The molecular epidemiology of Cryptosporidium and Giardia infections in coyotes from Alberta, Canada, and observations on some cohabiting parasites

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Abstract

Coyotes from southern Alberta and Saskatchewan, Canada, were examined for the presence of *Giardia* and *Cryptosporidium* and cohabiting helminths. *Toxascaris* was present in over 90% of the 70 animals examined, and *Taenia* sp. in 6.5–25% of the two groups of animals studied. *Giardia* (12.5–21.7%) and *Cryptosporidium* (0–17.4%) were also common and molecular characterisation revealed both zoonotic and host-adapted genotypes of *Giardia*, whereas the *Cryptosporidium* proved to be a variant of the canine species *C. canis*. The seasonal variation observed in the occurrence of *Cryptosporidium* may be related to stress-induced shedding of the parasite.

Keywords: Coyote; Alberta; *Giardia*; *Cryptosporidium*; Genotyping; *Toxascaris*

Introduction

Giardia is a common parasite of wildlife yet little is known about the species/genotypes of the parasites that occur in wildlife or the role wildlife plays in the maintenance of parasite transmission cycles (Thompson and Monis, 2004; Appelbee *et al*., 2005; Caccio *et al*., 2005 ; Kutz *et al*., 2008). Although more is known about the diversity of species and genotypes of *Cryptosporidium* in wildlife (Heitman *et al*., 2002; Appelbee *et al*., 2005; Caccio *et al*., 2005; Thompson *et al*., 2005), the epidemiology of such infections has not been investigated. The coyote (*Canis latrans*) inhabits nearly all of North America and is widely distributed in Canada (Long, 2003), and although much has been documented on their helminth fauna (e.g. Conder and Loveless, 1978; Samuel *et al*., 1978; Davidson *et al*., 1992; Van den Bussche *et al*., 1987; Gompper *et al*., 2003) there is little information on their enteric protozoa. Gompper et al. (2003) found *Giardia* in 14.9% of 145 coyotes sampled in New York and Heitman et al. (2002) identified *Giardia* in 5% of coyote scat samples collected from the North Saskatchewan river basin, but no information on species or genotype was obtained in either study and *Cryptosporidium* was not found. Concern has been expressed regarding

the potential role of coyotes as reservoirs of parasites of public health significance (Conder and Loveless, 1978; Bischof and Rogers, 2005), particularly in view of their adaptability and expansion into urban areas (Markovchick-Nichols et al., 2008; http://www.srd.gov.ab.ca/fishwildlife/wildlifeinalberta/coyotes/) and the role of wildlife as sources of environmental contamination associated with waterborne outbreaks of *Giardia* and *Cryptosporidium* infections (Smith et al., 2006). Both *Giardia* and *Cryptosporidium* have been isolated from coyotes in the USA and one of seven positive animals harboured zoonotic genotypes of *Giardia* (Xiao *et al*., 2002; Trout *et al*., 2006).

In the present study, two groups of coyotes collected opportunistically in southern Alberta and Saskatchewan, Canada, were examined for the presence of *Giardia*, *Cryptosporidium* and other intestinal parasites, and isolates of *Giardia* and *Cryptosporidium* genotyped to determine their potential zoonotic potential.

Materials and methods

Coyotes were collected by trappers and hunters in prairie regions of southern Alberta and Saskatchewan; one group in late spring and summer, and the other in winter of 2002. A qualitative assessment of the body condition of the coyotes was made based on the coat and signs of emaciation. The whole carcasses were frozen at −20 °C until examination, when they were thawed, the small intestine removed, divided into four equal sections and cut open longitudinally. The mucosal surface of each section was lightly scraped and the mucosal scrapings dispersed in water in a petri dish and examined under overhead light on a black background with the aid of a magnifier. Helminths and helminth-like structures were collected for more detailed microscopic examination. Faeces were collected from the rectum and prepared through sucrose flotation prior to examination by immunofluorescence of monoclonal antibodies specific for either *Giardia* (Giardi-a-Glo™, Waterborne Inc., New Orleans, LA) or *Cryptosporidium* (Crypt-a-Glo™, Waterborne Inc.), as described previously (Olson *et al*., 1997; Heitman *et al*., 2002).

