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TWO *THEILERIA CERVI* SSU RRNA GENE SEQUENCE TYPES FOUND IN ISOLATES FROM WHITE-TAILED DEER AND ELK IN NORTH AMERICA

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ABSTRACT: Two *Theileria cervi* SSU rRNA gene sequence Types, F and G, from white-tailed deer (*Odocoileus virginianus*) and elk (*Cervus elaphus canadensis*) isolates in North America were confirmed. Previously, nucleotide sequencing through a single variable (V4) region showed the presence of SSU rRNA gene Types F and G in *T. cervi* isolates from white-tailed deer and an elk. In this study, both sequence types were found in four *T. cervi* isolates (two from deer and two from elk). Microheterogeneity only appeared in the Type G gene, resulting in Subtypes G1, G2 and G3. Subtype G1 was found in two elk and one white-tailed deer *T. cervi* isolate; Subtypes G2 and G3 were found in a white-tailed deer *T. cervi* isolate. The Type F SSU rRNA genes were identical in nucleotide sequence in both elk and white-tailed deer *T. cervi* isolates. The high degree of conservation in the Type F variable regions may be exploited to design specific oligonucleotide primers for parasite detection by the polymerase chain reaction in cervine or tick hosts.

Key words: *Cervus elaphus canadensis*, elk, gene amplification, nucleotide sequence, *Odocoileus virginianus,* small subunit ribosomal RNA gene, *Theileria cervi,* white-tailed deer.

INTRODUCTION

Theileria spp., the causative agents of theileriosis, are cosmopolitan hemoprotozoan parasites of domestic and wild mammals. The prevalence of the organism is dependent upon the geographic range of the vector tick. Some *Theileria* spp., such as *Theileria parva* and *Theileria annulata* of cattle in Africa, are highly pathogenic and infections may result in devastating losses. Other less pathogenic species, such as *Theileria sergenti* of cattle in the Middle East, cause mild to moderate clinical theileriosis. In the United States, the *Theileria* spp. infecting domestic and wild ruminants are generally considered benign. Specifically, *Theileria cervi* infections in deer are generally considered nonpathogenic, although clinical disease does occur in hosts debilitated by other parasitic burdens or malnutrition (Kocan and Kocan, 1991).

In 1962 the *Theileria* sp. found in white-tailed deer (*Odocoileus virginianus*) in the United States was designated *Theileria cervi* (Schaeffler, 1961, 1962). *Theileria cervi* has since been identified in white-tailed deer in Texas, Oklahoma, Missouri, Arkansas, and Alabama (reviewed by Kingston, 1981). *Theileria* sp. organisms indistinguishable from *T. cervi* also have been reported in mule deer (*Odocoileus hemionus*) and in exotic axis (*Axis axis*) and sika (*Cervus nippon nippon*) deer in Texas (Laird et al., 1988; Waldrup et al., 1989). The primary tick vector, *Amblyomma americanum,* is widespread throughout the southeastern USA, and *T. cervi* probably occurs throughout the range of the vector tick (Kocan and Kocan, 1991).

Identification of the benign *Theileria* spp. of both deer and cattle in the USA has been primarily based on the mammalian host of origin. Not only are *T. cervi* and the bovine *Theileria* sp. morphologically indistinguishable, but serologic cross-reactions occur and infections with either parasite result in mild or non-existent clin-

TABLE 1. Summary of *Theileria cervi* isolates including year of acquisition, mammalian host source, geographic origin, health status of host regarding clinical signs attributable to *Theileria cervi* infection, Giemsa stained blood film observations, and SSU rRNA gene types identified in each.

Isolate	Year	Source	Clinical signs	Parasite identified by Giemsa stain	Parasite identified by SSU rRNA type
CNELK	1994	Free-ranging elk, Canada	No	*Theileria* sp.	*Theileria cervi* F, G, G1
OKELK1	1998	Free-ranging elk, Oklahoma	No	*Theileria* sp.	*T. cervi* F, G1
USWTD1	1994	Farmed WTD[a], Texas	Yes	*T. cervi*	*T. cervi* F, G, G1
USWTD2	1996	Farmed WTD, Texas	Yes	*T. cervi*	*T. cervi* F
USWTD3	1998	Wild-caught WTD, Oklahoma	No	*T. cervi*	*T. cervi* F, G2, G3

[a] WTD = white-tailed deer.

ical signs in an otherwise healthy host population. Attempts at tick or blood transfer of *T. cervi* from white-tailed deer to domestic ruminants have not been successful (reviewed by Kingston, 1981). To date, similar transfer experiments of the bovine *Theileria* spp. into cervine hosts have not been reported.

