



# ***GENETIC DIVERSITY OF WEST NILE AND TICK-BORNE ENCEPHALITIS VIRUSES: NEW GENETIC VARIANTS OF FLAVIVIRUSES IN THE ASIAN PART OF RUSSIA***

***State Research Center of Virology and Biotechnology  
Vector, Novosibirsk, Russia***

Irkutsk

September, 2009

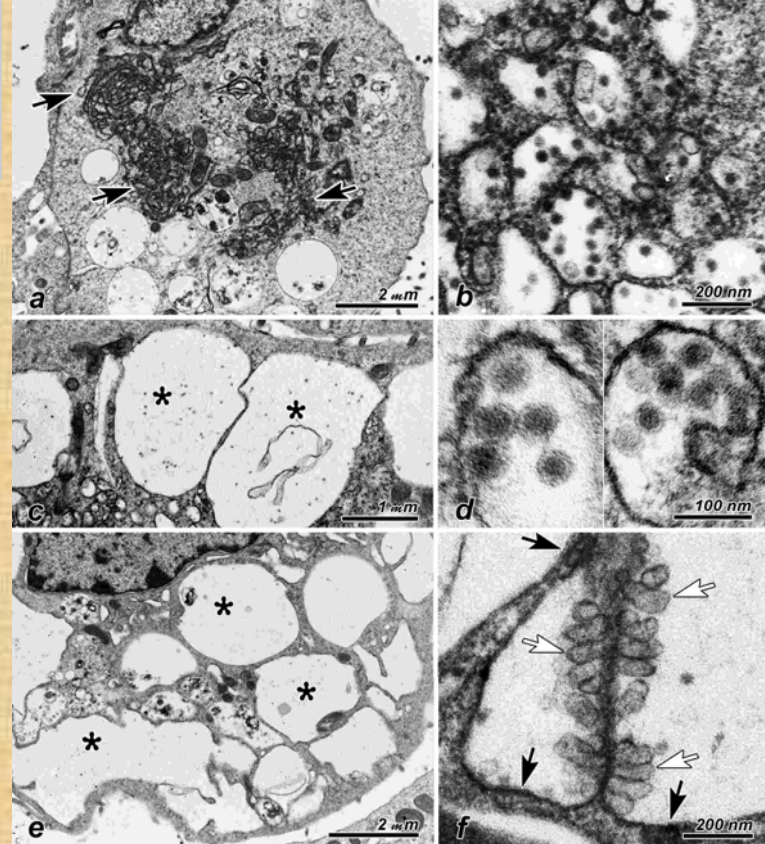


Первая дальневосточная экспедиция, открывшая вирус клещевого энцефалита. Июнь 1937 г. Исследование материала в пос.Обор. М.П.Чумаков (до заболевания клещевым энцефалитом) лаборант Г.Н.Зорина-Николаева и начальник северного отряда Е.Н.Левкович.

# Family. 00.026. *Flaviviridae*

## Genus:

- 00.026.0.01. *Flavivirus*
- 00.026.0.02. *Pestivirus*
- 00.026.0.03. *Hepacivirus*
- 00.026.0.00. *unassigned viruses*



|                           | Number: | species, | viruses, | putative |
|---------------------------|---------|----------|----------|----------|
| <i>Flavivirus</i>         |         | 53       | 74       | 2/2      |
| <i>Pestivirus</i>         |         | 4        | 13       | 1/1      |
| <i>Hepacivirus</i>        |         | 1        | 10       | 1/2      |
| <i>unassigned viruses</i> |         | 1        | 3        |          |

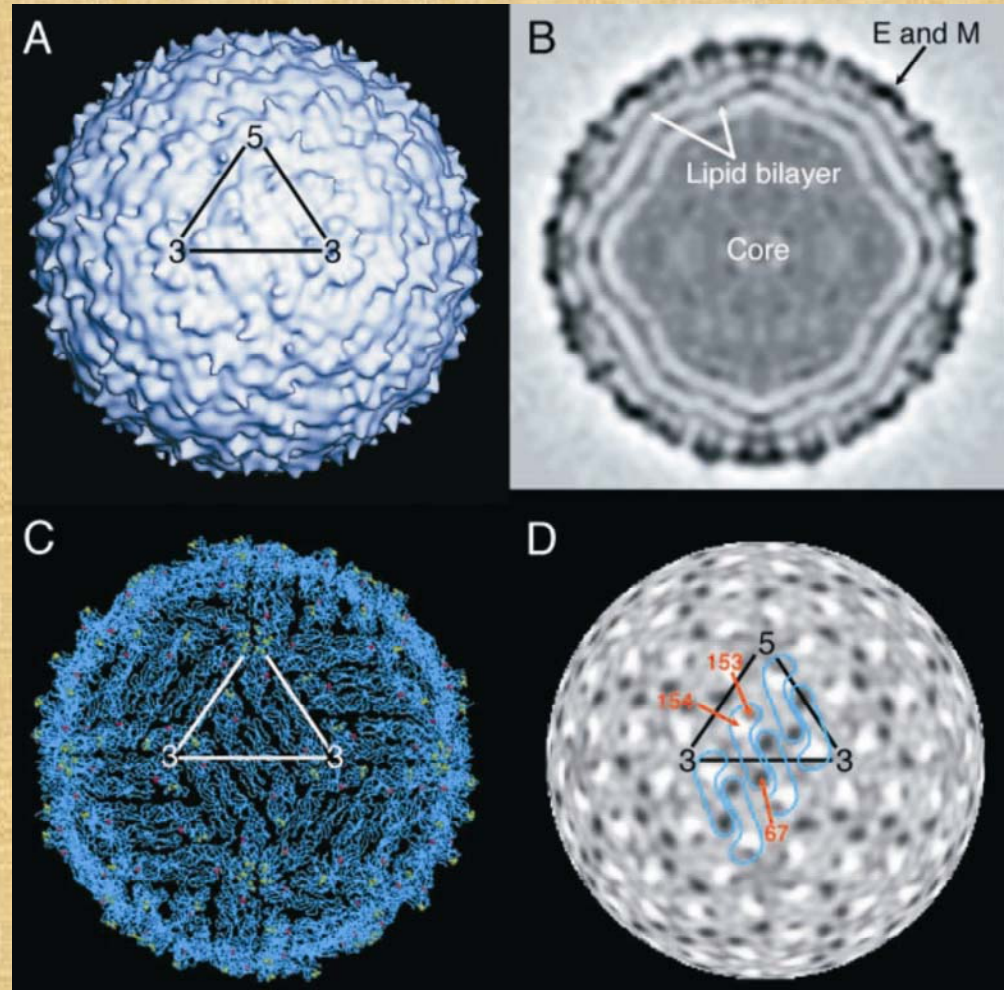


# **Main flaviviral infections for human**

- **Dengue**
- **Yellow fever**
- **Japanese encephalitis**
- **Tick-borne encephalitis**
- **West Nile fever**

# Flavivirus Genome

- ss (+) RNA genome
- Approximately 11 kb
- 5'-m<sup>7</sup>GpppAmp cap
- Lacks 3'-polyA tail
- Codes for
  - 3 structural proteins
    - Capsid (C), membrane (prM/M), envelope (E)
  - 7 non-structural proteins
    - NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5





# **Virus Taxonomy and new viruses**

**1999**

*The VII ICTV Virus Taxonomy Report provides information*

***3 Orders,  
56 families,  
9 subfamilies,  
233 genera,  
1550 species***

**2002**

*The VIII ICTV Virus Taxonomy Report provides information*

***3 orders of viruses,  
73 families,  
9 subfamilies,  
287 genera,  
1938 species***

**NOW**

***5 orders of viruses,  
82 families,  
11 subfamilies,  
307 genera  
2083 virus species***

# Flaviviruses is an emerging viruses

| Year          | Place  |  |
|---------------|--|--|
| 1988          | Sochi,<br>Russia   | New genotype of WNV, strain LEIV-Krnd88-190                                |
| 1993          | Japan  | Introduction of TBEV in Japan, Oshima 5-10                                 |
| 1994-<br>now  | Ceylon,<br>India, East<br>Africa,<br>tropical<br>countries of<br>America | Emergence and expansion of new hemorrhagic variant of dengue 3, genotype 3 |
| 1994          | Saudi Arabia   | Isolation new flavivirus – Alkhurma from outbreak hemorrhagic fever        |
| 1996<br>– now | Europe,<br>Asia,<br>America  | Global expansion WNV, genotype Ia  |



# Flaviviruses is an emerging viruses

|          |                      |  |
|----------|----------------------|--|
| 1997     | Czech                | Isolation new flavivirus Rabensburg                      |
| 1998     | Australia            | Introduction of Japanese encephalitis virus to Australia |
| 1998     | Australia            | Isolation new flavivirus New Mapoon                      |
| 1999     | West Siberia, Russia | Hemorrhagic form of TBEV                                 |
| 1999     | Kenya                | Isolation new flavivirus - Kamiti River                  |
| 2000     | Australia            | Detection new genotype for Kokobera virus                |
| 2000     | Australia            | Australian virus Kunjin is genovariant of WNV            |
| 2001-now | Austria, UK          | Introduction of Usutu virus (complex JEV) in Europe      |
| 2002     | Vietnam              | Discovery new flavivirus Quang Binh                      |

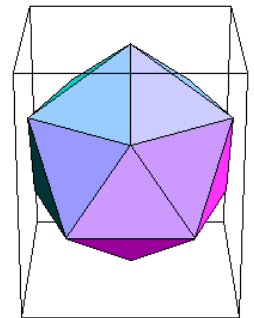
# Flaviviruses is an emerging viruses

|            |                     |   |
|------------|---------------------|---|
| 2002-2003  | China               | New genetic variant of TBEV - Senzhang                    |
| 2004       | Far Eastern, Russia | New genetic variant of TBEV - Glubinnoe/2004              |
| 2005 -2006 | India, Nepal        | Outbreak of Japanese encephalitis, new genotype G3 of JEV |
| 2005       | Turkey              | Turkish sheep encephalitis is European variant of TBEV    |
| 2005       | Spain               | Spanish sheep encephalitis is European variant of TBEV    |
| 2006       | Senegal             | Discovery new flavivirus - Ngoye                          |
| 2007       | Japan               | Discovery new flavivirus - Culex flavivirus (CxFV),       |
| 2009       | West Africa         | Discovery new flavivirus - Nounane (NOUV)                 |
| 2009       | Japan               | Discovery new flavivirus - Aedes flavivirus (AEFV)        |

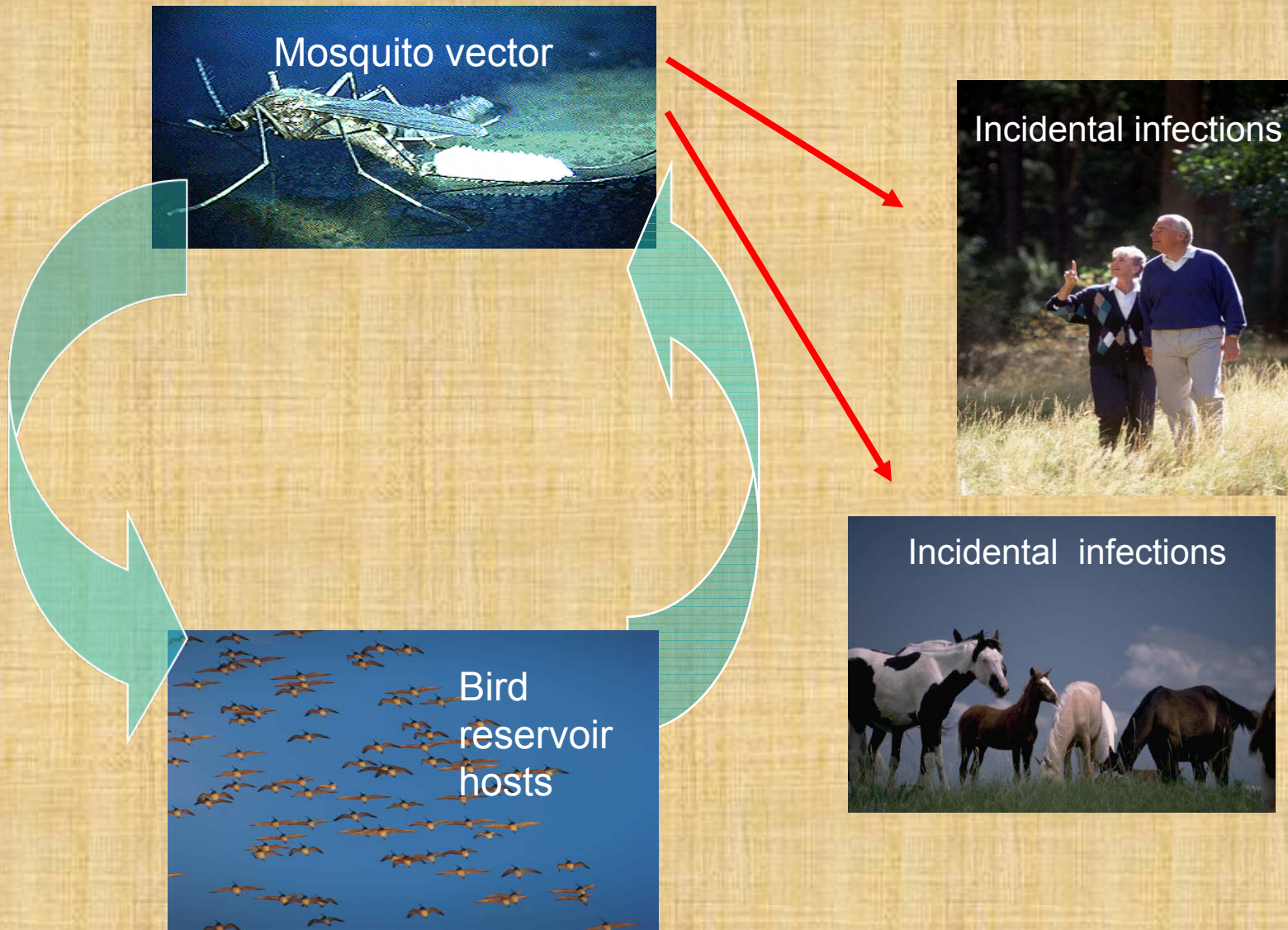


# West Nile Virus

- **Isolated in 1937 in Uganda.**
- **First human outbreaks were recorded in Israel in 1950 years.**
- **Detected in Africa, Europe, and Asia.**
- **In 1999, detected in USA.**



