Chapter 7

Genetic and Biological Properties of Original TBEV Strains Group Circulating in Eastern Siberia

I.V. Kozlova, M.M. Verkhozina, T.V. Demina, Yu.P. Dzhioev, S.E. Tkachev, L.S. Karan, E.K. Doroshchenko, O.V. Lisak, O.V. Suntsova, A.I. Paramonov, O.O. Fedulina, A.O. Revizor and V.I. Zlobin

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/54087

1. Introduction

In the XXI century tick-borne encephalitis (TBE) remains the most distributed severe natural foci infection transmitted by Ixodes ticks bite.

The causative agent of this infection is tick-borne encephalitis virus (TBEV). According to the modern classification it belongs to the group of mammal viruses transmitted by ticks, and is a member of the genus Flavivirus of the family Flaviviridae [23]. As a result of numerous studies devoted to TBEV genetic variability it was divided into three genotypes (subtypes): 1) genotype 1 (Far-Eastern (FE) subtype); 2) genotype 2 (European subtype); 3) genotype 3 (Siberian subtype). Each genotype is believed to distribute in its certain area where its absolute domination is observed [10, 20].

In Eastern Siberia the circulation of three TBE virus genotypes with the domination of genotype 3 was identified by the prior study. Moreover, the unique TBEV strains (886-84 and 178-79), which differed in genetic structure from all known TBEV genotypes, have been found on this territory [10].

Currently, with use of molecular hybridization of nucleic acids (MHNA) method with genotype-specific probes and sequencing of complete virus genome or its fragments we identified the group of 13 strains with high homology level to 886-84 strain that was

conventionally defined as "group 886" [6]. The obtained results confirm the validity of "group 886" certification as possible separate TBEV genotype.

The unique genetic structure of "group 886" strains also manifests in original phenotype pattern that is quite significant from the scientific point of view. "Group 886" strains can be tested as prototype candidate strains for the design of universal vaccines effective against strains of different serotypes (genotypes) and TBEV test-systems.

The aim of the study was to investigate genetic and biological properties of TBEV "886 group" strains circulating in Eastern Siberia (territories of Irkutsk region, Buryat Republic, Transbaikalia) for estimation of their potential as candidates for test-systems and vaccine development.

2. Materials and methods

2.1. TBE virus

Thirteen TBEV strains from the collection of FSSFE "Scientific Centre of Family Health and Human Reproduction Problems, Institute of Epidemiology and Microbiology SB RAMS", Irkutsk were investigated in the study. By MHNA genotyping and full genome or fragments sequencing they were classified as "group 886" strains. Detailed information about strains is presented in Table 1.

Strain	The year of isolation	Isolation source	The location of sample collection
886-84	1984	*Myodes (Clethrionomys) rutilus*	Irkutsk region, Ekhirit-Bulagatskiy district
711-84	1984	*Myodes rufocanus*	Buryat Republic, Barguzinskiy district
740-84	1984	*Myodes rufocanus*	Buryat Republic, Bichurskiy district
712-89	1989	*I. persulcatus*	Transbaikalia, Krasnochikoyskiy district
780-89	1989	*I. persulcatus*	Buryat Republic, Bichurskiy district
617-90	1990	*I. persulcatus*	Buryat Republic, Bichurskiy district
636-90	1990	*I. persulcatus*	Buryat Republic, Bichurskiy district
608-90	1990	*I. persulcatus*	Buryat Republic, Bichurskiy district
606-90	1990	*I. persulcatus*	Buryat Republic, Bichurskiy district
691-90	1990	*I. persulcatus*	Buryat Republic, Bichurskiy district
418-90	1990	*I. persulcatus*	Transbaikalia, Krasnochikoyskiy district
733-90	1990	*I. persulcatus*	Transbaikalia, Krasnochikoyskiy district
742-90	1990	*I. persulcatus*	Transbaikalia, Krasnochikoyskiy district

Table 1. Information concerning "group 886" strains of TBE virus, isolated on the Eastern Siberia territory.

http://dx.doi.org/10.5772/54087

2.2. Strains genotyping

We used MHNA with three panels of 40 deoxyoligonucleic probes complemented to fragments of 10 genes of different TBEV genotypes. The probe description and their localization in TBEV genome was presented earlier by Demina *et al*. [6].

The total RNA extraction from infected mice brains or porcine embryo kidney cells, applying RNA onto kapron or cellulose nitrate filters and hybridization with probes were performed by the common methods [16].

The amplification was carried out with primers complemented to 5'- UTR fragment, to C-prM-E-NS1 genes, E gene or E-NS1 genes fragments, synthesized in the Institute of Chemical Biology and Fundamental Medicine SB RAS (Novosibirsk, Russia). RT-PCR was performed according to the "BioSan" company (Novosibirsk, Russia) protocol.

The sequence analysis of PCR products was carried out with BigDye Terminators Cycle Sequencing Kit v.3.1 (Applied Biosystems, USA) in DNA Sequencing Center SB RAS, Novosibirsk, Russia. The obtained data was analyzed by Mega 5.0 program [28]. The gene fragments sequences of TBEV strains belonging to the different genetic types from GenBank database were used as a material for comparison. BLAST program (http://www.ncbi.nlm.nih.gov/blast/) was used for homology search of obtained nucleotide sequences with already known fragments of TBEV genomes.

The genome fragments sequences of "group 866" strains obtained during the study have been deposited into GenBank database with access numbers EF469662, EU878281-EU878283, JN936341, JN936347, JN936349-JN936350, JN936353-JN936355.

The sequencing of full genome of 886-84 strain has been performed by Karan *et al*. in Central Research Institute of Epidemiology of Rospotrebnadzor RF, Moscow, Russia.

