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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 [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 |
| 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.
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$_{50}$/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
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 became 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.
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.
| Genotype | Nucleotide Substitution Level (%) | Amino Acid Substitution Level (%) |
|----------|----------------------------------|----------------------------------|
| 1        | 4,3                              | 1,3                              |
| 2        | 16,4                             | 6,9                              |
| 3        | 14,4 15,2                        | 5,3 6,2                          |

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).

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 886-4 is marked with black.
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 | С | M | Е | 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 |

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 С, М, Е 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 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).
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 focus 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 Isolation source  | mNic (lgLD₅₀/мл) | mNsc (lgLD₅₀/мл) | mNic-mNsc II |
|-------------------------|------------------|------------------|--------------|
| I. persulcatus 711-84   | 8.58             | 7.16             | 1.2 ±        |
| I. persulcatus 740-84   | 10.2             | 9.4              | 0.8 ±        |
| I. persulcatus 617-90   | 6.64             | 3.8              | 2.84 ±       |
| I. persulcatus 636-90   | 7.06             | 4.35             | 2.71 ±       |
| I. persulcatus 608-90   | 7.9              | 4.86             | 3.04 -       |
| I. persulcatus 606-90   | 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 LD₅₀/мл while after subcutaneous inoculation (peripheral activity) were found to be from 3.8 to 9.8 lg LD₅₀/мл. The determined virus indexes of invasiveness (II) had medium and high values (from 0.8 to 3.04 lg LD₅₀/мл). 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 Krasnochikogkiy 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).
Table 5. The average life time and lethality percent of mice infected with TBEV “group 886” strains.

| Strain   | Source        | Life time (days ± m) | % of lethal cases (%) ± m |
|----------|---------------|----------------------|---------------------------|
| 711-84   | M. rufocanus  | 5.1±0.49             | 100                       |
| 740-84   | M. 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   | M. 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             | 70.0±3.65                 |

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 Republic [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 and meninx 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 |
|------------------------------------|-----------------|
| 256, depleted by a/g Aina          |                 |
| Aina, depleted by a/g Sofjin       |                 |
| Lesopark11, depleted by a/g Sofjin |                 |
| Sofjin, depleted by a/g 256        |                 |
| Aina, depleted by a/g 256          |                 |
| Sofjin, depleted by a/g Lesopark   |                 |
| Lesopark11, depleted by a/g 256    |                 |

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

| Strain Immune sera | Antigenic typing |
|--------------------|------------------|
| Prototype TBEV strains and viruses of TBEV complex |
| Sofjin            | 10240 1280 1280 F-E |
| Lesopark-11       | 5120 10240 1280 E-S |
| 256               | 10240 2560 5120 W |
| KFD               | 640 640 5120 |

TBEV “group 886” strains

| Strain     | 10240 1280 |
|------------|------------|
| 740-84     | 2560       |
| 711-84     | 2560       |
| 886-84     | 10240      |

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.
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 Isolation source | $lgTCD_{50}$/ml at 37˚С | $lgTCD_{50}$/ml at 50˚С | Difference 37-50˚С |
|-------------------------|-------------------------|------------------------|---------------------|
| 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 37ºС varied from 3.5 to 8.26. According to the thermoresistance 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 definite temperature limits. A number of authors investigating the proliferation of TBEV strains in cell cultures at different temperatures came to the conclusion that this marker could be the important phenotypical characteristics of the virus and closely connected with its virulence.

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

| Strain Isolation source | lgTCD\textsubscript{50}/ml at 37˚C | lgTCD\textsubscript{50}/ml at 42˚C | Difference \(37-42°С\) |
|-------------------------|---------------------------------|---------------------------------|-----------------|
| I. persulcatus 636-90   | 4.78                            | 5.78                            | -1.0 +/-         |
| I. persulcatus 608-90   | 4.5                             | 4.78                            | -0.28 +/-        |
| I. persulcatus 606-90   | 4.0                             | 6.83                            | -2.83 +/-        |
| I. persulcatus 691-90   | 4.84                            | 5.25                            | -0.41 +/-        |
| I. persulcatus 418-90   | 4.0                             | 3.0                             | 1.0 +/-          |
| Myodes rufocanus 740-84 | 7.18                            | 6.95                            | 0.23 +/-         |
| I. persulcatus 712-89   | 4.0                             | 2.23                            | 1.77 +/-         |
| I. persulcatus 733-90   | 4.25                            | 5.57                            | -1.32 +/-        |
| I. persulcatus 742-90   | 3.5                             | 3.0                             | 0.5 +/-          |
| Myodes rutilus 711-84   | 8.26                            | 5.90                            | 2.36 +/-         |
| I. persulcatus 617-90   | 8.0                             | 5.67                            | 2.33 +/-         |
| Myodes rutilus 886-84   | 7.08                            | 3.3                             | 3.78 -/+         |

Table 9. Rct\textsubscript{42} genetic marker for TBEV "group 886" strains.

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

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

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).

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.

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.
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