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A study of the systematics of *Theileria* spp. based upon small-subunit ribosomal RNA gene sequences

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Abstract The systematics of benign and moderately pathogenic *Theileria* isolates from cattle and deer originating from different geographic regions was undertaken by small-subunit ribosomal RNA (SSU rRNA) gene nucleotide-sequence analysis. A maximum-likelihood phylogenetic tree constructed from these sequences resulted in two major divisions, each with a common ancestor. One major division branches into four relatively divergent groups, including (1) bovine *Theileria* sp. Type D (USA and Korea), (2) *T. mutans* Intona and *Theileria* sp. MSD (Africa), (3) *T. cervi* (USA), and (4) well-characterized pathogenic *Theileria* spp. (Africa). The other major division branches into two groups: (1) *T. buffeli* Warwick and *T. buffeli* Marula and (2) a second branch of closely related isolates with SSU rRNA gene Types B, B1, C, E, and H. Putative geographically associated diversity was noted only in the Korean bovine *Theileria* spp. with SSU rRNA gene types C and H and in African *T. mutans* Intona and *Theileria* sp. MSD. The current results show that the United States bovine *Theileria* isolates are not *T. mutans* because they have *T. buffeli* Marula (Type A) and/or Type D (species undesignated) SSU rRNA gene sequences. The taxonomic separation of *T. buffeli* Warwick from African *T. mutans* is confirmed in this study.

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Introduction

Members of the genus *Theileria* are tick-borne piroplasms in the phylum Apicomplexa, which encompasses other diverse hemoprotozoan parasites of vertebrates (Levine 1985, 1988). Some members of this genus are pathogenic and infection results in theileriosis, a disease causing significant economic losses in domestic and wild animals throughout the world. Other members of this genus are considered moderately pathogenic or benign (Brown 1990; Brown et al. 1990). The taxonomic standings of the benign and moderately pathogenic *Theileria* spp. have been difficult to establish due to a number of factors, including: (1) similar morphology among isolates as determined by light microscopy, regardless of the mammalian host species; (2) incomplete life-cycle data [e.g., all of the tick vector(s) may not yet have been identified]; (3) available serological tests may not discriminate individual species due to the occurrence of mixed infections, antigenic variation within a species, and cross-reactions; and (4) difficulty in obtaining pure isolates for studies, especially in the case of benign organisms, where levels of circulating parasitemia may be very low.

Uilenberg et al. (1985) reviewed the status of the relationships among the members of the *T. orientalis/sergenti/buffeli* group and recommended that all three be grouped as *T. orientalis* on the basis of morphology, serology, and vector transmission experiments. However, the bovine *Theileria* sp. widespread in Korea, Japan, and Russia, commonly referenced as *T. sergenti* (Yakimoff and Dekhtereff 1930), is moderately patho-

genic to cattle. Thus, this species is clinically distinct from *T. buffeli* and *T. orientalis*, both of which are generally regarded as benign species (Levine 1985). *Theileria mutans*, too, is usually thought to be benign, although there have been reports of isolates that have been pathogenic (Young et al. 1978). Indeed, Levine (1985) categorizes the latter three species as synonymous. The classification of the moderately pathogenic/benign *Theileria* species is clearly in need of re-evaluation.

One aspect of a species reevaluation must be the renaming of *T. sergenti*, as the specific epithet is preoccupied by a sheep parasite originally named *Babesia sergenti* (Wenyon 1926). Now thought to be synonymous with *T. ovis* (Rodhain 1916), this parasite was meant to belong to the group of small *Babesia*, which now correspond to the accepted definition of *Theileria* (discussed by Morel and Uilenberg 1981; Stewart et al. 1996). As noted by Morel and Uilenberg (1981), "... even if it has no priority, the homonymous name *T. sergenti* Yakimov et Dekhterev, 1930 is preoccupied and cannot serve to designate the benign *Theileria* of cattle in Eurasia."

The objective of the current study was to investigate the systematics of geographically distinct isolates of moderately pathogenic and benign bovine and cervine *Theileria* spp. from Asia, Africa, and North America. The small-subunit ribosomal RNA (SSU rRNA) gene is widely used for characterization, taxonomic classification, and phylogenetic analysis, and the sequences of this gene from a wide variety of different organisms are available in a single data base, the Ribosomal Data-base Project (RDP; Maidak et al. 1997). This is currently the largest known data base for a single gene, containing over 2000 sequences from eukaryotic organisms alone. Consequently, SSU rRNA gene sequence analysis and an inferred phylogenetic tree from these data were used in this study to elucidate the taxonomic relationships among moderately pathogenic or benign bovine and cervine *Theileria* isolates and the well-characterized pathogenic bovine *Theileria* spp.