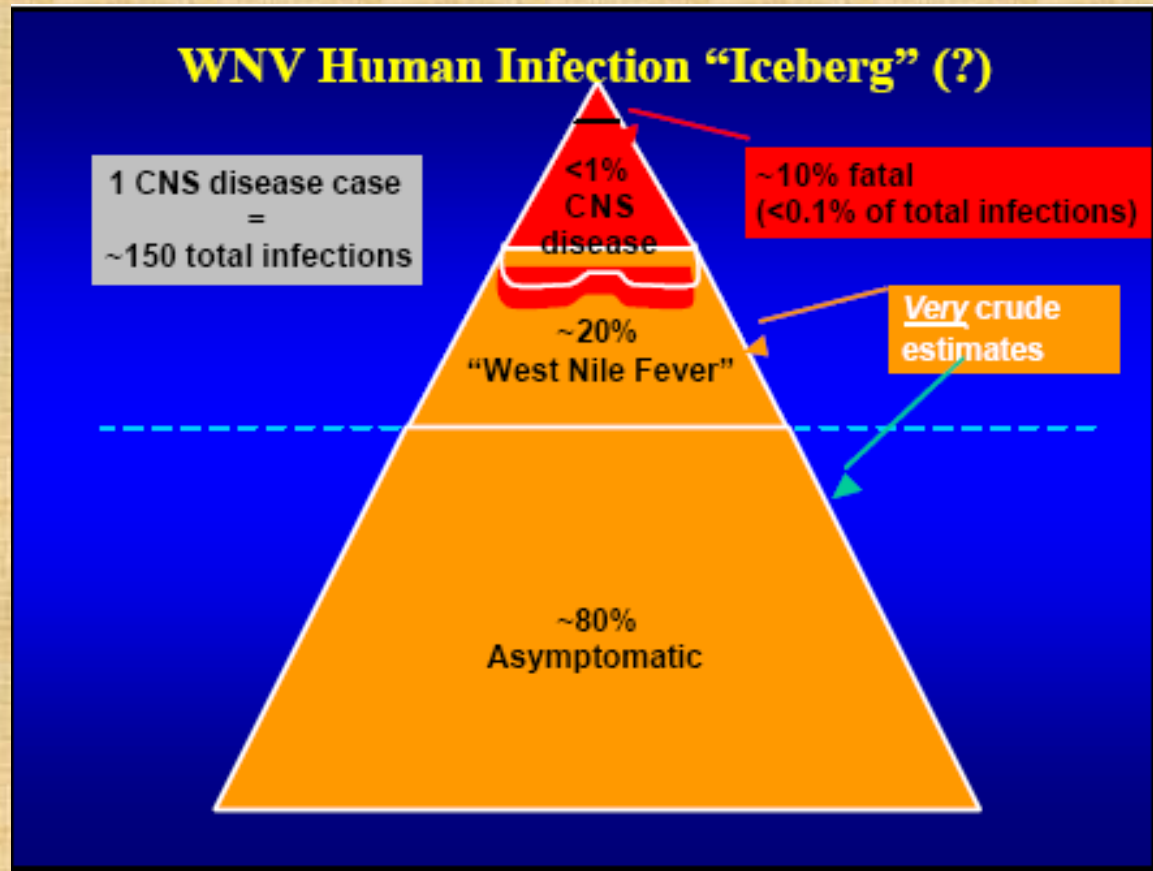
# West Nile Virus Transmission Cycle



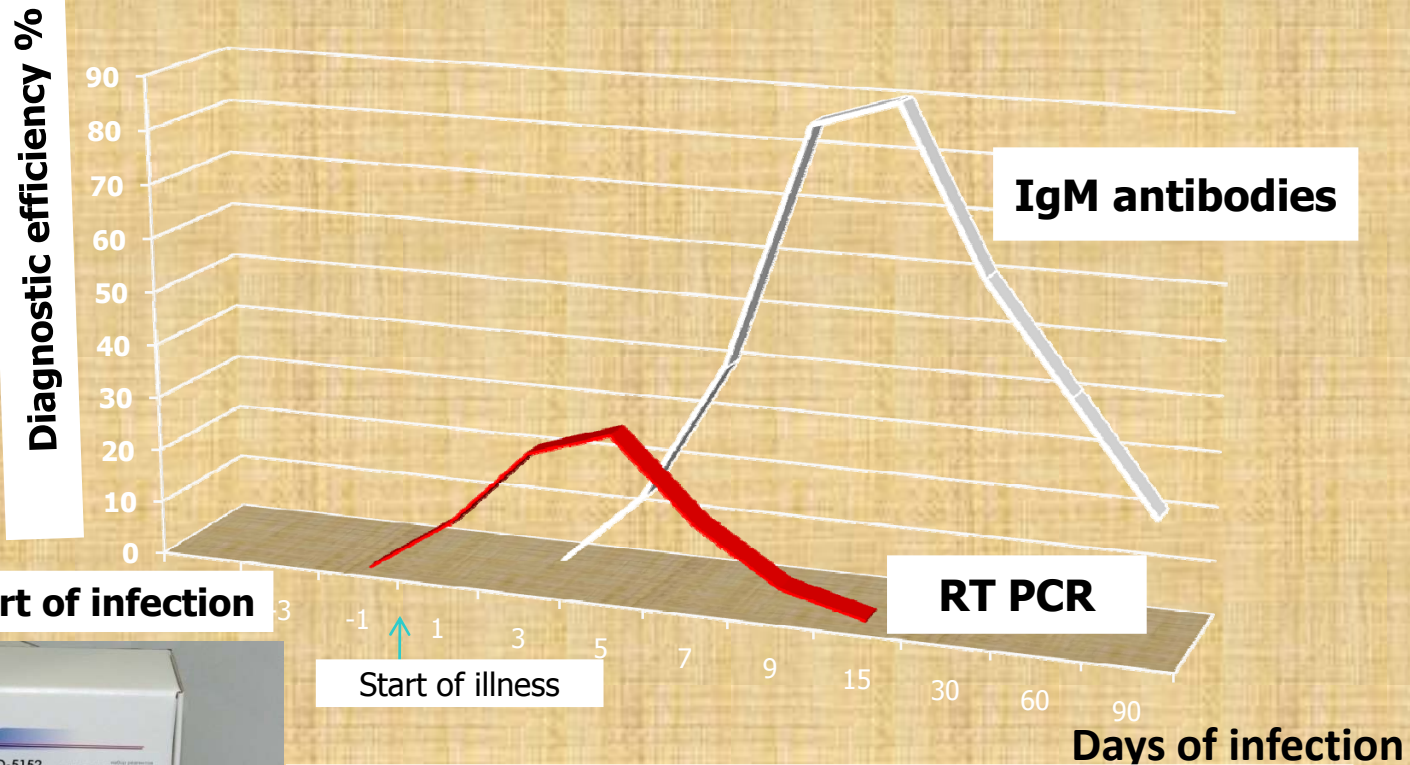


# WNV, vectors and hosts:

- > 60 species of mosquitoes
- > 300 species of birds
- > 20 species of mammals and reptilians
- - Human



# Diagnostic of flaviviral infection (WNF)



Fever

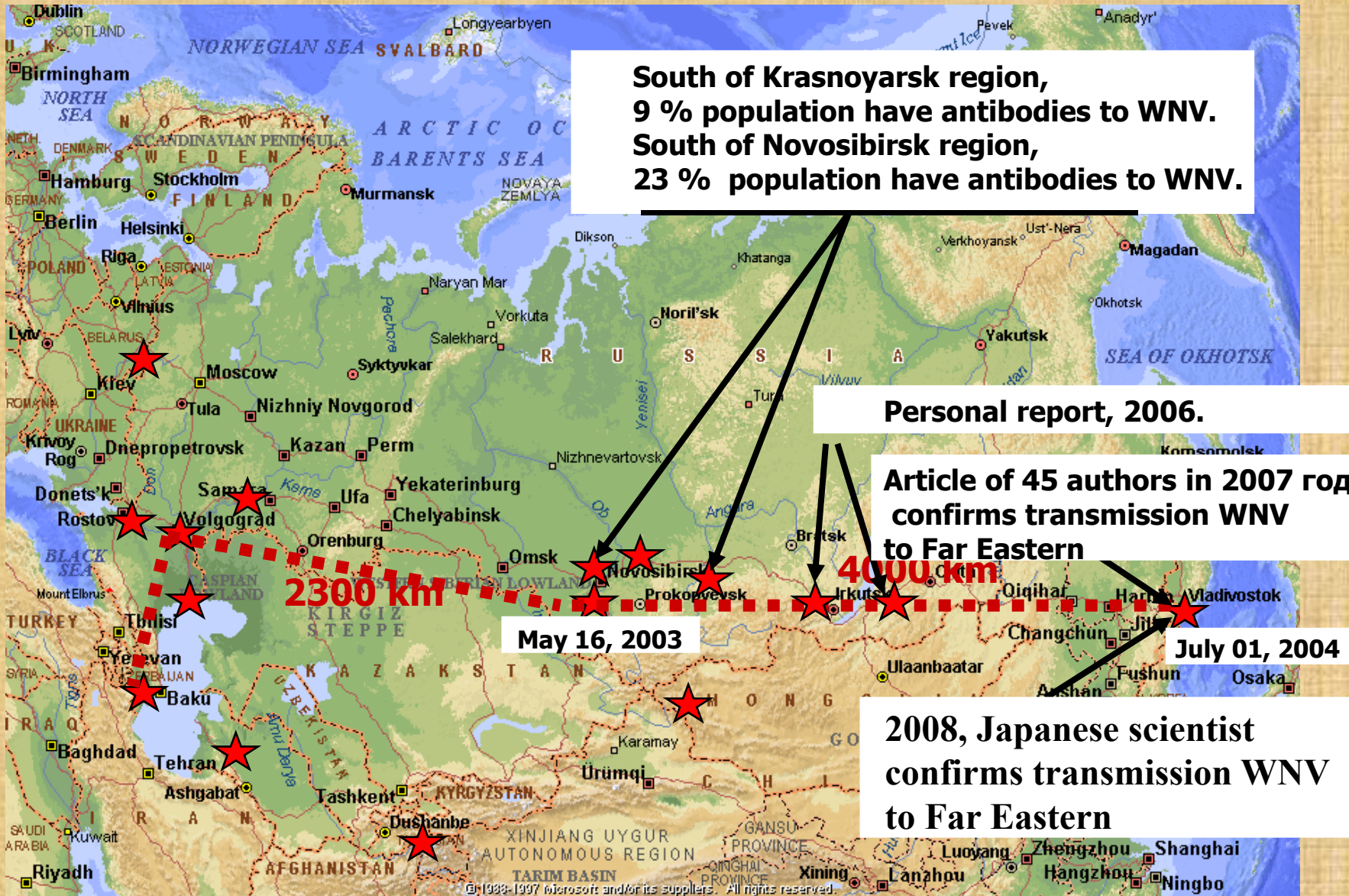
Encephalitis

Recovery





# WNV in Russia, 2003-2007







# Global transmission of WNV

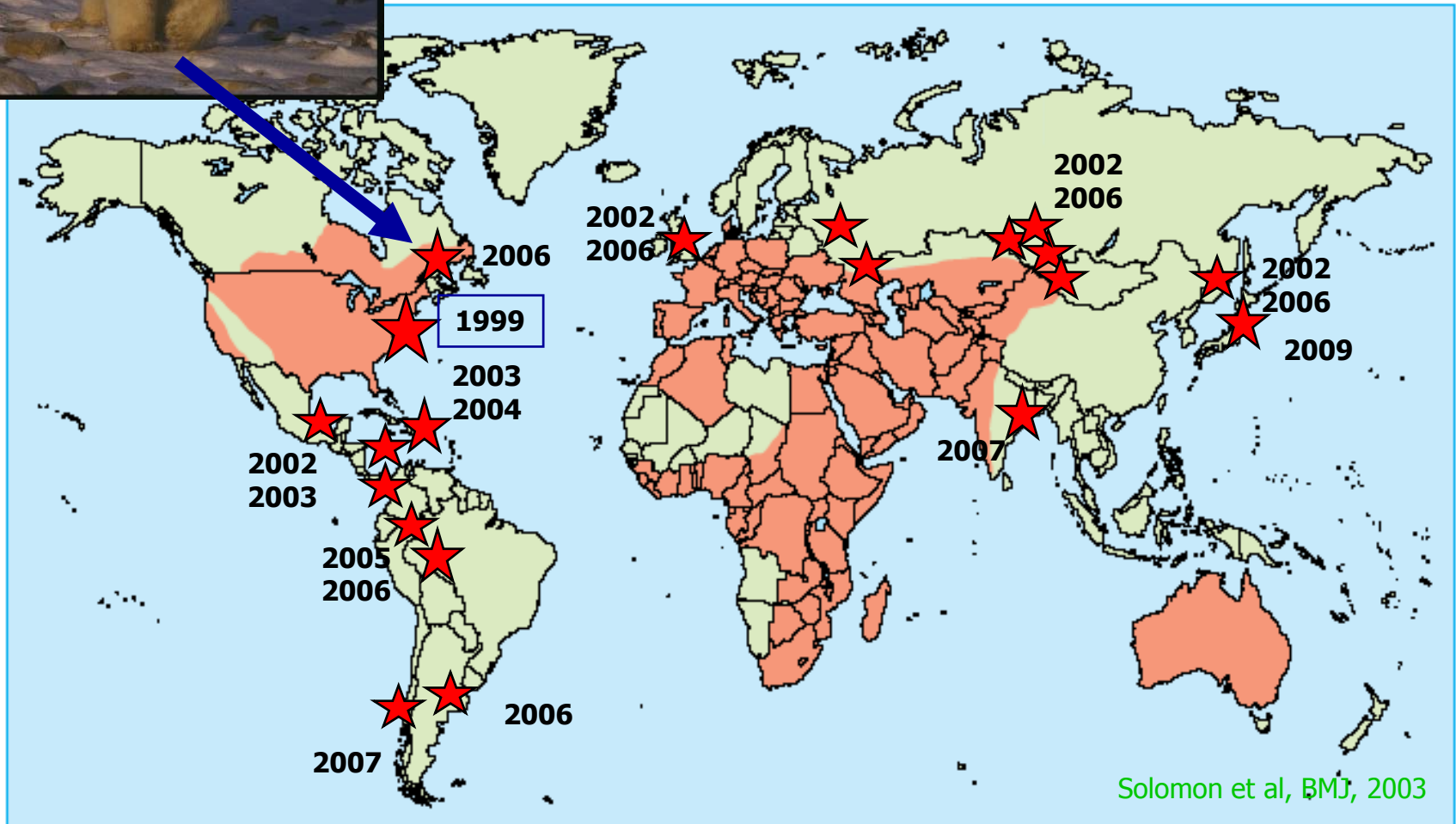
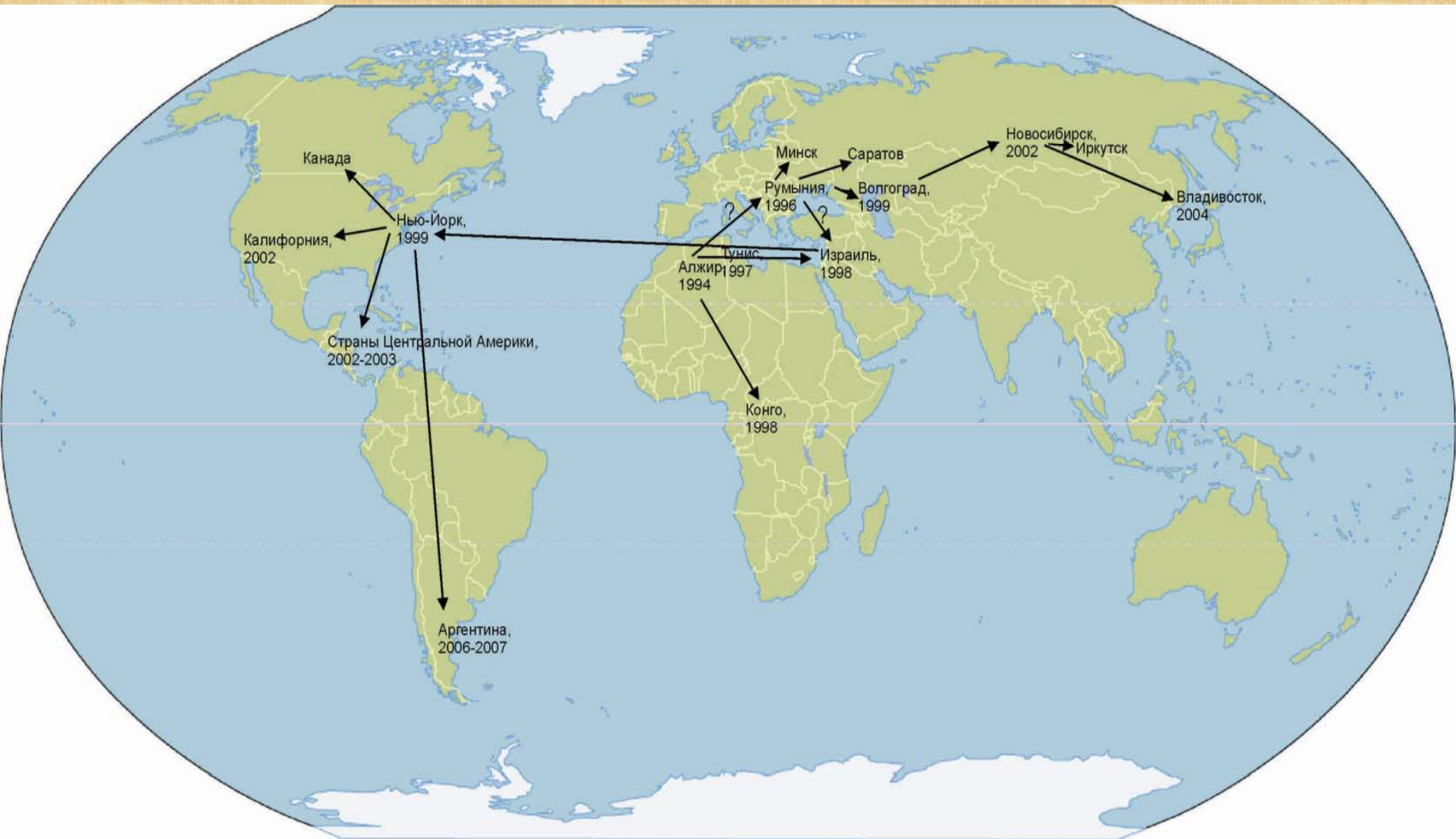
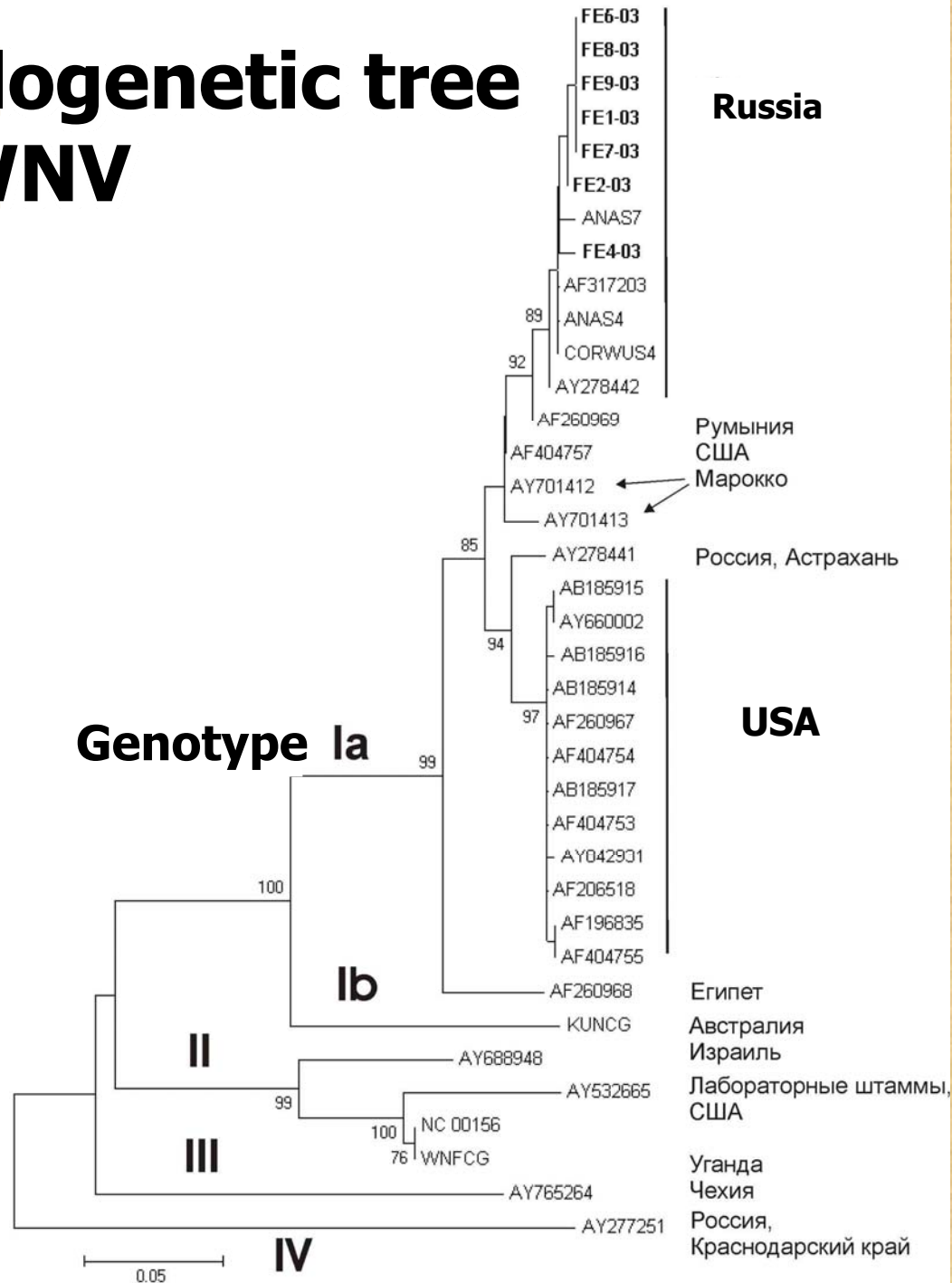


