Occurrence and molecular typing of *Giardia* isolates in pet rabbits, chinchillas, guinea pigs and ferrets collected in Europe during 2006–2012

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A total of 1180 faecal samples (528 from rabbits, 531 from chinchillas and 121 from guinea pigs) collected during 2006–2012 by veterinarians in Germany and in other European countries were submitted to a diagnostic laboratory for *Giardia* testing by means of coproantigen ELISA. Of these samples, 40 rabbits (7.6 per cent), 326 chinchillas (61.4 per cent) and five guinea pigs (4.1 per cent) were found to be positive. To gain insights into the genetic identity of *Giardia* in small mammals, ELISA-positive samples from 23 chinchillas, five ferrets, a rabbit, and a Desmarest’s hutia were investigated by PCR and sequencing of fragments of the small subunit ribosomal DNA (*ssu*), the triose phosphate isomerase (*tpi*) and the β-giardin (*bg*) genes. At the *ssu* locus, assemblage B was identified in 28 of 30 isolates, whereas assemblage A and D were each detected in one sample. The majority of isolates from chinchillas and those from ferrets had *Giardia duodenalis* sequences identical to sub-assemblages AI or BIV, based on either a single locus (*tpi* or *bg*) or multiple loci (*tpi* and *bg*). As sub-assemblies AI or BIV are associated with human infection, these results indicate that small mammals can act as reservoirs of cysts potentially infectious to humans.

**Introduction**
Small mammals are becoming increasingly popular among pet owners and their percentage in Small Animal practitioners’ patient material has also been increasing continuously over the past years. A common question arising is whether these animals that live in close contact to humans (particularly children) can be a source of zoonotic parasitic infections. Among zoonotic parasites, the unicellular flagellate *Giardia duodenalis* (syn. *Giardia intestinalis*, *Giardia lamblia*) is known to cause gastroenteritis in a wide range of vertebrates, including mammals and humans. Molecular and phylogenetic analyses of *G. duodenalis* isolates have identified eight distinct genetic groups (known as assemblages A–H), which differ in their host distribution (Monis and others 2009, Takumi and others 2012). Assemblages A and B are associated with human infection but they are also found in many other mammals; despite this, the role of animals in the epidemiology of human infection is still unclear (Sprong and others 2009).

Recent molecular findings have shown the presence of potentially zoonotic *G. duodenalis* assemblages (mainly B) in rabbits, guinea pigs and chinchillas (e.g., Lebbad and others 2010, Levecke and others 2011). *In vitro* cultivation of *Giardia* isolates from rabbit and chinchilla was established as early as 1965 (Meyer and Pope 1965), yet to date, few epidemiological and molecular studies have been conducted on these pet animals. During 2002–2004, faecal samples from 84 rabbits, 195 chinchillas and 27 guinea pigs, collected by veterinarians in Germany and other European countries, were examined for the presence of *Giardia* by means of coproantigen ELISA (Pantchev and others 2005). This survey revealed a prevalence of 5.9% (95% CI 2% to 13.3%) in rabbits, 66.7% (95% CI 59.6% to 73.2%) in chinchillas and 0% (95% CI 0% to 12.7%) in guinea pigs (Pantchev and others 2005). The present study reports the results of a similar study conducted from 2006 through 2012, using the same test (coproantigen ELISA), with samples from the same geographical areas, and with the inclusion of genotyping data from selected positive samples. Since a recent study suggested a significant increase in *Giardia* infection in ferrets from 2.9 per cent (2002–2004) to 13.3 per cent (2009–2010) (Pantchev and others 2011), genotyping of positive samples of ferrets was also undertaken. Furthermore, a sample of a Desmarest’s hutia was included for genotyping.

