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GIARDIA DUODENALIS CYSTS ISOLATED FROM WILD MOOSE AND REINDEER IN NORWAY: GENETIC CHARACTERIZATION BY PCR-RFLP AND SEQUENCE ANALYSIS AT TWO GENES

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ABSTRACT: There are few genotyping studies of *Giardia duodenalis* isolates from cervid hosts, although a previous study suggested that cervids may be a source of infection for humans and cattle. *Giardia duodenalis* isolates collected from wild moose (*Alces alces*) and reindeer (*Rangifer tarandus*) in Norway during 2002 and 2003 were characterized by polymerase chain reaction–restriction fraction length polymorphism (PCR-RFLP) at the β-giardin gene, and sequence analysis at both the β-giardin and glutamate dehydrogenase (*gdh*) genes. All results suggested that these isolates ($n=25$) belonged to assemblage A. Three different restriction patterns were obtained with PCR-RFLP, one of which has previously been associated with assemblage A. At the β-giardin gene, sequences from six reindeer isolates and one moose isolate were identical to a previously published assemblage A sequence from *G. duodenalis* cysts isolated from dairy calves. The other 10 moose isolates could be divided into five groups, with between two and 14 single nucleotide polymorphisms (SNPs) from the published genotype A2. At the *gdh* gene, three different sequences were obtained, differing from each other by between one and 15 SNPs and which have all been previously published as genotype A1, but with different specific hosts. Grouping of the isolates based on the sequences from both genes gave complex results; whereas all the *G. duodenalis* isolates from reindeer grouped together, two moose isolates, which had identical sequences at the β-giardin gene, had sequences that differed from each other by 15 SNPs at the *gdh* gene. The results of these studies, together with the large Norwegian populations of these cervids and the amount of fecal matter they produce, indicate that moose and reindeer may be significant reservoirs of *G. duodenalis* infection in Norway, which may be of importance to veterinary and public health.

Key words: Cervids, genotyping, *Giardia duodenalis*, moose, PCR-RFLP, reindeer, zoonoses.

INTRODUCTION

Although *Giardia duodenalis* is recognized as a relatively common parasite in a range of animal species and in humans, the zoonotic potential is not currently resolved, particularly with respect to wildlife infections. It is generally accepted that at least seven major assemblages exist in the *G. duodenalis* species complex (Thompson and Monis, 2004), most of which have distinct associations with particular host species. Only assemblages A and B appear to be associated with human infections (Thompson, 2000), but *G. duodenalis* isolates within these assemblages have also been identified from various animals, including cattle, dogs, and cats (Read et al., 2004; Lalle et al., 2005). Information on *G. duodenalis* genotypes from cervid hosts is less extensive.

The current known status of *G. duodenalis* in mammalian wildlife has been recently reviewed (Appelbee et al., 2005). Results from prevalence studies that are supported with molecular-genotyping, particularly in defined locations where the animal/environment interactions have been characterized will be of

paramount importance in furthering our understanding of transmission dynamics and *G. duodenalis* epidemiology (Appelbee et al., 2005). Animals within water catchments may contaminate water supplies and, therefore, represent a public health concern.

Large populations of wild cervids are widely distributed throughout Norway, including water catchment areas, and have a significant impact on the Norwegian environment. Population size is largely managed by hunting, which is extensive and is controlled by license distribution and hunting quotas; over 10,000 licenses for hunting cervids in Norway are issued annually (Statistics Norway, 2005).

During 2002 and 2003, fecal samples were collected from free-ranging wild moose (*Alces alces*) ($n=455$) and reindeer (*Rangifer tarandus*) ($n=155$) that were harvested in Norway during the hunting season (August to December); these were examined for *Cryptosporidium* spp. oocysts and *G. duodenalis* cysts (Hamnes et al., 2006). *Giardia duodenalis* cysts were detected in 56 (12.3%) of the moose samples and in 11 (7.1%) of the reindeer samples. As far as the authors were aware, this was the first report of *G. duodenalis* being detected in reindeer samples.

