lebbad, M., Karlsson, L., Mattsson, J. G., Svenungsson, B., 2013. Molecular epidemiology and clinical manifestations of human cryptosporidiosis in Sweden. *Epidemiol. Infect.* 141(5), 1009–1020.

Jiang, J., Alderisio, K.A., Xiao, L., 2005. Distribution of Cryptosporidium genotypes instorm event water samples from three watersheds in New York. *Appl. Environ.Microbiol.* 71, 4446–4454.

Krawczyk, A.I. van Leeuwen, A. D., Jacobs-Reitsma, W., Wijnands, L. M., Bouw, E., Jahfari, S., van Hoek, A. H. A. M., van der Giessen, J. W.B., Roelfsema, J. H., Kroes, M., Kleve, J., Dullemont, Y., Sprong, H., de Bruin, A., 2015. Presence of zoonotic agents in engorged ticks and hedgehog faeces from *Erinaceus europaeus* in (sub) urban areas. *Parasite Vector* 8(201), 4–9.

Kuczynska, E. Shelton, D.R. 1999. Method for Detection and Enumeration of Cryptosporidium parvum Oocysts in Feces, Manures, and Soils. *Appl. Environ. Microbiol.* 65(7), 2820–2826.

Kváč, M., Hofmannová, L., Hlásková, L., Květoňová, D., Vítovec, J., McEvoy, J., Sak, B., 2014a. *Cryptosporidium erinacei* n. sp. (Apicomplexa: Cryptosporidiidae) in hedgehogs. *Vet. Parasitol.* 201, 9–17.

Kvác, M., Sakov, K., Kvetonová, D., Kicia, M., Wesolowska, M., McEvoy, J., Sak, B., 2014b. Gastroenteritis caused by the *Cryptosporidium* hedgehog genotype in an immunocompetent man. *J. Clin. Microbiol.* 52(1), 347–349.

Laatamna, A.E., Wagnerová, P., Sak, B., Květoňová, D., Aissi, M., Rost, M., Kváč, M., 2013. Equine cryptosporidial infection associated with *Cryptosporidium* hedgehog genotype in Algeria. *Vet. Parasitol.* 197(1-2), 350–353.

Li, N., Xiao, L., Wang, L., Zhao, S., Zhao, X., Duan, L., Guo, M., Liu, L., Feng, Y., 2012. Molecular surveillance of *Cryptosporidium* spp., *Giardia duodenalis*, and *Enterocytozoon bieneus*i by genotyping and subtyping parasites in wastewater. *PLoS Negl. Trop. Dis.* 6(9): e1809.

Meredith, A.L., Milne, E.M., 2009. Cryptosporidial Infection in a captive European hedgehog (*Erinaceus europaeus*). *J. Zoo Wildl. Med.* 40(4),809–811.

Morgan, U.M., Constantine, C.C., Forbes, D.A., Thompson, R.C., 1997. Differentiation between human and animal isolates of *Cryptosporidium parvum* using rDNA sequencing and direct PCR analysis. *J. Parasitol.* 83(5), 825–830.

Nichols, G.L., Chalmers, R.M., Hadfield, S.J., 2014. Chapter 3 Molecular epidemiology of human cryptosporidiosis. In: Caccio, S.M., Widmer, G. (Eds). Cryptosporidium: parasite and disease. Springer-Verlag Wien pp. 81-149.

OIE, 2008. Cryptosporidiosis. In *OIE Terrestrial Manual*. Chapter 2.9.4. p. 1192–1215. <http://www.oie.int/fileadmin/Home/eng/Health\_standards/tahm/2.09.04\_CRYPTO.pdf>. Accessed 1 June 2015.

Pedraza-Diaz, S., Amar, C., Mclauchlin, J., 2000. The identification and characterisation of an unusual genotype of *Cryptosporidium* from human faeces as *Cryptosporidium meleagridis*. *FEMS Microbiol. Lett,* 189, 189–194.