**Materials and methods**

**Samples and coproantigen analysis**
A total of 1180 faecal samples (528 from rabbits, 531 from chinchillas and 121 from guinea pigs) were collected during 2006–2012 by veterinarians from Germany (approximately 85 per cent) and other European countries (approximately 15 per cent from Austria, Denmark, Hungary, Finland, France, Italy, Luxembourg,
the Netherlands, Norway, Poland, Portugal, Sweden), and were submitted to the author’s laboratory for *Giardia* diagnostics. Samples were screened using the ProSpecT Giardia Microplate Assay (Remel), which is officially registered for use in animals in Germany by the Friedrich Loeffler Institute. The test was performed according to the manufacturer’s instructions (optical density; measured by plate reader; see also Table 1). The coproantigen ELISA uses a monoclonal antibody for the qualitative detection of a *Giardia*-specific antigen (GSA 65; Rosoff and Stubbs 1996) in faecal samples.

## Molecular analysis

Faecal samples for molecular analysis were randomly selected from those with a positive coproantigen result and for which enough material was left for DNA extraction; they were labelled and stored at −20°C. The samples were shipped to the laboratories at Federal Institute for Risk Assessment, BfR (n = 8; Group A) during 2007–2008 (all from Germany except VB934778 from Luxembourg); analysis of *ssu* according to Hopkins and others (1997) which was performed according to Read and others (2002). PCR products were purified using spin columns (GE Healthcare) and sequenced from both strands. Sequences were edited using SeqMan 7.0 software (DNASTAR, Wisconsin, USA). Representative sequences of sub-assemblages A (strains Ad-1 and WB), B (strains KCS and Ad-2), BIII (strain BAH12) and BIV (strains Nij8 and Ad-28) were retrieved from GenBank and used as reference (Monis and others 1996, 1999, Homan and others 1998).

## Co-infection analysis

Whenever possible, faecal samples were analysed by standard bacteriological procedures (including culturing of Campylobacter, Yersinia and Salmonella), flotation or Cryptosporidium coproantigen ELISA (ProSpecT, Cryptosporidium Microplate ELISA Assay, Remel; tests for parasites were described by Pantchev and others 2005, see also Table 1).

## Statistical analysis

Differences between prevalence rates (coproantigen ELISA) of the first study performed (2002–2004; Pantchev and others 2005) and the present study (2006–2012), and differences among different animal species (rabbits, chinchillas and guinea pigs) of the present study were analysed for significance using the χ² test; differences were regarded as significant at a level of p < 0.05.

## Results

### Coproantigen test

*Giardia* coproantigen was detected using an ELISA assay in faecal samples from 40 of 528 rabbits (7.6 per cent; 95 per cent CI 5.5 to 10.2 per cent), 326 of 531 chinchillas (61.4 per cent; 95 per cent CI 57.1 to 65.6 per cent) and five of 121 guinea pigs (4.1 per cent; 95 per cent CI 1.4 to 9.4 per cent).

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**Table 1: Distribution of *Giardia duodenalis* assemblages in 30 small mammal isolates as inferred from genotyping at the *ssu*, *tpi* and *bg* genes**