Previously, SSU rRNA gene V4 variable region sequence analyses distinguished genes amplified from white-tailed deer and elk *T. cervi* isolates (sequence Types F and G) from genes of bovine *Theileria* spp. isolates (Chae et al., 1998). DNA probes to identify *Theileria* spp. have been designed based on specific SSU rRNA gene sequences (Allsopp et al., 1993). In the present study, entire Type F and G (and variations of G) SSU rRNA genes from white-tailed deer and elk *Theileria* sp. isolates were sequenced to confirm genes or gene regions specific to *T. cervi.*

MATERIALS AND METHODS

A male white-tailed deer housed at Oklahoma State University (Stillwater, Oklahoma, USA) was found by routine examination of a Giemsa-stained blood smear to be infected with *T. cervi* (designated USWTD3). The animal originated from Payne County (35°50′N, 97°00′W), Oklahoma, and was < 1-yr-old when the blood sample was drawn. During a routine epidemiological survey, a 2-yr-old bull elk (*Cervus elaphus canadensis*) residing at the Spavinaw Game Management Area (Spavinaw, Oklahoma; 36°20′N, 95°60′W) was found infected with *Theileria* sp. organisms (OKELK1). Two additional *T. cervi* isolates (USWTD1 and USWTD2) from Texas white-tailed deer and a Canadian elk *Theileria* sp. isolate (CNELK) used in this study have been previously described (Chae et al., 1998). A summary of the *Theileria* spp. isolates used in this study is given in Table 1.

DNA extraction and SSU rRNA gene amplification procedures for the USWTD1, USWTD2 and CNELK isolates have been previously reported (Allsopp et al., 1989; Chae et al., 1998). DNA purification and SSU rRNA gene amplification from the USWTD3 and the OKELK1 *Theileria* isolates were similarly executed. Appropriately sized amplicons were confirmed by electrophoresis in ethidium bromide stained 1% agarose gels which were then viewed by UV transillumination. The amplification product was directly ligated into the plasmid vector pCR® 2.1-TOPO and INVαF′ One Shot™ *Escherichia coli* transformed according to manufacturer's recommendations (TOPO TA Cloning Kit®; InVitrogen, San Diego, California, USA). Transformed clones were color-selected and tested by colony amplification to ensure that the correct-sized insert was present. A portion of an isolated colony for each clone to be tested was mixed into 4 μl of molecular reagent grade water (Sigma®, St. Louis, Missouri, USA) and 2 μl then added to amplification reaction mix to a final volume of 10 μl. Each 10 μl amplification reaction contained 1 pmol each of T7 and M13 reverse primer, 5 mM KCl, 1 mM Tris-HCl, pH 8.3, 1.5 mM $MgCl_2$ and 0.2 U TAQ polymerase. The amplification conditions initiated with denaturation at 96 C for 5 min, followed by 30 cycles of 92 C for 30 sec, 55 C for 30 sec and 72 C for 30 sec and terminated with a final extension at 72 C for 10 min and hold at 4 C until use. The insert amplicons were confirmed by electrophoresis in ethidium bromide stained 1% agarose gels viewed by UV transillumination.

Plasmid DNA for each selected clone was purified from overnight broth cultures by a modified alkaline lysis procedure (QIAprep Spin Miniprep Kit; Qiagen®, Valencia, California, USA). The plasmid DNA samples were then used to identify both Type F and Type G clones for further study. Sequencing reactions for each plasmid DNA sample (Dye Teminator Cycle Sequencing Ready Reaction; PE Applied Biosystem, Norwalk, Connecticut, USA) used primer 528F to determine the SSU rRNA gene V4 variable region nucleotide sequence as previously described (Chae et al., 1998). Sequencing was carried out in either an ABI PRISM Model 373A or ABA Model 377 automated sequencer with Version 1.2.2 or Version 2.1.1 software, respectively (Gene Technologies Laboratory, Institute of Developmental and Molecular Biology, Department of Biology, Texas A&M University, College Station, Texas, USA).