Plutzer, J., Karanis, P., 2007. Genotype and subtype analyses of *Cryptosporidium* isolates from cattle in Hungary. *Vet. Parasitol.* 146(3-4), 357–362.

Quílez, J., Torres, E., Chalmers, R. M., Hadfield, S. J., Del Cacho, E., Sánchez-Acedo, C., 2008. *Cryptosporidium* genotypes and subtypes in lambs and goat kids in Spain. *Appl. Environ. Microbiol.* 74(19), 6026–6031.

Robinson, I., Routh, A., 1999. Veterinary care of the hedgehog. *In Practice* 21(3), 128-137.

Silverlås, C., Bosaeus-Reineck, H., Näslund, K., Björkman, C., 2013. Is there a need for improved *Cryptosporidium* diagnostics in Swedish calves? *Int. J. Parasitol.* 43(2), 155–161.

Sturdee, A.P., Chalmers, R. M., Bull, S. A., 1999. Detection of Cryptosporidium oocysts in wild mammals of mainland Britain. *Vet. Parasitol.* 80, 273-280.

Sulaiman, I.M., Hira, P. R., Zhou, L., Faiza, M., Al-Shelahi, F. A., Shweiki, H. M., 2005. Unique endemicity of cryptosporidiosis in children in Kuwait. *J. Clin. Microbiol.* 43(6), 2805–2809.

Waldron, L.S., Ferrari, B.C., Power, M.L., 2009. Glycoprotein 60 diversity in *C. hominis* and *C. parvum* causing human cryptosporidiosis in NSW, Australia. *Exp. Parasitol.* 122(2), 124–127.

Wang, R., Wang, H., Sun, Y., Zhang, L., Jian, F., Qi, M., Ning, C., Xiao, L., 2011. Characteristics of *Cryptosporidium* transmission in preweaned dairy cattle in Henan, China. *J. Clin. Microbiol.* 49(3), 1077–1082.

Wang, L., Zhang, H., Zhao, X., Zhang, L., Zhang, G., Guo, M., Liu, L., Feng, Y., Xiao, L., 2013. Zoonotic *Cryptosporidium* species and *Enterocytozoon bieneus*i genotypes in HIV-positive patients on antiretroviral therapy. *J. Clin. Microbiol.* 51(2), 557–563.

Xiao, L. Fayer, R., Ryan, U., Upton, S. J., Xiao, L., Fayer, R., 2004. *Cryptosporidium* taxonomy: recent advances and implications for public health. *Clin. Microbiol. Rev.* 17(1), 72–97.

Xiao, L., 2010. Molecular epidemiology of cryptosporidiosis: an update. *Exp. Parasitol.* 124(1), 80–89.

Figure captions

**Figure 1**. Map depicting Great Britain and the location of centres (A-I) where faecal samples (n=108) were collected from adult hedgehogs (black dots). Centres A-H are wildlife centres. Centre I is the Institute of Zoology where post-mortem examinations were performed on three hedgehogs and distal large intestinal (LI) contents were collected: two hedgehogs were from Greater London and one was found dead in Northumberland. The number of positive samples (faecal and LI contents n=9) over the total number of samples (faecal and LI contents combined n=111) screened from each centre is denoted in brackets. Pie charts indicate the percentage of faecal samples that were positive for *Cryptosporidium* based on a PCR targeting the 18S rRNA gene, confirmed by sequencing. The *gp60* subtypes found at each centre are also presented. The asterisk (\*) indicates that this result is for the small intestinal tissue sample taken from a hedgehog admitted to this centre.

Figure 2. European hedgehog (*Erinaceus europaeus)* cross-section of small intestine (GI1XT0096-13) showing a large number of small round basophilic bodies (2-3.5 um diameter) characteristic of *Cryptosporidium* oocysts lining the epithelial surfaces. Haemotoxylin and Eosin stain. The black arrows indicate *Cryptosporidium* oocysts on the lining of the villi.

1. Note: Supplementary data associated with this article. [↑](#footnote-ref-1)