There are very few genotyping studies of *G. duodenalis* isolates from cervid hosts, although *Giardia* sp. cysts isolated from a white-tailed deer have previously been suggested as a potential source of infection for both humans and cattle (Trout et al., 2004a). To investigate whether the *G. duodenalis* isolated from the moose and reindeer samples in Norway might similarly be a potential source of infection for humans or domestic animals and thus of possible public and veterinary health significance, selected isolates of *G. duodenalis* cysts from these Norwegian cervids were characterized at a molecular level, by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) or by sequence analysis.

MATERIALS AND METHODS

Fecal samples

Fecal samples collected from moose and reindeer shot during the hunting season in 2002 (moose) and 2003 (moose and reindeer) were sent for analysis to the Parasitology Laboratory at the Norwegian School of Veterinary Science. Further details of sample collection and the initial analyses have been published (Hamnes et al., 2006).

Isolation of cysts from fecal samples

Fecal samples, purified by sucrose flotation (Hamnes et al., 2006), had been identified by microscopy as containing *G. duodenalis* cysts. Samples were selected that contained moderate to high numbers of cysts and that also had at least some cysts with nuclei (as demonstrated by inclusion of 4′6-diamidino-2-phenyl indole). Sixteen moose samples (29% of the positive samples) and nine reindeer samples (82% of the positive samples) were selected; details of the animals from which the samples were derived are given in Table 1. The cysts were purified further by immunomagnetic separation (IMS; GC-Combo, Dynal Biotech ASA, Oslo, Norway) using a modified procedure from that described by the manufacturer (Robertson et al., 2006).

DNA isolation

Cysts were resuspended in TE buffer, and placed in a heat block set at 100 C for 1 hr. DNA was isolated using a QIAamp DNA Mini Kit (Qiagen GmbH, Hilden, Germany). At the final step, two elutions in distilled water were performed. The isolated DNA was stored frozen until PCR amplification.

PCR, electrophoresis, RFLP, purification of PCR products, and sequencing

Two genes were used for molecular characterization: the β-giardin gene and the glutamate dehydrogenase (*gdh*) gene, using published methods and primers (Caccio et al., 2002; Read et al., 2004), with slight modifications to described protocols. Primers, PCR reaction mixture, and reaction conditions are described in Table 2. PCR products were electrophoresed on 1% agarose gels and stained with ethidium bromide.

For RFLP (Table 1), the PCR products from the β-giardin gene PCR were incubated overnight at 37 C with the restriction enzyme Hae III (10 μl PCR-product, 3 μl Hae III, 3 μl buffer, and 4 μl distilled water) and the

TABLE 1. Summary of information on moose and reindeer from which *Giardia duodenalis* cysts originated.[a]

	Moose samples	Reindeer samples
n	16	9
Age groups	All calves	7 calves, 2 adults
Sex	9 females, 7 males	3 females, 3 males, 2 unknown
Origin of samples in Norway by county	Nordland (*n*=1); Telemark (*n*=1); Vest Agder (*n*=3); Sør-Trøndelag (*n*=4); Oppland (*n*=7).	Sør-Trøndelag (*n*=2); Hedmark (*n*=5); unknown (*n*=2).
RFLP following PCR at β-giardin gene	12	6
Sequencing attempted following PCR at β-giardin and *gdh* genes	14 (10 of which had been analyzed by RFLP)	6 (3 of which had been analyzed by RFLP)
Sequencing successful following PCR at β-giardin gene	11 (8 of which had been analyzed by RFLP)	6 (3 of which had been analyzed by RFLP)
Sequencing successful following PCR at *gdh* gene	13	6

[a] RFLP = restriction fragment length polymorphism; PCR = polymerase chain reaction.

products electrophoresed on both 1% and 5% agarose gels and stained with ethidium bromide for visualization of products.

