Molecular identification of zoonotic and livestock-specific *Giardia*-species in faecal samples of calves in Southern Germany

Julia Gillhuber1*, Louise Pallant2, Amanda Ash2, RC Andrew Thompson2, Kurt Pfister1 and Miriam C Scheuerle1

Abstract

**Background:** *Giardia*-infection in cattle is often subclinical or asymptomatic, but it can also cause diarrhoea. The livestock-specific species *Giardia bovis* is the most frequently observed in cattle, however, the two zoonotic species *Giardia duodenalis* and *Giardia enterica* have also been found. Therefore calves are thought to be of public health significance. The aim of this study was to obtain current data about the frequency of the different *Giardia*-species in calves in Southern Germany.

**Findings:** Faecal samples of calves (diarrhoeic and healthy) in Southern Germany, diagnosed *Giardia*-positive by microscopy, were characterised by multi-locus PCR and sequencing. Of 152 microscopically *Giardia*-positive samples 110 (72.4%) were positive by PCR and successfully sequenced. *G. bovis* (Assemblage E) was detected in 101/110 (91.8%) PCR-positive samples, whilst *G. duodenalis* (Assemblage A) was detected in 8/110 (7.3%) samples and a mixed infection with *G. duodenalis* and *G. bovis* (Assemblage A+E) was identified in 1/110 (0.9%) samples. The sub-genotypes A1, E2 and E3 were identified with the β-giardin and the glutamate dehydrogenase genes. In the majority of diarrhoeic faecal samples a co-infection with *Cryptosporidium* spp. or *Eimeria* spp. was present, however, there were some in which *G. bovis* was the only protozoan pathogen found.

**Conclusions:** The results suggest that there is potentially a risk for animal handlers as calves in Southern Germany are, at a low percentage, infected with the zoonotic species *G. duodenalis*. In addition, it was found that *G. bovis* was the only pathogen identified in some samples of diarrhoeic calves, indicating that this parasite may be a contributing factor to diarrhoea in calves.

**Keywords:** PCR, Diarrhoea, Protozoan, *Giardia* assemblages, Cattle, *Giardia duodenalis* morphological group

Findings

**Background**

Worldwide the protozoan *Giardia* spp. is one of the most common intestinal parasites in humans (reviewed in [1,2]) and also a frequent enteric parasite in animals including companion animals, livestock and wildlife [2]. According to Monis *et al*. [3] there are eleven species within the genus *Giardia*. Six of them, formally known as Assemblages A-G of the *Giardia duodenalis* morphological group, are genetically but not morphologically distinguishable. They can infect humans and mammals, with some being host specific and others having low host specificity.