2.3. Strains immunotyping

The reaction of diffuse precipitation in agar (RDPA) was carried out by the method developed by Clark [12] with modifications by Rubin [27] and Bochkova [2]. We used immune sera against TBEV prototype strains of three serotypes (Sofjin – Far-Eastern serotype, 256 – Western serotype, Lesopark-11 and Aina/1448 – East-Siberian serotype) exposed to dosed adsorbtion with concentrated cultural antigens or cross-adsorbed sera against investigated strains [4].

The cytoplasmatic activity study was performed according to common methods. Virus titers were determined in tests on cell culture based on its cytopathic activity (CPA) by the full cumulative method (offered by Reed and Muench) and expressed as lg TCD_{50} [1]/ml [26].

2.4. Neuroinvasiveness

To estimate the neuroinvasiveness of TBEV strains we determined the index of invasiveness (II) – the difference between the virus titers after intracerebral (mNic) and subcutaneous

1 Tissue cytopathogenic dose

(mNsc) mice inoculation expressed as lg LD_{50}/ml [18]. Nonlinear mice (6-8 g in weight) were infected into brain with 0.03 ml or subcutaneously with 0.25 ml of inoculate. Animals infected intracerebrally were observed during 14 days while animals infected subcutaneously were observed during 21 days. Virus titers were detected by Reed and Muench method. The values of II 1-2.5 meant the high invasive activity of the virus, i.e. the ability of virus to overcome the blood-brain barrier to reach central nervous system (CNS) and propagate in it. The values of invasiveness index of equal or more than 3 indicated the lesser invasive activity of the virus strain.

2.5. Thermoresistance

Thermoresistance (T^{50}) of TBEV strains was tested by Ovchinnikova *et al.* method [17] using 24-hour cell culture grown in 96-hole plates at the presence of CO_2. The thermoresistance was determined by inactivation index – difference in lg of titers of virus samples heated at 50°C during 15 minutes or unheated (4°C). In case of titers difference equal or less than 2.0 lg the strain was characterized as T^{50+}, from 2.1 to 3.0 lg – as medium, equal or more than 3.1 lg – as T^{50-}.

2.6. Rct_{42}–feature

Rct_{42}-feature describes the ability of the virus to propagate at supraoptimal temperature. To determine rct_{42} the 24-hour cell culture grown in 96-hole plates was infected by different virus-containing suspensions (10^{-1} to 10^{-10}). One part of the cell cultures was infected with selected virus strain and incubated at 37°C, and other cells were infected with the same strain and incubated at 42°C at the presence of CO_2. Rct_{42} was determined on the sixth day after infection as a difference between lg of virus titers after the cultivation in cells at 37°C and 42°C. In case of titers difference equal or less than 2,0 lg the strain was characterized as rct_{42}^{-}, from 2.1 to 3.0 lg – as medium, equal or more than 3.1 lg – as rct_{42}^{+}.

2.7. S-feature

The cell culture was infected with TBEV strains undergone not more than 4 passages through the white mice brains and 3 cycles of cloning. The plaques appeared on the third or fourth day. The plaque size measuring was performed on the fifth day when they increased and become more sharp and transparent. S-feature was determined as S+ if plaque had the diameter (d)≥2.5 mm; S± at 2.5>d ≥2,0 mm; S– at 2.0>d ≥1,0 mm.

3. Results and discussion

For the first time the uniqueness of 886-84 strain was found during the investigation of its serological properties. Trukhina suggested that this strain takes the intermediate place between two TBEV serotypes – East-Siberian and Far-Eastern and shows the properties of both serotypes [21].

http://dx.doi.org/10.5772/54087

Then, 886-84 strain was described as a representative of the independent genotype according to criteria developed by our team after the comparing of difference level of 29 strains isolated on different territories of TBEV area [8]. In this study the fragment of E protein gene (positions 567-727 br) was used as a model. It was found that corresponding amino acid sequence of this fragment in 886-84 strain has Leu in position 206 as genotype 3 and Asp in position 234 as genotypes 1 and 2 [10]. At that time we did not find any homologous strains and isolates so the additional data were necessary to separate this TBEV strain into independent genotype.

Comparison of the strain 886-84 complete genome sequence (EF469662) with TBEV sequences available in GenBank has shown that it forms an independent branch and does not cluster with any strains of three main genotypes (Fig.1). It should be noted that nucleotide substitution level was close to the species separation border [11] (Table 2).

