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SUMMARY

Cryptosporidium spp. and *Giardia duodenalis* are important parasites infecting a wide range of domestic animals worldwide. The aim of the present study was to determine the occurrence of *Cryptosporidium* spp. and *Giardia* parasites in different domestic animals living in close contact with humans within rural/semi-urban communities in Kafue district in Zambia. A single faecal sample per animal was collected from pigs, goats, dogs, ducks, chickens and pigeons and analysed by Merifluor *Cryptosporidium/Giardia* immunofluorescence antibody assay for the simultaneous detection of these parasites. The faecal consistency was noted and scored as non-diarrhoeic or diarrhoeic. A total of 236 samples were collected. *Cryptosporidium* spp. oocysts were detected in pigs (11.5%, 17/148), goats (5.9%; 1/17), ducks (10.0%; 3/30) and chickens (14.3%; 2/14) while *Giardia* cysts were detected in pigs (8.1%; 12/148), goats (5.9%; 1/17), dogs (25.0%; 5/20) and ducks (6.7%; 2/30). Diarrhoea was not associated with either infection. Age was also not associated with either infection except in dogs where *Giardia* infection was only detected in animals aged less than six months (p=0.009). It is concluded from this study that *Cryptosporidium* spp. and *Giardia* parasites are prevalent among domestic animals reared within communities in Kafue district thereby constituting a potential source for zoonotic infections.

Key words: *Cryptosporidium* spp., *Giardia*, domestic animals, Zambia

INTRODUCTION

Cryptosporidium spp. and *Cryptosporidium* spp. And *Giardia duodenalis* are apicomplexan parasites infecting a wide range of vertebrates, including humans (Xiao and Fayer, 2008). They are reported to be the most common parasites of domestic animals (livestock, dogs and cats) (Fayer, 2004; Thompson, 2004). Both parasites share a broad host range, and both cryptosporidiosis and giardiasis are said to be zoonoses, although the status of giardiasis as a zoonosis is still debatable (Monis and Thompson 2003; Smith *et al*., 2006). The various species and genotypes of these parasites differ in their ability to infect one or more hosts. Each genus is comprised of a complex of species and genotypes, some of which are pathogenic, some specific to particular hosts and some zoonotic, thereby rendering them to be of public health significance (Xiao *et al*., 2004; Caccio *et al*., 2005; Smith *et al*., 2006).

Transmission occurs directly or indirectly by the ingestion of oocysts or cysts from infected individuals via contaminated water, food and pasture (Xiao *et al*., 1993; Xiao *et al*., 1994; Graczyk *et al*., 1997; Preiser *et al*., 2003; Castro-Hermida *et al*., 2005). The most important sources for human infection are believed to be contaminated drinking and recreational water, food, household animals and infected people (Dillingham *et al*., 2002). Household animals, such as dogs, cats and birds contribute to the transmission of intestinal parasites because of their close association with their owners. A study in Cote d'Ivoire investigating the genotypes and species of *Giardia* and *Cryptosporidium* in humans and free range domestic animals (dogs, goats, ducks, chickens) found *C. parvum* and *C. mealagridis* in both humans and chickens, highlighting the possibility of cross transmission between domestic animals and humans (Berrilli *et al*., 2012). Free-living animals may constitute a reservoir of infection in nature but also contribute to contamination of surface water, soil and food (Bajer, 2008).

In cryptosporidiosis, clinical manifestations vary and depend on the age and health status of the host, the infective dose and genetic background of the parasite (Xiao and Fayer, 2008). Stressed and immunologically compromised animals are more susceptible to infection, and disease incidence is high in such animals (Enemark *et al*., 2002; Ramirez *et al*., 2004). In young livestock, cryptosporidiosis can be debilitating and cause severe illness and/or death (de Graaf *et al*., 1999). Cryptosporidiosis in calves is often characterized by profuse watery diarrhoea of acute onset, anorexia, dehydration and weight loss (Fayer *et al*., 1998; de Graaf *et al*., 1999). In naturally infected pigs, infection is typically asymptomatic even in young animals (Ramirez *et al*., 2004, Maddox-Hyttel *et al*., 2006). However,

symptomatic infections do occur in young piglets less than three weeks of age. Inappetance, depression, vomiting, diarrhoea and mortality have been reported in such piglets (Rotkiewicz *et al*., 2001, Enemark *et al*., 2002; 2003). In companion animals, particularly dogs, over-crowding in breeding areas is a risk factor (Ramirez *et al*., 2004) and clinical signs have been reported in young puppies with other concurrent infections like parvovirus enteritis or canine distemper (Ramirez *et al*., 2004).

