

Multilocus phylogenetic analysis of *Cryptosporidium andersoni* (Apicomplexa) isolated from a bactrian camel (*Camelus bactrianus*) in China

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Abstract This is the first report of cryptosporidiosis in a bactrian camel (*Camelus bactrianus*) in China. Two *Cryptosporidium* isolates derived from the same bactrian camel (3-year-old) in November 2005 and April 2006 were characterized using sequence and phylogenetic analysis of the small-subunit rRNA (18S rRNA), 70-kDa heat shock protein (HSP70), actin and *Cryptosporidium* oocyst wall protein (COWP) genes. The sequences of the 18S rRNA and COWP were identical to all other *Cryptosporidium andersoni* isolates although minor differences were noticed between the isolates and the USA isolate at the actin locus (99.2% of similarity). The sequence of the HSP70 was identical to the Japanese *C. andersoni* isolate, with a minor difference from the Australian *C. andersoni* isolate (99.7% of similarity). Cross-transmission studies demonstrated that the *C. andersoni* isolates did not infect immunosuppressed or immunocompetent Kun-ming mice, severe combined

immunodeficiency mice, and immunosuppressed or immunocompetent calves. Among the *C. andersoni* isolates reported so far, only isolates from Japan could infect SCID mice. Thus, the *C. andersoni* isolates from the bactrian camel were biologically similar to most bovine *C. andersoni* isolates characterized so far, but are different from bovine isolates from Japan.

Introduction

Cryptosporidiosis is a zoonotic protozoan disease of worldwide distribution, affecting a wide range of vertebrate hosts. There are 16 valid species of *Cryptosporidium* so far, namely, *Cryptosporidium andersoni*, *Cryptosporidium baileyi*, *Cryptosporidium bovis*, *Cryptosporidium canis*, *Cryptosporidium felis*, *Cryptosporidium galli*, *Cryptosporidium hominis*, *Cryptosporidium meleagridis*, *Cryptosporidium molnari*, *Cryptosporidium muris*, *Cryptosporidium parvum*, *Cryptosporidium varanii*, *Cryptosporidium scophthalmi*, *Cryptosporidium serpentis*, *Cryptosporidium suis*, and *Cryptosporidium wrairi*. In addition, over 40 genotypes with no species names have been described (Xiao et al. 2004).

C. andersoni was separated from *C. muris* and was established as a new species based on molecular data and a transmission study (Lindsay et al. 2000; Xiao et al. 2004). To date, there are many reports of *C. andersoni* in cattle (Anderson 1991; Enemark et al. 2002; Fayer et al. 2006; Koyama et al. 2005; Kvac et al. 2004; Kvac and Vitovec 2003, 2006; Masuno et al. 2006; Matsubayashi et al. 2004, 2005; Robinson et al. 2006; Satoh et al. 2003; Xiao et al. 2000). Cross-transmission study showed *C. andersoni* isolates from different geographic region had different infection characteristics. *C. muris*-like oocysts from cattle

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in the USA were not infectious to mice or even cattle (Andersoni 1991). A Danish *C. andersoni* isolate was not transmissible to mice, but oocysts were detected in the feces of one experimentally infected calf (Enemark et al. 2002). *C. andersoni* oocysts from cattle in the Czech Republic were not transmissible to neonatal or adult BALB/C mice, SCID mice, common voles, bank voles, common field mice, desert gerbils, guinea pigs, rabbits, lambs, or goats; only cattle, Mongolian gerbils, and southern multimammate mice became infected (Koudela et al. 1998; Kvac et al. 2007). Contrary to other findings, *C. andersoni* isolates in Japan were found to be infective to SCID mice (Matsubayashi et al. 2004; Koyama et al. 2005; Satoh et al. 2003).

In the present study, we have characterized two *C. andersoni* isolates from a bactrian camel (*Camelus bactrianus*) in China by sequence analyses of four genes and by experimental infections of laboratory animals. Results of the study suggest that the Chinese isolates were biologically more similar to European and American *C. andersoni* isolates.