For sequencing (Table 1), the PCR-products were purified (High Pure PCR Product Purification Kit, Roche Diagnostics GmbH, Mannheim, Germany) following the manufacturer's protocol with minimal modifications. The air-dried purified products were sent for sequencing on both strands to MWG Biotech in Ebersberg, Germany. Chromatograms were examined using Chromas Lite (Cologne, Germany; http://www.technelysium.com.au) and sequences were examined using VectorNTI Invitrogen software. Sequence searches were conducted using BLAST

TABLE 2. Primer sequences and polymerase chain reaction (PCR) conditions used.[a]

Gene (product size)	Reference	Forward primer		Reverse primer		PCR reaction conditions
		Name	Sequence	Name	Sequence	
β-giardin (753 bp)	Caccio et al. (2002)	G7	AAG CCC GAC GAC CTC ACC CGC AGT GC	G759	GAG GCC GCC CTG GAT CTT CGA GAC GAC	Denaturation: 95 C for 15 min 50 cycles of 94 C for 30 sec, 66 C for 30 sec, 72 C for 30 sec Final extension: 72 C for 10 min
Gdh (432 bp)	Read et al. (2004)	GDHeF	TCA ACG TYA AYC GYG GYT TCC GT	GDHiR	GTT RTC CTT GCA CAT CTC C	Denaturation: 95 C for 15 min 50 cycles of 94 C for 45 sec, 54 C for 45 sec, 72 C for 45 sec Final extension: 72 C for 10 min

[a] For both genes the following PCR reaction mixture was used: 10 pmol of each primer, 0.4 µl bovine serum albumin (BSA) (20 mg/ml), 17.6 µl water, 25 µl HotStartTaqmaster (Qiagen), and 3 µl template (total reaction volume=50 µl). For each reaction set, negative (water) and positive (DNA from *G. duodenalis* trophozoites, assemblage A, Hyperion Research, Medicine Hat, Canada) controls were included.

TABLE 3. Restriction patterns from Hae III digestion of polymerase chain reaction (PCR) products of the β-giardin gene.

	Sizes of fragments in restriction patterns (bp)		
	Restriction pattern 1[a] 74, 126, 150, 201, 202	Restriction pattern 2 74, 126, 202, 351	Restriction pattern 3 74, 126, 150, 201, 202, 351
Moose	7 samples	3 samples	2 samples
Reindeer	1 sample	3 samples	2 samples

[a] This pattern is described for *G. duodenalis* assemblage A (Caccio et al., 2002).

(http://www.ncbi.nlm.nih.gov/BLAST/). Appropriate sequences for those isolates, which had also been examined by RFLP, were analyzed using NEBcutter (New England BioLabs, Frankfurt am Main, Germany; http://tools.neb.com/NEBcutter2/index.php) to determine the points where the sequences would have been cut with Hae III and the sizes of the resultant fragments.

New sequences were deposited in GenBank under the accession numbers: DQ648777-DQ648781.

RESULTS

RFLP

Three different restriction patterns were obtained by RFLP of the β-giardin sequence (Table 3). The first restriction pattern (74 bp, 126 bp, 150 bp, 201 bp, 202 bp) has been previously associated with *G. duodenalis* assemblage A (Caccio et al., 2002). The second pattern is similar but misses a cleavage between the fragments of 150 bp and 201 bp, thus these fragments are replaced by a single one of 351 bp. The third pattern contains all the fragments found in the previous two (i.e., six fragments in total).

Genotyping/sequence analysis

All the *G. duodenalis* isolates from both moose and reindeer were most similar to assemblage A at both genes. With the exception of one β-giardin sequence from *G. duodenalis* cysts isolated from a reindeer and one β-giardin sequence from cysts isolated from a moose (identified in Table 4), all the sequences described were clear, with single distinct peaks on the electropherograms.

Genotyping/sequence analysis at the β-giardin gene and comparison with RFLP

At the β-giardin gene, sequencing was successful for 11 moose samples (eight of which had been analyzed by RFLP) and all six reindeer samples and yielded six different sequences (Table 4). These were similar, but not identical, to sequences described as A2 (GenBank accession number: AY072723; Caccio et al., 2002) and A3 (GenBank accession number: AY072724; Caccio et al., 2002) but had between two and 14 single nucleotide polymorphisms (SNPs) from either A2 or A3 sequences, and all also had one SNP (at position 561) from both A2 and A3 (Table 4).

All the sequences from the reindeer isolates and from one moose isolate (seven isolates in total) were identical to one submitted from *G. duodenalis* cysts isolated from dairy calves in USA (GenBank accession number: AY655702; Trout et al., 2004b). The other 5 sequence types, each of which was represented by between one to four moose isolates, have not previously been described and have been submitted to GenBank (accession numbers: DQ648777-DQ648781).

