 DOI: 10.5114/ceji.2014.47732

Genetic diversity and phylogenetic analysis of Tams1 of *Theileria annulata* isolates from three continents between 2000 and 2012

JIAY WANG[1], XIANYONG YANG[2*], YUGE WANG[1*], ZHIHONG JING[3], KAI MENG[1], JIANZHU LIU[1], HUIJUN GUO[1], RUIXUE XU[1], ZIQIANG CHENG[1]*

[1]College of Veterinary Medicine, Research Center for Animal Disease Control Engineering Shandong Province, Shandong Agricultural University, Tai'an 271018, China
[2]Central Hospital of Tai'an City, Tai'an, Shandong, 271018, China
[3]College of Foreign Languages, Shandong Agricultural University, Tai'an 271018, China
[*]These authors contributed equally to the work.

Abstract

Theileria annulata, which is part of the Theileria sergenti/Theileria buffeli/Theileria orientalis group, preferentially infects cattle and results in high mortality and morbidity in the Mediterranean, Middle East, and Central Asia. The polypeptide Tams1 is an immunodominant major merozoite piroplasm surface antigen of T. annulata that could be used as a marker for epidemiological studies and phylogenetic analysis. In the present study, a total of 155 Tams1 sequences were investigated for genetic diversity and phylogenetic relationships through phylogenetic analysis. Results showed that the Tams1 sequences were divided into two major groups and that distribution for some isolates also exhibited geographic specificity. As targeting polymorphic genes for parasite detection may result in underestimation of infection, polymerase chain reaction (PCR) assay using two different probes targeting tams-1 genes of these two groups can be more credible. In addition, the direction of the spread of the disease was discovered to be from the Mediterranean or the tropical zone to the Eurasian peninsula, Middle East, Southern Asia, and Africa, particularly for Group 2. A similar occurrence was also found between the Ms1 gene of Theileria lestoquardi and the Tams1 gene of T. annulata, which explains cross-immunogenicity to a certain extent. However, no potential glycosylation site in the Tams1 of T. annulata was found in this study, which illustrated that instead of N-glycosylation, other modifications have more significant effects on the immunogenicity of the Tams1 protein.

Key words: *genetic diversity, phylogenetic analysis, Tams1, Theileria annulata, three continents.*

(Centr Eur J Immunol 2014; 39 (4): 476-484)

Introduction

Various species of *Theileria* bovine parasites are widespread in Southern Europe, North Africa, and Southern Asia, thus presenting a significant threat to livestock productivity [1]. Among these, the tick-borne protozoan parasite *Theileria annulata* is the causative agent of lymph proliferative theileriosis, an important disease with high mortality and morbidity. Recently, in an epidemiological survey of bovine *Babesia* and *Theileria* parasites, *T. annulata* has been found to be the most common blood parasite in cattle, buffalo, and sheep populations, which were bred in different geographical locations in Egypt [2]. *Theileria annulata* parasitizes the reticuloendothelial system and the red blood cells of cattle. The intermediate host then exhibits symptoms such as high fever (40°C to 42°C), depression, cough, runny nose and watery eyes, anemia, and jaundice. Given that the main vectors of ring *theileriosis* are *Hyalommadetritum*, *H. anatolicumanatolicum*, *H. anatolicumexcavatum*, *H. asiaticum*, *H. dromedarii*, and *H. marginatummarginatum* [3], the disease typically begins in May, with outbreaks occurring in June and July, and then gradually subsides.

The polypeptide Tams1 elicits a protective response as an immunodominant major merozoite piroplasm surface antigen against the protozoan parasite *T. annulata* [4, 5], and is considered as a candidate for inclusion in a sub-unit recombinant vaccine [6]. The Tams1-encoding gene has been developed for PCR-based assays, which could use bovine blood samples to detect *T. annulata* infections [1]. Tams1 protein has also been reported as a candidate to develop a diagnostic enzyme-linked immunosorbent assay

Correspondence: Prof. Jianzhu Liu, College of Veterinary Medicine, Research Center for Animal Disease Control Engineering Shandong Province, Shandong Agricultural University, Tai'an 271018, China, e-mail: liujz@sdau.edu.cn

(ELISA) because it exhibits significant sequence diversity and no geographic specificity [4]. However, sequencing and phylogenetic analyses of the Egyptian *T. annulata* showed that theTams-1 sequences are relatively diverse (87.8-100% identity values), dispersing themselves across several clades in the phylogenetic tree containing sequences from other countries [2]. Moreover, Tams1 diversity has been reported as being generated by the random mutation of nucleotides during asexual reproduction as well as by the selection of changes that confer a biological advantage instead of the differential expression of the members of a gene family [7, 8].