Materials and methods

C. andersoni isolates

Two fecal samples were collected from a bactrian camel (3-year-old) in the Wild Animals Rescue Centre of Henan Province in China in November 2005 and April 2006. Both samples were positive for *Cryptosporidium* by bright field microscopy under 400× and 1,000× after oocysts were concentrated by the Sheather's sugar flotation technique. Oocysts were purified from the samples by discontinuous sucrose gradient centrifugation as described previously (Arrowood and Sterling 1987; Arrowood and Donaldson 1996). The lengths and widths (to the nearest micrometer) of 50 oocysts (each isolate) were measured and the shape index (the ratio of length to width) of each oocyst was calculated. Oocysts were stored in 2.5% potassium dichromate solution at 4°C before they were used in experimental infections within 2 weeks.

DNA extraction

Genomic DNA was isolated from the purified oocysts using Mag Extractor-Genome kit (Toyobo, Osaka, Japan), based on chaotropic extraction followed by absorption onto silica-coated magnetic beads. Briefly, 50 µl of oocyst suspension was resuspended in 750 µl of lysis buffer. After five cycles of freeze-thaw (−80°C for 5 min and 37°C for 5 min), 40 µl of silica-coated magnetic beads were added to the oocyst lysate, and the tube was vortexed vigorously for 10 min. The magnetic beads were then separated from the suspension

using a magnet and washed twice in 900 µl of washing buffer and once in 900 µl of 70% ethanol. Afterwards, the magnetic beads were resuspended in 25–100 µl of double-distilled water, and the tube were vortexed vigorously for 10 min. The bead suspension was then centrifuged at 2,000×g for 2 min, and the supernatant was collected. The supernatant containing DNA was kept at −20°C before it was used in polymerase chain reaction (PCR) analysis.

18S rRNA and HSP70 genes amplification and sequencing

Primers and amplification conditions used in nested-PCR analysis of the partial 18S rRNA and HSP70 gene were previously described (Sulaiman et al. 2000; Xiao et al. 1999a). KOD-Plus amplification enzyme (Toyobo, Kita-ku, Osaka, Japan) was used for PCR amplification. The PCR products were examined by agarose gel electrophoresis and visualized after ethidium bromide staining. Direct sequencing of the PCR products was done by TaKaRa Biotechnology (Dalian, China) on an ABI PRISM™ 3730 XL DNA Analyzer (Applied Biosystems) using the Big Dye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). Sequence accuracy was confirmed by two-directional sequencing and by sequencing a new PCR product if necessary.

Actin and COWP genes amplification, cloning, and sequencing

Primers and amplification conditions used in nested-PCR analyses of the partial actin and COWP gene were previously described (Sulaiman et al. 2002; Xiao et al. 2000). TaKaRa Ex Taq® enzyme (TaKaRa Biotechnology) was used in PCR. PCR products were purified using a Mag Extractor-PCR and Gel Clean up-kit (Toyobo). Purified PCR products were cloned into the PMD19-T vector (TaKaRa Biotechnology) as recommended by the supplier. Positive clones were screened by PCR, and four to five recombinant plasmids per product were sequenced as described above. The nucleotide sequences of the partial 18S rRNA, HSP70, actin and COWP genes of *C. andersoni* from the bactrian camel were deposited in the GenBank database under accession numbers DQ989570 to DQ989577.

Phylogenetic analysis

Nucleotide sequences were aligned using the ClustalX 1.81 (Thompson et al. 1997), with manual adjustment. Phylogenetic analyses were performed using the software PHYLIP version 3.64 (Felsenstein 1989). Neighbor-joining trees were constructed based on the evolutionary distances calculated using the Kimura two-parameter model. Parsimony trees were constructed using the Program Dnapars

and the more thorough search. The reliability of these trees was assessed using the bootstrap analysis with 1,000 replicates, with values above 50% reported. Phylograms were drawn using the Tree View program version 1.65 (Page 1996).

Cross-transmission study

Fifty Kun-ming 18-day-old mice, six 5-week-old SCID mice (Shanghai SLAC Laboratory Animal, Shanghai, China), and two 8-day-old calves were inoculated with *C. andersoni* oocysts. Before the inoculation, fecal samples from all animals were examined daily for 10 days using Sheather's sugar flotation technique. Only *Cryptosporidium*-negative animals were used in the experimental infection. Immunosuppression was induced in Kun-ming mice using dexamethasone at 0.06 mg per day 10 days before and 45 days after the inoculation, while immunosuppression was induced in one calf using dexamethasone at 28 mg per day 10 days before and 14 days after the inoculation. The doses used in the experimental infections are shown in Table 1.

