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Ribosomal small-subunit RNA gene-sequence analysis of *Theileria lestoquardi* and a *Theileria* species highly pathogenic for small ruminants in China

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Abstract A fatal disease of sheep and goats in the northwestern part of China has been reported to be due to *Theileria lestoquardi* (syn. *T. hirci*). However, some characteristics of the causative agent are not in accordance with attributes ascribed to this parasite. We therefore determined the nucleotide sequence of the small-subunit ribosomal RNA (srRNA) gene of *T. lestoquardi* and the parasite identified in China and compared it with that of other *Theileria* and *Babesia* species. In the inferred phylogenetic tree the srRNA sequence of the Chinese parasite was found to be most closely related to *T. buffeli* and clearly divergent from *T. lestoquardi*, suggesting that it is an as yet unrecognized *Theileria* species. Extensive structural similarities were observed between the srRNA sequences of *T. lestoquardi* and *T. annulata*, revealing a close phylogenetic relationship between these two *Theileria* species. On the basis of the srRNA nucleotide sequence, polymerase chain reaction (PCR) primers were designed that specifically amplified genomic DNA of the Chinese *Theileria* species. These primers may be valuable tools in future epidemiology studies.

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Introduction

Theileria parasites are protozoans which predominantly infect ruminants (Brown et al. 1990). Due to the economic loss they cause, the most prominent representatives are the cattle-infecting species *T. parva* and *T. annulata*, which are the causative agents of East Coast fever and tropical theileriosis, respectively (Mehlhorn et al. 1993). Clinical symptoms of the infection include leukoproliferation in the early stage of the disease, which is later followed by a leukodestructive phase leading to leukopenia and anemia (Irvin and Morrison 1987). More recently, interest has arisen in sheep-infecting *Theileria* parasites (Brown et al. 1998). Several nonpathogenic *Theileria* species of sheep have been described, including *T. ovis*, *T. separata*, and *T. recondita* (Van Vorstenbosch et al. 1978; Uilenberg 1981; Alani and Herbert 1988a, b). The only one considered to be highly pathogenic, *T. lestoquardi*, frequently causes malignant theileriosis of sheep (Friedhoff 1997). The phylogenetic relationship between sheep-infecting *Theileria* species as well as between sheep- and cattle-infecting *Theileria* parasites is not well known. However, *T. lestoquardi* and *T. annulata* exhibit astonishing similarities with regard to serology and morphology (Brown et al. 1998) and during the preparation of this manuscript their close phylogenetic relationship was shown by srRNA gene comparison (Katzer et al. 1998).

Recently a *Theileria* species pathogenic for small ruminants was identified in the northwestern part of China (Jianxun and Yin 1997). The infectious agent was found to be transmitted by the tick *Haemaphysalis qinhaiensis*, and the geographic distribution of the tick is congruent with the distribution of the disease. Depending on the geographic region, the rate of sickness attributable to the Chinese *Theileria* parasite in sheep and goats varies between 18.8% and 65%, whereas the lethality lies within the range of 17.8–75.4%. Altogether, between 3.4% and 47% of sheep in the area investigated die due to the infection. The highest lethality is displayed by lambs and

imported exotic animals (Jianxun and Yin 1997; Yin et al. 1999).

Originally the pathogenic agent was reported as *T. lestoquardi*, yet there are findings indicating that this parasite may be a different *Theileria* species. First, *T. lestoquardi* is transmitted by ticks of the genus *Hyalomma*, not by *Haemaphysalis* (Hooshmand-Rad and Hawa 1973b). Second, in vitro cell culture of *T. lestoquardi* schizonts can be established with relative ease (Hooshmand-Rad and Hawa 1973a), whereas leukocytes could not be transformed by the Chinese parasite (Jianxun and Yin 1997).

