Molecular phylogenetic studies on clinical bovine piroplasmosis caused by benign *Theileria* in Shaanxi Province, China

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A group of benign *Theileria* species, which are often referred to as *T. orientalis*/*T. buffeli*/*T. sergenti* group, has low pathogenicity in cattle. Herein, we report on *Theileria* spp. in cattle on a farm from China. Based on phylogenetic analysis of the major piroplasm surface protein gene sequences, we detected 6 genotypes that were categorized as Types 1, 2, 3, 4, and 5 as well as an additional Type 9 genotype. The new epidemiological features of the *T. orientalis*/*T. buffeli*/*T. sergenti* parasites in China indicate a greater diversity in the genetics of these species than had been previously thought.

**Keywords:** MPSP gene, *Theileria* spp., genotype, phylogeny

A group of benign *Theileria* species transmitted by *Haemaphysalis* ticks is often referred to as *T. orientalis*/*T. buffeli*/*T. sergenti* group. The parasite’s presence is characterized by anemia, jaundice, depression, abortion, mortality, and the presence of *Theileria* in blood films [4]. Benign *Theileria* group parasites are widespread among cattle in subtropical and temperate zones [6], and the parasite can eventually lead to severe economic losses in endemic areas. For simplicity, *T. orientalis* is used throughout this paper to denote this benign *Theileria* group. Herein, we report on *Theileria* spp. in cattle on a farm in the Shaanxi Province, a region in which disease outbreaks have occurred.

The exact taxonomic status of *T. orientalis* has been debated for many years. The 18S rRNA data have shown that parasites in this group can be divided into at least 8 types, A, B, B1, C, D, E, H, and *T. buffeli* (Warwick). However, the number of identified types is increasing and there is a lack of consensus on their nomenclature [12,13]. Recently, the gene encoding the major piroplasm surface protein (MPSP) was considered a highly useful marker in revealing the phylogeny of *Theileria* parasites [3].

The nomenclature for the benign *Theileria* group has not reached consensus to date. In 1995, Kubota *et al.* [7] divided the group into at least four types, Ikeda (I), Chitose (C), Thai, and Buffeli (B), based on the allelic forms of the MPSP gene. However, Kim *et al.* [6] proposed a different classification approach based on MPSP, in which there were 6 genotypes, designated Types 1 to 6. Subsequently, 5 additional genotypes, including Types 7, 8, and N1 to N3, were added to the list of previously described genotypes [3,5].

*Theileriosis* caused by the benign *Theileria* group is widely reported from countries neighboring China. However, there are few reports on the occurrence of benign *Theileria* in China. Liu *et al.* [8] and He *et al.* [2] have reported that *Theileria* spp. are present in Hubei Province of China. In addition, surveys have revealed that at least five genotypes of *T. orientalis* (Types 2, 3, 6, 7, and 8) exist in China [3].

This study investigated the genetic diversity of the benign *Theileria* parasites in Huanglong county, which is located in the northern part of Shaanxi Province, China, by analyzing blood samples gathered from cattle at a farm where there was an outbreak of clinical piroplasmosis following a severe tick infestation (species unknown) in August 2013. Blood samples were collected from 10 cattle showing appropriate clinical signs: marked pallor in the oral and genital mucosa, anorexia, and fever (rectal temperature with high readings ≤ 42°C). The

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Fig. 1. Giemsa-stained blood smear from an infected calf. The arrows point out different polymorphisms of Theileria orientalis. Scale bar = 10 μm.

Table 1. Comparison of gene types based on 18S and major piroplasm surface protein (MPSP) genes

| Animal No. | 18S gene type/GenBank accession No. | MPSP gene type/GenBank accession No. |
|------------|-------------------------------------|--------------------------------------|
| 1          | –                                   | Type 5/KJ020551                       |
| 3          | –                                   | Type 3 (Buffeli)/KJ020552             |
| 5          | Type B (Ikeda)/KJ020548             | Type 2 (Ikeda)/KJ020553, KJ020549     |
| 6          | Type C (Buffeli)/KJ020546           | Type 3 (Buffeli)/KJ020555, Type 1 (Chitose)/KJ020554 |
| 7          | –                                   | Type 5/KJ020550                       |
| 8          | Type A (Chitose)/KJ020547           | Type 3 (Buffeli)/KJ020556, Type 5/KJ020557 |
| 9          | Type C (Buffeli)/KJ020545           | Type 5/KJ020560                       |
| 10         | Type B (Ikeda)/KJ020544             | Type 3 (Buffeli)/KJ020559, Type 9/KJ020558 |
| 4,045      | –                                   | Type 2 (Ikeda)/KJ020561, Type 4/KJ020562 |

Type 9, not detected.

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Fig. 2. Phylogenetic relationships among *Theileria* isolated in Shaanxi Province, China, based on major piroplasm surface protein (MPSP) gene sequences. The bold font indicates sequences obtained from this study. Bootstrap values are shown as percentages at each node based on 1,000 replicates. Branch lengths correlate to the number of substitutions inferred according to the scale shown.

The epidemiology of theileriosis in China has not been described. Basic information about the *Theileria* spp., such as their life cycles, vectors, modes of transmission, virulences, and host compatibilities require further study. Regardless, the present study demonstrates that infection by *T. orientalis* is a potentially serious problem in Shaanxi Province, China.
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Conflict of Interest

The authors declare no conflicts of interest.

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