*Giardia*-infection in cattle is often subclinical or asymptomatic, but this infection can also cause symptoms including acute or chronic diarrhoea, reduced weight gain and ill thrift in young calves [4,5]. Although the prevalence of *Giardia* in cattle around the world varies considerably (reviewed in [5,6]), longitudinal studies have shown cumulative infection rates in calves of 100% [7,8]. The two zoonotic species *G. duodenalis* (Assemblage A) and *G. enterica* (Assemblage B) and the livestock-specific species *G. bovis* (Assemblage E) are able to infect cattle with *G. bovis* being found most frequently followed by *G. duodenalis* [9-13]. Therefore, calves are thought to be of public health significance both as a source of waterborne
| Target gene | Number of reaction | Length of amplification (bp) | Primer | Cycle condition | Reaction volume | Reference |
|-------------|--------------------|------------------------------|--------|-----------------|-----------------|-----------|
| 18S rRNA    | Primary reaction   | 292                          | Forward primer: RH11 | a | Total volume 25 μl | [18] |
|             |                    |                              | $5'-\text{CATCCGGTCGATCCTGCC-3'}$ | 96°C, 45 s | d | 0.15 μl Taq-Ti hot start DNA polymerasee |
|             |                    |                              | Reverse primer: RH4 | 50°C, 30 s | b | 5% dimethyl sulfoxide (DMSO)f |
|             |                    |                              | $5'-\text{AGTCAACGCCCTTCCCGCCAGG-3'}$ | 72°C, 45 s | → 35 cycles | |
|             | Secondary reaction | 130                          | Forward primer: GiarF | a | 2 μl from the 1st-round PCR reaction | [19] |
|             |                    |                              | $5'-\text{GAGGCTTCCCCAAGGAC-3'}$ | 96°C, 45 s | | |
|             |                    |                              | Reverse primer: GiarR | 55°C, 30 s | | |
|             |                    |                              | $5'-\text{CTGGTACGCTGCTCG-3'}$ | 72°C, 45 s | → 35 cycles | |
| β-giardin   | Primary reaction   | 753                          | Forward primer: G7 | a | Total volume 25 μl | [20] |
|             |                    |                              | $5'-\text{AAGCCCGACGACCTCACCCGCAGTGC-3'}$ | 95°C, 30 s | d | 0.15 μl Tth Plus DNA polymerasee |
|             |                    |                              | Reverse primer: G759 | 50°C, 30 s | | |
|             |                    |                              | $5'-\text{GAGGCGCCCTGGATCTTGACAGGC-3'}$ | 72°C, 60 s | → 40 cycles | |
|             | Secondary reaction | 511                          | Forward primer: B-F | a | 2 μl from the 1st-round PCR reaction | [21] |
|             |                    |                              | $5'-\text{GAACGAACGAGATCGAGGTCCG-3'}$ | 96°C, 45 s | | |
|             |                    |                              | Reverse primer: B-R | 55°C, 30 s | | |
|             |                    |                              | $5'-\text{CTGACGAGCTTCGTGTT-3'}$ | 72°C, 45 s | → 35 cycles | |
| GDH         | Primary reaction   | not given                    | Forward primer: GDHeF | c | Total volume 25 μl | [19] |
|             |                    |                              | $5'-\text{TCAGCTTGYAYCGYGGYTTYTCCGT-3'}$ | 94°C, 30 s | d | 0.2 μl Tth Plus DNA polymerasee |
|             |                    |                              | Reverse primer: GDHiR | 50°C, 30 s | | |
|             |                    |                              | $5'-\text{GTTRTCCTTGACATCTCC-3'}$ | 72°C, 60 s | → 40 cycles | |
|             | Secondary reaction | 432                          | Forward primer: GDHiF | c | 2 μl from the 1st-round PCR reaction | [19] |
|             |                    |                              | $5'-\text{CAGTACAAYTGYGCCTTCCCG-3'}$ | 94°C, 30 s | | |
|             |                    |                              | Reverse primer: GDHiR | 60°C, 30 s | | |
|             |                    |                              | $5'-\text{GTTRTCCTTGACATCTCC-3'}$ | 72°C, 60 s | → 40 cycles | |

a: Initial activation step: 96°C, 5 min.
b: Final extension: 72°C, 7 min.
c: Initial activation step: 94°C, 5 min.
d: used substances: 2 μl diluted DNA template, 2.5 μl 10x Reaction Buffer, 2.5 μl MgCl₂ (25 mM), 1 μl dNTPs (5 mM) (Promega), 1 μl of each primer (10 μM), Water-ultra pure grade (Fisher Biotech Perth, Australia).
e: Fisher Biotech Perth, Australia.
f: Sigma–Aldrich St. Louis, Missouri.
outbreaks of giardiasis in humans and as a risk to in-contact animal handlers [2,14].

Current data on the occurrence of the different *Giardia* species in German calves is only available for 2–16 week-old calves from farms around Berlin. In that study (15) a commercially available monoclonal antibody-based ELISA was used and *Giardia* was detected in 100% of the farms and 51.2% of the animals sampled. Subsequent molecular characterisation ascertained *G. bovis* (Assemblage E) was the most common species present, but infections with *G. duodenalis* (Assemblage A) and mixed infections of *G. duodenalis* and *G. bovis* (Assemblage A+E) were also found [15].

Thus, the aim of this study was to obtain current data about the frequency of the different *Giardia* species in calves of a wider range of age in Southern Germany.

**Methods**

**Samples**

Faecal samples of calves from the southern federal states of Germany, Bavaria and Baden-Württemberg, were sent to the Diagnostic Laboratory of Comparative Tropical Medicine and Parasitology, LMU Munich, Germany for microscopy analysis. *Giardia* spp., *Cryptosporidium* spp. and *Eimeria* spp. were detected using the carbolfuchsin-stained direct faecal smear [16] and the merthiolate iodine formaldehyde concentration (MIFC) with the addition of Lugol’s solution [17]. Samples from 152 calves between 3 and 130 days of age (mean age: 50.7 days, n = 138) were diagnosed *Giardia*-positive by the MIFC-method between June 2011 and January 2013 and stored at −20°C. In February 2013 these samples were preserved in 70% ethanol and sent to the School of Veterinary and Life Sciences, Murdoch University, Australia, for molecular characterisation.