Results

Sequence characterizations of the two *C. andersoni* isolates

For each of the gene targets, sequences of the two *Cryptosporidium* isolates were aligned with those from other *C. andersoni* isolates using ClustalX 1.81 (Thompson et al. 1997). There were no sequence differences between the two *Cryptosporidium* isolates from the camel. The sequences of the 18S rRNA gene were identical to those

Table 1 The experimental design for cross-transmission studies of *C. andersoni* oocysts

Animals	Number	Group	Dosage of inoculated oocysts	Time of feces examination (day)
Kun-ming mice	10	IG ^a	1.0×10^6	45
	10	IG ^a	5.0×10^6	45
	10	NIG	1.0×10^7	45
	10	ICG	0	45
	10	NICG	0	45
SCID mice	4	IG ^b	5.0×10^6	60
	2	CG	0	60
Calf	1	NIG	3.0×10^7	20
	1	IG ^a	3.0×10^7	14

CG Control group, NIG non-immunosuppressed group, ICG immunosuppressed or immunocompromised control group, NICG non-immunosuppressed control group

^aImmunosuppressed or immunocompromised group

^bInfected group

obtained previously from *C. andersoni* in other countries (AF093496 for a USA isolate and AB089285 for Japanese isolate). Likewise, the COWP sequences obtained were also identical to those previously obtained from cattle (AF266262 for a Czech isolate and AB089289 for a Japanese isolate). The HSP70 sequences obtained were identical to a sequence obtained in Japan (AB089288), however, six nucleotide differences was observed with an Australia isolate (AF221542). In addition, there were eight nucleotide differences from a USA isolate (AF382352) in the actin gene (Fig. 1a,b).

Phylogenetic analysis

Neighbor-joining and parsimony trees were constructed from aligned partial 18S rRNA, HSP70, COWP, and actin sequences of these two *C. andersoni* isolates and those downloaded from the GenBank database (Fig. 2a,b,c,d). In the 18S rRNA, HSP70 and COWP loci, the two isolates formed a cluster with *C. andersoni*, which was supported by bootstrap analysis. While in the actin gene, the two isolates clustered together and formed a sister clade with *C. andersoni*.

Morphometric measurements

Oocysts of *C. andersoni* from the bactrian camel were ellipsoid and lacked sporocysts. The mean length and width of oocysts of the isolate 0511 were 7.0 and 5.1 μm , respectively, with a mean shape index of 1.37. The mean length and width of oocysts of the isolate 0604 was 7.2 and 5.2 μm , respectively, with a mean shape index of 1.39 (Table 2).

Cross-transmission results

All fecal samples from inoculated animals were negative for *Cryptosporidium* spp. as examined by the Sheather's sugar flotation technique.

Discussion

Results of the genetic characterizations suggests that the *Cryptosporidium* isolates derived from a bactrian camel in China belonged to *C. andersoni*. In phylogenetic analyses of the 18S rRNA, HSP70 and COWP genes, the *Cryptosporidium* isolates formed a cluster with *C. andersoni*, which was supported by bootstrap analysis. At the actin locus, the present *Cryptosporidium* parasites clustered together and formed a sister taxon with *C. andersoni*. Similarity analysis of nucleotide sequences obtained showed that the camel *Cryptosporidium* isolates and *C. andersoni* were identical at