On the other hand, *Giardia* infections in domestic animals are often asymptomatic, although clinical disease does occur in young animals (O'Handley *et al*., 1999; Robertson *et al*., 2000; Geurden *et al*., 2006a). In calves, *Giardia* mostly affects those between five to 10 weeks of age (Thompson, 2000) but infections may also occur in calves as young as four days (Xiao and Herd, 1994) and in adult cows (Maddox-Hyttel *et al*., 2006). Infection may result in numerous episodes of diarrhoea which in turn adversely affects production and results in economic losses for producers (Xiao, 1994). Other clinical signs may include chronic pasty diarrhoea, lethargy, weight loss and poor condition (Thompson, 2000). Clinical consequences of *Giardia* infections in dogs and cats are reported to be minimal (Thompson, 2000) but in young animals (puppies and kittens), the major complication is impairment of growth and development due to persistent infections (Farthing, 1999). In goats, clinical signs reported include apathy, reduced food intake and softening of faeces (Castro-Hermida *et al*., 2005).

Cryptosporidium spp. and *Giardia* in domestic animals have been reported in several African countries (Kambarage *et al*., 1996; Abd-El-Wahed 1999; Johnston *et al*., 2010; Berrilli *et al*., 2012). In Zambia, prevalence studies on *Cryptosporidium* spp. have been conducted in cattle, sheep and goats (Geurden *et al*., 2006b; Goma *et al*., 2007) and in intensively managed pigs (Siwila and Mwape, 2012), but no studies have been conducted in other domestic animals. Furthermore, to our knowledge, no studies have been made on *Giardia* in animals other than in pigs (Siwila and Mwape, 2012) in Zambia. As a preliminary attempt to evaluate the risk factors for human cryptosporidiosis and giardiasis in Zambia, the objective of the present study was to determine the occurrence of *Cryptosporidium* spp. and *Giardia* in different domestic animal hosts living in close contact with people in rural/semi-urban Zambian communities.

MATERIALS AND METHODS

Study area and study population

The study was implemented in four rural/semi-urban communities (Soloboni, Chawama, Kashelela and Mtendere) in the Kafue district. The district is located 45km south of Lusaka.

the capital of Zambia. The study population comprised of all domestic animals reared in the communities and these included pigs, goats, dogs, ducks, chickens and pigeons.

Domestic animals (pigs, goats and dogs,) and birds (ducks, chickens and pigeons) were reared free-range in all communities. The owners, however, had enclosures in the backyard where the animals (pigs and goats) were housed at night. In the morning, the animals were released and could roam the communities in search of food. Ducks and chickens on the other hand, were normally kept inside the family house and released during the day as for the other animals. Pigeons had a shelter in which they could go in and out anytime.

Sample collection and analysis

Samples were collected based on the animal owners' willingness to participate in the study and animals of all age groups were included in the study. Between January 2008 and January 2009, faecal samples were collected rectally from pigs, goats and dogs, and fresh droppings were collected from ducks, chickens and pigeons. A total of 236 samples were collected, 148 were from pigs, 17 from goats, 20 from dogs, 30 from ducks, 14 from chickens and 7 from pigeons.

The faecal consistency was noted as either diarrhoeic or non-diarrhoeic. The samples were transported in cool boxes packed with ice and were analysed within two days after collection at the University of Zambia, for the presence or absence of *Cryptosporidium* spp. and *Giardia*. The samples were processed by mixing one portion of faeces with three portions of 10% formalin and further analysed using a commercial immunofluorescence assay (Merifluor *Cryptosporidium/Giardia* IFA; Meridian Diagnostics Inc., Cincinnati, Ohio, USA) for the simultaneous detection of *Cryptosporidium* spp. and *Giardia*.

