Molecular and phylogenetic analysis of *Cryptosporidium muris* from various hosts

U. M. MORGAN[1]*, L. XIAO[2], P. MONIS[3], I. SULAIMAN[2], I. PAVLASEK[4], B. BLAGBURN[5], M. OLSON[6], S. J. UPTON[7], N. V. KHRAMTSOV[7], A. LAL[2], A. ELLIOT[1] *and* R. C. A. THOMPSON[1]

[1] *World Health Organization Collaborating Centre for the Molecular Epidemiology of Parasitic Infections and State Agricultural Biotechnology Centre, Division of Veterinary and Biomedical Sciences, Murdoch University, Murdoch, WA, 6150, Australia*
[2] *Division of Parasitic Diseases, Centres for Disease Control and Prevention, Atlanta, Georgia, 30341, USA*
[3] *Microbiology Unit, Australian Water Quality Centre, Hodgson Road, Bolivar, SA, 5110, Australia*
[4] *State Veterinary Institute Prague, Pathology and Parasitology Department 165 03 Prague 6, Czech Republic*
[5] *Department of Pathobiology, College of Veterinary Medicine, Auburn University, Auburn, Alabama, USA*
[6] *University of Calgary, Calgary, Canada*
[7] *Division of Biology, Kansas State University, Manhattan, KS 66506, USA*

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SUMMARY

Isolates of *Cryptosporidium muris* and *C. serpentis* were characterized from different hosts using nucleotide sequence analysis of the rDNA 18S and ITS1 regions, and the heat-shock (HSP-70) gene. Phylogenetic analysis confirmed preliminary evidence that *C. muris* is not a uniform species. Two distinct genotypes were identified within *C. muris*; (1) *C. muris* genotype A; comprising bovine and camel isolates of *C. muris* from different geographical locations, and (2) *C. muris* genotype B comprising *C. muris* isolates from mice, a hamster, a rock hyrax and a camel from the same enclosure. These 2 genotypes may represent separate species but further biological and molecular studies are required for confirmation.

Key words: *Cryptosporidium muris*, *Cryptosporidium serpentis*, 18S rDNA, ITS1, heat-shock gene, phylogenetic analysis.

INTRODUCTION

Cryptosporidium muris was first recognized and described in the stomach of mice by Tyzzer in 1907 (Tyzzer, 1907). The oocysts differed in size and site of infection to oocysts that Tyzzer later described in the mouse small intestine and assigned the name *C. parvum* (oocysts measured $7{\cdot}4 \times 5{\cdot}6$ μm for *C. muris* versus $5{\cdot}0 \times 4{\cdot}5$ μm for *C. parvum*). Since then, *C. muris*-like oocysts have been reported in other rodents such as voles (*Microtus* sp.), mice (*Mus musculus*) and rats (*Rattus* sp.) (Webster & MacDonald, 1995; Chalmers *et al*. 1997; *cf*. Fayer, Speer & Dubey, 1997; Sturdee, Chalmers & Bull, 1999). *Cryptosporidium muris*-like oocysts have also been reported in cattle (*Bos taurus*), a rock hyrax (*Procavia capensis* Pallas, 1966), desert hamsters (*Phodopus roborovskii* Satunin, 1903) and camels (*Camelus bactrianus*) (Upton & Current, 1985; Anderson, 1991; Fayer *et al*. 1991; Esteban & Anderson, 1995; Pavlasek, 1995; Pavlasek & Lavicka, 1995; Bukhari & Smith, 1996; Olson *et al*. 1997; Kaneta & Nakai 1998; Koudela, Modry & Vitovec, 1998).

Cryptosporidium muris is only known to infect the glands of the stomach (abomasum in cattle), and usually causing no overt illness but retards acid production. Histopathological examination of the gastric glands of infected mice revealed that most gastric glands were dilated and filled with numerous free or embedded parasites and that the gastric glands contained degenerated and atrophied epithelia cells (Ozkul & Aydin, 1994). In cattle, protein digestion in the abomasum is thought to be retarded, and milk production in cows that are chronically afflicted with *C. muris* can be reduced by about 13%. Growing calves may also be adversely affected (Anderson, 1998).

Preliminary molecular evidence suggests that there are genetic differences between *C. muris* isolates from a bovine host and a rock hyrax (Xiao *et al*. 1999*a*). The aim of the present study, was to conduct molecular and phylogenetic analysis on a range of *C. muris* isolates from different hosts and geographical origins at the 18S and the more variable ITS1 rDNA loci as well as the heat-shock gene (HSP-70) in order to understand more fully the extent of genetic diversity within *C. muris*.

* Corresponding author: Division of Veterinary and Biomedical Science, Murdoch University, Murdoch, WA, 6150, Australia. Tel: +08 9360 2457. Fax: +08 9310 4144. E-mail: morgan@numbat.murdoch.edu.au