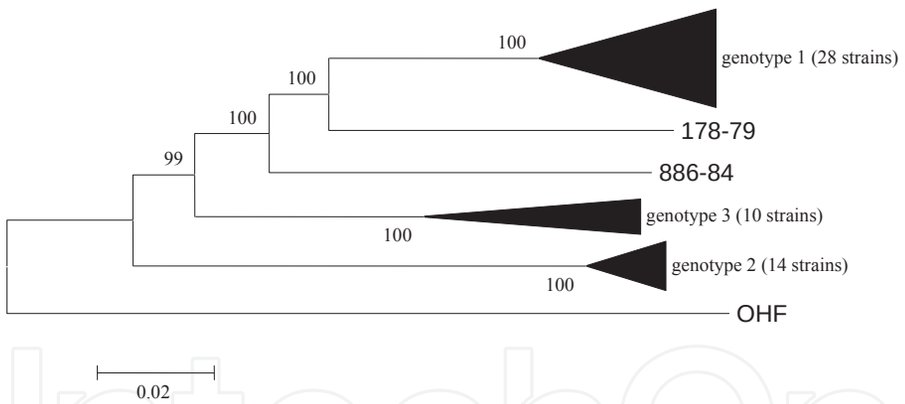

Figure 1. Phylogenetic tree demonstrating the genetic similarity level of 54 TBEV strains on the base of polyprotein coding region sequences (10242 nr). Genotype 1 cluster - Sofjin [25], AB022703, AB001026, DQ989336, AY182009, AY217093, JF316707, JF316708, FJ997899, EU816450-EU816455, AY169390, FJ906622, GQ228395, FJ402885, FJ402886, DQ862460, GU121642, HQ201303, HQ901367, HQ901366, HM859894, HM859895, JN003205; Genotype 2 cluster - TEU27495, TEU27491, TEU39292, AF091010, EU106868, DQ401140, GV266392, HM535610, HM535611, HM120875, GU183379-GU183381, GU183383; Genotype 3 cluster - L40361, AF527415, DQ486861, FJ968751, JN003206-JN003209, GU183382, GU183384. OHF – Omsk haemorrhagic fever virus.

Nucleotide substitution level (%) (coding region of polyprotein, 10242 nr)			
	genotype 1	genotype 2	genotype 3
genotype 1	4,3		
genotype 2	16,4	2,3	
genotype 3	14,4	15,2	5,4
178-79	11,0	16,0	14,1
886-84	12,5	15,6	13,7
Amino acid substitution level (%) (complete amino acid sequence of polyprotein, 3414 ar.)			
genotype 1	1,3		
genotype 2	6,9	0,9	
genotype 3	5,3	6,2	1,9
178-79	3,1	6,1	5,2
886-84	3,9	6,0	4,2

Comments: The level of nucleotide and amino acid substitutions within the genotype is marked with grey.

Table 2. Nucleotide and amino acid substitution level between different TBEV genotypes and strains 178-79 and 886-84.

The analysis of complete amino acid sequence of strain 886-84 polyprotein confirmed that it's the unique "mixture" of sequences common for genotypes 1, 2 and 3. For example, in the set of 22 positions which clearly differentiate all known TBEV strains into three genotypes the unique amino acids (alanine (A) in position C-108, serine (S) – NS2A-127 and glycine (G) – NS3-258) or interchange with amino acids typical for main TBEV genotypes were found in strain 886-84 polyprotein sequence [7] (Fig. 2).

protein	C		E		NS1			NS2A					NS3			NS4B			NS5			
Position number	3	108	206	317	54	141	285	100	127	174	175	225	126	258	376	21	28	96	18	297	671	832
Genotype 1	G	V/L/I	S	I/T	T	S/G	R	N	A	M/V	L	I/V	I	V	I/T	H	E	A/R	G	R	V/G	A/V/T
Genotype 2	K	I	V	A	S	Q	T	S	D/E	V	C	A	L/I	A	A	R/Q	S	T	N	E/A	L	M
Genotype 3	R	T	L	T	N	G	K	G/S	G	I/v	I/F	T	M/T	M/V/A	V	Q	G	S	S	G/R	I	T/A
178-79	R	V	S	T	T	S	K	N	G	M	L	T	I	V	V	Q	G	A	S	G	V	A
886-84	R	A	L	I	N	S	K	S	S	M	L	A	I	G	V	Q	G	A	S	G	V	A

Figure 2. The differences in 22 positions obtained by comparing of 54 TBEV strains polyprotein sequences; **Comments.** The cells marked with grey identify the amino acid residues corresponding to one of four TBEV genotypes. The unique amino acid for strain 886-84 is marked with black.

http://dx.doi.org/10.5772/54087

Thirty unique substitutions were detected in strain 886-84 polyprotein which could probably be the "genotype-specific" for "group 886" members. However, since studied polyprotein fragment for "group 886" strains was 1066 ar in length the "genotype-specific" uniqueness was confirmed only for 6 substitutions of 30 (Table. 3)

Protein	C		M	E		NS1
Position in polyprotein	98	108	270	688	735	898
Position in protein			158	408	455	122
genotype 1	A	V/L/I	V	K	L	S
genotype 2	A	I	V	K	L	S
genotype 3	T	T	V	K	L/M	S
178-79	A	V	V	K	L	S
"group 886"	V	A	A	R	I	A

Comments: genotype 1 is presented by translated nucleotide sequences X07755, AB022703, AB001026, DQ989336, AY182009, AY217093, JF316707, JF316708, FJ997899, EU816450-EU816455, AY169390, FJ906622, GQ228395, FJ402885, FJ402886, DQ862460, GU121642, HQ201303, HQ901367, HQ901366, HM859894, HM859895, JN003205; genotype 2 - TEU27495, TEU27491, TEU39292, AF091010, EU106868, DQ401140, GV266392, HM535610, HM535611, HM120875, GU183379- GU183381, GU183383; genotype 3 - L40361, AF527415, DQ486861, FJ968751, JN003206-JN003209, GU183382, GU183384. "Group 886" is presented by prototype strain sequence EF469662 (strain 886-84) and GenBank deposited (EU878281-EU878283) and non-deposited 617-90, 711-84 и 740-84 TBEV strains genome fragments.

Table 3. Unique substitutions in the polyprotein fragments (1066 ar) (proteins C, M, E and part of NS1) of TBEV "group 886" strains.

At present, using MHNA and sequencing methods we have found the group of 13 TBEV isolates with highly homologous genetic structure to the strain 886-84. For eight strains the genome fragments (1650 nr in length) coding proteins C, M, and E protein fragment were determined (GenBank accession numbers EF469662, EU878281-EU878283, JN936341, JN936347, JN936349-JN936350, JN936353-JN936355) (Fig.4). The homology level with the strain 886-84 genome sequence was 98.2-99.8% while the difference level with three main genotypes ranged from 13.1% (Sofjin) to 16.6% (Neudoerfl).