Statistical analysis

Statistical analyses were carried out using computer software STATA Version 10.1 (StataCorp, College Station, Texas, USA) and SPSS Version 11 (Statistical Package for Social Sciences). The data were reported as absolute values or percentages. Associations between faecal consistency, age and infection by the two protozoan parasites were compared by Fisher's exact test. All results were considered significant if $p<0.05$.

RESULTS

Table 1 shows the total number of samples assessed, those that were positive for *Cryptosporidium* spp. and *Giardia* by age and animal species. The total number of infected per animal species is also indicated.

The faecal consistency (diarrhoeic or

non-diarrhoeic) was noted for each animal species. Only 12 out of 148 pigs and one dog out of 20 had diarrhoea. However, faecal consistency was not significantly associated with either infection for any of the two animal species, i.e. *Cryptosporidium* spp. infection in pigs (p=0.63) and *Giardia* infection in dogs (p=0.43). *Giardia* infection was associated with age in dogs (p=0.009) as it was detected only in young animals less than six months.

Table 1. Animals from peri-urban communities in Kafue district of Zambia infected with *Cryptosporidium* spp. and *Giardia* according to animal species and age group.

Age group	≤2 months	>2-6 months	Adults [1]	Unknown	Total
Pigs (Total number[2])	32	44	15	57	148
Cryptosporidium+ n (%)	4 (12.5)	7 (15.9)	2 (13.3)	4 (7.0)	17 (11.5)
Giardia+ n (%)	1 (3.1)	3 (6.8)	3 (20.0)	5 (8.8)	12 (8.1)
Goats (Total number[2])	0	11	0	6	17
Cryptosporidium+ n (%)	0 (0.0)	1 (9.9)	0 (0.0)	0 (0.0)	1 (5.9)
Giardia+ n (%)	0 (0.0)	1 (9.9)	0 (0.0)	0 (0.0)	1 (5.9)
Dogs (Total number[2])	2	8	10	0	20
Cryptosporidium+ n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Giardia+ n (%)	2 (100.0)	3 (37.5)	0 (0.0)	0 (0.0)	5 (25.0)
Ducks (Total number[2])	0	12	0	18	30
Cryptosporidium+ n (%)	0 (0.0)	2 (16.7)	0 (0.0)	1 (5.6)	3 (10.0)
Giardia+ n (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (11.1)	2 (6.7)
Chickens (Total number[2])	0	0	0	14	14
Cryptosporidium+ n (%)	0 (0.0)	0 (0.0)	0 (0.0)	2 (14.3)	2 (14.3)
Giardia+ n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Pigeons (Total number[2])	0	0	0	7	7
Cryptosporidium+ n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Giardia+ n (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total sampled	34	75	25	102	236

[1] Adults are defined as animals above the age of 1 year; [2]Total number of samples collected, + n means number of positive samples

DISCUSSION

In the present study, *Cryptosporidium* oocysts and *Giardia* cysts were detected in the faeces of apparently healthy animals. In pigs, *Cryptosporidium* oocysts were detected more often in piglets ≤ 6 months than in adults. The age of several pigs was not known; however, the frequency of infection in this category was low. The occurrence of *Cryptosporidium* spp. in pigs in this study (11.5%) is comparable to that reported by Olson *et al.* (1997) (11%) in Canadian pigs. Furthermore, these authors reported a prevalence of 9% for *Giardia*, a result that is comparable to what we detected in the present study (8.1%), although there were differences in the analysis of samples. The present study did not incorporate a step to concentrate the oocysts or cysts. However, other studies have reported much higher prevalence; up to 100% for *Cryptosporidium* spp. and 84% for *Giardia* in Danish pigs (Maddox-Hyttel *et al.*, 2006). Likewise, a higher prevalence (21.9%) was found in pigs in north eastern Spain (Quilez *et al.*, 1996). These differences could be due to differences in the sample size and analysis of the samples. In the study by Maddox-Hyttel *et al.* (2006), the sample size was larger and oocysts and cysts were purified and concentrated before analysis. Differences in management systems and how the pigs are reared and/or differences in study designs in the two studies by Quilez *et al.* (1996) and Maddox-Hyttel *et al.* (2006) also might account for the differences with the present study. A relatively lower prevalence (7%) was reported in Ethiopia in extensively managed pigs (Tomass *et al.*, 2013).