Materials and methods

The benign or moderately pathogenic *Theileria* isolates used in this study are given in Table 1. Previously described isolates include Korean bovine *Theileria* spp. from Changsu, Chonbuk (KLS), Kimje, Chonbuk (KCB), Kyoungbuk (KKB), Chungnam (KCN), Kangwon (KKW), and Cheju (Jeju) Island (KCJ; Chae et al. 1998a); isolates from the United States – Texas (USET1 and USET2) and North Carolina (USNC) – and Japan – Shintoku, Hokkaido (JHS; Chae et al. 1998a); *Theileria* sp. Fukushima, Japan (Minami et al. 1980); *Theileria* sp. Ikeda, Japan (Minami et al. 1980); *T. cervi* isolates from the United States and Canada (USWTD1, USWTD2, USELK, CNELK; Chae et al. 1998a, 1999a); *T. mutans* Intona, Kenya (Morzaria et al. 1990); *T. buffeli* Warwick, Australia (Stewart et al. 1987) and *Theileria* sp. Essex, England (Morzaria et al. 1974). *Theileria* sp. MSD was obtained from blood collected from cattle at the Merck, Sharp & Dome experimental station near Pretoria, South Africa.

SSU rRNA genes for sequencing were obtained from *T. mutans* Intona, *Theileria* sp. MSD, *Theileria* sp. Fukushima, *Theileria* sp. Warwick, *Theileria* sp. Essex, *Theileria* sp. USET1 (Kuttler and Craig 1975), *Theileria* sp. USET2, and *Theileria* sp. USNC by specific amplification of the gene using primers A and B (Sogin 1990; Chae et al. 1998a). The amplicons were then ligated into appropriate plasmid vectors for sequencing and *Escherichia coli*-transformed. Full-length SSU rRNA gene sequences were obtained from *T. mutans* Intona and *Theileria* sp. MSD as described by Allsopp et al. (1989) ; from *Theileria* sp. Fukushima (JF), *T. buffeli* Warwick, and *Theileria* sp. Essex by the methods of Tanaka et al. (1993); and from previously reported cloned genes from *Theileria* sp. USET1, *Theileria* sp. USET2, and *Theileria* sp. USNC according to described methodology (Chae et al. 1998b, 1999a). SSU rRNA gene sequences for additional species used in the phylogenetic tree construction have previously been reported and are available from the GenBank data base (National Center for Biotechnology Information, National Institutes of Health). Included were SSU rRNA gene sequences for other *Theileria* spp.; the hemoprotozoan parasites *Babesia* spp. and *Cytauxzoon felis*; the protozoan parasites *Toxoplasma gondii* and *Sarcocystis muris*; and the outgroup species *Prorocentrum micans*, *Perkinsus* sp., and *Oxytricha nova*. *O. nova* served to root the tree. GenBank accession numbers for SSU rRNA gene sequences included in the tree are shown in Tables 1 and 2.

The initial basis for alignment of the SSU rRNA gene sequence data was provided by the RDP (Maidak et al. 1997). Introduced sequences were then manually adjusted using the Genetic Data Environment editor (GDE 2.2; Smith 1992). The final alignment included 28 hemoparasite isolates and is available upon request from the corresponding author. Phylogenetic trees were inferred using the maximum-likelihood algorithm implemented in the program fastDNAml (Felsenstein 1981; Olsen et al. 1994). The parameters for the analysis were as follows: rearrangements of partial trees were allowed across 8 branches; rearrangements of a full tree were allowed across 16 branches; empirical base frequencies were used; the transition/transversion ratio was set at 1.5. For confirmation of the species arrangement in the tree the sequence input order was jumbled and six different runs were performed.

The sequences obtained in this study were subjected to BLAST searches (Altschul et al. 1992) by the GenBank data base (National Center for Biotechnology Information, National Institutes of Health).