The *G. duodenalis* β-giardin gene sequences from reindeer isolates, which were analyzed by RFLP ($n=3$) (and were sequenced), all contained three cutting points for Hae III. The RFLP should have resulted in four fragments; two central fragments of 126 bp and 351 bp and two fragments at either end of >69 bp and >187 bp, with the actual sizes of the last two fragments dependent on sequence length. These are equivalent to the actual results obtained. The *G. duodenalis* β-giardin gene sequences from moose isolates, which had also been analyzed by both RFLP ($n=8$) and sequencing, all contained four cutting points for Hae III, which would result in five fragments;

TABLE 4. Sample groupings based on nucleotide changes[a] at the β-giardin gene as compared with published subgenotypes A2 (and A3).

Nucleotide positions[a]	A2 AY072723	A3 AY072724	A AY655702 6 reindeer, 1 moose	BG-cer1[e] 4 moose	BG-cer2[f] 2 moose	BG-cer3[g] 2 moose	BG-cer4[h] 1 moose	BG-cer5[i] 1 moose
39	G	G	G	G	G	G	G	T
81	C	C	T[b]	C	C	C	T	C
156	C	C	T[b]	C	C	C	T	C
176	T	T	T	T	T	T	C	T
249	C	C	C	T	C	C	C	T
324	C	C	C	C	C	C	C	C[c]
348	T	T	T	C	T	T	T	C
372	T	T	T	C	T	T	T	C
405[d]	C	C	T[b]	C	C	C	T	C
415	C	T	C	C	C	C	C	C
423	T	C	T	C	T	T	T	C
438	T	T	T	C	T	T	T	C
456	A	A	A	G	A	A	A	G
528	G	G	G	A	G	G	G	A
558	T	T	T	C	T	T	T	C
561	T	T	C	C	C	C	C	C
576	A	A	A	G	A	A	A	G
588	C	C	C[b]	C	C	T	C	C
644	T	T	T	T	T	T	C	T
651	T	T	T	C	T	T	T	C
684	G	G	G	G	A	G	G	G
696	A	A	A	G	A	A	A	G
702	C	C	C	T	C	C	C	T
704	A	A	A	A	A	A	G	A
TOTAL SNP FROM A2	—	2	4	13	2	2	7	14
TOTAL SNP FROM A3	2	—	6	13	4	4	9	14

[a] Nucleotide substitutions from A2 are underlined.

[b] One reindeer sample showed a double peak of T and C at these positions.

[c] A double peak (C/T) at this position.

[d] SNP at this position responsible for change in restriction fragment length polymorphism (RFLP) pattern as enzyme Hae III cuts at GGCC.

[e] Genbank accession number of sequence: DQ648777.

[f] Genbank accession number of sequence: DQ648778.

[g] Genbank accession number of sequence: DQ648779.

[h] Genbank accession number of sequence: DQ648780.

[i] Genbank accession number of sequence: DQ648781.

three central fragments of 126 bp, 150 bp, and 201 bp, and two fragments at either end of >54 bp and >89 bp, with the actual sizes of the last two fragments dependent on sequence length. For seven samples, these are equivalent to the actual results obtained, but for one sequence, four fragments were obtained similar to for the reindeer samples.

Genotyping/sequence analysis at the *gdh* gene

At the *gdh* gene, sequencing was successful for all but one of the samples analyzed (13 from moose, six from reindeer) and yielded three different sequences, each of which was identical to a previously published sequence (Table 5). All the isolates from reindeer and five of the isolates from moose were

TABLE 5. Sample groupings based on nucleotide changes[a] at the *gdh* gene compared with published subgenotype A1, GenBank accession number: AY178735.