a HSP70 gene

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cattle isolate TGATCAGGGTAATAGAACACCCCATCTTATGTTGCAITTAAGTCCGAACGTTAATTGGTATGATGAGCTAAGAATCAGGTTGCTCG 90
camel isolate .....G
cattle isolate TAACCCAGAAAATACTGTATTGATGCTAAACGTTTAAITGGTAGGAAATTTGATGATCAAGCTGTTCAAAGTGACATGACACATTGCCC 180
camel isolate .....G
cattle isolate ATTCAAAGGTTGCTCGTGGTCTTAAAGATAAACCAATTATTAATAATTAACCTACTTAGGTGAAACAAAGGAATTCATGCTGAAGAAAATTC 270
camel isolate .....G
cattle isolate AGCTATGGTCTTACAAAAGATGAAGGAGATTCTGAAGCTTACCTTGGTAAGACAGTTAAGAATGCTGTAGTTACAGTTCTGCTCATTT 360
camel isolate .....G
cattle isolate CAATGATTCACAACGTCAAGCTACAAGGATGCTGGTGAATTTGCTGGTTTGAACGTTATGCGTATTATTAATGAACCTACAGCTGCTGC 450
camel isolate .....G
cattle isolate TATAGCTTATGGTCTTGATAAGAAAGTACTGGTGAAGAAATGTCITGATCTTCGATTTAGGTGGTGCATTTGATGATCATTACT 540
camel isolate .....G
cattle isolate TACTATTGAAGATGGTATCTTCGAAGTAAAGGCAACAGCTGGTATACACATCTTGGTGGTGAAGACTTTGATAATAGATTAGTTGAATT 630
camel isolate .....G
cattle isolate CTGTGTCCAAGATTTAAGCGTAAGAATCGTGGTCTTGAATTAACCTACTAATGCCCGTCTTTGAGAAGATTAAAGACCCAGTGTGAACG 720
camel isolate .....G
cattle isolate TGCAAGAGAACACTTCTTCTACCTACACAAGCAACAATCGAATTGGATTCTTTGTTGCAAGGTATTGACTACTTTGTATCTATAAGTCCG 810
camel isolate .....G
cattle isolate TGCAAGATTTGAGGAACCTTGTCTGATTTTCCGTGACCATTAGCTCCAGTAGAGAAGGATTAAAGATTCTGGAATGGATAAGAG 900
camel isolate .....G
cattle isolate GTCAGTACATGATGTTGATATTAGTAGTGGTTCAACCCGATTTCCAAGGTACAACAATTAATTCAAGAAATCTTTAATGGTAAGGAACC 990
camel isolate .....G
cattle isolate ATGCAAGGCAATTAATCCAGATGAAGCTGTGCTTATGGTGTGCGATTCAGCAGCAATCTTAAATGGTGAACAGTCATCAGTAGTGCA 1080
camel isolate .....G
cattle isolate AGATTTATTGTTATGGATGTTGCTCCACTATCTTAAAGTTTGGAAACAGCAGGTGGTGTATGACAAAACCTTATTGAACGTAATACTAC 1170
camel isolate .....G
cattle isolate CATTCCAGCAAAAGAAAACCTCAAGTATTACACAACATATGCTGATAACAGAGTGGTGTACTTATTCAAGTATTGAAAGGTGAACGTGCAAT 1260
camel isolate .....G
cattle isolate GACAAAAGATAATCACTTACTAGGTAATTCATTTAGATGGTATTCCACCAGCTCCACGTGGTGTACCACAAAATGAAGTAACTTTGA 1350
camel isolate .....G
cattle isolate TATTGATGCAAAATGGCATTTTAAATGATCAGCAGTTGACAAAGATACCCGGTAAGAGCAGCAAGATTACGATTACTAATGATAAAGGTCG 1440
camel isolate .....G
cattle isolate TTTATCAAAGGAGATATTGAACGTATGGTTAATGATGCTGAGAAATATAAAAATGAGGATGAACAGAAATCGTGAAGGATTGAAGCTAA 1530
camel isolate .....G
cattle isolate GAATCTTTGGAAAATTAATCTGTACAATATGAGGAATACTATTCAAGAGACTAAAGTAAAAGAAAAGTTATCTCAAAGTGAAGTGAACA 1620
camel isolate .....G
cattle isolate AGCAGAGTCTAAAATTAAGGAGTACTTGATTGGTTAGAGCCTAATCAAAGTGCAGAGAAGGATGAATTTGAACACCAACAGAAGGAAT 1710
camel isolate .....G
cattle isolate TGAAGCTTTAATGAATCCATTAAATGATGAAGATTATGCTGCTGATGGTCTGCAGGTGGTATGCCAGGTGGTATGCCAGGTGGTATGCC 1800
camel isolate .....G
cattle isolate AGGAGGTATGCCAGGAGGTATGCCAGGAGGTATGCCAGGAGGTATGCCAGGTGGTATGCCAGGAGGTATGCTGA 1875

