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Molecular Detection of *Theileria* sp. in Wild Chinese Water Deer (*Hydropotes inermis argyropus*)

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ABSTRACT: Two cases of *Theileria* sp. infection in wild Chinese water deer (*Hydropotes inermis argyropus*) were diagnosed by microscopic examination of thin blood smears and by molecular typing. Two wild Chinese water deer were referred to the Veterinary Medical Center of Chungbuk National University in South Korea, presumably the result of traffic accidents. Thin blood smears, which were stained with Giemsa, revealed intraerythrocytic parasites; however, the deer had no remarkable findings on physical examination. Polymerase chain reaction and direct sequencing indicated that the parasite was nearly identical to the unidentified *Theileria* sp. previously found in China. Phylogenetic analysis showed that the *Theileria* sp. identified in these cases are closest to the group of unidentified *Theileria* spp. previously found in China and Japan.

Key words: Chinese water deer, phylogenetic analysis, South Korea, *Theileria* sp.

The genus *Theileria* consists of obligate, intracellular, protozoan parasites that can infect both wild and domestic *Bovidae*, *Cervidae*, and *Giraffidae*, as well as some rodents, throughout much of the world (BurrIDGE, 1975; Fujisaki et al., 1994; Garcia-Sanmartin et al., 2007). These parasites are transmitted by ixodid ticks and have complex life cycles in both vertebrate and invertebrate hosts (Ica et al., 2007). Unlike *Babesia* species which infect only erythrocytes, *Theileria* species can infect leukocytes and erythrocytes (Darja et al., 2008). The main clinical signs shown by infected cattle are fever and chronic anemia (Shiono et al., 2001). Chemotherapeutic agents are available to treat infection with *Theileria* species; however, the treatment does not completely eradicate the infection, leading to the development of carrier states in the hosts (Fujisaki et al., 1994).

In South Korea, theileriosis is one of the more important diseases of grazing cattle (Song and Sang, 2003; Jeong et al., 2005), and *Theileria sergenti* and *Theileria bufelli* infect cattle in South Korea (Choi et al., 1997; Onuma et al., 1998). No published information is available regarding infections of *Theileria* species in wildlife in South Korea.

This report describes two cases of *Theileria* sp. infection in wild Chinese water deer, as identified by microscopic examination of thin blood smears stained with Giemsa and by molecular typing.

In June (case 1) and December (case 2) 2008, two wild Chinese water deer were referred to the Veterinary Medical Center of Chungbuk National University in Cheongju in Chungbuk province (South Korea; 36°41'47"N, 127°39'26"E). The deer were found beside a road at day-break. On physical examination, both deer had many lacerations and ixodid ticks on their bodies. The deer exhibited no response to external stimuli, and the pupils of the eyes were pinpoint with no response to light. Based on the scene and the clinical signs, we presumed the deer suffered head trauma as the result of a traffic accident. The complete blood count (CBC) included no remarkable findings, and the serum biochemistry included elevations in creatine phosphokinase and aspartate aminotransferase. Radiographic examination revealed no abnormalities. Blood smears stained with Giemsa revealed ring-shaped intraerythrocytic parasites, which we identified by molecular typing. Using a Dynabeads® DNA Direct™ Universal kit (Invitrogen Life



FIGURE 1. Maximum parsimony phylogenetic analysis based on the 18S ribosomal RNA gene sequences in the GenBank databases. Asterisks indicate *Theileria* sp. identified in two Chinese water deer. The series of letters and numbers indicate GenBank accession numbers. Sequence alignments were performed using ClustalX v1.8; MEGA4 v4.02 was used for phylogenetic analyses. Bootstrap percentage values are shown on the branches.

Technologies, Inc., Carlsbad, California, USA), the genomic DNA of the parasites was extracted from 10 μ l of whole blood treated with ethylenediaminetetraacetic acid (EDTA). The genomic DNA encoding the small subunit ribosomal RNA gene (18S rDNA) region was amplified using primers RIB-19 and RIB-20, as previously described (Zahler et al., 2000). All ampli-

cons were bidirectionally sequenced using an ABI Prism™ BigDye™ Terminator Cycle Sequencing Ready Reaction kit V.3.1 (PE Applied Biosystems, Foster City, California, USA). When compared with sequences in the GenBank database, the sequence (1615 bp) of the parasite in case 1 was 98% similar, and the sequence (1645 bp) of the parasite in case 2 was 94%

similar, to the *Theileria* sp. sequences identified in China and deposited by the Research Center Borstel in Germany (GenBank accession AY262119 and AY262118, respectively). There was 95% similarity between the two isolated parasites. Thus, we identified the parasites from water deer as *Theileria* sp.

Infections by the genus *Theileria* have been reported in some wild animals, including red deer (*Cervus elaphus*), roe deer (*Capreolus capreolus*), chamois (*Rupicapra rupicapra*; García-Sanmartín et al., 2007), yak (*Bos grunniens*; Hong et al., 2004), and white-tailed deer (*Odocoileus virginianus*; Yabsley et al., 2005). These reports indicate the severity of the clinical signs ranges from severe anemia to abortive infection, depending on the *Theileria* species and the condition of the host. The water deer had no remarkable changes in CBC or serum biochemistry even though parasitemia was present.

Wild Chinese water deer live in hillocks and approach villages in South Korea at night to eat the crops. Ixodid ticks, which can act as vectors for *Theileria* spp., are prevalent in the deer population, regardless of the season. In this study, the ticks were identified as *Haemaphysalis leporispalustris* by molecular typing of the 28S rRNA gene of ticks by using 28SF and 28SR as previously described (Inokuma et al., 2003). In South Korea, this tick is widespread in several wild and domestic animals, including raccoon dog (*Nyctereutes procyonoides*) and domestic dog. Although this tick lives primarily on hares (*Lepus americanus*), it often infests other small mammals, several species of birds, and even humans (Keith and Cary, 1990). This suggests that deer that are infected with this tick and *Theileria* sp. can transmit the infections to domestic animals in South Korea. Additional investigation into the prevalence of *Theileria* spp. in wild Chinese water deer of South Korea is in progress.

Phylogenetic analysis was performed to determine which known *Theileria* species were similar to the unknown species found in water deer. The 18S rRNA gene sequences of the parasites isolated from cases 1 and 2 were deposited in the GenBank database under accession FJ595120 and FJ599640, respectively. Phylogenetic analysis, using the 18S rDNA sequences, revealed that the parasites in cases 1 and 2 were nearly identical to other unidentified *Theileria* species from China and Japan (Fig. 1). Thus, the unidentified *Theileria* species in water deer in South Korea may be a new subtype distributed in Northeast Asia.

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