Nucleotide positions[a]	A1 (specific host, *Homo sapiens*) AY178735 6 reindeer, 5 moose	A (specific host, roe deer) DQ100288 7 moose	A1 (specific host, ferret) AB159795 1 moose
74	T	C	T
83	C	G	C
95	T	C	T
134	C	T	C
197	C	A	C
215	T	C	T
221	A	G	A
347	G	C	G
356	C	T	C
359	A	G	A
395	C	T	C
428	T	C	T
446	C	C	T
458	C	T	C
461	T	C	T
Total SNP from AY178735	—	15	1

[a] Nucleotide substitutions as compared with A1 (AY178735) are underlined.

identical to a sequence described as A1, specific host *Homo sapiens* (GenBank accession number: AY178735); seven isolates from moose were identical to a sequence described as A, specific host roe deer (GenBank accession number: DQ100288; Van der Giessen et al., 2006), which is 15 SNPs from the previously mentioned sequence A1, host *Homo sapiens*. One isolate from a moose was identical to a sequence described as A1, specific host ferret (GenBank accession number: AB159795; Abe et al., 2005), which is one SNP from the previously mentioned A1, host *Homo sapiens*, sequence.

Comparison of results from sequence analysis at the *β-giardin* and *gdh* genes

The moose isolate that had grouped with the reindeer isolates at the β-giardin gene also grouped with the reindeer isolates at the *gdh* gene. Additionally, two moose isolates, which had grouped together at the *β-giardin* gene as Group BG-cer2 (Table 4), grouped with the reindeer isolates at the *gdh* gene. Similarly, the four moose isolates, which had grouped together at the β-giardin gene as Group BG-cer1 (Table 4), remained grouped together at the *gdh* gene, along with the single moose isolates in each of Groups BG-cer4 and BG-cer5. However, the two moose isolates in Group BG-cer3 (Table 4) at the β-giardin gene differed significantly from each other at the *gdh* gene with 15 SNPs between them, one of them being grouped with those of the BG-cer1, BG-cer4, and BG-cer5 group (at the *gdh* gene, identified as identical to assemblage A, specific host roe deer; GenBank accession number: DQ100288), whereas the other was alone in a separate group (at the *gdh* gene, identified as identical to assemblage A, specific host ferret; GenBank accession number: AB159795).

DISCUSSION

The zoonotic potential of *G. duodenalis* has been a matter of contention for many years, particularly when assessing whether animal infections may be the source of waterborne outbreaks of giardiasis. With the advent of molecular methods for investigating genetic differences between isolates from different host species, more detailed and extensive information has become available to add to the debate, but without any categorical resolution of the controversy.

Genotyping of *G. duodenalis* cysts isolated from a single white-tailed deer (*Odocoileus virginianus*) in USA (Trout et al., 2004a) at two gene loci (β-giardin and TPI) indicated that they belonged to assemblage A and the authors suggested that deer could be a potential source of cysts infectious for humans or other animals. In contrast, genotyping of *G. duodenalis* cysts isolated from a single roe

deer (*Capreolus capreolus*) in The Netherlands (Van der Giessen et al., 2006) at a single gene (*gdh*), indicated that although the isolate was in assemblage A, it did not cluster with the human isolates of that assemblage, and therefore, the authors suggested it might differ from isolates in humans. It is interesting to note that, to date, no *G. duodenalis* isolates from cervids have been demonstrated to belong to assemblage E, which has been identified relatively widely in other ruminants (cattle and sheep).

Comparison of the β-giardin sequence from the white-tailed deer (GenBank accession number: AY302561; Trout et al., 2004a) with the sequences obtained in this study demonstrates that it was most similar to the sequences obtained from the two moose isolates in Group BG-cer2 (GenBank accession number: DQ648778), but because it was some 250 bp shorter than the present moose isolate sequences, eight potential sites of SNPs could not be compared. For the remaining 16 SNP sites that could be compared, the sequence from the white-tailed deer only differed from Group BG-cer2 at nucleotide position 423, where a cytosine residue was reported. Additionally, the white-tailed deer sequence demonstrated a single SNP at nucleotide position 128, which did not occur with any of the samples isolated from *G. duodenalis* from moose or reindeer in any group and also does not occur in either of the documented sequences A2 and A3 (GenBank accession numbers: AY072723 and AY072724; Caccio et al., 2002).

