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Cryptosporidium sp. *Mrb*001 detected from *Myodes rufocanus bedfordiae,* an indigenous vole of Hokkaido, Japan

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北海道土着のエゾヤチネズミから分離されたクリプトスポリジウム種，*Mrb*001 株

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ABSTRACT

Cryptosporidium oocysts were detected from five of 111 grey red-backed voles (*Myodes rufocanus bedfordiae*) in a forestry area of Hokkaido, which is the northernmost island of Japan and is zoogeographically distinct from the mainland. The number of oocysts per gram of faeces from each vole ranged from 1.5×10^{6} to 1.8×10^{8}. A partial sequence of small subunit 18S rDNA (SSU rDNA) obtained in this study was registered as *Cryptosporidium* sp. *Mrb*001 (GenBank accession no. AB477098) and had the highest similarity to *Cryptosporidium* sp. 05. 1513.Rb (GenBank accession no. HM015880.1), which originated from drinking water in the United Kingdom (98.5%). Phylogenetic tree analysis indicated that *Cryptosporidium* sp. *Mrb*001 clustered with several waterborne *Cryptosporidium* isolates and other *Cryptosporidium* spp. that originated from voles that inhabit wetlands in Hokkaido. We provided information about this cryptosporidial parasite isolated from *Myodes* vole. (139 words)

KEYWORDS: Cryptosporidial parasite, small rodent, wetland, small subunit 18S rDNA

Cryptosporidium are infectious protozoan parasites in the gastrointestinal epithelium of humans and a variety of animals, and they can cause either no symptoms or mild to severe diarrhoea (Horčičková et al. 2018). Some species of cryptosporidial parasites can cause waterborne outbreaks of cryptosporidiosis in industrialised countries (Efstratiou et al. 2017). Host specificity is recognized in cryptosporidial parasites, and voles are reported to be natural hosts and/or reservoirs for at least 20 *Cryptosporidium* spp. and genotypes (Horčičková et al. 2018). Additionally, the vegetation type of areas inhabited by voles influences *Cryptosporidium* prevalence (Torres et al. 2000). The grey red-backed vole, *Myodes* (formerly *Clethrionomys*) *rufocanus bedfordiae,* is a subspecies of *M. rufocanus* found on Hokkaido and adjacent islands of Japan, and the southern Kurile Islands (Kaneko 1998). In this paper, we provided information on a *Cryptosporidium* sp. detected from indigenous voles captured from a natural forestry area in Hokkaido, which is the northernmost main island of Japan and is differentiated from mainland Japan by a zoogeographical border called Blakiston's line (McKay 2012).

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A total of 111 grey red-backed voles were captured at Nopporo Prefectural Forest Park in Hokkaido (latitude: 43°2′45″N, longitude: 141°31′25″E) from 2004 to 2006 with permission from the local government of Hokkaido Prefecture (Ishihara et al. 2011). *Cryptosporidium* oocysts obtained from faecal samples were observed under a light microscope at 400× magnification as previously described (Abe et al. 2002). After counting the oocysts in 100 microscopic fields of each sample, the oocysts per gram of faeces (OPG) were calculated. Cryptosporidial DNA was extracted from an aliquot of an oocyst sample as previously described (Gatei et al. 2002). A partial fragment of the *Cryptosporidium* SSU rDNA was amplified by nested PCR (Xiao et al. 1999). Purification and sequencing of the PCR amplicon were carried out as previously described (Uchida et al. 2014). Similarity to the sequences obtained in this study was analysed using BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis was performed in Clustal W (ver. 2.1, http://clustalw.ddbj.nig.ac.jp/index.php?lang=ja). The obtained neighbour-joining phylogenetic tree was edited using TreeView (ver. 1.6.6, https://treeview.software.informer.com/1.6/) with *Eimeria tenella* (GenBank accession no. AF026388) as an outgroup sequence. This study was carried out according to the guidelines of the Ethics Committee of the Graduate School of Dairy Science, Rakuno Gakuen University.

Five grey red-backed voles (4.5%; 5/111) were positive for the presence of *Cryptosporidium* oocysts. After observation of 100 microscopic fields in each slide, OPG in the voles were 1.5×10^6, 1.1×10^7, 1.2×10^7, 5.5×10^7, and 1.8×10^8 (average, 5.2×10^7).