Fig 1 Approximate global distribution of West Nile virus (or its subtype, Kunjin virus)

# Global transmission of WNV, genotype Ia

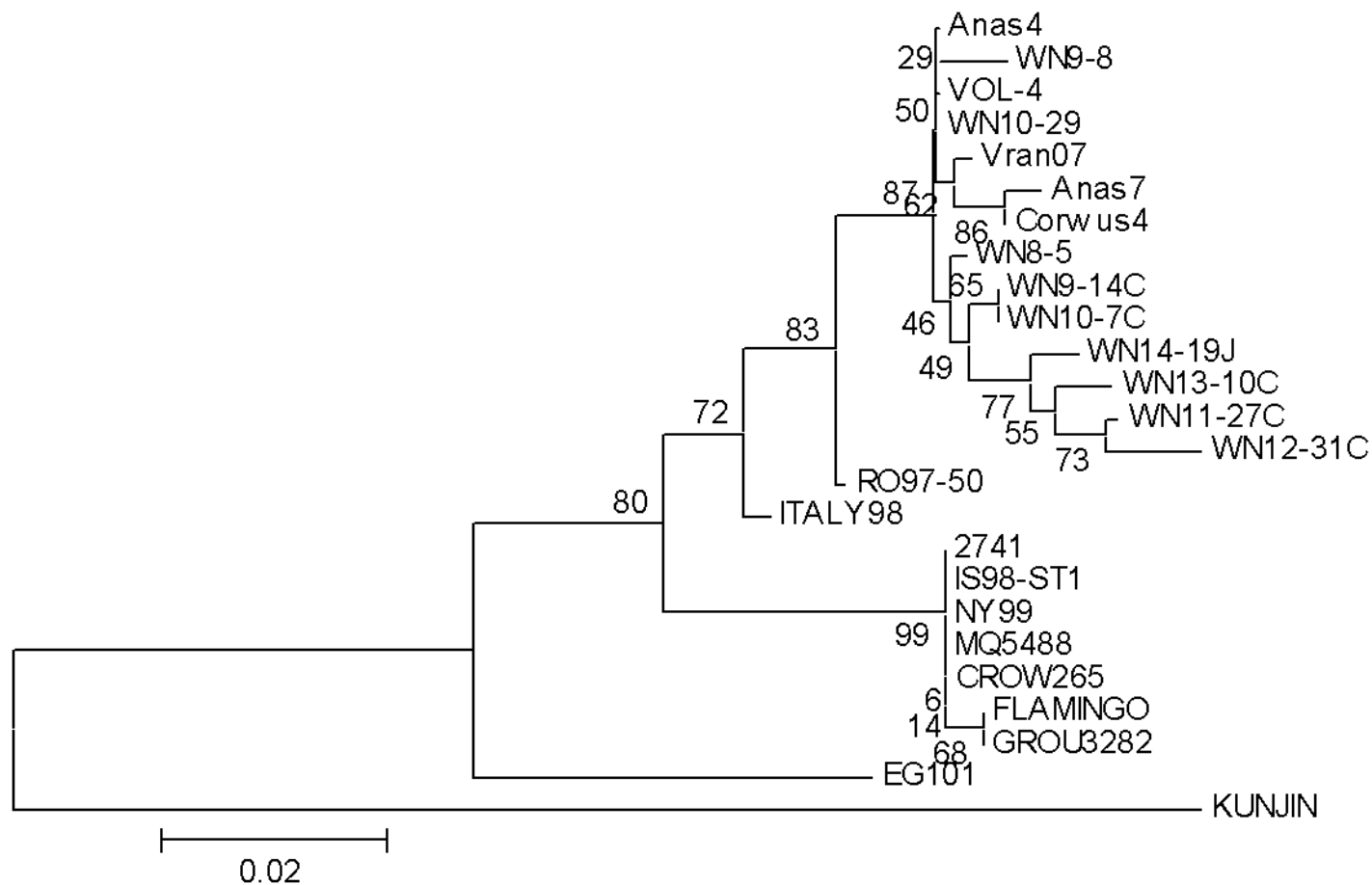


# Phylogenetic tree of WNV



2004





### Phylogenetic tree for Siberian variant WNV:

2002 - VOL-4 -strain LEIV-Vlg99-27889; Anas 4, Corvus 4 and Anas 7, Novosibirsk region;

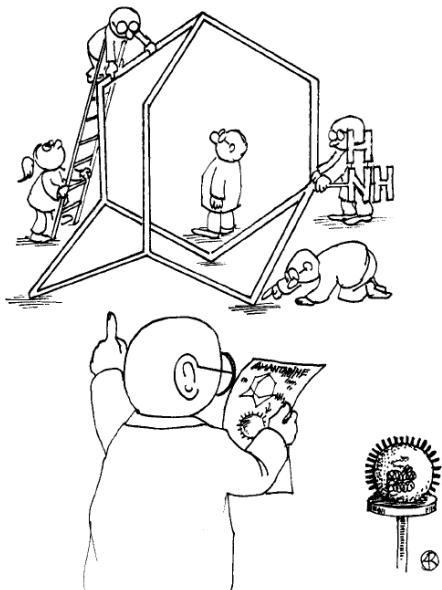
2003 - WN9-8 - crow, Mongolia; WN10-29 and WN8-5 – cormorant, Mongolia; Vran 07 – rook, Karasuk.

2004 - WN9-14C, WN10-7C, WN11-27C - Rook, Chany; WN12-31C and WN13-10C crow, Karasuk; WN14-19J – human.

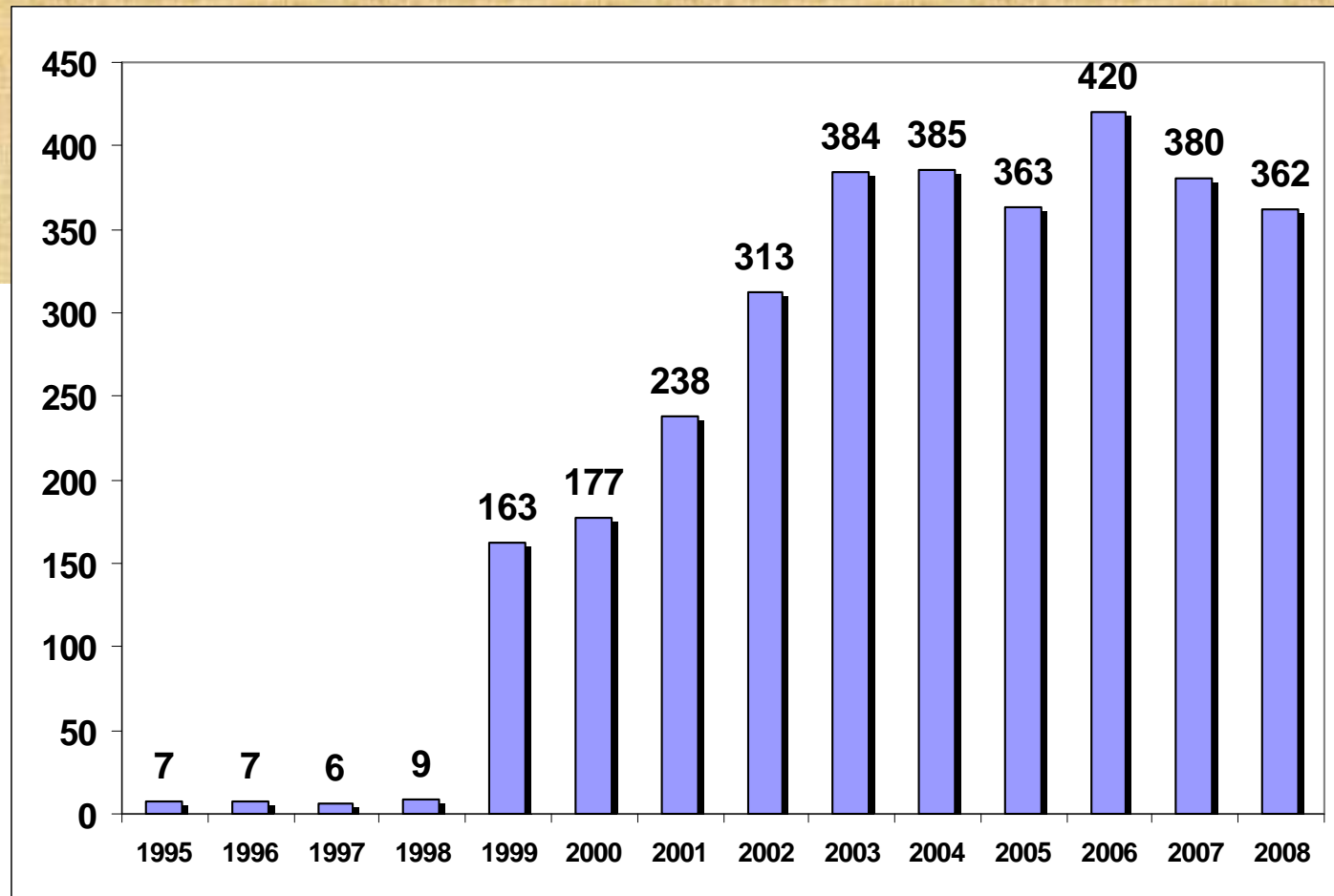
# **Novosibirsk WN fever, 2004**

- **Latent time – 14 days?**
- **Clinical picture – heavy fever**
- **Immunization against TBEV**
- **Titer against TBEV (IgG) – 1:400 (day 0 in hospital)**
- **Titer against WNV (IgG) – 1:1600 (day 14)**
- **RT PCR – positive (day 0 in hospital)**
- **Isolated fragment of cDNA (gene of protein E) – nucleotide sequence was shown that it is original variant of WNV**

# Number of publication for West Nile virus (Medline data)



THE CONSTRUCTION COMPANY





# 1937 – discovery of tick-borne encephalitis virus in Far Eastern of Russia

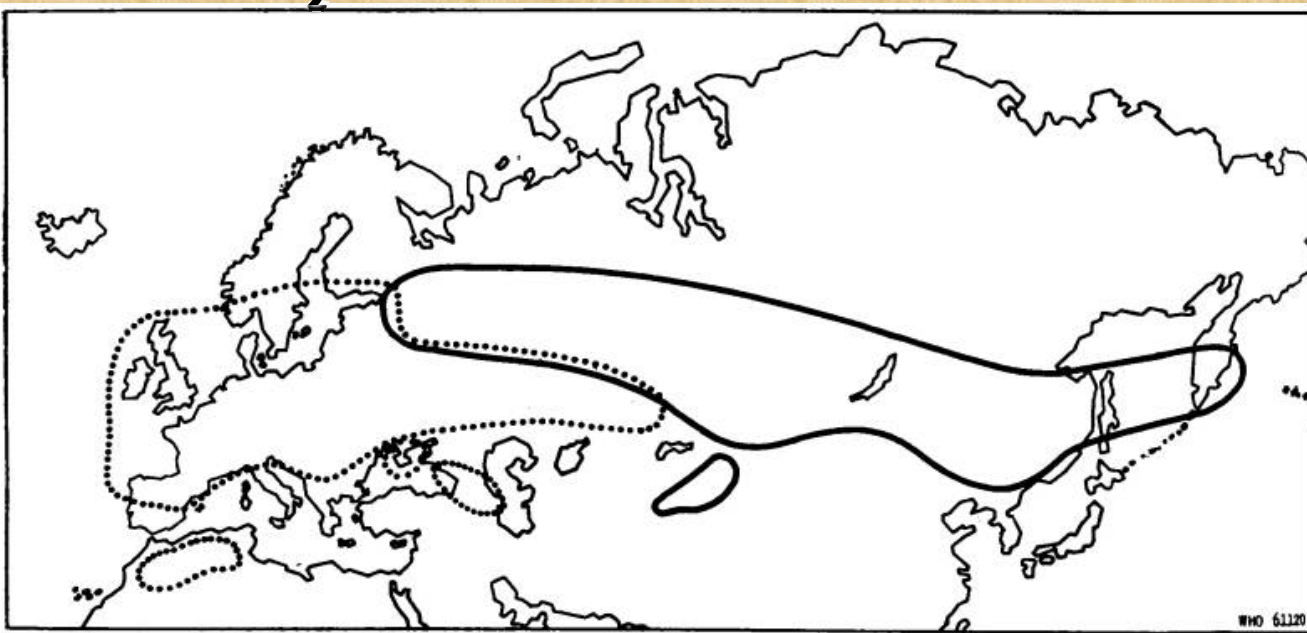


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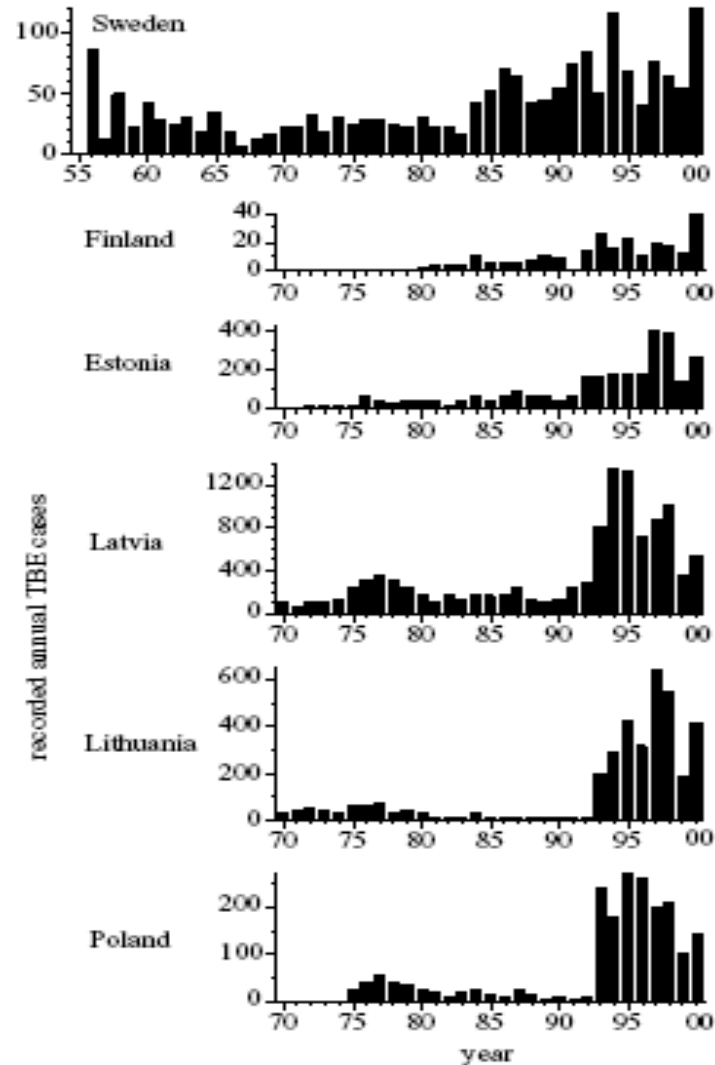