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Host</th>
<th><em>ssu</em></th>
<th><em>tpi</em></th>
<th><em>bg</em></th>
<th>OD value ELISA†</th>
<th>Co-infections</th>
</tr>
</thead>
<tbody>
<tr>
<td>VB935836</td>
<td>Chinchilla</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>0.56</td>
<td>Proteus species, <strong>flotation negative‡</strong></td>
</tr>
<tr>
<td>VB931161</td>
<td>Chinchilla</td>
<td>B</td>
<td>A</td>
<td>nc</td>
<td>2.33</td>
<td>Proteus species, <strong>flotation negative</strong></td>
</tr>
<tr>
<td>VB932208</td>
<td>Chinchilla</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>3.85</td>
<td><strong>Flotation negative</strong></td>
</tr>
<tr>
<td>VB935811</td>
<td>Ferret</td>
<td>B</td>
<td>nc</td>
<td>nc</td>
<td>3.58</td>
<td><strong>Escherichia coli</strong>, flotation negative</td>
</tr>
<tr>
<td>VB935615</td>
<td>Chinchilla</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>0.34</td>
<td>Bacterial culture/Cryptosporidium/flotation negative</td>
</tr>
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<td>VB935834</td>
<td>Chinchilla</td>
<td>B</td>
<td>nc</td>
<td>B</td>
<td>3.68</td>
<td><strong>Not known</strong></td>
</tr>
<tr>
<td>VB936855</td>
<td>Chinchilla</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>3.90</td>
<td><strong>Flotation negative</strong></td>
</tr>
<tr>
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<td>Chinchilla</td>
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<td>B</td>
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<td>3.73</td>
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<td>Chinchilla</td>
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<td>nc</td>
<td>B</td>
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<td><strong>E coli</strong></td>
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<td>B</td>
<td>B</td>
<td>3.55</td>
<td><strong>Not known</strong></td>
</tr>
<tr>
<td>VB931416</td>
<td>Chinchilla</td>
<td>B</td>
<td>nc</td>
<td>B</td>
<td>3.93</td>
<td><strong>Not known</strong></td>
</tr>
<tr>
<td>VB946656</td>
<td>Chinchilla</td>
<td>D</td>
<td>nc</td>
<td>nc</td>
<td>1.39</td>
<td>2°C</td>
</tr>
<tr>
<td>VB910878</td>
<td>Chinchilla</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>2.83</td>
<td><strong>Not known</strong></td>
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<td>VB91840</td>
<td>Chinchilla</td>
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<td>nc</td>
<td>B</td>
<td>3.64</td>
<td><strong>Not known</strong></td>
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<td>B</td>
<td>B</td>
<td>3.31</td>
<td>2°C</td>
</tr>
<tr>
<td>VB937281</td>
<td>Chinchilla</td>
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<td>nc</td>
<td>B</td>
<td>3.90</td>
<td><strong>Not known</strong></td>
</tr>
<tr>
<td>VB910878</td>
<td>Chinchilla</td>
<td>B</td>
<td>nc</td>
<td>B</td>
<td>3.64</td>
<td><strong>Not known</strong></td>
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<tr>
<td>VB91840</td>
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<td>B</td>
<td>B</td>
<td>B</td>
<td>1.14</td>
<td><strong>Not known</strong></td>
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<td>Chinchilla</td>
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<td>B</td>
<td>B</td>
<td>1.90</td>
<td><strong>Not known</strong></td>
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<td>VB917454</td>
<td>Chinchilla</td>
<td>B</td>
<td>nc</td>
<td>B</td>
<td>2.38</td>
<td><strong>Not known</strong></td>
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<td>VB945282</td>
<td>Chinchilla</td>
<td>B</td>
<td>B</td>
<td>nc</td>
<td>3.58</td>
<td><strong>Not known</strong></td>
</tr>
</tbody>
</table>

*Samples submitted 2007–2008 (all from Germany except VB934778 from Luxembourg); analysis of *ssu* according to Hopkins and others (1997)*

†Samples with OD value > 0.05 were considered positive; OD: optical density at 450 nm with reference wavelength of 620 nm

‡Flotation (zinc chloride/sodium chloride solution with specific gravity of 1.3) according to Pantchev and others (2005)

§Samples submitted 2011–2012 (all from Germany except VB906855 from Austria); analysis of *ssu* according to Read and others (2002)

nc: typing not successful; empty space: not conducted

The coproantigen ELISA uses a monoclonal antibody for the qualitative detection of a *Giardia*-specific antigen (GSA 65; Rosoff and Stubbs 1996) in faecal samples.