Table 1. Isolates of *Cryptosporidium* used in this study

Isolate code	Host	Species	Genotype	Oocyst dimensions (μm)	Geographical location	Source*
$AuCm_1$	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	7·5 × 5·1	Alabama, USA	AU
356	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	7·5 × 5·0	Calgary, Canada	UC
20	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	N.D.	Idaho, USA	CDC
22	Bactrian Camel (*Camelus bactrianus*)	*C. muris*	*C. muris B*	N.D.	Baltimore, USA	CDC
108735	Rock Hyrax (*Procavia capensis*)	*C. muris*	*C. muris B*	8·76 × 6·3	Baltimore, USA	CDC†
VS1742	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	8·3 × 6·2	Idaho, USA	KSU†
Cz-B1	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	7·2 × 5·6	Czech Republic	SVIP
Cz-B2	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	6·9 × 5·4	Czech Republic	SVIP
Cz-B3	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	7·3 × 5·1	Czech Republic	SVIP
Cz-B4	Cattle (*Bos taurus*)	*C. muris*	*C. muris A*	7·6 × 5·2	Czech Republic	SVIP
CZ-Caml	Camel (*Camelus bactrianus*)	*C. muris*	*C. muris A*	7·4 × 5·6	Czech Republic	SVIP
CZ-Cam2	Camel (*Camelus bactrianus*)	*C. muris*	*C. muris A*	7·5 × 5·0	Czech Republic	SVIP
Cz Ham1	Desert Hamster (*Phodopus roborovskii*)	*C. muris*	*C. muris B*	N.D.	Czech Republic	SVIP
SM 4	Mouse (*Mus musculus*)	*C. muris*	*C. muris B*	N.D.	Spain	LPUB
SM 6	Mouse (*Mus musculus*)	*C. muris*	*C. muris B*	7·3 × 5·2	Spain	LPUB
S11	Common death adder (*Acanthophis antarticus*)	*C. serpentis*	*C. serpentis*	6·2 × 5·4	SA	CVL
64	Amazon tree boa (*Corallus hortulanus*)	*C. serpentis*	*C. serpentis*	N.D.	Washington, DC	CDC
63	Savanna monitor (*Varanus exanthematicus*)	*C. serpentis*	*C. serpentis*	N.D.	Washington DC, USA	CDC

* AU, Auburn University, USA; CDC, Centers for Disease Control, Atlanta, USA; CVL, Central Veterinary Laboratories, Adelaide; KSU, Kansas State University, USA; LPUB, Laboratori de Parasitologica, University of Barcelona, Spain; SVIP, State Veterinary Institute, Prague, Czech Republic; UC, University of Calgary, Canada. N.D., Not determined.

† Note: ATCC 87666; ATCC 87715.

MATERIALS AND METHODS

Sources of parasite isolates, DNA purification and PCR

Sources of parasite isolates are listed in Table 1. Where possible, morphometric analysis was performed on isolates using an Optimas v 5.2 system at ×1000 magnification (Table 1). DNA was purified as previously described (Morgan *et al*. 1997).

18S rDNA gene amplification and sequencing

Primers and amplification conditions used to amplify a portion of the SSU ribosomal DNA were as previously described (Morgan *et al*. 1999*a*). TAQ Extender™ (Stratagene, La Jolla, CA) was included in all reactions to minimize PCR error. PCR products were purified using Qiagen spin columns (Qiagen, Hilden, Germany) and sequenced using an ABI Prism™ Dye Terminator Cycle Sequencing kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions except that the annealing temperature was raised to 60 °C. Sequences were analysed using SeqEd v 1.0.3. (Applied Biosystems, Foster City, CA). Additional *Cryptosporidium* 18S rDNA sequences were obtained from GenBank: 1 bovine *C. parvum* isolate C1 (AF108864), a human *C. parvum* isolate (AF108865), a 'mouse' genotype *C. parvum* isolate (AF108863), *C. wrairi* (U11440), *C. baileyi* (AF093495) and *C. serpentis* (AF108866). Sequences for *Cyclospora*, *Eimeria*, *Toxoplasma*, and *Neospora* species were also obtained from GenBank; *Cyclospora* sp. strain Gombe 22 (AF061566), *Cyclospora* sp. strain Gombe 34 (AF061567), *Cyclospora cayetanensis* (U40261), *E. acervulina* (U67115), *E. brunetti* (U67116). *E. maxima* (U67117), *E. mitis* (U67118), *E. mivati* (U76748), *E. necatrix* (U67119), *E. praecox* (U67120), *E. tenella* (U67121) (Barta *et al*. 1997), *T. gondii* (L24381), and *N. caninum* (L24380).

ITS1 amplification, cloning and sequencing

The primers, designated 18SF+ (5′-TGAATA TGCATCGTGATGG) and ITS R1 (5′-GAAT TATGCAGTTCACATTGC), were used to amplify the rDNA ITS1 locus as previously described except that the annealing temperature was lowered to 50 °C (Morgan *et al*. 1999*b*). TAQ Extender™ (Stratagene, La Jolla, CA) was included in all reactions to minimize PCR error. PCR products