Out of the 5 grey red-backed voles having oocysts, 2 voles were positive by nested PCR (Fig. 1). On the other hand, the remaining 3 voles were negative. The negative results may be reflected by the preparation of aliquots from the oocyst samples before DNA extraction. A 795-bp partial SSU rDNA sequence was obtained from one of the two PCR products. The SSU rDNA sequence was identified as *Cryptosporidium* by a BLAST search of the GenBank database (*Cryptosporidium* sp. *Mrb*001, GenBank accession no. AB477098). *Cryptosporidium* sp. *Mrb*001 showed the highest similarity to *Cryptosporidium* sp. 05.1513.Rb, which originated from drinking water in the UK and is not infectious to susceptible human hosts (GenBank accession no. HM015880.1; 98.5%, 786/798 bp, including three gaps) (Nichols et al. 2010). Although cryptosporidiosis in humans is mostly caused by *C. parvum* and *C. hominis*, 15 other *Cryptosporidium* species and four genotypes are also associated with human cases, albeit rarely; the species include *C. meleagridis*, *C. ubiquitum*, *C. fayeri*, *C. felis*, *C. suis*, *C. tyzzeri*, *C. muris*, *C. canis*, *C. cuniculus*, *C. andersoni*, *C. viatorum*, *C. scrofarum*, *C. xiaoi*, *C. erinacei*, and *C bovis*, and the genotypes include skunk, chipmunk I, horse, and mink (Xiao and Feng 2017). The *C.* sp. *Mrb*001 sequence did not correspond to any of the species or genotypes that are pathogenic to humans.

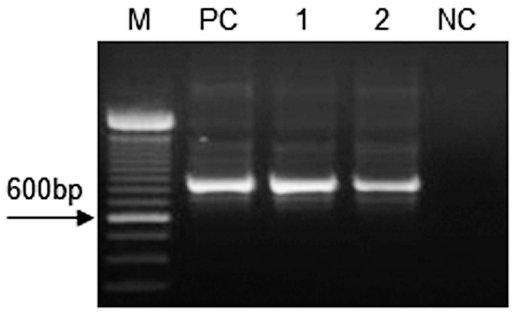

Fig. 1. Partial fragment of the *Cryptosporidium* 18S SSU rRNA gene detected by nested PCR. Amplicons were detected as 850-bp fragments from two grey red-backed voles. Lanes: M, a 100-bp DNA ladder (Invitrogen™; Life technologies, Tokyo, Japan); PC, positive control DNA which was extracted from a *C. parvum* isolate; lanes 1 and 2, genomic DNA samples extracted from the grey red-backed voles; NC, negative control (deionised distilled water). The arrow indicates a 600-bp DNA fragment.

Phylogenetic tree analysis revealed that *C.* sp. *Mrb*001 clustered with isolates that originated from drinking water, and another clade consisted of isolates that originated from *Microtus pennsylvanicus* (meadow vole) and several waterborne isolates (Fig. 2). Both meadow and grey red-backed voles inhabit wetlands such as swamps, bogs, and marshes. It is possible that wetland-inhabiting voles were infected by *Cryptosporidium* spp. that originated in the water systems in which they live.