#### Molecular analysis

Faecal samples for molecular analysis were randomly selected from those with a positive coproantigen result and for which enough material was left for DNA extraction; they were labelled and stored at −20°C. The samples were shipped to the laboratories at Federal Institute for Risk Assessment, BfR (n = 8; Group A) during 2007–2008 or University of Teramo (n = 8; Group A) during 2007–2008, and differences among different animal species (rabbits, chinchillas and guinea pigs) of the present study were analysed for significance using the χ² test; differences were regarded as significant at a level of p < 0.05.
Typing at a single locus

Typing at the ssu locus

The eight samples from group A and 20 of 22 samples from group B were classified as assemblage B (Table 1). Therefore assemblage B was identified in chinchillas (22 isolates), ferrets (four isolates), a rabbit and a Desmarest’s hutia (*Capromys pilorides*). The remaining two isolates from group B were classified as assemblage A (VB947588, from a ferret) and assemblage D (VB906856, from a chinchilla).

Typing at the tpi locus

From group A, five of eight samples were amplified, whereas from group B amplification was obtained from eight of the 22 samples. Sequencing revealed assemblage B in 10 samples and assemblage A in three samples. The sequence from isolates VB906836, VB929246 (chinchillas) and VB9225670 (from a ferret), all from group A, had 100 per cent homology to many assemblage A sequences, including that from the reference strain WB (GenBank L02120), and thus these isolates are identified as sub-assemblage AI (Table 2). The sequence from isolates VB910527, VB906835, VB946885, VB909588 (chinchillas) and VB911416 (from a ferret) had 100 per cent similarity with 17 sequences in GenBank, including that of the reference strain GS (L02116), and thus belong to sub-assemblage BIV. The sequence from isolates VB910466 and VB945282, both from chinchillas, had 99 per cent similarity (one difference over 505 bp) to a number of assemblage B sequences, including isolates of human origin (eg, GenBank EU272153). The sequence from isolate VB945280 and VB922147 (chinchillas) was identical to the sequence from a human isolate (HM165219) and a beaver isolate (DQ789114). Finally, the sequence from isolate VB916523 had five mixed positions and therefore could only be assigned to the level of assemblage B (Table 2).

Typing at the bg locus

From the group A samples, amplification was obtained from four of the eight isolates, all from chinchillas, whereas from the group B samples, amplification was obtained from eight of 22 isolates, namely seven from chinchillas and one from a ferret (Table 1). Sequencing revealed assemblage A in three isolates from group A chinchillas, whereas all other isolates (from groups A and B) were classified as assemblage B. Blast comparison allowed identification of sub-assemblage AI in two isolates (VB906836 and VB922147), while the isolate VB929246 had a sequence identical to those from a cat (GenBank EU769205) and a deer isolate (A03570114). Finally, the sequence from isolate VB911416 from a Desmarest’s hutia (*Capromys pilorides*) was identical to that from a human isolate (HQ616628 from a lemur). Finally, in two isolates from a chinchilla (VB946885) and a ferret (VB911416) the sequence was characterised by two overlapping nucleotides (double peaks) at two positions, and therefore could only be assigned to the level of assemblage B (Table 2).

### Multi-locus genotyping data

Sequence data for the three investigated loci were obtained from four chinchillas from group A and from six chinchillas and one ferret from group B samples. In three of the four chinchillas from group A, a non-concordant assignment to assemblage was evident (Table 1). Indeed, assemblage B was identified at the ssu locus, but two isolates had assemblage A at the tpi and bg loci, and one sample had assemblage A at the bg locus and assemblage A at the tpi locus (Table 1). For group B samples, all isolates had assemblage B at the three loci. From two other isolates, information was available for two loci: the isolate VB925670 from a ferret was identified as assemblage B at the bg locus, but as assemblage AI at the tpi locus, whereas assemblage B was found at the same two loci in the case of the chinchilla isolate VB945282. Two chinchilla isolates that were typed at the tpi/bg loci revealed sequence identity to the human reference strain WB (sub-assemblage BIV, isolate VB906836) and GS (sub-assemblage BIV, isolate VB906855). Another two isolates (VB909588 and VB910887) with assemblage B at ssu locus showed identical sequences to each other at the tpi and bg locus (Table 2).