Table 1 List of *Theileria* isolates from which full-length SSU rRNA genes were sequenced. The SSU rRNA gene type, corresponding GenBank accession number, and country of origin for each isolate are given

Isolate	SSU rRNA gene/GenBank no.	Country of origin
Theileria mutans Intona	*T. mutans*/AF078815	Kenya
Theileria sp. Essex	Identical to *T. buffeli* Warwick/AB000272*	Great Britain
Theileria sp. Fukishima	*Theileria* sp. Type A/AB016074	Japan
Theileria sp. Ikeda	*Theileria* sp. Type B/U97048	Japan
Theileria sp. MSD	*Theileria* sp. MSD/AF078816	South Africa
Theileria sp. USET1	*Theileria* sp. Type A/U97047	United States
Theileria sp. USET2	*Theileria* sp. Type D/U97052	United States
Theileria sp. USNC	*Theileria* sp. Type A/U97047	United States
Theileria sp. Warwick	Identical to *T. buffeli* Warwick/AB000272[a]	Australia

[a] GenBank accession number for *T. buffeli* Warwick SSU rRNA gene sequence submitted by Chansiri K, Kawazu S, KamioT, Uthaisang W, Tananyutthawongse C, and Sarataphan N (unpublished data)

Results

Complete forward- and reverse-strand sequences of SSU rRNA genes were obtained for *Theileria* sp. Warwick, *Theileria* sp. Essex, *Theileria* sp. Ikeda, *Theileria* sp. Fukushima, *Theileria* sp. MSD, *T. mutans* Intona, *Theileria* sp. USET1 (Kuttler and Craig 1975), *Theileria* sp. USET2, and *Theileria* sp. USNC. The complete SSU rRNA gene sequences of the latter six isolates were submitted to the GenBank data base. Corresponding GenBank accession numbers for SSU rRNA sequences and the sequence types obtained are shown in Table 1.

Both *Theileria* sp. Warwick and *Theileria* sp. Essex SSU rRNA genes were 1742 bp in length and shared identical nucleotide sequences. These sequences were also identical to the SSU rRNA gene sequence previously reported for *T. buffeli* Warwick (GenBank accession number AB000272). Hereafter, for the sake of clarity, we shall refer to both of the "Warwick" sequences as *T. buffeli* Warwick.

T. mutans Intona, Kenya (GenBank accession number AF078815) and *Theileria* sp. MSD, South Africa (GenBank accession number AF078816) had SSU rRNA sequences that were distinct from each other and from those of other *Theileria* isolates in this study. Gene lengths for *Theileria* sp. MSD and *T. mutans* Intona were 1734 and 1737 bp, respectively. Differences in 24 nucleotide positions were noted between these 2 isolates; 15 of these were observed in the variable (V4) region between positions 621 and 674. *T. mutans* Intona and *Theileria* sp. MSD share an identity value of 98.6% (ALIGN program GeneStream, Institut de Genetique Humaine, Montpellier, France).

Two *Theileria* SSU rRNA gene sequence types were predominant among the United States bovine isolates. *Theileria* sp. USET2 possessed the Type D SSU rRNA gene sequence (GenBank accession number U97052), which has also been found in bovine *Theileria* isolates and associated ticks in Missouri (Chae et al. 1999b; GenBank accession numbers AF060211–AF060216). *Theileria* sp. USNC and *Theileria* sp. USET1 (Kuttler and Craig 1975) had SSU rRNA gene Type A (GenBank accession number U97047).

SSU rRNA gene sequence Type A was also found in *Theileria* sp. Fukushima (GenBank accession number AB010674). The Type A sequence is identical to that previously reported for *T. buffeli* Marula (GenBank accession number Z15106).

The SSU rRNA gene sequence noted for *Theileria* sp. Ikeda was identical to that observed for Type B (GenBank accession number U97048) and differed in two nucleotide positions from the SSU rRNA gene sequence found for *T. sergenti* Ikeda as reported in the GenBank data base (GenBank accession number AB000271). The North Carolina bovine *Theileria* isolate described above was also found to have SSU rRNA gene Type B1 (GenBank accession number U97049), which differs from Type B in five nucleotide positions (Chae et al. 1998b) and has previously been shown in the Korean isolate *Theileria* sp. KCJ.

Putative geographically associated diversity was noted only in African bovine *T. mutans* Intona and *Theileria* sp. MSD among the isolates sequenced in this study.

A phylogenetic tree of full-length SSU rRNA gene sequences from 28 *Theileria* isolates was inferred by maximum-likelihood analysis (Fig. 1). Because the DNAml algorithm examines a relatively small proportion of all possible trees, there is a very real chance that the search procedure might not find the tree topology with the highest likelihood. Thus, alteration of the order of taxon addition and comparison of the trees found is a fairly efficient method for testing convergence (Olsen et al. 1994). Each of six separate examinations of the data using different input orders resulted in the inference of trees having the same topology.

