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

JIAYI WANG<sup>1\*</sup>, XIANYONG YANG<sup>2\*</sup>, YUGE WANG<sup>1\*</sup>, ZHIHONG JING<sup>3</sup>, KAI MENG<sup>1</sup>, JIANZHU LIU<sup>1</sup>, HUIJUN GUO<sup>1</sup>, RUIXUE XU<sup>1</sup>, ZIGIANG CHENG<sup>1</sup>

<sup>1</sup>College of Veterinary Medicine, Research Center for Animal Disease Control Engineering Shandong Province, Shandong Agricultural University, Tai'an 271018, China

<sup>2</sup>Central Hospital of Tai'an City, Tai'an, Shandong, 271018, China

<sup>3</sup>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 Tams I 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 subunit 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. 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.

#### 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-GTAACCTTTAAAAAC-GT-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

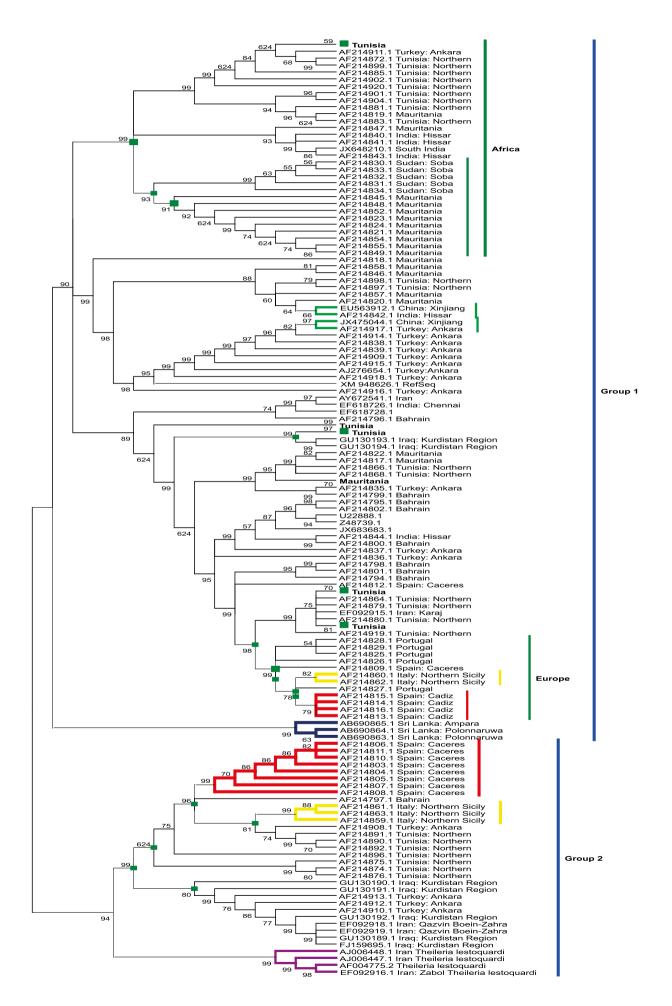
## J. Wang et al.

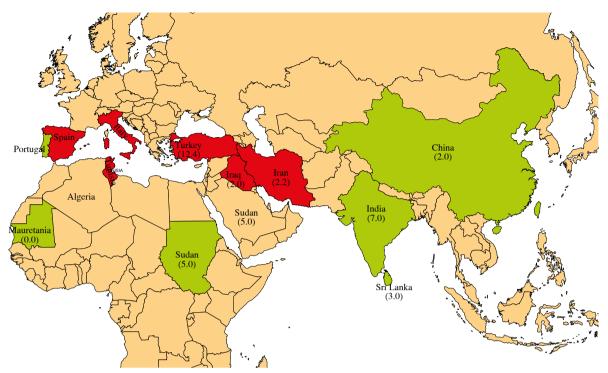
No.	Country	Year	Accession no.	No.	Country	Year	Accession no.
1	Spain	2000	AF214813.1	42	India	2000	AF214844.1
2	Spain	2000	AF214816.1	43	Tunisia	2000	AF214868.1
3	Spain	2000	AF214814.1	44	Tunisia	2000	AF214866.1
4	Spain	2000	AF214815.1	45	Mauritania	2000	AF214817.1
5	Spain	2000	AF214827.1	46	Mauritania	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. Origin of Tams1 isolates used in the present study