Nucleotide sequence data (tpi and bg locus; see Table 2) reported in this paper are available in the GenBank database under the accession numbers KF843897-KF843920.

### Discussion

In the present survey, *Giardia* coaptrogonietis was found in 7.6 per cent (95 per cent CI 5.5 to 10.2 per cent), 61.4 per cent (95 per cent CI 57.1 to 65.6 per cent) and 4.1 per cent (95 per cent CI 1.4 to 9.4 per cent) of the faecal samples of rabbits, chinchillas and guinea pigs, respectively, which were collected during 2006–2012. Compared with the results of a similar survey conducted from 2002 to 2004 (Pantchev and others 2005, see above), the difference in prevalence rates (first time period vs second time period) were not statistically significant (P = 0.60 for rabbits, P = 0.19 for chinchillas and P = 0.22 for guinea pigs). In the present study, there was also no statistical difference in occurrence between rabbits and guinea pigs (P = 0.15), but significantly more positive reactions (P < 0.001) were identified in chinchillas. The prevalence of *Giardia* in chinchillas observed in this study is comparable with the results of a recent study from Belgium (Levecke and others 2011), which reported *Giardia* cysts in 53 of 80 (66.3 per cent) faecal samples from chinchillas by means of a sedimentation-flotation technique. That study also showed that young animals and those participating in shows were more at risk of being infected by *Giardia*. A lower rate of infection (59.4 per cent; 41/104) was found in a recent report from Italy in which a direct immunofluorescence assay was used (Veronesi and others 2012). This variability in prevalence rates may be due to the sensitivity of different diagnostic methods, difference in sample preservation methods and the criteria used for sample inclusion.

For rabbits, similar results were shown in a recent study from China (Zhang and others 2012), in which *Giardia* cysts were detected in 28 of 378 (7.4 per cent) tested samples. The same study showed, based on the analysis of the tpi gene, that rabbits were infected with assemblage B, with one sequence (B-I) largely predominant (18/28). The occurrence of assemblage B in rabbits was first reported by Sulaiman and others (2003), and further supported by a multi-locus analysis at the bg, tpi and glutamate dehydrogenase (*gdh*) genes of a Swedish isolate (Lebbad and others 2010). Of note, the owner of this pet rabbit was previously treated for giardiasis, but no samples were available for comparison; however the sequences of the three genes from the rabbit

### Table 2: Classification of 14 isolates at the level of the ssu-assemblages as inferred at the tpi and bg loci

<table>
<thead>
<tr>
<th>Sample code</th>
<th>Host</th>
<th>tpi</th>
<th>bg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VB906836</td>
<td>Chinchilla</td>
<td>AI (100% to WB, L02120)</td>
<td>AI (100% to WB, EU03494)</td>
</tr>
<tr>
<td>VB922147</td>
<td>Chinchilla</td>
<td>B (100% to HM10711, †)</td>
<td>AI (100% to WB, EU03494)</td>
</tr>
<tr>
<td>VB929248</td>
<td>Chinchilla</td>
<td>AI (100% to WB, L02120)</td>
<td>A‡</td>
</tr>
<tr>
<td>VB915823</td>
<td>Chinchilla</td>
<td>B§</td>
<td>BIV (100% to GS, EU03494)</td>
</tr>
<tr>
<td>VB925670</td>
<td>Ferret</td>
<td>AI (100% to WB, L02120)</td>
<td>nc</td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VB911416</td>
<td>Ferret</td>
<td>BV</td>
<td>B§</td>
</tr>
<tr>
<td>VB906855</td>
<td>Chinchilla</td>
<td>BV (100% to GS, L02116)</td>
<td>BV (100% to GS, EU03494)</td>
</tr>
<tr>
<td>VB909588</td>
<td>Chinchilla</td>
<td>B (99% to EU72153)</td>
<td>B (100% to HM162519)</td>
</tr>
<tr>
<td>VB918740</td>
<td>Chinchilla</td>
<td>nc</td>
<td>B (100% to HM162519)</td>
</tr>
<tr>
<td>VB946885</td>
<td>Chinchilla</td>
<td>B§</td>
<td>BIV (100% to GS, EU03494)</td>
</tr>
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<td>VB910887</td>
<td>Chinchilla</td>
<td>BV (100% to GS, L02116)</td>
<td>B (100% to HM162519)</td>
</tr>
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<td>VB945280</td>
<td>Chinchilla</td>
<td>B (100% to HM10711, †)</td>
<td>B (100% to HM162519)</td>
</tr>
<tr>
<td>VB945282</td>
<td>Chinchilla</td>
<td>B (99% to EU72153)</td>
<td>nc</td>
</tr>
</tbody>
</table>