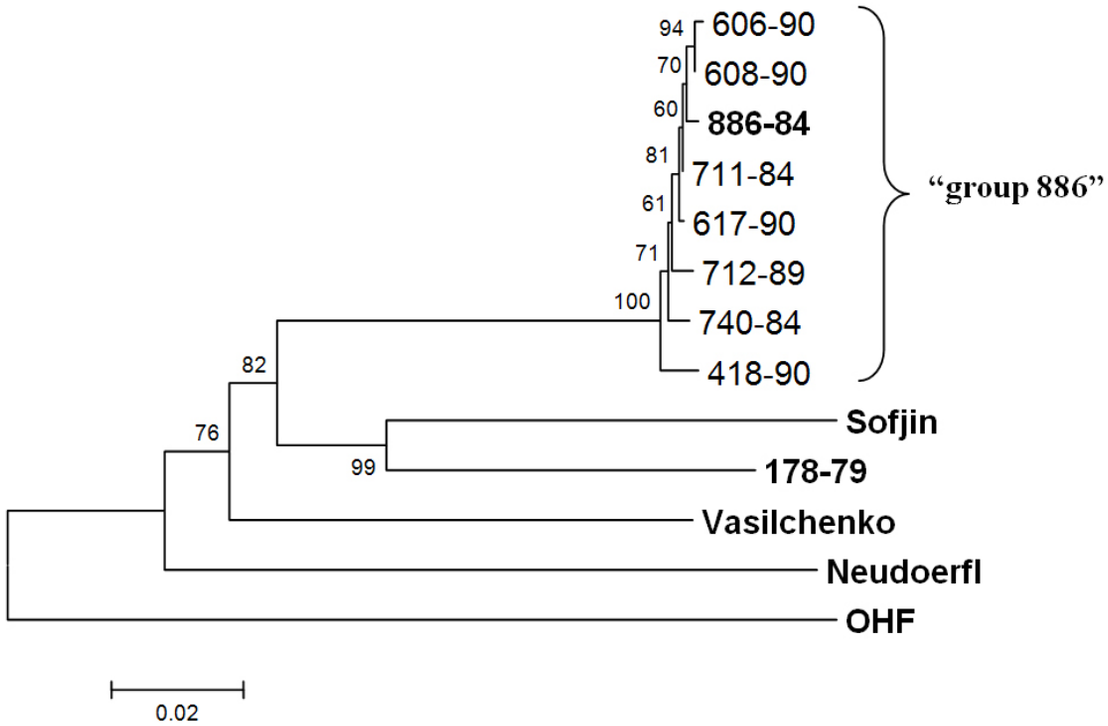

Figure 3. Phylogenetic tree (NJ, Kimura 2), based on TBEV genome fragments sequences (1650 nr in length) (proteins C, M, and E protein fragment).

Our study determined specific areas of habitat for TBEV "group 886" (Fig. 4).

Figure 4. TBEV "group 886" area of habitat.

http://dx.doi.org/10.5772/54087

The strains forming this TBE virus variant were isolated from samples collected in Irkutsk region, Buryat Republic and Transbaikalia in 1984-1990. Also it was recently reported about two "group 886" strains isolated on the territory of National Park "Alkhanai" in Duldurginskiy district, Transbaikalia from *I. persulcatus* tick (in 1999) and one strain from *Myodes rutilus* (in 2010) [1]. Moreover, the case of meningoencephalitis with lethal outcome was described in Bulganskiy aimak in Mongolia caused by TBEV isolate with genome fragment sequence similar to strain 886-84 [24].

The common feature of above-listed territories is the presence of several landscape forms that could provide rich biodiversity of flora and fauna. The combination of forest landscapes with steppe areas is typical for Ekhirit-Bulagatskiy district of Irkutsk region. Bichurskiy district of Buryat Republic is presented by mountain forest ecosystems as well as submountain and mountain-valley areas including submountain landscapes with local pine woods and steppe-meadows. Barguzinskiy district is located from Barguzin river mouth along Barguzin river valley in mountain-forest zone. Its middle part corresponds an "island" of steppe and forest-steppe landscapes in isolated mountain valley surrounded by mountain-forest area. Krasnochikoiskiy district of Transbaikalia is the eastern frontier of South-Siberian mountain landscape territory. The basic components of foci territories are similar to ones from Irkutsk region and Buryat Republic south, where the combination of mountain-forest, forest-steppe and steppe landscapes could be observed. The landscape of National Park "Alkhanai" is also very diverse and includes steppes, meadows, forests and rocky mountains. The National Park location on the border of Eurasia boreal forests and Dauria steppes has the special biospheric importance and results in the significant biodiversity because of flora and fauna interpenetration. Bulganskiy aimak of Mongolia located in Selenga river basin is characterized by forest-steppe, steppe, dry steppe zones and river valleys landscapes.

Also for "group 886" strains we obtained the data concerning their ecological connections with all elements of transmissible chain. Thus, strains 712-89, 418-90, 606-90, 608-90, 617-90, 636-90, 691-90, 733-90 and 742-90 were isolated from *I. persulcatus* ticks, and 711-84 and 740-84 from gray-sided vole brain.

The case of meningoencephalitis with lethal outcome described in Bulganskiy aimak in Mongolia was caused by TBEV isolate highly homologous to strain 886-84. So it demonstrates that this TBEV variant may play the role in human infectious pathology. Also the strains isolation during the long period of time (since 1984 to 2010) confirms the stability of its circulation on Eastern Siberia territory.