**DNA extraction**

DNA was extracted from faecal samples using the Maxwell® 16 Tissue DNA Purification Kit (Promega, Madison, USA) with the Maxwell® 16 Instrument (Promega). In addition to the recommended protocol, 1 μl of the final elution was further diluted by adding 4 μl of Water-ultra pure grade (Fisher Biotech Perth, Australia). Both neat and dilute templates were used in PCRs.

**PCR amplification**

For the amplification of the 18S rRNA gene and the β-giardin gene a nested PCR was carried out and for the amplification of the glutamate dehydrogenase (GDH) gene a semi-nested PCR was performed. Details of primers and cycling conditions are listed in Table 1.

**DNA sequencing**

PCR products were purified using Agencourt AMPure XP magnetic beads (Beckman Coulter, Beverly, USA) as per the manufacturer’s instructions. Sequence reactions were performed using the Big Dye Terminator Version 3.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's instructions. PCR products were sequenced with the second round primers (1 μl [2.5 μM]). The cycling conditions for nucleotide sequencing are: 1 cycle of 96°C for 2 min and 25 cycles at 96°C for 10 s, 50°C for 5 s and 60°C for 4 min. Reactions were electrophoresed on an ABI 3730 48 capillary machine.

**Species identification**

Sequences were analysed using Sequencher 4.8 (Gene Codes, Ann Arbor, MI, USA) and compared to published sequences (Table 2) to identify species and sub-genotype information.

**Results**

Of the 152 samples, diagnosed *Giardia*-positive by microscopy, 110 (72.4%) were positive by PCR and successfully sequenced. Sequence analysis identified the presence of *G. bovis* (Assemblage E) in 101/110 (91.8%) PCR-positive samples, *G. duodenalis* (Assemblage A) in 8/110 (7.3%) samples and a mixed template of *G. duodenalis* and *G. bovis* (Assemblage A+E) in 1/110 (0.9%) samples. Using the β-giardin and GDH genes it was possible to identify sub-genotypes within the species *G. bovis* (E2 and E3) and *G. duodenalis* (A1) (Table 3).

Of the 110 PCR-positive samples 94 (85.5%) samples amplified at one locus, whereas 12/110 (10.9%) and 4/110 samples amplified at two loci.

**Table 2 GenBank accession numbers used for alignment with *Giardia* sequences**

| 18S rRNA | β-giardin | GDH    |
|---------|-----------|--------|
| AI      | AF199445  | A1     | X14185 | A  | DQ100288 |
| AI      | MS4878    | A2     | AYS45649 | A  | M84604  |
| III     | AF199446  | A2     | FN386482 | A1 | DQ14242  |
| III     | AF199447  | A5     | AYS45643 | A2 | L40510  |
| B       | U09491    | B8     | AYS45649 | B  | AY826193 |
| B       | U09492    | B      | AY072728 | B3 | AF069059 |
| C       | AF199449  | B      | AY647266 | B4 | AY178750 |
| D       | AF199443  | C      | AYS45646 | C  | U60982  |
| E       | AF199448  | C      | FJ009206 | D  | U60986 |
| E       | DQ157272  | D      | AYS45648 | E  | AY178741 |
| F       | AF199444  | E      | EU189375 | F  | AF069057 |
| G       | AF199450  | E1     | AY072729 | G  | AF069060 |
|         |           |        | AYS45650 | E2 |        |
|         |           |        | AY653159 | E3 |        |
samples amplified at 2 and 3 loci, respectively. 18S amplified most frequently (106/152 samples, 69.7%), whereas β-giardin and GDH amplified comparatively rarely (16/152, 10.5%; 8/152, 5.3%) (Table 3).

Table 4 shows that in the majority of the calves with diarrhoea a co-infection with Cryptosporidium spp. or Eimeria spp. was present.

Discussion
The results of this study reveal that the livestock-specific species G. bovis (Assemblage E) is the most frequent species (91.8%) in calves in Southern Germany. The zoonotic species G. duodenalis (Assemblage A) was found in a low number of samples (7.3%), while a mixed infection of G. duodenalis and G. bovis was identified in only one sample (0.9%). G. enterica (Assemblage B), the second zoonotic species, was not detected in this study.