Several attempts have been made to analyze the phylogenetic characteristics of the Tams1 gene of *T. annulata* [8]. However, no distinct classification for all reported Tams1 sequences obtained from three continents (Asia, Africa, and Europe) is yet available. In the present study, 155 complete Tams1 genes of *T. annulata* occurring over a wide geographical range were first sequenced. Given that the taxonomic status and epidemiology of *T. annulata* remain undefined [9], studying the phylogenetic variability and molecular genetic characterization of Tams1 will help provide an understanding of the relationship between the molecular evolutionary history of *T. annulata* and the emergence, breakout, and spread of new *T. annulata* epidemics.

In addition, attempts were made to analyze the phylogenetic diversity and distribution of *T. annulata*. A phylogenetic tree was constructed and analyzed. Comparisons and additional analyses were also performed, including predicting potential glycosylation sites as well as finding volatile regions and evolutionary regular patterns [10].

Material and methods

Theileria sampling, DNA extraction, polymerase chain reaction amplification, and sequencing

A total of 81 adult ticks were collected from 61 cattle in a randomly selected dairy farm in Xinjiang, a northwestern city in China, in 2012. DNA extraction, as well as polymerase chain reaction (PCR) detection and amplification, were performed by Meng *et al.* [3]. In brief, a previously reported primer set (Forward 5-GTAACCTTTAAAAACGT-3, Reverse 5-CAGTTACGAACATGGGTTT-3) was used to detect Tams1 DNA specifically [1, 11]. The recombinant plasmid pMD18-T vector (Takara Bio Inc., Japan) was transformed into *Escherichia coli* TOP10 competent cells after purified DNA fragments were cloned and inserted. Then, the *E. coli* that was cultured overnight were purified and sent to a private company [Sangon Biotech (Shanghai) Co., Ltd., China] for sequencing. The results were identified by comparing the obtained sequences with the registered sequences in GenBank through BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi). From the clinical isolate, an identical sequence with the Tams1 gene of *T. annulata* was found (GenBank accession no. JX475044).

Sequence alignment and phylogenetic analysis

Tams1 gene sequences published in GenBank were also included. Blasting was performed at the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The amplified sequences and Tams1 were both blasted to ensure that all known Tams1 genes of *T. annulata* were brought forward. Consequently, a total of 155 isolates were discovered (Table 1) including RefSeq (Turkey, 1), Spain (14), Portugal (5), Italy (5), Tunisia (46), Iran (4), Bahrain (9), Turkey (18), India (7), Mauritania (22), Iraq (7), China (2), Sudan (5), Sri Lanka (3), unknown origin (4), and isolates of *T. lestoquardi* (4) [11].

Multiple sequence alignments were performed using the ClustalW algorithm [12]. The phylogenetic tree was constructed through the neighbor-joining method [13] and a bootstrap value of 1000 replicates, using the MEGA 5.1 software [14].

Predicting N-glycosylation sites

Glycosylation sites have an important role in determining the properties of the concerned protein, such as antigenic properties, among others [15, 16]. Considering that the glycosylation sites of the protein were formed after processing, only potential glycosylation sites were predicted at the amino acid level. In this investigation, referring to the phylogenetic tree, 39 isolates (including XM948626.1, AF214906.1, AF214872.1, AF214920.1, AF214904.1, AF214819.1, AF214840.1, JX648210.1, AF214832.1, AF214852.1, AF214849.1, AF214818.1, AF214898.1, EU563912.1, JX475044, AJ276654.1, EF618726.1, AF214869.1, AF214900.1, GU130193.1, AF214866.1, AF214856.1, AF214835.1, U22888.1, AF214800.1, AF214801.1, AF214812.1, AF214879.1, EF092915.1, AF214825.1, AF214815.1, AB690864.1, AF214805.1, AF214797.1, AF214863.1, AF214875.1, GU130190.1, EF092918.1, FJ159695.1) on behalf of 155 isolates were selected, followed by the submission of the amino acid sequence of the protein to NetNGlyc Server (http://www.cbs.dtu.dk/services/NetNGlyc/) [17], and the prediction of the *N*-glycosylation sites in the amino acid sequence of the protein.

