

Group B Betacoronavirus in Rhinolophid Bats, Japan

Jin SUZUKI¹⁾, Ryota SATO²⁾, Tomoya KOBAYASHI¹⁾, Toshiki AOI²⁾ and Ryô HARASAWA^{1)*}

¹⁾Department of Veterinary Medicine, Faculty of Agriculture, Iwate University, Morioka 020–8550, Japan

²⁾Department of Wildlife Management, Faculty of Agriculture, Iwate University, Morioka 020–8550, Japan

(Received 6 January 2014/Accepted 9 May 2014/Published online in J-STAGE 27 May 2014)

ABSTRACT. We report group B *Betacoronavirus* infection in little Japanese horseshoe bats in Iwate prefecture. We then used reverse-transcription PCR to look for the coronavirus RNA-dependent RNA polymerase gene in fecal samples collected from 27 little Japanese horseshoe bats and found eight were provisionally positive. We had a success in the nucleotide sequencing of six of the eight positive samples and compared them with those of authentic coronaviruses. We found that these six samples were positive in coronavirus infection, and they belonged to the group B *Betacoronavirus* by phylogenetic analysis. Virus isolation using the Vero cell culture was unsuccessful. Pathogenic trait of these bat coronaviruses remained unexplored.

KEY WORDS: bat, *Betacoronavirus*, SARS

doi: 10.1292/jvms.14-0012; *J. Vet. Med. Sci.* 76(9): 1267–1269, 2014

Viruses in the genus *Betacoronavirus* include pathogens that cause emerging pandemic diseases, such as severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS), in humans. It has been suggested that bats are major reservoirs for these zoonotic pathogens [9]. This justifies surveillance for coronaviruses (CoVs) in bats throughout the world. In Japan, CoVs belonging to the genus *Alphacoronavirus* have been detected in intestinal and fecal specimens of Eastern bent-winged bats (*Miniopterus fuliginosus*) in Wakayama Prefecture [8]. Here, we report the first demonstration of group B betacoronaviruses in little Japanese horseshoe bats (*Rhinolophus cornutus*) in Iwate Prefecture, Japan.

A total of 38 fecal samples were collected individually from captured insectivorous bats, including 27 little Japanese horseshoe bats (*R. cornutus*), six greater horseshoe bats (*R. ferrumequinum*), four Japanese large-footed bats (*Myotis macrodactylus*) and one Ussuri tube-nosed bat (*Murina ussuriensis*) from 2 separate caves in Morioka, Iwate Prefecture with permission from the local administration, during 2013. We designated specimens as “Og” or “Is” based on the respective originating cave name, Ogayu (39.6019N, 141.2508E) and Isari (39.6771N, 141.0881E), and we also assigned them numbers. The Ogayu cave is an abandoned gold mine called Manju. The Isari cave is located at the Atago shrine, which is approximately 16 km away from the Ogayu cave. Feces were homogenized with sterile phosphate buffered-saline solution containing penicillin, streptomycin and amphotericin B. Viral RNA was extracted from feces homogenates by the single-step guanidium isothiocyanate-

phenol-chloroform method [1]. cDNA synthesis and PCR were performed as described previously [3]. The PCR primers IN-6 and IN-7 were used to amplify the RNA-dependent RNA polymerase (*RdRp*) gene of CoV as described elsewhere [5]. We also performed a second-step PCR using the IN-6 primer and a reverse primer (5'-ATCAGATAGAATCAT-CATAGAGA-3') described previously [2]. PCR products of expected size were obtained in eight of 38 samples, after the second-step PCR. Five of the eight positive samples were derived from bats roosting in the Isari cave, and the rest were from the Ogaya cave. These positive PCR products derived only from the samples of little Japanese horseshoe bats were subjected to nucleotide sequencing as described previously [3]. CoV RNA was not detected in the other three bat species. Virus isolation using the Vero cell culture derived from an African green monkey was unsuccessful, probably due to inappropriate choice of cell culture.