DNA was extracted from microscopy-positive samples using the QIAmp DNA Stool Mini Kit (Qiagen Pty Ltd., Australia, Cat. No. 51504) with some modifications. Five cycles of freeze/thaw in ASL buffer were included to aid oocyst destruction. In addition, DNA was eluted in 50 µl of AE buffer rather than 200 µl as recommended. DNA was stored at −20 °C. *Giardia* DNA was amplified at the 18S rDNA locus using nested PCR primers as previously described (Read et al., 2002). Amplification conditions for these primers were modified by including 5% DMSO in the primary reaction and dropping the annealing temperature of the secondary PCR to 53 °C. Sequencing was carried out using the internal reverse primer GiarR at 53 °C. Isolates were grouped into assemblages based on the sequence of a 130 bp fragment according to Read et al. (2002). *Giardia* isolates were also amplified and sequenced at a second locus, the glutamate dehydrogenase (*gdh*) gene following the method described by Read et al. (2004). Once sequenced the isolates were genotyped into assemblages by multiple alignment with a range of reference strains with the aid of web based Clustal X (Thompson et al., 1997).

Cryptosporidium isolates were genotyped by amplification and sequencing of the 18S rDNA and HSP70 genes using primers and conditions previously described by Ryan et al. (2003) with slight modification of the annealing temperature from 58 to 56 °C. Multiple alignments for phylogenetic analysis of the *Cryptosporidium* isolates at the 18S rDNA were done using 13 *Cryptosporidium* sequences retrieved from Genbank with the following accession nos., *C. parvum* DQ656355, *C. hominis* DQ286403, *C. meleagridis* DQ656356, *C. suis* AF108861, *C. canis* AB210854, *C. canis ex* coyote DQ385545, *C. felis* AF112575, *C. saurophilum* AY382172, '*C. blagburni*' AY120911, *C. baileyi* AF093495, *C. muris* CM18SR221, *C. Anderson* AF093496 and *C. serpentis* AF093501. *Plasmodium cathemerium* 18S rDNA sequence (Accession no. AY625607) was used as the out-group. The sequences were aligned using Clustal W (Thompson et al., 1994) and the aligned sequences were then converted to mega format for use in MEGA 2.1 (Kumar et al., 2001). The phylogenetic trees were constructed using neighbour Joining analysis of distance matrices calculated by Kimura-2 parameter distance method and maximum parsimony in MEGA with a bootstrap value of 1000.

Results

Seventy coyotes were examined for parasites. All the coyotes collected in winter (Group 1; *n* = 46) were judged to be in poor condition, whereas those collected in summer (Group 2; *n* = 24) were in moderate to excellent condition. The latter group commonly had fur and small mammal bones in their gut, whereas the gut contents of winter collected coyotes were devoid of animal remains and in contrast, signs of opportunistic/atypical feeding were evident on occasion with pieces of plastic and other non-animal material recovered.

Toxascaris was the most common parasite in both groups of coyotes (Group 1: 45/46 = 97.8%; Group 2: 22/24 = 91.7%), as determined on the basis of worms recovered from the intestine and eggs in faeces. The prevalence of *Giardia* and *Cryptosporidium* was higher in coyotes in Group 1, which were sampled in winter (10/46 = 21.7% and 8/46 = 17.4%) compared to Group 2 (3/24 = 12.5% and 0/24 = 0%). It was not possible to determine the species of *Taenia* recovered (Group 1: 3/46 = 6.5%; Group 2: 6/24 = 25%) due to the poor condition of the worms, recovered from the intestine all of which were devoid of hooks.

Samples positive for *Giardia* by microscopy were amplified and sequenced at both the 18S rDNA and the *gdh* genes. Sequence information was obtained for 11 of the 16 microscopy-positive *Giardia* isolates. Eight isolates were amplified and sequenced at the 18S rDNA, of these three aligned with the potentially zoonotic assemblage A and five with assemblage D. At the *gdh* locus sequence information was obtained for nine isolates of which five were from assemblage A and two were assemblage D. There were also two mixed isolates one with assemblage A and C and one with assemblage A and D. Sequence information was obtained for six isolates of *Giardia* at both loci but in only two of these isolates did the genotyping results correlate with each other which may be due to the preferential amplification of one genotype over another at the different loci (see Wielinga and Thompson, 2007). Only two of the microscopy-positive *Cryptosporidium* samples could be amplified by PCR and characterised by sequencing at the 18S rDNA and HSP70 loci. Using Clustal X they were compared to a range of known genotypes and were found to be most similar to *C. canis* with only one