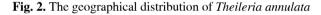
No.	Country	Year	Accession no.	No.	Country	Year	Accession no.
83	Tunisia	2000	AF214885.1	120	Turkey	2000	AF214912.1
84	Iran	2004	AY672541.1	121	Turkey	2000	AF214913.1
85	China	2012	JX475044.1	122	Iraq	2010	GU130191.1
86	Turkey	2000	AF214917.1	123	Iraq	2010	GU130190.1
87	Turkey	2000	AF214914.1	124	Italy	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				

Table 1. Cont
---------------





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.



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-

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

**Table 2.** Numbers of *Theileria annulata* isolates in groups1 and 2 in each country

	LOV	TLT	SCEN	VTF	KNV	DP	NTT	ELI	TV	K D	GFI	RFI	C T L	KV	G D I	C T L	FNVD	1 5 1	CHTP	VOA
21.AF214047.1 Maultania												1.1	STOL .	1.9.1			121212121			1
2 2 104_948626.1 RefSeg					1000	. 5	1000			A	. Y.			1.1.1						
2 3 AF214958 1 Mautania			0				1000			A .	Y									
# 4 AF214827.1 Portugal		A .		A	D R		. 5 V	٧		PE	Y			1				G		κ.
2.5. AF214826.1 Portugal		A		A	D R		S V	V		AE	Y							6		κ.
¥ 6. AF214845.1 Maultania			1 2 2 4				1000			100	122									
7. AF214920.1 Tunkia Nothern					100		- Calles													
2 8. AF214060.1 Maly Northern Sicily		A	2.2.2.2	A .	D . R		5.4	V .		A				1212						K .
9. 9 AF214881.1 Tunicia: Northern																				
¥ 10 AF214834.1 Suday Soba	C. L. L.								1	8			100							
211 AF214313.1 Tunkie Nothern		A		A	0 R		S V	v	A	AE	Y							6		K
2 12 AF214739.1 Bahan				100	DTR		A V	V		1.	H				1	N				
213.AF214794.1 Bahsan					DTR		5 4	v .		4.5	H									
214 AF214003.1 Tunicia Nothern		LA			8 V .		T			P	100									
2 15 AF214817 1 Mautania					DIR	1.0	A 4	V												K
216 AF2148121 Spain Caceres					0 8		5 V	v I		AE	Y									
17. AB690864.1 Sri Lanka Poloman		4					-			NE	10									
18 AB690865.1 Sri Lanka Ampara			1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.		Y	5	100		1	NE				100						3 A.
✓ 13. AF214796.1 Bahain			1 1 1 2 1 1		D B		S V	V		A	H			1.1.						
20 AF214902 1 Bahain		A			DTR		AV	V.		1						N				K.
2 21 FJ1500351 key Kudates Repo				v	HTL	1.5								1111						
2 22 AF21 6113.1 Turkey Arkins				¥.	HIR		. Caller													
2 23 AF214976 1 Tuning Northern				v	HTR															
24 AF214295 1 Turnin Nothers				v	HIR	100					-8								1.1.1	
V 25 AF214891.1 Turkin Northern	1.1.1.1.1			v	NTR	5			111					1.1.						1. A.
28 AF2149081 Turkey Arkers				v	NTR	1														
2 27 M 2149231 Baly Northern Sick				v	HIR	1.1						111								
2 20 AF214707.1 Babean				v	HIB															-
2 29 M/2149081 Spain Casmer	1 1 1 1 1	LA		v	HTR															
V 30 AF214910 1 Seven Conserv	1.1.1.1.1.1		5	v	HIR			1				101	111	1011			1. 1. 1. 1.			
COLUMN NUMBER	<b>K S A K</b>	ELD	APKFE	O P	ALFI	S A	NFG	TGK	( K	Y T F		DF	K P S	ĸv	LFE	KK	EVGKP	NN	AFYL	DVF
921.AF214947.1 Maultania	K S A K	ELD		0 P	A L F T		NFG	TGK		YTF	K K	DF	K P S	ĸv		K K	E V G X P	N N	A K Y L	
9 1. AF214047.1 Mauitania 9 2.554_540626.1 RelSec	K S A K	ELD		0 P	A L F 1	ŝ	N F G	TGK	H	Y T F	K K	D F	K P S	 	L F E	K K	EVGKP	H N	A K Y L	e i v
R1.AF214047.1 Maultania	K S A K	E L D		- T	5	ŝ	A	T G K	м	Y T F	K K	D F C . C .	K P S	 	L F E	K K  	E V G K P	NN	A K Y L	
2 1. AF214547.1 Maultaria 9 2. XM_540525.1 RelSec 9 3. AF2145611 Maultaria	K S A K	E L D		. t . t . T	5	5	A	T G K	м	V T F	K K		K P S	 	L F E	K K    	E V G K P	N N	A K Y L    	e i v
H 1. AF214847.1 Mauitaria H 2. MJ, S48025.1 Reflec H 2. AF224895.1 Mauitaria H 4. AF214827.1 Potugal H 5. AF214825.1 Potugal H 5. AF214825.1 Potugal H 6. AF214845.1 Mauitaria	K S A K	E L D      		- T - T - T - T - T	5	5 5 5 5	A	T G K	м	VTF	K K		K P S	5 K V	L F E	K K     	E V G K P	H N	A K Y L   	e i v
V 1. A/2149473 Maxitania V 2. VAL 5405251 Restlera V 2. A/2240531 Maxitania V 5. A/2240521 Maxitania V 5. A/2240521 Potugal V 5. A/2240551 Maxitania V 7. A/2240511 Maxitania V 7. A/2240511 Turkite Northwan	K S A K	E L D       		- T - T - T - T - T - A	5		A	T G K	м	Y T F	K K   	0 F C . C . C . C . C .	K P S	5 K V	L F E	K K       	E V G K P	NN	A F Y L 	e i v
11.7216973 Maxitania 2.204,540251 RetSeq 2.47214953 Maxitania 2.47214923 Potogai 2.5.47214923 Potogai 2.5.472149251 Potogai 2.5.472149251 Turkin Norbean 2.6.472149251 Turkin Norbean 2.6.472149251 Mg/ Norbean Sch	K S A K	E L D	\$ 5 5 5 7 5 7 5	· · · · · · · · · · · · · · · · · · ·	5				м	Y T F	<b>K K</b>		K P S	5 K V	L F E	K K       	E V G K P	H N	A F Y L	e i v