Results

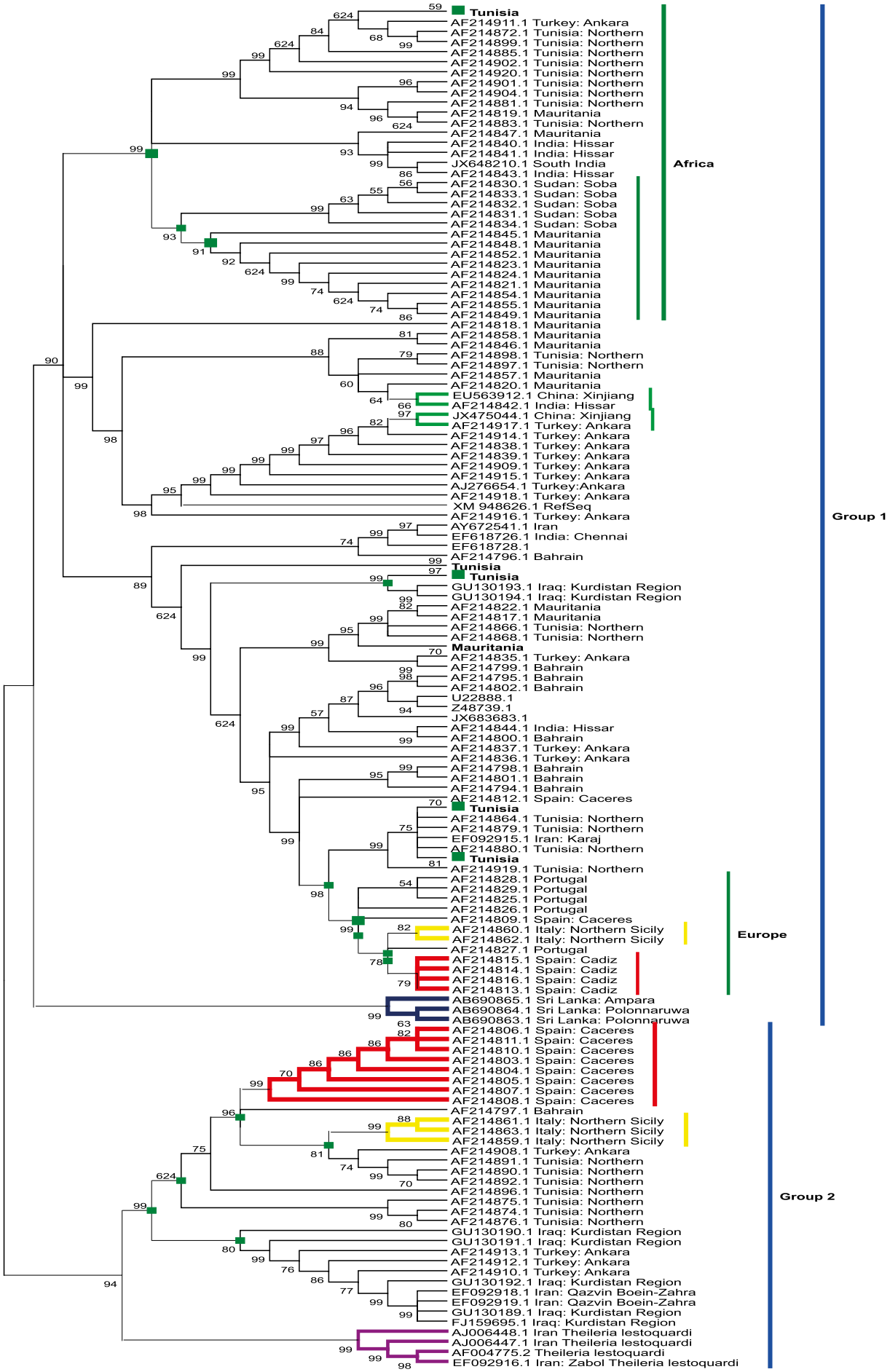
Based on the multiple sequence alignments and the neighbor-joining method, the sequence obtained from the clinical isolate and the 154 sequences (including four Ms1 genes from *T. lestoquardi*) in GenBank of Tams1 isolates derived from the three continents (Asia, Africa and Europe) were assigned to two groups (Fig. 1). Sequences