We had a success in the nucleotide sequencing of six of the eight positive samples and have deposited partial nucleotide sequences of the *RdRp* gene to the DNA databases under the accession numbers AB889995 to AB890000. The 2 other samples unsuccessful in nucleotide sequencing were considered to be false-positive in PCR. The six sequences (426 nt) showed more than 98% nt identity to each other. Comparison between the six CoVs from little Japanese horseshoe bats and those from DNA databases revealed that the nucleotide sequences had the highest identity (about 91% nt) to bat SARS CoV strain Rp3 (GenBank accession no. DQ071615), which was isolated from fecal swabs of Pearson's horseshoe bats (*R. pearsonii*) in Guangxi Province, China in 2005 [4]. Nucleotide sequences aligned using Clustal W [10] were analyzed by the neighbor-joining method [7]. The phylogenetic tree indicated that the six CoV sequences detected in little Japanese horseshoe bats have a monophyletic relationship to the SARS CoV in the same lineage of the group B betacoronavirus (Fig. 1). Bat SARS CoV or SARS-like CoV has been detected in rhinolophid bats on the Eurasian continent [4, 6].

Our results indicate that betacoronaviruses closely related

*CORRESPONDENCE TO: HARASAWA, R., The Iwate Research Center for Wildlife Diseases, Morioka 020–0816, Iwate, Japan.
e-mail: harasawa@iwate-u.ac.jp

©2014 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License <<http://creativecommons.org/licenses/by-nc-nd/3.0/>>.

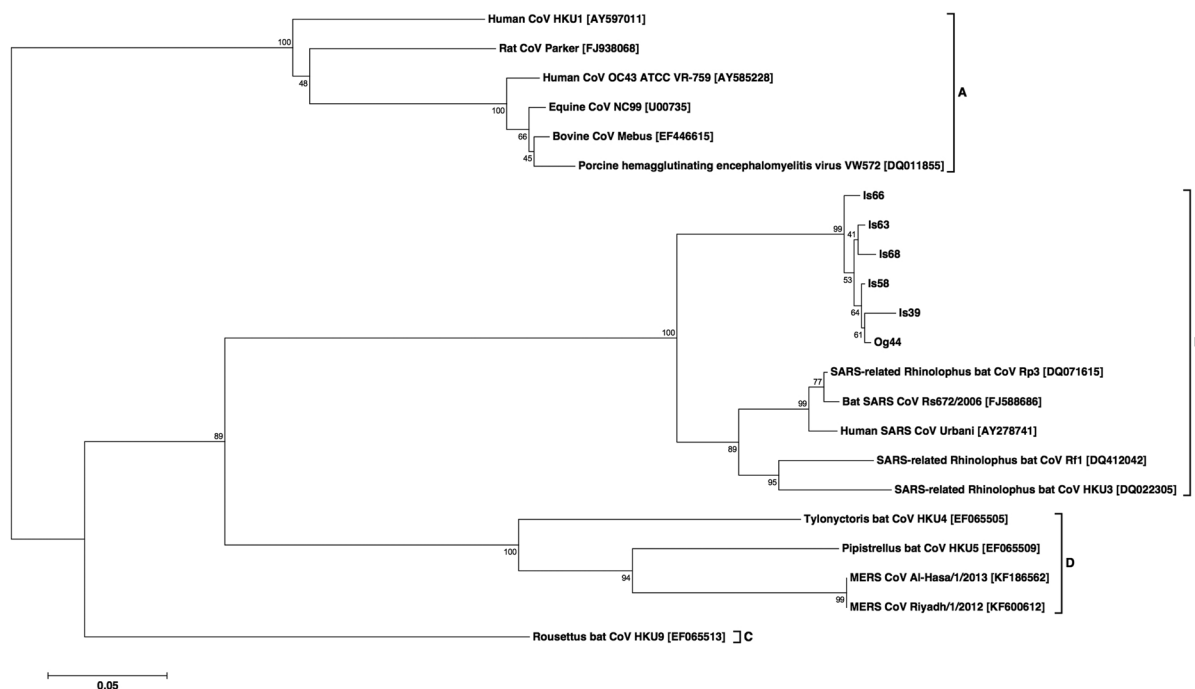


Fig. 1. A phylogenetic analysis of *RdRp* gene sequences among the *Betacoronavirus* strains (accession numbers are given in brackets). Genetic distances were computed with CLUSTAL W. Four groups (A through D) in the genus *Betacoronavirus* are indicated. Bat *Betacoronavirus* strains Is39, Is58, Is63, Is66, Is68 and Og44 detected in Japan were included in the group B betacoronavirus, along with the SARS-related CoV strains. Numbers in the relevant branches refer to the values of bootstrap probability of 1,000 replications. The scale bar indicates the number of nucleotide substitutions per site.