**Approximately 700 million people in more than 30 countries of Europe and Asia (excluding China) live in the areas where TBEV is endemic. The estimated annual incidence of tick-borne encephalitis (TBE) is 14000 cases (excluding China). From this number 11000 TBE cases occur in Russia (Gritsun et al. 2003a). Data on TBE incidence in China are scarce, the only report describes 3500 cases in 1994 (Suss, 2003, Lu et al. 2008).**

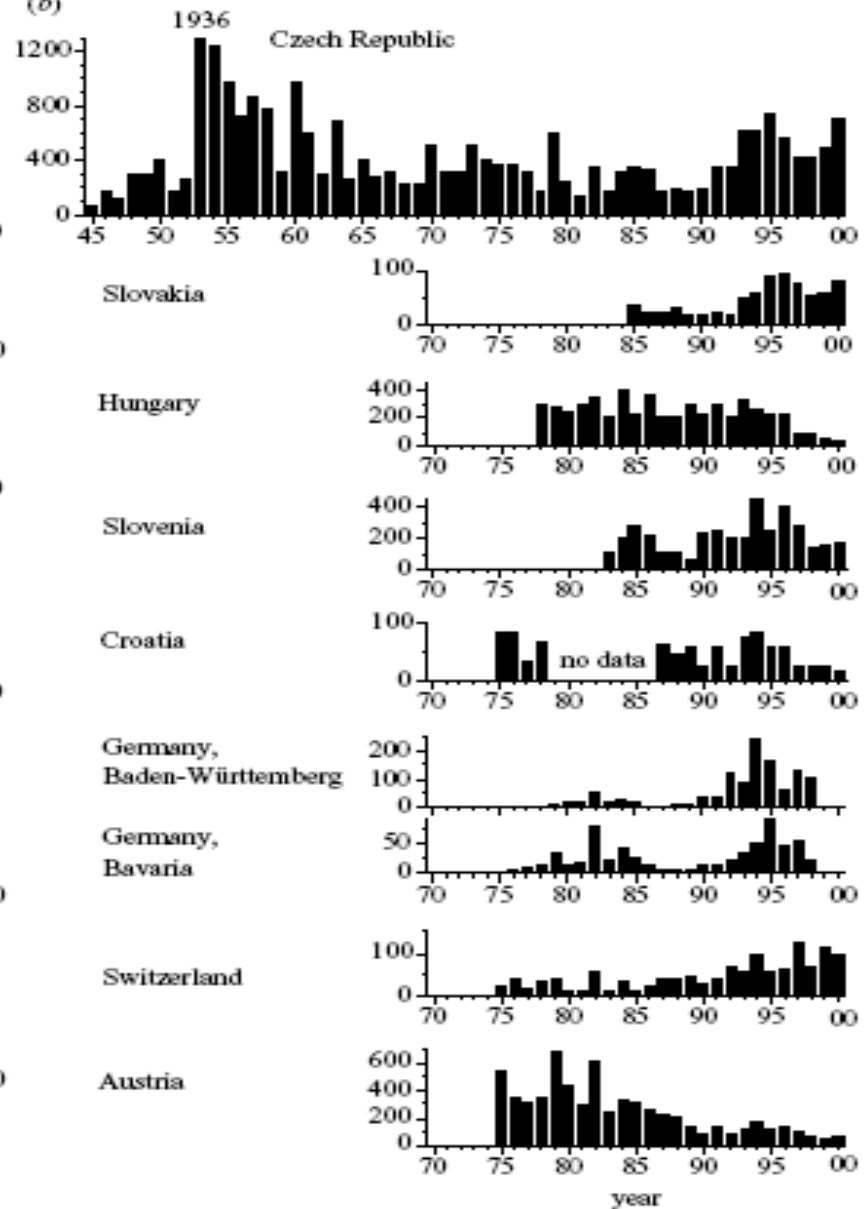


**Distribution of *Ixodes ricinus* (vector of European subtype of TBEV and louping ill virus; *dotted line*) and *Ixodes persulcatus* (vector of Siberian and Far Eastern subtypes of TBEV; *solid line*).**

(a)



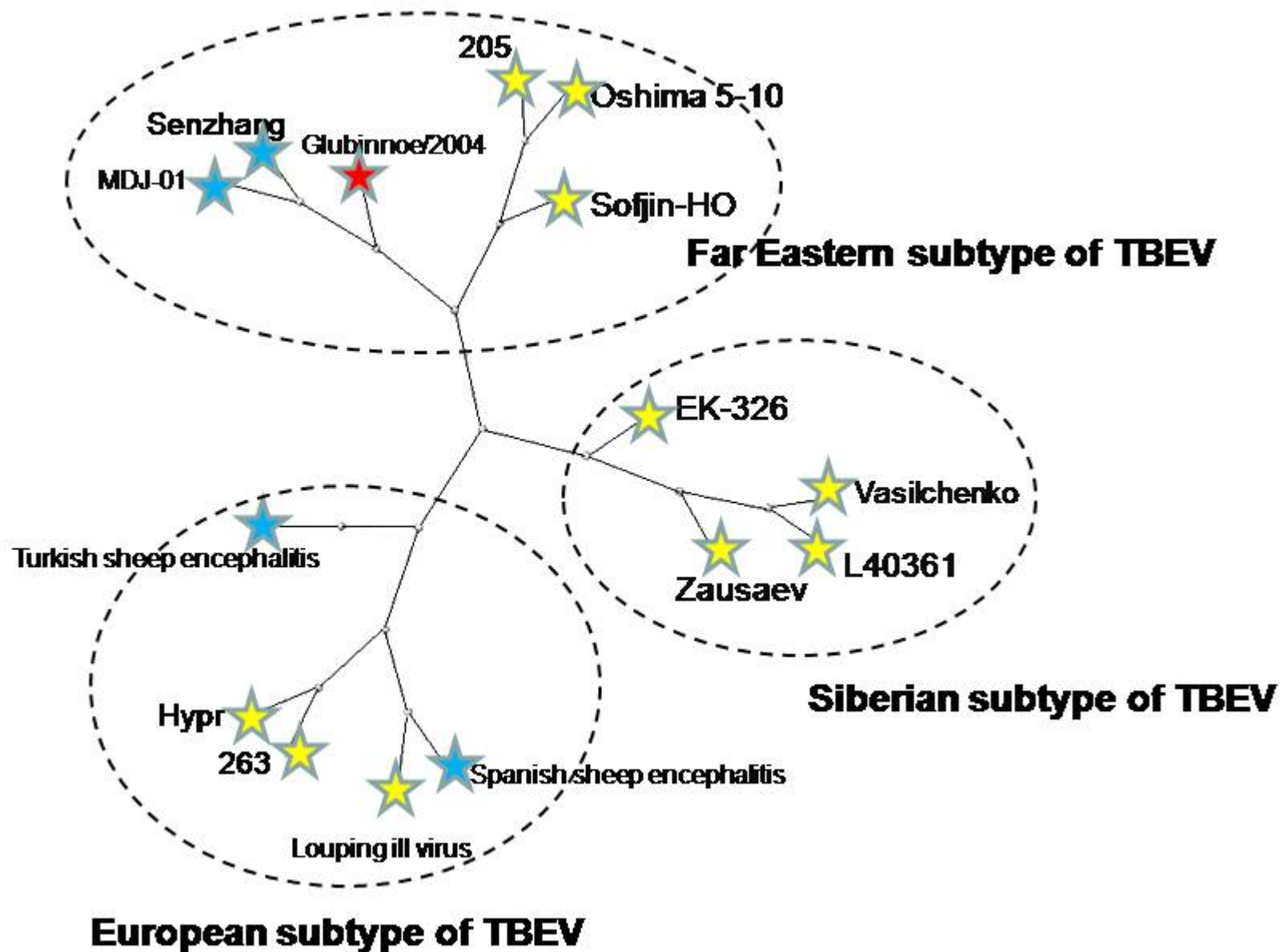
(b)



# Tick borne encephalitis in Europe.



## Phylogenetic tree based on complete polyprotein sequences of TBEV

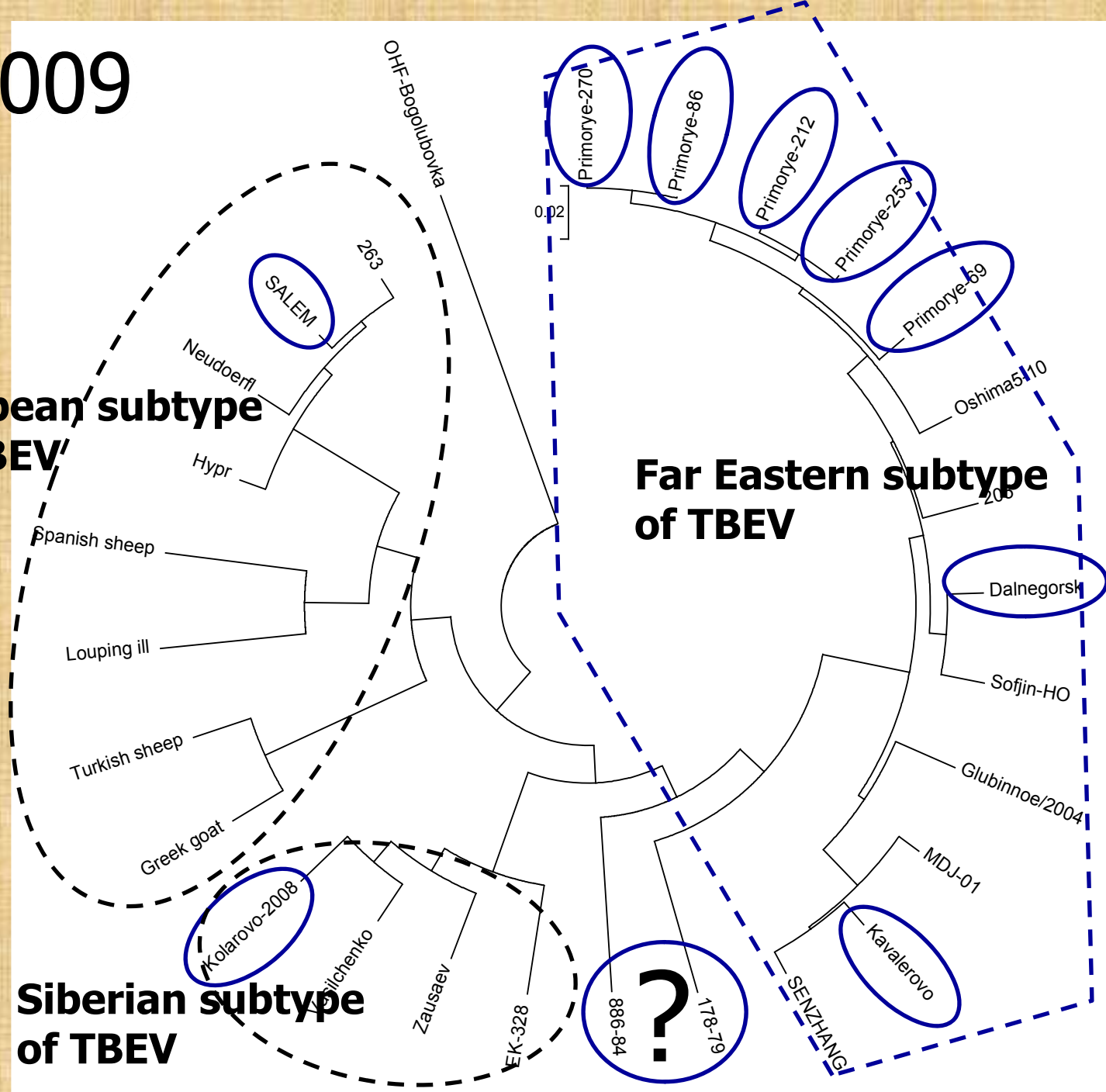


May, 2009

**European subtype  
of TBEV**

**Far Eastern subtype  
of TBEV**

**Siberian subtype  
of TBEV**



# Nucleotide (Nt) and amino acid (A.a) identity (%)

|             |    | Far Eastern Subtype |        |             | Siberian Subtype |         | European Subtype |            |
|-------------|----|---------------------|--------|-------------|------------------|---------|------------------|------------|
| A.a.        | Nt | 205                 | Sofjin | Oshima 5-10 | Vasilchenko      | Zausaev | Hypr             | Neuerdorfl |
|             |    |                     |        |             |                  |         |                  |            |
| 205         |    |                     | 91     | 89          | 81               | 82      | 80               | 78         |
| Sofjin      |    | 94                  |        | 93          | 85               | 85      | 83               | 81         |
| Oshima 5-10 |    | 92                  | 96     |             | 84               | 83      | 81               | 82         |
| Vasilchenko |    | 90                  | 94     | 92          |                  | 93      | 84               | 83         |
| Zausaev     |    | 91                  | 93     | 91          | 97               |         | 85               | 82         |
| Hypr        |    | 89                  | 91     | 90          | 91               | 93      |                  | 94         |
| Neuerdorfl  |    | 87                  | 90     | 90          | 91               | 90      | 95               |            |



# **Hemorrhagic tick-borne encephalitis (Novosibirsk, 1999)**

|                                    |                      |
|------------------------------------|----------------------|
| <b>Age of patient:</b>             | <b>44 - 69 years</b> |
| <b>Latent period</b>               | <b>12,8 days</b>     |
| <b>Start of clinical symptoms:</b> |                      |
| <b>Fever symptoms</b>              | <b>1 day</b>         |
| <b>First hemorrhagic symptom</b>   | <b>7 day</b>         |
| <b>CNS manifestation</b>           | <b>10 day</b>        |
| <b>Massive hemorrhagic</b>         | <b>13-14 days</b>    |
| <b>Death</b>                       | <b>16 day</b>        |



**Homology (%) between the nucleotide acid sequences of  
prototype strains for various subtypes of TBEV and OHF virus**

| Subtypes          | 1<br>European | 2<br>Far<br>Eastern | 3<br>Siberian |              |
|-------------------|---------------|---------------------|---------------|--------------|
|                   | neud          | sofjin              | botsad        | omskhf       |
| neud              | 100.00        | 81.94               | 84.72         | 80.28        |
| sofjin            | 81.94         | 100.00              | 85.28         | 77.78        |
| botsad            | 84.72         | 85.28               | 100.00        | 78.89        |
| <i>koltsovo1</i>  | <i>81.67</i>  | <i>99.72</i>        | <i>85.00</i>  | <i>78.06</i> |
| <i>koltsovo19</i> | <i>80.28</i>  | <i>92.50</i>        | <i>83.33</i>  | <i>76.67</i> |
| <i>koltsovo23</i> | <i>78.61</i>  | <i>91.39</i>        | <i>82.50</i>  | <i>75.00</i> |
| <i>koltsovo29</i> | <i>80.83</i>  | <i>92.78</i>        | <i>83.61</i>  | <i>77.50</i> |
| omskhf            | 80.28         | 77.78               | 78.89         | 100.00       |



## Novel Variant of Tickborne Encephalitis Virus, Russia

Vladimir A. Ternovoi,\* Elena V. Protopopova,\*  
Eugene V. Chausov,\* Dmitry V. Novikov,†  
Galina N. Leonova,‡ Sergey V. Netesov,\*  
and Valery B. Loktev\*

We isolated a novel strain of tickborne encephalitis virus (TBEV), Glubinnoe/2004, from a patient with a fatal case in Russia. We sequenced the strain, whose landmark features included 57 amino acid substitutions and 5 modified cleavage sites. Phylogenetically, Glubinnoe/2004 is a novel variant that belongs to the Eastern type of TBEV.