†Sequence identical to those from a cat (EU769205) and a deer isolate (AY302561). ‡Sequence containing mixed positions.

§Sequence identical to that from various mammals (eg, 100 per cent to HQ616628 from a lemur).

nc: typing not successful.
isolate were identical to those reported from humans in other studies, providing support for zoonotic potential (Lebbad and others 2010). In the present study, one rabbit sample was typed as assemble B at the ssu locus, but genotyping at other loci was unsuccessful (Table 1).

In chinchillas, different G. duodenalis assemblages have been identified. Typing isolates at a single locus (ssu or gdh; Karanis and Ey 1998, Veronesi and others 2012) provided only one identical sequence (ssu and gdh; Soares and others 2011). Assemble B was the most common, but also mixed infection with assemblages A, C and E were identified at the tpi locus using assemble-specific primers (Levecke and others 2011). Interestingly, attempts to infect a chinchilla with an isolate from a dog failed, but no molecular typing was possible at the time of this study (Shelton 1954b). It cannot be excluded that the finding of the dog-specific assemble D was the result of a mechanical passage of cysts in an uninfected chinchilla (isolate VB906556).

In the present study, the zoontic assemblages A and B were detected in isolates from chinchillas, with assemble B identified at ssu in 22 of 25 isolates; in at least three isolates the multi-locus analysis resulted in different assemblages being assigned at the three loci (Table 1). Similar to these results, potentially zoontic sub-assemblages AI, AII, BII and BIV were identified in chinchillas by Levecke and others (Table 1), and sub-assemble BIV by Soares and others (2011). In the present study, sequences of most chinchilla isolates were identical to human-adapted Giardia (eg, AI or BIV; Table 2) at a single locus. Nevertheless, it should be stressed that only two isolates had identical sequences at two loci (tpi/gdh) to a human strain of assemble A (WB; originally isolated from a human in Afghanistan) or assemble B (GS; originally isolated from a human in Alaska) (reviewed by Jerlström-Hultqvist and others 2010). For G. duodenalis, sequence heterogeneity was also observed within each sub-assemble, and those genetic variants are referred to as subtypes (Sprong and others 2009).

In molecular studies using single assemble typing, potentially zoontic assemblages A and B are commonly identified, but after multilocus analysis (eg, by combining sequence data from the ssu, bg, tpi and tpi loci to define multi-locus genotypes [MLGs]), only few of them, mainly from assemble A, proved to have a real zoontic potential (Sprong and others 2009). Mixed genotypes in a single sample could be the result of mixed infections with different field isolates (Takumi and others 2012), and/or especially for sub-assemble B, also of alleleic sequence heterozygosity of a single isolate (Ankarklev and others 2012). Interestingly, two chinchilla samples (VB909588 and VB910687) collected in geographically different regions, had identical assemble A sequences at the ssu, tpi and bg loci, and harbour a MLG with full homology to human sequences (Table 2). Surprisingly, assemblage B was detected in chinchillas of group B despite the only significant difference between the two groups was the time of sampling. One explanation could be the presence or absence of symptoms in the sampled animals, as observed in another study by Read and others (2002). They found a correlation between the genotype of G. duodenalis and diarrhoea with assemble A more common in symptomatic children. Several other studies have also found a correlation between assemble A and diarrhoea, but others found the opposite, as for example, correlation between assemble A and diarrhoea, or no correlation at all (reviewed by Monis and others 2009). But it is also likely that the outcome of infection is a complex phenotype and that host factors and co-infections (eg, other parasites or bacteria) will also affect the development of disease (Monis and others 2009, Lebbad and others 2011). Two out of three chinchillas in group A with mixed assemblages (A and B) were also infected with Proteus species (Table 1; see above).

