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Molecular epidemiology of *Cryptosporidium* in livestock animals and humans in the Ismailia province of Egypt

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ABSTRACT

The zoonotic potential of *Cryptosporidium* was studied in one of the most densely populated provinces of Egypt regarding livestock and people. In a representative survey, faecal samples from cattle, buffalo and stool samples from diarrhoeic children (<10 years) were investigated. Parameters assumed to be related to cryptosporidiosis were recorded for animals and children. Animal samples (804) were examined by the Copro-antigen *RIDA® QUICK* test, followed by PCRs targeting the 18S rDNA and *gp60* genes for antigen-positive and 10% randomly selected negative samples. All 165 human samples were tested by both methods. The overall estimated prevalence of *Cryptosporidium* in ruminants was 32.2%, without significant difference between animal species. PCR identified 65.7% *Cryptosporidium parvum*, 11.8% *Cryptosporidium ryanae*, 4.1% *Cryptosporidium bovis*, and combinations of *C. parvum* plus *C. ryanae* (11.2%), *C. parvum* plus *C. bovis* (5.3%) and of *C. parvum* plus *Cryptosporidium andersoni* (1.8%), also without significant differences in species occurrence between cattle and buffalos. The human *Cryptosporidium* spp. prevalence was 49.1%, of which 60.5% were *Cryptosporidium hominis*, 38.2% *C. parvum* and 1.2% *C. parvum* plus *C. bovis*. Analysis of *gp60* variants allocated *C. parvum* found in animals to the zoonotic subtype family IIa (18.9%, subtype IIaA15G1R1 only) and to IId (81.1%, mostly IIdA20G1). In humans 50% were classified as subtype family IIa (IIaA15G1R1 and IIaA15G2R1) and 50% were IIdA20G1. *C. andersoni* occurred only in cattle older than 1 year. In contrast, mono-infections with one of the three single *Cryptosporidium* species and the three combinations with *C. parvum* were more prevalent in cattle and buffaloes younger than 1 year, particularly in those younger than 3 months, and were predominantly subtype family IId. In human samples no *Cryptosporidium* were identified in children younger than 7 months. Neither place of residence nor the source of drinking-water had measurable effects on prevalence. Remarkably, however, all children with *C. parvum* subtype family IIa and 86% with subtype family IId had contact to animals. High prevalence and identical genotypes of *C. parvum* in animals and humans indicate zoonotic transmission due to contact with animals, involving IIdA20G1 as the most frequent subtype.

1. Introduction

Cryptosporidium are ubiquitous enteric protozoan parasites infecting a wide range of host species including mammals, birds, reptiles and fish (Smith et al., 2007). They are a major cause of diarrhoeal disease, nutritional

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deficiencies and impair the development of children in developing countries (Ahs et al., 2010; Dillingham et al., 2002). In 2004 the WHO included cryptosporidiosis in its neglected diseases initiative in order to reduce its impact through applying better control methods in humans and reduction of transmission from reservoir animals.

In cattle *Cryptosporidium parvum* is frequently associated with mild to severe diarrhoea and weight loss particularly in neonates (Fayer et al., 2006; Feng et al., 2007). In humans clinical consequences of *Cryptosporidium* infections are most commonly associated with an immune-compromised health status (e.g. acquired immune deficiency syndrome, leukemia, immunosuppressive therapy) (Current and Garcia, 1991; Hassanein et al., 2012), undernourished patients (Neira-Otero et al., 2005), occupational exposure (e.g. veterinarians, paraveterinarians, farmers, etc.) (Konkle et al., 1997; Levine et al., 1988), and/or children (Smith et al., 2007). Symptoms of cryptosporidiosis range from self-limiting gastrointestinal illness in immune-competent people to chronic or fulminant disease with fatal outcome in immune-compromised persons (Chen et al., 2002; Dillingham et al., 2002). Humans can acquire *Cryptosporidium* infections either by person-to-person transmission or from animals (zoonotic transmission) or by ingestion of contaminated food or water (Xiao, 2010). The relative importance of either transmission route is not entirely clear, largely due to the inability of traditional diagnostic tools to differentiate parasite species and genotypes, important to understand transmission pathways and dynamics.

