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No molecular epidemiological evidence supporting household transmission of zoonotic *Giardia duodenalis* and *Cryptosporidium* spp. from pet dogs and cats in the province of Álava, Northern Spain

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ABSTRACT

The role of pet dogs and cats as suitable source of human infections by the diarrheagenic protozoan parasites *Giardia duodenalis* and *Cryptosporidium* spp. has been a topic of intense debate for long time and still remains a largely unsolved problem. In this cross-sectional molecular epidemiological survey we attempted to investigate whether zoonotic (or zooanthroponotic) disease transmission was occurring among humans and domestic dogs and cats sharing the same spatial and temporal setting in both rural and urban areas of the province of Álava, Northern Spain. A total of 268 (including 179 human, 55 canine, and 34 feline) individual faecal specimens were obtained from 63 family households during February–March and November–December 2014. Detection of *G. duodenalis* cysts and *Cryptosporidium* spp. oocysts was achieved by direct fluorescence microscopy (DFAT) and PCR-based methods targeting the small subunit (*SSU*) ribosomal RNA gene of the parasites. *Giardia*-positive isolates were subsequently sub-genotyped at the glutamate dehydrogenase (*GDH*) and β-giardin (*BG*) genes. Overall, *G. duodenalis* infections were identified in 3.4% (6/179) of humans, 29% (16/55) of dogs, and 5.9% (2/34) of cats, respectively. *Cryptosporidium* spp. infections were detected in 1.1% (2/179) of humans, 5.5% (3/55) of dogs, and 8.8% (3/34) of cats, respectively. Simultaneous infections in human and canine/feline hosts by *G. duodenalis* or *Cryptosporidium* spp. were only demonstrated in a single household in which a cat and its owner tested positive for *Cryptosporidium* by DFAT, but this result could not be confirmed by *SSU*-PCR. Infections were homogeneously distributed among the studied human or animal populations irrespectively of their sex, age group, or geographical region of origin. Inadequate washing of raw vegetables and fruits was the only risk factor significantly associated to a higher likelihood of having human giardiosis/cryptosporidiosis. Molecular characterization of *G. duodenalis* isolates revealed the presence of sub-assemblage BIV in a single human isolate. All dog ($n = 3$) and cat ($n = 2$) isolates successfully genotyped were assigned to canine- and feline-specific assemblages C and F, respectively. No mixed assemblage or sub-assemblage infections could be demonstrated. Regarding *Cryptosporidium*, *C. canis* was found infecting dogs ($n = 2$), and *C. felis* a single cat. Attempts to amplify and characterize *Cryptosporidium* human isolates failed repeatedly. Our results suggest that pet dogs and cats do not seem to play a significant role as suitable reservoirs of human giardiosis or cryptosporidiosis in the province of Álava. We conclude, therefore, that zoonotic transmission of giardiosis or cryptosporidiosis among pet dogs and cats and their owners in this geographical region is very likely a rare event.

1. Introduction

The protozoan parasites *Giardia duodenalis* and *Cryptosporidium* spp. are among the most frequently reported enteric pathogens causing infection and gastrointestinal illness in humans and many

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other vertebrate species, including companion animals. Dog and cat pets have become an integral part of families globally, developing strong bonds with their owners and exerting beneficial effects on their physical and psycho-emotional health (Hodgson et al., 2015). Despite their unquestionable qualities, dogs and cats may also serve as sporadic sources of human infections by a wide range of bacteria, viruses, and parasites, particularly if untreated and not properly cared for (Chomel, 2014). Among them, *G. duodenalis* and *Cryptosporidium* spp. are common causes of both symptomatic and asymptomatic infections in dog and cat pets worldwide (Esch and Petersen, 2013).

Giardia duodenalis consists of eight genetic variants (assemblages A–H) with marked differences in host range and specificity. Cats and dogs are infected by canine-specific (C–D) or feline-specific (F) assemblages, whereas humans are almost exclusively infected by zoonotic assemblages A and B (Ryan and Cacciò, 2013). Human cases harbouring assemblages C, D, and F have been sporadically reported primarily in immunocompromised individuals (Ryan and Cacciò, 2013). Regarding *Cryptosporidium*, this genus comprises at least 30 valid species and over 70 genotypes, of which host-specific *C. canis* and *C. felis* cause the vast majority of infections in dogs and cats, respectively (Fayer, 2010). Both species are considered of low zoonotic risk to humans, who are primarily infected by *C. hominis* and *C. parvum* (Ryan et al., 2014).