The DNAml phylogenetic tree based on SSU rRNA gene sequences placed the group of benign and moderately pathogenic *Theileria* spp. infecting cattle and deer into two major divisions, each with a common ancestor. One major division consists of two groups, one being composed of the closely related isolates *T. buffeli*

Table 2 List of species included in the phylogenetic tree constructed from SSU rRNA gene sequences obtained from the GenBank data base. GenBank accession numbers and contributors are given

Species	GenBank no.	Reference
Babesia bigemina	X59604	Reddy et al. 1991
B. bovis	L19077	Allsopp et al. 1994
B. caballi	Z15104	Allsopp et al. 1994
B. canis	L19079	Allsopp et al. 1994
B. divergens	U16370	Holman 1994
B. equi	Z15105	Allsopp et al. 1994
B. microti	U09833	Allsopp et al. 1994
B. odocoilei	U16369	Holman 1994
Babesia sp.	U09834	Allsopp 1994
Cytauxzoon felis	L19080	Allsopp 1994
Oxytricha nova	M14601	Elwood et al. 1985
Perkinsus sp.	X54863	Goggin and Barker 1993
Prorocentrum micans	M14649	Herzog and Maroteaux 1985
Sarcocystis muris	M64244	Gajadhar et al. 1991
Theileria annulata	M64243	Gajadhar et al. 1991
T. buffeli Marula	Z15106	Allsopp et al. 1994
T. cervi Type F	U97054	Chae et al. 1999
T. cervi Type G	U97055	
T. cervi Type G1	U97056	
T. parva	L02366	Allsopp et al. 1993
Theileria sp.Type A	U97047	Chae et al. 1998b
Theileria sp.Type B	U97048	Chae et al. 1998b
Theileria sp.Type B1	U97049	Chae et al. 1998b
Theileria sp.Type C	U97051	Chae et al. 1998b
Theileria sp.Type D	U97052	Chae et al. 1998b
Theileria sp.Type E	U97053	Chae et al. 1998b
Theileria sp.Type H	U97050	Chae et al. 1998b
Theileria sp.	L19081	Allsopp et al. 1994
T. taurotragi	L19082	Allsopp et al. 1994
Toxoplasma gondii	X68523	Long et al., unpublished data

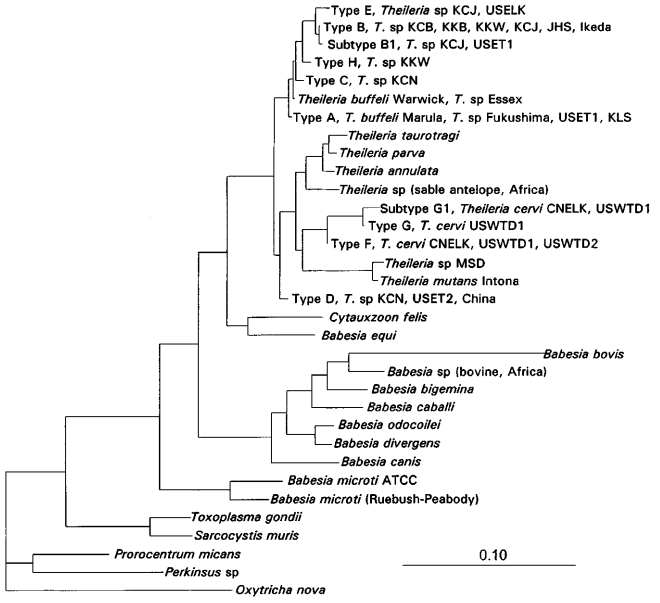

Fig. 1 Phylogenetic tree inferred using fast DNAml (maximum likelihood algorithm). Twenty eight SSU rRNA gene sequences from hemoparasite isolates were included. Strain designations are listed for isolates indicated by SSU rRNA gene sequence Type. Representative Type A isolates are listed above; others include *T.* sp. KCB, *T.* sp. KCN, *T.* sp. KCJ, and *T.* sp. USNC. A 1067 gene fragment from *Theileria* sp. China (GenBank Accession Number AF036336) shows 100% identity with Type D. The scale bar corresponds to 10 changes per 100 nucleotide positions.