21 AP210073 Maximie 22 304,500261 Refleq 23 AP214003 Maximie 24 AP214003 Potopii 25 AP214003 Potopii 25 AP214051 Potopii 25 AP214051 Turisis Nortwan 26 AP214051 Turisis Nortwan 26 AP214051 Turisis Nortwan 26 AP214051 Turisis Nortwan 26 AP214051 Turisis Nortwan	K S A K	E L D           	\$ 5 5 5 5 5 7 5 7 5 7 5 7 5 7 7 5 7 7 5 7	- T - T - T - T - T - T - T - T	5			T G K	м	Y T F	<b>K K</b>	0 F C C C C C C	K P S	<b>K V</b>	L F E - 0 1 -	K K 	E V G K P	H N	A F Y L 	e i v
1.4.2140/1.1 Maultania 2.244,540321.1 Maultania 2.4.47248931.1 Maultania 2.4.47248921.1 Portugal 2.4.47248921.1 Portugal 2.5.47248821.1 Maultania 2.7.472489251.1 Maultania 2.472148861.1 May Northern Scily 2.5.4724881.1 Turvisa Northern 2.10.47214881.1 Sudan Soba	K S A K	E L D   			5		A + + + + + + + + + + + + + + + + + + +		м	Y T F 	K K      		K P 5	K V       	L F E - 0 1 . 0	K K       	E V G K P + + + + + + + + + + + + + + + + + + +	N N	A         K         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -	e i v
11.47214071 Maufania 2.244, 546251 Reflec 2.347214091 Maufania 3.47214091 Potogai 3.47214091 Potogai 3.47214091 Potogai 4.5.47214091 Tuniae Norhem 2.7.47214091 Tuniae Norhem 2.10.47214091 Tuniae Norhem 2.10.47214091 Sudar Sola 2.11.47214091 Sudar Sola	K S A K	E L D 			5		A		м	Y T F 	K K 		K. P. S	K V       	L F E - 0 - 0 - 0 - 0 - 0 - 0 - 0 - 0	K K       	E V G K P		A         K         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -	e i v
11.472149073 Maultania 22.344,540923 Maultania 23.472349931 Maultania 24.472349931 Perkagai 25.47234923 Perkagai 25.472349053 Maultania 25.472349053 Maultania 25.472349053 Maultania 25.472349053 Maultania 25.472349053 Maultania 25.472349053 Maultania 25.472349053 Maultania 21.472349533 Maultania 21.472349533 Maultania 21.472349533 Maultania	K S A K	E L D 		E D P . T . T . T . A . A . A . A . A	5		A = 0 A = 0		м	Y T F 	<b>K K</b>		K P 5		L F E 1 . 0 1 . 0 1 . 0 1 . 0 1 . 0 1 . 0 1 . 0		E V G R P 	N N		e i v
York 2014071 Maximia     X 2014071 Maximia     X 2014071 Maximia     X 2014071 Maximia     X 2014071 Portugal     X 47214071 Portugal     X 47214071 Portugal     X 47214071 Portugal     X 47214071 Maximia     X 47214071     X 4721407     X 47214     X 4721407     X 47214     X 47214     X 47214     X 4721     X 4721	K S A K	E         L         D           -         -         - <th></th> <th>E D P </th> <th>5</th> <th></th> <th>A</th> <th></th> <th>м</th> <th>Y T F </th> <th><b>K K</b></th> <th></th> <th>K P S</th> <th></th> <th>L F E</th> <th></th> <th>· · · · · · · · · · · · · · · · · · ·</th> <th>DK</th> <th>A         F         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -</th> <th>e i v</th>		E D P 	5		A		м	Y T F 	<b>K K</b>		K P S		L F E		· · · · · · · · · · · · · · · · · · ·	DK	A         F         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -	e i v
11.47214907.3 Maxilania V2.344.590521 Million V2.344.590521 Million V2.472349201 Potogal V5.472349251 Potogal V5.472349251 Maxilania V5.472349251 Maxilania V5.472349251 Maxilania V5.472349251 Maxilania V5.472349251 Maxilania V1.04721493431 fundia Nothern V1.04721493431 fundia Nothern V1.04721493431 fundia Nothern V1.04721493431 fundia Nothern V1.0472149341 Maxilania V1.0472149341 Maxilania V1.0472149341 Maxilania V1.0472149341 Maxilania V1.0472149341 Maxilania	K S A K 	E         L         D           4         4         4           5         6         4           5         6         4           5         6         4           5         6         6           7         7         7         7           6         7         7         7           7         7         7         7           8         7         7         7           9         7         7         7           9         7         7         7           9         7         7         7           9         7         7         7           9         7         7         7           9         7         7         7           9         7         7         7           9         8         7         7           9         8         8         7           9         8         8         7           9         8         8         8           9         8         8         8           9         8         8		· T · T · T · A · T · A · T · A · T · A · T · A · T · A · T · T · · T · · T · · · T · · · · · ·	5		A = 0 A = 0		м	Y T F 			K. P. S		L F E T . 0 T . 0	K, K        -	· · · · · · · · · · · · · · · · · · ·		A F Y L 	e i v