Plasmid clones containing the entire SSU rRNA genes from USWTD1, USWTD2, and CNELK from previous work (Chae et al., 1998) were used for complete gene sequencing for these isolates. Since previously only one plasmid clone containing the WTD2 SSU rRNA gene insert was obtained (Chae et al., 1998), transformation of the original ligated plasmid into INVαF′ One Shot™ *E. coli* was repeated. Putative transformed colonies were selected and tested by colony amplification as described above. Those with the correct-sized insert were then checked by sequence analysis of the SSU rRNA gene V4 variable region.

The complete forward and reverse strands of clones of SSU rRNA gene Type F in USWTD1, USWTD2, and CNELK were sequenced. The Type G genes from CNELK and USWTD1, Subtype G1 (USWTD1 and CNELK) and Subtype G2 genes (USWTD3) also were sequenced. A primer complementary to the T7 promotor region of the plasmid vector (Stratagene®, La Jolla, California, USA) and a series of previously described internal primers (Elwood et al., 1989) were used for automated sequencing reactions and sequencing was carried out as described above. The CLUSTAL W (Version 1.60) multiple sequence alignment program (Thompson et al., 1994) and MACAW multiple alignment construction and analysis workbench (Version 2.05 Win 32I; Schuler et al., 1991) were used to facilitate sequence alignment and comparison. Sequence Types F and G and Subtype G1 also were aligned with corresponding *Theileria* spp. SSU rRNA gene sequences from the GenBank database including *T. buffeli* (GenBank Accession No. Z15106; Allsopp et al., 1994), *Theileria annulata* (GenBank Accession No. M64243, M34845; Gajadhar et al., 1991), *Theileria parva* 18S rRNA gene (GenBank Accession No. L02366; Allsopp et al., 1993), *T. parva* 16S rRNA gene (GenBank Accession No. L28999, Kibe et al., 1994), *Theileria taurotragi* (GenBank Accession No. L19082; Allsopp et al., 1994) and identity values were obtained by the ALIGN program (GeneStream, Centre de Recherche en Biochimie Macromoleculaire, Montpelier, France).

Nucleotide sequence data reported in this paper have been submitted to the GenBank™ data base with the accession numbers U97054, U97055, U97056, AF86804, AF86805.

RESULTS

Amplification with SSU rRNA gene primers A and B resulted in a single band of approximately 1.8 kb for the *Theileria* spp. isolates USWTD3 and OKELK1 as observed by ethidium bromide stained agarose gel electrophoresis (not shown). *Theileria cervi* SSU rRNA gene sequence Types F (GenBank Accession No. U97054) and G Subtypes G1, G2 and G3 (GenBank Accession Nos. U97056, AF086804, and AF086805, respectively) were identified from these isolates (Table 1). Three additional plasmid clones from USWTD2 were identified with the correct-sized insert. These inserts were determined to be Type F by SSU rRNA gene V4 variable region sequence analysis.