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b actin gene

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cattle isolate TGGTTCGGGTATGGTCAAGGCAGGATTTGCTGGTATGATGCTCCTCGCTGTGTTTCCCATCAATTTGACGAAGACCAAGGATGCCAGGTA 92
camel isolate .....G
cattle isolate TTATGGTAGGTATGGATCAGAAAATTTGTTATGTTGGTATGAGGCACAGTCCAAAAGAGGATTCTAACTTTAAAGTATCCAATTGAACAT 184
camel isolate .....G
cattle isolate GGTATTGTTACAAATTTGGGAAGATATGGAGAAGATATGGCACCATACATTTTACAATGAATTCGCTGTAGCCCTTGAAGAATCCAGTTTT 276
camel isolate .....G
cattle isolate ATTAACAGAAGCTCCAATGAATCCAAGGCAAAACCGTGAAGAAATGACTCAAAATGATGTTTGAACCTTTCAATGTACCCGCAATGTATGTAA 368
camel isolate .....G
cattle isolate ATATACAAGCTGTTCTGCTTTTGTATGATCTGGACGTAAGTACTGAAATTTGACTTGTATGATGGTGTATGGTGTATCACAATACAGTTCCTATT 460
camel isolate .....G
cattle isolate TATGAGGTTATGCTCTTCTCATGCCAATATGAGATTAGATCTAGCTGGTCTGATCTAACTGACTATCTAATGAAAATTTCTACATGATCG 552
camel isolate .....G
cattle isolate TGGTTACAGCTTTACTACAACCTGCTGAAAAGAGAAATAGTTAGAGATAITTAAGGAAAAGTTATGTTACATTGCTTTAGAGTATGAAGATGAAT 644
camel isolate .....G
cattle isolate TAAAGAAGGCCAGGAATCTTCTGAAATGAGAAAACATATGAATACCAGATGGTAATGTTATTACTGTAGGTAGCGAGAGATTTAGATGT 736
camel isolate .....G
cattle isolate CCAGAAGCAGCTTTTCCAACCAATCTATTAGTAAAGGAGCTGCTGGTGTGGTGAAGTACTTTCCAATCTATTATGAAATGTGATCTTGA 828
camel isolate .....G
cattle isolate CATCCGTAAGGATTTGTATGCAAAATGTTGCTCTTCTGGTGGTACTACAATGTATCCTGGACTTGGTGAAGAAATGACTAAAGAATTTACTT 920
camel isolate .....G
cattle isolate GCTTAGCTCCTTCTACTATGAAGATTAAGTTGTTGCCCTCTGAGAGAAAATATTCAGTATGGATTGGAGGATCCATATTTATCATCTTTA 1012
camel isolate .....G
cattle isolate TGTACGTTCCAGCAGATGTGGATT-CAAAGGAGGAA 1047
camel isolate .....G

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Fig. 1 Sequence differences between the *C. andersoni* camel isolate in this study and other *C. andersoni* cattle isolates in the partial HSP70 (a) and actin (b) genes. The cattle reference sequences used are

AF221542 for the HSP70 gene of an Australian isolate and AF382352 for the actin gene of an American isolate. Dots denote sequence identity to the bovine sequence. Dashes represent nucleotide deletions

the 18S rRNA and COWP loci, 100% with Japanese isolates, and 99.7% with an Australian isolate at the HSP70 locus and 99.2% at actin locus. The size of oocysts from the two isolates were also very close to the reported size for *C. andersoni* (mean $7.4 \times 5.5 \mu\text{m}$; range $6.0\text{--}8.1 \times 5.0\text{--}6.5 \mu\text{m}$; shape index 1.35; Lindsay et al. 2000). This is the first report of *Cryptosporidium* in bactrian camel in China, although *Cryptosporidium* spp. have been reported in bactrian camels in other countries (Morgan et al. 2000; Sulaiman et al. 2000; Xiao et al. 1999b).