Table 1. Origin of Tams1 isolates used in the present study

No.	Country	Year	Accession no.	No.	Country	Year	Accession no.
1	Spain	2000	AF214813.1	42	India	2000	AF214844.1
2	Spain	2000	AF214816.1	43	Tunisia	2000	AF214868.1
3	Spain	2000	AF214814.1	44	Tunisia	2000	AF214866.1
4	Spain	2000	AF214815.1	45	Mauritania	2000	AF214817.1
5	Spain	2000	AF214827.1	46	Mauritania	2000	AF214822.1
6	Italy	2000	AF214862.1	47	Iraq	2010	Gu130194.1
7	Italy	2000	AF214860.1	48	Iraq	2010	Gu130193.1
8	Spain	2000	AF214809.1	49	Tunisia	2000	AF214869.1
9	Iran	2006	EF092915.1	50	Tunisia	2000	AF214871.1
10	Tunisia	2000	AF214879.1	51	Tunisia	2000	AF214870.1
11	Tunisia	2000	AF214864.1	52	Bahrain	2000	AF214796.1
12	Tunisia	2000	AF214903.1	53	Bahrain	2007	EF618728.1
13	Tunisia	2000	AF214882.1	54	India	2007	EF618726.1
14	Tunisia	2000	AF214865.1	55	Tunisia	2000	AF214878.1
15	Spain	2000	AF214812.1	56	Tunisia	2000	AF214877.1
16	Spain	2012	JX683683.1	57	Tunisia	2000	AF214887.1
17	Spain	2006	Z48739.1	58	Tunisia	2000	AF214867.1
18	Spain	1995	U22888.1	59	Tunisia	2000	AF214900.1
19	Bahrain	2000	AF214802.1	60	Tunisia	2000	AF214889.1
20	Bahrain	2000	AF214795.1	61	Tunisia	2000	AF214888.1
21	Bahrain	2000	AF214799.1	62	Turkey	2000	AF214909.1
22	Turkey	2000	AF214835.1	63	Turkey	2000	AF214915.1
23	Mauritania	2000	AF214851.1	64	Turkey	2010	AJ276654.1
24	Mauritania	2000	AF214856.1	65	Turkey	2000	AF214918.1
25	Mauritania	2000	AF214853.1	66	Turkey	2008	XM948626.1
26	Mauritania	2000	AF214850.1	67	Turkey	2000	AF214916.1
27	Portugal	2000	AF218426.1	68	Mauritania	2000	AF214858.1
28	Portugal	2000	AF218425.1	69	Mauritania	2000	AF214846.1
29	Portugal	2000	AF218429.1	70	Tunisia	2000	AF214898.1
30	Portugal	2000	AF218428.1	71	Tunisia	2000	AF214897.1
31	Tunisia	2000	AF214919.1	72	Mauritania	2000	AF214854.1
32	Tunisia	2000	AF214895.1	73	Mauritania	2000	AF214821.1
33	Tunisia	2000	AF214893.1	74	Mauritania	2000	AF214824.1
34	Tunisia	2000	AF214894.1	75	Mauritania	2000	AF214823.1
35	Tunisia	2000	AF214880.1	76	Mauritania	2000	AF214848.1
36	Bahrain	2000	AF214794.1	77	Mauritania	2000	AF214845.1
37	Bahrain	2000	AF214801.1	78	Mauritania	2000	AF214819.1
38	Bahrain	2000	AF214798.1	79	Tunisia	2000	AF214883.1
39	Turkey	2000	AF214836.1	80	Tunisia	2000	AF214881.1
40	Turkey	2000	AF214837.1	81	Tunisia	2000	AF214901.1
41	Bahrain	2000	AF214800.1	82	Tunisia	2000	AF214920.1

Table 1. Cont.