11.472148013 Mauliania 2.234 (2008) Mauliania 2.3.47234803 Mauliania 2.4.47234803 Mauliania 2.4.47234803 Pentagai 2.4.47234803 Mauliania 2.5.47234803 Mauliania 2.5.47234803 Tunian Konhem 2.6.47234880 Tunian Konhem 2.10.47234803 Tunian Konhem 2.11.47234803 Tunian Konhem 2.12.47234803 Tunian Konhem 2.12.47234803 Tunian Konhem 2.13.47234803 Tunian Konhem 2.14.47234803 Tunian Konhem		E L D 		0 0 7 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	5		A = 0 A = 0		м	Y T F	E. K.		K. P. S		L F E T . 0 T . 0			DK		e i v
11.47214907.3 Maxilania V2.344.590521 Million V2.344.590521 Million V2.472349201 Potogal V5.472349251 Potogal V5.472349251 Maxilania V5.472349251 Maxilania V5.472349251 Maxilania V5.472349251 Maxilania V5.472349251 Maxilania V1.04721493431 fundia Nothern V1.04721493431 fundia Nothern V1.04721493431 fundia Nothern V1.04721493431 fundia Nothern V1.0472149341 Maxilania V1.0472149341 Maxilania V1.0472149341 Maxilania V1.0472149341 Maxilania V1.0472149341 Maxilania	K         S         A         K           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -           -         -         -         -         -	E         L         D			5		A = 0 A = 0		м				K.P.S	K         V           1         2           2         3           2         3           2         3           2         3           2         3           3         3           3         3           3         3           3         3           3         4           3         4	L F E 1 . 0 1		1         2         2         2           4         4         4         4         4           4         4         4         4         4           4         4         4         4         4           4         4         4         4         4           4         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4           1         4         4         4         4	0 K 0 K	A         E         Y         L           -         -         -         -           -         -         -         -           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -         -         -         -         -         -         -         -           -	e i v
11.472149011 Maufania 2.214,246025 RefSec 2.347214901 Maufania 2.47214901 Potagai X.6.47214901 Potagai X.6.47214901 Potagai X.6.47214901 Naviania 2.6.47214901 Naviania 2.10.47214901 Naviania 2.10.47214901 Studen Soba 2.10.47214903 Studen Soba 2.10.47214903 Studen Soba 2.10.47214903 Studen Soba 2.10.47214903 Studen Soba 2.10.47214931 Balania 2.10.47214931 Balania 2.10.47214931 Studen Soba		E         L         D		· · · · · · · · · · · · · · · · · · ·	5		A						K. P. S			K         K           -         -		0 K 0 K	A         F         Y         L	e i v
VI. A2214921 X Musiliania     Z. 204, 940825 X Musiliania     Z. 204, 940825 X Musiliania     Z. A22349821 Perkugal     X. 4. A2244921 Perkugal     X. 4. A2244925 X Musiliania     X. 4.		E         L         D           1         1         2           2         2         2           3         2         2           4         2         2           5         2         2           7         2         2           8         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           9         2         2           10         3         3           10         3         3           10         3         3           10         4 <tr< th=""><th></th><th>· · · · · · · · · · · · · · · · · · ·</th><th>5</th><th></th><th>A</th><th></th><th></th><th></th><th></th><th></th><th>K. P. S</th><th></th><th>L F E T . 0 T . 0</th><th>K         K           -         -</th><th>2         2</th><th>0 K 0 K</th><th></th><th>e i v</th></tr<>		· · · · · · · · · · · · · · · · · · ·	5		A						K. P. S		L F E T . 0 T . 0	K         K           -         -	2         2	0 K 0 K		e i v