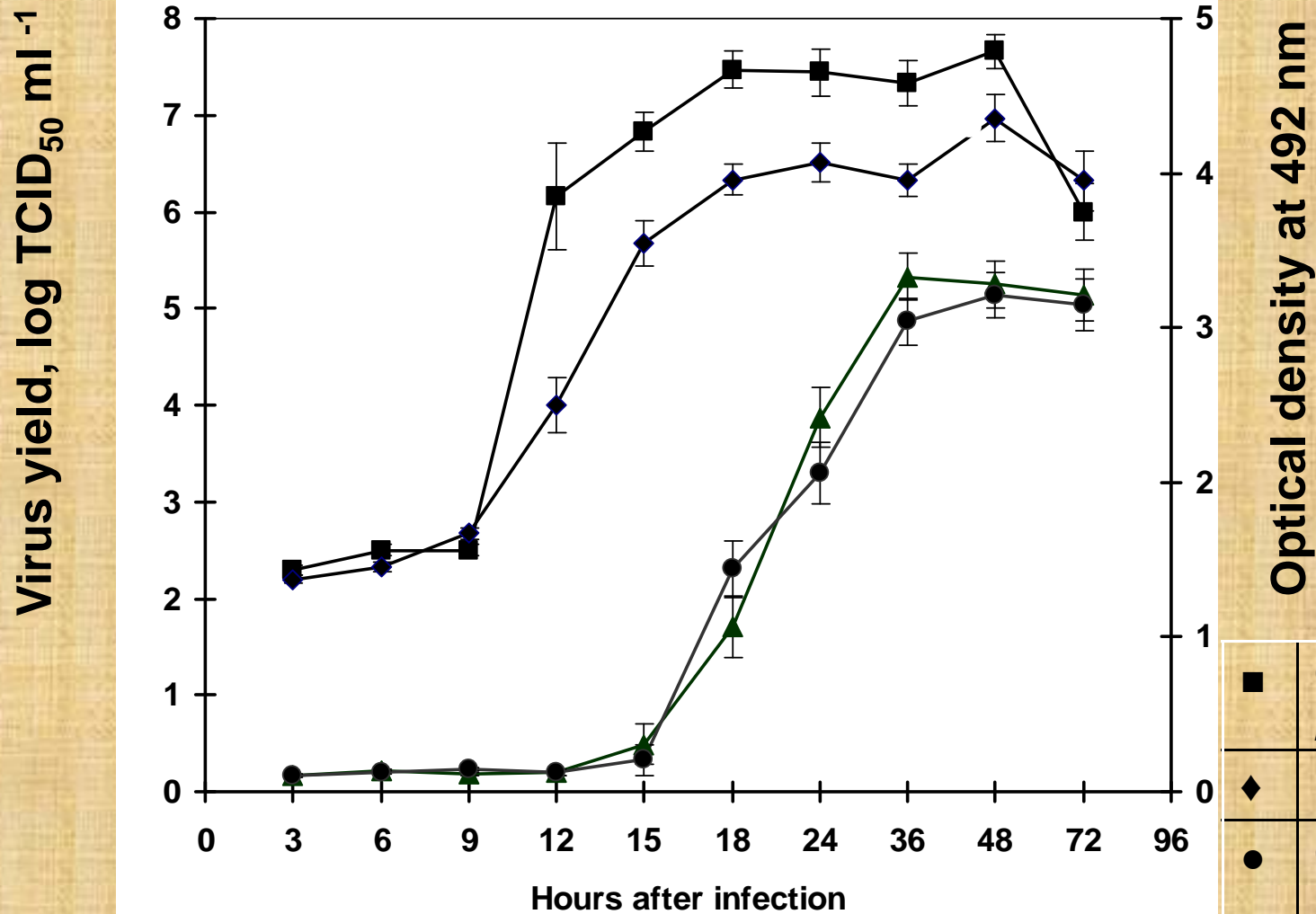
Place of isolation TBEV, 1937

Place of infection,  
Glubinnoe/2004

# Main clinical dates for Glubinnoe/2004

|  |                     |
|--|---------------------|
| <b>Age of patient:</b>   | <b>15 years</b>     |
| <b>Date of byte</b>  | <b>30 May, 2004</b> |
| <b>Latent period</b>   | <b>8 days</b>       |
| <b>Start of clinical symptoms:</b>   |                     |
| <b>Fever symptoms</b>  | <b>1 day</b>        |
| <b>First CNS symptom</b>   | <b>2 day</b>        |
| <b>CNS manifestation</b> (Transportation to<br>Vladivostok by emergency aviation on day 4) | <b>4 day</b>        |
| <b>Coma and respiratory<br/>dysfunction</b>  | <b>5 day</b>        |
| <b>Death (heart failure)</b>   | <b>10 day</b>       |

# Growth curves and E protein synthesis of Glubinnoe/2004 and 205 in PKE cells



|   |                                 |
|---|---------------------------------|
| ■ | Glubinnoe/2004<br>, virus yield |
| ◆ | 205, virus yield                |
| ● | Glubinnoe/2004<br>, ELISA, OD   |
| ▲ | 205, ELISA, OD                  |



## Serological characteristics of Glubinnoe/2004 and 205 with panel MAbs to TBEV.

| MAb               | Target protein<br>(region* of protein E) | Specificity of MAbs  | Titer in ELISA (reverse value) |                |         |        |
|-------------------|--|----------------------|--------------------------------|----------------|---------|--------|
|                   |  |                      | 205                            | Glubinnoe/2004 | WNV     | JEV    |
| 7F10              | N-end                                    |                      | 656100                         | 656100         | -       | -      |
| 6B9               | N-end                                    |                      | ≥656100                        | ≥656100        | -       | -      |
| 10H10             | N-end                                    | HI,<br>groupspecific | ≥656100                        | ≥656100        | ≥656100 | 900    |
| 4F6               | N-end                                    |                      | 656100                         | 656100         | -       | 300    |
| 13F6              | C-end                                    |                      | ≥656100                        | ≥656100        | -       | -      |
| E6B               | C-end                                    | HI, Nt               | ≥656100                        | ≥656100        | -       | -      |
| EB1               | C-end                                    | HI                   | ≥656100                        | ≥656100        | -       | -      |
| 12C7              | C-end                                    |                      | 2700                           | 2700           | -       | -      |
| 7D3               | C-end                                    |                      | 72900                          | 24300          | -       | -      |
| 3C3               | C-end                                    |                      | 8100                           | 24300          | -       | -      |
| 4C7               | ND                                       | groupspecific        | 8100                           | 2700           | 2700    | 2700   |
| 13D12             | ND                                       | groupspecific        | 656100                         | 218700         | 218700  | 218700 |
| E4A               | ND                                       | groupspecific        | 2700                           | 2700           | 300     | 900    |
| 8C6               | ND                                       | groupspecific        | 8100                           | 8100           | 2700    | 2700   |
| Normal mouse sera | -  |                      | -                              | -              | -       | -      |

# Nucleotide (Nt) and amino acid (A.a) identity (%)

| Nt<br>A.a          | Glubinnoe/<br>2004 | 205 | Sofjin | Oshima<br>5-10 | Vasilchenko | Zausaev | Hypr | Neuerdorfl |
|--------------------|--------------------|-----|--------|----------------|-------------|---------|------|------------|
| Glubinnoe<br>/2004 |                    | 91  | 94     | 92             | 85          | 85      | 83   | 81         |
| 205                | 94                 |     | 91     | 89             | 81          | 82      | 80   | 78         |
| Sofjin             | 97                 | 94  |        | 93             | 85          | 85      | 83   | 81         |
| Oshima 5-<br>10    | 95                 | 92  | 96     |                | 84          | 83      | 81   | 82         |
| Vasilchen<br>ko    | 93                 | 90  | 94     | 92             |             | 93      | 84   | 83         |
| Zausaev            | 93                 | 91  | 93     | 91             | 97          |         | 85   | 82         |
| Hypr               | 91                 | 89  | 91     | 90             | 91          | 93      |      | 94         |
| Neuerdorfl         | 90                 | 87  | 90     | 90             | 91          | 90      | 95   |            |

# Amino acid substitutions of Glubinnoe/2004 and 205/Sofjin-HO viruses

C CTHD<sub>pr</sub> M E NS1 NS2a NS2b NS3 NS4a 2K NS4b NS5

|    |    |    |    |     |     |     |     |     |     |    |     |     |
|----|----|----|----|-----|-----|-----|-----|-----|-----|----|-----|-----|
| 96 | 20 | 89 | 75 | 496 | 353 | 229 | 131 | 621 | 126 | 23 | 252 | 903 |
|----|----|----|----|-----|-----|-----|-----|-----|-----|----|-----|-----|

## Comparison with strain 205

|   |   |   |   |   |   |   |   |    |   |   |   |    |         |
|---|---|---|---|---|---|---|---|----|---|---|---|----|---------|
| 0 | 5 | 0 | 1 | 4 | 4 | 5 | 1 | 10 | 2 | 0 | 5 | 16 | Tot: 53 |
| 3 | 5 | 0 | 3 | 4 | 2 | 6 | 1 | 12 | 2 | 0 | 3 | 16 | Tot: 57 |

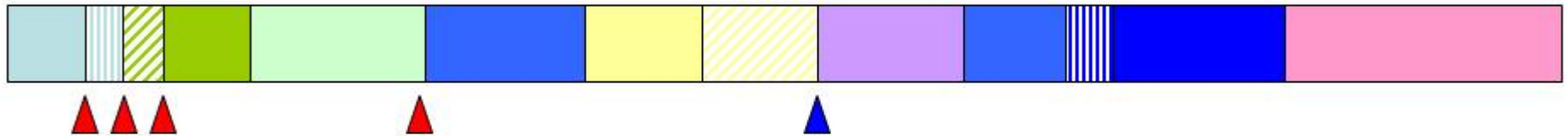
## Comparison with Sofjin-HO

CTHD - C-terminal hydrophobic domain




# Changed putative cleavage sites of Glubinnoe/2004

C CTHD pr M E NS1 NS2a NS2b NS3 NS4a 2K NS4b NS5



 – Putative host cell signalase cleavage site

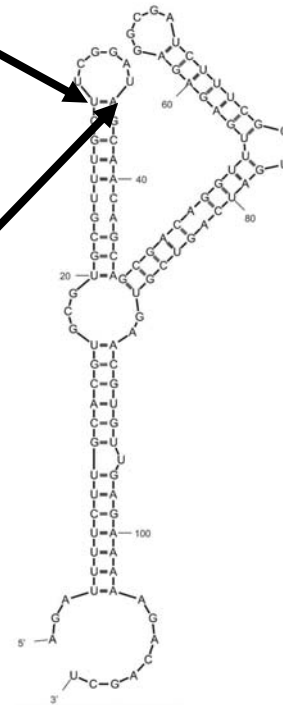
 – Putative viral NS3 protease cleavage site

# Siberian subtype of TBEV

## Putative Site for RNA-RNA pol.

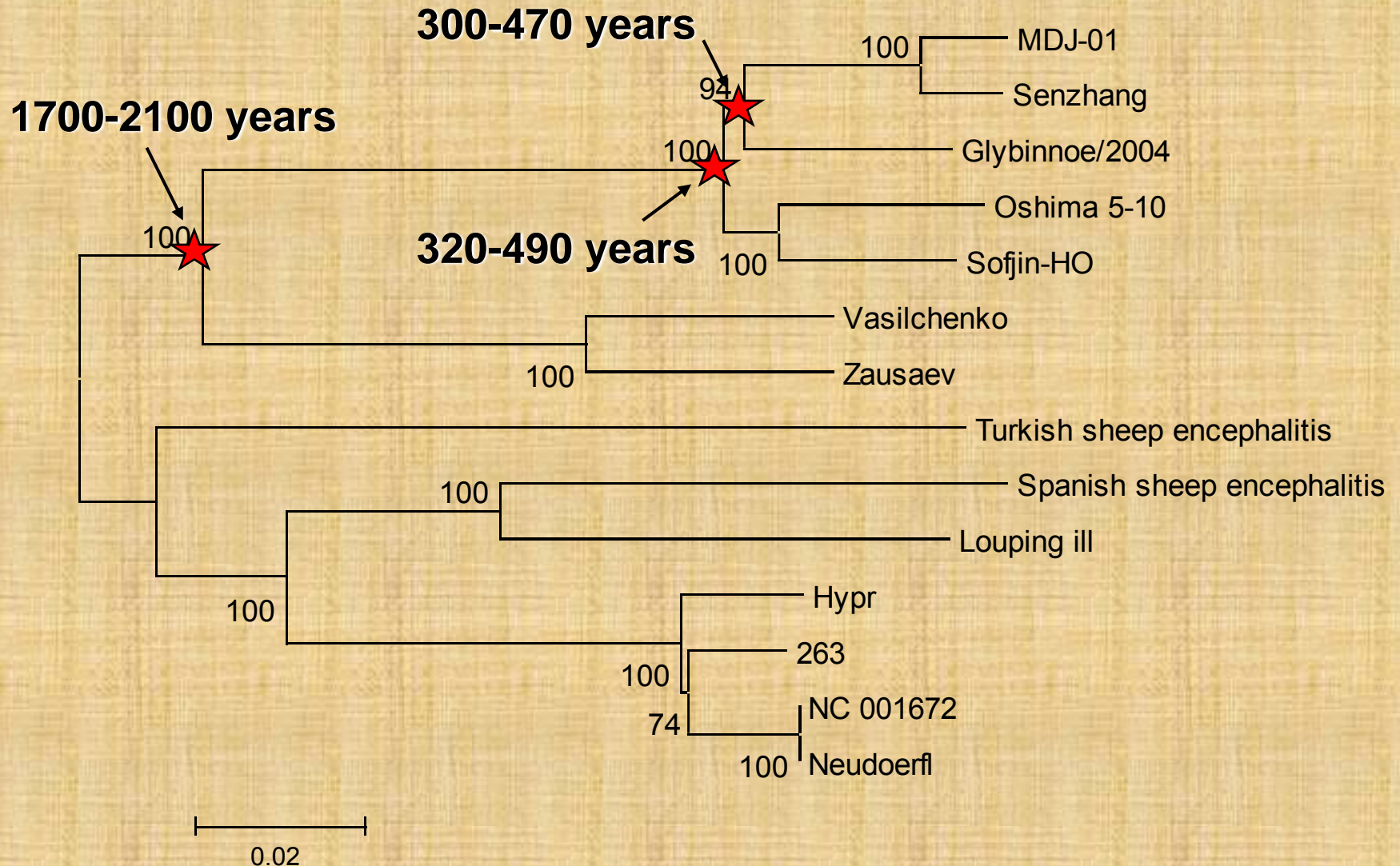
|                             | A1                                       | B1                           | B2                                   | C1      | C2             | A2                     |      |
|-----------------------------|--|------------------------------|--------------------------------------|---------|----------------|------------------------|------|
|                             | *  | *                            | *                                    | *       | *              | *                      |      |
|                             | 20                                       |                              |                                      | 60      | 80             | 100                    |      |
| Zauzaev                     | AGATTTTCTTGCACGTCGCTGCGTTTGCCTTCGGATAGCA | TCACCGCGCAGGTTCCGGAAGAGACATC | CTCTCGTTTCTACTAGTCGTGAACUTGTTGAGAAAG |         |                |                        | 106  |
| Vasilchenko                 | ---                                      | ---                          | ---                                  | ---     | ---            | ---                    | 5    |
| ek328                       | ---                                      | TA                           | C                                    | C       | T              | T                      | 11   |
| EU715139*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 6    |
| EU715140*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715141*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715142*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715143*                   | A T T T                                  | A                            | A                                    | C       | T              | T                      | 9    |
| EU715144*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715145*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715148*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715149*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 3    |
| EU715150*                   | T  | A                            | A                                    | C       | T              | T                      | 4    |
| EU715151*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 2    |
| EU715152*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 6    |
| EU715174*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715154*                   | A  | A                            | A                                    | C       | T              | T                      | 3    |
| EU715155*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 6    |
| EU715156*                   | T  | A                            | A                                    | C       | T              | T                      | 3    |
| EU715157*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 2    |
| EU715158*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 1    |
| EU715159*                   | TA                                       | A                            | A                                    | C       | T              | T                      | 8    |
| EU715160*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 4    |
| EU715161*                   | TAG                                      | A                            | A                                    | C       | T              | T                      | 8    |
| EU715163*                   | ---                                      | A                            | A                                    | C       | T              | T                      | 1    |
| EU715146**                  | ---                                      | A                            | A                                    | C       | T              | T                      | 6    |
| EU715147**                  | ---                                      | A                            | A                                    | C       | T              | T                      | 5    |
| EU715153**                  | ---                                      | A                            | A                                    | C       | T              | T                      | 3    |
| EU715162**                  | A  | A                            | A                                    | C       | T              | T                      | 10   |
| Prot3                       | A  | A                            | A                                    | CC AG G | A              | C C AC                 | 23   |
| Far Eastern subtype of TBEV | ---                                      | ---                          | ---                                  | ---     | ---            | ---                    | 4    |
| 205                         | ---                                      | ---                          | ---                                  | ---     | ---            | ---                    | 17   |
| Glubinnoe                   | ---                                      | C                            | C                                    | AC      | A              | ---                    | 19   |
| ADU-V1                      | ---                                      | ---                          | ---                                  | ---     | ---            | ---                    | -    |
| Oshima5-10                  | A  | ---                          | ---                                  | AC      | A              | C                      | 17   |
| Senzhang                    | ---                                      | ---                          | ---                                  | ---     | ---            | ---                    | -    |
| SofjinNO                    | ---                                      | ---                          | ---                                  | AC      | A              | ---                    | 16   |
| EU715164*                   | ---                                      | ---                          | ---                                  | AC      | A              | ---                    | 17   |
| Prot2                       | T  | GC G AG G                    | C                                    | C       | C              | ---                    | 15   |
| EU715166*                   | T  | A                            | C                                    | AC      | A              | ---                    | 21   |
| EU715167*                   | T  | A                            | C                                    | AC      | A              | ---                    | 21   |
| EU715168*                   | ---                                      | A                            | C                                    | AC      | A              | ---                    | 20   |
| Prot1                       | A  | C                            | C                                    | A       | T AG GT        | ---                    | 8    |
| EU715173*                   | TT                                       | T                            | A                                    | AC      | A              | T AG GT ATCCT -C TG TC | 23   |
| EU715165**                  | TT                                       | A                            | C                                    | AC      | A              | T AG GT ATCCT -C TG TC | 22   |
| EU715169**                  | T  | T                            | T AG GT ATCCT -C T TC                | T       | T T            | ---                    | 24   |
| EU715170**                  | T  | A                            | AA                                   | C       | AC             | A                      | 23   |
| EU715171**                  | T  | A                            | C                                    | AC      | A              | T AG GT ATCCT -C TG TC | 21   |
| EU715172**                  | T  | A                            | C                                    | AC      | A              | T AG GT ATCCT -C TG TC | 22   |
| European subtype of TBEV    | ---                                      | ---                          | ---                                  | ---     | ---            | ---                    | ---  |
| Kypr                        | G  | ---                          | C                                    | TT      | T A A T TC T T | T C                    | 14   |
| 263                         | ---                                      | A                            | C                                    | TTT     | T A T TC T T   | C                      | 13   |
| k23                         | ---                                      | G                            | C                                    | TT      | T A T TC T T   | C C                    | 14   |
| Neudoerfl                   | ---                                      | A                            | C                                    | TT      | T A T TC T T   | C                      | 12   |
| BE83                        | ---                                      | ---                          | ---                                  | ---     | T A TAT T T    | C                      | 53   |
| BE100                       | ---                                      | ---                          | ---                                  | ---     | T A TAT T T    | AGTCGTGAG GTGTAGAG     | GA 3 |
| BE105                       | ---                                      | ---                          | ---                                  | ---     | T A TAT T T    | C                      | 3    |