Whereas some sequences obtained at the β-giardin were new, all the sequences at the *gdh* gene had previously been recorded by other research groups. A *gdh* sequence reported from a roe deer (GenBank accession number: DQ100288) was one of the three sequences identified and occurred in isolates from seven moose. However, from all the reindeer and the other six moose, the sequences were either more similar to, or identical to, a *gdh* sequence associated with human infections.

One *gdh* sequence from *G. duodenalis* isolated from a moose was identical to one reported from a ferret-derived isolate in Japan (GenBank accession number: AB159795; Abe et al., 2005). As the DNA from the ferret isolate had also been analyzed at the *β-giardin* gene, this *β-giardin* sequence (GenBank accession number: AB159797) was compared with that obtained from the moose isolate (GenBank accession number: DQ648779). Although the comparison was limited by the relatively short sequence length from the ferret isolate (some 240 bp shorter), the sequences differed by at least two SNPs. Indeed, the β-giardin sequence from the ferret isolate was actually more similar to that from another cervid isolate group (BG-cer4; GenBank accession number: DQ648780), having only one SNP from this sequence, although at the *gdh* gene the sequence from this isolate was 15 SNPs different from that isolated from the ferret.

A further complexity is added when comparing the results of group sequence analyses at both genes with each other. Whereas six groups were obtained at the β-giardin gene and only three at the *gdh* gene, in general the different isolates remained similarly grouped, such that all the isolates from two groups at the β-giardin gene (those identical to assemblage A, Genbank accession number: AY655702 and those in group BG-cer2) were in the same group at the *gdh* gene, and all the isolates from three groups at the β-giardin gene (all those in group BG-cer1 and those from BG-cer4 and BG-cer5) were also together at the *gdh* gene. However, another two isolates from moose, which were identical at the β-giardin gene (in group BG-cer3; GenBank accession number: DQ648779) differed from each other at the *gdh* gene by 15 SNPs.

This complexity when grouping isolates following sequencing at several gene loci

has previously been encountered in other *G. duodenalis* genotyping studies. Thus, 21 human isolates, originating from a waterborne outbreak of giardiasis, also showed a lack of concurrence at the β-giardin and *gdh* gene (Robertson et al., 2006). Similarly, genotyping of *G. duodenalis* isolates from cats and dogs at two genes (Read et al., 2004) demonstrated a lack of concurrence in results from each gene, with some isolates being described as potentially zoonotic at one locus (i.e., from assemblage A or B) but nonzoonotic at another locus. These authors suggested that mixed infections with several genotypes may be one explanation with different genotypes being preferentially amplified over others at different loci. However, because of one sequence (at the *gdh* gene) being less conserved and longer than the other sequence, these authors also suggested that the information from the *gdh* gene may be more reliable. Conversely, in the study of *G. duodenalis* isolates from cervids described here, the *gdh* sequence (432 bp) is both more conserved and shorter than the β-giardin sequence (753 bp). Rather than suggest that this may indicate that the data from the β-giardin sequences is more reliable, we believe that this highlights the complexity of the system and that caution must be used when attempting to group isolates from sequence data, particularly if data from only a single gene is available. Changes or polymorphisms may occur at different rates in different genes, and thus, isolates that have similar sequences in one locus may be less similar at other loci. Additionally, allelic sequence heterozygosity (ASH) may occur, as well as mixed infections with several genotypes. The use of different classification systems at different genes is suggested as one approach to simplify the complexity around the present system.

Although sequence data provides more information than PCR-RFLP, use of an established PCR-RFLP system as described here may be useful in providing crude information on isolate similarity. Additionally, when combination patterns are obtained (as in the third pattern here), this may provide evidence of mixed-genotype infections (or ASH within a single genotype infection), which might not otherwise be apparent, particularly if one genotype in a mixed infection predominates. Our limited PCR-RFLP results suggest that for four *G. duodenalis* isolates (two from reindeer and two from moose), infections either were mixed or contained ASH within a single infection. Unfortunately, only for one of these mixed-pattern isolates was the PCR product sequenced, but the electropherogram did not demonstrate multiple peaks. Additionally, it was unfortunate that the single reindeer isolate that demonstrated a different PCR-RFLP pattern to that of the other reindeer isolates could not be sequenced because that would be expected to show a different sequence to those from the other reindeer isolates (notably a cytosine residue rather than a thymine residue at nucleotide position 405). One inconsistency between the PCR-RFLP and sequencing results was for one isolate from a moose, which was in Group BG-cer1, and, therefore, had a cytosine residue at nucleotide position 405. Therefore, it should have produced a RFLP pattern of five bands; however, only four bands were demonstrated, indicating a lack of digestion of the 351 bp band. Whether this is indicative of a mixed infection, which showed up on neither the sequencing nor RFLP individually, or is indicative of a failure of enzymic digestion for methodologic reasons cannot be ascertained. However, it does demonstrate that the use of PCR-RFLP may indicate associations between isolates that are not necessarily correct and, therefore, that PCR-RFLP results should always be treated with caution and supported with sequencing data.