Therefore, "group 886" strains seem to possess all necessary characteristics to separate them into the independent TBEV genotype. Earlier we suggested that two TBEV strains 178-79 and 886-84 are not the members of three known genotypes forming their own branch on phylogenetic tree and may be the representatives of genotypes 4 and 5 [8, 9]. The presented data confirm and develop this hypothesis and also allow to separate "group 886" strains into the new genotype 5 on the base of their properties described in our work.

Along with the original genome sequences of "group 886" strains we investigated their phenotypic characteristics which are the essential part of the virus nature and properties study and useful for practical virology.

The pathogenic properties of viruses are the most important biological characteristics. Taking into account that TBEV "group 886" strains play the role in human infectious pathology [24] it was extremely important to study their pathogenic potential.

We have estimated the virulence level of TBEV "group 886" strains according two parameters: the average infectious virus titers after intracerebral or subcutaneous inoculation of mice. Peripheral virus activity was characterized by index of invasiveness (II) (Table 4).

Strain	Isolaion source	mNic (lgLD$_{50}$/мл)	mNsc (lgLD$_{50}$/мл)	mNic-mNsc	II
691-90	*I. persulcatus*	7,02	5,1	1,92	+
418-90	*I. persulcatus*	9¸72	7,8	1,52	+
886-84	*Myodes rutilus*	8,58	7,16	1,2	+
711-84	*Myodes rufocanus*	6,75	4,6	1,0	+
740-84	*Myodes rufocanus*	10,2	9,4	0,8	+
712-89	*I. persulcatus*	10,9	9,8	1,1	+
617-90	*I. persulcatus*	6,64	3,8	2,84	±
636-90	*I. persulcatus*	7,06	4,35	2,71	±
608-90	*I. persulcatus*	7,9	4,86	3,04	-
606-90	*I. persulcatus*	7,02	4,02	3,0	-

Table 4. The index of invasiveness (II) for TBEV "group 886" strains.

The virus titers after intracerebral inoculation of mice ranged from 6.64 to 10.9 lg LD_{50}/ml while after subcutaneous inoculation (peripheral activity) were found to be from 3.8 to 9.8 lg LD_{50}/ml. The determined virus indexes of invasiveness (II) had medium and high values (from 0.8 to 3.04 lg LD_{50}/ml). According to obtained results, six strains from "group 886" had high invasive properties that mean their ability to overcome the blood-brain barrier, penetrate into CNS and propagate in it. The highest invasive properties were observed in three strains isolated from rodents and in one strain isolated from tick collected in Krasnochikoiskiy district of Transbaikalia. Two strains (606-90 and 608-90) from Bichurskiy district, Buryat Republic had the lower neuroinvasive activity.

Additionally, to characterize the virulence of TBEV "group 886" strains we determined the average life time and lethality percent of infected mice (Table 5).

http://dx.doi.org/10.5772/54087

Strain №	Isolaion source	Life time (days ± m)	% of lethal cases (% ± m)
711-84	*Myodes rufocanus*	5,1±0,49	100
740-84	*Myodes rufocanus*	5,2±0,24	100
712-89	*I. persulcatus*	5,3±0,79	100
780-89	*I. persulcatus*	6,6±0,45	100
691-90	*I. persulcatus*	6,0±0,89	100
418-90	*I. persulcatus*	6,3±0,06	100
733-90	*I. persulcatus*	5,0±0,45	100
742-90	*I. persulcatus*	6,8±0,76	100
886-84	*Myodes rutilus*	6,1±0,35	93,8±1,91
606-90	*I. persulcatus*	5,9±0,27	93,3±1,98
636-90	*I. persulcatus*	5,3±0,44	88,9±2,5
608-90	*I. persulcatus*	6,4±0,89	77,8±3,3
617-90	*I. persulcatus*	5,3±1,03	70±3,65

Table 5. The average life time and lethality percent of mice infected with TBEV "group 886" strains.

The value of mice average life time after infection with different strains ranged from 5.0±0.45 to 6.8±0.76 days and lethality percent varied from 70±3.65 to 100%. It should be noted that the strains isolated from *I. persulcatus* ticks collected in Krasnochikoiskiy district caused the lethal outcome in laboratory animals in 100%.

Earlier, we have noted that registered and described severe clinical cases of TBE were found in the foci where "group 886" strains were isolated. The foci are located in Krasnochikoiskiy district of Transbaikalia and Bichurskiy district of Buryat Repblic [5, 15, 22].

Recently, the case of meningoencephalitis with lethal outcome was described in Mongolia caused by TBEV isolate possessed 98.5% homology level with strain 886-84 genome sequence. The infection of patient occurred after tick bite in Bulganskiy aimak bordering from south with four natural foci where TBEV "group 886" strains were isolated from collected samples. The patient was hospitalized on 11th day after the tick bite with diagnosis "meningoencephalitis" and died on 11th day of the disease. The presence of TBEV RNA in macromyelon samples, in core andmeninx vasculosa indicates the multilevel localization of lesions which are typical to the most severe forms of acute TBE resulting in lethal outcome or disability [19].

Taking into account the genetic and antigenic properties of 886-84 strain, the strain itself and the strains of the group could be considered as a candidates for development of universal vaccines and test-systems effective for different TBEV serotypes (genotypes). So we investigated "group 886" strains according the complex of criteria suggested by L.S. Vereta and M.S. Vorob'eva for the strains – candidates for vaccine prototypes [3].