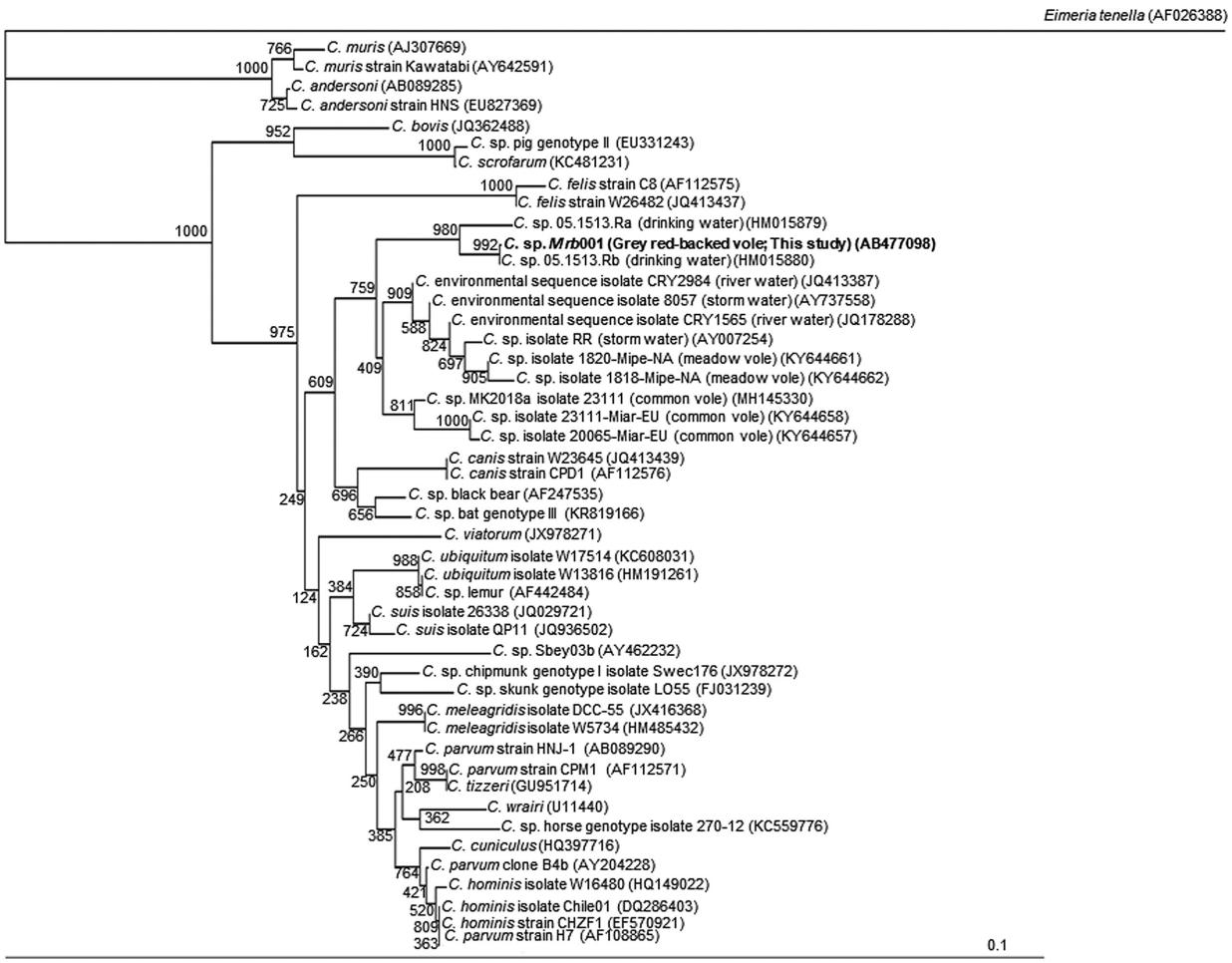

Fig. 2. Phylogenetic analysis on 18S SSU rDNA sequences of *Cryptosporidium* sp. obtained in this study and known *Cryptosporidium* microorganisms. *Cryptosporidium* spp. and genotypes used as references for the phylogenetic tree analysis were as follows (derivations) (GenBank accession nos.): *C. muris*, *C. muris* strain Kawatabi, *C. andersoni*, *C. andersoni* strain HNS, *C. bovis*, *C.* sp. pig genotype II, *C. scrofarum*, *C. felis* strain C8, *C. felis* strain W26482, *C.* sp. 05.1513.Ra, *C.* sp. 05.1513.Rb, *C.* environmental sequence isolate CRY2984, *C.* environmental sequence isolate 8057, *C.* environmental sequence isolate CRY1565, *C.* sp. isolate RR, *C.* sp. isolate 1820-Mipe-NA, *C.* sp. isolate 1818-Mipe-NA, *C.* sp. MK2018a isolate 23111, *C.* sp. isolate 23111-Miar-EU, *C.* sp. isolate 20065-Miar-EU, *C. canis* strain W23645, *C. canis* strain CPD1, *C.* sp. black bear, *C.* sp. bat genotype III, *C. viatorum*, *C. ubiquitum* isolate W17514, *C. ubiquitum* isolate W13816, *C.* sp. lemur, *C. suis* isolate 26338, *C. suis* isolate QP11, *C.* sp. Sbey03b, *C.* sp. chipmunk genotype I isolate Swec176, *C.* sp. skunk genotype isolate LO55, *C. meleagridis* isolate DCC-55, *C. meleagridis* isolate W5734, *C. parvum* strain HNJ-1, *C. parvum* strain CPM1, *C. tyzzeri*, *C. wrairi*, *C.* sp. horse genotype isolate 270–12, *C. cuniculus*, *C. parvum* clone B4b, *C. hominis* isolate W16480, *C. hominis* isolate Chile01, *C. hominis* isolate CHZF1 and *C. parvum* strain H7.