For clarification of the identity and phylogenetic classification of the Chinese parasite and for its distinguishment from *T. lestoquardi*, the nucleotide sequence of the small-subunit rRNA (srRNA) gene was analyzed and compared with that of other *Theileria* and *Babesia* species. The deduced srRNA sequence of *T. lestoquardi* enables us to address the question of its phylogenetic relationship to *T. annulata* and the Chinese *Theileria* species. Furthermore, on the basis of the srRNA gene sequence of the Chinese isolate, species-specific polymerase chain reaction (PCR) primers were designed for future epidemiology studies on the distribution of this parasite.

Materials and methods

Parasites and DNA extraction

The *Theileria*-transformed bovine, ovine, and caprine cell lines designated *T. annulata* Ankara 288, *T. annulata* sheep, *T. annulata* goat and *T. parva* Muguga 803 have been characterized by Ahmed et al. (1989), Steuber et al. (1986), and Dobbelaere et al. (1988). Erythrocytes were isolated from venous blood of sheep infected with the Chinese *Theileria* species originating from the Gansu district of China as described elsewhere (Shayan et al. 1998). DNA used as a control in subsequent experiments was isolated from venous blood taken from uninfected sheep and cattle. DNA from cell lines, erythrocytes, and venous blood was isolated using a genomic DNA extraction kit (Qiagen) according to the manufacturer's instructions. Genomic DNA of the *T. lestoquardi*-infected cell line originating from sheep from the geographic area of Fars, Iran, was provided by S. Rahbari. Piroplasm DNA of *T. sergenti* (isolate from Shintoku, Hokkaido, Japan) was kindly provided by M. Onuma.

DNA amplification

PCR amplification was performed in a final volume of 30 µl containing 100 ng of genomic DNA, 10 µ*M* of each primer, PCR buffer (10 m*M* TRIS-HCl, 50 m*M* KCl, 1.5 m*M* $MgCl_2$, 0.01% gelatin), 200 µ*M* of each deoxynucleoside triphosphate (dNTP), and 1.5 U of Taq polymerase in an automatic DNA thermocycler (Eppendorf). The reaction mix was incubated at 94 °C for 3 min, which was followed by 30 incubation cycles carried out at 94 °C for 30 s, at annealing temperature (T_A; see values below for the different primers used) for 30 s, and at 72 °C for 1 min, with a final extension step being carried out at 72 °C for 5 min. The annealing temperature for primer pairs was set as follows for amplification of DNA fragments covering the srRNA gene region of *Theileria* species: 989/990, $T_A = 60$ °C (amplifies an srRNA gene fragment of *Theileria* species; Allsopp et al. 1993); 989/rDNA-AS, $T_A =$ 55 °C (specific for a region of the *Theileria* srRNA gene; Medlin et al. 1988; Allsopp et al. 1993); and rDNA-S/990, $T_A = 55$ °C (specific for a region of the *Theileria* srRNA gene; Medlin et al. 1988; Allsopp et al. 1993). The primer pairs Tc1-S/Tc-AS ($T_A = 57$ °C) and Tc2-S/Tc-AS ($T_A = 57$ °C) were designed on the basis of the deduced Chinese *Theileria* srRNA gene and specifically amplified genomic DNA of this parasite (Tc1-S = TTTCTGAC-CTATCAGCTTGA, Tc2-S = GTAGGGTATTGGCC-TACTGA, Tc-AS = CGT-ACTAAAGATTACCCAGA). Primers Tc1-S, Tc2-S, and Tc-AS bind to highly conserved rDNA regions and were compared against all sequences present in the ARB data base (Ludwig and Strunk 1999). They exhibited at least a one-nucleotide difference from nontarget sequences.

Cloning and sequencing of amplified DNA fragments

PCR products of 989/rDNA-AS and rDNA-S/990 primer pairs, resulting in a 1453- and a 1379-bp fragment, respectively, were directly ligated, transformed, and cloned using the TA cloning kit according to the manufacturer's instructions (Invitrogen). Sequencing of qualified clones was performed according to standard procedures on an automatic sequencer (Applied Biosystems). Coding and noncoding strands of at least four clones obtained in three independent PCR reactions were determined. The nucleotide sequence data determined for *T. lestoquardi* srRNA and for the srRNA of the Chinese *Theileria* species are available in the EMBL, GenBank, and DDJB data bases under the accession numbers AF081135 and AF081136, respectively.