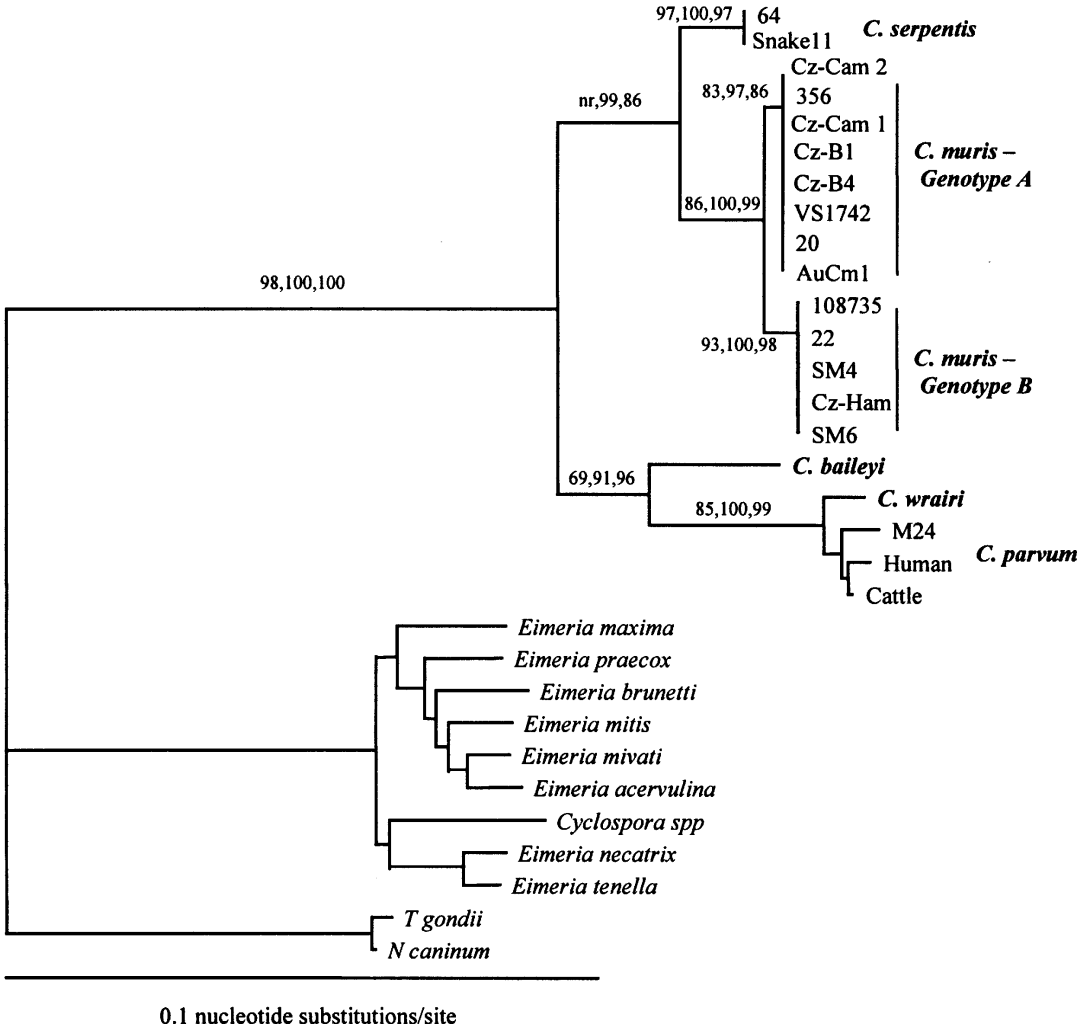

Fig. 1. Phylogram depecting evolutionary relationships among species of *Cryptosporidium* inferred by Neighbor joining analysis of Tamura Nei distances derived from the 18S rDNA sequence data. Percentage bootstrap support (from 1000 replicates) is indicated at each node (maximum likelihood, neighbor joining, parsimony). Nr, branch not recovered.

were purified as described above, cloned into the PCR 2000™ T-vector (Invitrogen, Carlsbad, CA), and transformants screened by PCR. Recombinant plasmids were sequenced as described above. At least 3 clones of each PCR product were sequenced in both directions. Sequences were analysed using SeqEd v 1.0.3. (Applied Biosystems, Foster City, CA).

HSP70 gene amplification

A 2-step nested PCR protocol was used to amplify the HSP70 gene (Khramtsov *et al.* 1995) from genomic DNA of selected *Cryptosporidium* isolates for nucleotide sequencing. For the primary PCR, a PCR product of ~2015 bp was amplified using a forward (5′-ATG TCT GAA GGT CCA GCT ATT GGT ATT GA-3′) and reverse primer (5′-TTA GTC GAC CTC TTC AAC AGT TGG-3′). The PCR reaction consisted of 50 ng genomic DNA, 200 μM each of dNTP, 1 × PCR buffer (Perkin–Elmer), 3·0 mM $MgCl_2$, 5·0 units of *Taq* polymerase (Gibco–BRL), and 40 ng of forward and reverse primers in a total of 100 μl reaction. Thirty-five PCR cycles (94 °C for 45 sec, 55 °C for 45 sec, 72 °C for 60 sec) were carried out in a Perkin–Elmer Gene Amp PCR 9700 thermocycler with an initial hot start (94 °C for 5 min) and a final extension (72 °C for 10 min). For the secondary PCR, a fragment of ~1950 bp was amplified using 2·5 μl of primary PCR product and nested forward (5′-TA/CT TCA TG/CT GTT GGT GTA TGG AGA AA-3′) and nested reverse (5′-CAA CAG TTG GAC CAT TAG ATC C-3′) primers. The PCR condition for the secondary PCR was identical to the primary PCR, except that the annealing temperature was 45 °C. Secondary PCR products were sequenced directly in both directions. Each isolate was sequenced at least twice.

Phylogenetic analyses

Nucleotide sequences were aligned using Clustal X (Thompson *et al.* 1997). (Sequence alignments can