No.	Country	Year	Accession no.	No.	Country	Year	Accession no.
83	Tunisia	2000	AF214885.1	120	Turkey	2000	AF214912.1
84	Iran	2004	AY672541.1	121	Turkey	2000	AF214913.1
85	China	2012	JX475044.1	122	Iraq	2010	GU130191.1
86	Turkey	2000	AF214917.1	123	Iraq	2010	GU130190.1
87	Turkey	2000	AF214914.1	124	Italy	2000	AF214859.1
88	Turkey	2000	AF214838.1	125	Italy	2000	AF214863.1
89	Turkey	2000	AF214839.1	126	Italy	2000	AF214861.1
90	Mauritania	2000	AF2w14857.1	127	Bahrain	2000	AF214797.1
91	Mauritania	2000	AF214820.1	128	Spain	2000	AF214808.1
92	China	2008	EU593912.1	129	Spain	2000	AF214807.1
93	India	2000	AF214842.1	130	Spain	2000	AF214805.1
94	Mauritania	2000	AF214818.1	131	Spain	2000	AF214804.1
95	Mauritania	2000	AF214849.1	132	Spain	2000	AF214803.1
96	Mauritania	2000	AF214855.1	133	Spain	2000	AF214810.1
97	Sudan	2000	AF214834.1	134	Spain	2000	AF214811.1
98	Sudan	2000	AF214831.1	135	Spain	2000	AF214806.1
99	Sudan	2000	AF214832.1	136	Sri Lanka	2012	AB690865.1
100	Sudan	2000	AF214833.1	137	Sri Lanka	2012	AB690864.1
101	Sudan	2000	AF214830.1	138	Sri Lanka	2012	AB690863.1
102	India	2000	AF214843.1	139	Iran	2006	EF092916.1
103	South India	2012	JX648210.1	140	Iran	2000	AF004775.2
104	India	2000	AF214841.1	141	Iran	1999	AJ006447.1
105	India	2000	AF214840.1	142	Iran	1999	AJ006448.1
106	Mauritania	2000	AF214847.1	143	Iraq	2010	FJ159695.1
107	Tunisia	2000	AF214899.1	144	Tunisia	2000	AF214876.1
108	Tunisia	2000	AF214872.1	145	Tunisia	2000	AF214874.1
109	Turkey	2000	AF214911.1	146	Tunisia	2000	AF214875.1
110	Tunisia	2000	AF214873.1	147	Tunisia	2000	AF214896.1
111	Tunisia	2000	AF214886.1	148	Tunisia	2000	AF214892.1
112	Tunisia	2000	AF214906.1	149	Tunisia	2000	AF214890.1
113	Tunisia	2000	AF214907.1	150	Tunisia	2000	AF214891.1
114	Tunisia	2000	AF214905.1	151	Turkey	2000	AF214908.1
115	Tunisia	2000	AF214884.1	152	Mauritania	2000	AF214852.1
116	Iran	2006	EF092919.1	153	Tunisia	2000	AF214904.1
117	Iran	2006	EF092918.1	154	Tunisia	2000	AF214902.1
118	Iraq	2010	GU130192.1	155	Iraq	2010	GU130189.1
119	Turkey	2000	AF214910.1				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AF214899.1 Tunisia: Northern
AF214885.1 Tunisia: Northern
AF214902.1 Tunisia: Northern
AF214920.1 Tunisia: Northern
AF214901.1 Tunisia: Northern
AF214904.1 Tunisia: Northern
AF214881.1 Tunisia: Northern
AF214819.1 Mauritania
AF214883.1 Tunisia: Northern
AF214847.1 Mauritania
AF214840.1 India: Hissar
AF214841.1 India: Hissar
JX648210.1 South India
AF214843.1 India: Hissar
AF214830.1 Sudan: Soba
AF214833.1 Sudan: Soba
AF214832.1 Sudan: Soba
AF214831.1 Sudan: Soba
AF214834.1 Sudan: Soba
AF214845.1 Mauritania
AF214848.1 Mauritania
AF214852.1 Mauritania
AF214823.1 Mauritania
AF214824.1 Mauritania
AF214821.1 Mauritania
AF214854.1 Mauritania