Most human cryptosporidiosis cases are caused by one of the five *Cryptosporidium* species, *Cryptosporidium hominis*, *Cryptosporidium parvum*, *Cryptosporidium meleagridis*, *Cryptosporidium felis*, and *Cryptosporidium canis* (Xiao, 2010; Xiao and Fayer, 2008). *C. hominis* and *C. parvum* are by far the most common species responsible for the majority of human infections, especially in industrialised nations, although in some areas *C. meleagridis* infection rates comparable to those of *C. parvum* were reported (Cama et al., 2007, 2008). A few other *Cryptosporidium* species and genotypes are occasionally found in humans, including *Cryptosporidium muris*, *Cryptosporidium suis*, *Cryptosporidium andersoni, Cryptosporidium bovis*, *Cryptosporidium ubiquitum*, *Cryptosporidium cunniculus*, as well as horse, skunk and chipmunk genotypes (Bushen et al., 2007; Ng et al., 2012; Robinson et al., 2008; Xiao, 2010).

Traditional direct microscopic diagnosis of *Cryptosporidium* from faecal or stool samples using acid-fast stains is insensitive, time consuming and requires skilful personnel to identify the organism (Morgan et al., 1998; Ridley and Olsen, 1991; Webster et al., 1996) but nevertheless still remains the gold standard in many laboratories worldwide. Immuno-chromatographic tests that incorporate antibodies for the detection of *Cryptosporidium* spp. in human stool samples are fast, simple, versatile and adequately sensitive and specific but do not allow discrimination of species or subtypes. Polymerase chain reaction (PCR) is more sensitive and in particular allows species identification and subtyping of *Cryptosporidium* spp. (Xiao, 2010) which is required for identification of transmission pathways between animals and humans. Sequence analyses of the polymorphic 60 kDa glycoprotein gene (*gp60*) indicated specific variations with discriminatory power to differentiate several subtypes within *C. parvum* and *C. hominis* (Jex and Gasser, 2010; Xiao, 2010). Subtype families IIa and IId are frequently isolated from humans and several animal species whereas other subtype families have been exclusively isolated from humans up to now, with genotype family IIc observed most often (Jex and Gasser, 2010; Xiao, 2010).

Limited knowledge on *Cryptosporidium* is available in developing countries, particularly concerning the relative occurrence of particular species and subtypes. In Egypt, for instance, numbers of investigations are limited, information is mostly discontinuous and the majority of studies concentrated only on humans. Of those studies some were based on microscopy only (El Naggar et al., 1999; Khashba et al., 1989; Safar et al., 1996; Samn et al., 2012; Soliman, 1992), others on combinations of microscopy and serology or serology only (Abdel-Messih et al., 2005; Abdel Hameed et al., 2008; Antonios et al., 2010; Boghdadi, 1996; El-Kadi et al., 2006; El-Moamly and El-Sweify, 2012; El-Sibaei et al., 2003; Elshazly et al., 2007; Hassanein et al., 2012; Nora et al., 2012) or on combinations of microscopy, serology and molecular techniques (Abd El Kader et al., 2012; Abdel-Hafeez et al., 2012; El-Hamshary et al., 2008; El-Shazly et al., 2002; Helmy et al., 2004, 2006). There is a single study on human samples exclusively employing molecular techniques but the experiments focused predominantly on pathological changes of isolated subtypes on experimentally infected mice (Eida et al., 2009).

The only three investigations on animals all used microscopic techniques (Abd el-Wahed et al., 1999; Amer et al., 2010c; El-Khodery and Osman, 2008) although (Amer et al., 2010c) also performed molecular analyses. So far, in Egypt only four investigations were published on both, animal and human samples, all only using microscopic diagnosis (Abou-Eisha, 1994; Abou-Eisha et al., 2000; El-Sherbini and Mohammad, 2006; Shoukry et al., 2009).

The scope of our study was to improve the understanding of the epidemiology of *Cryptosporidium* in Egypt by studying the prevalence and genetic diversity of *Cryptosporidium* in both populations of farm animals and humans from the same area. Molecular diagnostic results were co-analysed with epidemiological information.

2. Materials and methods

2.1. Sample collection

The study area was the Ismailia province of Egypt, one of the most densely populated provinces regarding livestock and people, with its arable belt along the province's canal system, particularly the Suez Canal. For a cross-sectional survey sample sizes necessary to capture expected prevalences were calculated. In the selected province an estimated number of 6700 farms are located of which 98% have less than 0.4 ha with an average herd size <10 animals. Most cattle are low producing native cross-breeds, often in mixed herds with buffalos. As randomisation of samples was not possible (due to missing