V 1. A221407.1 Maufanie     V. 2. Na., Skille S. 1. Maufanie     V. 2. Na., Skille S. 1. Maufanie     V. 3. A2734893.1 Maufanie     V. 4. A2724893.1 Portugal     V. 5. A2724895.1 Portugal     V. 5. A2724895.1 Portugal     V. 5. A2724895.1 Portugal     V. 5. A2724895.1 Turkias Northen     V. 4. A2724895.1 Turkias Northen     V. 3. A2724895.1 Turkias Northen		E         L         D           -		· · · · · · · · · · · · · · · · · · ·	5		A						K. P. S				2         2	0 K 0 K	A         F         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -         -         -         -         -         -         -         -           -	e i v
York 201407.1 Maximia     X 201407.1 Maximia     X 201407.1 Maximia     X 201407.1 Maximia     X 201407.1 Portugal     X 47214807.1 Maximia     X 47214807.1 Maximia     X 47214807.1 Maximia     X 47214807.1 Maximia     X 47214807.1 Surjan     X 47214807.1 S		E         L         D           -		· · · · · · · · · · · · · · · · · · ·	5		A						K P 5				2         2	0 K 0 K	A         F         Y         L           -	e i v
VI. A2214907.3 Maximie     V. 2. 204, 94092.5 Maximie     Z. 204, 94092.5 Maximie     Z. 204, 94092.5 Markine     VI. A221499.3 Maximie     VI. A221499.3 Maximie     VI. A221499.5 Maximie     VI. A42149.2 Maximie     VI. A42149.2 Maximie     VI. A420149.5 Maximie     VI. A42149.2 Maximie     VI. A420149.5 Maximie     VI. A4		E         L         D		· · · · · · · · · · · · · · · · · · ·	5		A A A						K P 5				2         2	0 K 0 K	A         F         Y         L           -	e i v
York 201407.1 Maufania     Z. 2014, 540325.1 Maufania     Z. 2014, 540325.1 Maufania     Z. 47274892.1 Maufania     X. 4 4721482.1 Protogal     X. 4721483.1 Trutogal     X. 4721483.1 Trut		E         L         D	8         -           5         -           6         -           5         -           5         -           7         5           8         -           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5           9         5	· · · · · · · · · · · · · · · · · · ·	5		A								L F E T - 0 T	L         L         L           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           2         2         2         2           3         2         2         2           4         2         2         2           5         2         2         2           4         2         2         2           5         2         3         2           6         2         2         2           7         3         3         3           8         3         3         3           9         3         4         3           9         3         3	2         2	0 K 0 K	A         K         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -	e i v
York 201407.3 Maufania     York 201407.3 Maufania     Zong Subliks 1 Maufania     Zong Subliks 1 Maufania     Zong Subliks 1 Perkagal     Xe 24724827.1 Turkins Monthem     Xe 24724828.1 South Concrete     Xe 244828.1 South Concrete     Xe 24724828.1 South Concrete     Xe 24724828.1 South Concrete     Xe 24724828.1 South Concrete     Xe 24724828.1 Southem     Xe 24724828.1 Turken     Xe Xee		E         L         D		· · · · · · · · · · · · · · · · · · ·	5		A A A						<u>K P</u> 5			K         K         K           -         -         -         -           -         -         -         -         -           -         -         -         -         -         -           -	2         2	0 K 0 K	A         K         Y         L           -	-         -
York 201407.1 Maufania     Z. 2014, 540325.1 Maufania     Z. 2014, 540325.1 Maufania     Z. A. 272 4899.1 Maufania     Z. 4. A72 4899.1 Maufania     Z. 4. A72 4892.1 Protugal     X. 4. A72 4892.1 Protugal     X. 5. A72 4852.1 Maufania     Z. 7. A72 4852.1 Maufania     Z. 7. A72 4852.1 Turinia: Northern     Z. 4. A72 4891.1 Turinia: Northern     Z. 14. A72 4891.1 Turinia: Northern     Z. 4. A72 4891.1 Turinia: Northern     Z. 4. A72 4891.1 Turinia: Northern     Z. 4. A72 4891.1 Turinia: Northern     Z. 14. A72 4891.1 Turinia: Northern     Z. 24. A72 4892.1 Turinia: Northern     Z. 24. 472 4892.1 Solaris     Z. 24. 472 4892.1 Solaris     Z. 24. 472 4892.1 Turinia: Northern     Z		E         L         D		· · · · · · · · · · · · · · · · · · ·	5		A A A						<u>K P</u> 5		L F 6 T - 0 T	K         K           -         -	2         2	0 K 0 K	A         F         Y         L           -	-         -