Warwick and *T. buffeli* Marula (and other isolates with SSU rRNA gene Type A, including *Theileria* sp. USET1, *Theileria* sp. USNC, *Theileria* sp. Fukushima, *Theileria* sp. JHS, *Theileria* sp. KLS, *Theileria* sp. KCB, *Theileria* sp. KCN, and *Theileria* sp. KCJ) and the other consisting of closely related bovine isolates with Type B (and subtype B1) SSU rRNA genes (*Theileria* sp. KCB, *Theileria* sp. KKB, *Theileria* sp. KKW, *Theileria* sp. KCJ, *Theileria* sp. JHS, and *Theileria* sp. USNC); Type C SSU rRNA genes (*Theileria* sp. KCN); Type E SSU rRNA genes (*Theileria* sp. KCJ and *Theileria* sp. USELK); or Type H SSU rRNA genes (*Theileria* sp. KKW). The latter group is composed primarily of Korean isolates.

The other major division is composed of four relatively divergent groups, including (1) well-characterized pathogenic *Theileria* spp., *T. annulata*, *T. parva*, *T. taurotragi*, and *Theileria* sp. (antelope); (2) *T. cervi*; (3) *T. mutans* Intona and *Theileria* sp. MSD from Africa; and (4) *Theileria* isolates with SSU rRNA gene Type D (*Theileria* sp. USET2 and *Theileria* sp. KCN) from the United States and Korea (also reported from a bovine *Theileria* sp. from China – GenBank accession number AF036336).

All the branches in this tree were assessed to be significantly positive ($P < 0.01$), the exception being the branches ending with *T. cervi* Type F and *T. buffeli* Warwick.

Discussion

The maximum-likelihood phylogenetic tree generated from hemoparasite SSU rRNA sequences shows a common ancestor for all the *Theileria* spp. sequenced to date. The pathogenic *Theileria* spp. are clearly separated taxonomically from those species generally considered benign or moderately pathogenic. Furthermore, *T. mutans* Intona and *Theileria* sp. MSD are clearly separated from *T. buffeli* (Marula and Warwick) and from isolates from Japan and Korea generally regarded as *T. sergenti* (SSU rRNA gene sequence Types B, C, E, and H). The placement of *T. cervi*, considered a benign parasite of deer in the Northern hemisphere, in the tree shows it to be distinct from the United States bovine *Theileria* spp. as well as from other bovine *Theileria* spp.

The maximum-likelihood program separates the bovine isolates with the Type A SSU rRNA gene (*T. buffeli* Marula, *Theileria* sp. Fukushima and *Theileria* sp. USET1) and *Theileria* sp. Type D (species undesignated as yet), but the genetic distance between them is small as indicated by their branch lengths. Indeed, the actual sequence similarity between them is in excess of 98% (ALIGN program). The similarity value between *T. annulata* and *T. parva*, two well-characterized, clearly distinct species, is also in excess of 98% and reveals a similarly short genetic distance between them in the tree. However, unlike the case for *T. annulata* and *T. parva*, clinical data are not presently available that would support the conclusion that A and D are separate species.

The interpretation of the current results in terms of speciation is confounded by the finding that many *Theileria* isolates possess SSU rRNA genes with different nucleotide sequences. In some cases these isolates appear to consist of mixed populations. For example, three SSU rRNA sequence types – A, C, and D – were found in the Korean KCN isolate (Chae et al. 1998b), each of which may presumably represent a distinct species (Fig. 1). Alternatively, the existence of multiple SSU rRNA gene sequence types may represent genes from multiple copy units within a parasite as shown in *T. parva* (Kibe et al. 1994). Previously we reported the presence of two *T. cervi* SSU rRNA gene sequence types within the same isolates (Types F and G; Fig. 1; Chae et al. 1999a). Transcript regulation of distinct stage-specific SSU rRNAs has been shown in *Plasmodium* spp. (Waters et al. 1989; Corredor and Enea 1994;Li et al. 1997), and it is not unlikely that SSU rRNA genes of varied sequence may be expressed differentially according to the *Theileria* sp. life-cycle stages as well. It is also possible that *Theileria* spp. SSU rRNA genes may be organized similarly to those of *Cryptosporidium parvum*. This parasite possesses five copies of rRNA, not tandemly arrayed but located on at least three chromosomes with two structurally distinct types of rRNA unit (LeBlancq et al. 1997). Determination of the copy number and organization of rRNA units in the benign

Theileria spp. is needed for clarification of the source of SSU rRNA gene heterogeneity.