(18S rDNA) and four (HSP70) base pair mutations between the coyote isolates and the published reference *C. canis* 18S rDNA (Xiao et al., 2002). A phylogenetic tree constructed from 18S rDNA sequences confirmed the close relationship between the coyote isolates and *C. canis*, and a previously reported isolate from a coyote in the USA, DQ385545 (Trout et al., 2006) (Fig. 1).

Discussion

The high prevalence of *Toxascaris leonina* in the coyotes from Alberta complements previous studies in North America (Conder and Loveless, 1978; Samuel *et al*., 1978 ; Henke *et al*., 2002) and the apparent lack of any seasonal influence suggests that coprophagy may be more important than paratenic hosts in the transmission and maintenance of infection. In contrast, the lower prevalence of *Taenia* in coyotes collected in winter does reflect the reduced availability of intermediate hosts. Coyotes are relatively gregarious and live in family groups for most of the year (Long, 2003).

The absence of *Echinococcus*, particularly *E. multilocularis* was unexpected given the role of the coyote as a definitive host in North America (Rausch, 1995) and must reflect lack of access in the prairies of Southern Alberta and Saskatchewan to infected intermediate hosts of either *Echinococcus granulosus* (by scavenging) or *E. multilocularis* (by predation). The emerging problem of cystic echinococcosis in farmed elk in Alberta (Thompson et al., 2006; http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex8833?open document) raises the possibility of coyotes contracting infection from scavenging carcasses near areas where elk are farmed.

Evidence of seasonal variation in the occurrence of *Giardia* and *Cryptosporidium* was found but was particularly marked with *Cryptosporidium*. This is unlikely to be related to increased environmental exposure to infective stages in the harsh winter environment. Since this variation correlates with the fact that coyotes sampled in winter were in poor condition and nutritionally compromised, this stress may have promoted the expression of infection as evidenced by the detection of oocysts in the faeces.

The suppression of host immunity as a result of disease, e.g. HIV, or stress associated with poor living conditions and overcrowding (Current, 1998; Newman *et al*., 1999) is associated with relapses of *Cryptosporidium* infections in humans and other animals (Okhuysen and White, 1999). Such relapses are thought to be due to latent infections with *Cryptosporidium* (Holley and Thiers, 1986; Tatar *et al*., 1995; Maggi *et al*., 2000), a phenomenon that may also be associated with repeated shedding in other animals (e.g.Warren *et al*., 2003; Atwill *et al*., 2004). In humans, seroepidemiological data suggest that *Cryptosporidium* infections may be more common in most regions than faecal oocyst surveys have indicated (Current, 1998). Evidence of such latent infections lends support to the importance of host cell invasion by *Cryptosporidium* as an immunological defence mechanism (Thompson *et al*., 2005; Borowski *et al*., 2008).

Host-adapted species/genotypes of *Cryptosporidium* (*C. canis*) and *Giardia* (*G. duodenalis* assemblage D) were recovered from the coyotes, indicating their maintenance within coyote populations. Zoonotic genotypes of *Giardia* (*G. duodenalis* assemblage A) were also recovered from some coyotes demonstrating their role as possible reservoirs of a parasite of public health significance. *Giardia* and *Cryptosporidium* infections in coyotes may also represent spillover infections from scavenging on human waste, as there was evidence of close association with humans in these study animals. The adaptability of coyotes to areas inhabited by humans where they may serve as reservoirs for zoonotic as well as common canid diseases has been emphasised (Grinder and Krausman, 2001; Bischof and Rogers, 2005) and should be considered in parasite control efforts.

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Fig. 1. Phylogenetic tree constructed from the 18S rDNA sequences of *Cryptosporidium* isolates using Kimura-2 parameter distance estimation method clustered by the NJ method in MEGA 2.1 and evaluated by 1000 bootstraps. Percentage bootstrap support of >50% analyzed by NJ and parsimony methods are shown respectively. *ns* node not supported by method. Since both coyote samples from this study were identical only one is included in the tree.