5' NCR район,  
Глубинное/2004



Strain 205, dG = -31,5 kcal/mole  
Far Eastern subtype

# Phylogenetic tree based on complete polyprotein sequences of TBEV and times of divergence





# Genetic variability 5'NCR of TBEV in individual ticks *Ix.persulctus* and *Ix.pavlovskyi*, Western Siberia, Tomsk

VBZ-2009-0064-Chausov\_1P.3D 08/12/09 4:13pm Page 1

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Type: research-article

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DOI: 10.1089/vbz.2009.0064

Variability of the Tick-Borne Encephalitis Virus Genome  
in the 5' Noncoding Region Derived from Ticks *Ixodes  
persulcatus* and *Ixodes pavlovskyi* in Western Siberia

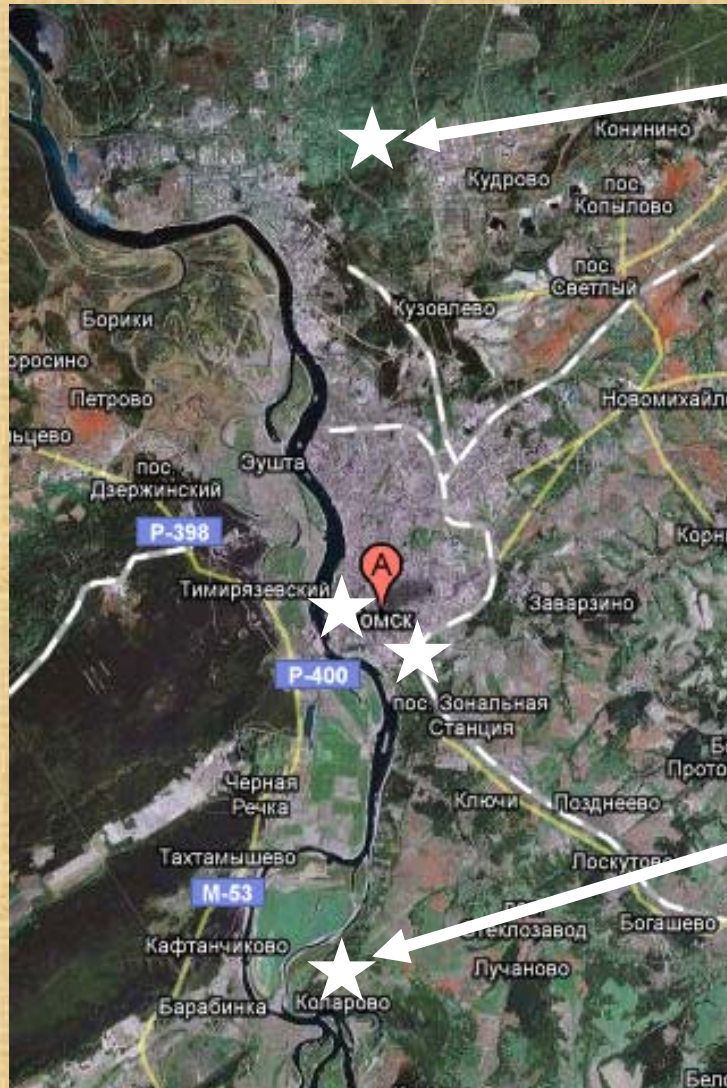
Eugene V. Chausov,<sup>1</sup> Vladimir A. Ternovoi,<sup>1</sup> Elena V. Protopopova,<sup>1</sup> Julia V. Kononova,<sup>1</sup>  
Svetlana N. Kononova,<sup>1</sup> Natalia L. Pershikova,<sup>1</sup> Vladimir N. Romanenko,<sup>2</sup> Nadezda V. Ivanova,<sup>2</sup>  
Natalia P. Bolshakova,<sup>2</sup> Nina S. Moskvitina,<sup>2</sup> and Valery B. Loktev<sup>1</sup>

# Tomsk, places of ticks collection

N

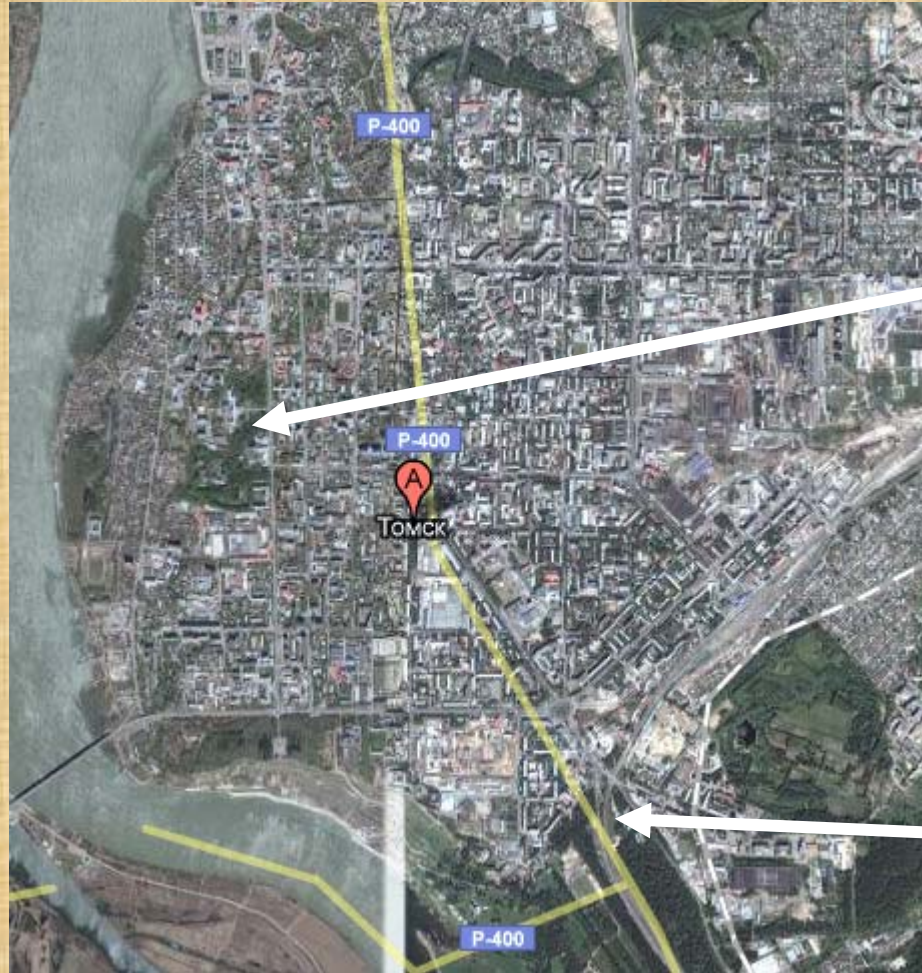


S



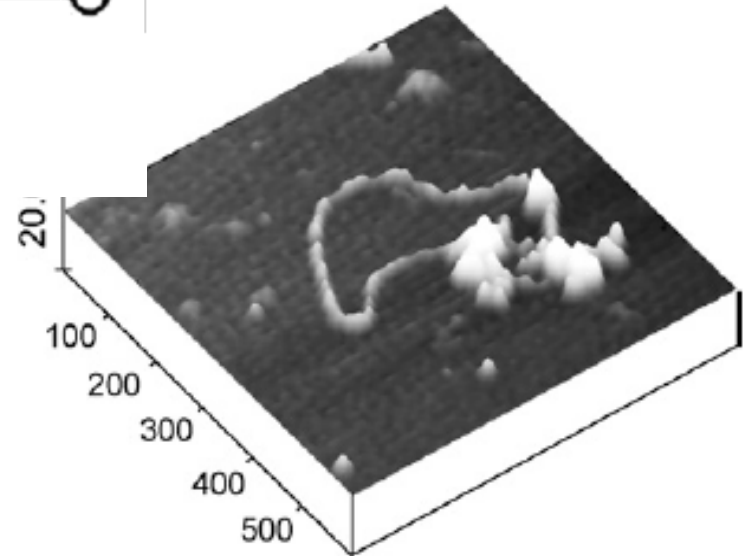
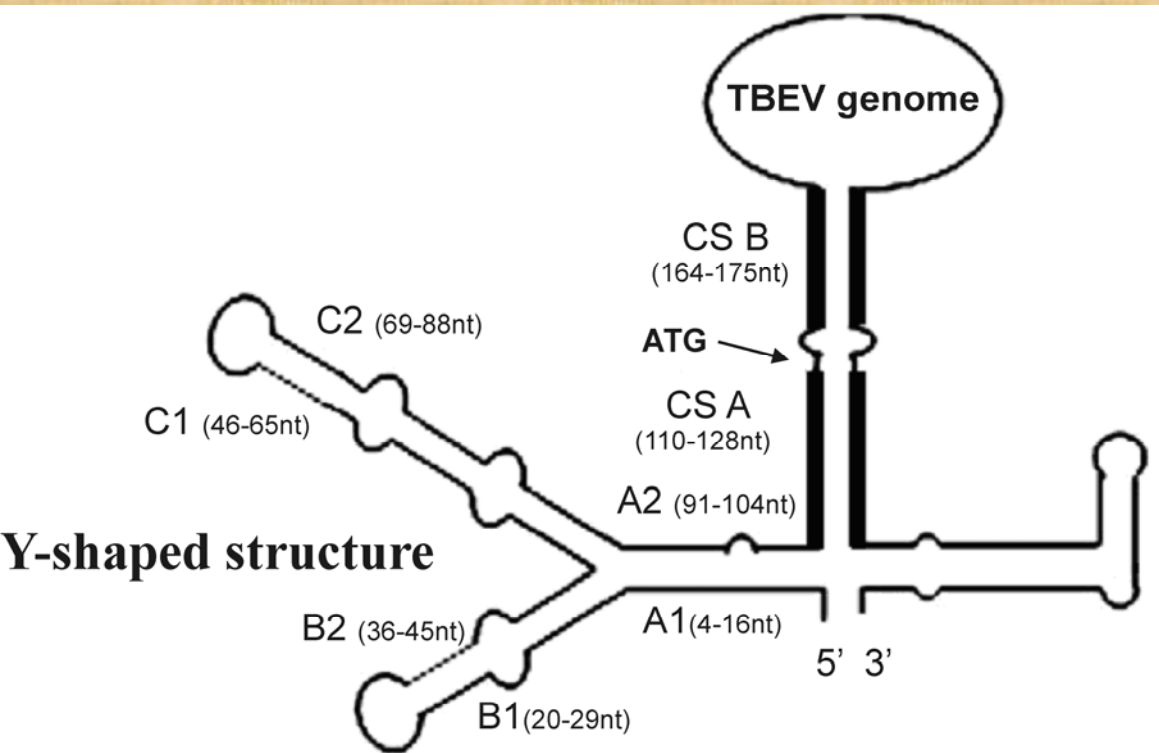