Entire SSU rRNA gene sequences for Types F (USWTD1, USWTD2, and CNELK) and G (GenBank Accession No. U97055) (USWTD1 and CNELK) and Subtypes G1 (CNELK and USWTD1) and G2 (USWTD3) were obtained (Fig. 1). Partial gene sequences were obtained for Type F (USWTD3 and OKELK1) and Subtype G3 (USWTD3). USWTD3 and OKELK1 Type F sequences (3 clones checked for each) were identical to the V4 variable region sequences of USWTD1, USWTD2, and CNELK. Subtype G3 was found in three clones from the USWTD3 isolate, but as it differed from G2 only in one nucleotide position (635) in the V4 region (Fig. 1) the entire gene was not sequenced. The *T. cervi* Type F sequence, based on alignment of the V4 variable region, was found in all isolates tested (USWTD1, USWTD2, USWTD3, CNELK and OKELK1); *T. cervi* Type G was found in USWTD1 and CNELK (Table 1). Sub-

```
            1             15 16-180  181            195 196-210 211           225 226           240
T parva     AACCTGGTTGATCCT ........ ATTTGGCGGCGTTTA ........ AACCGCTTG-CGGTG  TCCGGTGATTCATAA
T.cervi
  Type F    AACCTGGTTGATCCT ........ TTCGGGCGGCGTTTA ........ AACCGCTTG-CGTCG  TACGGTGATTCATAA
  Type G    AACCTGGTTGATCCT ........ TTCGGGCGGCGTTTA ........ AACCGCTTG-CGTCG  TACGGTGATTCATAA
  G1        AACCTGGTTGATCCT ........ GGTTGGCTGCGTTTA ........ ATTCGAATGTCGAAA ACCGGTGATTCATAA
  G2        AACCTGGTTGATCCT ........ TTTGGGCTGCGTTTA ........ ATTCGAATGTCGAAC AC -GGTGATTCATAA
  G3        ------------------------------------------------------------------------------------------

            241           255  256            270 271-465 466           480 481-615 616 ⇒      630
T parva     TAAATATGCGAATCG  T- ACTTAG - - - TGCG ........ ACGGGGCTTAAAGTC ........ ATTTCTGCTGCATCG
T cervi
  Type F    TAAACTTGCGAATCG  CGGCTTCGG- CTGCG ........ ACGGGGCTTAATGTC ........ ATTTCTGCTGCTCCG
  Type G    TAAACTTGCGAATCG  CGGCTTCGG- CTGCG ........ ACGGGACGTA -TGTT ........ ATTTCAGCTGCTCCG
  G1        TAAACTTGCGAATCG  CGGCTTAGGGCTGCG ........ ACGGGACGTA -TGTT ........ ATTTCTGCTGCTCCG
  G2        TAAACTTGCGAATCG  TGGCTTAGG -CTGCG ........ ACGGGACGTA -TGTT ........ ATTTCAGCTGCTCCG
  G3        ---------------------------------------------------------------------ATTTCAGCTGCTCCG

            631           645 646          660 ⇐661          675 676           690 691↓        705
T parva     C -TTGT - GTCCCTTC  - -GGGGTCTCTGCA-  TGTGGCTTATTTCGG ACGGAGTTC-GCTTT GTCTGGATGTTTACT
T cervi
  Type F    CATT - - - TTCCCTTT GAGGGGTTTTTGCGC TGTGGCTTATTTCGG ACTGTGTTATGCACT GTCCGGATGTTTACT
  Type G    CACTATCTTCCCGTT ATGGAGGTTTTGCGC TGTGGCTTATTTCGG ACTGTGTTATGCACT GTCCGGATGTTTACT
  G1        CACTATCTTCCCGTT ATGGAGGTTTTGCGC CGTGGCTTATTTCGG ACTGTGTTATGCACT GTCCGGATGTTTACT
  G2        CACTATCTTCCCGTT ATGGAGGTTGTGCGC TGTGGCTTATTTCGG ACTGTGTTATGCACT GTCCGGATGTTTACT
  G3        CACTTTCTTCCCGTT ATGGAGGTTGTGCGC TGTGGCTTATTTCGG ACTGTGTTATGCACT GTCCGGATGTTTACT

          706-35 736            750 751-810 811          825  826           840 841-915 916          930
T parva   ........ TTTGCCTTGAATAGT ........ AATGGTTAATAGGAA CAGTTGGGGGCATTC ........ TTCATTAATCAAGAA
T cervi
  Type F  ........ TTTGCCTTGAATAGT ........ AATGGTTAATAGGAA CAGTTGGGGGCATTC ........ TTCATTAATCAAGAA
  Type G  ........ TTTGCCTTGAATAGT ........ AATGGTTAATAGGAG CAGTTGGGGGCATTC ........ TTCATTAATCAAGAA
  G1      ........ TTTGGCTTGAATAGT ........ AATGGTTAATAGGAG CAGTTGGGGGCATTC ........ TTCATTAATCAAGAA
  G2      ........ TTTGGCTTGAATAGT ........ AATGGTTAATAGGAG CAGTTGGGGGTATTC ........ TTCATTAACCAAGAA
  G3      ........ TTTGGCTTGAATAGT ........ AATGGTTAATAGGAA CAGTTGGGGGCATTC ........ TTCATTAACCAAGAA

       931-75 976            990 991-1035 1036          1050 1051-70 1171          1185 1186          1200
T parva ........ ACCATAAACAATGCC ........ TTGAGAGAAATCAAA ........ TCCAGACAAAGGAAG  GATTGACAGATTGAT
T cervi
 Type F ........ ACCATAAACTATGCC ........ TTGAGAGAAATCAAA ........ TCCAGACAAAGGAAG  GATTGACAGATTGAT