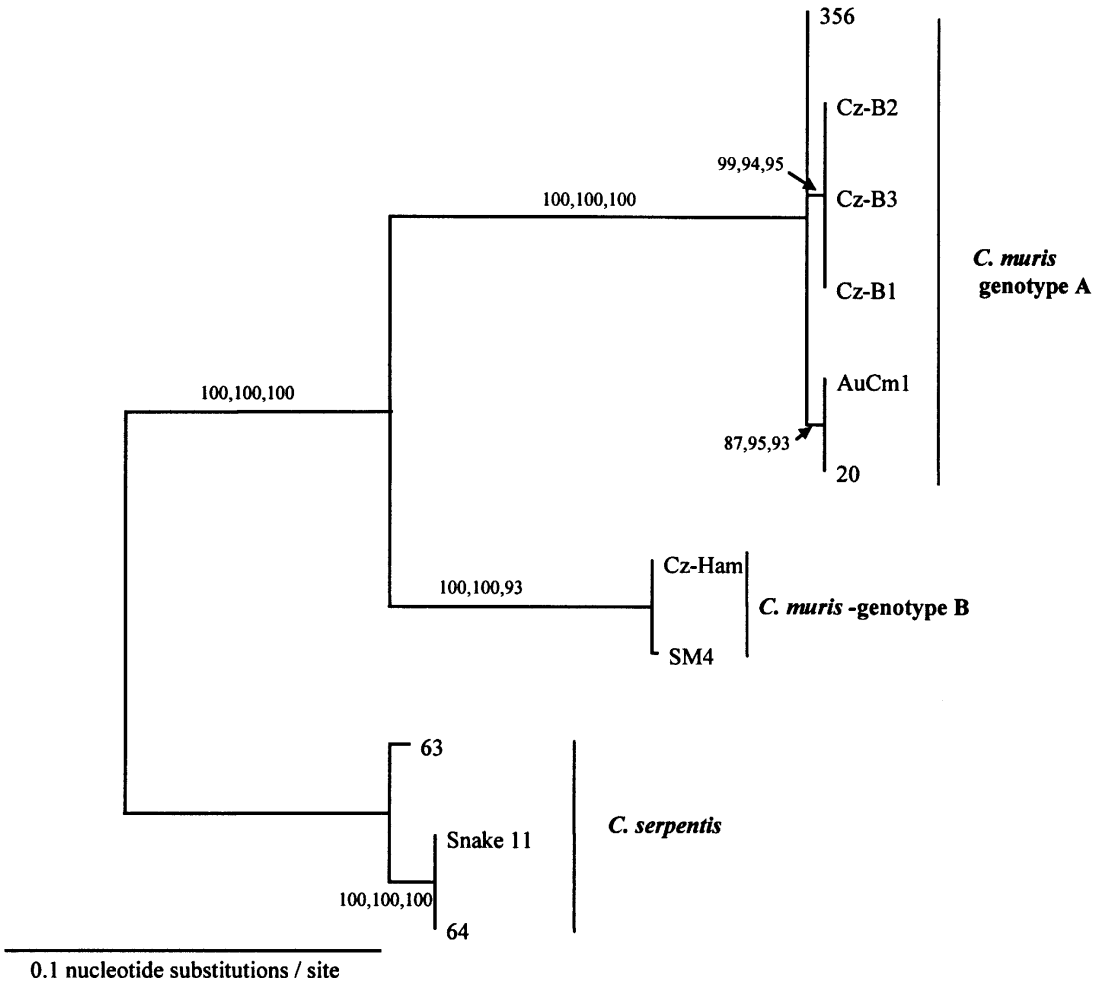

Fig. 2. Phylogram depecting evolutionary relationships among isolates of *Cryptosporidium muris* and *C. serpentis* inferred by maximum likelihood analysis of ITS1 rDNA sequence data. Percentage bootstrap support (from 100 replicates) is indicated at each node (maximum likelihood, neighbor joining, parsimony).

be obtained from the authors upon request.) Distance-based and parsimony analysis was performed using PAUP* (Swofford, D.L. 1999. PAUP*). Phylogenetic Analysis Using Parsimony (*and Other Methods), (Version 4.0b2 Sinauer Associates, Sunderland, MA). Maximum likelihood analyses were performed using PUZZLE (version 4.1, Strimmer & von Haeseler, 1996). Distance-based analyses were conducted using Tamura–Nei distance estimates and trees were constructed using the Neighbor Joining algorithm. Parsimony analyses were conducted using either the branch and bound or heuristic search option of PAUP. Bootstrap analyses were conducted using 1000 replicates. Phylograms were drawn using the Tree View program (Page, 1996).

RESULTS

Morphology

All *C. muris* isolates measured (see Table 1), conformed to the accepted dimensions for *C. muris* (Upton & Current, 1985).

Sequence analysis of the 18S rDNA gene

Partial sequences of the 18S rDNA gene were obtained from 6 bovine-derived *C. muris* isolates (AuCm1, 20, VS1742, Cz-B1, Cz-B4 and 356), 3 camel-derived *C. muris* isolates (Cz-Cam-1, Cz-Cam-2, 22), 3 rodent-derived *C. muris* isolates (Cz-Ham1, SM4, SM6) and a rock hyrax (108735). No type B ribosomal units were detected. A total of 12 *C. muris* isolates were analysed at this locus and compared with 2 *C. serpentis* isolates, *C. baileyi*, *C. wrairi* and a number of *C. parvum* isolates. Additional *Cryptosporidium* sequence information was obtained from GenBank as described above.

Phylogenetic analysis of the 18S rRNA sequences

Phylogenetic analysis of the 18S rRNA sequence data by distance-based and parsimony methods produced trees with identical topologies (Fig. 1, Neighbor Joining tree illustrated). All of the nodes of these trees were strongly supported by bootstrap