X1 AF214971 Maultanie     X2 XM 244921 Maultanie     Z XM 244921 Maultanie     Z XM 244921 Maultanie     X4 AF214921 Portugal     X4 AF214921 Portugal     X5 AF214921 Portugal     X5 AF214921 Tovinia Monthem     X6 AF214921 Tuvinia Monthem     X6 AF214921 Tuvinia Monthem     X6 AF214921 Tuvinia Monthem     X1 AF214921 Tuvinia Monthem     X2 AF		E         L         D		· · · · · · · · · · · · · · · · · · ·	5	555555 5555 555775 555 5 <b>PP PAPA PANNA PPP A</b>	A A						<u>K P</u> 5		L F E T - 0 T	K         K         K           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           3         -         -           2         -         -           3         -         -           4         -         -           5         -         -           6         -         -           7         -         -           8         -         -           9         -         -           10         -         -           11         -         -           12         -         -           13         -         -           14         -         - <th>2         2</th> <th>0 K 0 K</th> <th>A         K         Y         L           -</th> <th>-         -</th>	2         2	0 K 0 K	A         K         Y         L           -	-         -
York 201407.3 Maximie     York 201407.3 Maximie     Z. 2014, 94032.5 Maximie     Z. 2014, 94032.5 Marking     Z. 2014, 94032.5 Marking     Z. 2014, 9403.5 Maximie     Z. 4721498.5 Maximie     X. 4721482.5 Maximie     Z. 4721498.5 Maximie     Z. 4721492.5 Maximie     Z. 4721492.5 Maximi     Z. 4721492.5 Maxim     Z. 4721492.5 Maximi     Z. 4721492				· · · · · · · · · · · · · · · · · · ·	5	5555555 8555 8555 85 PP PAPA PANNA PPP 44	I         I         I           A         I         I						K P S			K         K         K           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           2         -         -           3         -         -           4         -         -           5         -         -           6         -         -           6         -         -           7         -         -           8         -         -           9         -         -           10         -         -           11         -         -           12         -         -           13         -         -           14         -         -           15         -         -           16         -         -<	2         2	0 K 0 K	A         F         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -	-         -
11. A721490.7.1 Maufania 2.2 MJ, SABDES I. Marking 2.3 A723499.3.1 Maufania 2.4 A723499.3.1 Maufania 2.4 A723492.1 Portugal 2.5 A721492.1 Portugal 2.5 A721492.1 Portugal 2.7 A721492.1 Turkis Northen 2.7 A721492.1 Turkis Northen 2.8 A723491.3 Units Northen 2.10 A721492.1 Sudan Soba 2.11 A721492.1 Sudan Soba 2.11 A721492.1 Sudan Soba 2.13 A721479.1 Bahasin 2.14 A721492.1 Sudan Soba 2.14 A721492.1 Sudan Soba 2.14 A721492.1 Sudan Soba 2.15 A721479.1 Bahasin 2.16 A721492.1 Sudan Soba 2.17 A400004.1 SicLaka Potomato 2.16 A721492.1 Sudan Soba 2.17 A400015.1 SicLaka Anomato 2.18 A721479.1 Bahasin 2.18 A721479.1 Bahasin 2.18 A721479.1 Bahasin 2.17 A721479.1 Bahasin 2.17 A721479.1 SicLaka Potomato 2.21 A721479.1 SicLaka Potomato 2.21 A721479.1 SicLaka Potomato 2.21 A721479.1 SicLaka Potomato 2.21 A721479.1 Turkin Mothem 2.21 A721479.1 Turkin Mothem 2.21 A721479.1 Turkin Mothem 2.21 A721479.1 Turkin Mothem				· · · · · · · · · · · · · · · · · · ·	5		A A						K P 5		L F E T . C T		2         2	0 K 0 K	A         K         Y         L           -         -         -         -         -           -         -         -         -         -         -           -         -         -         -         -         -         -           -	-         -
VI. A221490.7 Maximie     V. 2. 204, SM025.1 Maximie     V. 2. 204, SM025.1 Marking     V. 2. A273499.1 Maximie     V. 4. A221492.1 Protugal     V. 4. A221492.1 Protugal     V. 5. A221495.1 Protugal     V. 5. A221495.1 Protugal     V. 5. A221495.1 Turkine Northern     VI.5. A221495.1 Solven     VI.5. A221495.1 Turkine Northern     VI.5. A22149				· · · · · · · · · · · · · · · · · · ·	5	5555555 8555 8555 85 PP PAPA PANNA PPP 44	I         I         I           A         I         I						K P S				2         2	0 K 0 K	A         K         Y         L           -	-         -