The proteins of virus envelope are responsible for hemagglutination and antigenic activity, thermostability and some other properties. All TBEV "group 886" strains have shown the hemagglutination activity (titers in hemagglutination reaction 1:1280-1:10240) in the reaction with goose erythrocytes. In RDPA with cross-adsorbed strain-specific sera 886-84 strain demonstrated the same level of similarity with all TBEV subtypes. The high level of antigenic cross-reaction with East-Siberian and Far-Eastern subtypes was observed in RDPA and neutralization reaction (NR) tests for 886-84, 711-84 and 740-84 strains [4]. The results of antigenic typing are presented in Tables 6 and 7.

	Strain antigen	Serum against strain							*antigenic typing results
		256, depleted by a/g Aina	**Aina, depleted by a/g Sofjin**	**Leso-park11, depleted by a/g Sofjin**	**Sofjin, depleted by a/g 256**	**Aina, depleted by a/g 256**	**Sofjin, depleted by a/g Lesopark 11**	**Aina, depleted by a/g Lesopark 11**	
Prototype Strains	Aina/1448	0	4	4	2	4	0	0	E-S
	Sofjin	0	0	0	4	0	4-8	0	F-E
	256	2-4	0	2	0	0	0	0	W
	Lesopark -11	2	4	2-4	0	2	0	0	E-S
"group 886" strains	*740-84*	0	4	4	0	0	0	0	E-S
	711-84	0	4-8	4	2-4	0	4	0	E-S F-E
	886-84	4	4	4	2	2	4-8	0	W, E-S, F-E

Comments: the reverse titers of precipitating antibodies are shown; 0-negative result at sera dilution 1:32; * marks: W – Western antigenic variant; E-S – East-Siberian; F-E – Far Eastern; a/g – antigen.

Table 6. Immunotyping of TBEV "group 886" strains by RDPA test.

	Strain	Immune sera				Antigenic typing results
		Sofjin	**Lesopark**	**256**	**KFD**	
Prototype TBEV strains and viruses of TBEV complex	Sofjin	**10240**	1280	1280	1280	F-E
	Lesopark-11	5120	**10240**	10240	1280	E-S
	256	10240	2560	**10240**	5120	W
	KFD	640	640	640	**5120**	KFD
TBEV "group 886" strains	740-84	**10240**	**10240**	2560	1280	F-E, E-S
	711-84	**5120**	**5120**	2560	1280	F-E, E-S
	886-84	**5120**	**10240**	2560	1280	E-S, F-E

Comments: reversed antibody titers are presented in the table; the significant values of neutralization marked with bold (the significant titers were corresponded to neutralization values of prototype strain with the same sera in homologous system). KFD – prototype strain of Kyasanur Forest disease virus. W – TBEV Western antigenic variant; E-S – East-Siberian; F-E – Far Eastern.

Table 7. TBEV "group 886" strains neutralization reaction test with antisera against prototype TBEV strains and viruses of tick-borne encephalitis virus complex.

http://dx.doi.org/10.5772/54087

Thermostability is one of the most important genetic features to which the attention should be paid during the selection of TBEV strains for inactivated vaccines production. The results of inactivation index determination for 13 TBEV "group 886" strains at temperature 50°C (T^{50}) are shown in Table 8.

Strain	Isolaion source	lgTCD$_{50}$/ml at 37˚C	lgTCD$_{50}$/ml at 50˚C	Difference 37-50°C	T^{50}
636-90	*I. persulcatus*	4,78	3,0	1,78	+
606-90	*I. persulcatus*	4,0	2,23	1,77	+
691-90	*I. persulcatus*	4,84	3,43	1,41	+
418-90	*I. persulcatus*	4,0	2,22	1,78	+
886-84	*Myodes rutilus*	7,08	6,9	0,18	+
711-84	*Myodes rufocanus*	8,26	7,07	1,2	+
712-89	*I. persulcatus*	4,0	2,23	1,77	+
733-90	*I. persulcatus*	4,25	2,5	1,75	+
742-90	*I. persulcatus*	3,5	2,23	1,27	+
608-90	*I. persulcatus*	4,5	2,0	2,5	±
287-83	*I. persulcatus*	5,33	2,5	2,83	±
740-84	*Myodes rufocanus*	7,18	4,78	2,4	±
617-90	*I. persulcatus*	8,0	3,67	4,33	-

Table 8. The results of thermal resistance determination for TBEV "group 886" strains.

The values of lgTCD$_{50}$/ml at 37ºC varied from 3.5 to 8.26. According to the thermoresistanse feature all tested strains were divided into three group: thermostable (T^{50+}) - nine strains; thermolabile (T^{50-}) – one strain; strains with medium thermoresistance – three strains. It should be noted that all strains isolated from *I. persulcatus* ticks collected in Krasnochikoiskiy district of Transbaikalia were thermostable.

The genetic features linked with intracellular TBEV proliferation are the cytopathic activity, plaque size and type in cells culture under agar layer (S-feature) and ability to proliferate at different temperatures (rct- or ts- feature).

All "group 886" strains caused the destruction of infected cells monolayer on 4th-6th day. The plaque size and type in cells culture under the agar layer differed depending on certain strain. Thus, strain 740-84 formed large plaques 3.0 mm in diameter (S+), strain 886-84 formed plaques of medium size (d=2.0 mm) (S±) and strain 711-84 formed small plaques with diameter 1-1.5 mm (S-).

The most of the viruses proliferate and form mature virus particles in sensitive cells in definite temperature limits. A number of authors investigating the proliferation of TBEV strains in cell cultures at different temperatures (rct-feature) came to the conclusion that this marker could be the important phenotypical characteristics of the virus and closely connected with its virulence [13, 14].

We investigated rct_{42} genetic marker for 12 TBEV "group 886" strains. The results are presented in Table 9.