Sequence alignment and analysis

The srRNA nucleotide sequences of the Chinese *Theileria* species (AF081136), *T. lestoquardi* (AF081135), some *Babesia* spp., and all *Theileria* species (srRNA gene sequences accessible in the GenBank data base) were inserted into an alignment of more than 10,000 homologous sequences in the ARB data base according to primary and higher-order structural similarities. The alignment was done and optimized using the respective software tools of the ARB program package (Ludwig and Strunk 1999). Distance-matrix, maximum-parsimony, and maximum-likelihood methods were applied for phylogenetic analyses and tree creation as implemented in the ARB software package. Different data sets varying with respect to included outgroup reference organisms (sequences) as well as alignment positions were analyzed. By inclusion or exclusion of alignment positions according to their degrees of conservation, their influence on the topology of the trees was evaluated and minimized (Neefs et al. 1990; Ludwig et al. 1998).

Results

Sequence alignment and phylogenetic analysis

The sequences of the srRNA gene of *Theileria lestoquardi* and the Chinese *Theileria* parasite were determined from the overlapping contiguous sequences of two PCR fragments generated by amplification with the primer pairs 989/rDNA-S and rDNA-AS/990 (data not shown). Only srRNA amplification products of *Theileria* species were generated because primers 989 and 990 exclusively bind sequence motifs of *Theileria*-srRNA target sequences and do not bind those of host cells. Besides the binding site of primers rDNA-S and rDNA-AS at the 5′- and 3′-terminal constant regions of the srRNA gene, the whole sequence could be determined.

All tree analyses using the aligned srRNA sequences as specified in Materials and methods supported a monophyletic *Theileria* group, including the Chinese *Theileria* parasite (Fig. 1). Identity values obtained after the establishment of a similarity matrix (data not shown) ranged from 96.5% to 99.7% between *Theileria* species, whereas the identity between *Babesia* and *Theileria* species never exceeded 91%. In the inferred phylogenetic tree the branch of *Theileria* sequences split into two monophyletic clades. One of these clades contained *T. annulata*, *T. parva*, *T. taurotragi*, and *T. lestoquardi* rRNA gene sequences, with the srRNA gene sequence of *T. lestoquardi* being most closely related to *T. annulata* (identity 99.7%). This was the highest degree of identity determined and was followed by an identity of 99.0% with the *T. parva* sequence. The *T. lestoquardi* sequence had an identity of 96.5% with the Chinese *Theileria* species. This was the lowest degree of identity determined between the *Theileria* species examined in this study. The other clade included *T. sergenti*, *T. buffeli*, and the Chinese *Theileria* isolate, with the latter two being most closely related. The Chinese *Theileria* parasite exhibited an identity of 97.4% with *T. buffeli*, followed by 97.1% identity with *T. sergenti*. The lowest identity values were observed for the sequence of *T. lestoquardi* (96.5%), *T. annulata*, and *T. taurotragi* (96.6%), respectively.

Structural analysis of srRNA sequences

Comparison of the srRNA gene sequences of the Chinese *Theileria* species and of *T. lestoquardi* with those of *Babesia sensu strictu*, *Babesia sensu latu*, and other *Theileria* species was done. A 2-nucleotide deletion at position 333–334 (Fig. 2A; this position and the following positions are given according to Allsopp et al. 1994) is present in the Chinese *Theileria* srRNA and is shared only with *Babesia sensu strictu* species (Allsopp et al. 1994). In contrast, a 4-nucleotide deletion at position 2016–2019 was observed in both the Chinese *Theileria* parasite and the *T. lestoquardi* srRNA gene (Fig. 2B). The 4-nucleotide position was restricted to *Theileria* species and was not present in *Babesia* parasites. Both the 2- and the 4- nucleotide deletion were not existent in *Babesia sensu latu* parasites. Additionally, two nucleotide patterns located proximal to the 4-nucleotide deletion, one at position 2008 ("T") and the other at position 2022–2023 ("TT"), were observed in the Chinese *Theileria* species. They were shared with most *Babesia* parasites (Fig. 2B). The nucleotide exchange at position 2008 was present in *Babesia sensu strictu* parasites (*B. caballi*, *B. canis*, *B. bigemina*, *B. divergens*) and in *Babesia sensu latu* parasites (*Cytauxzoon felis*, *B. equi*). The sequence motif 2022–2023 was