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-GTAACCTTTAAAAAC-GT-3, Reverse 5-CAGTTACGAACATGGGGTTT-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 distribution, 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, 23]. Cross-immunity studies conducted by Leeman [22] 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).

#### References

- Santos M, Soares R, Costa P, et al. (2013): Revisiting the Tams1-encoding gene as a species-specific target for the molecular detection of Theileria annulata in bovine blood samples. Ticks Tick Borne Dis 4: 72-77.
- Elsify A, Sivakumar T, Nayel M, et al. (2014): An epidemiological survey of bovine Babesia and Theileria parasites in cattle, buffaloes, and sheep in Egypt. Parasitol Int [Epub ahead of print].
- Meng K, Li Z, Wang Y, et al. (2014): PCR-based detection of Theileria annulata in Hyalomma asiaticum ticks in northwestern China. Ticks Tick Borne Dis 5: 105-106.
- Gubbels MJ, Katzer F, Hide G, et al. (2000): Generation of a mosaic pattern of diversity in the major merozoite-piroplasm surface antigen of Theileria annulata. Mol Biochem Parasitol 110: 23-32.
- 5. Gharbi M, Darghouth MA, Weir W, et al. (2011): Primeboost immunisation against tropical theileriosis with two

parasite surface antigens: Evidence for protection and antigen synergy. Vaccine 29: 6620-6628.