Similarly in another study on German calves, the same species were detected and G. bovis was also found most frequently; however, there was a higher proportion of infection with G. duodenalis as well as with mixed infections than observed in this study [15].

Finding G. bovis in the majority of Giardia-infections in calves and G. duodenalis in only some cases also concurs with the results of former studies on cattle [10-12,21]. In some studies G. bovis was the only species identified in calves [9,25]. G. enterica was not detected in this study, which is in accordance with the results of many previous studies although several did find this genotype in cattle [10,12,13,21]. One study diagnosed G. enterica more frequently than G. bovis [26] whereas studies in New Zealand found only infections with G. duodenalis and G. enterica, but not with G. bovis [27-29].

The finding of sub-genotypes E2 and E3 within the species G. bovis (Assemblage E) is similar to former studies [11,14,21]. According to Xiao and Fayer [30] and Feng and Xiao [1] A1 and A2 are the most common sub-genotypes of G. duodenalis (Assemblage A), with humans being mostly infected with A2 and animals with A1. This agrees with former results [14,22,23] and with the results of this study, as A1 was the only sub-genotype of G. duodenalis diagnosed. However, others have found one or more of the sub-genotypes A1-A4 in cattle [10-12,21,24]. Therefore it is possible that calves can be infected with a variety of sub-genotypes of G. duodenalis, all of which have also been identified in humans [21]. This suggests that there may be an interaction between the human and livestock transmission cycle [3]. Cattle have long been assumed to be of public health significance as a source of waterborne outbreaks of giardiasis in humans due to contamination of ground and surface water, although, there is no evidence incriminating infected cattle in any of the 132 documented waterborne outbreaks [2]. However, it has been shown, that animal handlers can be in danger of zoonotic transmission of G. duodenalis from infected cattle [14], and in reverse anthropozoonotic transmission of G. duodenalis from animal handlers to cattle is also possible [13]. Thus, transmission of the zoonotic species, which

| Table 3 Genotypic characterisation of Giardia spp. isolates at different loci |
|-----------------|----------|-----|-------|-------|-------|
| 18S rRNA | β-giardin | GDH | 18S rRNA and β-giardin | 18S and GDH | 18S rRNA, β-giardin and GDH |
| A (5) | A1 (1) | A1 (1) | E, E (1) | E, A1 (1) | A, A1, A (1) |
| E (85) | E3 (1) | E (1) | E, E2 (1) | E, E (1) | E, E3, E (3) |

| Table 4 Distribution of mono- and mixed infections of Giardia-positive calves in relation to faecal consistency |
|-----------------|----------|-------|-------|-------|
| Total | Monoinfection with Giardia spp. | Coinfection with Cryptosporidium spp. | Coinfection with Eimeria spp. |
| MIFC positive | Total | 152 | 66 | 15 | 71 |
| With diarrhoea | 62 | 25 | 10 | 27 |
| Without diarrhoea | 90 | 41 | 5 | 44 |
| PCR: G. duodenalis | Total | 8 | - | 3 | 5 |
| With diarrhoea | 4 | - | 2 | 2 |
| Without diarrhoea | 4 | - | 1 | 3 |
| PCR: G. bovis | Total | 101 | 48 | 8 | 45 |
| With diarrhoea | 38 | 17 | 6 | 15 |
| Without diarrhoea | 63 | 31 | 2 | 30 |
| PCR: G. duodenalis + G. bovis | Total | 1 | 1 | - | - |
| With diarrhoea | - | - | - | - |
| Without diarrhoea | 1 | 1 | - | - |

Gillhuber et al. Parasites & Vectors 2013, 6:346 http://www.parasitesandvectors.com/content/6/1/346
was detected in this study, could in principle be possible between animal handlers and cattle.

The role of *Giardia* as a cause of diarrhoea in calves is still unclear, as there are conflicting results from a number of studies, some demonstrating an association and others not. Furthermore, the presence of species-specific pathogenicity in calves poses further difficulties in the evaluation and has not been determined in another bovine study [11]. The role of the particular *Giardia*-species in mixed-infections in diarrhoeic calves could not be clarified either. However, the identification of some diarrhoeic samples, where *G. bovis* was the only pathogen detected, may suggest that this species does contribute to diarrhoea in calves. Whether these results are indicative or not remains unclear. Further studies will show whether differences in the clinical outcomes can occur due to the various sub-genotypes as has been established in human medicine [2].