Strain	Isolaion source	$lgTCD_{50}$/ml at 37˚C	$lgTCD_{50}$/ml at 42˚C	Difference 37-42°C	rct_{42}
636-90	*I. persulcatus*	4,78	5,78	-1,0	+
608-90	*I. persulcatus*	4,5	4,78	-0,28	+
606-90	*I. persulcatus*	4,0	6,83	-2,83	+
691-90	*I. persulcatus*	4,84	5,25	-0,41	+
418-90	*I. persulcatus*	4,0	3,0	1,0	+
740-84	*Myodes rufocanus*	7,18	6,95	0,23	+
712-89	*I. persulcatus*	4,0	2,23	1,77	+
733-90	*I. persulcatus*	4,25	5,57	- 1,32	+
742-90	*I. persulcatus*	3,5	3,0	0,5	+
711-84	*Myodes rufocanus*	8,26	5,90	2,36	±
617-90	*I. persulcatus*	8,0	5,67	2,33	±
886-84	*Myodes rutilus*	7,08	3,3	3,78	-

Table 9. Rct_{42} genetic marker for TBEV "group 886" strains.

"Group 886" strains demonstrated the high heterogeneity in proliferation ability at 42°C. Five strains propagated more effectively at supraoptimal temperature (42º). Moreover, eight of nine strains isolated from ticks had $rct_{42±}$ feature. The strains isolated from rodents were more heterogenic: strain 740-84 had rct_{42+} feature, strain 886-84 had rct_{42-} feature and strain 711-84 had $rct_{42±}$ feature. All TBEV "group 886" strains isolated from ticks collected in Krasnochikoiskiy district of Transbaikalia actively propagate at 42ºC.

4. Conclusions

1. The new data concerning original TBE virus variant circulating on the territory of Eastern Siberia have been obtained. We have demonstrated the unique genetic structure of

"group 886" strains that is the "mixture" of amino acid sequences typical for genotypes 1, 2 and 3.

2. This TBEV variant can be considered as an independent TBEV genotype 5 (high level of genetic difference compared to other genotypes – more than 12%, the existence of its own natural foci, the ecological connection with all elements of transmissive chain, the role in infectious pathology, stability and durational circulation in nature).
3. The ability to cause focal forms of tick-borne encephalitis with lethal outcome and laboratory results of virulence level evaluation testify the high pathogenic potential of TBEV "group 886" strains. During the study of the genetic markers connected with virus intracellular reproduction we have found that "group 886" strains have high adaptive ability and can easily accommodate to circulation in different biocenosises and in variety of landscape-geographical zones.
4. Some studied "group 886" strains possess the wide spectrum of antigenic properties, hemagglutination and neutralizing activity, high virulence and thermotolerance. They match the basic criteria of strains-candidates chosen for diagnostic and vaccine development.

Acknowledgements

The authors are greatly appreciated to all colleagues collected the field materials and to the staff of Natural-Foci Infection Department: E.V. Arbatskaya, I.V. Voronko, O.Z. Gorin, N.A. Gusarova, G.A. Danchinova, V.M. Kogan, S.I. Lipin, O.V. Melnikova, A.G. Trukhina. Also we acknowledge the partial financial support by Integration interdisciplinary project grant No. 135 from the Siberian Branch of the Russian Academy of Sciences.

Author details

I.V. Kozlova[1], M.M. Verkhozina[2], T.V. Demina[3], Yu.P. Dzhioev[1], S.E. Tkachev[4], L.S. Karan[5], E.K. Doroshchenko[1], O.V. Lisak[1], O.V. Suntsova[1], A.I. Paramonov[1], O.O. Fedulina[1], A.O. Revizor[3] and V.I. Zlobin[3]

1 FSSFE Scientific Centre of Family Health and Human Reproduction Problems SB RAMS, Irkutsk, Russia

2 Centre for Epidemiology and Hygiene in Irkutsk region, Irkutsk, Russia

3 Irkutsk State Medical University of Russian Ministry of Heath, Irkutsk, Russia

4 Institute of Chemical Biology and Fundamental Medicine SB RAS, Novosibirsk, Russia

5 Central Research Institute of Epidemiology of Rospotrebnadzor RF, Moscow, Russia

References

[1] Andaev, E.I., Sidorova, E.A., Borisova, T.I. et al. (2011). Tick-borne encephalitis in the Trans-Baikal region, and molecular-biological characteristics of the pathogen // "National priorities of Russia" (special issue). №2 (5), pp. 148-150 (Russian).

[2] Bochkova, N.G., Zhezmer, V.Y., Trukhina, A.G. et al. (1985) The study of Aina/1448 serotype of tick-borne encephalitis virus //Vopr. Virusol. № 5. pp. 572-575 (Russian).

[3] Vereta, L.A., Vorobyova, M.S. (1990). Natural heterogeneity and the purposeful selection of strains of tick-borne encephalitis virus. - M.: Medicine - 124 p. (Russian).

[4] Verkhozina, M.M. (2000). Molecular epidemiology and genetic characteristics of the regional population of tick-borne encephalitis virus in Eastern Siberia: PhD thesis. – Irkutsk. - 163 p. (Russian).

[5] Gorin, O.Z., Mungalova, N.P., Leonov, V.A. et al. (1992). The situation on tick-borne encephalitis in the west of the Chita region. In.: etiology, epidemiology and diagnosis of infectious diseases in East Siberia: comp. scientific works for the IEM RAMS 80th anniversary. - Irkutsk, pp. 44-53 (Russian).