Some of the observed variation in gene sequences may be explained by SSU rRNA gene microheterogeneity, where minor nucleotide sequence differences occur in homologous regions of the gene, particularly in the V4 variable region (Chae et al. 1998). Such microheterogeneity may account for differences evident among the *Theileria* SSU rRNA genes sequenced to date. A number of *Theileria* isolate SSU rRNA gene sequences available in the GenBank data base were found to be similar to the *Theileria* SSU rRNA gene sequences included in our study. For example, the SSU rRNA gene sequence for the Thung Song *Theileria* isolate from Thailand (GenBank accession number AB000270) is nearly identical to the *Theileria* sp. Type D SSU rRNA gene sequence found in our study, differing at only four nucleotide positions, two of which are in the V4 region. Similarly, Japanese *T. sergenti* Ikeda (GenBank accession number AB000271) differs at only two nucleotide positions from the corresponding *Theileria* sp. Type B and the Japanese *Theileria* sp. Ikeda isolate sequenced in our study; *Theileria* sp. Malaysia-Ipoh (GenBank accession number AB000273) differs at only eight nucleotide positions from the corresponding *Theileria* sp. Type E; and *Theileria* sp. Indonesia-Medan (GenBank accession number AB000274) differs from the *T. buffeli* Warwick and *Theileria* sp. Essex SSU rRNA gene sequence at only six nucleotide positions. These closely similar SSU rRNA gene sequences were not included in the inferred tree; in trial analyses they always paired with their corresponding SSU rRNA gene Type (or species), and each pair subsequently behaved as a single operational taxonomic unit (OTU). They were therefore omitted to preserve clarity in the tree.

Uilenberg et al. (1985) concluded that a more pathogenic bovine *Theileria* stock isolated on Jeju Island, Korea, was serologically and morphologically indistinguishable from benign bovine *Theileria* isolates from Iran, Australia (*T. buffeli* Warwick), Great Britain (*Theileria* sp. Essex), Japan (*Theileria* sp. Fukushima), and the United States (*Theileria* sp. USET1; Kuttler and Craig 1975). The latter three isolates were among those analyzed in the current study, which found *Theileria* sp. Type A SSU rRNA genes in isolates from Korea [*Theileria* sp. KCJ from Cheju (Jeju) Island], Japan (*Theileria* sp. Fukushima) and the United States (*Theileria* sp. USET1). *T. buffeli* Warwick and *Theileria* sp. Essex shared the same SSU rRNA gene sequence, which differed from that of *Theileria* sp. Type A (and *T. buffeli* Marula) in only nine nucleotide positions (five of which were in the V4 region). Our data suggest that the isolates identified as *T. buffeli* Warwick, *Theileria* sp. Essex, and *T. buffeli* Marula, and others with the SSU rRNA Type A gene, may indeed be the same species.

At present there is no consensus on how much SSU rRNA gene sequence variation must exist for the source organisms to be considered as different species, but we would suggest that the *T. buffeli*-like sequences (Warwick, Essex, Marula, Fukushima, and other Type A isolates) represent at least one *Theileria* species separate from the collective *Theileria* spp. Types B, C, E, and H.

It is unclear at this point whether the Australian isolate cited in the Uilenberg et al. (1985) study corresponds to the Warwick isolate used in our study. Also, we could not include an isolate from Iran. However, if the *Theileria* isolates from the United States, Japan, Great Britain, Australia, Iran, and Korea examined in the study by Uilenberg et al. (1985) possessed SSU rRNA Type A genes, then our genetic results would support their conclusions, with one caveat: the Cheju Island Korea isolate in our study was found to contain both Type A and Type B SSU rRNA genes. The latter may correlate with the moderate pathogenicity associated with Korean bovine theileriosis. Thus, the Korean isolate may not be a pathogenic nosodeme of the benign *Theileria* sp. (*T. orientalis*), as suggested by Uilenberg et al. (1985), but may indeed represent a mixed population of benign (Type A) and more pathogenic (Type B) *Theileria* spp.