to SARS CoV were prevalent among little Japanese horse-shoe bats in Iwate Prefecture, Japan. Currently, pathogenic trait of these betacoronaviruses is unknown. Although there is no evidence of human infection by these bat CoVs, the existence of group B betacoronaviruses in bats may be predictive of the emergence of a disease, such as SARS, since the Isari cave is also used as a den for civets, which is another potential intermediate species. During this study, we marked the bats with aluminum bands to identify them for further investigation of their habitat. This may provide further information, so we can achieve a better understanding of the relationship between rhinolophid bat behavior and betacoronavirus ecology in the future.

ACKNOWLEDGMENT. We thank Mitsuru Mukoyama of the NPO Bat Conservation Society of Japan for his support in collecting bat samples and identifying bat species.

REFERENCES

- Chomczynski, P. and Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**: 156–159. [[Medline](#)] [[CrossRef](#)]
- Chu, D. K. W., Poon, L. L. M., Chan, K. H., Chen, H., Guan, Y., Yuen, Y. and Peiris, J. S. 2006. Coronaviruses in bent-winged bats (*Miniopterus* spp.). *J. Gen. Virol.* **87**: 2461–2466. [[Medline](#)] [[CrossRef](#)]
- Harasawa, R., Giangaspero, M., Ibata, G. and Paton, D. J. 2000. Giraffe strain of pestivirus: Its taxonomic status based on the 5'-untranslated region. *Microbiol. Immunol.* **44**: 915–921. [[Medline](#)] [[CrossRef](#)]
- Li, W., Shi, Z., Yu, M., Ren, W., Smith, C., Epstein, J. H., Wang, H., Crameri, G., Hu, Z., Zhang, H., Zhang, J., McEachem, J., Field, H., Daszak, P., Eaton, B. T., Zhang, S. and Wang, L. F. 2005. Bats are natural reservoirs of SARS-like coronaviruses. *Science* **310**: 676–679. [[Medline](#)] [[CrossRef](#)]
- Poon, L. L. M., Chu, D. K., Chan, W. K. H., Wong, O. K., Ellis, T. M., Leung, Y. H., Laaua, S. K., Woo, P. C., Suen, K. Y., Yuen, K. Y., Guan, Y. and Peiris, J. S. 2005. Identification of a novel coronavirus in bats. *J. Virol.* **79**: 2001–2009. [[Medline](#)] [[CrossRef](#)]
- Rihtaric, D., Hostnik, P., Steyer, A., Grom, J. and Toplak, I. 2010. Identification of SARS-like coronaviruses in horseshoe bats (*Rhinolophus hipposideros*) in Slovenia. *Arch. Virol.* **155**: 507–514. [[Medline](#)] [[CrossRef](#)]
- Saitou, N. and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* **4**: 406–425. [[Medline](#)]
- Shirato, K., Maeda, K., Tsuda, S., Suzuki, K., Watanabe, S., Shimoda, H., Ueda, N., Iha, K., Taniguchi, S., Kyuwa, S., Endoh, D., Matsuyama, S., Kurane, J., Saijo, M., Morikawa, S., Yoshikawa, Y., Akashi, H. and Mizutani, T. 2012. Detection of bat coronaviruses from *Miniopterus fuliginosus* in Japan. *Virus Genes* **44**: 40–44. [[Medline](#)] [[CrossRef](#)]

9. Tang, X. C., Zahng, J. X., Zhang, Y. S., Wang, P., Fan, X. H., Li, L. F., Li, G., Dong, B. Q., Liu, W., Cheung, C. L., Xu, K. M., Song, W. J., Vilaykrishna, D., Poon, L. L., Peiris, J. S., Smith, G. J., Chen, H. and Guan, Y. 2006. Prevalence of genetic diversity of coronaviruses in bats from China. *J. Virol.* **80**: 7481–7490. [\[Medline\]](#) [\[CrossRef\]](#)
10. Thompson, J. D., Higgins, D. G. and Gibson, T. J. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**: 4673–4680. [\[Medline\]](#) [\[CrossRef\]](#)