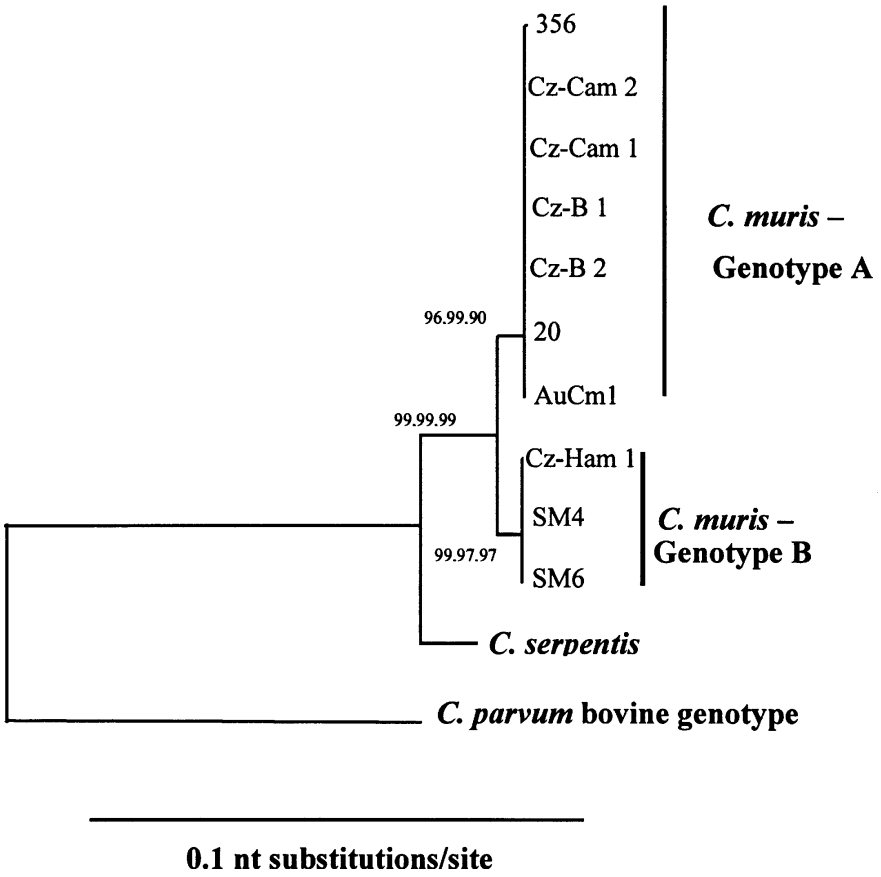

Fig. 3. Phylogram depecting evolutionary relationships among isolates of *Cryptosporidium muris* and *C. serpentis* inferred by Neighbor joining of Tamura Nei's distance derived from HSP-70 sequence data. Percentage bootstrap support (from 1000 replicates) is indicated at each node (maximum likelihood, neighbor joining, parsimony).

analysis. The genus *Cryptosporidium*, which was found to be monophyletic, comprised 2 main clusters, one containing *C. muris* and *C. serpentis* and the other containing *C. baileyi*, *C. wrairi* and *C. parvum*. The isolates of *C. muris* were divided into 2 distinct clusters; (1) *C. muris* genotype A, which contained bovine and camel-derived isolates, and (2) *C. muris* genotype B which contained rodent, camel and a rock hyrax derived isolates. The tree inferred by maximum likelihood analysis was similar, with the only difference being the placement of *C. serpentis* as a sister taxon to the *C. baileyi*, *C. wrairi*, *C. parvum* cluster rather than as a sister taxon to *C. muris* (data not shown).

Sequence analysis at the ITS1 rDNA locus

ITS1 sequence information was obtained for 3 *C. serpentis* isolates (63, 64 and Snake 11), 6 bovine derived *C. muris* isolates (AuCm1, 356, 20, Cz-B1, Cz-B2, Cz-B3) and 2 rodent derived *C. muris* isolates (Cz-Ham-1, SM4). Due to the limited amounts of DNA available, it was not possible to analyse all isolates at all 3 loci.

Phylogenetic anlaysis of the ITS1 rDNA locus

Additional *Cryptosporidium* genotypes and distantly related coccidia were not included in the ITS1 rDNA analysis because the high level of variability exhibited by these genotypes/species for this region, adversely affected the sequence alignment, and was likely to be too great to be phylogenetically informative. rDNA ITS sequence data were analysed using distance-based, parsimony and maximum likelihood methods. All 3 methods produced trees with the same topology (Fig. 2, maximum likelihood tree illustrated). Bootstrap analysis found strong support for all nodes of the tree. The same 2 clusters of *C. muris* isolates identified in the 18S rRNA analysis were also recovered by the ITS analysis. Minor differences, which appeared to correlate with the geographical locality of the isolates, were observed between bovine derived genotypes at the rDNA ITS1 locus. Isolates from the same locality were placed into the same cluster (AuCm1 and 20 were bovine-derived isolates from the USA, isolates Cz-B1, Cz-B2 and Cz-B3 were from the Czech republic and isolate 356 was a bovine-derived isolate from Canada).

HSP-70 locus

Partial HSP-70 sequence information was obtained for 5 bovine-derived *C. muris* isolates (AuCm1, 20, 356, Cz-B1, Cz-B2), 2 camel isolates (Cz-Cam 1, Cz-

Cam 2), 3 rodent-derived isolates (Cz-Ham 1, SM 4, SM6) as well as *C. serpentis* (64) and a *C. parvum* bovine genotype isolate (USA-H6).

Phylogenetic analysis of the HSP-70 locus

Phylogenetic analysis of the HSP-70 locus using maximum likelihood, distance-based and parsimony methods also provided strong support for 2 distinct groups within *C. muris*; bovine-derived isolates, AuCm1, Cz-Cam1, Cz-Cam2, Cz-B1, Cz-B2, 20 and 356 (genotype A) grouped together and were distinct from the rodent-derived isolates, Cz-Ham1, SM4, SM6 (genotype B) (Fig. 3, Neighbor Joining tree illustrated). All three methods recovered trees with identical topologies. The monophyly of *C. muris*, as well as the existence of the 2 genetic groups comprising *C. muris*, were strongly supported by bootstrap analysis ($\geqslant 90\,\%$).

