

Preview

Louping Ill Virus (LIV) in the Far East: A Perspective

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Abstract | This study focused on finding, culturing, and identifying the biological and genetic characteristics of three Louping ill virus (LIV) strains in the south of the Russian Far East isolated from *Ixodes persulcatus* ticks and one strain was isolated from the blood of a person after a tick bite. Phylogenetic analysis showed that these strains are related to LI/A and LI/K strains isolated in England and Scotland.

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The discovery of tick-borne encephalitis (TBE) in 1937 in the Far East resulted in the emergence of a new concept in the tick-borne viral infection pathology (Zilber, 1939). In the 1960s, new viruses were discovered in different countries, which had similar antigenic, ecological and biological characteristics as that of TBE. The TBE complex viruses were assigned to the *Flavivirus* genus of the *Flaviviridae* family, consisting of 70 classified members that share common physical properties and a common genome structure and organization (Westaway et al., 1985). According to the latest classification (King et al., 2012), this complex list has changed and the following virus groups were included into the genus *Flavivirus* (mammalian tick-borne viruses): Gadgets Gully virus (GGYV); Kyasanur Forest disease virus (KFDV); Langat virus (LGTV); Louping ill virus (LIV); Omsk hemorrhagic fever virus (OHFV); Powassan virus (POWV); Royal Farm virus (RFV); Tick-borne encephalitis virus (TBEV); Meaban virus (MEAV); Saumarez Reef virus (SREV); Tyuleniy virus (TYUV); Kadam virus (KADV). Among the TBE viruses of the Far East, we described a group of Powassan virus strains circulating in the North American continent (L'vov et al., 1974; Leonova et al., 1991). The complete sequencing

of the genome of these strains showed that they were genetically related to the virus Powassan virus strains (Leonova et al., 2009).

In the middle of the 20th century, another TBE complex virus, Negishi virus, drew the attention of researchers, which was isolated in 1948 in Tokyo from a patient with symptoms similar to those of Japanese encephalitis (Ando et al., 1952). The Negishi virus was grouped into the tick-borne encephalitis virus complex upon performing hemagglutination inhibition (HI) and complement fixation (CF) tests (Okuno et al., 1961). After cross-neutralization experiments (Calisher et al., 1989), the Negishi virus was classified as an independent representative of the TBE complex group viruses. At the same time, this virus showed a close unilateral antigenic connection with Louping ill virus. Comparative molecular and genetic research on Negishi virus and Louping ill virus established the phylogenetic affinity of these representatives of the TBE complex group viruses (Venugopal et al., 1992). Negishi virus is currently considered an independent representative strain of the LIV virus.

In this review we present the characteristics of three

strains of the TBE complex group viruses isolated in the Russian Far East, showing affinity with the Negishi virus and Louping ill virus. Two strains (Primorye-155-77 and Primorye-20-79) were isolated from *Ixodes persulcatus* ticks; strain Primorye-185-91 was isolated from the blood of a patient on the second day after tick bite. Ticks infected with Primorye-155-77 and Primorye-20-79 were found in the Spassky district (a plain adjacent to the western slope of Sikhote Alin), and the ticks infected with Primorye-185-91 were found in the suburban forest zone of Vladivostok. These three strains had weak antigenic connections with antibodies in sera against the TBE virus. The cytopathic activity of the virus strains based on the model of intracerebral infection in white outbred mice varied from 6.9 to 8.0 lg LD₅₀/mL. However, after subcutaneous infection of white mice, these three strains had very low levels: 2.9, 2.9 and 3.2 lg LD₅₀/mL. The nucleotide sequences of these fragments were determined and it was found that the analyzed strains are close to Louping ill virus. A new set of primers for amplification of the full genomes analogous to that described (Belikov et al., 2010) was constructed based on sequences of strains 369/T2 and Penrith.

In Western Europe (England, Scotland, Norway, etc.) the Louping ill virus circulates, another virus from TBE complex and the following subtypes were included into the LIV list: British subtype (LIV-Brit), Irish subtype (LIV-Ir), Spanish subtype (LIV-Spain), Turkish sheep encephalitis virus subtype (TSEV) and Greek goat encephalitis virus subtype (GGEV) (King et al., 2012). Whereas TBE viruses widely circulating in Eurasia pose a threat to human health, LIV is most dangerous to sheep as it causes cerebromeningitis in this species. It also infects birds (hazel grouses, black grouses) (Marston et al., 2013) and, to a lesser degree, humans (Ando et al., 1952). There are descriptions of complete genomes only for two LIV strains in the literature. The complete genome of the 369/T2 LIV strain, which was isolated in Scotland for the first time from an *Ixodes ricinus* tick in 1963, was sequenced after a long passage history in the late 1990s (Gritsun et al., 1997). The complete genome of the second LIV strain isolated from the spinal cord of a dead sheep near Penrith, England, in 2009, was published in 2013 (Gao et al., 1994). The Penrith genome is 10,875 nucleotides long with 95.6% identity to the genome of strain 369/T2, despite the different time, host of isolation and passage history.

The time lags between isolating those two LIV strains the Negishi and Primorye-185-91 are 46 years and 43 years, respectively, which allows for evaluating the mutation of this virus circulating under diverse environmental conditions in virtually equal intervals after the discovery of their ancestral forms. Isolation of the Negishi virus in Japan and the identification of a natural focus of LIV in Primorye can probably be attributed to a single period (up to 1950), when there was an active migration of repatriated soldiers, who presumably were infected by a virus Negishi in Manchuria (currently a cross-border region of Russia and China) (Heinz, 2003). The evolution rate of LIV strains on the British Isles and in the Far East do not differ significantly. The similar speed of mutation accumulation in LIV variations in their natural habitat and in introduced Negishi-like variations shows that the evolution of such viruses does not depend on differences in environmental conditions or on the time of infection center emergence. That the distance between LIV strains isolated in their natural habitat is significantly greater than that between the strains introduced in Primorye. After the only case of isolating the Negishi virus from an ill person, Japanese researchers made repeated but unsuccessful attempts to process the circulation of this virus in natural foci on the country's islands (Gaunt et al., 1997). Such research resulted in discovering another affiliated TBE virus on Hokkaido (Smith et al., 1981; Mandl, 2005; Gao et al., 1997), which was not Negishi virus. During tick-borne infections, an ill person does not constitute an epidemiological threat to the wider public, as viral circulation in the foci of natural-focus diseases takes place independently from humans; the human is a biological drop point for the virus (Ogawa et al., 1973). This is probably why human infection caused by Negishi virus did not recur in Japan (Ando et al., 1952).

We do not exclude a similar possibility regarding introduction of the Louping ill virus. Both in Japan and in Primorsky Krai, Negishi-like strains are represented by a single subtype of the Louping ill virus, the British subtype (LIV-Brit), which is indicative of a single introduction of the virus into the Far Eastern region from a single natural focus. Therefore, we have discovered and characterized a group of Negishi-like strains of the Louping ill British subtype virus (LIV-Brit) in Primorye, Far East. The introduced Louping ill viruses together with the Powassan virus described by us earlier are uncommon in natural foci of Pri-

morsky Krai. Obviously, they occupy a very limited ecological niche and not compete with TBEV strains that have existed in this area for a long time.

Apparently, these cases of virus detection in the new territories, uncharacteristic for their usual habitats are no exception to LIV and require further studies using a combination of classical methods of verification with modern molecular genetic research methods.

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