To date, *Cryptosporidium andersoni* oocysts were not transmissible to neonatal or adult BALB/C mice, SCID mice, common voles, bank voles, common field mice, desert gerbils, guinea pigs, rabbits, lambs, or goats; only cattle, Mongolian gerbils and southern multimammate mice became infected (Anderson 1991; Enemark et al. 2002; Koudela et al. 1998; Kvac et al. 2007). In contrast, bovine *C. andersoni* isolates in Japan were infectious to SCID mice, thus was named as a novel type of *C. andersoni* (Matsubayashi et al. 2004; Koyama et al. 2005; Satoh et al. 2003).

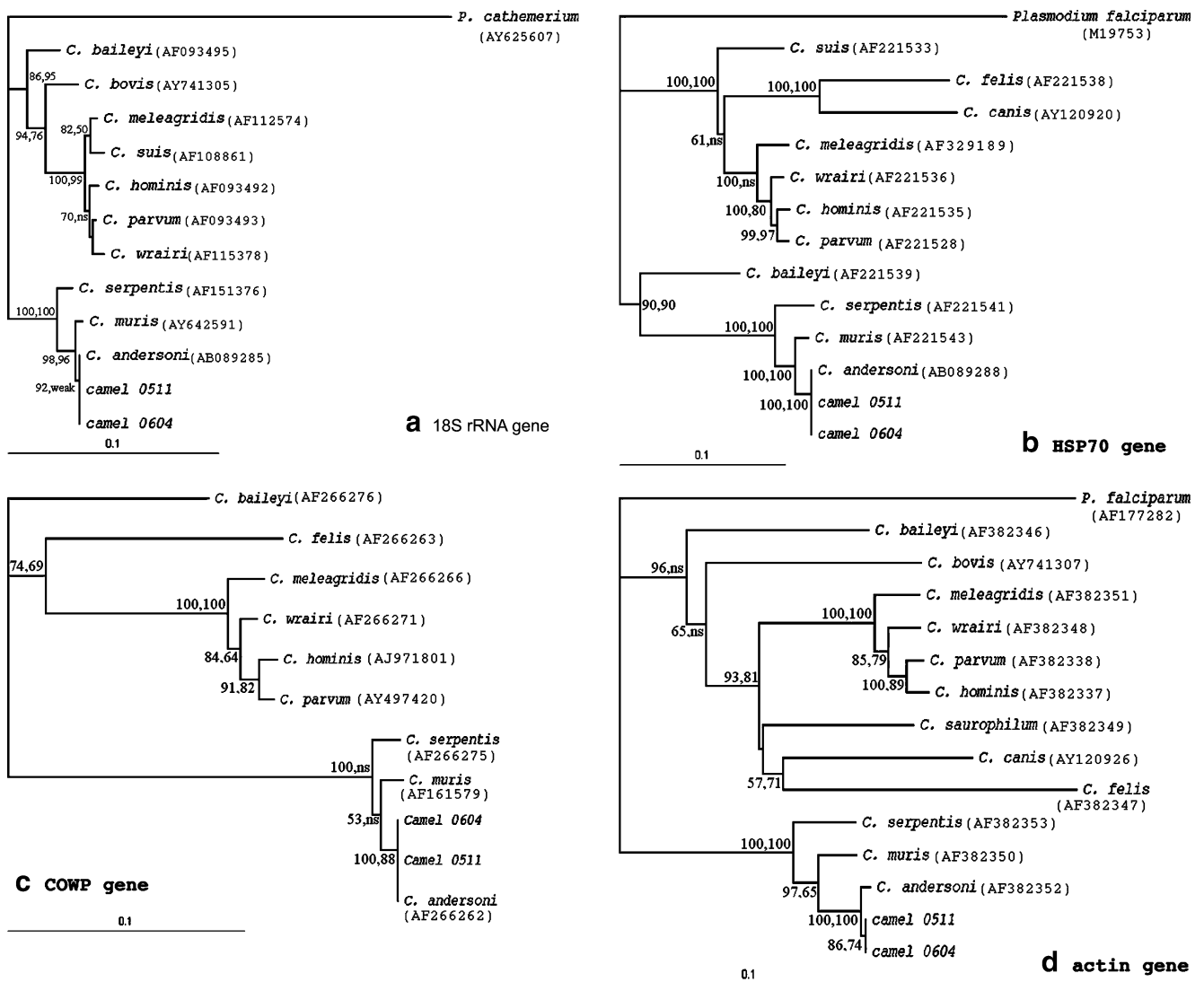


Fig. 2 Phylogenetic relationship of *Cryptosporidium* parasites inferred by neighbor-joining analysis of the 18S rRNA (a), HSP70 (b), COWP (c), and actin genes (d) based on Kimura 2-parameter model. Bootstrap values (in percentage) above 50% from 1,000 pseudo-replicates are shown for both the neighbor-joining (the first value) and