 Type G ........ ACCATAAACTATGCC ........ TTGAGAGAAATCAAA ........ GCAGGACAAAGGAAG GATTGACAGATTGCG
 G1     ........ ACCATAAACTATGCC ........ TTGAGAGAAATCAAA ........ GCAGGACAAAGGAAG GATTGACAGATTGCG
 G2     ........ ACCATAAACTATGCC ........ TTGAGAGAAATCAAA ........ GCAGGACAAAGGAAG GATTGACAGATTGCG
 G3     ........ ACCATAAACTATGCC ........ TTGAGAGAA ----------------------------------------------------

          1201          1215 1216-1305 1306          1320 1321          1335 1336          1350 1351-1365
T parva   AGCTCTTTCTTGATT ........ GGGTACGGGAATAAG  CTCTCGCTGTCCCGT CATCGCTTCTTAGAG  ........
T cervi
  Type F  AGCTCTTTCTTGATT ........ GGGTACGGGAATAGG  CTTCGGCTGTCCCGT GATCGCTTCTTAGAG  ........
  Type G  CGCCCTTTCTTGATT ........ GCTCACGGGAATAGG  TTAAGACCGTCCCCT GGATGCTTCTTAGAG  ........
  G1      CGCCCTTTCTTGATT ........ GCTCACGGGAATAGG  TTAAGACCGTCCCCT GGATGCTTCTTAGAG  ........
  G2      CGCCCTTTCTTGATT ........ GCTCACGGGAATAGG  TTAAGACCGTCCCCT GGATGCTTCTTAGAG  ........
  G3      ---------------------------------------------------------------------------------------

          1366          1380 1381-1470 1471          1485 1486-1500 1501          1515 1516          1530
T parva   AAATCGCAAGGAAGT........ GAGGCCCGGGTAATC  ........ GATGGGGATCGATTA TTGCAATTGTTAATC
T cervi
  Type F  AAATCGCAAGGAAGT........ GAGGCCCGGGTAATC  ........ GATGGGGATCGATTA TTGCAATTATTAATC
  Type G  AAATCGCAAGGAGGT........ GAGGTTTGGGTAATC  ........ GATGGGGATCGATTA TTGCAATTATTAATC
  G1      AAATCGCAAGGAAGT........ GAGGT - -GGGTAATC  ........ GATGGGGATCGAATA TTGCAATTATTAATC
  G2      AAATCGCAAGGAAGT........ GAGGT - -GGGTAATC  ........ GATGGGGATCGAATA TTGCAATTATTAATC
  G3      --------------------------------------------------------------------------------------

          1531-1545   1546          1560   1561-1753
T parva   ........    CATCAGCTTGTGCAG      ........ 1742
T cervi
  Type F  ........    CACCAGCTTGTGCAG      ........ 1748
  Type G  ........    CACCAGCTTGTGCAG      ........ 1750
  G1      ........    CACCAGCTTGTGCAG      ........ 1750
  G2      ........    CACCAGCTTGTGCAG      ........ 1748
  G3      --------------------------------------------
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FIGURE 1. Nucleotide sequence of *Theileria cervi* SSU rRNA gene types and subtypes aligned with the *Theileria parva* SSUrRNA gene nucleotide sequence. Gaps represented by dashes (-) were introduced into the aligned sequences to maximize homology. Nucleotide positions differing from those of *T. parva* are designated by bold type. The V4 variable region is delineated by arrows (nucleotide positions 621–661). Gaps where the sequence is identical in all genes are designated by dotted lines (········). The unsequenced portion of Subtype G3 is shown by a broken line (– – –). The SSU rRNA gene sequence shown for *T. parva* was obtained from the GenBank data base, Accession Number M67476. *Theileria cervi* SSU rRNA gene GenBank Accession Numbers are as follows: Type F, U97054; Type G, U97055; Subtype G1, U97056; Subtype G2, AF86804; Subtype G3, AF86805.

type G1 was found in both deer and elk isolates, USWTD1 and OKELK1. Subtypes G2 and G3 were identified in USWTD3.

The total SSU rRNA gene lengths were: Type F, 1748 bp; Type G, 1750 bp; Subtype G1, 1750 bp; Subtype G2, 1748. G1, G2 and G3 are considered subtypes of

TABLE 2. Number of nucleotide position differences among the *Theileria cervi* SSU rRNA gene types. Top matrix shows differences found in the entire gene sequence; bottom matrix shows differences found only in the V4 variable region of the gene (nucleotide positions 621–661).

	Nucleotide differences among *Theileria cervi* SSU rRNA gene types				
Type	G	G1	G2	G3	F
G	0	23	19	ND[a]	34
G1	2	0	11	ND	45
G2	1	3	0	ND	54
G3	2	4	1	0	ND
F	10	10	11	11	0

[a] ND = not done. Entire *Theileria cervi* G3 SSU rRNA gene not sequenced.