**Conclusions**

The results of this study show that although the livestock specific species *G. bovis* has been diagnosed most frequently, the potential zoonotic species *G. duodenalis* is also present in calves in Southern Germany and thus might be a risk for animal handlers. Furthermore the results indicate that *G. bovis* might contribute to diarrhoea, as it was the only pathogen found in a proportion of the samples from diarrhoeic calves.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

JG prepared the samples, analysed and interpreted the data and drafted the manuscript, AA and LP carried out the PCR and the sequence analysis, AT JG prepared the samples, analysed and interpreted the data and drafted the manuscript, MC prepared the samples and reviewed the manuscript, AA and LP carried out the PCR and the sequence analysis, AT JG prepared the samples, analysed and interpreted the data and drafted the manuscript. All authors read and approved the final manuscript.

**Acknowledgements**

We thank our colleagues in the lab, especially Elisabeth Kiess, Kathrin Simon and Tim Tiedemann for their contribution to the study.

**Author details**

1. Comparative Tropical Medicine and Parasitology, Faculty of Veterinary Medicine, Ludwig-Maximilians-Universität München, Leopoldstr. 5, Munich 80802, Germany.
2. School of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia, Australia.

Received: 21 October 2013 Accepted: 4 December 2013

**References**

1. Feng Y, Xiao J. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. *Clin Microbiol Rev* 2011; 24:110–140.
2. Thompson RC, Monis P. *Giardia*-from genome to proteome. In Advances in Parasitology. Volume 78. Edited by Rollinson D, Hay St. London: Elsevier; 2012:57–95.
3. Monis PT, Caccio SM, Thompson RC. Variation in *Giardia*: towards a taxonomic revision of the genus. *Trends Parasitol* 2009; 25:93–100.
4. Geurden T, Vercruysse J, Claerebout E: Field testing of a fenbendazole treatment combined with hygienic and management measures against a natural *Giardia* infection in calves. *Parasitol Res* 2006; 102:87–91.
5. Geurden T, Vercruysse J, Claerebout E: Is *Giardia* a significant pathogen in production animals? *Exp Parasitol* 2010; 124:88–106.
6. Xiao L. *Giardia* infection in farm animals. *Parasitol Today* 1994; 10:436–438.
7. O’Halloran TA, Cockwill C, McAllister TA, Jealosi M, Morck DM, Olson ME: Duration of naturally acquired giardiosis and cryptosporidiosis in dairy calves and their association with diarrheaa. *J Am Vet Med Assoc* 1999, 214:391–396.
8. Ralston BJ, McAllister TA, Olson ME: Prevalence and infection pattern of naturally acquired giardiosis and cryptosporidiosis in range beef calves and their dams. *Parasitol Res* 2003; 94:113–122.
9. Becher KA, Robertson JD, Fraser DM, Palmer DG, Thompson RC: Molecular epidemiology of *Giardia* and Cryptosporidium infections in diary calves originating from three sources in Western Australia. *Parasitol Res* 2004, 102:131–139.
10. Mendonca C, Almeida A, Castro A, de Lurdes DS, Soares S, da Costa JM, Canada N: Molecular characterization of *Cryptosporidium* and *Giardia* Isolates from cattle from Portugal. *Parasit Res* 2007, 100:47–50.
11. Geurden T, Geldhof P, Levecke B, Martens C, Berkvens D, Caiaret S, Vercruysse J, Claerebout E: Mixed *Giardia duodenalis* assemblage A and E infections in calves. *Int J Parasitol* 2008, 38:259–264.
12. Ng J, Yang R, McCarthy S, Gordon C, Hijjawi N, Ryan U: Molecular characterization of *Cryptosporidium* and *Giardia* in pre-weaned calves in Western Australia and New South Wales. *Parasitol Res* 2011, 107:45–150.
13. Dixon B, Parriington L, Cook A, Pintar K, Pollati F, Kelton D, Farber J: The potential for zoonotic transmission of *Giardia* duodenalis and Cryptosporidium spp. from beef and dairy cattle in Ontario, Canada. *Parasitol Res* 2011, 107:20–36.