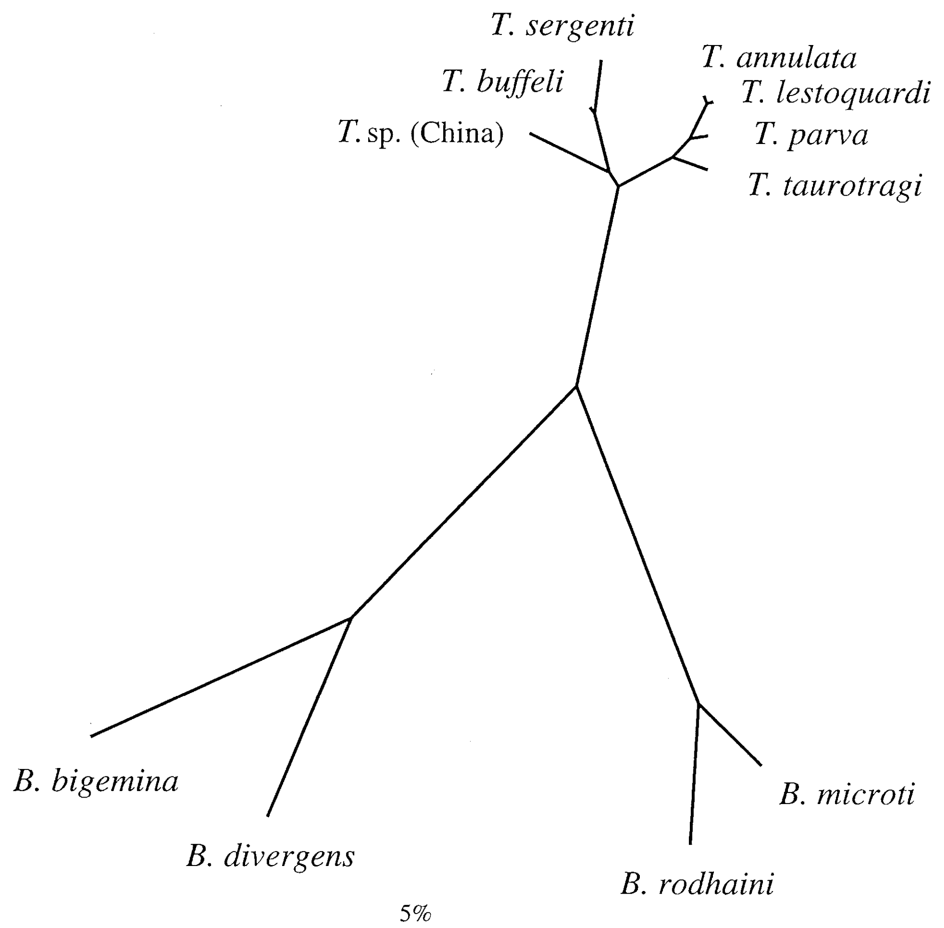

Fig. 1 The tree is based on a maximum-likelihood analysis of srRNA gene sequences from *Theileria* species and a selection of closely as well as moderately related organisms: *T. annulata* (M64243), *T. parva* (AB000271), *T. taurotragi* (L19082), *T. sergenti* (Ab000271), *T. buffeli* (AB000272), *T. lestoquardi* (AF081135), Chinese *Theileria* species (AF081136), *Babesia rodhaini* (M87565), *B. microti* (U09833), *B. bigemina* (X59605), and *B. divergens* (Z48751). The tree topology is supported by the results of distance-matrix and parsimony analysis of all available almost-complete srRNA sequences from *Eucarya*. Only sequence positions that shared invariant residues in at least 50% of all *Theileria* species were included for calculation. The *bar* indicates 5% estimated sequence divergence