In the current study the *T. buffeli*/Type A isolates were ancestral to, and clearly separated taxonomically from, the moderately pathogenic isolates from Korea and Japan (Type B SSU rRNA genes), traditionally but incorrectly termed *T. sergenti* (Yakimoff and Dekhtereff 1930; Morel and Uilenberg 1981). This taxonomic separation is also supported by serologic dissimilarities revealed in comparative enzyme-linked immunoassays (ELISAs) and Western blots from two-dimensional polyacrylamide gel electrophoresis (PAGE; Sugimoto et al. 1991; Kawazu et al. 1992a, b). Furthermore, Fujisaki et al. (1994) suggested that Japanese "*T. sergenti*" and Australian *T. buffeli* are separate species as based on comparative studies of the piroplasm major antigens (p33/34) and transmission studies identifying their respective vector ticks. We conclude that the *Theileria* sp. Type B (*T. sergenti* Ikeda) sequence is representative of Korean and Japanese "*T. sergenti*" and must be assigned an acceptable binomial. We suggest the designation *Theileria* ikeda for isolates with Type B SSU rRNA gene sequences, as *T. sergenti* Ikeda is a well-characterized isolate and possesses only the Type B SSU rRNA gene (Fujisaki et al. 1988, 1993; Kawazu et al. 1988, 1991, 1992a–c; Shimizu et al. 1988, 1990; Kamio et al. 1990; Yoshihara et al. 1990; Sugimoto et al. 1991, 1992; Tanaka et al. 1993; Sato et al. 1994; Table 1). Still unresolved is whether the genetic distances among the SSU rRNA gene Types B (and subtype B1), C, E, and H represent heterogeneity within the same *Theileria* sp., i.e., subspecies, or whether they represent separate species.

Although *T. mutans*, *T. buffeli*, and *T. orientalis* are considered synonymous by Levine (1985), the current data clearly separate *T. mutans* Intona and *Theileria* sp. MSD from *T. buffeli*. The *Theileria* sp. MSD isolate was derived from a natural infection in cattle at the Merck, Sharp & Dome experiment center in Hartebeestpoort, Pretoria, South Africa. Two SSU rRNA gene sequences

were recovered from this isolate, one being identical to that found in the Intona *T. mutans* isolate. The other component, represented by our designation *Theileria* sp. MSD, was suspected to be from the benign organism *T. velifera*, which is transmitted by *Amblyomma hebraeum* ticks, as is *T. mutans*. However, the SSU rRNA nucleotide sequence recently reported for a Tanzanian *T. velifera* isolate (GenBank accession number AF097993) is dissimilar from that of *Theileria* sp. MSD throughout the V4 region of the gene (BLAST homology search, NCBI). Therefore, this conclusion was not corroborated, and further studies are needed to clarify the identity of *Theileria* sp. MSD.

Benign bovine *Theileria* isolates from Australia, Great Britain, and the United States were at first designated *T. mutans*, primarily on the basis of pathogenicity similar to that of African *T. mutans*. Later morphologic and serologic comparisons have shown the African *T. mutans* to be distinct from the British and Australian isolates (Morzaria et al. 1977; Uilenberg et al. 1977; Joyner et al. 1979). *Theileria mutans* Essex (*Theileria* sp. Essex) was originally isolated from a farm in the county of Essex in Southeast England by allowing field-collected *Haemaphysalis punctata* ticks to feed on a splenectomized calf (Morzaria et al. 1974). The organism was subsequently found to be transstadially transmitted by this tick species (Brocklesby et al. 1975). Characterization of *Theileria* sp. Essex by serologic comparison with six isolates of *T. mutans* from eastern and southern Africa suggested that this organism "could be distinct from African *T. mutans*" (Morzaria 1977). The current results now make that suggestion quite certain. The current results also clearly show that the United States *Theileria* isolates are not *T. mutans* because they have Type D or Type A (*T. buffeli* Marula) SSU rRNA gene sequences. Finally, the taxonomic separation of *T. buffeli* Warwick from African *T. mutans* was also confirmed in this study.

SSU rRNA gene sequence Type A (*T. buffeli* Marula) has been identified in bovine *Theileria* isolates from the United States, Korea, Japan, and South Africa. As noted above, the SSU rRNA gene sequences from *T. buffeli* Marula differ from *T. buffeli* Warwick and *Theileria* sp. Essex in only nine base positions. Thus, we have found that isolates with the Type A-like sequence are cosmopolitan. Global movement of cattle without regard to infection by the benign *Theileria* species has probably dispersed the agents worldwide such that geographic isolation exists only in relation to the distribution of a competent vector tick. Thus, it would be unwise to conclude at this time that any of these isolates is limited geographically.

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