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著者	Sato Rintaro, Takahashi Tomoya, Numazu Keiji, Wakatsuki Takushi, Morita Yasuhiro, Komura Akimasa, Tada Chika, Fukuda Yasuhiro, Nakai Yutaka
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Phylogenetic Analysis of *E. zuernii* and *E. bovis* with Nuclear 18S rRNA and Mitochondrial CO1 Genes

**Rintaro Sato¹, Tomoya Takahashi², Keiji Numazu², Takushi Wakatsuki³,
Yasuhiro Morita³, Akimasa Komura³, Chika Tada¹,
Yasuhiro Fukuda¹ and Yutaka Nakai¹**

¹Tohoku University, Japan

²NOSAI Miyagi, Japan

³NOSAI Okayama, Japan

Intoroduction

Coccidiosis is an infectious disease caused by intestinal parasites of genus *Eimeria*, and 14 species are registered as bovine parasites in the world. Of these, *E. bovis* and *E. zuernii* have been regarded as highly pathogenic. In our recent epidemiological study, the two pathogenic species were frequently detected from calves without showing any clinical state. In this study, we collected *E. bovis* and *E. zuernii* from calves showing different symptoms and analyzed their genetic relationship and clinical states in their host.

Material and Methods

Fecal samples were collected from calves under 6 months old which showed clinical and subclinical of coccidiosis, and samples were collected in Tsuyama city, Okayama prefecture in 2012 and Osaki city, Miyagi prefecture in 2010-2011. Species of collected and purified oocysts were identified morphologically. Single oocysts identified as *E. bovis* and *E. zuernii* were isolated and transferred into PCR tube. They were treated with freezing and thawing cycle in order to extract their genomic DNA. For genotyping analysis, nuclear 18S rRNA gene and 0.8 kbp of mitochondrial CO1 gene were amplified by nested-multiplex PCR. Nucleotide sequences of obtained PCR products were determined by direct sequencing. To analyze their genetic polymorphisms within the species, we compared molecular phylogenetic relationships in nucleotide sequences.

Results and Discussions

Oocysts of *E. bovis* and *E. zuernii* were collected from 23 calves, 11 of which shed bloody stool, and the rest showed no clinical features. The nested-multiplex PCR was successfully conducted in 40 oocysts, and we determined their nucleotide sequences. According to constructed phylogenetic trees with 18S rRNA gene, each two *Eimeria* species formed highly supported single clusters. Besides, no relationship implying host symptoms was observed in the tree. Similar trend was observed in a tree from CO1 gene. This result suggested that their molecular genetic diversity did not reflect on their pathogenicity and other factors such as infection sites might cause symptoms.