DISCUSSION

Phylogenetic analysis of *Cryptosporidium* species resulted in 2 broad groupings, with *C. serpentis* and *C. muris* forming 1 group and *C. baileyi*, *C. wrairi* and *C. parvum* forming the second group. This supports earlier preliminary analysis, which indicated that *C. muris* is not a uniform species (Xiao *et al*. 1999*a*, *b*). Phylogenetic analysis at the 18S rDNA locus has shown that *C. muris* is the most divergent species of *Cryptosporidium* and is most closely related to *C. serpentis* (Morgan *et al*. 1999*a*; Xiao *et al*. 1999*a*, *b*). Results of this study using sequence analysis at the rDNA 18S and ITS1 loci and also the HSP-70 locus, has also confirmed that while *C. muris* and *C. serpentis* are each other's closest relative, they are distinct species. Their validity as separate species is further confirmed by recent cross-transmission studies where *C. muris* isolates from mice, calves and Bactrian camels did not produce infections in rat snakes (*Elaphe obsoleta*), whereas all snakes challenged with *C. serpentis* oocysts isolated from reptiles became infected (Graczyk & Cranfield, 1998).

Preliminary data also suggest that lizard and snake-derived isolates of *C. serpentis* differ genetically. At the ITS 1 rDNA locus, snake derived *C. serpentis* isolates from different geographical locations (snakes 11 and 64) were identical, whereas an isolate from a Savanna monitor (63) exhibited only 98·4 % similarity to the snake-derived *C. serpentis* isolates. Previous analysis at the 18S rDNA locus also revealed genetic differences between snake and lizard-derived *C. serpentis* isolates (Morgan *et al*. 1999*a*). Future studies, on a wider range of isolates are required in order to confirm this.

This study has confirmed that *C. muris* is not a uniform species. Two distinct genotypes were identified amongst *C. muris* isolates from different hosts. Phylogenetic analysis of all 3 loci demonstrated that *Cryptosporidium muris*-like isolates from bovine and camel hosts were a distinct genotype, referred to herein as *C. muris* genotype A. This genotype appeared to be conserved across geographical areas as bovine and camel-derived *Cryptosporidium* isolates from the US, Canada and the Czech Republic all exhibited very similar or identical genotypes. Similarly, *C. muris*-like oocysts from mice from Spain, a hamster from the Czech Republic, a camel and a rock hyrax were virtually identical and genetically very distinct from *C. muris* genotype A isolates, and are referred to here as *C. muris* genotype B. Analysis of the ITS1 locus revealed genetic variation among *C. muris* genotype A isolates that appeared to correlate with their geographical origin, with isolates from the same country possessing identical sequences. However, more isolates would need to be examined to validate this observation.

Phylogenetic data provided strong support for 2 distinct groupings within *C. muris* and all 3 loci produced similar results. At the 18S rDNA locus, the genetic similarity between *C. muris* (genotype A/genotype B) and *C. serpentis* (isolate 64) was approx 97·4 % and 97·07 % respectively. The genetic similarity between the *C. muris* genotypes (A and B) was 99·14 %. This is less than the similarity between the 'human' and 'cattle' *C. parvum* genotypes at the same locus (99·7 %). At the rDNA ITS1 locus the genetic similarity between the *C. muris* genotype A (AuCm1) and genotype B (SM41) was only 84 %. This is less than the similarity between *T. gondii* and *N. caninum* at the same locus (approx. 89 %). *C. serpentis* exhibited approx. 79 % similarity with both *C. muris* genotypes (A/B) at the ITS1 locus, further confirming its validity as a separate species. At the HSP-70 locus the genetic similarity between *C. muris* genotype A (AuCm1) and *C. muris* genotype B (SM4) was approx. 99 %. The similarity between *C. muris* (A/B) and *C. serpentis* (64) was much less at 96·2 % and 95·5 % respectively.

In addition to the distinct genetic differences between *C. muris* genotype A and B isolates, there are also biological differences as bovine-derived *C. muris* (genotype A), does not appear to readily infect mice. For example, a recent study, revealed that *C. muris* oocysts from the rock hyrax isolate (108735) (genotype B) produced infections in mice but bovine-derived *C. muris* oocysts (isolate 20) (genotype A) did not (Xiao *et al*. 1999*a*). In an earlier study, *C. muris*-like oocysts from cattle had also been shown to produce no evidence of infection in mice (Anderson, 1991). In another study, a bovine isolate of *C. muris* failed to produce infections in neonatal BALB/c mice, adult BALB/c mice, SCID mice, common voles (*Microtus arvalis*), bank voles (*Clethrionomys glareolus*), common field mice (*Apodemus sylvaticus*), desert gerbils (*Gerbilus gerbilus*), guinea-pigs, rats, rabbits and goats (Koudela *et al*. 1998). Only Mongolian gerbils (*Meriones unguiculatus*) were

susceptible to the infection and discharged *C. muris* oocysts in their faeces (Koudela *et al*. 1998). Based on these results, the authors suggested that significant differences in host specificity of individual *C. muris* isolates exist, and that wild rodents are not reservoirs for *C. muris* infection in cattle (Koudela *et al*. 1998). In a similar study, oocysts of *Cryptosporidium muris*, directly isolated from the stomach of experimentally infected laboratory mice were orally inoculated into rats, gerbils, guinea-pigs, dogs, and rabbits. Only weaned rats developed patent *C. muris* infection. No signs of clinical illness were detected in mice and rats. Laboratory raised suckling rabbits, guinea-pigs, gerbils and dogs fed *C. muris* rarely developed patent infections and they were considered not a true host for *C. muris* (Aydin & Ozkul, 1996).