Only one goat out of 17 was found to be infected with *Cryptosporidium* oocysts. This is in contrast to other studies that have reported higher prevalence with morbidity approaching 100% in kids less than six months (Johnson *et al.*, 1999). Our results are, however, comparable with those reported by Matos-Fernandez *et al.* (1993) where the prevalence of *Cryptosporidium* spp. infection was 11% in goats aged less than five months and the results reported by Kambarage *et al.* (1996) where *Cryptosporidium* was only detected in 0.8% of the goats examined. *Giardia* cysts were detected in one goat. The low occurrence in the present study falls within the range (0-20%) reported by Castro-Hermida *et al.* (2005) in adult goats from Western France. However, the age of the infected goat in the present study was less than one year. The sample size in the present study was small, a factor which most likely contributed to differences in occurrence compared to other studies for example, Johnston *et al.* (2010). Furthermore, even though the results in the present study were comparable to those reported by Matos-Fernandez *et al.* (1993), caution must be taken when comparing these results as the sample sizes were different.

No *Cryptosporidium* oocysts were detected in the 20 dogs examined in

the present study. Lower infection rates are expected in household dogs compared to other domestic animals because they live in less overcrowded conditions. Nevertheless, dogs in the present study were roaming freely and in contact with other animals and could therefore, probably, have been exposed to infection from other animals. The no-infection status in the present study could be because the dogs were not infected or not shedding oocysts at the time of sampling. In houses with dogs, there were on average only two dogs per household. However, low infection rates have been reported in household dogs where a larger sample size was used compared to that in the present study (Claerebout *et al*., 2009). Normally, infection rates are higher in breeding kennels/areas due to overcrowding and high number of puppies in such areas (Claerebout *et al*., 2009), with infection rate being higher in dogs aged less than 12 months. On the other hand, *Giardia* cysts were detected in young dogs less than six months old, a finding that is consistent with previous reports of infection being common in young animals with or without symptoms (Thompson, 2000). Other studies have also reported *Giardia* to be the most common enteric parasite of dogs. For example, in Australia, *G. duodenalis* was the most common enteric parasite found in domestic dogs (Bugg *et al*., 1999), and a study in Belgium also reported *Giardia* to be the most frequently isolated parasite (Claerebout *et al*., 2009).

In the present study, *Cryptosporidium* oocysts were detected in two ducks (aged between 2-6 months) and two chickens of unknown age. There is little information on the prevalence of the parasite in domestic ducks and chickens. However, the parasite has been detected in wild ducks in Southern Mexico (Kuhn *et al*., 2002) and aquatic ducks in Hungary (Plutzer and Tomor, 2009). No *Giardia* cysts were found in birds in these studies.

The findings of the present study may have implications for the health of the people considering the closeness with which they associate with their animals and birds. The finding of *Cryptosporidium* spp. in chickens that are housed in the same house as the owners mean that the latter are at greater risk of infection with the avian species of *Cryptosporidium* spp. which have been reported to infect humans (Morgan *et al*., 2000). The sample size in the present study was small because not all animal owners or households with animals were willing to participate in the study.

To our knowledge, this is the first description of *Cryptosporidium* spp. in ducks and chickens and the first description of *Giardia* in dogs in Zambia. The results indicate that both parasites appear to be common among domestic animals in Zambia, however, further studies are needed to determine the true prevalence and species/genotype variation of these protozoa, and the potential risk they pose to humans living in close contact with animals.

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