Assessing the risk for zoonotic transmission of *G. duodenalis* and *Cryptosporidium* spp. from pet animals (mainly dogs) is difficult. The task is generally tackled by genotyping analyses of the obtained parasite isolates at a variety of suitable genetic markers (Ryan and Cacciò, 2013; Ryan et al., 2014). Although useful to ascertain the genetic diversity of these pathogens and generate baseline molecular epidemiological information, these data alone should not be used to infer the occurrence of zoonotic transmission (Ballweber et al., 2010). In doing so, three confounding factors may lead to partial or unproven conclusions, including the general lack of molecular data at the sub-genotype level, the possible existence of reverse zoonosis, and the unrecognized potential of companion animals to act as passive carriers of parasite (oo)cysts of anthroponotic origin (Gil et al., 2017). Therefore, household- or community-based surveys simultaneously sampling companion animals and humans in close proximity should be preferred when attempting to obtain convincing evidence of zoonotic transmission. It is also necessary to considerer that even these concurrent studies may have limitations and do not preclude, for instance, the existence of a common source of infection such as contaminated drinking water.

In Spain, canine giardiosis and cryptosporidiosis have been detected in the range of 1–38% and 7–15%, respectively, whereas *Cryptosporidium* infections have been reported only in cats at a prevalence rate of 15% (reviewed in Navarro-i-Martinez et al., 2011; Carmena et al., 2012). Very few studies have been aimed to investigate the genotypic diversity of these parasites in Spanish dog and cat populations (Dado et al., 2012; Ortuño et al., 2014), whereas demonstration of transmission between dogs/cats and humans closely sharing the domestic environment has never been attempted to date. This work is the rational continuation of a series of community-based and field studies conducted by our research group to characterize the prevalence, molecular diversity and transmission dynamics of *G. duodenalis* and *Cryptosporidium* spp. in selected human and animal populations in the province of Álava, Northern Spain (Carmena et al., 2007; Cardona et al., 2011, 2015; Cano et al., 2016; Gil et al., 2017). In the present study we aimed to document potential animal-to-human (or human-to-animal) disease transmission between household family members and their dog and/or cat pets in this geographical region.

2. Material and methods

2.1. Ethical statement

Written informed consent was obtained from all participants, or their parents or legal tutors in the case of children, who volunteered to participate in this study. Gathered socio-demographic or epidemiological data were coded prior to any analysis to preserve the identity of the participants. This study and the procedures involved, including the data collection spreadsheets used, have been approved by the Research Ethics Committee of the Carlos III Health Institute (reference number: CEI PI 30_2012).

2.2. Study area and faecal sample collection

Álava is one of the three provinces conforming the Autonomous Region of the Basque Country in Northern Spain. It encompasses 51 municipalities distributed in seven administrative regions, covering an area of 2963 km^2. The 2015 census recorded the population at 321,932, of which 243,918 inhabitants were living in the capital city, Vitoria-Gasteiz. Two cross-sectional studies were conducted in February–March and November–December 2014 among households with pet dogs and cats in rural (Añana, Ayala, Campezo-Montaña Alavesa, Gorbeialdea, and Salvatierra) and urban (Vitoria-Gasteiz) regions of Álava. Local school principals were personally contacted and informative meetings were held with school committee members and parentís representatives to explain the aim of the study and the procedures involved. Families with children and pet dogs and cats were asked to provide individual faecal samples from each member of the household including dogs and cats. Consenting participants were provided with a pre-labelled sampling kit including sterile polystyrene flasks and instructions on how to take and identify the samples safely. Standardised data collection spreadsheets were also developed and distributed in order to gather socio-demographic data (age, gender, area of residence), water-use practices (source of drinking water, washing hands, raw fruits and vegetables before eating, aquatic sports), contact with livestock, known episodes of diarrhoea affecting any member of the family or classmates during the previous month and traveling abroad during the last 3 months. Collection of stool samples and epidemiological information was organized in collaboration with the schools, reviewed for matching and completeness, and shipped to the Spanish National Centre for Microbiology (Majadahonda, Spain). Stool samples were kept at −20 °C with no preservative solutions until further diagnostic and molecular analyses.

2.3. Direct fluorescent antibody test

A direct fluorescent antibody test (DFAT) was used to detect *Giardia* cysts and *Cryptosporidium* oocysts by fluorescence microscopy. Briefly, ∼1 g of faecal material was processed using the concentration system PARASEP Midi® (Grifols Movaco, Barcelona, Spain) according to the manufacturer's instructions. Five μL of concentrated faecal material were placed on welled slides. Smears were air-dried, methanol fixed, stained with fluorescein-labelled mouse monoclonal antibodies (Crypto/Giardia Cel, Cellabs, Sydney, Australia), and examined at 400× magnification. Total (oo)cyst counts per well were used as estimation of the parasitesí burden.

2.4. DNA extraction and purification

Total DNA was extracted from a fresh aliquot (∼200 mg) of each faecal sample using the QIAamp® DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's instructions. Purified DNA samples (200 μL) were stored at −20 °C for further