Interestingly, 2 of the camel isolates examined in this study exhibited the *C. muris* genotype A (Cz-Cam-1, Cz-Cam-2) whereas the third camel isolate (22) exhibited the *C. muris* genotype B. This latter isolate was derived from a camel that was housed in the same enclosure as the rock hyrax (isolate 108735) at the National Park Zoo in Baltimore. As both animals were found to be infected with *Cryptosporidium*, it is therefore possible that the camel acquired its *C. muris* genotype B infection from the rock hyrax. The rock hyrax belongs to the Order Hyracoidea and is one of the most taxonomically unique mammal species. Belonging to ungulates and most closely related to elephants, these small rodent-like mammals are widely distributed throughout Egypt, Syria, Lebanon, Israel, Jordan, Sinai, Libya and parts of Africa. Preliminary data indicate therefore that ungulates are capable of being infected with both genotype A and B of *C. muris* as both genotypes were found in camels and genotype B was detected in the rock hyrax. Supportive evidence for cattle being capable of being infected with both genotypes comes from a recent study by Kaneta & Nakai, (1998), in which *Cryptosporidium* oocysts measuring 7·0–7·9 × 5·3–6·1 μm from adult cattle produced infections in mice and rats when inoculated orally (Kaneta & Nakai, 1998), suggesting that the cattle were infected with *C. muris* genotype B. In contrast, it appears that rodents are susceptible only to *C. muris* genotype B as evidence to date indicates that the *C. muris* genotype B is very host specific. However, further studies are required in order to confirm the susceptibility of different hosts to *C. muris* genotypes A and B.

The prevalence of *C. muris* in bovine hosts has not been extensively studied but is important as *C. muris* infections in cattle tend to be chronic and can affect milk production in cows and growth rates in calves (Anderson, 1998). In the USA, the prevalence is approx. 4·7% (Anderson, 1991). In a European study, *C. muris*-like oocysts were detected in 4·5% of heifers imported into the Czech Republic from France and in 7·9% of those from Germany (Pavlasek, 1995). On one farm, 57·9% out of 19 animals were positive for *C. muris* and 1 bull shed oocysts for longer than 18 months (Pavlasek, 1995). In Japan, a prevalence of 4·7% was reported for adult cattle (24/512) (Kaneta & Nakai, 1998). In Canada, a prevalence of 2% for *C. muris* was reported for 386 male and female Holstein calves (newborn to 24 weeks) in 20 dairies located in the lower Fraser river valley area of British Columbia (Olson *et al*. 1997). Unlike *C. parvum* infections that were predominant in calves 2–4 weeks of age, *C. muris* was demonstrated in calves older than 4 weeks (Olson *et al*. 1997). In a Brazilian study, 53 (17·3%) of 307 calf faecal samples examined were positive for *C. muris* (Pena, Kasai & Gennari, 1997). The mean persistency of oocyst shedding in faeces was 4 ± 3 months.

Many of the bovine-derived *C. muris* isolates used in this study were from chronically infected cattle. For example, the Bactrian camel (isolate 22) from the National Park Zoo in Baltimore, is still positive for *C. muris* after initial diagnosis in early 1990 (Xiao *et al*. unpublished observations). The Canadian isolate (356) was from a chronically infected steer, which has been shedding *C. muris* oocysts for several years. The US isolate (AuCm1) was obtained following a faecal survey of 238 lactating dairy cattle from east central Alabama, USA. This cow (806) was first examined and determined to be shedding oocysts of *C. muris* on 30 August 1997 and had been passaging oocysts for approximately 2 years.

The results of this study indicate that *C. muris* is not a uniform species but is composed of 2 distinct groups that differ both biologically and genetically. The *C. muris* genotype A infects predominantly cattle, while the *C. muris* genotype B infects predominantly rodents, however, preliminary data suggest that ungulates are capable of being infected with both genotypes of *C. muris* (A/B). Both genotypes are also conserved across geographical areas. The genetic differences between the 2 *C. muris* genotypes (A/B), particularly at the rDNA ITS1 locus, coupled with biological differences indicate that they may in fact be separate species; however, further characterization studies on a wider range of isolates are required in order to confirm this.

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REFERENCES

ANDERSON, B. C. (1991). Experimental infection in mice of *Cryptosporidium muris* isolated from a camel. *Journal of Protozoology* **38**, 16S–17S.

ANDERSON, B. C. (1998). Cryptosporidiosis in bovine and

human health. *Journal of Dairy Science* **81**, 3036–3041.

AYDIN, Y. & OZKUL, I. A. (1996). Infectivity of *Cryptosporidium muris* directly isolated from the murine stomach for various laboratory animals. *Veterinary Parasitology* **66**, 257–262.

BARTA, J. R., MARTIN, D. S., LIBERATOR, P. A., DASHKEVICK, M., ANDERSON, J. W., FEIGHNER, S. D., ELBRECHT, A., PERKINS-BARROW, A., JENKINS, M. C., DANFORTH, H. D., RUFF, M. D. & PROFOUS-JUCHELKA, H. (1997). Phylogenetic relationships among eight *Eimeria* species infecting domestic fowl inferred using complete small subunit ribosomal DNA sequences. *Journal of Parasitology* **83**, 262–271.

BUKHARI, Z. & SMITH, H. V. (1996). Detection of *Cryptosporidium muris* oocysts in the faeces of adult dairy cattle in Scotland. *Veterinary Record* **138**, 207–208.

CHALMERS, R. M., STURDEE, A. P., BULL, S. A., MILLER, A. & WRIGHT, S. E. (1997). The prevalence of *Cryptosporidium parvum* and *C. muris* in *Mus domesticus*, *Apodemus sylvaticus* and *Clethrionomys glareolus* in an agricultural system. *Parasitology Research* **83**, 478–482.

ESTEBAN, E. & ANDERSON, B. C. (1995). *Cryptosporidium muris* – prevalence, persistency, and detrimental effect on milk production in a drylot dairy. *Journal of Dairy Science* **78**, 1068–1072.