AF214855.1 Mauritania
AF214849.1 Mauritania
AF214818.1 Mauritania
AF214858.1 Mauritania
AF214846.1 Mauritania
AF214898.1 Tunisia: Northern
AF214897.1 Tunisia: Northern
AF214857.1 Mauritania
AF214820.1 Mauritania
EU563912.1 China: Xinjiang
AF214842.1 India: Hissar
JX475044.1 China: Xinjiang
AF214917.1 Turkey: Ankara
AF214914.1 Turkey: Ankara
AF214838.1 Turkey: Ankara
AF214839.1 Turkey: Ankara
AF214909.1 Turkey: Ankara
AF214915.1 Turkey: Ankara
AJ276654.1 Turkey:Ankara
AF214918.1 Turkey: Ankara
XM 948626.1 RefSeq
AF214916.1 Turkey: Ankara
AY672541.1 Iran
EF618726.1 India: Chennai
EF618728.1
AF214796.1 Bahrain
Tunisia
Tunisia
GU130193.1 Iraq: Kurdistan Region
GU130194.1 Iraq: Kurdistan Region
AF214822.1 Mauritania
AF214817.1 Mauritania
AF214866.1 Tunisia: Northern
AF214868.1 Tunisia: Northern
Mauritania
AF214835.1 Turkey: Ankara
AF214799.1 Bahrain
AF214795.1 Bahrain
AF214802.1 Bahrain
U22888.1
Z48739.1
JX683683.1
AF214844.1 India: Hissar
AF214800.1 Bahrain
AF214837.1 Turkey: Ankara
AF214836.1 Turkey: Ankara
AF214798.1 Bahrain
AF214801.1 Bahrain
AF214794.1 Bahrain
AF214812.1 Spain: Caceres
Tunisia
AF214864.1 Tunisia: Northern
AF214879.1 Tunisia: Northern
EF092915.1 Iran: Karaj
AF214880.1 Tunisia: Northern
Tunisia
AF214919.1 Tunisia: Northern
AF214828.1 Portugal
AF214829.1 Portugal
AF214825.1 Portugal
AF214826.1 Portugal
AF214809.1 Spain: Caceres
AF214860.1 Italy: Northern Sicily
AF214862.1 Italy: Northern Sicily
AF214827.1 Portugal
AF214815.1 Spain: Cadiz
AF214814.1 Spain: Cadiz
AF214816.1 Spain: Cadiz
AF214813.1 Spain: Cadiz
AB690865.1 Sri Lanka: Ampara
AB690864.1 Sri Lanka: Polonnaruwa
AB690863.1 Sri Lanka: Polonnaruwa
AF214806.1 Spain: Caceres
AF214811.1 Spain: Caceres
AF214810.1 Spain: Caceres
AF214803.1 Spain: Caceres
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AF214805.1 Spain: Caceres
AF214807.1 Spain: Caceres
AF214808.1 Spain: Caceres
AF214797.1 Bahrain
AF214861.1 Italy: Northern Sicily
AF214863.1 Italy: Northern Sicily
AF214859.1 Italy: Northern Sicily
AF214908.1 Turkey: Ankara
AF214891.1 Tunisia: Northern
AF214890.1 Tunisia: Northern
AF214892.1 Tunisia: Northern
AF214896.1 Tunisia: Northern
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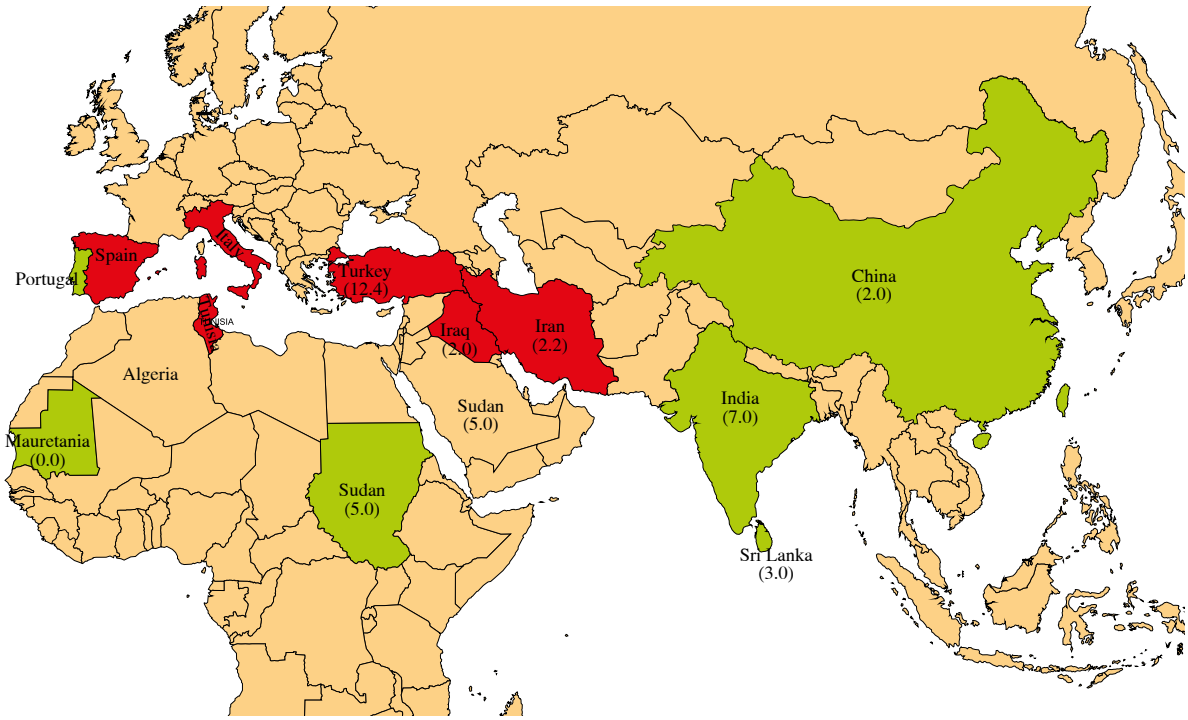
GU130191.1 Iraq: Kurdistan Region
AF214913.1 Turkey: Ankara
AF214912.1 Turkey: Ankara
AF214910.1 Turkey: Ankara
GU130192.1 Iraq: Kurdistan Region
EF092918.1 Iran: Qazvin Boein-Zahra
EF092919.1 Iran: Qazvin Boein-Zahra
GU130189.1 Iraq: Kurdistan Region
FJ159695.1 Iraq: Kurdistan Region
AJ006448.1 Iran Theileria lestoquardi
AJ006447.1 Iran Theileria lestoquardi
AF004775.2 Theileria lestoquardi
EF092916.1 Iran: Zabol Theileria lestoquardi
Africa
Europe
Group 1
Group 2