14. Khan SM, Deb Nath C, Pramanik AK, Xiao L, Nozaki T, Ganguly S: Molecular evidence for zoonotic transmission of *Giardia* duodenalis among dairy farm workers in West Bengal, India. *Parasitol Res* 2011, 107:342–345.
15. Geurden T, Vanderstichel R, Pothie H, Ehsan A, van Sonom-Himmelstjerna G, Morgan ER, Camuset P, Capelli G, Vercruysse J, Claerebout E: A multicentre prevalence study in Europe on *Giardia* duodenalis in calves, with molecular identification and risk factor analysis. *Parasitol Res* 2012, 109:383–390.
16. Heine J: Eine einfache Nachweismethode für Kryptosporidien im Kot. *Zentralbl Veterinaermed Reihe B* 1991, 38:405–409.
17. Thornton SA, West AH, DuPont HL, Mehlhorn KW, Mehlhorn KH, Wetherall JD, Reynolds JA, Thompson RC: Ribosomal RNA sequencing reveals differences between the genotypes of *Giardia* isolates recovered from humans and dogs living in the same locality. *J Parasitol* 1997, 83:94–101.
18. Read CM, Monis PT, Thompson RC: Discrimination of all genotypes of *Giardia* duodenalis at the glutamate dehydrogenase locus using PCR-RFLP. *Infect Genet Evol* 2004, 4:125–130.
19. Caccio SM, De Giacomo M, Pozio E: Sequence analysis of the beta-giardin gene and development of a polymerase chain reaction restriction fragment length polymorphism assay to genotype *Giardia duodenalis* cysts from human faecal samples. *Int J Parasitol* 2002, 32:1023–1030.
20. Lalle M, Pozio E, Capelli G, Bruschi F, Crotti D, Caccio SM: Genetic heterogeneity at the beta-giardin locus among human and animal isolates of *Giardia duodenalis* and identification of potentially zoonotic subgenotypes. *Int J Parasitol* 2005, 35:207–213.
21. Langkjær RB, Vige H, Enemark HL, Maddox-Hyttel C: *C. pylori* and *H. pylori* phylogenetic characterization of *Cryptosporidium* and *Giardia* from pigs and cattle in Denmark. *Parasitol Res* 2007, 104:339–350.
22. Souza SL, Gennari SM, Righi Filho AS, Pena HF, Furuda MR, Cortez A, Gregori F, Soares RM: Molecular identification of *Giardia duodenalis* from humans, dogs, cats and cattle from the state of Sao Paulo, Brazil, by sequence analysis of fragments of glutamate dehydrogenase (gdh) coding gene. *Parasitol Res* 2007, 104:258–264.
23. Feng Y, Ortega L, Cama V, Terel J, Xiao L, High intragenotypic diversity of *Giardia* duodenalis in dairy cattle on three farms. *Parasitol Res* 2008, 103:87–92.
24. Benlifl D, Chavez D, De Liberato C, Franco A, Saramazzino P, Orecchio P: Genotype characterisation of *Giardia duodenalis* isolates from domestic and farm animals by SSU-rRNA gene sequencing. *Parasitol Res* 2004, 92:193–199.
25. Cokkin T, Farber J, Parriington L, Dixon B: Prevalence and molecular characterization of *Giardia* duodenalis and *Cryptosporidium* spp. in dairy cattle in Ontario, Canada. *Parasitol Res* 2007, 103:297–305.
27. Winkworth CL, Learmonth JJ, Matthaei CD, Townsend CR. Molecular characterization of *Giardia* isolates from calves and humans in a region in which dairy farming has recently intensified. *Appl Environ Microbiol* 2008, 74:5100–5105.

28. Learmonth JJ, Ionas G, Pita AB, Cowie RS. Identification and genetic characterisation of *Giardia* and *Cryptosporidium* strains in humans and dairy cattle in the Waikato Region of New Zealand. *Water Sci Technol* 2003, 47:21–26.

29. Hunt CL, Ionas G, Brown TJ. Prevalence and strain differentiation of *Giardia intestinalis* in calves in the Manawatu and Waikato regions of North Island, New Zealand. *Vet Parasitol* 2000, 91:7–13.

30. Xiao L, Fayer R. Molecular characterisation of species and genotypes of *Cryptosporidium* and *Giardia* and assessment of zoonotic transmission. *Int J Parasitol* 2008, 38:1239–1255.

doi:10.1186/1756-3305-6-346

Cite this article as: Gillhuber et al.: Molecular identification of zoonotic and livestock-specific *Giardia*-species in faecal samples of calves in Southern Germany. *Parasites & Vectors* 2013 6:346.