This appears to be the first identification of assemble B in ferrets, since only assemble A was detected in the few isolates tested so far. Abe and others (2010) analysed G. duodenalis from two ferrets in Japan by typing at four loci (ssu, bg, tpi, gdh) and found two different assemble A MLGs. The tpi, bg and gdh sequences from these isolates (GF2 and GF3) have also been identified in several other mammals (including humans). The isolate GF2-for example, showed an identity of 99 per cent at the partial tpi, bg and gdh sequences to a beaver isolate Be-2 (ssu sequences of the latter are not available in the GenBank), so this MLG from ferrets might be less ferret specific and more zoonotic than originally suggested (Abe and others 2010). In the present study, Giardia isolates from ferrets were shown to be identical to either human reference strain WB (AI) or GS (BIV) at the tpi locus (Table 2), and had at least one identical sequence (VB923670) to the previously described ferret isolate GF-3 (Abe and others 2010). No obvious reason could be identified for the increase in the number of ferrets positive for Giardia as observed by Pantchev and others (2011). Further studies with more samples should show whether this may be due to the fact that these animals are susceptible to assemblages A and B. In one sample from a Desmaretz’s hutia (Capromys pilorides), G. duodenalis assemble B was identified at the ssu locus. This sample, and samples from one ferret and four chinchillas, revealed a simultaneous growth (aerobic bacterial culture) of Enterobacteriaceae (Proteus species, Escherichia coli) (Table 1), which could represent an interesting result from the clinical perspective, as Pseudomonas species was previously associated with giardiasis and death in chinchillas (Shelton 1954a). Another parasite associated with giardiasis and death of a chinchilla in the latter study was the dwarf tapeworm Hymenolepis nana. Interestingly, co-infections or repeated infections are discussed as predisposing factors of disease in chinchillas, where the outcome of experimental infections range from asymptomatic to severe diseases with anorexia, soft discoloured amorphous faeces, watery diarrhoea, weight loss and even death (Shelton 1954a).

It can be concluded that the coproantigen ELISA is a reliable method for detecting Giardia infection in small mammals. Chinchillas show a very high infection level, with approximately two-thirds being positive, and ferrets are also increasingly tested positive. Although sequence identities at one (ferrets) or more (chinchillas) loci were identified, further studies (eg, multi-locus genotyping of isolates obtained from humans and pets living together) are necessary to assess the real risk of zoonotic transmission. Rabbits, despite showing a lower prevalence, may also pose a zoonotic risk based on sequence identities and coincidence of infection in the same host (reviewed by Monis and others 1998, LEBBAD and others 2010). Guinea pigs show a comparable low prevalence and to date only one unique isolate has been typed (LEBBAD and others 2010), so further studies are necessary to prove potential transmission of cross-infective Giardia isolates. Finally, molecular analysis revealed that mixed assemblages were detected by multi-locus analysis, although further studies are needed to provide proper explanations of this finding.

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Compliance statement

All investigations comply with the current laws of the countries in which they were performed.

Competing interests

None.

References


Occurrence and molecular typing of *Giardia* isolates in pet rabbits, chinchillas, guinea pigs and ferrets collected in Europe during 2006–2012

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