FAYER, R., PHILLIPS, L., ANDERSON, B. C. & BUSH, M. (1991). Chronic cryptosporidiosis in a Bactrian camel (*Camelus bactrianus*). *Journal of Zoo and Wildlife Medicine* **22**, 228–232.

FAYER, R., SPEER, C. A. & DUBEY, J. P. (1997). The general biology of *Cryptosporidium*. In *Cryptosporidium and Cryptosporidiosis* (ed. Fayer, R.), pp. 1–42. CRC Press: Boca Raton.

GRACZYK, T. K. & CRANFIELD, M. R. (1998). Experimental transmission of *Cryptosporidium* oocyst isolates from mammals, birds and reptiles to captive snakes. *Veterinary Research* **29**, 187–195.

KANETA, Y. & NAKAI, Y. (1998). Survey of *Cryptosporidium* oocysts from adult cattle in a slaughter house. *Journal of Veterinary Medical Science* **60**, 585–588.

KHRAMTSOV, N. V., TILLEY, M., BLUNT, D. S., MONTELONE, B. A. & UPTON, S. J. (1995). Cloning and analysis of a *Cryptosporidium parvum* gene encoding a protein with homology to cytoplasmic form HSP70. *Journal of Eukaryotic Microbiology* **42**, 416–422.

KOUDELA, B., MODRY, D. & VITOVEC, J. (1998). Infectivity of *Cryptosporidium muris* isolated from cattle. *Veterinary Parasitology* **76**, 181–188.

MORGAN, U. M., CONSTANTINE, C. C., FORBES, D. A. & THOMPSON, R. C. A. (1997). Differentiation between human and animal isolates of *Crytosporidium parvum* using rDNA sequencing and direct PCR analysis. *Journal of Parasitology* **83**, 825–830.

MORGAN, U. M., XIAO, L., FAYER, R., GRACZYK, T. K., LAL, A. A., DEPLAZES, P. & THOMPSON, R. C. A. (1999*a*). Phylogenetic analysis of 18S rDNA sequence data and RAPD analysis of *Cryptosporidium* isolates from captive reptiles. *Journal of Parasitology* **85**, 525–530.

MORGAN, U. M., SARGENT, K. D., DEPLAZES, P., FORBES, D. A., SPANO, F., HERTZBERG, H., ELLIOT, A. & THOMPSON, R. C. A. (1999*b*). Sequence and PCR-RFLP analysis of the internal transcribed spacers of the rDNA repeat unit in isolates of *Cryptosporidium* from different hosts. *Parasitology* **118**, 49–58.

OLSON, M. E., GUSELLE, N. J., O'HANDLEY, R. M., SWIFT, M. L., MCALLISTER, T. A., JELINSKI, M. D. & MORCK, D. W. (1997). *Giardia* and *Cryptosporidium* in dairy calves in British Columbia. *Canadian Veterinary Journal* **38**, 703–706.

OZKUL, I. A. & AYDIN, Y. (1994). Natural *Cryptosporidium muris* infection of the stomach in laboratory mice. *Veterinary Parasitology* **55**, 129–132.

PAGE, R. D. M. (1996). TREEVIEW: An application to display phylogenetic trees on personal computers. *Computer Applications in the Biosciences* **12**, 357–358.

PAVLASEK, I. (1995). Findings of cryptosporidia and of other endoparasites in heifers imported into the Czech Republic. *Veterinarni Medicina* **40**, 333–336.

PAVLASEK, I. & LAVICKA, M. (1995). The first findings of natural *Cryptosporidium* infection of stomach in desert hamsters (*Phodopus roborovskii* Satunin, 1903). *Veterinarni Medicina* **40**, 261–263.

PENA, H. F. D., KASAI, N. & GENNARI, S. M. (1997). *Cryptosporidium muris* in dairy cattle in Brazil. *Veterinary Parasitology* **73**, 353–355.

STRIMMER, K. & VON HAESELER, A. (1996). Quartet puzzling: a quartet maximum likelihood method for reconstructing tree topologies. *Molecular and Biological Evolution* **13**, 964–969.

STURDEE, A. P., CHALMERS, R. M. & BULL, S. A. (1999). Detection of *Cryptosporidium* oocysts in wild mammals of mainland Britain. *Veterinary Parasitology* **80**, 273–280.

THOMPSON, J. D., GIBSON, T. J., PLEWNIAK, F., JEANMOUGIN, F. & HIGGINS, D. G. (1997). The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **25**, 4876–4882.

TYZZER, E. E. (1907). A sporozoan found in the peptic glands of the common mouse. *Proceedings of the Society for Experimental Biology and Medicine* **5**, 12–13.

UPTON, S. J. & CURRENT, W. L. (1985). The species of *Cryptosporidium* (Apicomplexa: Cryptosporidiidae) infecting mammals. *Journal of Parasitology* **71**, 625–629.

XIAO, L. H., ESCALANTE, L., YANG, C. F., SULAIMAN, I., ESCALANTE, A. A., MONTALI, R. J., FAYER, R. & LAL, A. A. (1999*a*). Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Applied and Environmental Microbiology* **65**, 1578–1583.

XIAO, L., MORGAN, U., LIMOR, J., ESCALANTE, A., ARROWOOD, M., SCHULAW, W., THOMPSON, R. C. A., FAYER, R. & LAL, A. A. (1999*b*). Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. *Applied and Environmental Microbiology* **65**, 3386–3391.

WEBSTER, J. P. & MACDONALD, D. W. (1995). Parasites of wild brown rats (*Rattus norvegicus*) on UK farms. *Parasitology* **111**, 247–255.