Type G because they differ from G in only one or two nucleotide positions through the V4 region (nucleotide positions 623–663) (Fig. 1 and Table 2) and share sequence identity in nucleotide positions 1171–1174, 1199–1204, 1307–1309, 1319–1328, and 1334–1339 (Fig. 1). In contrast, Type F does not share sequence identity in these positions (Fig. 1) and also differs from Type G in 10 nucleotide positions through the V4 region (Fig. 1 and Table 2).

Differences in the V4 region sequences among the subtypes range from one nucleotide substitution between G and G2, and G2 and G3 to four substitutions between G1 and G3 (Table 2). Subtypes G2 and G3 share substitutions distinguishing them from G1: G2 and G3 have guanine (G) instead of thymidine (T) at position 657; G2 and G3 have cytosine (C) instead of thymidine (T) at 926 (Fig. 1).

Differences found in the entire gene sequences among the various *T. cervi* SSU rRNA genes are summarized in Table 2. Total Type G and Subtype G1 genes showed variation in 23 positions; Type G and Subtype G2 showed variation in 19 positions. Variation was only found in 11 positions between Subtypes G1 and G2. In contrast, differences at 33 positions were noted between Types F and G. No microheterogeneity was found among the Type F sequences.

Identity values determined among the *Theileria* spp. and Types F and G and Subtype G1 showed Type G and Subtype G1 to share the highest identity among the obtained sequences, 98.8% (Table 3). The percent identity between *T. cervi* Type F and Type G was 97.8. When compared to SSU rRNA gene sequences reported for other *Theileria* spp., identity values for Type F ranged from 96.7 to 97.5%, and for Type G from 95 to 95.6%. These percentages reflect 43–57 position differences in Type F and 76–96 position differences in Type G when compared to other *Theileria* spp. (Table 3). *Theileria cervi* SSU rRNA sequence Types F and G (and subtypes) were closest in sequence homology to that of *T. parva* (GenBank Accession Nos. L02366 and L28999).

DISCUSSION

SSU rRNA data indicate that both white-tailed deer and elk harbor *T. cervi,* as supported by this study and previous work (Chae et al., 1998). Complete nucleotide sequences from SSU rRNA genes amplified from cervine blood infected with *Theileria* spp. confirmed that two sequence Types, F and G, were present in the same isolate population. Microheterogeneity in Type G was observed in isolates from two white-tailed deer and two elk, and subtypes G1, G2, and G3 were designated. Considering that Type G SSU rRNA gene heterogeneity was the norm rather than the exception among the small number of isolates in this study, it is likely that additional divergent Type G sequences will be found among other *T. cervi* isolates. Thus, the Type G gene and G subtypes likely comprise a polymorphic family of G SSU rRNA genes.

SSU rRNA Type G or a G subtype gene was not found in one isolate, USWTD2. Amplification of the SSU rRNA gene from this isolate was very difficult and the original ligation and transformation yielded only one plasmid clone with the SSU

TABLE 3. ALIGN program sequence homology of *Theileria* spp. SSU rRNA genes. SSU rRNA gene sequences from the bovine *Theileria* spp., *Theileria annulata, Theileria parva,* and *Theileria taurotragi,* and from the cervine *Theileria* spp., *Theileria* sp. from sable antelope and *Theileria cervi* SSU rRNA gene Types F and G and Subtype G1. Upper matrix shows percent identity between sequences. Lower matrix shows the number of nucleotide differences in bold print and the number of overlapping nucleotides in parentheses.

SSU rRNA[a]	*T. annulata*	*T. parva*	*T. taurotragi*	*Theileria* sp.	*T. buffeli*	*T. cervi*-F	*T. cervi*-G	*T. cervi*-G1
T. annulata	100 (1,744)	98.4	97.8	96.1	96.3	96.8	95.2	94.7
T. parva	**28** (1,744)	100 (1,742)	98.5	96.6	96.6	97.5	95.6	95.2
T. taurotragi	**39** (1,744)	**27** (1,742)	100 (1,737)	96.6	96.4	97.0	95.4	95.0