Fig. 2A, B Regions of the *Theileria* srRNA alignment of positions 324–342 and 2007–2025 (positions are given according to Allsopp et al. 1994) are shown with the corresponding sequence positions of srRNA sequences of the *Babesia sensu latu* species (Mehlhorn and Schein 1984) *B. equi* (Z15105), *B. microti* (U09833), *B. rodhaini* (M87565), and *Cytauxzoon felis* (L19080); of the *Babesia sensu strictu* species (Mehlhorn and Schein 1984) *B. bigemina* (X59605), *B. bovis* (L19077), *B. caballi* (Z15104), *B. canis* (L19079), and *B. divergens* (Z48751); and of the *Theileria* species *T. annulata* (M64243), *T. buffeli* (AB000272), *T. lestoquardi* (AF081135), *T. parva* (AB000271), *T. sergenti* (Ab000271), *T. taurotragi* (L19082), and Chinese *Theileria* species (AF081136). The alignment shows **A** a 2-nucleotide deletion in *Babesia sensu strictu* species and the Chinese *Theileria* species at position 333–334 and **B** a 4-nucleotide deletion in *Theileria* species at position 2016–2019. Sequence patterns "T" at position 2008 and "TT" at position 2022–2023 are *enclosed in boxes*

	A	B
B. microti	TATCAGCTTTGGACGGTAG	ACGTGGATTCGTCCTTCGT
B. rodhaini	TATCAGCTTTGGACGGTAG	ACTTAGATTCGTCTAAAGT
C. felis	TATCAGCTTTGGACGGTAG	ATCTAA*TTCGTTAGATAC
B. equi	TATCAGCTTTGGACGGTAG	TTCCTT*TTCTTTGGAGAC
T. annulata	TATCAGCTTTGGACGGTAG	TCCCGT*CA****GGGAAC
T. parva	TATCAGCTTTGGACGGTAG	TCCCGA*CA****GGGAAC
T. sergenti	TATCAGCTTTGGACGGTAG	TCCCGT*TA****GGGAAC
T. buffeli	TATCAGCTTTGGACGGTAG	TCCCGT*TA****GGGAAC
T. taurotragi	TATCAGCTTTGGACGGTAG	TCCCGT*CA****GGGAAC
T. lestoquardi	TATCAGCTTTGGACGGTAG	TCCCGT*CA****GGGAAC
T. sp. (China)	TATCAGCTT**GACGGTAG	TTCCGT*AA****GGTTAC
B. caballi	CATCAGCTT**GACGGTAG	TTCCGA*TTCGTCGGTTTT
B. canis	CATCAGCTT**GACGGTAG	TTCCGA*TTCGTCGGTTTT
B. bigemina	CATCAGCTT**GACGGTAG	TTCCGA*TTCGTCGGTTTT
B. divergens	CATCAGCTT**GACGGTAG	TTCCGA*TTCGTCGGCTTG
B. bovis	CATCAGCTT**GACGGTAG	GCGCG***TCGTCGCCGCT

existent in the *Babesia sensu strictu* parasites *B. caballi*, *B. canis*, and *B. bigemina*.

Species-specific amplification of *Theileria* parasites and the Chinese *Theileria* species with oligonucleotide primers

Theileria-specific srDNA primers 989 and 990, which have been described by Allsopp et al. (1993), amplified the expected 1098-bp fragment of genomic DNA of all *Theileria* species examined, including the Chinese *Theileria* species and *T. lestoquardi* (Fig. 3A). The integrity of the isolated genomic DNA employed could thus be confirmed. The specificity for parasitic DNA of this and all other primers was confirmed since neither the use of DNA from cattle nor that of DNA from sheep resulted in the generation of PCR products. Previously published, well-defined *T. annulata*-, *T. parva*-, and *T. sergenti*-specific primers reacted only with their respective DNA and did not react with DNA of *T. lestoquardi* or with that of the Chinese *Theileria* species (data not shown). The properties of these species-specific oligonucleotide primers have been described by Shayan et al. (1998) and Kubota et al. (1994).