In general, relatively little information has been compiled to date on the zoonotic potential of *G. duodenalis* infections in

cervid populations. However, in some localities, including much of Norway, deer may be an important potential reservoir of infection. Not only are there large populations of cervids in Norway (estimates made both from observations and from records of hunted deer indicate summer populations in Norway of approximately 30,000 to 35,000 wild reindeer and 145,000 to 165,000 moose; additionally there are large populations of roe deer and red deer and approximately 185,000 semidomesticated reindeer; Solberg et al., 2003, 2006; Statistics Norway, 2005) but also, because they are large herbivores, they produce substantial quantities of feces on a daily basis. It has been estimated (Hjeljord et al., 1994; Persson et al., 2000) that a single moose produces between 2 kg and 5 kg (dry weight) of feces daily, weighing about 14 kg wet (extrapolation from these estimates indicates that the combined annual fecal output of the Norwegian moose population is sufficient to cover Greater New York City to a depth of more than 1 cm). Additionally, cervids have been frequently reported in water catchment areas in Norway (particularly moose and roe deer but also red deer and reindeer; Robertson et al., 2001), although in that study no association was detected between the occurrence of these animals in catchment areas and *G. duodenalis* cysts in the associated water supply (Robertson et al., 2001). These data described here suggest that we cannot exclude cervids in Norway as being a reservoir of *G. duodenalis* infection for both people and domestic animals and also that cervids may be susceptible to *G. duodenalis* infections from humans and domestic animals. Whereas some of our data, in conjunction with that from a previous publication (Van der Giessen et al., 2006), suggest the possibility of a *G. duodenalis* isolate that is exclusive to cervids, other data from our studies indicate that those genotypes of *G. duodenalis* detected in moose and reindeer may also occur among other mammals, including ferrets (Abe et al., 2005), cattle (Trout et al., 2004b), and humans (Caccio et al., 2002). The majority of *G. duodenalis* isolates from Norwegian cattle investigated in our laboratory have been of assemblage E, although genotypes similar to A2 at the β-giardin gene (GenBank accession number: AY072723), and similar to A1 (GenBank accession number: AY178735) or B3 (GenBank accession number: DQ090534) at the *gdh* gene have also been identified (Norges Veterinærhøgskole, Parasitology Laboratory, unpubl. data). Genotyping at two gene loci (β-giardin and *gdh*) of an anomalous *G. duodenalis* isolate from a Norwegian reindeer sample (host information missing) in our laboratory (but not included in the data described here) identified it as being from assemblage B and with sequences identical to those associated with a waterborne giardiasis outbreak in Norway in the winter of 2004 (GenBank accession numbers: DQ090523 and DQ090534; Robertson et al., 2006).

The majority of semidomesticated reindeer in Norway live geographically distant from the wild reindeer (most semidomesticated reindeer are in northern Norway, particularly Finnmark county, which is >1,000 km from the location of the wild reindeer populations of Norway), but in some areas, particularly Hedmark and Oppland, it may be possible for wild reindeer and semidomesticated reindeer to come into contact with each other or share grazing land. It would, therefore, also be of interest to investigate the occurrence and genotypes of *G. duodenalis* in the semidomesticated reindeer population, as well as among the associated reindeer herders, in the different regions of Norway. It would also be of interest to attempt to determine whether any *G. duodenalis* infections in cervids are symptomatic.

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