[6] Demina, T.V., Dzioev, Yu.P., Verkhozina, M.M., Kozlova, I.V., Tkachev, S.E., Doroshchenko, E.K., Lisak, O.V., Zlobin, V.I. (2009). Genetic variability and genotyping of tick-borne encephalitis virus with desoxyoligonucleotide probes. Vopr. Virusol. №3. pp. 33-42 (Russian).

[7] Demina, T.V., Dzhioev, Yu.P., Kozlova, I.V. et al. (2012). Genotypes 4 and 5 of tick-borne encephalitis virus: structural features of genomes and a possible scenario for their formation. Vopr. Virusol. № 4. V. 57. P. 13-19 (Russian).

[8] Zlobin, V.I., Demina, T.V., Mamaev, L.V. et al. (2001). Analysis of genetic variability of TBEV strains on the primary structure of a cover protein's gene fragment. Vopr. Virusol. №1. pp. 12-16 (Russian).

[9] Zlobin, V.I., Demina, T.V., Belikov, S.I. et al. (2001). Genetic typing of TBEV strains in terms of homology analysis of an envelope protein's gene fragment. Vopr. Virusol. №1. pp. 17-22 (Russian).

[10] Zlobin, V.I,. Belikov, S.I., Dzioev, Yu.P. et al. (2003). Molecular epidemiology of tick-borne encephalitis. - Irkutsk: RRC ESSC SB RAMS, 2003. - 271 p. (Russian).

[11] Karan, LS, Malenko, G.V., Bochkova, N.G. et al. (2007). The use of molecular genetic techniques to study the structure of tick-borne encephalitis virus strains. Bull. RAMS. №4. pp. 34-40 (Russian).

[12] Clark, D. (1964). Further studies of antigenic relationships between group B arboviruses. Bull. WHO. T. 31. №1. pp. 50-67.

[13] Levkovich, E.N., Karpovich, L.G., Loginova, N.V. (1964). The study of tick-borne encephalitis virus strains grown in tissue culture at different temperatures // In.: Tick-

borne encephalitis, Kemerovo tick fever, hemorrhagic fever and other arboviral infections. - M., pp. 52-54 (Russian).

[14] Libikova, E., Stanchek, D. (1965). Characterization of three different variants of the same strain of tick-borne encephalitis // Acta Virol. V. 9. pp. 481-487 (Russian).

[15] Lochov, M.G., Blinnikova, I.A. (1986). To structural and functional characterization of foci of tick-borne encephalitis in the Bichursky area of Buryat Autonomous Republic. In.: Nidal infections in Eastern Siberia: comp. scientific works - Irkutsk: Irkut. Med. Inst, pp. 44-51 (Russian).

[16] Clinical Molecular Diagnostics: method / EdC. Herrington, J McGee. - Academic Press, 1999. 558 p.

[17] Ovchinnikov, E.A., Karpovich, LG., Levkovich, E.N. (1967). The study of thermal resistance of strains of tick-borne encephalitis complex having different neurovirulence for laboratory animals. Vopr. Virusol. №5. P. 607.

[18] Pogodina, V.V., Savinov, A.P. (1964). Variation in the pathogenicity of viruses of the tick-borne encephalitis complex for different animal species. I. Experimental infection of mice and hamsters. Acta virologica. №8. pp. 424-434.

[19] Pogodina, V.V., Levina, L.S,. Karan, L.S. et al. (2009). Fatal cases of tick-borne encephalitis caused by the Siberian subtype of the pathogen in the European part of Russia and in the Urals. Med. Virology. V. XXVI. pp. 121-122 (Russian).

[20] Pogodina, V.V., Bochkova, N.G., Karan, L.S. et al. (2004). Siberian and Far Eastern subtypes of tick-borne encephalitis in the European and Asian parts of Russia: genetic and antigenic characterization of strains. Vopr. Virusol. №3. pp. 20-25.

[21] Trukhina, A.G. Features of TBE pathogen circulation in the zone of the two serotypes distribution in the Baikal region: PhD thesis. - Irkutsk, 1989. – 176 p.

[22] Shasaitov, S.S., Domaev Yu.A. On the tick-borne encephalitis in the Chita region. In.: Zonal Conf. Ministry of Health of the Russian Federation "The influence of climatic factors on the functional state of the person." - Chita, 1980. pp. 97-99.

[23] Heinz, F.X., Collet, M.S., Purcell, R.H. et al. Family Flaviviridae // Virus taxonomy: classification and nomenclature of viruses: 7 report of the International committee of taxonomy of viruses. – San Diego, 2000. pp. 1217-1225.

[24] Khasnatinov, M.A., Danchinova, G.A., Unursaikhan, U. et al. (2009). Characterizaition of tick borne encephalitis virus that caused the lethal meningoencephalitis human in Mongolia. Inter. Conference Zoonotic infections desease and tourism. – Ulaanbaatar, pp. 88-93.

[25] Pletnev, A.G., Yamshikov, V.F., Blinov, V.M. (1990). Nucleotide sequence of the genome and complete amino acid sequence of the polyprotein of tick-borne encephalitis virus. Virology. V. 174. pp. 250–263.

[26] Reed, L., Muench, H.A. (1938). A Simple Method of Estimating Fifty Per Cent Endpoints. Am. J. Hyg.. № 27. pp. 493-497.

[27] Rubin, S.G., Chumakov, M.P. (1980). New data on the antigenic types of tick-borne encephalitis (TBE) virus. Arboviruses in the Mediterranen Countries. – Stutgart, New York, pp. 231-236.

[28] Tamura, K, Peterson, D, Peterson, N, Stecher, G., Nei, M, and Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Molecular Biology and Evolution. 2011. V. 28. №10. pp. 2731-2739.