maximum parsimony analyses (the second value). *Weak* Node supported by method but very weak (<50%), *ns* node not supported by method. *Scale bar* indicates an evolutionary distance of 0.1 substitutions per site in the sequence

Table 2 Morphometric measurements (micrometers) of two *C. andersoni* isolates from a bactrian camel

Parameter	Isolate 0511 (n=50)		Isolate 0604 (n=50)	
	Mean	95% confidence limits	Mean	95% confidence limits
Length	7.0	6.2–7.7	7.2	6.2–7.7
Width	5.1	4.6–5.4	5.2	4.9–5.4
Shape index	1.37	1.20–1.50	1.39	1.19–1.50

n Number of oocysts measured

In this study, *C. andersoni* derived from a bactrian camel was not infectious to immunosuppressed or immunocompetent Kun-ming mice, SCID mice, immunosuppressed or immunocompetent calves. Such results were similar to other *C. andersoni* isolates from Europe and America, although there were a few nucleotide differences between the isolate in this study and the USA isolate (Fig. 1b) at the actin locus. In contrast, the results were different from the *C. andersoni* isolates from cattle in Japan in that the latter could infect SCID mice. Interestingly, there were no sequence differences among these isolates, Europe, America, and Japan isolates at 18S rRNA, HSP70, and COWP loci. On the other hand, the transmission study in Australia was unavailable, although some nucleotide differences were found between the isolates in this study and the Australia

isolates in the HSP70 gene (Fig. 1a). The reason for the different transmission behavior is therefore unknown.