In the green parts, only *T. annulata* of group two can be found, while in the red areas *T. annulata* of both groups can be found. And the numbers in the brackets means isolate number in group 1 and isolate number in group 2.

Fig. 2. The geographical distribution of *Theileria annulata*

from Spain, Italy, Tunisia, Iran, Bahrain, Turkey and Iraq were found in both groups, while in Portugal, India, Mauritania, China, Sudan and Sri Lanka, sequences were only found in Group 1. In the phylogenetic tree, isolates from the same continents (Africa and Europe) got-together in Group 1 (Fig. 2). The N-glycosylation sites' prediction by online software showed that no potential glycosylation sites were found.

Discussion

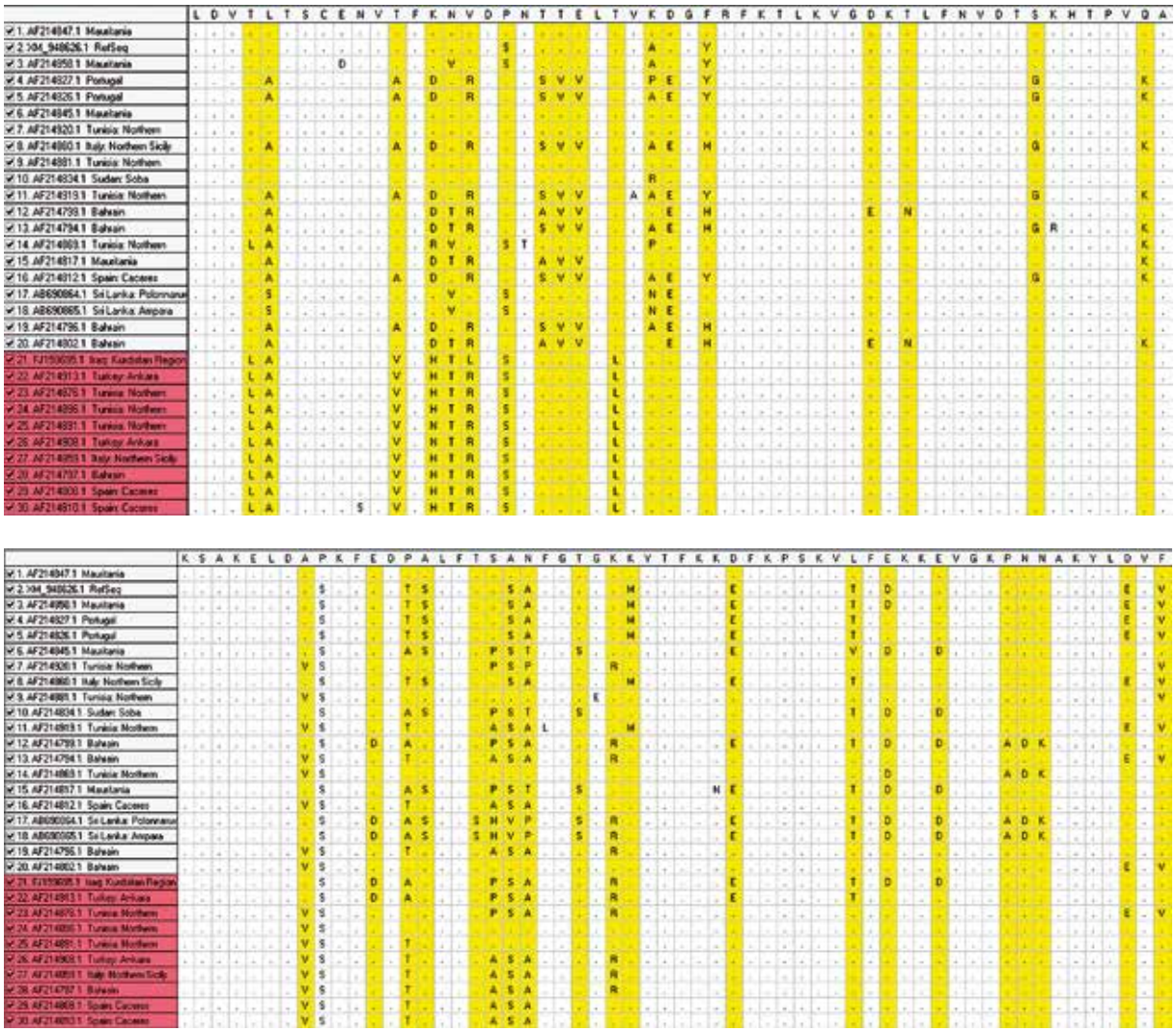
The phylogenetic tree constructed with 155 Tams1 gene sequences (including four Ms1 genes from *T. lestoquardi*) shows that *T. annulata* isolates were divided into two major groups, which were called Groups 1 and 2. This discovery was very important and significant, as almost every research about Tams1 would bring up some similar guesses [18]. Habibi [19] classified 17 Tams1 gene sequences into 2 clusters. However, classifying all reported Tams1 sequences obtained from three continents (Asia, Africa, and Europe) has not yet been performed.

Several studies found that the Tams1 gene is highly polymorphic, raising questions concerning the suitability of Tams1 gene-targeted primers to detect all *T. annulata* isolates [1]. And especially in clinical practice, as targeting polymorphic genes for parasite detection may result in un-

Table 2. Numbers of *Theileria annulata* isolates in groups 1 and 2 in each country

Country	Group 1	Year	Group 2	Year
Spain	6	2000	8	2000
Portugal	5	2000	0	
Italy	2	2000	3	2000
Tunisia	39	2000	7	2000
Iran	1	2004	2	2006
	1	2006		
Bahrain	8	2000	1	2000
Turkey	11	2000	4	2000
	1	2010		
India	5	2000	0	
	2	2007		
	1	2012		
Mauritania	22	2000	0	
Iraq	2	2010	5	2010
China	1	2001	0	
	1	2012		
Sudan	5	2000	0	
Sri Lanka	3	2012	0	

Fig. 1. Phylogenetic tree for the Tams1 gene of the 155 Theileria annulata isolates



The first 20 sequences were from Group 1, while the last 10 sequences marked in red were from Group 2. The volatile sites and regions in Tams1 gene were marked in yellow in the figure.

Fig. 3. Analysis and comparison of amino acid mutations in Tams1

derestimation of infection, PCR assay using two different probes targeting tams-1 genes of these two groups, for example the primers (Forward 5-GTAACCTTTAAAAACGT-3, Reverse 5-CAGTTACGAACATGGGTTT-3) [3] and primers (Forward 5-ATGTGTCCAGGACCACCC-3, Reverse 5-GGGTTTTAAAGGAAGTAAAGG-3) [4], can be more credible than just relying on one primer pair [18]. The sequences from Spain, Italy, Tunisia, Iran, Bahrain, Turkey, and Iraq were found in both groups, whereas the sequences from Portugal, India, Mauritania, China, Sudan, and Sri Lanka were only found in Group 1. In this group, isolates from the same continents (Africa and Europe) were obviously clustered in the phylogenetic tree (Fig. 1).