- 6. Boulter N, Hall R (1999): Immunity and vaccine development in the bovine theilerioses. Adv Parasitol 44: 41-97.
- Weir W, Karagenç T, Baird M, et al. (2010): Evolution and diversity of secretome genes in the apicomplexan parasite Theileria annulata. BMC Genomics 11: 42.
- Esmaelizad M, Niaraki S J, Fesharaki R H (2011): Molecular and phylogenetic analysis of the partial tams1 gene sequence of a vaccine strain of Theileria annulata. Braz Arch Biol Tech 54: 1109-1116.
- Kim SJ, Tsuji M, Kubota S, et al. (1998): Sequence analysis of the major piroplasm surface protein gene of benign bovine Theileria parasites in East Asia. Int J Parasitol 28: 1219-1227.
- Katzer F, McKellar S, Kirvar E, Shiels B (1998): Phylogenetic analysis of Theileria and Babesia equi in relation to the establishment of parasite populations within novel host species and the development of diagnostic tests. Mol Biochem Parasitol 95: 33-44.
- 11. Pan W, Gao Y, Qin L, et al. (2012): Genetic diversity and phylogenetic analysis of glycoprotein GP85 of ALV-J isolates from Mainland China between 1999 and 2010: coexistence of two extremely different subgroups in layers. Vet Microbiol 156: 205-212.
- Thompson JD, Higgins DG, Gibson TJ (1994): CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673-4680.
- Saitou N, Nei M (1987): The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.
- Tamura K, Dudley J, Nei M, Kumar S (2007): MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24: 1596-1599.
- 15. Chen XL, Shi T, Yang J, et al. (2014): N-glycosylation of effector proteins by an  $\alpha$ -1,3-mannosyltransferase is required for the rice blast fungus to evade host innate immunity. Plant Cell 26: 1360-1376.
- He P, Srikrishna G, Freeze HH (2014): An N-glycosylation deficiency reduces ICAM-1 induction and impairs inflammatory response. Glycobiology 24: 392-398.
- Auguste AJ, Carrington CV, Forrester NL, et al. (2014): Characterization of a novel Negevirus and a novel Bunyavirus isolated from Culex (Culex) declarator mosquitoes in Trinidad. J Gen Virol 95: 481-485.
- Ghoneim AM, El-Fayomy AO (2014): Targeting tams-1 gene results in underestimation of Theileria annulata infection in diseased cattle in Egypt. Acta Parasitol 59: 85-90.
- Habibi G (2013): A comparative phylogenetic analysis of Theileria spp. by using two "18S ribosomal RNA" and "Theileria annulata merozoite surface antigen" gene sequences. Arch Razi 68: 47-52.
- Sivakumar T, Hayashida K, Sugimoto C, Yokoyama N (2014): Evolution and genetic diversity of Theileria. Infect Genet Evol 27: 250-263.
- 21. Katzer F, McKellar S, Ferguson MA, et al. (2002): A role for tertiary structure in the generation of antigenic diversity and molecular association of the Tams1 polypeptide inTheileria annulata. Mol Biochem Parasitol 122: 55-67.
- 22. Thumbi SM, Bronsvoort BM, Poole EJ, et al. (2014): Parasite co-infections and their impact on survival of indigenous cattle. PLoS One 9: e76324.

- Leemans I, Brown D, Hooshmand-Rad P, et al. (1999): Infectivity and cross-immunity studies of Theileria lestoquardi and Theileria annulata in sheep and cattle: I. In vivo responses. Vet Parasitol 82: 179-192.
- Cooke CL, Huff JL, Solnick JV (2005): The role of genome diversity and immune evasion in persistent infection with Helicobacter pylori. FEMS Immunol Med Microbiol 45: 11-23.