On the basis of the srRNA gene sequence of the Chinese *Theileria* species, three specific oligonucleotide primers were deduced. The primer sets Tc1-S/Tc-AS (1180 bp) and Tc2-S/Tc-AS (1198 bp) selectively amplified genomic DNA obtained from the blood of sheep infected with the Chinese isolate (Fig. 3B, C). Neither DNA of *T. lestoquardi*, DNA of any other *Theileria* species tested, nor DNA isolated from uninfected ovine or bovine blood reacted with these primers.

Discussion

The phylogenetic analysis of *Theileria* and *Babesia* srRNA genes clearly demonstrates the affiliation of the Chinese *Theileria* parasite and of *T. lestoquardi* with the genus *Theileria*. This observation is in accordance with our finding that primers amplifying the DNA of different cattle-infecting *Theileria* parasites (*T. annulata*,

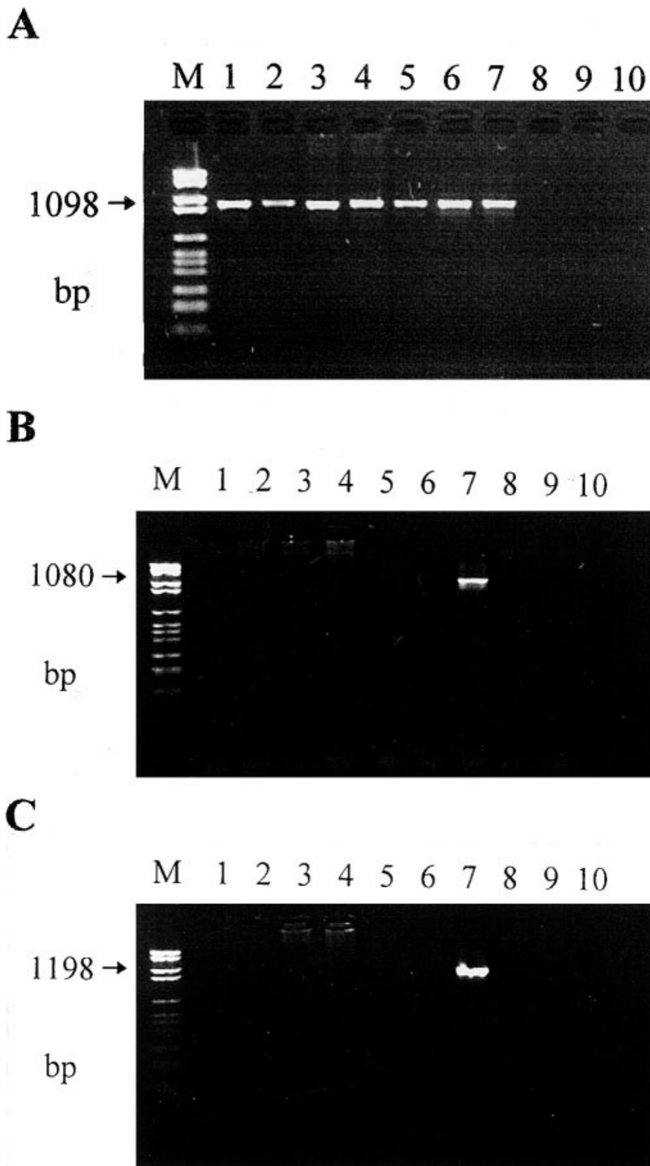

Fig. 3A–C Agarose-gel electrophoresis of amplification products obtained from genomic DNA of different *Theileria* species using *Theileria*-specific primers and primers specific for the Chinese *Theileria* species. Amplification was performed with **A** primer pair 989/990 (positive for *Theileria* parasites), **B** primer pair Tc1-S/Tc-AS, and **C** primer pair Tc2-S/Tc-AS (specific for the Chinese *Theileria* parasite) [*Lane M* Molecular-size markers VI (pBR 328 cleaved with *Bgl*I and *Hin*fI), *lane 1 T. annulata* (cattle), *lane 2 T. annulata* (goat), *lane 3 T. annulata* (sheep), *lane 4 T. parva* (cattle), *lane 5 T. sergenti* (cattle), *lane 6 T. lestoquardi* (sheep), *lane 7 Theileria* isolate from China (sheep), *lane 8* PBL (cattle), *lane 9* PBL (sheep), *lane 10* negative control]