Theileria sp.	**68** (1,746)	**59** (1,746)	**59** (1,746)	100 (1,746)	96.8	96.7	95.2	94.8
T. buffeli	**65** (1,744)	**59** (1,742)	**62** (1,740)	**55** (1,746)	100 (1,740)	96.9	95.0	94.7
T. cervi-F	**56** (1,748)	**43** (1,748)	**52** (1,748)	**57** (1,748)	**54** (1,748)	100 (1,748)	97.8	96.8
T. cervi-G	**84** (1,750)	**76** (1,750)	**80** (1,750)	**84** (1,750)	**87** (1,750)	**38** (1,750)	100 (1,750)	98.6
T. cervi-G1	**92** (1,750)	**83** (1,750)	**88** (1,750)	**91** (1,750)	**93** (1,750)	**56** (1,750)	**24** (1,750)	100 (1,750)

[a] GenBank Accession Numbers for SSU rRNA gene sequences: *Theileria annulata,* M64243 and M34845; *Theileria parva,* L02366 and L28999; *Theileria taurotragi,* L19082; *Theileria* sp., L19082; *Theileria buffeli,* Z15106; *Theileria cervi* Type F, U97054; *Theileria cervi* Type G, U97055; *Theileria cervi* Subtype G1, U97056.

rRNA gene insert (Chae et al., 1998). In this study, bacterial transformation by the original ligated plasmid preparation was repeated in an effort to obtain additional plasmid clones to sequence. Only three more clones containing the gene were obtained and all three contained inserts of the Type F sequence. Unfortunately, additional DNA was not available to repeat the entire protocol. Clearly, there is a possibility that this isolate possessed other SSU rRNA gene types that we did not find.

The existence of multiple *T. cervi* SSU rRNA gene sequence types may represent genes from mixed populations of parasites or multiple copy units within a parasite as shown in *T. parva* (Kibe et al., 1994). Inas-much as both Types F and G (or G subtypes) were found in all isolates, with the exception of USWTD2, it appears likely that individual *T. cervi* parasites possess both gene types. It is not unlikely that different SSU rRNA genes may be expressed during different developmental stages of the *Theileria* life cycle. Transcript regulation of distinct stage specific SSU rRNAs has been shown in *Plasmodium* spp. (Li et al., 1997; Waters et al., 1989; Corredor and Enea, 1994). Further studies are needed to determine if both gene types are represented in multiple gene clusters in *T. cervi*, whether both types produce functional rRNAs, and whether transcription of different SSU rRNA gene types is stage dependent in *T. cervi.* It is also possible that the microheterogeneity observed in the Type G sequence reflects the presence of pseudogenes.

SSU rRNA Type G microheterogeneity previously reported based on V4 region data (Chae et al., 1998) was confirmed by the present study. Subtype G1 was found in both white-tailed deer and elk isolates, USWTD1, CNELK, and OKELK1. Subtypes G2 and G3, which are nearly identical through the V4 region, were found in a white-tailed deer isolate, USWTD3. Alternatively, no microheterogeneity was found among the Type F SSU rRNA gene sequences regardless of host or geographic origin. Thus, the Type F gene appears highly conserved among the *T. cervi.* Although a single base substitution in the V4 region has been reported (Chae et al., 1998), the Type F genes identified in this study did not show this substitution.

The conservation of the Type F SSU rRNA gene sequence among *T. cervi* isolates provides a base of reference when investigating new isolates and presents an opportunity to develop specific DNA primer sequences which could be used in amplification-based assays for detecting *T. cervi* infections in deer or vector ticks. Such assays could be used to screen ticks for the presence of the parasite to identify potential vectors aside from the known vector, *A. americanum,* although ultimately transmission experiments would be necessary to confirm the ability of a tick species to vector the parasite. Sensitive molecular methods could facilitate epidemiological studies in ticks, deer, and other suspected hosts of the parasite.

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