Previous studies have reported that no geographic specificity was observed and nearly identical sequences occurred in different geographic areas. Moreover, a panmictic (from panmixia, that is, the ability of individuals in a population to move freely within their habitat, and thus breed with other members of the population) population structure was suggested by the results of several studies [4]. In the present study, however, the comparison of the Tams1 gene sequences obtained from three continents revealed that particular sequence types belong to definitive regions, such as Sudan, Sri Lanka, and so on (marked in dark green and purple in Fig. 1). Nearly no identical Tams1 sequence was found in widely separated regions.

By referring to Fig. 2 and Table 2, the direction of the spread of the disease, particularly for Group 2, can be speculated. Based on the time (as the recording time reflects the isolates' outbreak time in some degree) and dis-

tribution, the following conclusions can be drawn. Isolates from both groups were found in the European peninsula in 2000, in Iran in 2006, and in Iraq in 2010. However, only Group 1 isolates were found in China, India, and Sri Lanka. Group 2 isolates eventually spread to Iraq. The overall spread direction was from the Mediterranean or the tropical zone to the Eurasian peninsula, Middle East, Southern Asia, and Africa.

Theileria annulata has the ability to transform the leukocytes of host animals and been called transforming *Theileria*. The evolution of the transforming *Theileria* has been accompanied by drastic changes in its genetic makeup, such as acquisition or expansion of gene families, which are thought to play critical roles in the transformation and the immune escapement of host cells [20]. Immune escape in *Theileria* is facilitated by genetic diversity in its antigenic determinants, which potentially results in a loss of T cell receptor recognition in its host. And in the case of Asn-Xaa-Ser/Thrsequons (incl. Asn-Pro-Ser/Thr), no potential glycosylation site was discovered. This positive verified the suspect of Katzer in 2002 that there were no N-glycosylation sites on Tams1 genes [21]. Consequently, Tams1 protein easily combined with the antibody as a stable protein antigen. These results indicate that compared with *N*-glycosylation, other modifications have more significant effects on the immunogenicity of the Tams1 protein.

Cows in the aforementioned three continents have been reported to have been infected with mixed populations of geographically variable *Theileria* parasites [9, 22]. Cross-immunity studies conducted by Leeman [23] suggested that partial cross-immunity from *T. annulata* to *T. lestoquardi*, and vice versa, developed in sheep, thus indicating a close relationship among parasite species. As the tree may not be a true representation of the evolutionary relationship of these sequences/species, however, Tams1 sequences for *T. annulata* would be more likely to cluster together than with sequences from a distinct species. Coincidentally, four *T. lestoquardi* Ms1 gene isolates (Query cover: 86% to 95%, ident: 85% to 86%) were obtained by blasting the Tams1 gene of *T. annulata* and were included in Group 2 through phylogenetic assay. This finding provides evidence of a significant similarity between the Ms1 gene of *T. lestoquardi* and the Tams1 gene of *T. annulata*, thus explaining the immunogenicity of the Tams1 protein to a certain extent.

Given that it enables evasion from host immune response, diversity is generally believed to be a positively selected result [24]. In Fig. 3, several variable areas were marked. These areas represent the generation of Tams1 diversity that enabled *T. annulata* to escape from host immunity. This finding provides valuable information on the antigenic structure of *T. annulata* and may be helpful in designing vaccines.

Conclusions

Tams1, as an immunodominant surface antigen, has been used to investigate genetic diversity and vaccine purposes for antigenicity [4]. In the present study, all 155 Tams1 isolates were classified into two major groups in the phylogenetic tree, which explained why PCR assay using one primer pair targeting tams-1 gene failed to detect infection of some animals. Geographic specificity was observed when comparing Tams1 gene sequences. In addition, based on the geographic distribution and recording time of *T. annulata*, the spread direction of the disease, particularly for Group 2, was hypothesized to be from the Mediterranean or the tropical zone to the Eurasian peninsula, Middle East, Southern Asia, and Africa. A similarity on immunodominant major surface antigen gene was also found between the Ms1 gene of *T. lestoquardi* and the Tams1 gene of *T. annulata*, which explains cross-immunogenicity to a certain extent. However, no potential glycosylation site was found in this study, thus instead of *N*-glycosylation, other modifications have been hypothesized to have more significant effects on the immunogenicity of the Tams1 protein.

The authors declare no conflict of interest.

This work was supported by Shandong Modern Agricultural Technology & Industry System (No. SDAIT-13-011-04), the Natural Science Foundation of Shandong Province (ZR2012CQ001), the Research Fund for the Doctoral Program of Higher Education (20123702120008).

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