# Tomsk, places of ticks collection, urban foci



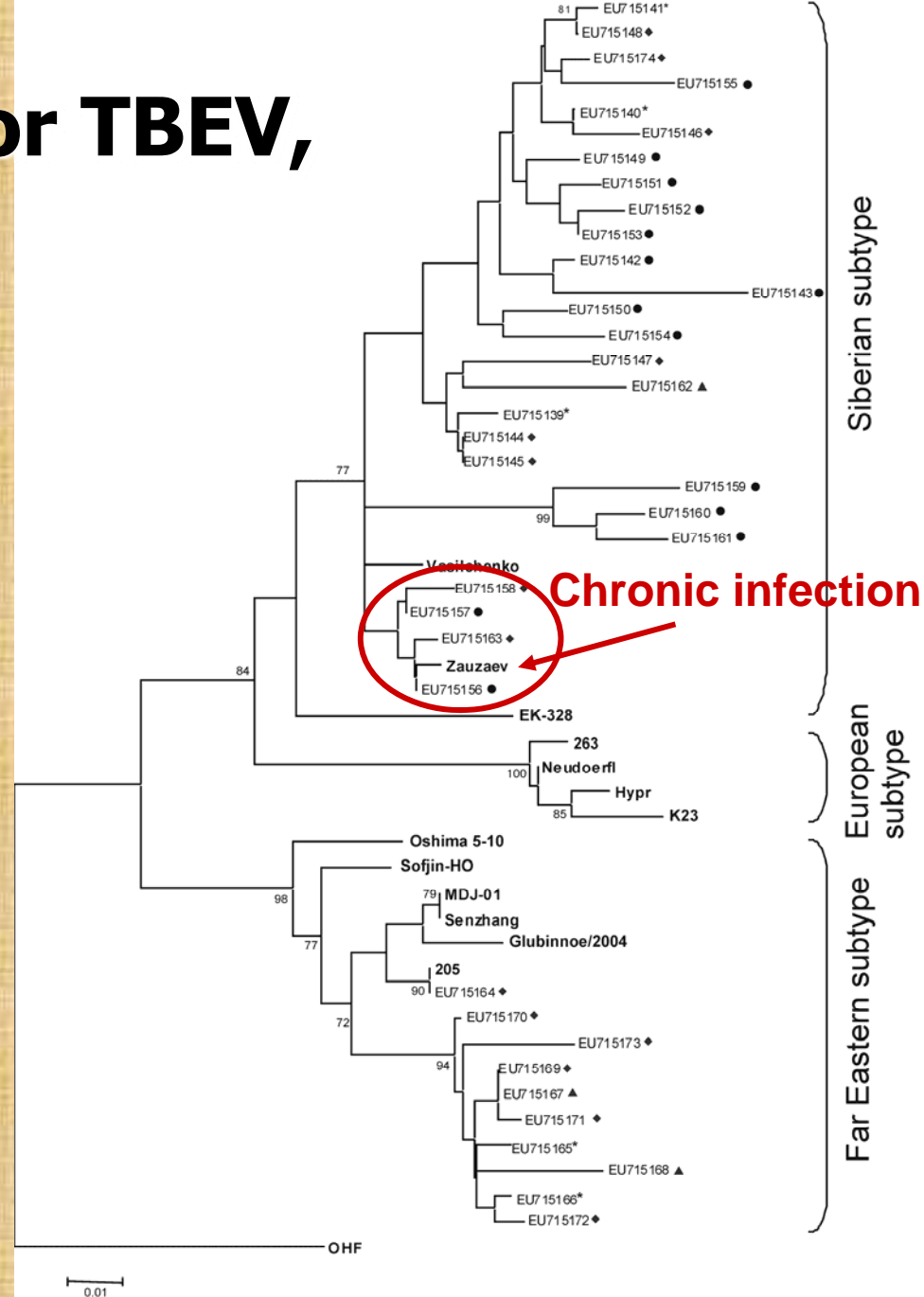


# 5' NCR of TBEV genome

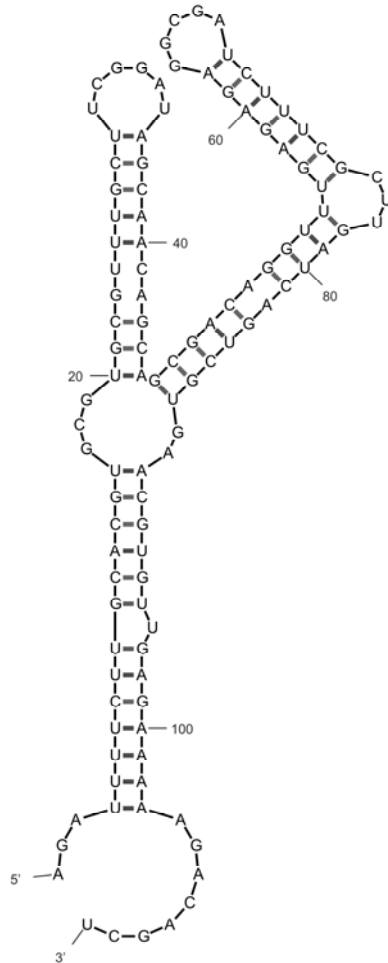


# Phylogenetic tree for TBEV, Tomsk, 2006-2008

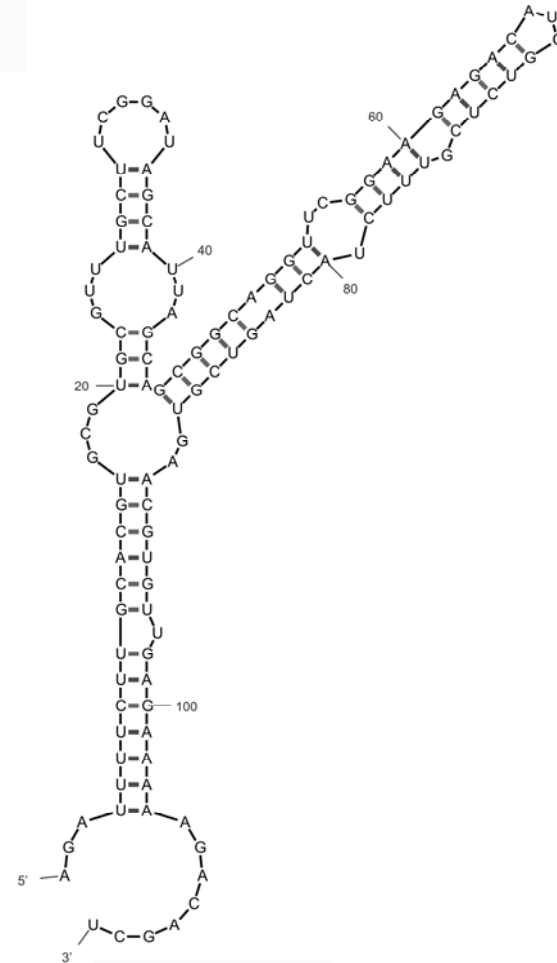
Genotyping of the virus showed that Siberian subtype (89,5%) is predominant in individual ticks of Tomsk suburbs, however, the proportion of Far Eastern subtype in two urban sites reached 47%.



# The Y-shaped structures of 5'-NCR for the different subtypes of TBEV



Strain 205,  $dG = -31,5$  kcal/mole  
**Far Eastern subtype**



Strain Zausaev,  $dG = -32,3$ ; Neudoerf,  $dG = -31,50$   
**Siberian and European subtype**



# Variability of the 5'NCR elements in TBEV from individual ticks

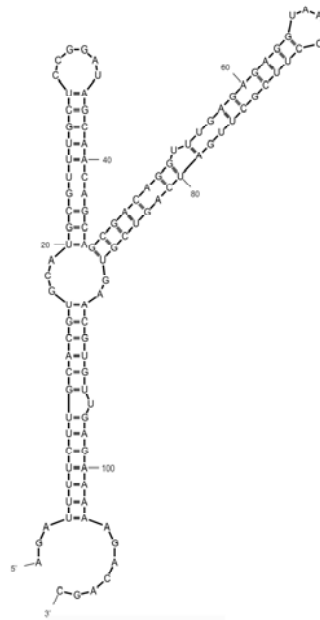
TABLE 1. VARIABILITY OF THE 5'-NONCODING REGION ELEMENTS IN TICK-BORNE ENCEPHALITIS VIRUS FROM INDIVIDUAL TICKS

| <i>5'-NCR elements</i>                 | <i>Percentage of sequences from individual ticks with substitutions</i> | <i>Number of sequences of Siberian/Far Eastern subtype</i> | <i>Number of ticks of Ixodes persulcatus/Ix. pavlovskyi species</i> |
|--|---|--|---|
| Y-Shaped structure                     |   |  |   |
| A1                                     | 13.9  | 3/2  | 4/1   |
| A2                                     | 55.5  | 19/1   | 15/5  |
| B1                                     | 2.8   | 0/1  | 0/1   |
| B2                                     | 0   | 0/0  | 0/0   |
| C1                                     | 80.6  | 18/9   | 19/8  |
| C2                                     | 33.3  | 3/9  | 6/6   |
| Putative site for viral RNA polymerase | 72.2  | 17/9   | 18/8  |
| CS and start codon                     |   |  |   |
| CS A                                   | 8.0   | 3/0  | 3/0   |
| CS B                                   | 22.2  | 6/2  | 6/2   |
| ATG                                    | 0   | 0/0  | 0/0   |

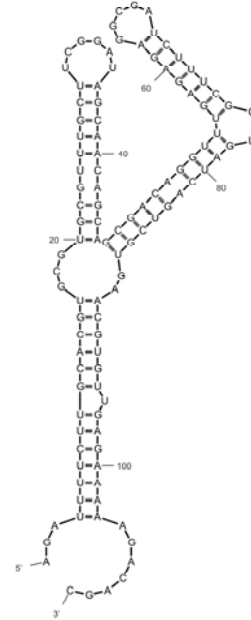
Number of substitutions was calculated by comparing with strain Zausaev for Siberian subtype and with strain 205 for Far Eastern subtype of TBEV.

TBEV, tick-borne encephalitis virus; 5'-NCR, 5' noncoding region; CS, conserved sequence.

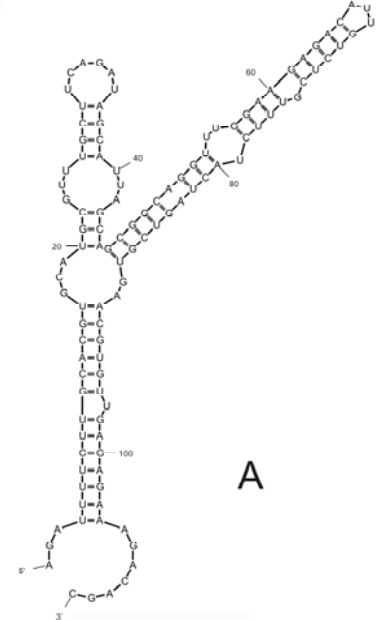
# The Y-shaped structures of the field-isolated (a) and PKE-adapted (b) variants of TBEV.



EU 715168, dG = -29,80

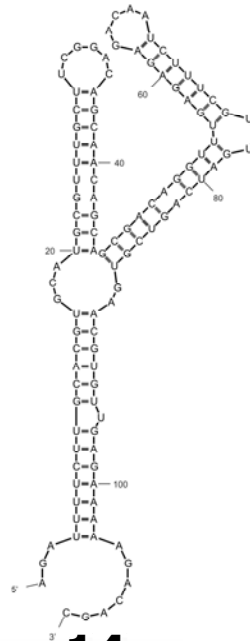


EU 715164, dG = -27,79



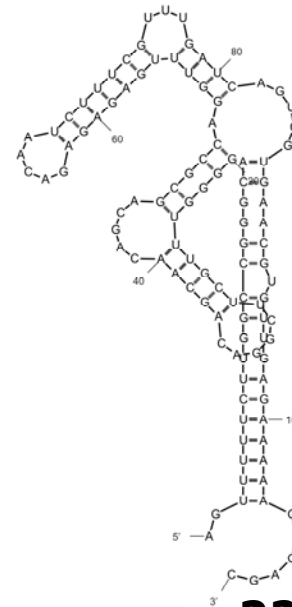
EU 715162, dG = -33,00

A



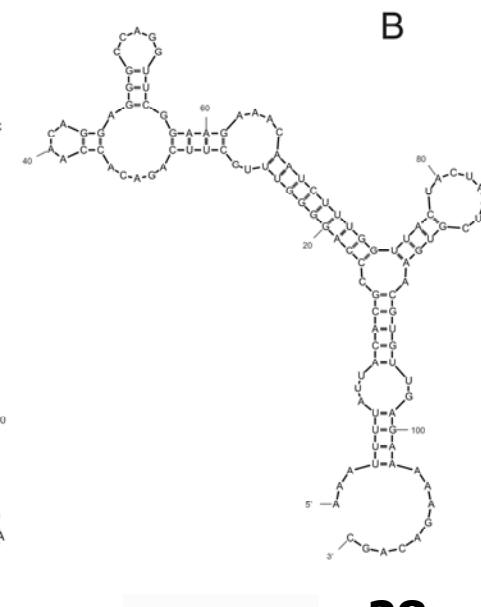
**14**

Prot1, dG = -30,90



**22**

Prot2, dG = -30,90



**28**

Prot3, dG = -20,40

B

Number of substitutions

# **Powassan virus in Far Eastern of Russia**

Arch Virol (2009) 154:811–820  
DOI 10.1007/s00705-009-0376-y

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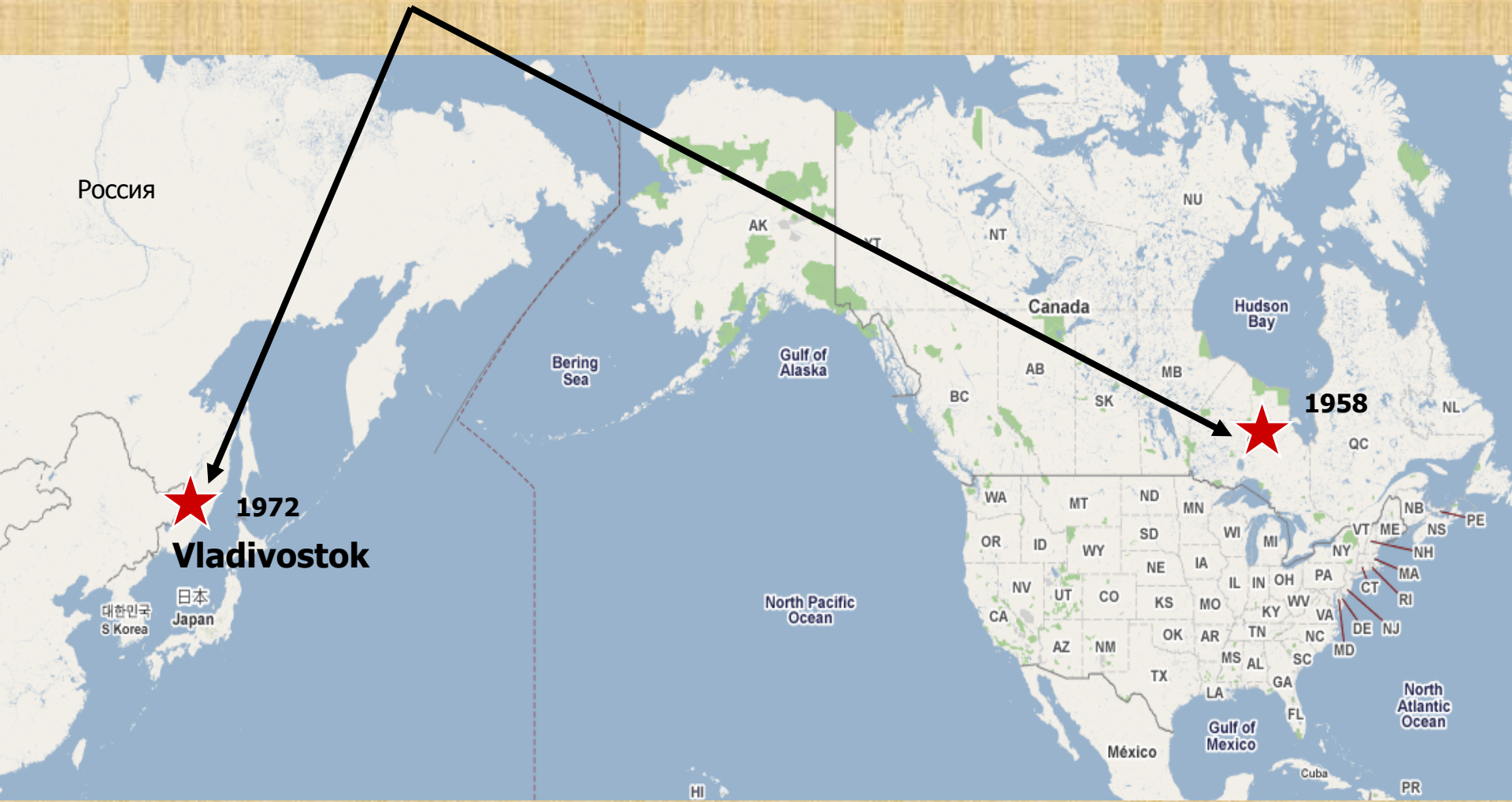
ORIGINAL ARTICLE

## **Characterization of Powassan viruses from Far Eastern Russia**

**Galina N. Leonova · Ilia G. Kondratov · Vladimir A. Ternovoi · Elena V. Romanova ·  
Elena V. Protopopova · Eugene V. Chausov · Elena V. Pavlenko ·  
Elena I. Ryabchikova · Sergey I. Belikov · Valery B. Loktev**



# Powassan virus



# Strains of Powassan virus

**Table 1** Powassan virus strains used in the study

| Strain                      | Place of origin in Primorsky krai   | Year of isolation | Number of passages | Source of isolation                                  | GenBank no. |
|-----------------------------|-------------------------------------|-------------------|--------------------|--|-------------|
| Primorye-40 (P-40)          | Khasan region                       | 1972              | 18                 | <i>Haemaphysalis longicornis</i> Neumann, 1901       | EU978948    |
| Spassk-9 (Sp-9)             | Spassk region                       | 1975              | 4                  | <i>Dermacentor silvarum</i>                          | EU770575    |
| Primorye-109 (P-109)        | Spassk region                       | 1978              | 6                  | <i>Dermacentor silvarum</i>                          | EU978946    |
| Primorye-555 (P-555)        | Island "Russian" (near Vladivostok) | 1978              | 7                  | Blood of infected individual                         | EU978947    |
| Spassk-333 (Sp-333)         | Spassk region                       | 1979              | 9                  | <i>Haemaphysalis japonica douglasii</i> Nutt et Werb | EU978945    |
| Lubitovka-1979 (Lub-1979)   | Lubitovka, Dalnerechinsk region     | 1979              | 4                  | Human brain, lethal case                             | EU978949    |
| Nadezdinsk-1991 (Nad-1991)  | Nadezdinsk region                   | 1991              | 5                  | Blood of infected person                             | EU670438    |
| Partizansk/2006 (Part/2006) | Partizansk region                   | 2006              | 3                  | Blood of infected person                             | EU543649    |