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REFERENCES

Abe N., Kimata I., Iseki M. 2002: Identification of genotypes of *Cryptosporidium parvum* isolates from a patient and dog in Japan. J. Vet. Med. Sci. 64: 165–168.

Efstratiou A., Ongerth J. E., Karanis P. 2017: Waterborne transmission of protozoan parasites: review of worldwide outbreaks - An

update 2011–2016. Water Res. 114: 14–22.

Gatei W., Ashford R.W., Beeching N.J., Kamwati S.K., Greensill J., Hart C. A. 2002: *Cryptosporidium muris* infection in an HIV-infected adult, Kenya. Emerg. Infect. Dis. 8: 204–206.

Horčičková M., Čondlová Š., Holubová N., Sak B., Květoňová D., Hlásková L., Konečný R., Sedláček F., Clark M., Giddings C., McEvoy J., Kváč M. 2018: Diversity of *Cryptosporidium* in common voles and description of *Cryptosporidium alticolis* sp. n. and *Cryptosporidium microti* sp. n. (Apicomplexa: Cryptosporidiidae). Parasitology 146: 220–233.

Ishihara K., Kanamori K., Asai T., Kojima A., Takahashi T., Ueno H., Muramatsu Y., Tamura Y. 2011: Antimicrobial susceptibility of *Escherichia coli* isolates from wild mice in a forest of a natural park in Hokkaido, Japan. J. Vet Med. Sci. 73: 1191–1193.

Kaneko Y., Nakata K., Saitoh T., Stenseth N. C., Bjφrnstad O.N. 1998: The biology of the vole *Clethrionomys rufocanus*: a review. Res. Popul. Ecol. 40: 21–37.

McKay B. D. 2012: A new timeframe for the diversification of Japan's mammals. J. Biogeogr. 39: 1134–1143.

Nichols R.A.B., Connelly L., Sullivan C.B., Smith H.V. 2010: Identification of *Cryptosporidium* species and genotypes in Scottish raw and drinking waters during a one-year monitoring period. Appl. Environ. Microbiol. 76: 5977–5986.

Torres J., Gracenea M., Gómez M.S., Arrizabalaga A., González-Moreno O. 2000: The occurrence of *Cryptosporidium parvum* and *C. muris* in wild rodents and insectivores in Spain. Vet. Parasitol. 92: 253–260.

Uchida L., Heriyanto A., Thongchai C., Hanh T. T., Horiuchi M., Ishihara K., Tamura Y., Muramatsu Y. 2014: Genetic diversity in the prion protein gene (*PRNP*) of domestic cattle and water buffaloes in Vietnam, Indonesia and Thailand. J. Vet. Med. Sci. 76: 1001–1008.

Xiao L., Feng Y. 2017: Molecular epidemiologic tools for waterborne pathogens *Cryptosporidium* spp. and *Giardia duodenalis*. Food Waterborne Parasitol. 8-9: 14–32.

Xiao L., Morgan U. M., Limor J., Escalante A., Arrowood M., Shulaw W., Thompson R. C. A., Fayer R., Lal A.A. 1999: Genetic diversity within *Cryptosporidium parvum* and related *Cryptosporidium* species. Appl. Environ. Microbiol. 65: 3386–3391.