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References

- Anderson BC (1991) Prevalence of *Cryptosporidium muris*-like oocysts among cattle populations of the United States: preliminary report. *J Protozool* 38:14s–15s
- Arrowood MJ, Sterling CR (1987) Isolation of *Cryptosporidium* oocysts and sporozoites using discontinuous sucrose and isopycnic percoll gradients. *J Parasitol* 73:314–319
- Arrowood MJ, Donaldson K (1996) Improved purification methods for calf-derived *Cryptosporidium parvum* oocysts using discontinuous sucrose and cesium chloride gradients. *J Eukaryot Microbiol* 43:89
- Efron B, Halloran E, Holmes S (1996) Bootstrap confidence levels for phylogenetic trees. *Proc Natl Acad Sci USA* 93:13429–13434
- Enemark HL, Ahrens P, Lowery CJ, Thamsborg SM, Enemark JM, Bille-Hansen V, Lind P (2002) *Cryptosporidium andersoni* from a Danish cattle herd: identification and preliminary characterization. *Vet Parasitol* 107:37–49
- Fayer R, Santin M, Trout JM, Greiner E (2006) Prevalence of species and genotypes of *Cryptosporidium* found in 1–2-year-old dairy cattle in the eastern United States. *Vet Parasitol* 135:105–112
- Felsenstein J (1989) PHYLIP: Phylogeny Inference Package (Version 3.2). *Cladistics* 5:164–166
- Koudela B, Modry D, Vitovec J (1998) Infectivity of *cryptosporidium muris* isolates from cattle. *Vet Parasitol* 76:181–188
- Koyama Y, Satoh M, Maekawa K, Hikosaka K, Nakai Y (2005) Isolation of *Cryptosporidium andersoni* Kawatabi type in a slaughterhouse in the northern island of Japan. *Vet Parasitol* 130:323–326
- Kvac M, Vitovec J (2003) Prevalence and pathogenicity of *Cryptosporidium andersoni* in one herd of beef cattle. *J Vet Med B Infect Dis Vet Public Health* 50:451–457
- Kvac M, Ditrich O, Kouba M, Sak B, Vitovec J, Kvetonova D (2004) Failed attempt of *Cryptosporidium andersoni* infection in lambs. *Folia Parasitol (Praha)* 51:373–374
- Kvac M, Kouba M, Vitovec J (2006) Age-related and housing-dependence of *Cryptosporidium* infection of calves from dairy and beef herds in South Bohemia, Czech Republic. *Vet Parasitol* 137:202–209
- Kvac M, Ondrackova Z, Kvetonova D, Sak B, Vitovec J (2007) Infectivity and pathogenicity of *Cryptosporidium andersoni* to a novel host, southern multimammate mouse (*Mastomys coucha*). *Vet Parasitol* 143:229–233
- Lindsay DS, Upton SJ, Owens DS, Morgan UM, Mead JR, Blagburn BL (2000) *Cryptosporidium andersoni* n. sp. (Apicomplexa: Cryptosporiidae) from cattle, *Bos taurus*. *J Eukaryot Microbiol* 47:91–95
- Masuno K, Yanai T, Hirata A, Yonemaru K, Sakai H, Satoh M, Masegi T, Nakai Y (2006) Morphological and immunohistochemical features of *Cryptosporidium andersoni* in cattle. *Vet Pathol* 43:202–207
- Matsubayashi M, Kimata I, Abe N, Tani H, Sasai K (2004) The detection of a novel type of *Cryptosporidium andersoni* oocyst in cattle in Japan. *Parasitol Res* 93:504–506
- Matsubayashi M, Kimata I, Iseki M, Hajiri T, Tani H, Sasai K, Baba E (2005) Infectivity of a novel type of *Cryptosporidium andersoni* to laboratory mice. *Vet Parasitol* 129:165–168
- Morgan UM, Xiao L, Monis P, Sulaiman I, Pavlasek I, Blagburn B, Olson M, Upton SJ, Khramtsov NV, Lal A, Elliot A, Thompson RC (2000) Molecular and phylogenetic analysis of *Cryptosporidium muris* from various hosts. *Parasitol* 120:457–464
- Ng J, Pavlasek I, Ryan U (2006) Identification of novel *Cryptosporidium* genotypes from avian hosts. *Appl Environ Microbiol* 72:7548–7553
- Page RDM (1996) TREVIEW: An application to display phylogenetic trees on personal computers. *Comput Appl Biosci* 12:357–358
- Robinson G, Thomas AL, Daniel RG, Hadfield SJ, Elwin K, Chalmers RM (2006) Sample prevalence and molecular characterisation of *Cryptosporidium andersoni* within a dairy herd in the United Kingdom. *Vet Parasitol* 142:163–167
- Satoh M, Hikosaka K, Sasaki T, Suyama Y, Yanai T, Ohta M, Nakai Y (2003) Characteristics of a novel type of bovine *Cryptosporidium andersoni*. *Appl Environ Microbiol* 69:691–692
- Sulaiman IM, Morgan UM, Thompson RC, Lal AA, Xiao L (2000) Phylogenetic relationships of *Cryptosporidium* parasites based on the 70-kilodalton heat shock protein (HSP70) gene. *Appl Environ Microbiol* 66:2385–2391
- Sulaiman IM, Lal AA, Xiao L (2002) Molecular phylogeny and evolutionary relationships of *Cryptosporidium* parasites at the actin locus. *J Parasitol* 88:388–394
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 24:4876–4882
- Xiao L, Escalante L, Yang C, Sulaiman I, Escalante AA, Montali RJ, Fayer R, Lal AA (1999a) Phylogenetic analysis of *Cryptosporidium* parasites based on the small-subunit rRNA gene locus. *Appl Environ Microbiol* 65:1578–1583
- Xiao L, Morgan UM, Limor J, Escalante A, Arrowood M, Shulaw W, Thompson RC, Fayer R, Lal AA (1999b) Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. *Appl Environ Microbiol* 65:3386–3391
- Xiao L, Limor J, Morgan UM, Sulaiman IM, Thompson RC, Lal AA (2000) Sequence differences in the diagnostic target region of the oocyst wall protein gene of *Cryptosporidium* parasites. *Appl Environ Microbiol* 66:5499–5502
- Xiao LH, Fayer R, Ryan U, Upton SJ (2004) *Cryptosporidium* Taxonomy: recent advances and implications for public health. *Clin Microbiol* 17:72–97