# Cross reactivity for Powassan virus and other flavivirus

**Table 3** Serological comparison of the strain Partizansk/2006 to TBEV, WNV and JEV with MAbs to TBEV and WNV

| MAb              | Target region of protein E | MAb specificity    | Titer in EIA          |        |        |        |
|------------------|----------------------------|--------------------|-----------------------|--------|--------|--------|
|                  |                            |                    | POWV, Partizansk/2006 | TBEV   | WNV    | JEV    |
| MAbs to TBEV     |                            |                    |                       |        |        |        |
| 7F10             | N-ter.                     | HI, Group-specific | 8100                  | 72900  | —      | —      |
| 6B9              | N-ter.                     |                    | —                     | 218700 | —      | —      |
| 10H10            | N-ter.                     |                    | 656100                | 656100 | 656100 | 900    |
| 4F6              | N-ter.                     |                    | —                     | 218700 | —      | —      |
| 13F6             | C-ter.                     |                    | —                     | 656100 | —      | —      |
| E6B              | C-ter.                     | HI, Nt             | —                     | 656100 | —      | —      |
| EB1              | C-ter.                     | HI                 | —                     | 656100 | —      | —      |
| 12C7             | C-ter.                     | Group-specific     | 8100                  | 24300  | —      | —      |
| 7D3              | C-ter.                     |                    | 2700                  | 8100   | —      | —      |
| 3C3              | C-ter.                     |                    | 2700                  | 24300  | —      | —      |
| 4C7              | NR                         |                    | 8100                  | 8100   | 2700   | 2700   |
| 13D12            | NR                         |                    | 24300                 | 656100 | 218700 | 218700 |
| E4A              | NR                         | Group-specific     | 656100                | 656100 | 300    | 900    |
| 8C6              | NR                         | Group-specific     | 72900                 | 218700 | 2700   | 2700   |
| Negative control |                            |                    | —                     | —      | —      | —      |
| MAbs to WNV      |                            |                    |                       |        |        |        |
| 8G8              | N-ter.                     | Group-specific     | 24300                 | —      | 72900  | 72900  |
| 6G5              | N-ter.                     |                    | 8100                  | 24300  | 72900  | 24300  |
| 2H5              | N-ter.                     |                    | —                     | 2700   | 72900  | —      |
| 2B9              | N-ter.                     |                    | —                     | —      | 72900  | —      |
| 2H3              | N-ter.                     |                    | 2700                  | —      | 24300  | 24300  |
| 3A6              | C-ter.                     |                    | —                     | —      | 72900  | 2700   |
| 6H4              | C-ter.                     |                    | —                     | —      | 24300  | 24300  |
| Negative control |                            |                    | —                     | —      | —      |        |



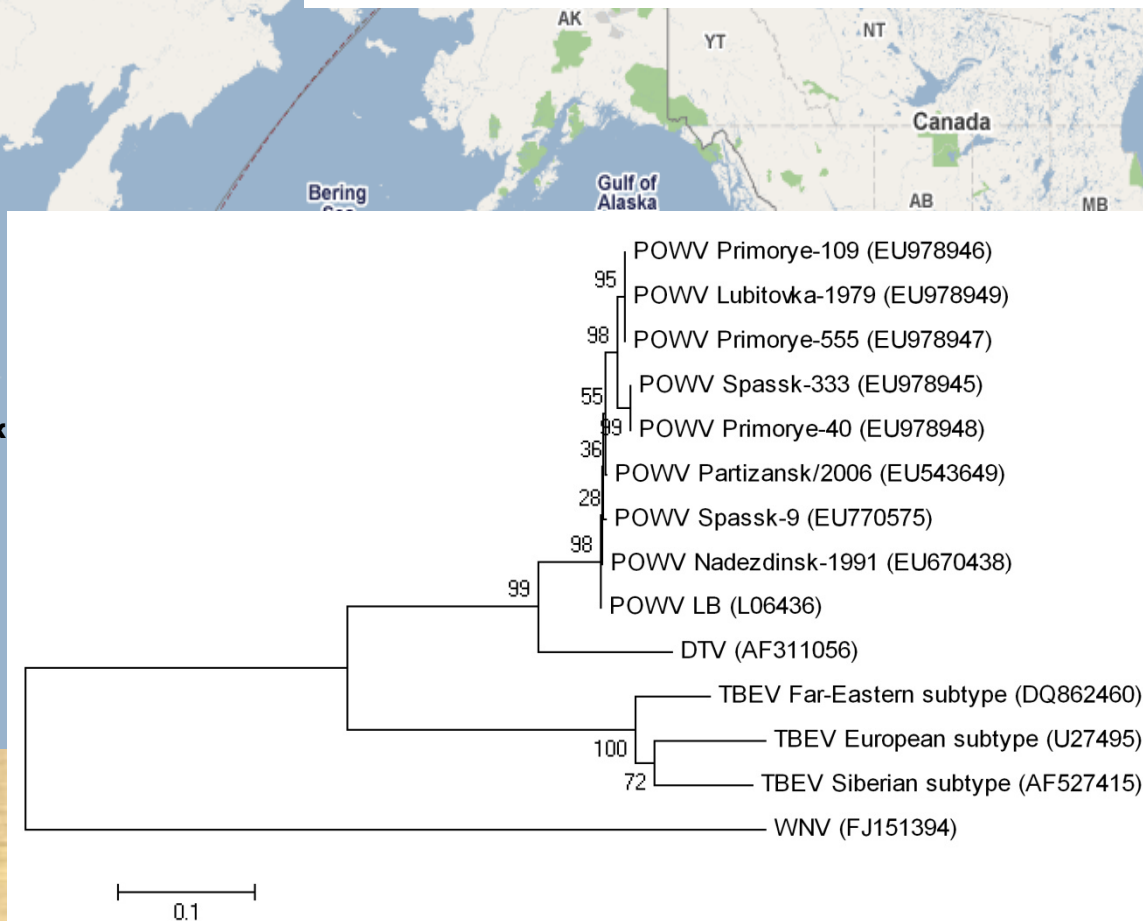
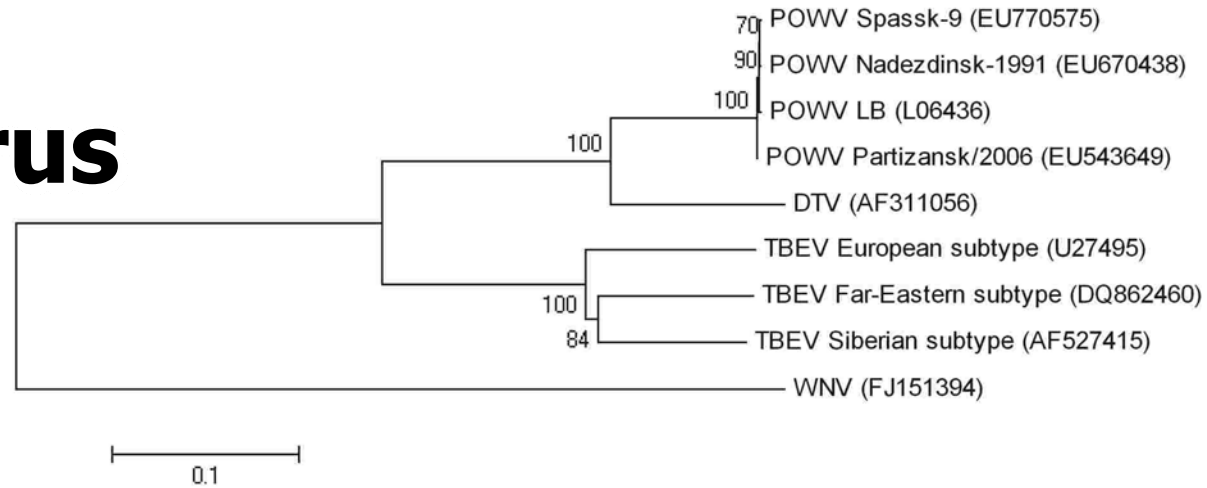
# Powassan virus

**Number of substitution for complete genome in comparison with strain LB (Canada).**

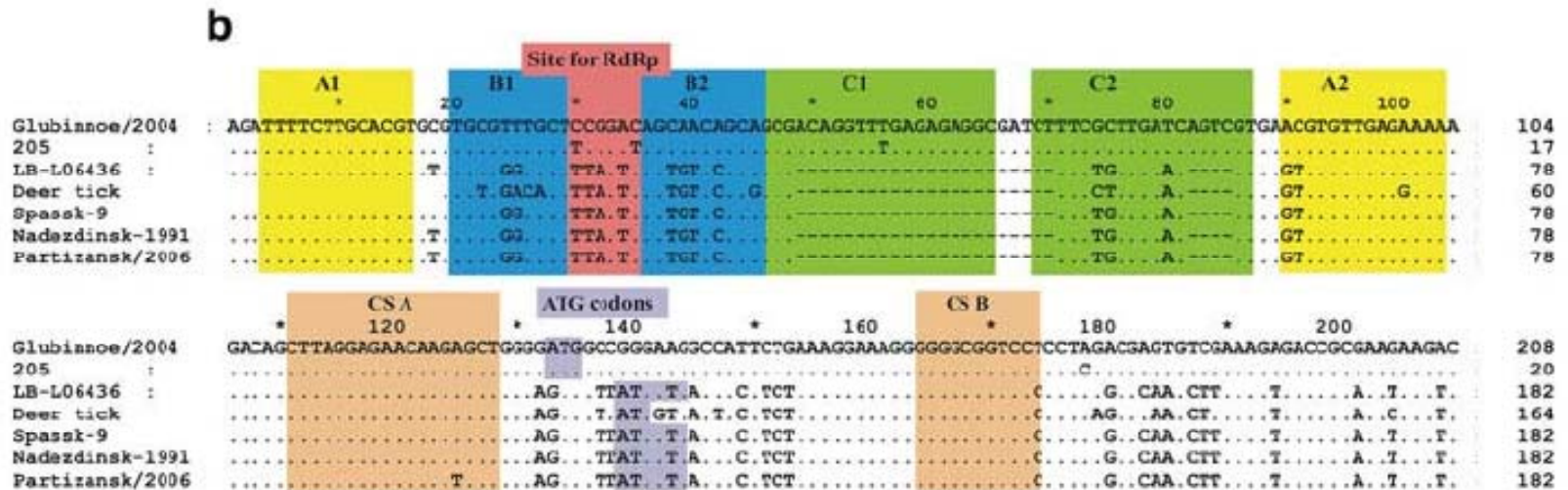
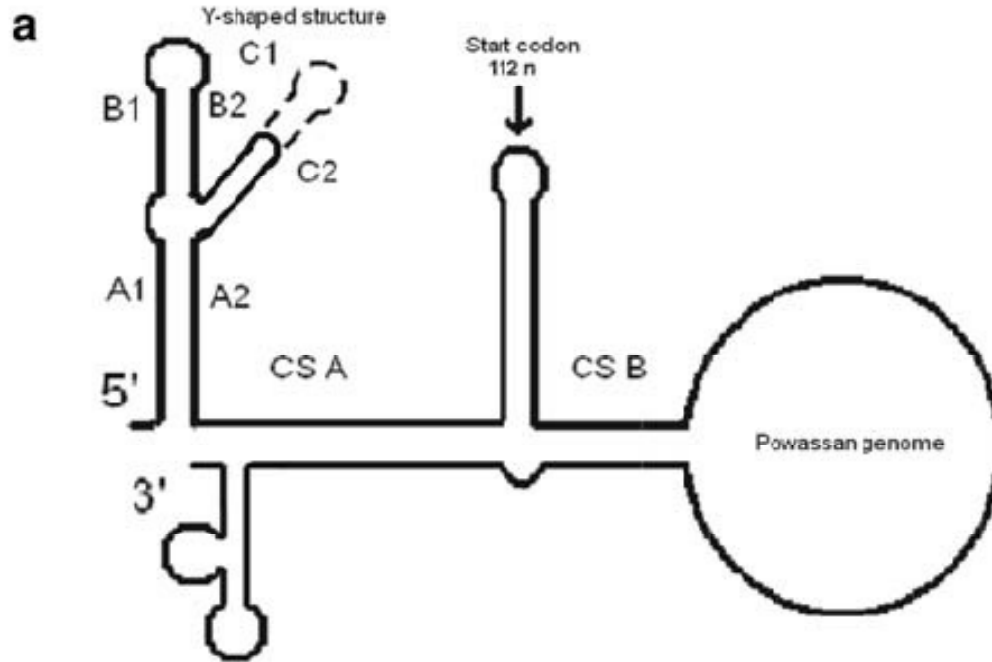
|               | Spassk-9 (1975Г.) |            | Nadezdinsk-1991 |            | Partizansk/2006 |            |
|---------------|-------------------|------------|-----------------|------------|-----------------|------------|
| Substitutions | Nucleotide        | Amino acid | Nucleotide      | Amino acid | Nucleotide      | Amino acid |
| Number        | <b>19</b>         | <b>6</b>   | <b>20</b>       | <b>5</b>   | <b>22</b>       | <b>8</b>   |

**Genome has 10839 nucleotides. Level of homology is near 99,8% for isolates from Russia and Canada**

# Powassan virus

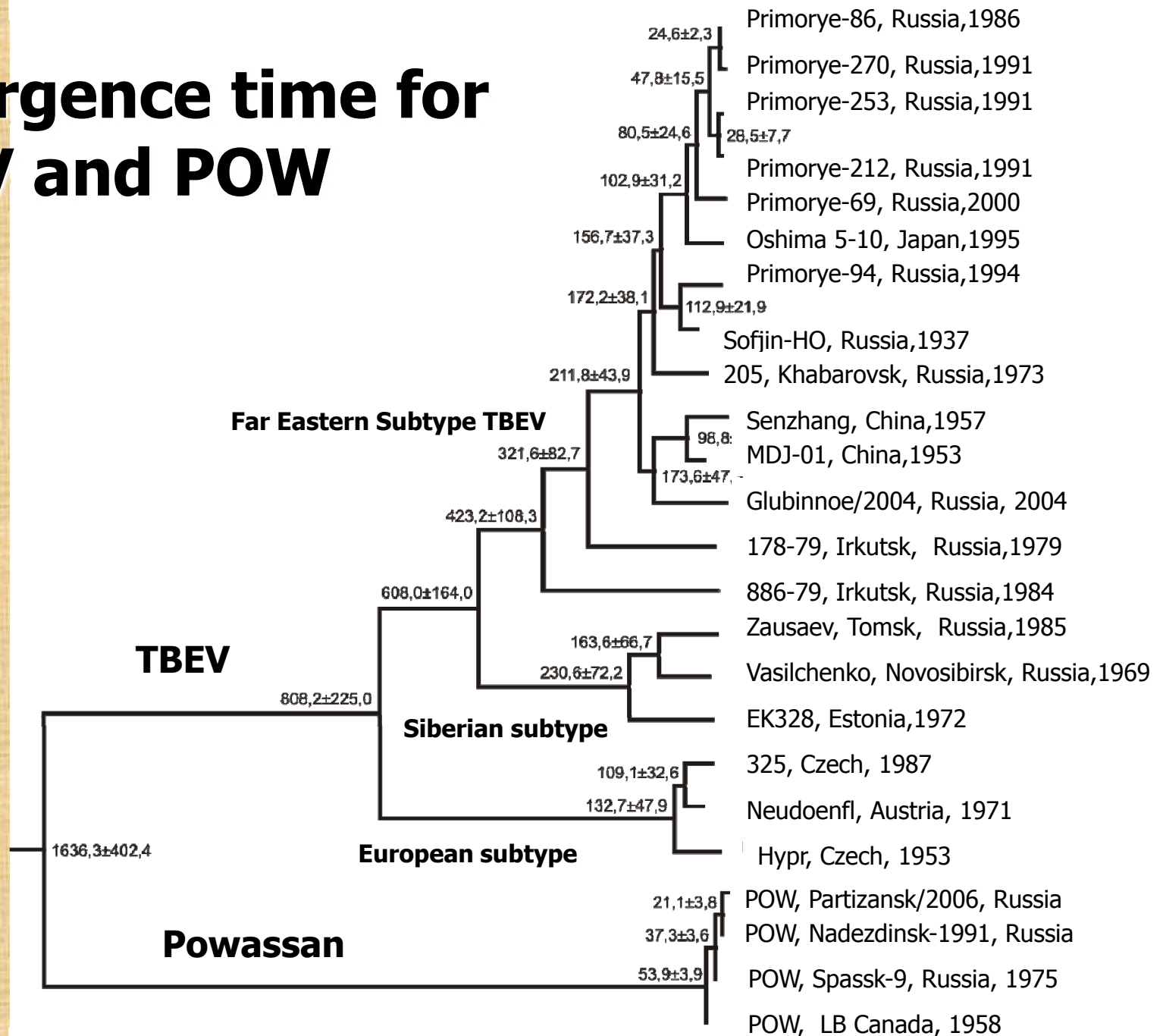


# Powassan virus, 5'NCR region





# Divergence time for TBEV and POW





# Conclusion

- **New genetic variants of flaviviruses and new flaviviruses were found in different regions of World.**
- **Unusual transmission and new variants of WMV, TBEV, and POW were discovered for Asian part of Russia.**
- **A new genetic variants of flaviviruses have become an important factor influencing the organization of the control for these viruses.**

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Novikov D.V.  
Pavlenko E.V.  
Slonova R.A.**

## **1<sup>st</sup> Clinical Municipal Infectious Hospital, Novosibirsk**

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# Thank you for your attention!!!



DON'T WORRY—IT'S ONLY A VIRUS!