T. parva, *T. sergenti*, *T. taurotragi*, *T. buffeli*, *T. mutans*, and *T. velifera*; Allsopp et al. 1993; d'Olivera et al. 1995) reacted with DNA of the Chinese isolate as well as with that of *T. lestoquardi*. It is noteworthy that these primer pairs did not amplify DNA of the *Babesia* parasites *B. bigemina* or *B. bovis*. The phylogenetic tree established for *Theileria* srRNA genes splits into two monophyletic branches, one branch comprising *T. annulata*, *T. parva*, *T. taurotragi*, and *T. lestoquardi*, with the latter being most closely related to *T. annulata*, and the other branch including *T. sergenti*, *T. buffeli*, and the Chinese isolate. Thus, apart from other attributes of the Chinese *Theileria* parasite (no transformation of leukocytes, different tick vector), the phylogenetic analysis presented evidence that it cannot be characterized as *T. lestoquardi*. Instead, it seems to be a distinct *Theileria* species, being most closely related to *T. buffeli* and next most closely related to *T. sergenti*. It should be noted in this context that *T. buffeli* and *T. sergenti* cannot presently be subspecified and may be regarded as variants of a single species (*T. buffeli*/*T. sergenti* group; Katzer et al. 1998). The group consisting of *T. annulata*, *T. lestoquardi*, *T. parva*, and *T. taurotragi* represents *Theileria* parasites with a marked intraleukocytic phase, in contrast to the group formed by *T. sergenti* and *T. buffeli* (Allsopp et al. 1994). The Chinese *Theileria* species segregated into the latter group, suggesting that it also exhibits a less marked leukocytic phase. This would be in accordance with the observation that the Chinese *Theileria* species, like *T. sergenti*/*T. buffeli*, cannot transform cells in vitro (Yin et al. 1999).

To date, only *T. lestoquardi* has been considered to be highly pathogenic for small ruminants (Hooshmand-Rad and Hawa 1973a). Although we did not include *T. ovis* in our study, the Chinese isolate does not seem to belong to this species, since *T. ovis* is nonpathogenic and is transmitted by *Rhipicephalus* tick species (Uilenberg 1981). For several reasons the Chinese parasite does not seem to be identical to two other sheep-infecting *Theileria* parasites, namely, *T. separata,* which is distributed in some areas of eastern and southern Africa, and *T. recondita*, which has been reported in Wales and Turkey (Uilenberg 1981; Alani and Herbert 1988a, b). Both *T. separata*, which is transmitted by *Rhipicephalus* sp. ticks, and *T. recondita*, which is transmitted by *Haemaphysalis punctata* ticks, are nonpathogenic and exhibit a rather limited geographic distribution. Therefore, it can be assumed that, distinct from *T. lestoquardi*, the Chinese *Theileria* parasite is an additional sheep-infecting *Theileria* species capable of causing theileriosis in small ruminants. Although the sheep-pathogenic parasite *T. lestoquardi* has not yet been described in China, its occurrence cannot be excluded. The primer pairs described in the present paper, together with a recently developed *T. lestoquardi* species-specific primer pair, would be a useful tool for epidemiology studies (Kirvar et al. 1998). They would allow a precise discrimination between the two sheep-pathogenic *Theileria*, *T. lestoquardi* and the Chinese *Theileria* species, if both of them indeed occur in China.

Recently, sequence heterogeneity in the V4 region of srRNA within single isolates of bovine-infecting *Theileria* parasites (*T. buffeli*) was observed (Chae et al. 1998). It was supposed that different srRNA genotypes represented either multiple species (subspecies) or genetic variation within a species. We did not observe heterogeneity of the srRNA gene sequences determined

for the *Theileria* isolate from China and the *T. lestoquardi* isolate, excluding the possibility that these isolates were composed of multiple species and implying the absence of srRNA gene variation. Structural analysis between srRNA gene sequences of *Theileria* and *Babesia* species revealed that the Chinese *Theileria* species displayed a 2-nucleotide deletion at position 333–334, reported only for *Babesia sensu strictu* parasites, and a 4-nucleotide deletion at position 2016–2019, found only in *Theileria* parasites (Allsopp et al. 1994). In the context of the phylogenetic analysis performed and of the simultaneous presence of the 4-nucleotide deletion restricted to *Theileria* species, it becomes evident that the 2-nucleotide deletion is not a quality restricted to *Babesia sensu strictu* parasites. Further comparison of aligned srRNA gene sequences of *Theileria* species with those of the Chinese *Theileria* species revealed some single nucleotide exchanges that were unique to the Chinese *Theileria* parasite and were not present in any of the other *Theileria* sequences (data not shown). As is apparent in the phylogenetic tree, this seems to mirror the relatively early divergence of this parasite from a putative common *T. buffeli*/*T. sergenti* ancestor, resulting in a more prolonged and individual evolution of this *Theileria* parasite.

In accordance with Katzer et al. (1998), the srRNA gene sequence of *T. lestoquardi* was found to be strikingly similar to that of *T. annulata* (identity 99.7%). Not surprisingly, these two parasites have many features in common; they share a similar morphology (Kirvar et al. 1998), they are serologically cross-reactive (Leemans et al. 1997), they display an overlapping geographic distribution (Neitz 1957; Hooshmand-Rad and Hawa 1973a), and they share the same tick vector, *Hyalomma anatolicum anatolicum* (Hooshmand-Rad and Hawa 1973b). All these observations imply a close evolutionary relationship and suggest that *T. annulata* and *T. lestoquardi* evolved very recently from a common ancestor (Katzer et al. 1998). In analogy to *T. lestoquardi* and *T. annulata*, the Chinese *Theileria* species and *T. buffeli*/*T. sergenti* also share a common ancestor. Variation within the *T. buffeli*/*T. sergenti* group seems to be great, improving the likelihood that a suitable variant might cross the host-species border (Chae et al. 1998). Such a variant may be represented by the Chinese parasite. This would be in accordance with the observations that the *T. buffeli*/*T. sergenti* group and the Chinese parasite are transmitted by ticks of the same genus (*Haemaphysalis* sp.) and that their geographic distribution overlaps (Jianxun and Wenshun 1997). A host change by *Theileria* parasites, then, must have occurred in at least two independent evolutionary events. It follows that the two sheep-infecting *Theileria* species, *T. lestoquardi* and the Chinese *Theileria* parasite, do not represent a monophyletic group. Accordingly, the identity between their srRNA genes was the lowest observed in a comparison of different *Theileria* species (96.5%).

In summary, our results indicate that the Chinese *Theileria* parasite does not correspond to *T. lestoquardi* but is an as yet unknown sheep-infecting *Theileria* species that is closely related to *T. buffeli*. srRNA comparison shows that it shares sequence features that have been reported to be unique to *Babesia sensu strictu* parasites. Segregation of the srRNA genes of the *T. lestoquardi* and the Chinese *Theileria* species into two different phylogenetic lineages suggests that at least these two sheep-infecting parasites do not represent a monophyletic group but seem to have evolved independently. The pathogenic mechanism of the Chinese *Theileria* parasite remains to be resolved. In contrast to that of other pathogenic *Theileria* parasites, it does not seem to be based on the capacity to transform leukocytes, suggesting that its potential to cause disease might parallel that of the *T. buffeli*/*T. sergenti* group and/or that of *Babesia* species.

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